POLYMORPHISM IN HAWKMOTH CATERPILLARS - AN ECOLOGICAL AND BIOCHEMICAL STUDY OF CRYPSIS IN SMERINTHUS OCELLATA (L.) AND LAOTHOE POPULI (L.)

by

JOY C. GRAYSON B.Sc.

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DECLARATION

The work presented in this thesis was carried out in the School of Applied Biology, Lancashire Polytechnic (formerly Biology Division of Preston Polytechnic). Unless otherwise stated it is the original work of the author.

While registered as a candidate for the degree of Doctor of Philosophy, for which submission is now made, the author has not been a registered candidate for another award of the C.N.A.A. or of a University. This thesis has not been submitted in whole, or in part for any other degree.

J. GRAYSON

POLYMORPHISM IN HAWKMOTH CATERPILLARS - AN ECOLOGICAL AND BIOCHEMICAL STUDY OF CRYPSIS IN SMERINTHUS OCELLATA (L.) AND LAOTHOE POPULI (L.)

Extensive field surveys in Lancashire and Merseyside have shown that for both Smerinthus ocellata (the eyed hawkmoth) and Laothoe populi (the poplar hawkmoth) there is a correlation between larval and foodplant coloration.

For L. populi, experiments have shown that foodplant determines the colour of full grown caterpillars. Siblings reared on Salix fragilis L. (which has green leaves) became yellow-green, intermediate-green or dull-green whereas those reared on Populus alba L. became either white or green. Further experiments have shown that reflected light intensity may be the vital cue which determines whether a caterpillar becomes white or green. Thus for this species the polymorphism is environmentally rather than genetically determined. However, the genetic background appears to have some effect on the proportion of dull-green and intermediate-green morphs in broods.

For S. ocellata, both laboratory and field experiments have shown that the coloration of caterpillars is also determined by some environmental factors related to light. There is no simple genetic or nutritional control of larval colour in this species.

Pigment extraction and analysis have shown that the proportions of different carotenoids in the main foodplants are very similar, as are the chlorophyll a to b ratios and carotenoid to chlorophyll ratios. The principal carotenoid in the food of both species of caterpillar, lutein, is sequestered by the insects in the integument and contributes to the animal's coloration. Cis-lutein is also present in small quantities in the integuments of both L. populi and S. ocellata caterpillars. Yellow-green L. populi larvae contain more lutein in the integument than dull-green morphs and in white caterpillars this carotenoid is barely detectable.

Field predation experiments indicated that white caterpillars of L. populi are at a selective advantage compared with yellow-green morphs on the white undersurface of P. alba leaves. However, both white and green morphs suffered similar predation on S. fragilis bushes.

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CHAPTER 1

INTRODUCTION

1.1. Crypsis and Colour Change in Animals

An animal is said to be cryptic when it cannot easily be distinguished from its background in its natural environment (Cott, 1940). Well known examples of cryptic animals are green phytophagous insects, such as caterpillars and grasshoppers, resting on green leaves or grass and white arctic species, such as the ptarmigan (Lagopus mutus) and the snowshoe hare (Lepus americanus), resting on snow.

The advantage of being cryptic in terms of reduced predation has been demonstrated by various workers (Di Cesnola, 1904; Eisentraut, 1927; Isley, 1938; (all summarised in Edmunds, 1974), Sumner, 1935; Kettlewell, 1956; Baker, 1970; Wiklund, 1975).

There are, however, several limitations to crypsis as a means of anti-predator defence in animals. Firstly, to remain undetected a cryptic animal must not move and this may conflict with essential activities such as feeding and reproduction. To overcome this, cryptic animals tend to move slowly by day and often limit feeding and mating to night-time.

Secondly, predators which hunt by searching image may quickly learn to find cryptic prey. This, however, is unlikely to occur if the prey species is widely spaced and therefore rarely encountered. Polymorphism is a method by which scattered cryptic animals may become more common without suffering increased predation (Croze, 1970).

A third limitation of crypsis is that an animal may be very conspicuous if it rests in the wrong place. Thus, many cryptic animals have behavioural mechanisms which ensure that they rest in the places where they are best camouflaged (Kettlewell and Conn, 1976; Gillis, 1982; earlier work summarised in Edmunds, 1974).

One way by which an animal can be cryptic in a number of different environments is by changing its colour. Some cephalopods, lizards and teleost fish can change their colour and patterning rapidly and reversibly to match different substrates. A number of arctic birds and mammals show seasonal changes in colour with a brown summer plumage, cryptic on earth and sparse vegetation, and a white winter plumage, cryptic on snow (Cott, 1940). Many insects are also able to change colour but this can only usually occur at moulting (Edmunds, 1974).

Background colour matching can be achieved in a variety of different ways. For some animals it appears to be the quality of reflected light which determines colour. For example, the female of the crab spider Misumena vatia (Clerck) changes from white to yellow when placed on yellow paper and back to white if placed on white paper (Gabritshevsky, 1927). Colour change in the larvae of the grasshopper Acrida turrita is also initiated by a change of substrate colour (Ergene, 1950, summarised in Edmunds, 1974). However, these animals may be responding to a difference in the intensity of reflected light rather than its colour. Various frogs change their hue in response to different coloured backgrounds (Cott, 1940). Nielson (1979, 1980) has shown that green morphs of Rana esculenta L. and

Hyla arborea L. adapt to the lightness of the background rather than its hue. He suggests that these frogs may lack colour vision.

For some grasshoppers, the critical factor determining whether an animal changes colour is the quality of incident radiation rather than reflected radiation (Rowell, 1971). In other species, a change in colour is caused by a change in humidity. For example, in the grasshopper Syrbula admirabilis L., green nymphs can be induced to turn brown if placed in a dry environment or if fed on a diet with low moisture content (Otte and Williams, 1972). The hercules beetle Dynastes hercules L. also changes colour in response to changes in ambient humidity, but this may be of adaptive significance in terms of thermoregulation rather than crypsis (Hinton and Jarman, 1972).

The alpine grasshopper Kosciusola tristis Sjöst. shows a physiological colour change under the control of temperature (Key and Day, 1954) which is probably also of ecological significance in terms of thermoregulation.

Many mantids are dimorphic for colour in the nymphal stage of development. The two most important environmental factors which determine whether an insect becomes green or brown are relative humidity and incident radiation. The relative importance of these two factors has been shown to vary in different species (Barnor, 1972, in Edmunds, 1974).

Colour change in eolids such as Favorinus branchialis (Rathke) and Aeolidia papillosa (L.) is simply due to a change in diet. Hence Favorinus appears yellow a few hours after feeding on yellow snail eggs and pale red after feeding on red eggs (Haefelfinger, 1969). A. papillosa

appears red after feeding for a few days on the red anemone Actinia equina (L.) and is then cryptic amongst a colony of Actinia (Edmunds, 1974).

1.2. Polymorphism

Polymorphism in body colour and pattern occurs in a wide variety of animals from crustaceans to mammals (Mayr, 1963). Many cryptic animals are polymorphic, and in species which occur at very high density, such as the sandy beach bivalve Donax it is difficult to find two individuals of the same pattern (Moment, 1962). Polymorphism can be regarded as a means of increasing the density of a population in a particular habitat without increasing the numbers killed by predators (Edmunds, 1974). Alternatively, polymorphism may be of adaptive significance in terms of thermoregulation. Watt (1968) investigated pigment polymorphism in Colias butterflies and suggested that the darkening due to melanin deposition may be an adaptation for increased efficiency of absorption of solar radiation for heating.

In many species of Lepidoptera that rely on being cryptic for their protection against visually hunting predators, the larvae, pupae and/or adults may be polymorphic in colour. The morphs may be either genetically or environmentally determined. In moths with industrial melanic forms such as the peppered moth, Biston betularia (L.), colour is determined genetically and the morph frequencies are maintained by selective predation (Kettlewell, 1956).

Some mimetic butterflies are also polymorphic for

colour. For example, the female of the tiger swallowtail, Papilio glaucus L. is either yellow or black. Whilst the former morph resembles the male of the species the latter is a Batesian mimic of the aposematic butterfly Battus philenor (L.). The butterfly Limenitis arthemis astyanax Fabr. is also dimorphic, mimicking B. philenor in regions where the latter is common but where Battus does not occur Limenitis is non-mimetic and disruptively cryptic in colour (summarised in Edmunds, 1974).

The aposematic African monarch (Danaus chrysippus L.) is polymorphic having four genetically determined morphs. Consequently, its Batesian mimic, the female of Hypolimnas misippus (L.) also has four colour morphs each one resembling closely a morph of D. chrysippus (summarised in Edmunds, 1974).

In the butterfly Colias philodice Godart, a colour mutation has been shown to affect all four stages in the life cycle, but is most pronounced in the larval stage where three discontinuous morphs have been recognized (Gerould, 1921; 1926).

The next two sections (1.3 and 1.4) review pupal and larval polymorphisms in Lepidoptera.

1.3. Pupal Polymorphism

The role of environmental factors in the determination of pupal colour in relation to camouflage in Lepidoptera has been studied by many workers. Poulton (1886a, 1887, 1888 and 1892) showed that pupal colour in Vanessa urticae L., Pieris brassicae L. and P. rapae L. varies with the colour of the substrate. Further work by Merrifield and

Poulton (1899) showed that many polymorphisms for green or brown forms are environmentally determined by the colour of the background on which they rest.

In Pieris brassicae pupae, coloration varies from green to grey-black. Various workers have shown that pigmentation is determined by light stimuli during a sensitive period in the last larval instar whilst the caterpillar is settling for pupal moulting (Oltmer, 1968; Brecher, 1919; Kayser-Wegmann, 1973).

Further research on this species has shown that wavelength, intensity and the direction of incident light to which a prepupa is exposed all affect pupal melanization (Angersbach and Kayser, 1971; Kayser and Angersbach, 1974; 1975; Angersbach, 1975). Smith (1980) showed that although light stimuli are the most important factors in determining pupal colour in various pierid species, other factors such as relative humidity and temperature may also have influence on colour. He interpreted his results in terms of defence against visually hunting predators whereas Kayser and Angersbach (1974) suggested that pigment deposition in P. brassicae may be a mechanism for protecting the pupae against short wavelength radiation.

Work with various species of swallowtail butterflies has also shown that factors other than light may be important in determining pupal colour. Ohnishi and Hidaka (1955) showed that green pupae of Papilio xuthus L. and P. protenor demetrius Cram. are formed amongst the green leaves of the foodplant and brown pupae on dead branches even when pupation occurs in darkness. Ishizaki and Kato

(1956) showed that for P. xuthus low relative humidity increases the production of brown pupae. They also reported that low temperature, darkness during larval life and low humidity at the time of pupation increases the production of orange-type pupae in this species.

In P. protenor demetrius the diameter, curvature and surface texture of the pupational substrate, relative humidity (Honda, 1981) and odour of the foodplant (Honda, 1979) have all been shown to affect pupal coloration.

The environmental determinants of pupal colour in Papilio machaon L. have been shown to include the colour of the pupational substrate (Gardiner, 1974) the wavelength of light to which the prepupa is exposed (Wiklund, 1972) and the texture and geometry of the pupational substrate (West and Hazel, unpublished observations cited by Wiklund, 1975).

In Papilio polytes L. and Papilio demoleus L. pupal coloration appears to be considerably influenced by the texture and to a lesser extent by colour of the pupational substrate the effects being modified by relative humidity, temperature, photoperiod, and perhaps chemical influences (Smith, 1978). Differences in the relative importance of texture and background colour in these two species were interpreted in relation to the time of day a larva selects its pupation site. Thus, non-visual stimuli appear to be more important in P. polytes which tends to pupate at night-time than in P. demoleus which tends to select its pupational site in daylight.

In the multivoltine swallowtail Papilio polyxenes Fabr., photoperiod during the late larval stages determines

whether the ensuing pupa will be dimorphic. Autumn photoperiod causes an almost exclusive production of brown pupae irrespective of larval substrate. Midsummer photoperiod, however, permits the prepupa to respond to variation in the substrate with the appropriate pupal colour. Thus, on thin (green or brown) twigs pupae tend to be green whereas on thicker (usually brown) branches pupae tend to be brown. It is the "thinness" of the twig rather than its colour that is important in determining the colour of the chrysalis in this light regime (West et al., 1972).

Hazel (1977) studied the genetic basis of pupal colour dimorphism in P. polyxenes and concluded that pupal colour in this species is a function of the environmental stimuli and the genetic ability of the prepupal larva to interpret these stimuli and respond with the appropriate colour. More recently, Hazel and West (1982) have arrived at similar conclusions for the swallowtail butterfly Eurytides marcellus Cr.. Their selection experiments illustrated that the genetic basis of pupal colour dimorphism lies in differences among individuals in their tendencies to produce green and brown pupae. They suggested that these differences are maintained in nature by weak stabilizing selection.

Although several environmental factors have been found to influence pupal colour in Pieris and Papilio spp. the general result is the same, i.e. that pupae tend to be cryptic against their natural pupational sites. The importance of camouflage to the survival of butterfly pupae has been demonstrated in predation experiments for both

Pieris (Baker, 1970) and Papilio (Wiklund, 1975).

1.4. Polymorphism in Caterpillars

Very few detailed studies have been made on polymorphisms in lepidopterous larvae and the approach in each was quite different. For these reasons each study is reviewed here separately.

1.4.1. The Citrus Swallowtail Papilio demodocus Esper.

The early instars of P. demodocus are monomorphic and resemble bird droppings. However, final instar caterpillars are polymorphic for colour pattern. The commonest morph is bright green with disruptive brown and white marks and is found on Citrus and other Rutaceae throughout most of tropical Africa. In parts of South Africa P. demodocus larvae are also found on fennel and other Umbelliferae and here the final instar is polymorphic. Although a minority have the normal citrus-type pattern, most larvae have a more complex black and green pattern.

Van Son (1949) maintains that the food of the larvae determines colour pattern in the final instar. However, experiments in which sibling larvae were reared on either Citrus or fennel showed a seggregation for pattern on both foodplants (Clarke, Dickson and Sheppard, 1963). Moreover, Clarke et al. found no evidence that the "citrus" form survived better on Citrus or the "umbellifer" form on fennel. Controlled breeding experiments showed the polymorphism to be genetically determined with the "umbellifer" form dominant or semi dominant. The intermediate forms produced in some broods were believed to be

heterozygotes. Similar intermediate forms were also found in the wild on umbellifers. In general these much more closely resembled the "umbellifer" than the "citrus" morphs. They then investigated whether there are selective pressures operating on the two morphs in the wild. Random samples of full grown and early instar larvae were collected from both Citrus trees and Umbellifers. The immature larvae were reared to maturity in the laboratory to determine their final instar colour. The different proportion of forms amongst early and final instars strongly suggested that there is selection against the umbellifer- and intermediate-type caterpillars on Citrus and possibly against the citrus-type pattern on umbellifers. Clarke et al. were further able to show that there is predation on final instar larvae by birds and they suggested that these predators may be responsible for eliminating conspicuous morphs.

1.4.2. The Mediterranean Flour Moth Ephestia kühniella (Zeller)

Caterpillars of E. kühniella are pests in flour mills, barley mills and feed mixing factories. Fifth instar larvae of this species show a continuous colour variation ranging from white to deep pink. Because colour variation is continuous this is not, strictly, a true polymorphism which implies discrete colour classes, but it is nevertheless convenient to discuss it here. In 1979, Imura and Shibuya showed that colour variation is due to a difference in the amount of xanthommatin present in the epidermis of each individual larva. The results of controlled breeding

experiments (Imura, 1980) indicated that about 87% of phenotypic variance can be explained by an additive genetic factor, dominance being absent or balanced. Imura attributed the remainder to environmental factors and went on to examine these in 1982 using genetically pure strains of larvae. He showed that the degree of integumental pigmentation correlated fairly well with the tryptophan content of the food medium on which larvae were reared. Other environmental factors affecting larval pigmentation were temperature, relative humidity, starvation and crowding.

1.4.3. Pine Looper Caterpillars Bupalus piniarus L.

Pine looper caterpillars are dimorphic for colour in their later instars. In addition to the normal green larval form which is well camouflaged against pine needles, a yellow morph occurs in nature at very low frequencies (a few per cent at the maximum). Boer (1971) showed that polymorphism in this species is controlled by a simple Mendelian inheritance, the yellow character being dominant over the green. A recessive blue phenotype and an even rarer white morph were also reared in the laboratory, but neither of these colours have been seen in wild populations. Boer went on to investigate the various selective pressures operating on green and yellow morphs and their importance to the survival of yellow caterpillars in nature. He found no differences between adult moths from green or yellow caterpillars with respect to mating preference, copulation success, fecundity of females, the hatching of eggs or larval growth rates. But he did show that there are

differential pressures operating on the two larval morphs of B. piniarus through predation. The colour of caterpillars is important in protecting them from predation by birds (Parus spp.) but their behaviour and smell are also important with respect to wasp and spider predators.

1.4.4. The New World Hawkmoth Errinyis ello L.

Caterpillars of the New World hawkmoth Errinyis ello are polymorphic for colour in their last three instars (Curio, 1965). In Jamaica, the green, blue and green-grey final instar larvae feed and rest on the leaves of Euphorbia (Poinsettia) pulcherrima. By contrast, the brown final instar larvae rest on the trunk and branches during the day and move to the foliage at night to feed (Curio, 1970 a, b and c). Hence, all four larval morphs of this species are cryptic in their day-time resting positions. Curio demonstrated by selection experiments in the natural environment that mortality is independent of larval coloration but he showed that larvae resting amongst the foliage suffered greater mortalities than those resting on the trunk. The high selective advantage (88%) for the trunk resting habit was attributed to predation by wasps which search exclusively amongst the foliage. Curio went on to investigate what pressures are preventing non-brown morph from resting on the wasp-free trunk sites. He showed that the anoline lizard, Anolis lineatopus L., which searches only on the trunk and main branches, exerts a selection pressure sufficiently high to force non-brown final instar larvae to stay in the foliage.

1.5. Physico-Chemical Basis of Colour in Insects

Colour in insects may be caused by pigments or be of structural origin. Structural colours include iridescence and Tyndall blues. Iridescence is caused either by diffraction as in *Serica* beetles (Fox and Vevers, 1960) or by interference as in the wings of *Morpho* butterflies (Mason, 1927). Tyndall blues are caused by the scattering of short wavelengths of light by small particles. Colour of this origin is found in some dragonfly species (Fox and Vevers, 1960).

Pigments in insects are either derived from plants or formed *de novo* by the metabolism of amino acids (see review by Thomson, 1960). Exogenous pigments include carotenoids, which are present in the haemolymph and other body tissues of many insects, and flavones, which occur in low concentrations in the cocoons of *Bombyx mori* L. Endogenous pigments include pteridines and melanins which have both been found in the wings of many butterflies, ommochromes, which occur as eye pigments in *Drosophila* and bile pigments, which have been isolated from various green insects (Thomson, 1960). Other reviews of colour in insects have been made by Cott (1940), Fox (1953), Fox and Vevers (1960), Fuzeau-Braesch (1972) and Hinton (1973).

1.6. Green Insect Pigmentation

One of the commonest groups of pigments utilised by insects for body coloration is the carotenoids. The presence of carotenoids in insects was first demonstrated by Wackenroder in 1831 (in Feltwell and Harborne, 1978).

Poulton (1885) was one of the earliest researchers to study the pigments present in Lepidoptera. He identified the green and yellow in Smerinthus ocellata as chlorophyll and 'xanthophyll' and showed that they were derived directly from the foodplant since the spectra of pigment in the plants correlated with that found in the 'blood' of the caterpillar. The word 'xanthophyll' was used as a broad descriptive term in early works and included not only the true xanthophylls (carotenoids containing at least one oxygen atom) but also the carotenes (hydrocarbons).

Poulton continued his work in 1887 to investigate the pigments responsible for coloration in Pieris brassicae. He believed that 'xanthophylls' were present in the ova and the subcutaneous tissue of this species and that chlorophyll 'or some modification of it' passed through the digestive tract and gave green colour to the haemolymph.

Podiapolsky (cited in Feltwell and Harbourne, 1978) stated that the absorption spectrum of the green pigment of Tettigonia (Locusta) viridissima (L.) was similar to that of chlorophyll, but later experiments on various insect species showed that the formation of green pigment was independent of the amount of chlorophyll in the diet (Toumanoff, 1927; Giersberg, 1928).

Przibram and Lederer (1933) showed that the green colour of the haemolymph of the common stick insect Carausius morosus Br. is due to a mixture of yellow and blue pigments rather than a green pigment such as chlorophyll. Junge (1941) went on to show that these pigments both occurred as chromoproteins, the prosthetic group of

the yellow component being a carotenoid and that of the blue one a bile pigment which he called glaucobilin but which was later renamed mesobiliverdin (Lemberg and Legge, 1949). He obtained similar results when investigating the skins of several other Orthoptera and also of the hawkmoth Sphinx ligustri L. For these mixtures of pigment he suggested the name 'insectoverdin'. Hackman (1952) identified similar chromoprotein mixtures in the haemolymph of larvae of Pieris rapae L., Cacoecia australana (Lew.), and Amphipyra sanguinipuncta (Guér.) (all Lepidoptera). Reviews of early literature on green pigments in insects are given by Okay (1947) and Cromartie (1959).

Although it is now generally recognised that the green colour of insects is often due to 'insectoverdins', Needham (1974) believes that chlorophyll is also present in the bodies of most herbivorous insects, while Vuillaume (1968) believes it is the bile pigments alone which produce the green colour.

1.7. Pigments in Lepidoptera

Steche (1912) found that in Pieris brassicae, haemolymph from the female larvae contained slightly modified chlorophyll only, whereas the male haemolymph had xanthophylls only, the chlorophylls having been already degraded. However, he could not determine whether carotenes were synthesized in the larvae or were of plant origin, but he did show that the larval carotene had similar chemical properties to carotene from carrots. Meyer (1930) was the first biologist to identify β-carotene in the gut and

haemolymph of P. brassicae. The bile pigment, biliverdin, has since been identified in this species (Weiland and Tartter, 1940; Lemberg and Legge, 1949; Rüediger et al, 1968; 1969).

Feltwell and Valadon (1972) and Feltwell (1973) showed that the carotenoids present in all stages of the life cycle of P. brassicae reflected the carotenoid composition of the cabbage on which it had been fed. Fourteen carotenoids identified in Brassica oleracea var. capita L. were also present in the adult butterfly. β-carotene, 5,6-monoepoxy-β-carotene and lutein were found in the ova, only β-carotene was identified in the first three larval instars; β-carotene, lutein, 5-6 monoepoxy lutein, 5,6-monoepoxy β-carotene and chrysanthemaxanthin were present in the full grown larvae. These results indicate that P. brassicae does not modify the structure of carotenoids ingested from the foodplant.

Ohtaki and Ohnishi (1967) investigated the pigments in the pupal integuments of Pieris rapae crucivora Boisduval. They demonstrated that in green pupae, the yellow pigment (lutein) is mainly deposited in the cuticle whilst the blue pigment (probably mesobiliverdin) is confined to the underlying tissues. Lutein was also isolated from the cuticle of the brown-type pupae, but in smaller amounts.

In 1977, Rothschild, Valadon and Mummery compared the carotenoids in pupae of the small white butterfly (Pieris rapae) and the large white butterfly (P. brassicae) by rearing the larvae on portions of the same cabbage leaves. They showed that each has a characteristic carotenoid storage pattern. P. brassicae stores considerably more

carotenoid and contains β-carotene as the dominant pigment, whereas P. rapae follows the pattern found in the cabbage so that lutein is the principal carotenoid in its tissue.

Ohnishi (1959) identified both lutein and β-carotene in the pupal cuticles of green Papilio xuthus and green P. protenor demetrius, β-carotene being the dominant carotenoid in the former whilst lutein was dominant in the latter species. Brown pupae, however, contained an astaxanthin-like carotenoid and two unknown carotenoids which Ohnishi termed "papilioerythrins". He suggested that these carotenoids were oxidized products of dietary β-carotene and/or lutein.

In 1975, Mummery, Valadon and Rothschild measured the carotenoids in the larvae of the hawkmoth Manduca sexta (Johan) and the silkmoth Hyalophora cecropia (L.) together with plants on which they were reared. There was some suggestion of selective storage in both species, of lutein in the former and zeaxanthin in the latter. No carotenoids were found in the three blue coloured hawkmoth larvae reared on an artificial diet. The blue mutant of H. cecropia in the fifth instar stored only $2.4 \mu g g^{-1}$ of carotenoids compared with $157.6 \mu g g^{-1}$ in normal green heterozygous siblings. The fact that twice the concentration of carotenoids was found in the frass of the blue larvae compared with that from the normal green form suggests that there is poor assimilation in the blue mutant.

Valadon et al. (1975) continued this research by comparing carotenoids in the blue mutant and normal green pupae of H. cecropia specimens reared on apple leaves.

Pupae derived from blue larvae contained 3.78 µg of carotenoids per individual compared with 266.0 µg in pupae derived from normal green larvae. Only two carotenoids were found in the mutant pupae: lutein which was also found in the green pupae, and 5,6-monoepoxy β-carotene which was not. (However, only very small amounts of the two pigments were present, being insufficient to impart a green colour to the pupae.) Since the blue mutants were fed on the same apple leaves as the green siblings, the mutation presumably blocks some step involved in carotenoid assimilation or deposition. The dominant carotenoid in the green pupae was β-carotene; others present were lutein, cryptoxanthin, zeaxanthin and flavoxanthin, the latter only being found in field-collected specimens.

The absence of carotenoids in artificial diets causes blueness in various lepidopterous larvae e.g. M. sexta and H. cecropia (Riddiford, 1968; Dahlman, 1969). Clark (1971) succeeded in returning blue larvae of H. cecropia to their normal coloration by adding commercial vegetable lutein to the artificial diet on which they had been reared; the lack of this carotenoid had evidently induced the blue coloration. Clark noted, however, that the addition of β-carotene added pale yellow and pink coloration to the dorsal and abdominal tubercules only, it did not affect the colour of the cuticle and epidermis.

Feltwell and Rothschild (1974) examined the carotenoids in 38 species of Lepidoptera and showed that although there is some selective storage, Lepidoptera chiefly store the carotenoids present in their food plants unchanged. In the

adult of Laothoe populi they identified lutein, β-carotene and 5,6-mono-epoxy-β-carotene. However, the carotenoids from the foodplants of this species were not analysed.

Although some moths and Papilio species contain carotenoids which are not present in their foodplant, these are presumed to be produced by a transformation of diet derived C_{40}-precursors (summarised by Kayser, 1982). To date there is no convincing report of any de novo formation of carotenoids by animals.

1.8. The Present Work

1.8.1. Introduction

Several species of hawkmoth have polymorphic caterpillars e.g. Errinyis ello and Agrius (Herse) convolvuli (L.) (Curio, 1965, 1970; Edmunds, 1974). There is probably selective advantage in having different colour morphs in terms of camouflage and reduced predation but the genetic, biochemical and environmental basis of the morphs has not been established in any species of hawkmoth.

1.8.2. Aim

The aim of the present work was to investigate the factors which determine and maintain larval colour polymorphism in two species of hawkmoths. The specific objectives were:-

1) To determine the relationship between the colour of foodplant and colour of caterpillar in the field.

2) To determine the environmental and nutritional factors which cause a caterpillar to change colour. Further, to examine whether there is a genetic component to coloration

in the caterpillars.

3) To establish whether plant pigments in the food directly affect the body colour of the caterpillar.

4) To ascertain whether there is selective predation of different coloured caterpillars depending on their background.

1.8.3. The Species

The hawkmoths chosen for study were Smerinthus ocellata (L.) (the eyed hawkmoth) and Laothoe populi (L.) (the poplar hawkmoth). The main reasons for choosing these animals were:-

1) Eyed and poplar hawkmoths are locally common in Lancashire.

2) Survival of both species is reasonably good under laboratory conditions. Preliminary experiments have shown that the eyed hawkmoth mates and lays eggs readily in captivity and survival of pupae over winter is good. The poplar hawkmoth also mates and survives well in the laboratory (Williams, 1966).

There is no applied aspect to the work since neither of the insects being studied is normally a pest, although caterpillars of the eyed hawkmoth do occasionally infest apple trees. However, other hawkmoths such as Manduca sexta (the tobacco hornworm) and Acherontia atropos (L.) (the deaths head hawkmoth) are pests. Any conclusions relating to the eyed and poplar hawkmoths may be of relevance to these species since they also have polymorphic caterpillars.

1.8.4. Organisation of the Thesis

The results of extensive field surveys in Lancashire and Merseyside are presented and discussed in Chapter 2 (objective 1).

The methods of rearing larvae are described in Chapter 3. This chapter also investigates differences in growth, survival and fecundity of *Laothoe populi* reared as larvae on either *Populus alba* or *Salix fragilis*.

The major rearing programmes of *L. populi* and *Smerinthus ocellata* caterpillars on different foodplants and under different environmental conditions are outlined in Chapters 4 and 5 respectively (objective 2).

Chapter 6 describes and discusses the field predation experiments carried out using *L. populi* larvae (objective 4).

The biochemical analyses of plant and larval pigments are described and the results discussed in Chapter 7 (objective 3).

The conclusions reached from the study as a whole are discussed in Chapter 8.

CHAPTER 2

FIELD SURVEYS

2.1. Introduction

Preliminary field work in 1978, 1979 and 1980 indicated that in Smerinthus ocellata and Laothoe populi there may be a correlation between species of foodplant and colour of caterpillar (Edmunds, unpublished data). The aim of the present work is to determine the relative frequencies of occurrence of the different colour morphs on each species of foodplant and to see if the commonest morphs are best camouflaged (as judged by the human eye).

2.2. Method

During August and September of each year from 1978 until 1983, individual plants of Populus spp. and Salix spp. were searched carefully by eye and each final instar caterpillar of L. populi and S. ocellata was scored for colour. The presence or absence of red spots was also recorded.

A time interval of at least ten days was allowed between consecutive visits to each site. This avoids recording data for the same individual twice, since final instar larvae found on the previous visit will have pupated.

2.3. The Sites

2.3.1. Cuerden Valley Park, Lancashire SD 567239 (FIG. 2.1)

This area is underlain by Triassic Bunter Sandstone. The valley bottom and lower slopes have impeded drainage

indicating that they are underlain by clay.

Various species of willow and poplar trees have recently been planted along the banks of the River Lostock, upstream of the Clayton Brook tributary. These plants include Salix fragilis, Salix caprea, Salix viminalis and Populus alba and at the time of the survey many were of a suitable height (< 2 m high) for searching. This site was visited in 1982 and 1983: full grown eyed hawkmoth larvae were observed on S. caprea in both years, poplar hawkmoth larvae were found on P. alba bushes in 1982 only.

2.3.2. Lytham St. Annes, Lancahsire (FIG. 2.2)

2.3.2.1. SITE A SD 311306 (FIG. 2.3)

This nature reserve is composed of a series of irregular sand dunes underlain by peat which gives rise to waterlogging of the two areas known as the Large and Small Slacks.

The prevailing sea winds result in slow bushy growth of the willow species present on the reserve. Low spreading bushes of Salix atrocinerea grow within the "Large Slack". Larger bushes of S. fragilis and P. alba grow at the west end of this area. Eyed hawkmoth larvae were common here in 1978-1981, however, populations tended to decline in the following two years.

Salix repens is common on the dunes but due to its height and structure, searching of this plant was time consuming and usually fruitless.

S. alba bushes grow at the east of the reserve and were populated with eyed hawkmoth larvae in all years of the survey.

FIG. 2.1. Location of Cuerden Valley and Haslam Park field sites (O.S. 1:50 000)

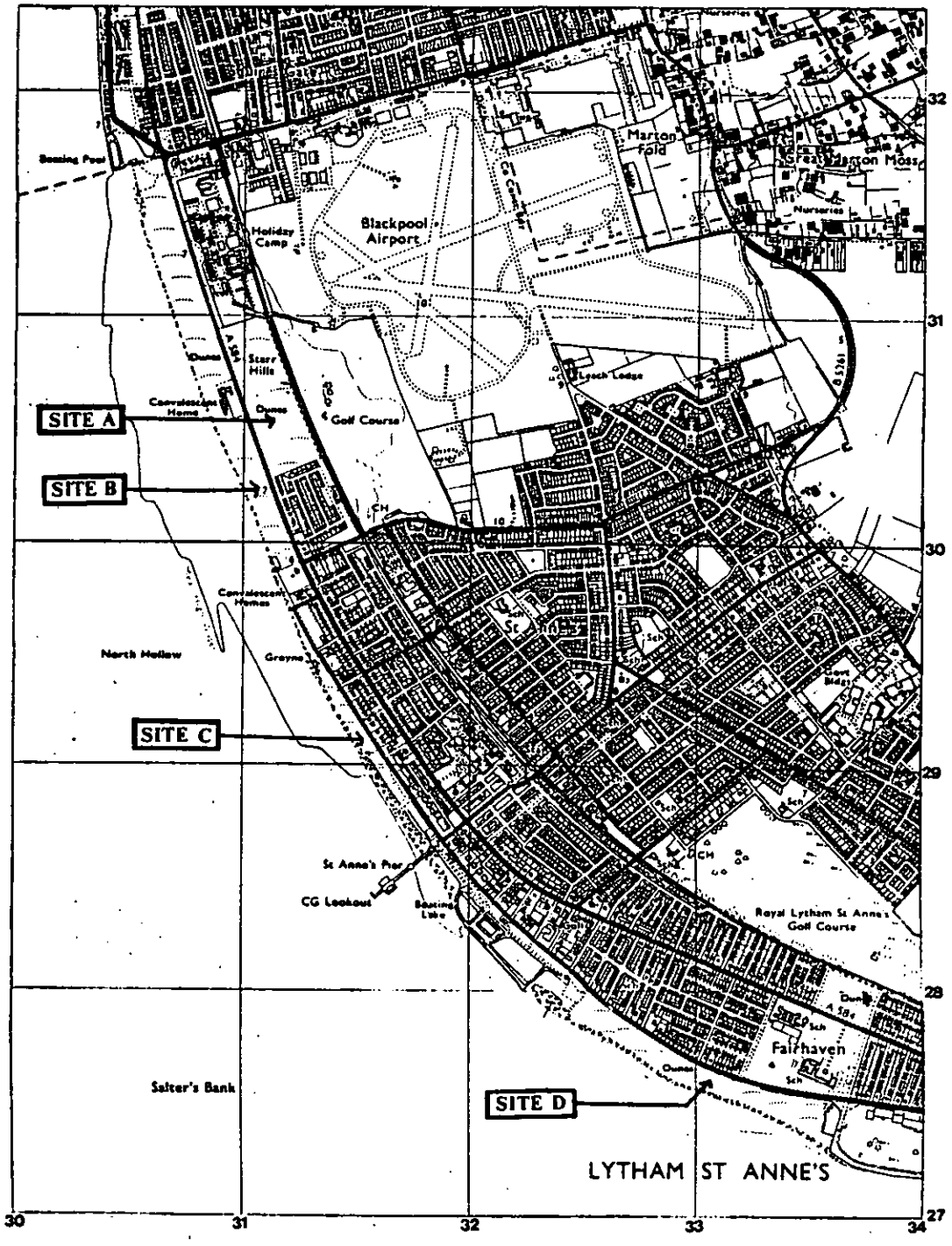


FIG. 2.2. Location of field sites at Lytham St. Annes (O.S. 1:25 000)

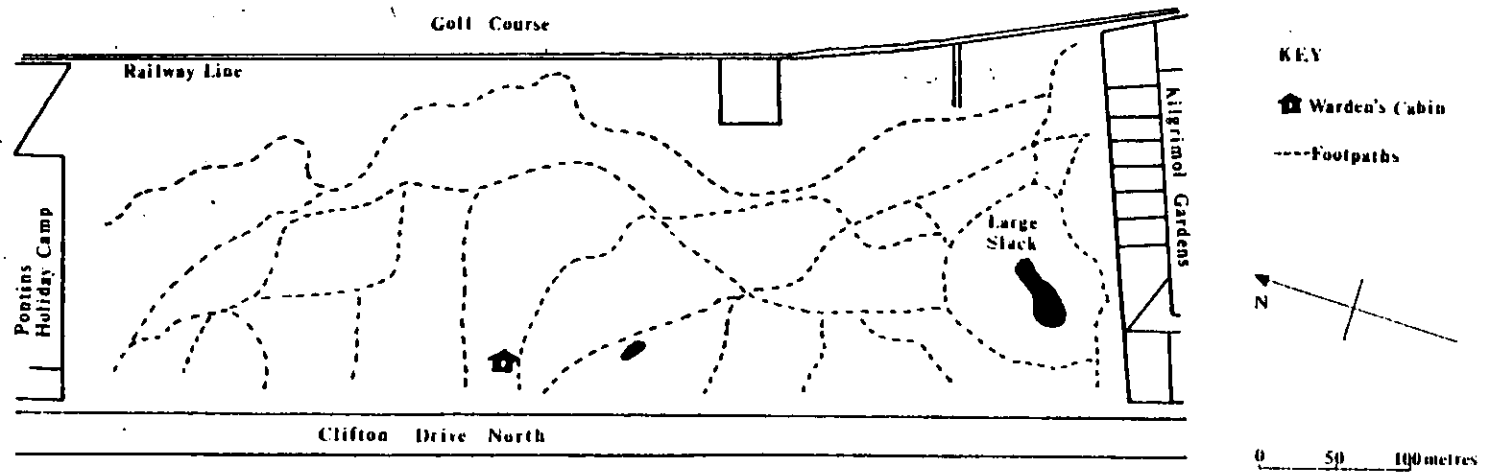


FIG. 2.3. Lytham St. Annes Nature Reserve (The Starr Hills)

2.3.2.2. SITE B SD 310304 to 311301

S. viminalis, P. alba and S. repens grow on these dunes. Eyed hawkmoth larvae were found to be common on S. viminalis.

2.3.2.3. SITE C SD 317289 to 314295

S. viminalis and P. alba grow along this stretch of dunes. Poplar hawkmoth larvae were common on P. alba in 1981 but only a few individuals were found in the following years.

2.3.2.4. SITE D SD 330276

Although many willow trees and bushes grow in this area of dunes, only five poplar hawkmoth larvae were found at this location on Salix daphnoides in 1981.

2.3.3. Formby Point Nature Reserve, Merseyside SD 275075 (FIG. 2.4)

Formby Point is an area of sand dunes on the Merseyside coast between Liverpool and Southport. Visits were made to this site in 1979 to 1982 only. The large white poplar trees which grow around the car park and Pinetree Caravan Park were difficult to search effectively due to their size (>4 m); only one poplar hawkmoth caterpillar was found on P. alba in the four years of study.

S. repens is common on the fixed dunes. Eyed hawkmoth caterpillars were found on this foodplant in 1979 and in 1982.

2.3.4. Haslam Park, Preston, Lancashire SD 515310 (FIG. 2.1)

An occasional hawkmoth caterpillar was found during collection of foodplant material (S. fragilis and P. alba) for the summer breeding experiments.

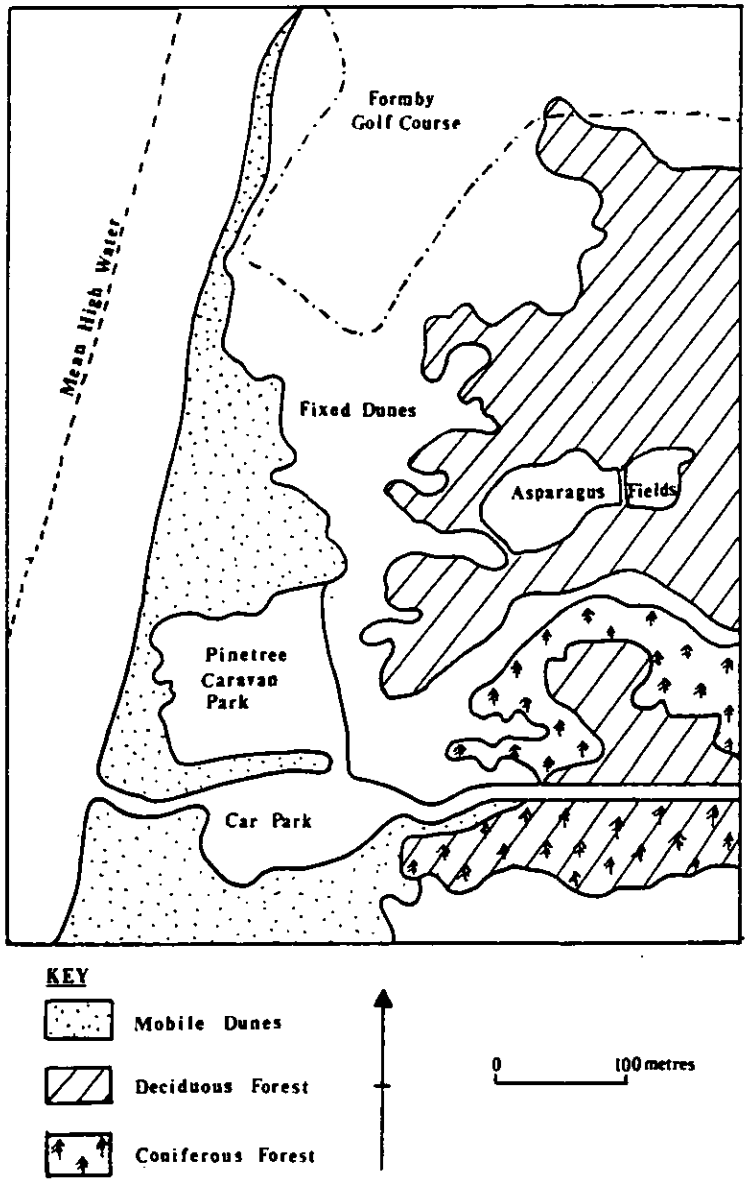


FIG. 2.4. Formby Point Nature Reserve

2.3.5. Mill Lane, Goosnargh, Lancashire SD 560380

S. fragilis and S. atrocinerea grow in the roadside hedge along Mill Lane, Goosnargh. In 1980, 27 L. populi larvae were found on S. fragilis. In 1983, three S. ocellata larvae were found on the same bush.

2.4. The Foodplants

The following are descriptions of the leaves of the main foodplants of eyed and poplar hawkmoth larvae - from Flora of The British Isles 2nd ed. Clapham, Tutin & Warburg.

1. Populus alba L.

Leaves varying from 3-10 cm long, "ovate-orbicular" to "palmately lobed"..."dark green above,...white tomentose beneath".

2. Salix alba L.

Leaves 5-10 cm "lanceolate"..."covered with white silky appressed hairs on both surfaces".

3. S. caprea L.

"Lvs 5-10 cm,"..."oval to ovate-oblong or obovate"... "dark green and finally glabrous or glabrescent above (pubescent at first), persistently softly and densely, almost woolly, grey-tomentose beneath".

4. S. cinerea L.

"Lvs 2.5-7(-10) cm",..."obovate, oblanceolate or rarely elliptic"..."pubescent above at least when young, persistently pubescent or tomentose beneath".

5. S. atrocinerea Brot.

"Lvs usually buff or reddish-pubescent above when young; beneath thinly and shortly pubescent mainly on the

veins, at maturity glaucous and with some or all of the hairs rust-coloured, not soft to the touch as in S. caprea..."

6. S. fragilis L.

"Lvs 6-15 x 1.5-4 cm.,"..."lanceolate"..."thinly silky when young, soon glabrous, bright green above, glaucous or less often paler green beneath."

7. S. repens L.

"Lvs 0.5-4.5 cm.,"..."very variable in shape, oval, oblong, elliptic or lanceolate,"..."appressed silvery-silky on both sides when young, persistently so beneath, becoming glabrous above or not, prominently reticulate-veined beneath, often blackening when dried."

8. S. viminalis L.

"Lvs 10-25 cm.,"..."lanceolate or linear-lanceolate, gradually narrowing to the apex"..."dark green and glabrous above, silvery silky-tomentose beneath."

9. S. daphnoides Vill.

"Lvs 5-10 cm.,"..."linear-lanceolate or oblong-ovate,"..."glandular-serrulate, soon glabrous, dark green and shining above, glaucous beneath."

2.5. Colour and Resting Behaviour of Poplar and Eyed Hawkmoth Larvae

Two colour categories were defined for the eyed hawkmoth: green and grey. These colours refer to the sides of the caterpillars. (See PLATES 1 and 2.) Although these two morphs are not discrete, caterpillars can normally be assigned to one or the other group. The dorsal surface of green eyed hawkmoth larvae varies from yellow-green to

grey-green while that of grey larvae is typically grey-green or white-green. The final instar caterpillar normally rests on the lower side of a stem or petiole with the head closely apposed to the leaf on which it is eating. The stem is often in a direct line between the caterpillar and the sun and the caterpillar shows reversed countershading with the dorsal (lower) surface pale and ventral (upper) surface dark. (See FIG. 2.5.)

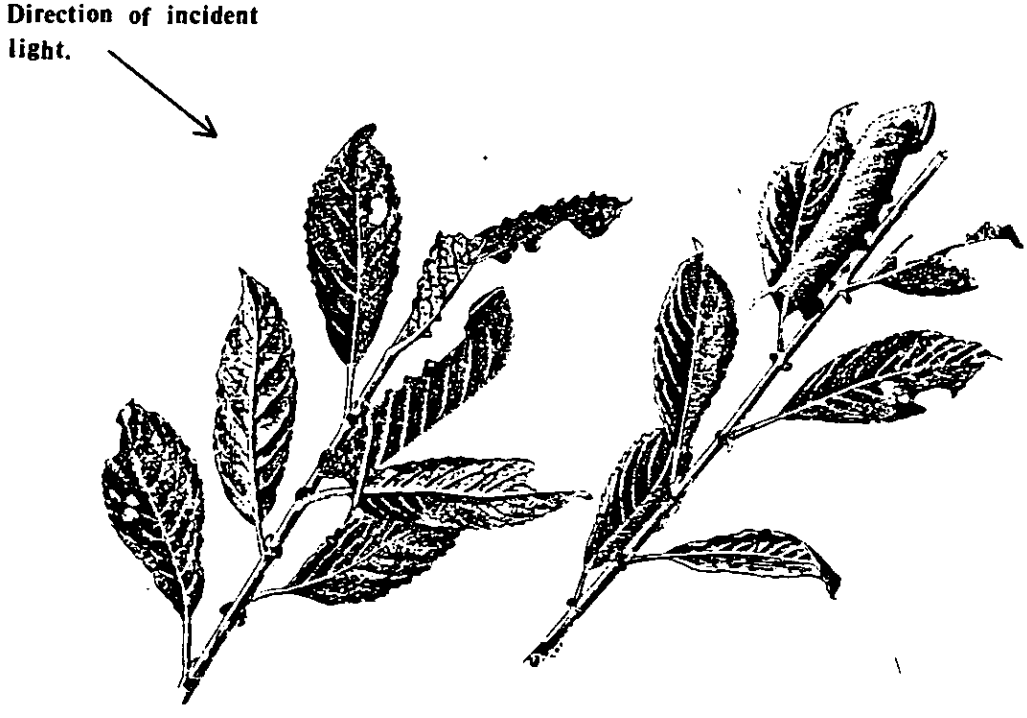


FIG. 2.5. Smerinthus ocellata larvae illustrating reversed countershading. Left: natural resting position showing the obliterative effect of reversed countershading. Right: unnatural position, showing strong relief and solid appearance. (From Cott, 1940).

For final instar poplar hawkmoth larvae three colour categories were recognized: yellow-green, dull (blue)-green and white. (See PLATES 3, 4 and 5.) However, occasionally white morphs were slightly greyer than the one illustrated in PLATE 5. These are referred to as white-grey (WG) in APPENDIX 2 but have been combined with the white category in subsequent analyses. Reversed countershading does not occur in the poplar hawkmoth. Its ventral surface is usually slightly paler than the rest of the body in yellow-green and dull-green morphs. White caterpillars show no signs of countershading. This difference between the two species is perhaps associated with the fact that poplar caterpillars typically rest beneath leaves in the shade whereas full grown eyed hawkmoth caterpillars rest on stems and petioles where countershading is more effective in concealing the insect.

Both species may have up to ten spots running laterally along the sides of the caterpillar. Further, in individuals with red spots, the spiracles are usually redder than in those with no red spots. This is particularly noticeable in larvae having nine or ten red spots. (See PLATES 1 and 6). In white morphs of poplar hawkmoth larvae the spots (if present) are pink. In dull-green poplar hawkmoth larvae and grey eyed hawkmoth larvae the red spots are less bright than in yellow-green morphs.

2.6. Results and Discussion

2.6.1. Eyed Hawkmoth

The results of the surveys are presented in APPENDIX 1. The Kolmogorov-Smirnov heterogeneity test was applied to

these data (TABLE 2.1). There are no significant differences in the ratios of green to grey caterpillars in the different years on each of the five relevant foodplants. The data for the different years were therefore combined for each foodplant (TABLE 2.2). This table shows that willow species with green leaves have a high proportion of green caterpillars, species with greyish leaves have a high proportion of grey caterpillars and species such as S. viminalis whose leaves are yellow-green above and grey-green below have more or less equal numbers of the two colour morphs. Thus the majority of caterpillars are well camouflaged on all six species of foodplant on which they were found.

The data on red spots for this species are also presented in APPENDIX 1. By applying the Kolmogorov-Smirnov heterogeneity test (see TABLE 2.3) it was shown that there is no significant difference between years in the relative frequency of occurrence of red spots for larvae on S. viminalis. However, the data for S. atrocinerea is heterogeneous between years. For P. alba there is clearly no difference between the years so no test was applied to these data. The data for different years were thus combined for S. viminalis and P. alba only.

TABLE 2.4 presents the results of comparing the relative frequency of occurrence of red spotted larvae on the three foodplants. For comparisons made with S. atrocinerea, data for 1981 was tested separately, this being the year in which most observations were made. Data for the other foodplants were limited and therefore have

TABLE 2.1. Comparison of relative frequencies of green and grey caterpillars of Smerinthus ocellata on foodplants in different years of study using the Kolmogorov-Smirnov two sample heterogeneity test

Foodplant	Frequency of each morph n_1 (green)	n_2 (grey)	Largest difference D	$n_1 n_2 D$	Critical value for $n_1 n_2 D$ at 5% level two-tailed	Probability
Salix alba	2	30	0.367	22.0	59.6	n.s.
S. atrocinerea	96	7	0.263	177.0	357.8	n.s.
S. viminalis	50	43	0.095	204.0	608.1	n.s.
S. repens	10	32	0.22	70.0	157.7	n.s.
S. caprea	12	4	0.25	12.0	36.0	n.s.

n.s. - not significant

TABLE 2.2. Colour of Smerinthus ocellata caterpillars found on different foodplants in Lancashire and Merseyside

Foodplant (Salix spp)	Leaf colour above / below	Number of larvae green	Number of larvae grey	Comparison of numbers of larvae of 2 colours on different foodplants: fragilis	atrocinerea	viminalis	repens	alba
fragilis	yellow-green / green	12	0					
atrocinerea	green / grey-green	96	7	-				
viminalis	yellow-green / grey	50	43	**	***			
repens	green / grey	10	32	***	***	**		
alba	grey-green / grey	2	30	***	***	***	-	
caprea	green / grey-green	12	4	-	-	-	**	***

Comparison made using 2 x 2 χ^2 test where applicable:- -, not significant.
*, $p < 0.05$
**, $p < 0.01$
***, $p < 0.001$

Comparison between S. fragilis and S. caprea made using the Fisher exact probability test, since $e < 5$.

TABLE 2.3. Kolmogorov-Smirnov heterogeneity test for the relative frequency of occurrence of red spots on Smerinthus ocellata larvae in different years

Food plant Salix spp	Frequency of larvae with spots n_1	Frequency of larvae with no spots n_2	Largest difference D	n_1n_2D	Critical value for n_1n_2D at 5%. 2-tailed	Heterogeneous
S. atrocinerea	49	54	0.438	1159	710	YES
S. viminalis	20	73	0.16	236	501	NO
S. alba	0	32	-	-	-	NO (by inspection)

TABLE 2.4. The relative frequency of occurrence of red spotted Smerinthus ocellata caterpillars on different foodplants

Foodplants (*Salix* spp)	Years	No. of caterpillars with red spots	No. of caterpillars with no red spots	χ^2 (1)	Probability
S. alba	1978-1983	0	32	6.67	$p \approx 0.01$
S. viminalis		20	73		
S. alba	1981	0	5	*	$p < 0.001$
S. atrocinerea		26	5		
S. atrocinerea	1981	26	5	23.30	$p < 0.001$
S. viminalis		12	32		
S. atrocinerea	1981-1983	49	54	13.44	$p < 0.001$
S. viminalis		20	73		
S. atrocinerea	1981-1983	49	54	61.57	$p < 0.001$
S. alba		0	32		

* Fisher's exact probability test used since $e < 5$.

been omitted from these statistical analyses.

The results of the analyses show that the relative number of red spotted caterpillars is different for larvae feeding on different foodplants. No caterpillars found on S. alba had red spots whereas up to 84% (1981) of larvae on S. atrocinerea had red spots. On S. viminalis an average of 21.5% of observed larvae had red spots. The significance of the red spots is unclear since they appear to make these otherwise cryptic larvae conspicuous (as judged by the human eye). However, on the leaves of S. atrocinerea and S. fragilis trees red galls are often present. It is possible that the red spots are an adaptation to becoming better camouflaged on these trees.

2.6.2. Poplar Hawkmoth

The results of the surveys are presented in APPENDIX 2. The data for different years show no evidence of heterogeneity although for most foodplants the number of caterpillars found is very small. TABLE 2.5 shows that poplar hawkmoth caterpillars are well camouflaged in their normal resting places; white morphs on the white downy undersides of the leaves of P. alba, but yellow-green on the stems, petioles and leaf bases of S. fragilis which has yellow-green young leaves and older leaves which are bright green above and glaucous beneath.

The data on red spots for the poplar hawkmoth are presented in APPENDIX 2. The Kolmogorov-Smirnov heterogeneity test was applied to the data for P. alba and S. fragilis (see TABLE 2.6). In both cases there was no heterogeneity due to different years. The chi-squared

TABLE 2.5. Colour of Laothoe populi caterpillars found on Salix fragilis and Populus alba in Lancashire

Foodplant	Leaf colour above / below	Number of caterpillars YG	DG	W	$\chi^2_{(1)}$ YG against the rest
S. fragilis	YG / G	31	0	0	$p < 0.001$
P. alba	DG / W	0	1	53	

YG - Yellow-green
DG - Dull-green
W - White
G - Green

test was applied to the combined data ($\chi^2_{(1)} = 2.8$, $p \approx 0.1$). Thus there appears to be no significant difference in the proportion of caterpillars with red spots on P. alba and on S. fragilis. The data for other foodplants were not analysed due to limited observations but by inspection, the proportion of larvae with red spots does not appear to be different from on S. fragilis or P. alba (see APPENDIX 2).

TABLE 2.6. Kolmogorov-Smirnov test for heterogeneity between years of the relative frequency of occurrence of red spots on Laothoe populi larvae

Foodplant	Frequency of larvae with red spots	Frequency of larvae with no red spots	Largest difference D	n_1n_2D	Critical value of n_1n_2D at 5%, 2-tailed	Heterogeneous
Populus alba	42	12	0.107	54.0	224.4	NO
Salix fragilis	18	13	0.308	72.0	115.8	NO

CHAPTER 3

BREEDING PROGRAMME

3.1. Introduction

This chapter includes miscellaneous data related to the rearing of caterpillars.

3.2. Rearing Methods

Adult hawkmoths from captive larvae emerged in June and July and were mated to obtain several hundred eggs. Whenever available, moths from similar coloured caterpillars were crossed in order to keep any genetic interaction as simple as possible. Eyed and poplar hawkmoths mate and lay eggs readily under laboratory conditions. Mating begins at night and lasts about 24 hours. The female begins laying eggs during the night following mating, over half the final total being laid within 48 hours (Williams, 1965). Female moths were released after four to five days, while male moths were kept for a second and sometimes third mating before being released. The eggs were laid either singly, or, more often, in clusters on the sides of the cages and on twigs. Each day they were carefully removed and counted before being placed in labelled sterile petridishes in a cool place. The eggs were observed daily for signs of hatching; just prior to hatching the eggs become paler and more opaque. Newly hatched larvae were transferred to a feeding cage (FIG. 3.1) using a soft camel-hair paintbrush. It was more practical to feed first instar larvae on rooted,

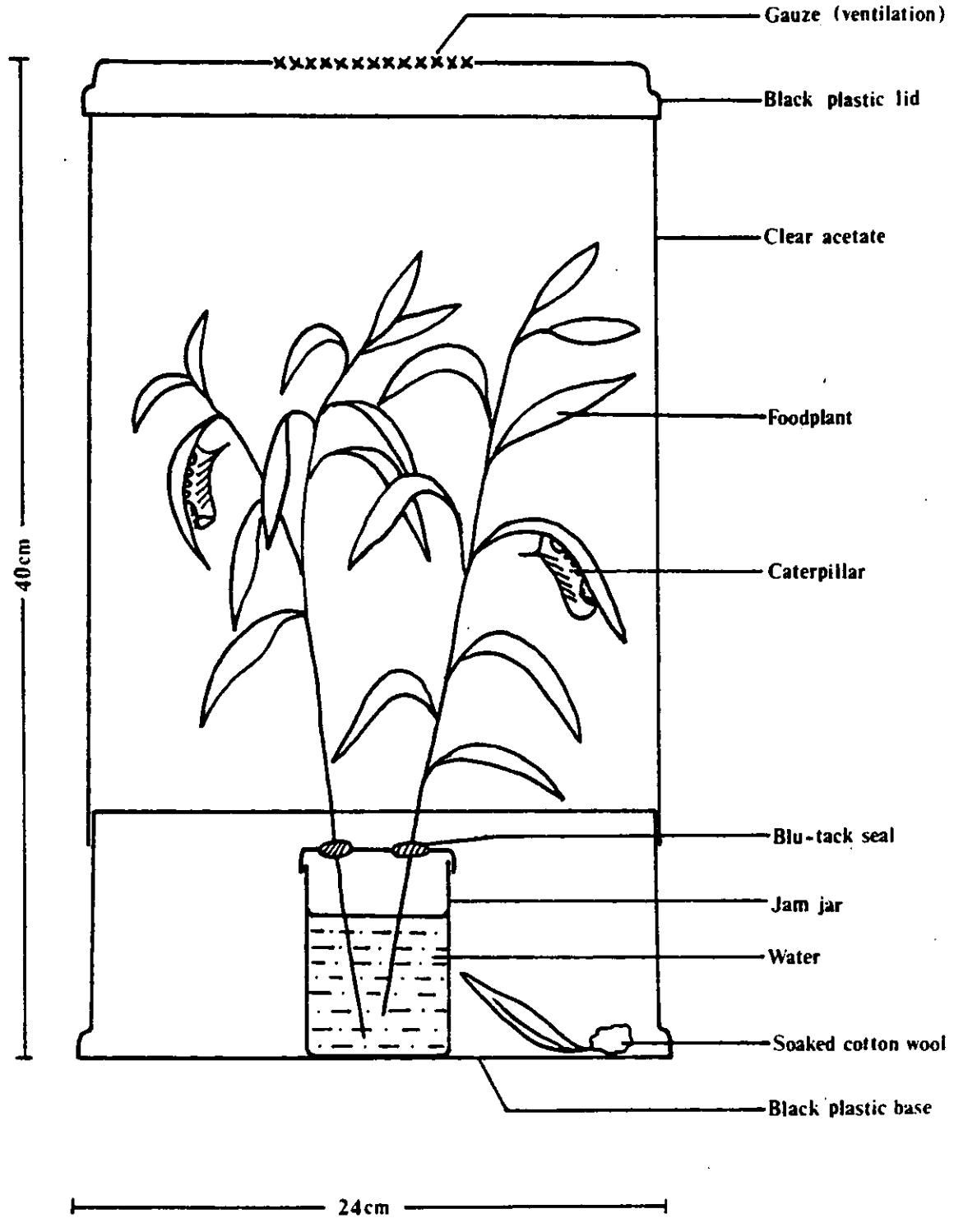


FIG. 3.1. Caterpillar rearing cage

growing plants since cut twigs required frequent replacement due to wilting. This minimised handling of the larvae in the early sensitive stages. The plants used were Salix fragilis, S. viminalis, S. atrocinerea and S. repens. All attempts to root Populus alba were unsuccessful. Later instar larvae were fed on cut twigs, fresh food being provided every second day. (The twigs were cut in water and stored under plastic bags in jars of water until required.) Hawkmoth larvae cling to the plant by means of claspers, thus irrepairable damage may result if force is employed in their removal. When changing food, the uneaten leaves were removed from old twigs, and these twigs (plus caterpillars) were put with the fresh food, thus allowing the larvae to self-transfer. Moulting larvae were never disturbed.

The cages were cleaned daily as frass becomes mildewed exceedingly quickly. This mildew can spread rapidly to the foodplant and become the precursor of disease. In order to prevent disease spreading, infected larvae (recognised by browning of the cuticle and diarrhoea) were removed from the cages. Also the number of larvae per cage was kept as low as possible.

The colours and stages of development of the larvae were recorded weekly and sometimes more frequently.

At the time of pupation, larvae become a shade duller, lose their grip and spend a period of time wandering around the floor of the cage. Separate pupation cages containing damp sand were provided for the different coloured larvae. These cages were stored in a cool place

over winter (ambient temperatures but sheltered from rain).

In 1981, rearing experiments were performed at Lancashire Polytechnic. However, ventilation in the laboratory was inadequate and overheating of the cages was probably the cause of quickly wilting plants and high caterpillar mortality.

In 1982 and 1983, experiments were carried out in a cool but poorly illuminated shed at Mill House, Goosnargh, near Preston.

3.3 Establishment of the Number of Instars in Laothoe populi and Smerinthus ocellata Caterpillars

3.3.1. Introduction

In insects, development is punctuated by a series of moults or ecdyses, this being a condition determined by the properties of the cuticle. In many insects, the amount of growth which is achieved between each developmental stage (or instar) is predictable from certain empirical laws (Dyar, 1890; Tessier, 1936). According to Dyar (1890) the head capsule of a caterpillar grows in a regular geometric progression in successive instars by a ratio of about 1.2 to 1.4, this growth ratio being constant for a given species. Further work has shown that this rule can be applied to other body dimensions such as the pharynx (Cameron, 1934 and also to other insect orders (Tessier, 1936). For species with a very constant growth ratio such as Haematopota (Tabanidae), it is possible to use Dyar's law to calculate the actual number of instars even when

data are incomplete (Cameron, 1934). In other species such as Heliothis (Lepidoptera) and Papillia (Coleoptera) there are so many departures from Dyar's rule that it cannot be used for establishing the number of instars. Simple rules which relate growth with moulting also break down for species in which the number of instars is variable. Where extra instars occur, the condition may be hereditary, as in the silkworm Bombyx mori (Ogura, 1933). In other species instar number is sex-linked, the female usually having more moults than the male, as in Sphodromantis (Orthoptera) (Przibram and Megusar, 1912), Dermestes (Coleoptera) (Kreyenberg, 1929), Tineola (Lepidoptera) and many other insects (Titschack, 1926).

Inadequate nutrition leading to a prolonged larval growth period may increase the number of moults as in the clothes moth Tineola. When kept on mediums of varying nutritional quality, the larval period may vary from 26 days with four moults (rich diet) to 900 days with 40 moults (poor diet). In the latter case Tineola may even gradually grow smaller during stages in the moult-intermoult cycle (Titschack, 1926).

Temperature has also been shown to be an influence on larval development: larvae of Pieris brassicae have been shown to have five moults when kept at 14 to 15°C, four at 15 to 20°C and only three moults when reared at 22 to 27°C (Klein, 1932). Temperature has the reverse effect on Ephestia kühniella, this species having four instars at 18°C and five at 25°C (Gierke, 1932).

Caterpillars reared under crowded conditions may

develop quicker with reduced numbers of instars compared with solitary larvae (Long, 1953).

3.3.2. Method

During the course of the breeding programme, many larvae died, particularly during the early most sensitive instars. In 1983, these were stored in 70% alcohol. L. populi caterpillars reared on Salix fragilis and Populus alba were stored separately. The head capsule widths (H.C.W.s) of the samples were measured either by using a calibrated dissecting microscope (for early instars) or by using a micrometer screw gauge (for penultimate and final instars). Attempts to measure H.C.W.s of live larvae were unsuccessful since the movement of larvae made measurements inaccurate. These early data have therefore been excluded from this report.

This method was used to establish the number of instars of both poplar and eyed hawkmoth larvae.

3.3.3. Results and Discussion

3.3.3.1. Poplar Hawkmoth

The head capsule width measurements are presented in APPENDICES 3a, b and c. Size-frequency histograms of H.C.W. measurements were plotted for L. populi on S. fragilis and on P. alba (FIGS. 3.2 and 3.3). For L. populi reared on S. fragilis FIG. 3.2 clearly shows that there are four larval instars. However, FIG. 3.3 shows that the number of instars of L. populi larvae reared on P. alba is not clearly defined. Between the first and last instars there could be either two or three instar groups. Dyar's law was applied to these data to extrapolate the number of

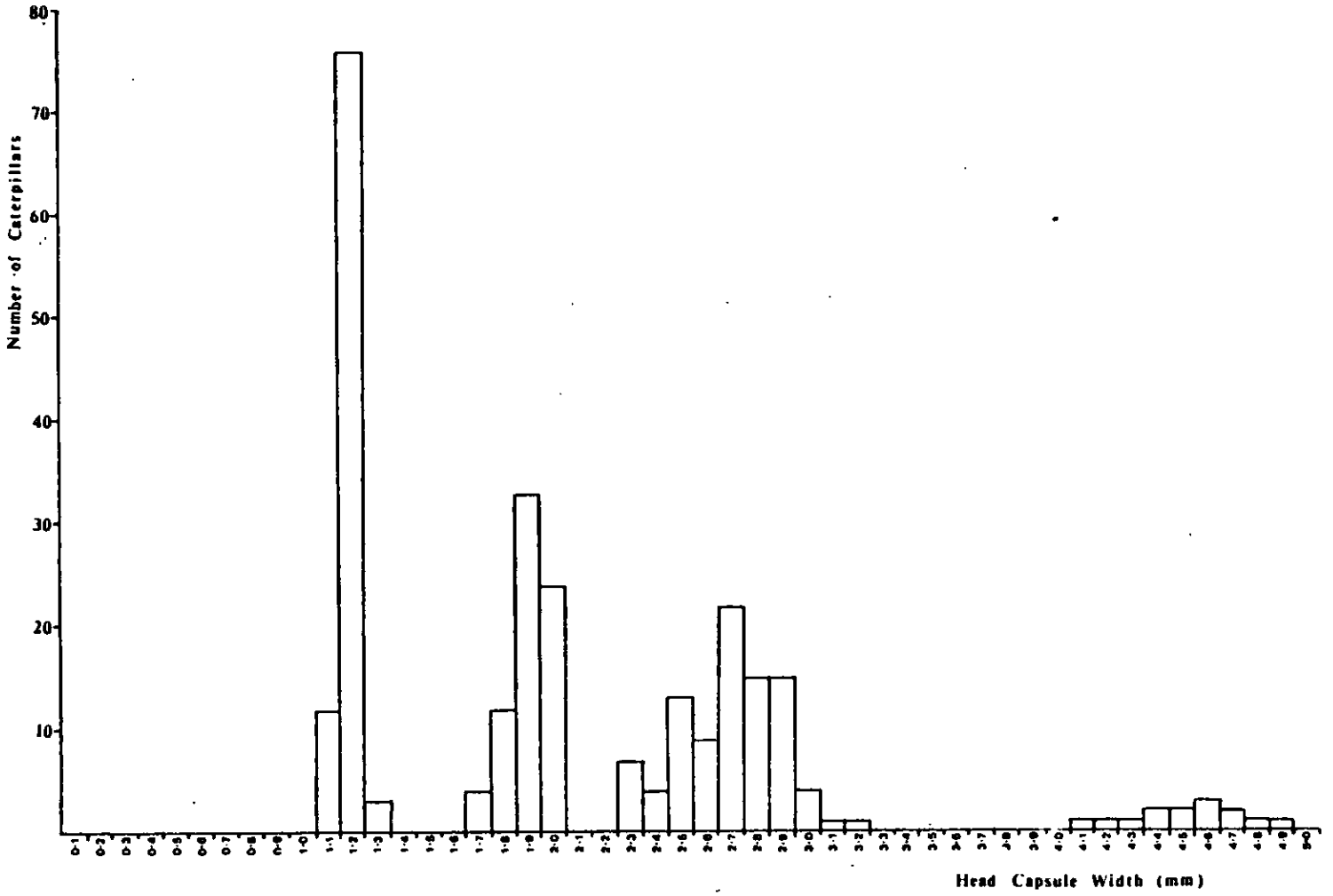


FIG. 3.2. Size-frequency histogram of head capsule widths of Laothoe populi larvae reared on Salix fragilis (showing four distinct instars)

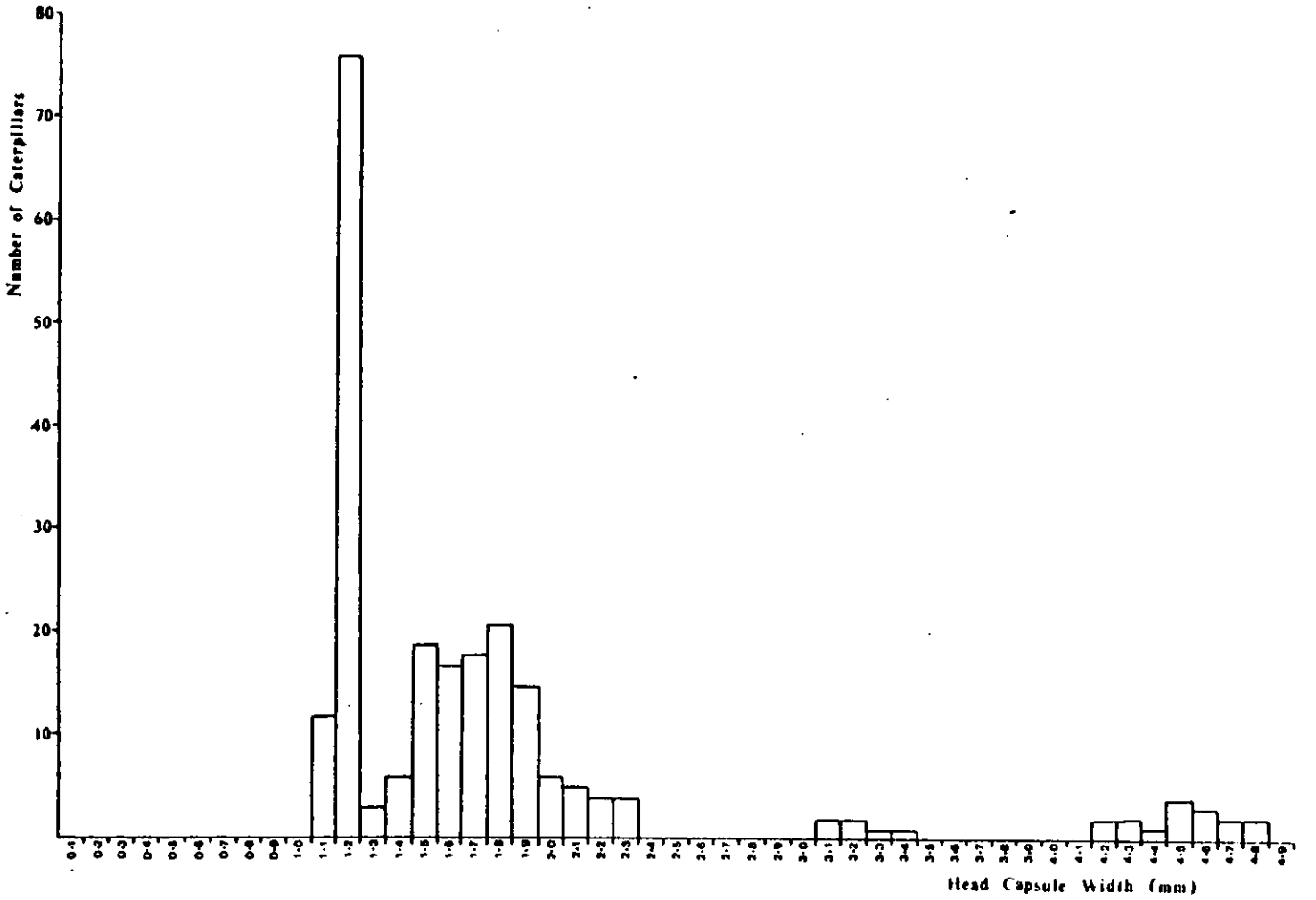


FIG. 3.3. Size-frequency histogram of head capsule widths of Laothoe populi larvae reared on Populus alba

instars by calculating log. mean H.C.W. of the first, penultimate and final instars (TABLES 3.1a and b) and plotting these figures against instar number (FIG. 3.4). For comparison, TABLE 3.2 and FIG. 3.5 present comparable data for L. populi reared on S. fragilis. By regressing log. H.C.W. against instar number and drawing in the calculated line, the mean H.C.W. of the missing instar(s) can be extrapolated. These values have been plotted onto FIG. 3.4 and included in TABLES 3.1a and b, marked with an asterisk. It is still unclear from these results whether larvae have four or five (or even a mixture of four and five) larval instars on P. alba since both regressions give correlation coefficients approximating one. Nevertheless, direct observation of isolated caterpillars feeding on P. alba has shown that in some cases there are five larval instars, however insufficient data are available to conclude that this is always the case. Furthermore, since there are not five discrete peaks in FIG. 3.2 it seems more likely that some L. populi larvae have four instars and some have five instars when reared on P. alba. This extra instar is probably related to the fact that L. populi larvae are slower to develop on P. alba than on S. fragilis (TABLE 3.4) which indicates that the former plant is less nutritious than the latter.

3.3.3.2. Eyed Hawkmoth

The head capsule width measurements of eyed hawkmoth larvae are presented in APPENDIX 4. No final instar caterpillars were available since in the year when H.C.W. measurements were made all full grown caterpillars

TABLE 3.1. Mean head capsule widths (H.C.W.) ($\bar{x}$ mm), $\log_{10}\bar{x}$ and standard deviation of $\log_{10}\bar{x}$ values for Laothoe populi larvae reared on Populus alba

a) With four larval instars

Instar	Mean H.C.W. $\bar{x}$ mm	$\log_{10}\bar{x}$	$SD_{\alpha}n-1$ ($\log_{10}\bar{x}$)	Standard error	n
1	1.19	0.075	0.0122	1.28×10^{-3}	91
2	*1.86	*0.27	-	-	-
3	3.22	0.507	0.0157	6.39×10^{-3}	6
4	4.51	0.654	0.0186	4.66×10^{-3}	16

b) With five larval instars

Instar	Mean H.C.W. $\bar{x}$ mm	$\log_{10}\bar{x}$	$SD_{\alpha}n-1$ ($\log_{10}\bar{x}$)	Standard error	n
1	1.19	0.075	0.0122	1.28×10^{-3}	91
2	*1.66	*0.22	-	-	-
3	*2.32	*0.365	-	-	-
4	3.22	0.507	0.0157	6.39×10^{-3}	6
5	4.51	0.654	0.0186	4.66×10^{-3}	16

Asterisks indicate extrapolated values

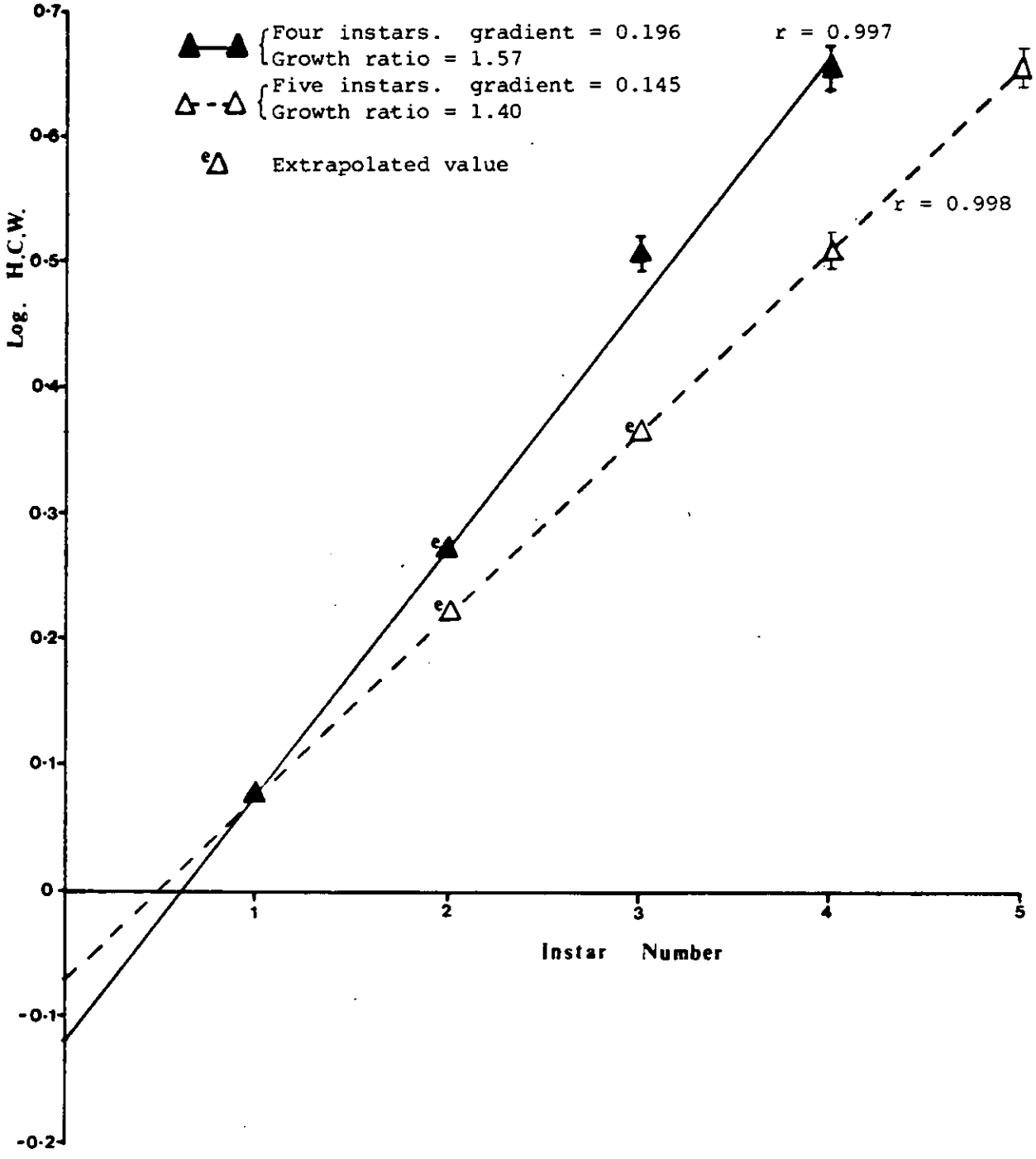


FIG. 3.4. Log. head capsule width (H.C.W.) versus instar number for Laothoe populi larvae reared on Populus alba (either four or five instars)

TABLE 3.2. Mean head capsule widths (H.C.W.) ($\bar{x}$ mm) $\log_{10}\bar{x}$ and standard deviation of $\log_{10}\bar{x}$ values for Laothoe populi larvae reared on Salix fragilis

Instar	Mean H.C.W. ($\bar{x}$ mm)	$\log_{10}\bar{x}$	SD$_{n-1}$ ($\log_{10}\bar{x}$)	Standard error	n
1	1.19	0.075	0.0122	1.28×10^{-3}	91
2	1.905	0.28	0.0196	2.30×10^{-3}	73
3	2.69	0.43	0.0327	3.43×10^{-3}	91
4	4.52	0.655	0.0219	5.84×10^{-3}	14

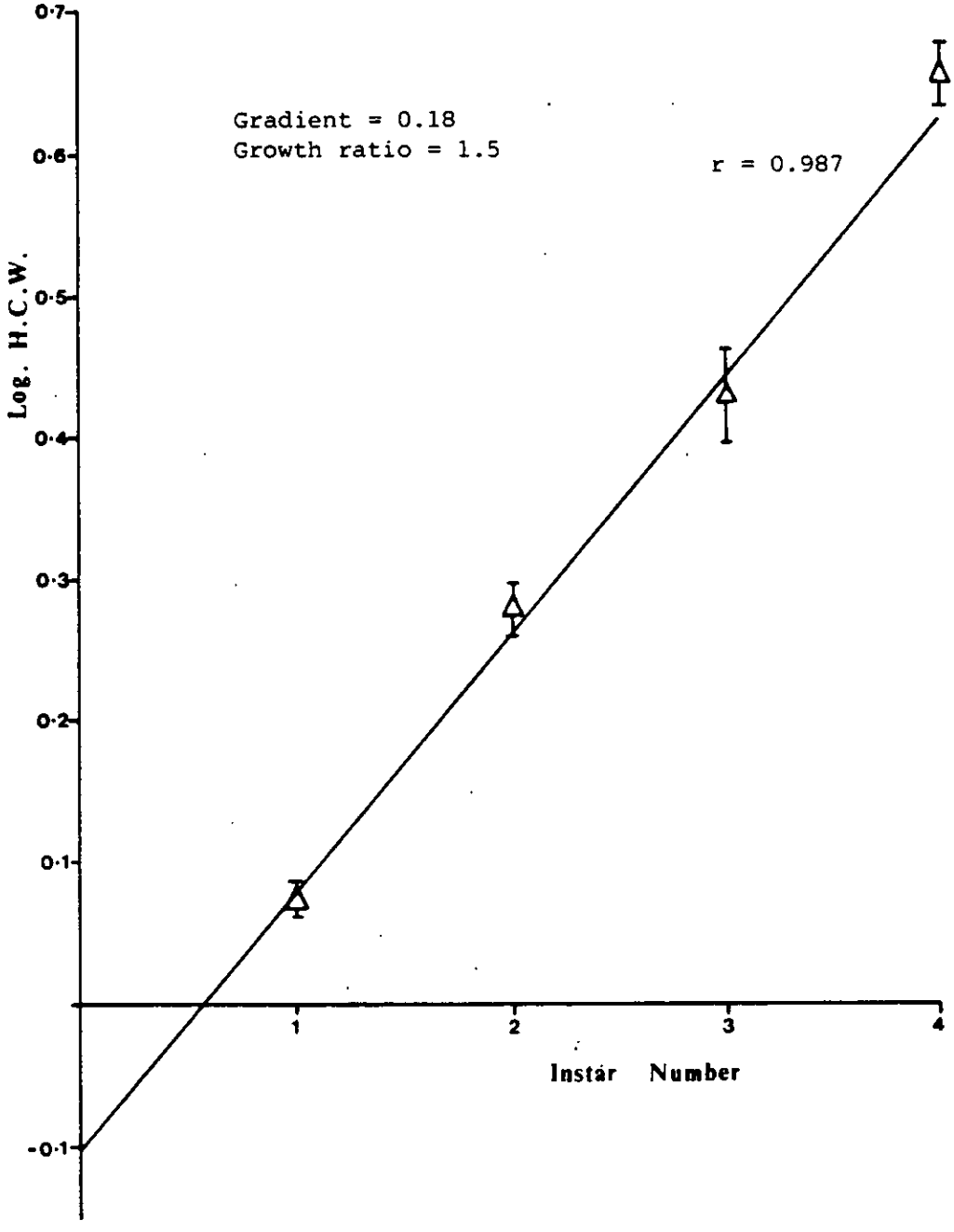


FIG. 3.5. Log. head capsule width (H.C.W.) versus instar number for Laothoe populi larvae reared on Salix fragilis

survived until pupation. A size frequency histogram of H.C.W. measurements was plotted (FIG. 3.6) which shows four distinct instar groupings. FIG. 3.7 (plotted from the H.C.W. data summarised in TABLE 3.3) shows that Dyar's law applies to this species since log. H.C.W. plotted against instar number produces a straight line. The expected H.C.W. of fifth instar larvae has been extrapolated from the regressed line and has been included in TABLE 3.3, marked with an asterisk. Thus in this species there are clearly five larval instars.

3.4. Growth, Development and Survival of Poplar Hawkmoths on S. fragilis and P. alba

3.4.1. Introduction and Aims

Field surveys have shown that L. populi larvae feeding on S. fragilis and P. alba differ in their integumental coloration. The aims of this section of the project are:

1. To compare mortalities of L. populi larvae reared on S. fragilis and P. alba under laboratory conditions
2. To compare the following growth parameters of L. populi larvae reared on the two foodplants:

a) Larval developmental time

b) Pupal weights

c) Adult wingspan

d) Egg production

3.4.2. Method

In summer 1983, three broods of larvae were divided, half the siblings from each being reared on S. fragilis, half on P. alba. Numbers of surviving larvae were counted

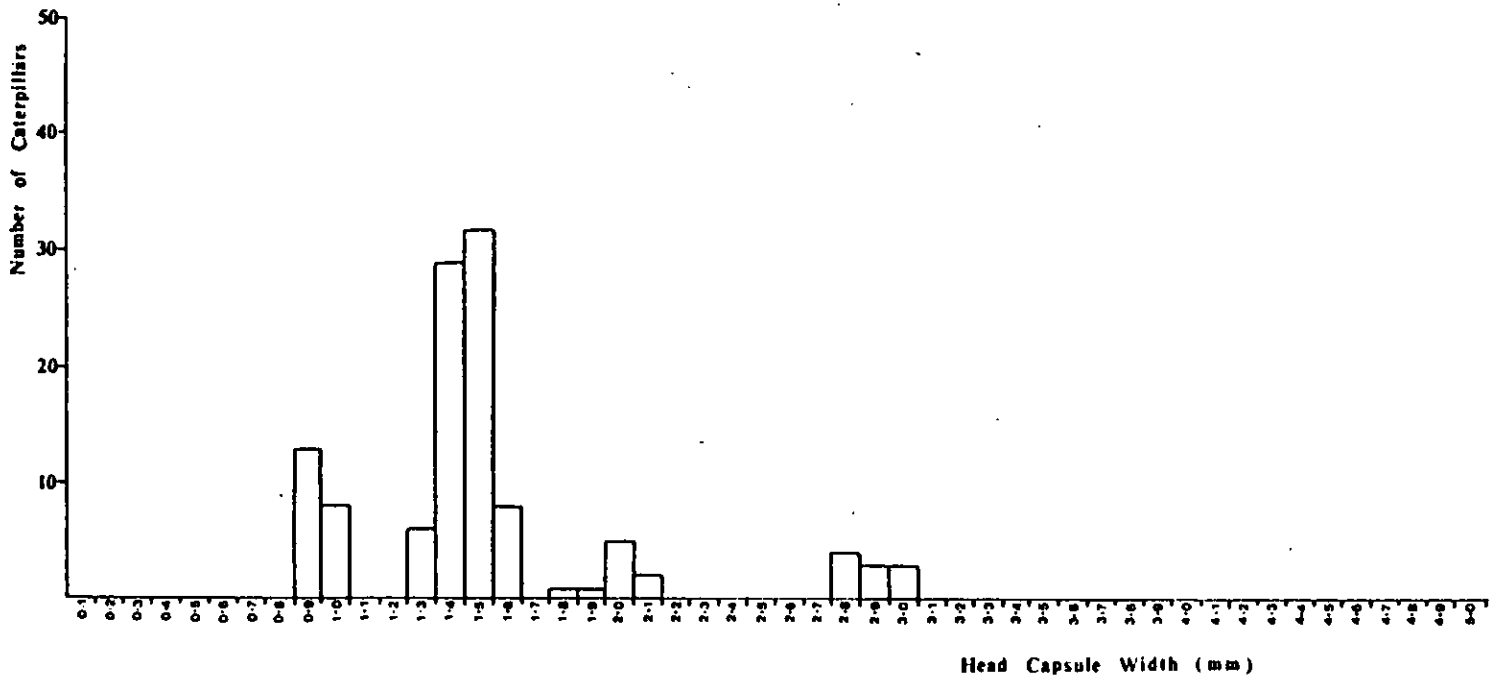


FIG. 3.6. Size-frequency histogram of head capsule widths of Smerinthus ocellata larvae

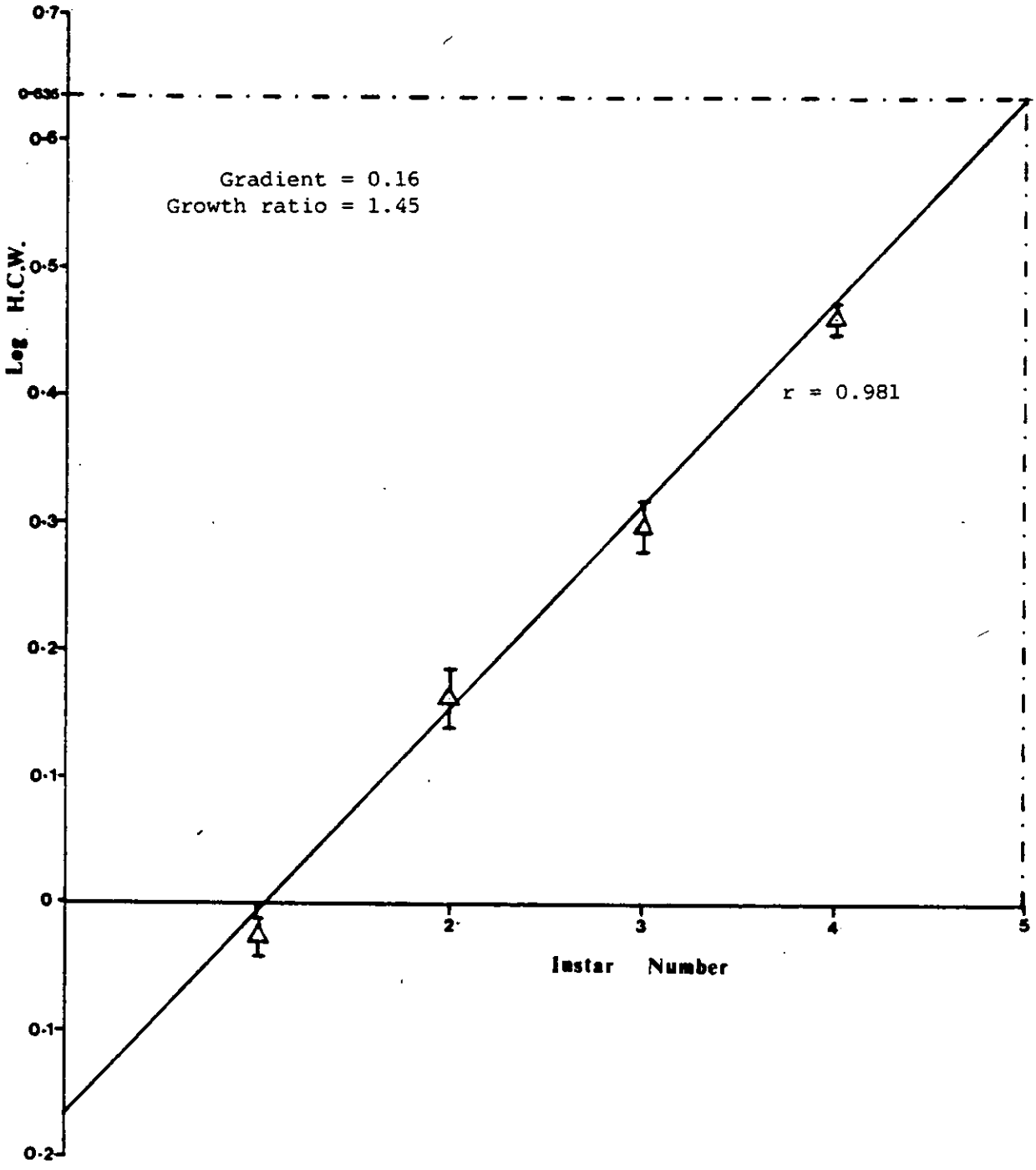


FIG. 3.7. Log. head capsule width (H.C.W.) versus instar number for Smerinthus ocellata larvae with regression line extrapolated to give fifth instar point

TABLE 3.3. Mean head capsule width (H.C.W.) ($\bar{x}$ mm), $\log_{10}\bar{x}$ and standard deviation of $\log_{10}\bar{x}$ values for Smerinthus ocellata caterpillars

Instar	Mean H.C.W. $\bar{x}$ mm	$\log_{10}\bar{x}$	SD_{n-1} ($\log_{10}\bar{x}$)	Standard error
1	0.945	-0.025	0.015	3.21×10^{-3}
2	1.456	0.162	0.024	2.74×10^{-3}
3	1.99	0.298	0.021	6.88×10^{-3}
4	2.89	0.461	0.013	4.14×10^{-3}
5	*4.3	*0.635	-	-

*Extrapolated results from FIG 3.7

at one to four day intervals. Dates of hatching and pupation of larvae were recorded. In spring 1984, pupae from these batches were removed from the sand and weighed. The dates of emergence, wingspan, and sex of adult hawk-moths were noted. For each female moth, the number of eggs laid was counted and finally these animals were dissected to establish total egg production.

3.4.3. Results and Discussion

3.4.3.1. Mortality of L. populi Larvae on S. fragilis and P. alba

Survivorship curves for sibling L. populi larvae on the two foodplants were plotted (FIGS. 3.8a, b and c) using the data presented in APPENDICES 5a, b and c.

Survival was clearly better on S. fragilis than on P. alba in all three broods. The leaves of P. alba are relatively thick due to their downy undersurface thus making biting difficult for small first instar larvae. This could possibly lead to inadequate nutrition and consequently explain the high initial mortality of larvae on this foodplant relative to that of larvae fed on S. fragilis. Williams (1966) failed to rear any larvae of this species on P. alba and suggested that surface texture was the determining factor.

Mortality on S. fragilis was also heaviest during the first instar possibly due to difficulty in feeding and overcrowding. Larvae surviving the first moult tended to survive until the final instar on both foodplants, however during the final instar, mortality increased slightly.

a) BROOD 1

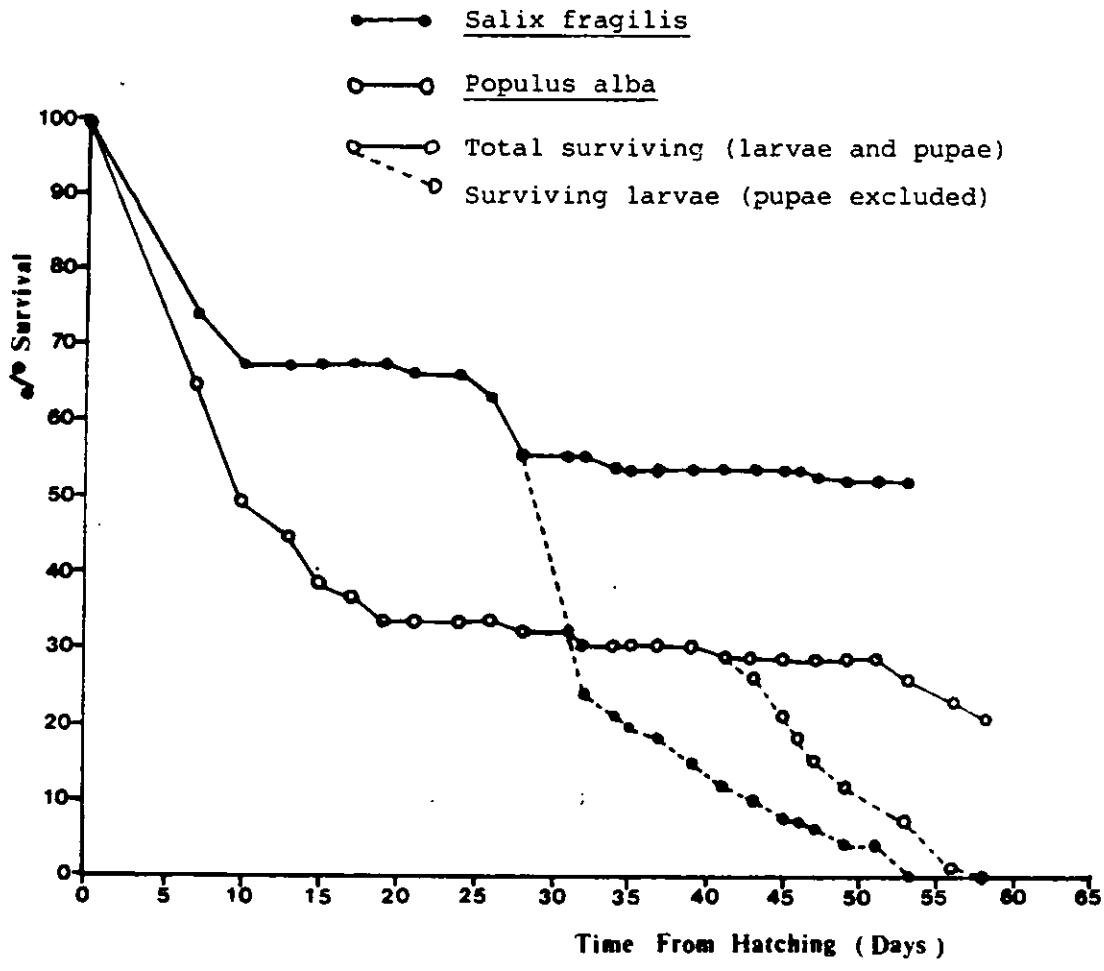


FIG. 3.8. Survivorship curves for sibling Laothoe populi larvae on Salix fragilis and on Populus alba

b) BROOD 2

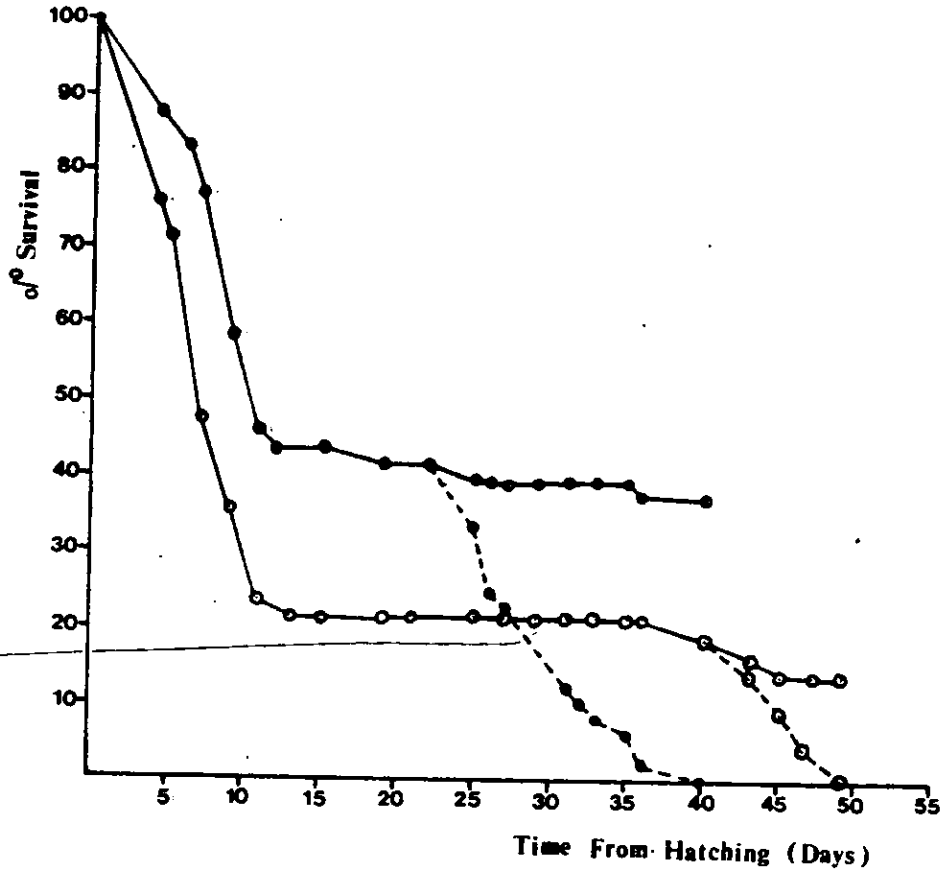


FIG. 3.8. continued

c) BROOD 3

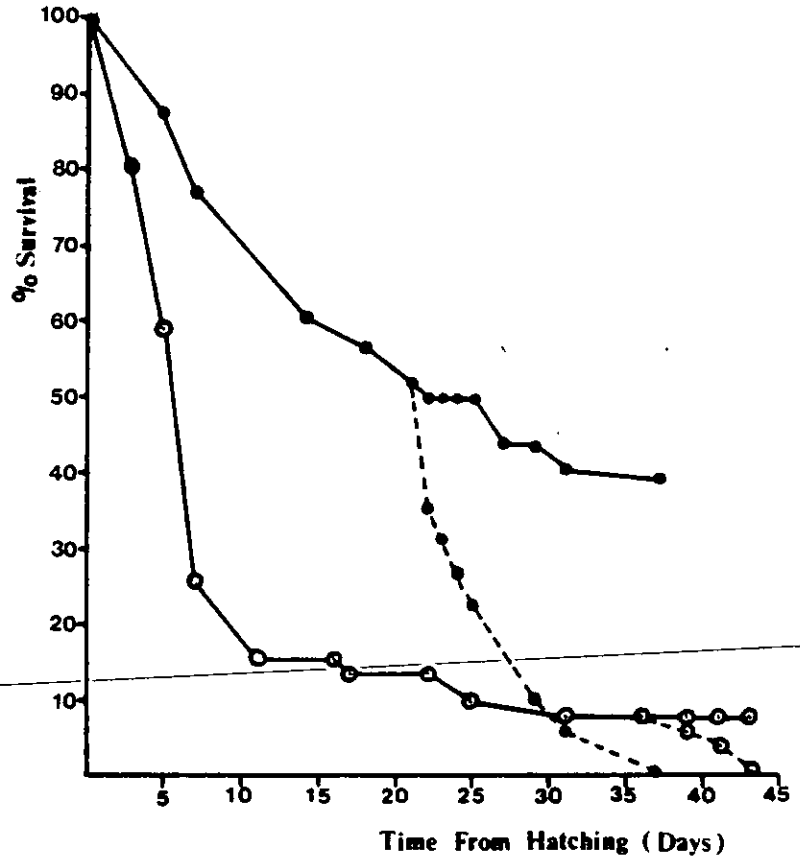


FIG. 3.8. continued

3.4.3.2. Larval Developmental Time

The number of days spent in the larval development phase for each larva was taken as the time interval between the date when the first eggs of the batch hatched to the date the larva burrowed into the sand to pupate. Larval development times for L. populi larvae on S. fragilis and P. alba for the three laboratory broods are presented in APPENDICES 6a, b and c. TABLE 3.4 shows that sibling larvae reared on S. fragilis develop significantly quicker than on P. alba in the laboratory. This difference probably results from the fact that much of the ingested P. alba leaf is indigestible downy fibres, thus making this plant less nutritious than S. fragilis which has very few fine hairs.

TABLE 3.4. t-test to compare the mean rate of larval development of Laothoe populi siblings reared on Salix fragilis and on Populus alba

Brood	Foodplant	t-test comparing mean no. of days spent as larvae on the two foodplants			
		n	$\bar{x}$	t	p(2-tailed)
1	S. fragilis	34	36.23	5.50	$p < 0.001$
	P. alba	14	47.86		
2	S. fragilis	18	29.72	4.76	$p < 0.001$
	P. alba	6	46.67		
3	S. fragilis	20	24.65	11.64	$p < 0.001$
	P. alba	4	41.50		

3.4.3.3. Pupal Weights, Adult Wingspan and Egg Production of L. populi Reared as Larvae on S. fragilis or P. alba

The results are presented in APPENDICES 7a, b and c. TABLE 3.5 shows that for Brood 3 caterpillars feeding on S. fragilis there is a significant difference between male and female mean pupal weights. TABLE 3.6 shows that mean adult wingspan of male moths is significantly different from that of female moths in this batch. Due to these differences and also limited data for males (none of the three batches had males surviving on both foodplants) the following analyses were carried out on data for females only.

t-tests comparing Broods 2 and 3 with Brood 1 showed there to be no significant differences in any of the three parameters between broods, except between adult wingspans in Broods 3 and 1 on P. alba ($t = 4.54$, $d.f = 13$, $p < 0.001$). It is possible that caterpillars in Brood 3 on P. alba were starved. This would explain the poor survival on this plant, (only three larvae survived to maturity compared to 17 siblings on S. fragilis) and also the smaller than average wingspans of the three survivors. Therefore the data for Brood 3 have been excluded from the following analyses.

TABLE 3.7 shows there are significant differences in pupal weights, adult wingspans and egg production of female poplar hawkmoths when reared on the different foodplants.

TABLE 3.8 shows there is a correlation between pupal

TABLE 3.5. Comparison of mean pupal weights of male and female poplar hawkmoths from Brood 3, reared as larvae on Salix fragilis

Sex	n	$\bar{x}$	t	p(2-tailed)
♀	10	2.151	3.89	0.01 > p > 0.001
♂	7	1.844		

TABLE 3.6. Comparison of mean adult wingspans of male and female poplar hawkmoths from Brood 3, reared as larvae on Salix fragilis

Sex	n	$\bar{x}$	t	p(2-tailed)
♀	10	3.654	2.87	0.02 > p > 0.01
♂	5	3.266		

TABLE 3.7. t-Test comparing growth parameters of sibling female poplar hawkmoths on Salix fragilis and Populus alba

Parameter	Foodplant	n	t-test $\bar{x}$	t	p(2-tailed)
Pupal weight	S. fragilis	33	2.02	10.23	< 0.001
	P. alba	17	2.70		
Adult wingspan	S. fragilis	31	3.52	9.50	< 0.001
	P. alba	15	4.04		
Egg production	S. fragilis	31	107.4	2.19	0.05 > p > 0.02
	P. alba	15	135.5		

TABLE 3.8. Spearman rank correlation tests to determine whether there is any correlation between pupal weight, adult wingspan and egg production in poplar hawkmoths (females only)

Parameters being compared	Foodplant	N	r_s	p
Pupal weight vs adult wingspan	Salix fragilis	41	0.610	< 0.01
	Populus alba	15	0.417	> 0.05
Pupal weight vs egg production	S. fragilis	40	0.345	< 0.05
	P. alba	14	0.044	> 0.05
Adult wingspan vs egg production	S. fragilis	40	0.491	< 0.01
	P. alba	14	0.362	> 0.05

weight, adult wingspan and egg production of female poplar hawkmoths reared on S. fragilis but not on P. alba.

3.5. Summary of Sections 3.3 and 3.4

1. Poplar hawkmoth larvae feeding on Salix fragilis have four instars whereas on Populus alba larvae may have either four or five instars. Eyed hawkmoth larvae have five instars under laboratory conditions. Dyar's law applies to both species.
2. Survival was better on S. fragilis than on P. alba in the laboratory. Mortality was heaviest during the first instar on both foodplants.
3. Larvae reared on S. fragilis developed significantly quicker than those reared on P. alba.
4. Female larvae reared on P. alba developed into larger pupae and subsequently larger adults than those reared on S. fragilis. Egg production was also significantly higher in the former.
5. There was a correlation between pupal weight, adult wingspan and egg production of female poplar hawkmoths reared on S. fragilis but not on P. alba.

CHAPTER 4

LABORATORY REARING PROGRAMME - LAOTHOE POPULI

4.0. Aim

Experiments have been developed to answer the following questions:-

1. Is the polymorphism genetically determined?
2. Is the polymorphism nutritionally determined?
3. Is a light stimulus necessary to elicit different colours of caterpillars and if so is it light intensity or quality which is important?
4. Does texture of the substrate affect the colour of caterpillars?

4.1. Experiment 1

4.1.0. Aim

To compare the colours of sibling larvae reared on different coloured foodplants. If the polymorphism is genetic, the colour ratios produced on the different plants should not differ significantly from one another.

4.1.1. Method

Moths from similar coloured caterpillars were crossed and the offspring divided into two groups. One group was reared on a plant which produces white caterpillars in the field (Populus alba), the other on a plant which produces green caterpillars in the field (Salix fragilis). The colours of the different groups of larvae were monitored until pupation.

All hatching Laothoe populi caterpillars were

yellow-green irrespective of their foodplant or parental background. Final instar larvae reared on S. fragilis were either yellow-green in colour (see PLATE 3) or dull-green (see PLATE 4). However, since these morphs were not discrete in the laboratory, a third category, intermediate-green was introduced (see PLATE 6). Dull-green and intermediate-green caterpillars were only usually distinguishable in the final instar.

Caterpillars reared on P. alba began to change colour from yellow-green to white after about 13 days under the laboratory conditions. However, some larvae became white after the final moult and others remained green on this foodplant. Thus, final instar caterpillars reared on white poplar cuttings were either white (see PLATE 5), yellow-green, intermediate-green or dull-green in colour.

4.1.2. Results and Conclusion

The results presented in TABLE 4.1 show that the colours of final instar sibling larvae on the two plants are significantly different. There are significantly more white caterpillars produced on P. alba than on S. fragilis in all the replicates. This indicates that the colour polymorphism is not under simple genetic control.

4.2. Experiment 2

4.2.0. Aim

For the poplar hawkmoth, preliminary work in 1980 indicated that caterpillars can change colour if their foodplant is changed. This further indicates that there is not a simple genetic basis to coloration in this

TABLE 4.1. Colours of final instar sibling Laothoe populi larvae reared on either Salix fragilis or Populus alba

Colour of both parents as larvae	Foodplant	Number of larvae of each colour YG	IG	DG	W	χ^2 (1) White: non white	p
Yellow-green	S. fragilis	30	3	3	0	15.88	< 0.001
	P. alba	10	1	2	10		
Yellow-green	S. fragilis	30	0	6	0	43.70	< 0.001
	P. alba	2	0	0	18		
Yellow-green	S. fragilis	11	2	2	0	20.61	< 0.001
	P. alba	0	0	1	12		
Dull-green	S. fragilis	13	4	8	0	*	< 0.001
	P. alba	1	0	1	5		
Dull-green	S. fragilis	10	17	33	0	*	< 0.001
	P. alba	0	0	0	10		
Dull-green	S. fragilis	4	4	4	0	*	< 0.001
	P. alba	0	2	0	7		
White	S. fragilis	18	0	2	0	*	< 0.001
	P. alba	0	0	0	9		

YG - Yellow-green
IG - Intermediate-green
DG - Dull-green
W - White

* χ^2 test not applicable as e < 5.

The Fisher exact probability test used.

species. The aim of this experiment is to determine during which instars the stimulus to become green or white is effective, by transferring caterpillars from one plant to another at different stages.

4.2.1. Experiment 2a

4.2.1.1. Method

Hatching larvae were fed on S. fragilis in the 'laboratory'. At the late first instar and mid second instar, batches of twenty caterpillars were transferred to P. alba. The colours of the various groups of larvae were monitored until pupation.

4.2.1.2. Results and Conclusion

The results presented in TABLE 4.2 show that larvae reared initially on S. fragilis have the ability to become white if transferred to P. alba before the first moult, since there are significantly more white caterpillars produced when transferred to P. alba during the first instar compared to when reared on S. fragilis throughout.

However, the results in TABLE 4.3 show that no caterpillar transferred to P. alba after the first moult became white. These results suggest that the stimulus for a caterpillar to become white is only effective if present before the first moult.

4.2.2. Experiment 2b

4.2.2.1. Method

In June 1982, 160 green second instar larvae, all reared on S. fragilis were released onto small white poplar, and crack willow trees in Cuerden Valley Park, near Chorley, Lancashire (see FIG. 2.1). The colours of

TABLE 4.2. Colour of final instar sibling Laothoe populi caterpillars reared on Salix fragilis throughout or transferred from S. fragilis to Populus alba at the late first instar

Colour of both parents as caterpillars	Food from late first instar	Number of larvae of each colour			Fisher exact probability test white vs non white
		Yellow-green	Dull-green	White	
Yellow-green	S. fragilis	30	6	0	p = 0.0013
	P. alba	6	0	4	
Dull-green	S. fragilis	7	29	0	p < 0.001
	P. alba	2	2	7	
White	S. fragilis	46	7	0	p < 0.001
	P. alba	5	3	4	

TABLE 4.3. Colour of final instar sibling Laothoe populi caterpillars reared on Salix fragilis throughout or transferred from S. fragilis to Populus alba during the second instar

Colour of both parents as caterpillars	Food from second instar	Number of larvae of each colour			Significance white vs non-white (by inspection)
		Yellow-green	Dull-green +intermed.	White	
Yellow-green	S. fragilis	30	6	0	n.s.d.
	P. alba	9	1	0	
Dull-green	S. fragilis	7	29	0	n.s.d.
	P. alba	1	6	0	
White	S. fragilis	46	7	0	n.s.d.
	P. alba	4	1	0	

n.s.d. - no significant difference

these larvae were monitored weekly until full grown.

4.2.2.2. Results and Conclusion

TABLE 4.4. Colour of final instar sibling Laothoe populi caterpillars reared on Salix fragilis until the second instar, then transferred to wild S. fragilis or Populus alba

Foodplant from second instar	No. of larvae of each colour: Yellow-green	Intermediate & dull-green	White	$\chi^2_{(2)}$	p
S. fragilis	14	3	0		
P. alba	1	15	15	33.0	<0.001

The results presented in TABLE 4.4 show that there is a significant difference between colours of final instar siblings reared on different coloured food plants in the field from the second instar. Thus, the stimulus necessary for a caterpillar to become white is effective during the second instar. However, no caterpillar changed colour when transferred from S. fragilis to P. alba during the second instar in experiment 2a, which implies that the laboratory conditions may differ from field conditions in a critical way.

4.2.3. Experiment 2c

4.2.3.1. Method

Hatching larvae were fed on P. alba in the laboratory. During the second instar (when all caterpillars are still yellow-green) twenty larvae were transferred to S. fragilis. During the third instar, ten white caterpillars

were transferred to S. fragilis. The colours of the various groups were monitored until pupation.

4.2.3.2. Results and Conclusion

TABLE 4.5. Colour of final instar Laothoe populi larvae reared on Populus alba throughout or transferred from P. alba to Salix fragilis at the second instar

Colour of parents as caterpillars	Foodplant from 2nd instar	Number of larvae of each colour YG	I	DG	W	χ^2 (1) white: green	p
White	P. alba	2	3	7	26	19.4	< 0.001
	S. fragilis	7	2	8	0		

YG - Yellow-green
I - Intermediate
DG - Dull-green
W - White

The results in TABLE 4.5 show that significantly fewer white larvae were produced when transferred from P. alba to S. fragilis at the second instar compared to those reared on P. alba throughout the experiment.

One white larva transferred to S. fragilis during the third instar became yellow-green after the final moult and two became dull-green (TABLE 4.6). Thus larvae can change colour if their foodplant is changed during the third instar. This also shows that colour change in L. populi caterpillars is reversible.

4.2.4. Conclusion for Experiment 2

The results from experiments 2a, b and c suggest that

TABLE 4.6. Colour of Laothoe populi larvae transferred from Populus alba to Salix fragilis at the third instar

	No. of larvae of each colour		
Instar	Yellow -green	Dull-green	White
iii	-	-	10
v	1	3	3

colour in poplar hawkmoth caterpillars is determined environmentally and not genetically since a colour change can be induced by changing their foodplant. The results from experiment 2a suggest that the sensitive period for receiving the stimulus to become white is during the first instar. However, in the field (experiment 2b) the stimulus is still effective during later instars.

The results from experiment 2c show that the colour change is reversible. White caterpillars can receive and respond to a stimulus to become green even after the second moult.

4.3. Experiment 3

4.3.0. Preamble and Aim

Although Experiments 1 and 2 have shown that there is not a simple genetic basis to colour polymorphism in poplar hawkmoth larvae, observations during the breeding programme have nevertheless indicated that some aspects of

colour development in caterpillars may be influenced by the larval colour of the parents. Firstly, in some batches reared on S. fragilis there appeared to be relatively high numbers of dull-green and "intermediate" coloured caterpillars compared with yellow-green ones. Secondly, in some batches a large number of larvae had red spots, whereas in other batches there were no red-spotted caterpillars. The aim of this section is to determine whether there is a genetic basis to the formation of dull-green and/or red spotted morphs.

4.3.1. Method

Throughout the laboratory experiments, moths from caterpillars of similar coloration were paired, thereby keeping any genetic influence on caterpillar coloration as simple as possible. For the same reason, moths from caterpillars with red spots were paired whenever possible. The colours of final instar caterpillars, and the number of caterpillars with red spots were recorded.

4.3.2. Results and Conclusion

4.3.2.1. Frequency of Dull-Green and Intermediate-Green Morphs

The results are presented in TABLE 4.7. The Kolmogorov-Smirnov test for heterogeneity was applied to data in each of the three groups from different parental backgrounds. There was no heterogeneity within each group and so the replicates were summed. TABLE 4.8 presents the results of comparisons made between the three groups using the chi-squared test.

The ratio of dull-green (including intermediates) to

TABLE 4.7. Colours of final instar Laothoe populi caterpillars from parents of different larval coloration, raised on Salix fragilis

Colour of both parents as caterpillars	Number of caterpillars		Kolmogorov-Smirnov test		Hetero-geneous
	YG	DG +I	largest n_1n_2D	$1.36\sqrt{Nn_1n_2}$	
Yellow-green	30	6			
	10	2			
	24	0			
	30	6	768	1341	NO
	11	4			
	22	6			
	19	5			
	7	3			
	6	0			
Dull-green	10	50			
	13	12			
	4	8	710	910	NO
	7	29			
White	18	2			
	46	7	34.6	279	NO

YG - Yellow-green
DG - Dull-green
I - Intermediates

TABLE 4.8. Comparison of the colours of fourth instar Laothoe populi caterpillars (reared on Salix fragilis) from parents of different larval coloration

Colour of both parents as caterpillars	No. of caterpillars of each colour		χ^2 (1)	p
	YG	DG (+I)		
Yellow-green	159	32	105.9	< 0.001
Dull-green	34	99		
White	64	9	70.4	< 0.001
Dull-green	34	99		
Yellow-green	159	32	0.49	0.5 > p > 0.3
White	64	9		

YG - Yellow-green
DG - Dull-green
I - Intermediates

yellow-green caterpillars is significantly different in offspring from dull-green parents compared with offspring from both yellow-green and white parents. However the relative proportion of dull-green (+ intermediates) to yellow-green caterpillars is similar in broods from yellow-green and white parents.

Thus parental background does appear to influence the relative proportion of dull-green and intermediate caterpillars in broods reared on S. fragilis.

4.3.2.2. Frequency of Red Spotted Morphs

The results are presented in TABLE 4.9. The relative proportion of spotted caterpillars in broods from parents which had spots as larvae are similar and so the three replicate sets of data have been summed. However, the proportion of larvae with red spots from parents which did not have spots as larvae varies from zero to 57%. A Kolmogorov-Smirnov test applied to these data shows heterogeneity between broods.

TABLE 4.10 shows that significantly more red-spotted larvae occur in broods from parents which had red spots during the larval stage. Thus it appears that red spots are an inherited character in eyed hawkmoth larvae. The fact that some spotted caterpillars were produced by parents which lacked spots as larvae suggests that at least one gene for red spotting must be dominant.

4.4. Experiment 4

4.4.0. Aim

Both field and laboratory results have shown that for

TABLE 4.9. Frequency of Laothoe populi larvae with red spots in broods from different parental backgrounds

Did parents have red spots as caterpillars?	Foodplant of larvae	No. of larvae with red spots	No. of larvae with no red spots	Proportion of larvae with red spots	Kolmogorov-Smirnov test
YES	*Salix fragilis*	27	9	0.75	
	Populus alba	18	2	0.90	
YES	*S. fragilis*	14	6	0.70	Homogeneous
	P. alba	7	2	0.78	$1.36\sqrt{Nn_1n_2}$ = 678.1
YES	*S. fragilis*	11	4	0.73	largest n_1n_2D = 274
	P. alba	11	2	0.85	
	TOTALS	88	25	0.78	
NO	*S. fragilis*	3	9	0.25	
	P. alba	2	7	0.22	Heterogeneous
NO	*S. fragilis*	5	20	0.20	$1.36\sqrt{Nn_1n_2}$
	S. caprea	7	17	0.29	= 1221.75
NO	*S. fragilis*	0	60	0.00	largest n_1n_2D
	P. alba	0	10	0.00	= 2040
NO	*S. fragilis*	13	12	0.52	
	P. alba	4	3	0.57	

TABLE 4.10. Comparison of the frequencies of red spotted larvae in broods from parents with or without red spots

Did parents have red spots as larvae?	No. of offspring with red spots	No. of offspring with no red spots	χ^2 (1)	p
YES	88	25	21.90	$p < 0.001$
NO	5	16		
YES	88	25	39.00	$p < 0.001$
NO	12	37		
YES	88	25	6.46	$0.02 > p > 0.01$
NO	17	15		
YES	88	25	101.9	$p < 0.001$
NO	0	70		

the poplar hawkmoth there is a relationship between colour of larva and foodplant. This experiment was designed to test whether the colour of final instar caterpillars is determined by the chemical nature or texture of the food, or whether the colour is determined by some stimulus perceived by the caterpillar's photoreceptors.

4.4.1. Method

Sibling caterpillars were split into two groups, one group being reared on S. fragilis in continuous darkness, the other in a normal day/night regime. Trial experiments using P. alba as the foodplant resulted in very high mortalities in the dark, so later broods were not split.

4.4.2. Results and Conclusion

The results presented in TABLE 4.11. show that the proportion of dull-green and intermediate-green coloured larvae is significantly higher in batches reared in continuous darkness compared to in batches reared in a normal day/night regime on Salix fragilis. It was unusual that in one brood reared on this foodplant in permanent darkness a white final instar larva was produced. Throughout the breeding experiments, no white caterpillar was produced on this foodplant in a normal day/night regime.

The results for caterpillars reared on Populus alba in continuous darkness are presented in TABLE 4.12. A control brood of similar parental background to the experimental broods but reared in a normal day/night regime was used for comparison.

The proportion of dull-green and intermediate

TABLE 4.11. Colours of final instar Laothoe populi larvae reared in continuous darkness compared to in a normal day/night regime (fed on Salix fragilis)

Colour of parents as caterpillars ♀	♂	Colour of larvae in normal day/night regime YG	DG+I	W	Colour of larvae reared in continuous darkness YG	DG+I	W	p (DG+I vs rest)
White	Yellow-green	13	4	0	0	18	0	< 0.001 (χ^2 = 18.74)
Yellow-green	Yellow-green	19	5	0	1	5	0	0.009 (F.e.)
Yellow-green	Yellow-green	*11	*4	0	1	42	0	< 0.001 (F.e.)
White	White	*46	*7	0	8	21	0	< 0.001 (χ^2 = 26.65)
White	White	*46	*7	0	1	10	0	< 0.001 (F.e.)
Yellow-green	Dull-green				1	13	1	

* Day/night controls drawn from TABLE 4.7 since broods in darkness were not split.

YG - Yellow-green I - Intermediate-green F.e. - Fisher's exact test
DG - Dull-green W - White applied since e < 5

TABLE 4.12. Colours of final instar Laothoe populi larvae reared in permanent darkness compared to in normal day/night conditions (fed on Populus alba)

Lighting condition	Colour of parents as caterpillars ♀	♂	Colour of caterpillars YG	DG(+I)	W	Comparison with control p(dull-green+intermediates vs other colours)	p(white vs non-white)
Permanent darkness	Dull-green	Yellow-green	0	6	0	< 0.001	0.004
	Yellow-green	Dull-green	0	5	0	< 0.001	0.01
	Yellow-green	Dull-green	1	2	0	Not significant (small sample)	not significant (small sample)
CONTROL Normal day/night regime	Yellow-green	Dull-green	10	7	30		

YG - Yellow-green
DG - Dull-green
I - Intermediate-green
W - White

coloured caterpillars is significantly higher in two out of the three batches reared in continuous darkness compared to in the control. In the third brood the number of larvae surviving is presumably too low to show any difference. No white caterpillars were produced on P. alba when reared in permanent darkness, whereas over half the caterpillars in the control on this foodplant became white.

Since the colour ratios produced in the two light regimes were different (both on S. fragilis and on P. alba) this suggests that neither the chemical nature of the food nor the texture of the leaf surface are responsible for determining caterpillar coloration. It appears that some stimulus associated with light is necessary to elicit the normal development of both yellow-green and white colours.

4.5. Experiment 5

4.5.0. Aim

The aim of this experiment was to determine whether different coloured substrates affect larval coloration.

4.5.1. Method

Hatching larvae were reared in darkness for seven days. Salix fragilis was used as the foodplant since in the previous year (1981), broods reared in permanent darkness on this plant survived relatively well. From day eight, caterpillars were fed only during the night (i.e. in the dark) and were placed in rearing cages with either white, green or black paper backgrounds (but no food) during the day. Larvae were given fresh food each night.

In the daytime, the substrate leaf was carefully cut from around each larva which was then left to self-transfer onto the appropriate coloured background. The 'colour-cages' were placed in a well lit place out of direct sunlight for six to eight hours. Larvae (plus their paper substrate) were then returned to darkness to feed. The colours of larvae in the various groups were recorded daily.

4.5.2. Results and Conclusion

Although survival was poor, the results in TABLE 4.13. indicate that caterpillars reared on a white background tend to become white whereas those reared on a green or black background become green.

4.6. Experiment 6

4.6.0. Aim

The results obtained in Experiment 5 could be either due to caterpillars perceiving different colours or different intensities of reflected light. The aim of Experiment 6 is to determine whether the intensity of reflected light affects caterpillar coloration.

4.6.1. Method

Experiment 5 was repeated using white and grey papers. The latter was selected to have a similar reflected light intensity value to the green paper using a Minilux Photoelectric Photometer P1 light meter. Perspex boxes (19 x 11 x 7 cm) were covered on the outside with either white or grey paper and a similar coloured piece of paper was placed inside each box. The transparent lids

TABLE 4.13. Colour of Laothoe populi caterpillars reared on Salix fragilis at night but kept in coloured cages without food during the day

Colour of parents as larvae			Colour of caterpillars in Green cage			Black cage			White cage		
Female	Male	Instar	YG	DG+I	W	YG	DG+I	W	YG	DG+I	W
YG	I	iii	4	3	0				0	0	6
		iv	4	2	0				0	0	5
YG	YG	iii				7	0	0	0	1	3
		iv				0	2	0	0	0	4
YG	YG	ii				4	0	0	3	2	3
unknown	unknown	iii	8	0	0				0	0	6

YG - Yellow-green DG - Dull-green I - Intermediate-green
W - White

were left uncovered. These colour cages were more suitable than the regular rearing cages since the latter have black bases and lids (which were more impractical to cover).

Larvae were reared in darkness on Salix fragilis until the early second instar. They were then introduced into the light-boxes, initially for four hours each day, but gradually increasing the time spent in the light-boxes to eight hours daily by the third instar.

The loose paper in each colour cage was renewed frequently and the boxes were cleaned each day to minimise the spread of infection. The colours of the various groups of larvae were monitored daily.

4.6.2. Results and Conclusion

The results are presented in TABLE 4.14. Larvae only survived until the third instar in this experiment, probably as a consequence of frequent handling and long daily periods with no food. However, all 17 caterpillars surviving on the grey background in daylight developed a normal yellow-green colour whereas 19 out of 26 larvae placed on a white background became white (see PLATE 7). In both experimental broods, the ratios of white to non-white larvae produced were significantly different on the two backgrounds. These results suggest that it is the intensity of reflected light rather than its colour which is the cue for a caterpillar to become white or yellow-green.

TABLE 4.14. Colour of Laothoe populi caterpillars reared on Salix fragilis at night but kept on either grey or white backgrounds (with no food) during the day

Colour of both parents as caterpillars	Instar of caterpillars	Colour of caterpillars in Grey cage			Colour of caterpillars in white cage			p white:non white in grey and white cages (Fisher exact test)
		YG	DG+IG	W	YG	DG+IG	W	
White	Third	10	0	0	4	2	8	0.004
Yellow-green	Third	7	0	0	1	0	11	< 0.001

YG - Yellow-green
DG - Dull-green
IG - Intermediate-green
W - White

4.7. Experiment 7

4.7.0. Aim

The results from Experiment 6 suggest that reflected light intensity may be the vital cue determining whether poplar hawkmoth caterpillars become yellow-green or white.

The aims of this experiment are:

1. To compare the spectral properties of Populus alba and Salix fragilis leaves.
2. To compare the spectra of the green, white and grey papers used in Experiments 5 and 6 with those of the two foodplants.

4.7.1. Method

The spectra were run on a modular fluorimeter using a 150 Watt tungsten iodide light source.

For the plants, a 4 cm x 1 cm section of leaf (free from large veins) was cut from a fully expanded mature leaf and placed in a cuvette at a 45° slope. The spectra of light reflected from the lower surface of S. fragilis and P. alba leaves were recorded. The spectra of light reflected from the green, white and grey papers were recorded in a similar way.

4.7.2. Results and Conclusion

The spectra of reflected light from the green, grey and white papers and from the lower leaf surfaces of P. alba and S. fragilis are shown in FIG. 4.1. The area under each spectrum is directly proportional to the intensity of light reflected from that particular substrate. The relative intensity values of light reflected from each substrate compared with the white paper are

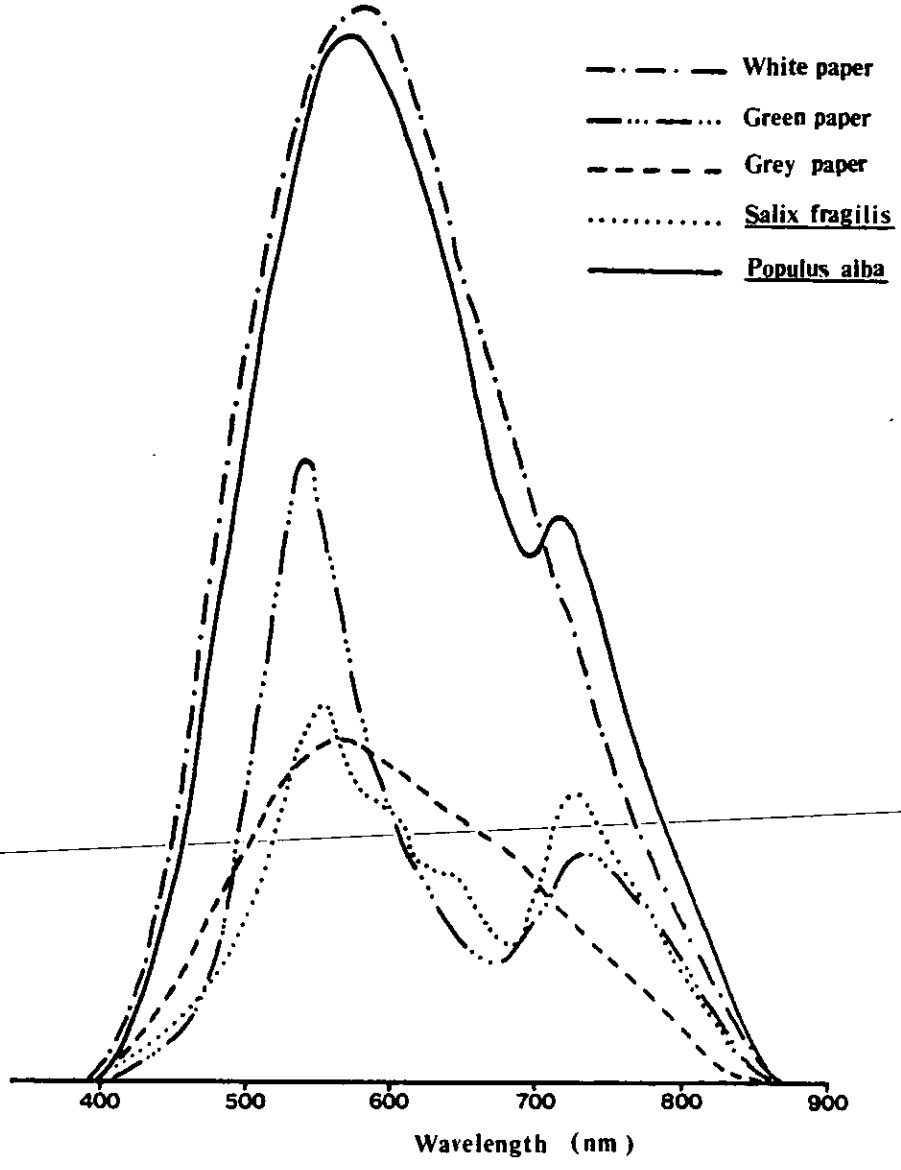


FIG. 4.1. Comparison of reflected light from green, white and grey papers used in Experiments 5 and 6 and from the lower leaf surfaces of Populus alba and Salix fragilis (using a tungsten iodide light source

presented in TABLE 4.15. The following conclusions can be drawn from the results:-

1. The white paper used in Experiments 5 and 6 is a good 'colour' representation of the white downy lower leaf surface of P. alba since both the spectra and relative intensities of reflected light from these two substrates are very similar.

2. The green paper used in Experiment 5 is a good 'colour' representation of the lower leaf surface of S. fragilis since the intensity peaks in the spectra of these two substrates occur at similar wavelengths (see FIG. 4.1). The intensity of light reflected from the green paper is slightly greater than from the S. fragilis leaf. However the colour of S. fragilis leaves varies and the spectrum obtained represents only one leaf.

3. The light spectrum of the grey paper has a similar wavelength distribution to that of the white paper and P. alba leaf but reflects a similar intensity of light as the green paper and S. fragilis leaf. Therefore it is a suitable substrate for determining whether intensity rather than colour of light is the cue for colour development in L. populi larvae.

4. Approximately three times as much light is reflected from the P. alba lower leaf surface compared with the S. fragilis lower leaf surface.

4.8. Experiment 8

4.8.0. Introduction

In the field surveys (Chapter 2) 53 out of 54 final

TABLE 4.15. Relative intensity and wavelength peaks of light reflected from the coloured papers used in Experiments 5 and 6 and from the lower leaf surfaces of Populus alba and Salix fragilis (Tungsten iodide light source)

Substrate	Area under curve (cm^2)	Relative Intensity	Wavelength Peaks (nm)
White paper	122	1	572
Grey paper	40	0.33	570
Green paper	46	0.38	531 732
Populus alba leaf (downy undersurface)	118	0.97	554
Salix fragilis leaf (lower leaf surface)	40	0.33	551 724

instar poplar hawkmoth larvae found on white poplar bushes were white and therefore were well camouflaged against the white downy leaf undersurface of this plant. However, in batches of L. populi reared on P. alba in the laboratory, up to 55% of the final instar larvae were green (TABLES 4.1. and 4.5.). This discrepancy could be due to the larvae in the breeding cages receiving 'false' signals by resting in the 'wrong' position. In the field, all L. populi (including some in the early instars) were resting on the undersurface of leaves.

4.8.1. Aim

The aim of this experiment is to determine on what part of the foodplant a poplar hawkmoth caterpillar rests in the laboratory conditions.

4.8.2. Method

In 1982, the resting positions of L. populi larvae on both P. alba and S. fragilis were recorded during each larval instar. The main resting sites were the upper and lower leaf surfaces and the stalks. However, the occasional larvae on P. alba was in its feeding position on the edge of a leaf.

4.8.3. Results and Conclusion

The results presented in APPENDICES 8a and 8b show that the majority of larvae rest on the leaves of the foodplants. Since the larvae on the stalks tended to be moving (presumably to a new leaf) and not actually resting, these data have been omitted from the subsequent analyses. The few larvae on the sides of P. alba leaves were feeding rather than resting so have also been disregarded.

The Kolmogorov-Smirnov heterogeneity test was applied to the data for caterpillars resting on the upper and lower leaf surfaces of both foodplants (TABLE 4.16a and b). There is no heterogeneity within or between instars on S. fragilis (see TABLE 4.16a). A chi-squared test applied to the summated data on this foodplant shows that significantly more caterpillars are resting on the lower compared with the upper leaf surfaces (χ^2 = 206, $p << 0.001$ compared with a 50:50 distribution).

For the larval resting site data on P. alba there is heterogeneity between the first instar replicates only. In three of the first instar replicates (asterisked in APPENDIX 8b) there are significantly higher proportions of larvae resting on the upper leaf surfaces compared with in the later instars (* χ^2 = 6.46, $p \approx 0.01$, ** χ^2 = 62.43, $p < 0.001$, *** χ^2 = 18.9, $p < 0.001$). This difference in resting behaviour amongst some batches of first instar larvae may have been a result of overcrowding in the cages. Although the density of caterpillars was normally kept to below 20 per cage, newly hatching batches (of up to 150 larvae) were usually kept in only one or two cages for the duration of their first instar.

A chi-squared test applied to the summed data for second to final instar larvae on P. alba shows that significantly more larvae choose to rest on the lower rather than the upper leaf surfaces (χ^2 = 211, $p < 0.001$ compared with a 50:50 distribution).

Further the proportion of larvae resting on the lower surface of S. fragilis and P. alba are similar (χ^2 = 0.49,

TABLE 4.16. Kolmogorov-Smirnov heterogeneity tests on the resting site data of Laothoe populi larvae on a) Salix fragilis leaves and b) Populus alba leaves

a) S. fragilis

Instar	Position of larvae		Largest		Hetero-
	Leaf-upper	Leaf-under	n_1n_2D	$1.36\sqrt{Nn_1n_2}$	geneous
i	23	139	731	979	NO
ii	24	129	651	936	NO
iii	18	139	415	852	NO
iv	17	155	368	916	NO
i-iv	82	562	3156	7409	NO

b) P. alba

Instar	Position of larvae		Largest		Hetero-
	leaf-upper	Leaf-under	n_1n_2D	$1.36\sqrt{Nn_1n_2}$	geneous
i	72	256	5184	3344	YES
ii	20	153	252	989	NO
ii+iii	19	92	537	599	NO
iii	16	195	658	1104	NO
iv and v	13	95	172	497	NO
ii-v	68	535	4700	6370	NO

$p \approx 0.5$, omitting first instar data on P. alba). Thus it seems that most L. populi larvae choose to rest on the under surface of leaves. This is probably an innate behavioural habit which has evolved because it gives better protection against predators.

Experiment 6 has shown that the colour of a final instar caterpillar depends on the intensity of light reflected from its substrate. Thus, a larva which rests on the upper surface of a P. alba leaf during its early instars may be receiving a visual signal to become green. The relatively high proportion of green caterpillars on P. alba in some laboratory batches compared to in the field was probably a result of many larvae in overcrowded batches resting on the leaf upper surface rather than on the lower surface in their initial instar.

CHAPTER 5

LABORATORY REARING PROGRAMME - SMERINTHUS OCELLATA

5.0. Introduction

The overall aim of this section of the project is to determine the environmental and possible genetic factors which influence coloration of eyed hawkmoth caterpillars.

5.1. Experiment 1

5.1.1. Aim

To investigate the effect of foodplant on caterpillar coloration in the laboratory and determine whether the ratios of green to grey morphs are comparable to those found in the field surveys.

5.1.2. Method

Larvae from parents of known caterpillar coloration were reared on either Salix atrocinerea, S. fragilis or S. repens and during the fourth and fifth instars the colour of each caterpillar was recorded. Three colour categories were recognized: green, intermediate and grey.

5.1.3. Results and Discussion

TABLE 2.2 shows that in the field, the majority of eyed hawkmoth caterpillars found on S. atrocinerea were green thus being well camouflaged amongst the green leaves of this plant. However, the results presented in TABLE 5.1 show that in the laboratory the majority of caterpillars reared on this foodplant became grey and none became green (irrespective of the colour of the parents as caterpillars). A number of caterpillars, however, were slightly

TABLE 5.1. Colours of laboratory reared Smerinthus ocellata caterpillars fed on various foodplants

Colour of parents as caterpillars ♀	♂	Foodplant of offspring	Colour of offspring larvae			Instar
			Green	Intermediate	Grey	
Grey	Grey	S. atrocinerea	0	0	13	iv
Grey	Grey	S. atrocinerea	0	0	1	v
Grey	Grey	S. atrocinerea	0	0	8	iv
Grey	Grey	S. atrocinerea	0	0	10	iv
Grey	Grey	S. atrocinerea	0	2	1	v
Grey	Grey	S. atrocinerea	0	1	3	v
Grey	Grey	S. atrocinerea	0	0	5	iv
Green	Grey	S. atrocinerea	0	0	4	iv
Green	Grey	S. atrocinerea	0	4	1	iv
Grey	Grey	S. fragilis	0	1	7	v
Green	Grey	S. fragilis	0	1	3	v
Grey	Green	S. fragilis	0	0	8	iv
Grey	Grey	S. repens	0	0	1	v

greener than the normal grey category and these were recorded as "intermediates". Similar results were obtained when S. ocellata larvae were reared on S. fragilis, another species which produces green larvae in the field (TABLE 2.2). Survival on S. repens was very poor; only one larva from a total of three egg batches survived to the final instar, so this foodplant was not used in further experiments.

The results from this experiment indicate that the stimulus for a caterpillar to become green or grey is neither nutritional nor textural since plants which normally produce green larvae in the field produced only grey and intermediate larvae in the laboratory. Furthermore, caterpillar colour is unlikely to be under simple genetic control otherwise at least some of the grey/green matings would be expected to produce some green caterpillars. However, the experiment can be criticised on the grounds that lighting in the cages in both the laboratory and shed was poor and not comparable with that in the field, so it is possible that the low intensity of illumination prevented the formation of green caterpillars.

5.2. Experiment 2

5.2.1. Aim

Failure to obtain any green caterpillars in experiment 1 was possibly a result of poor illumination in the "laboratory" during rearing. The aim of the following experiment was to investigate the effect of duration and intensity of incident light on larval coloration in the

eyed hawkmoth.

5.2.2. Experiment 2a

5.2.2.1. Method

Siblings from known parents were divided and reared on S. atrocinerea under the following light regimes:-

a) Permanent darkness:- the cages were covered with black plastic.

b) Permanent bright light:- achieved by placing the cages under fluorescent strip lights.

c) Normal day/night regime:- the cages were placed in a well lit position in the "laboratory".

The colours of the three groups were monitored until pupation. The experiment was repeated using S. fragilis as the foodplant.

5.2.2.2. Results and Discussion

TABLE 5.2. Colours of final instar Smerinthus ocellata caterpillars reared in a) Permanent darkness, b) Permanent bright light and c) A normal day/night regime

Colour of both parents as caterpillars	Foodplant of larvae	Number of caterpillars of each colour in light regime								
		a) dark			b) light			c) Light/Dark		
		G	I	Gr	G	I	Gr	G	I	Gr
Green	Salix atrocinerea	0	0	6	0	0	2	0	1	6
Green	S. fragilis	0	1	2	0	0	6*	0	4	4

G - Green
I - Intermediate
Gr - Grey
* - third instar larvae

The results presented in TABLE 5.2 show that no green caterpillars were produced in any light regime: all eight larvae surviving in bright light were grey. The method of lighting used, however, was not ideal: the lids of the rearing cages are black (with a 9 cm diameter metal mesh) thus lights positioned alongside the cages would have been more effective than overhead lights. Poor survival in this condition (only two larvae survived to the final instar) was possibly due to the quick wilting of the foodplants under the heat of the fluorescent light tubes.

These results however, do indicate that the polymorphism is not under simple genetic control since no larva became green despite being from parents which were green as caterpillars.

5.2.3. Experiment 2b

5.2.3.1. Method

A batch of eggs was divided four ways and larvae fed on S. atrocinerea under the following light regimes:-

a) Permanent darkness

b) A normal day/night regime in a well lit position in the shed

c) A normal day/night regime in a dimly lit position in the shed

d) A normal day/night regime on small bushes in the field.

The colours and developmental stages of larvae in the four groups were recorded throughout the experiment.

5.2.3.2. Results and Discussion

Under laboratory conditions (a, b and c) no larvae survived beyond the third instar. No larvae released onto

S. atrocinerea in the field were refound. The section of hedge onto which they were released was grazed by cattle shortly after release of the caterpillars. Unfortunately no further batches of eggs were available to repeat the experiment.

Due to the poor survival of eyed hawkmoth caterpillars in the "laboratory" and a failure to obtain any green morphs, the following experiments were carried out in the field. If a single batch of eggs produces green caterpillars in the field but only grey ones in the laboratory, this would suggest that the stimulus to become green is not effective under laboratory conditions and would further confirm that the polymorphism is not under simple genetic control.

5.3. Experiment 3

5.3.1. Aim

To compare the colours of sibling eyed hawkmoth larvae reared on various foodplants in the field with the colours produced in the "laboratory".

5.3.2. Method

Twenty sibling first instar larvae were released onto each of the following bushes in the field:-

a) S. fragilis, Cuerden Valley Park, near Chorley, Lancashire (FIG. 2.1)

b) S. viminalis, Cuerden Valley park, near Chorley, Lancashire

c) S. caprea, Cuerden Valley Park, near Chorley, Lancashire

d) S. atrocinerea, Mill Lane, Goosnargh, Lancashire.

Two control groups of twenty larvae were reared in the shed on S. atrocinerea. The colours of larvae in the six groups were recorded weekly.

5.3.3. Results and Discussion

The larvae released onto bushes in the field only survived up to the second instar, no larva was refound after this stage. Sixteen larvae reared in the 'laboratory' survived to the fourth instar, two being intermediate in colour, the rest being grey.

This experiment was repeated using S. viminalis and S. atrocinerea bushes only, with S. atrocinerea as a control in the laboratory, however no larvae survived past the second instar. Poor survival of larvae in the field could be a result of heavy predation by birds due to the high density of larvae on each bush. Alternatively it is possible that poor survival was due to successive years of inbreeding.

5.4. Experiment 4

5.4.1. Aim

To produce green S. ocellata caterpillars by rearing them in a protected environment in the field.

5.4.2. Method

Sibling larvae from "green" parents were reared on either S. fragilis or S. viminalis trees at Goosnargh, Lancashire. In order to protect the caterpillars from predation by birds, the branches onto which larvae were released were enclosed in white muslin fine mesh sleeves.

Frass was removed from the sleeves, once a week at first and every other day when larvae were full grown. Larvae were transferred to fresh branches when necessary. The colours of the two groups were monitored during each instar.

5.4.3. Results and Discussion

TABLE 5.3. Colours of sibling Smerinthus ocellata caterpillars reared in white muslin sleeves on either Salix fragilis or Salix viminalis in the field

Colour of parents as caterpillars	Foodplant of larvae	Number of fifth instar larvae		
		Green	Grey	White-green
Green	S. viminalis	0	0	15
	S. fragilis	0	0	17

The results presented in TABLE 5.3 show that all larvae reared in the white muslin sleeves in the field became white-green by the final instar on both S. fragilis and S. viminalis. While this white colour was not as pale as white morphs of poplar hawkmoth larvae, it was a much paler shade than the normal grey eyed hawkmoth caterpillars reared in the laboratory or found in the field. The green first instar larvae became grey by the second instar and finally became whiter than any previous individual of this species. TABLE 2.2 shows that in the wild, green and grey morphs occur on S. viminalis in similar proportions whereas only green morphs were found on S. fragilis. The results

of the present experiment suggest that the white netting is causing the larvae to become white-green and thus that some quality of light perceived by the caterpillar is a critical factor contributing to colour formation in eyed hawkmoth caterpillars. Since the larvae in this experiment were from parents which were green as caterpillars it is unlikely that the colour polymorphism in this species is genetic.

It was planned to repeat this experiment in 1983 using both green and white netting but unfortunately, no eyed hawkmoths emerged in that year.

CHAPTER 6

FIELD PREDATION EXPERIMENTS

6.1. Aim

To ascertain whether there is selective predation on different coloured caterpillars depending on their conspicuousness against their foodplants.

6.2. Experiment 1

6.2.1. Method

In 1982 Laothoe populi caterpillars were reared to the second instar on Salix fragilis in the shed at Goosnargh. These green larvae were released onto Populus alba, S. fragilis and S. caprea bushes of similar heights (1.5 to 2 metres high) in Cuerden Valley park (see FIG. 2.1 for location). Each of the bushes used was initially searched and native caterpillars (not only L. populi) were transferred to nearby trees. Ten reared larvae were then released onto each of the selected bushes. At intervals of seven days, the experimental bushes were searched and the surviving larvae counted and scored for colour.

6.2.2. Results and Discussion

The results are presented in APPENDIX 9. Survival on six of the eight S. fragilis bushes (APPENDIX 9, replicates 1-6) was unexpectedly poor and the four larvae actually surviving on these plants after seven days appeared to be very unhealthy. Subsequent laboratory observations showed that healthy second instar larvae placed on cuttings from these plants became flaccid and

sickly after two to three days. This plant is probably an unknown cultivar of S. fragilis which is clearly indigestible to L. populi caterpillars. In TABLE 6.1 which summarises the results and on FIG. 6.1 this plant is referred to as 'S. fragilis - unnamed cultivar'.

A further problem was that the green second instar larvae placed on P. alba bushes tended to become cryptic with time. About 50% had become white or slightly greeny-white by week three when most larvae were in their final instar (see TABLE 4.4).

FIG. 6.1 presents survivorship curves for L. populi larvae on the four different foodplants. Survival was best on S. fragilis and poorest on the cultivated variety of this species. The green larvae were well camouflaged (to the human eye) on both of these foodplants however on the latter the high mortality was probably due to starvation rather than predation (see above).

Survival was better on P. alba than on S. caprea even before caterpillars on the former plant had become camouflaged. The caterpillars released onto the S. caprea plants were probably more exposed to predators due to the upright structure of the branches and leaves and also the relatively small leaf size.

The results of a chi-squared test (TABLE 6.1) show that survival of L. populi larvae on P. alba, S. fragilis and S. caprea was significantly different at all observation stages. A comparison on the week 4 data was not carried out since at this stage some larvae had possibly pupated. These differences in survival on the three

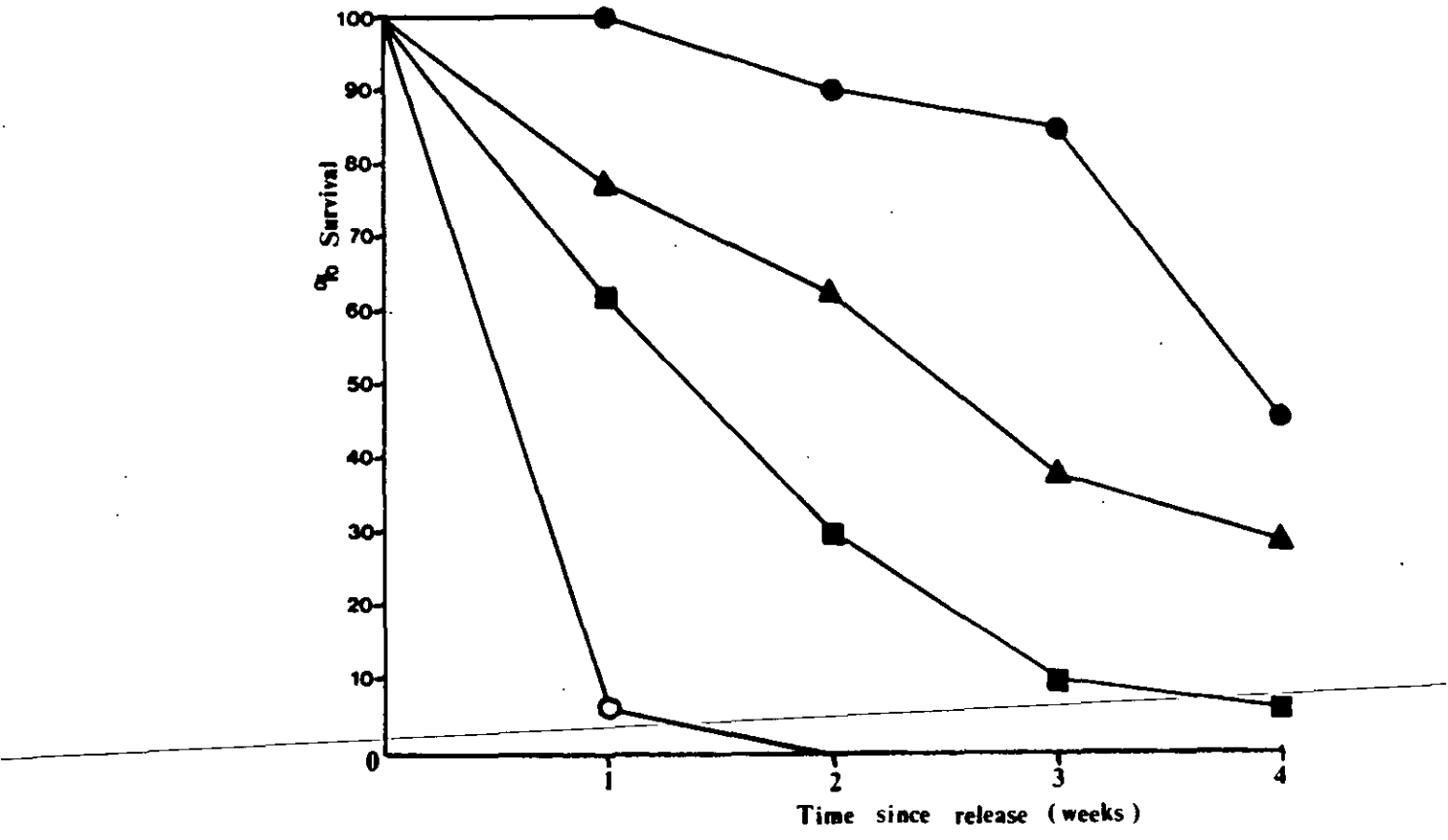


FIG. 6.1. Survival of green Laothoe populi larvae on various foodplants in the field

TABLE 6.1. Survival of Laothoe populi larvae on various foodplants in the field

Time from release (weeks)	Number of larvae surviving on				Comparison of survival on P. alba, S. fragilis and S. caprea		
	Populus alba	Salix fragilis	S. fragilis "unnamed cultivar"	S. caprea	χ^2 (2)	df	p
0	80	20	60	50	-	-	-
1	62	20	4	31	11.53	2	0.01 > p > 0.001
2	50	18	0	15	24.37	2	p < 0.001
3	30	17	0	5	36.10	2	p < 0.001
4	23	9	0	3	*	*	*

* Test not carried out since some larvae may have pupated.
df - Degrees of freedom

foodplants were probably due to differential predation although none of the predators of L. populi larvae were observed.

6.2.3. Conclusion

Survival of green L. populi larvae differed significantly on S. fragilis, S. caprea and P. alba bushes. However, this may have been due to the differences in the structure of the three plants (and hence the resting sites and exposure of larvae to predators) rather than whether the caterpillars were camouflaged or not. Thus, in the following experiment the former variable was eliminated by releasing cryptic and non-cryptic larvae onto the same bushes.

Since green larvae released onto P. alba bushes at the second instar tended to become white by the final instar, it was decided to release caterpillars at a later stage. Consequently more frequent observations will be necessary due to a shorter time span between release and pupation.

6.3. Experiment 2

6.3.1. Method

In 1983 both green and white morphs were obtained by rearing L. populi larvae on P. alba. Additional green morphs were taken from broods reared on S. fragilis. Three white and three green penultimate instar larvae of similar sizes were released onto each of 12 S. fragilis and 12 P. alba bushes in Cuerden Valley Park. As a control six green larvae were released onto each of ten S. fragilis

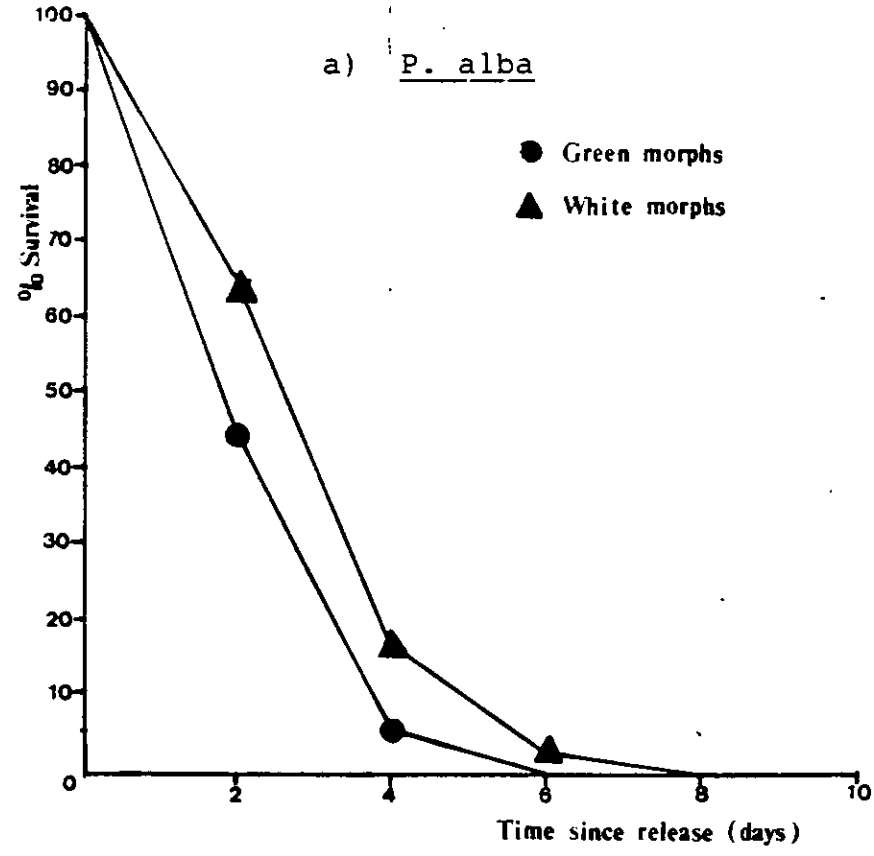
bushes. A similar control experiment on P. alba bushes was not carried out due to the limited availability of white larvae. The numbers and colours of the surviving caterpillars were recorded every 48 hours.

6.3.2. Results and Discussion

The results are presented in APPENDICES 10(a and b) and 11 and illustrated as survivorship curves in FIGs. 6.2a and b. On white poplar bushes, the survival of white morphs is slightly better than that of green morphs (FIG. 6.2a). The results of a t-test (TABLE 6.2a) show that the average survival time of a white morph on this plant is significantly higher than that of a green morph, presumably because the former is less conspicuous on the white downy undersurface of P. alba leaves. A median test was also applied to these data since it appears that there may be a skewed distribution. The results of this test, however, show no difference between the average survival times of the two morphs.

FIG. 6.2b compares survival of white and green larvae on S. fragilis bushes. The analyses in TABLE 6.2a show that the average survival times of the two morphs are not significantly different on this plant (either by t-test or median test). Nevertheless, there is a trend in the predicted direction, green caterpillars surviving on average slightly longer than white caterpillars.

TABLES 6.2b and c show that on P. alba there was a tendency for green larvae to be taken first and also for all green morphs to be eaten before all the white morphs. However on S. fragilis there was no comparable tendency.



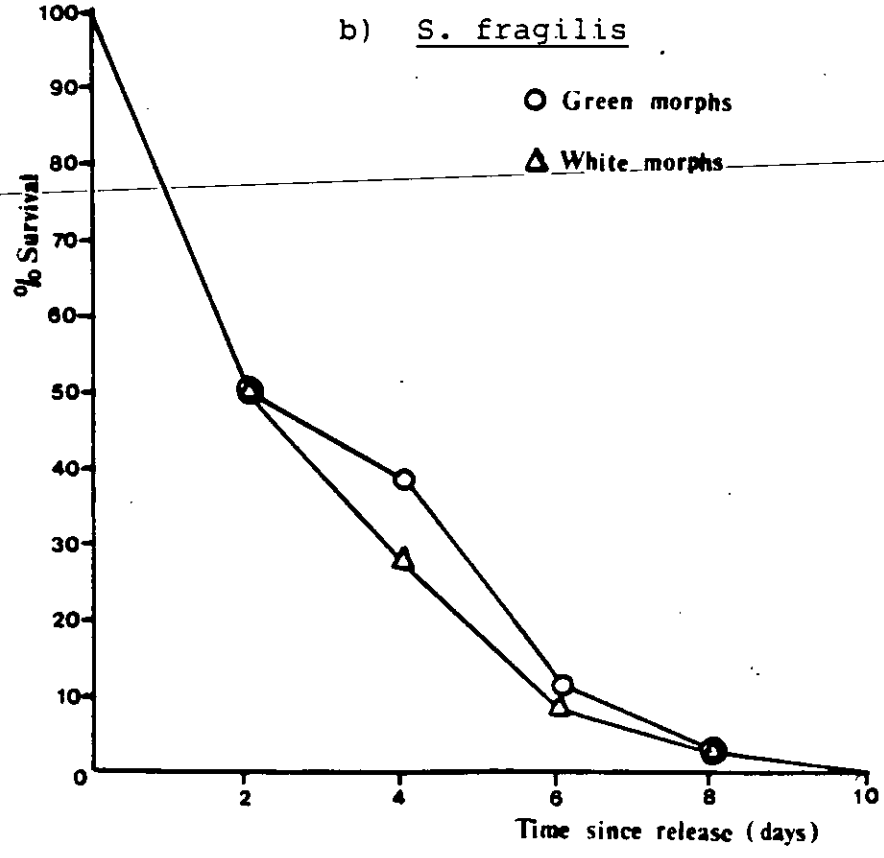


FIG. 6.2. Survival of green and white Laothoe populi larvae on a) Populus alba and b) Salix fragilis

TABLE 6.2. Treatment of the 1983 field predation results

a) Average survival times

Foodplant	Larval colour	Number of larvae surviving 0	2	4	6	8 days	Survival time (days) Total for 36 larvae	Average	d.f.	t	p	Median test χ^2	p
Populus alba	Green	20	14	2	-	-	36	1.0	70	2.039	< 0.05	2.01	0.2 > p > 0.1
	White	13	17	5	1	-	60	1.67					
Salix fragilis	Green	18	4	10	3	1	74	2.05	70	0.517	n.s.	0.055	0.9 > p > 0.8
	White	18	8	7	2	1	64	1.78					

d.f. - degrees of freedom
n.s. - not significant

TABLE 6.2. b) Colour of the first caterpillar to be eaten

Foodplant	No. of replicates in which colour of first larva eaten was:		
	Green	White	Not known
P. alba	4	0	8
S. fragilis	3	4	5

c) Colour of caterpillars to be all eaten first

Foodplant	No. of replicates in which colour to be all eaten first was:		
	Green	White	Not known
P. alba	4	0	8
S. fragilis	5	5	2

TABLE 6.2d Average survival times of green larvae on Salix fragilis in the presence and absence of white caterpillars

Number of larvae per bush		No. of bushes	Number of green larvae surviving							Survival time (days) of green larvae		t	d.f.	p	Median test	
Green	White		0	2	4	6	8	10	12 days	Total	Average				χ^2	p
6	0	10	26	19	3	1	7	3	1	154	2.567	0.805	94	n.s.	1.45	n.s.
3	3	12	18	4	10	3	1	0	0	74	2.055					

d.f. - degrees of freedom.
n.s. - not significant.

The results in TABLE 6.2d show that the average times survived by a green larva in the presence and absence of white larvae are not significantly different. Thus, there is no suggestion that the non-cryptic white morphs are attracting predators to the camouflaged green larvae on S. fragilis bushes.

Clearly, in order to demonstrate any selective advantage of camouflaged larvae, experiments would need to be carried out over a number of years with a greater number of replicates and preferably with fewer animals per bush. Poor survival in these experiments may have been due to the high density of larvae released onto each bush. In terms of analysing the data, it would have been better to have released the larvae in pairs (i.e. one cryptic and one non-cryptic caterpillar per bush) and also to have made more frequent observations. Nevertheless, the results of this work indicate that there is probably a selective advantage of white compared with green morphs on P. alba.

CHAPTER 7

BIOCHEMICAL ANALYSIS OF PLANT AND LARVAL PIGMENTATION

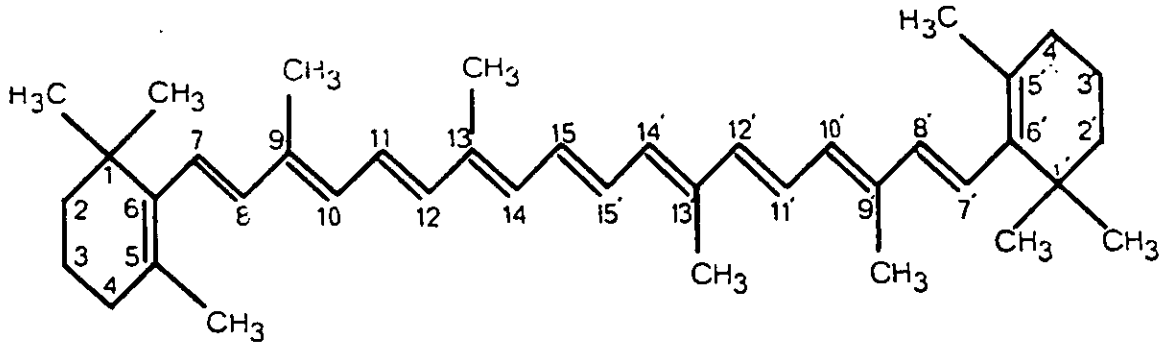
7.1. Introduction

Carotenoid pigments are widely distributed in plants but their presence is often masked by chlorophyll. Carotenoids are polyene pigments usually containing forty carbon atoms and are built up of eight isoprene residues. FIG. 7.1 illustrates the chemical structures of β-carotene and lutein. Over 400 carotenoids are now known (Weedon, 1980) and the number is growing as more sensitive techniques for analysis are developed. Their function in photosynthetic membranes has been extensively researched and it is probable that they protect the chloroplast against photo-oxidative damage, but they may also have a light harvesting function (Junge, 1977; Krinsky, 1966).

Carotenoids have also been isolated from various animal tissues, the Insecta and Crustacea being amongst the most well researched groups. However, there is no convincing report of any de novo formation of carotenoids by animals and it is believed that they derive these pigments either directly or indirectly from plants or microbes (Britton et al, 1977). After ingestion by an animal, carotenoid pigments may be stored unchanged either selectively or indiscriminately, structurally modified or totally rejected. Thus, knowledge of the feeding habits of an insect is a prerequisite in considering the origin of an insect carotenoid (Kayser, 1982).

One of the main functions of carotenoids in insects is

β-CAROTENE



LUTEIN

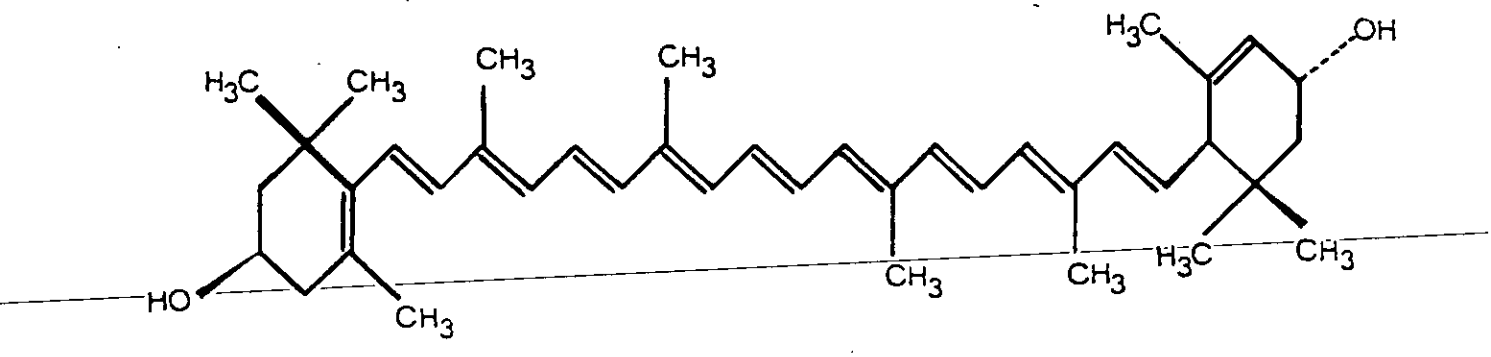


FIG. 7.1. Chemical structure of β-carotene and lutein

to provide body coloration either protective such as relatively dull cryptic coloration and bright aposematic warning coloration or attractive (sexual) signals (Rothschild, 1975). Carotenoids have also been demonstrated to be the precursors of vitamin A (Goodwin, 1963; Goodman and Olson, 1969) which plays an important role in insect vision (Briggs, 1961; Goldsmith, 1958; 1964; Wolken et al, 1960).

Other functions which have been discussed are roles in reproduction (Goodwin, 1950; Loughton and West, 1965), growth (Dadd, 1973; Gilmour, 1961), toxicity (Rothschild and Feltwell, 1974) and photoperiod induction (Shimizu and Kato, 1984).

7.2. Aim

The aim of the biochemical analyses is to see if caterpillar coloration is determined directly by the uptake and storage of plant pigments. The results are discussed in the light of the three most likely fates of ingested foodplant pigments, namely:

1) Unaltered and unspecific sequestration of plant pigments.
2) Selective absorption and deposition of pigment.
3) Chemical or structural transformation of the plant pigments by the larvae.

7.3. Methods

7.3.1. The Larvae

In 1981, fully grown larvae surplus to the breeding

experiments were starved for 24 hours and killed by immersing in liquid nitrogen. Specimens were stored at -15°C until required for pigment analysis. Samples of yellow-green and dull-green morphs of Laothoe populi and grey Smerinthus ocellata larvae were available in the first year of study.

In 1982, white morphs of L. populi larvae were available in addition to the two green morphs. Only third instar green and final instar white-green S. ocellata (from Experiment 4, Chapter 5) larvae were available in this year. Larvae were starved for 48 hours, since in the previous year, 24 hours was found to be an insufficient length of time to empty the gut completely. Prior to pigment analysis, the frozen larvae were allowed to thaw and the integument was dissected off to be analysed separately from the rest of the body. It was impossible to differentiate the body tissues any further in these samples since multiple lesions in the body walls occurred during freezing and thawing. The cuticles were washed before extracting the pigments to remove contamination from haemolymph and other body tissues.

In 1983, starved poplar hawkmoth larvae were killed by ethyl acetate vapour and dissected by making an incision along the entire dorsal surface, pinning out the integument and cutting away the connecting tissue. The gut was removed by tying both ends with cotton, cutting the ends free and carefully lifting from the integument. A small area of bright yellow (possibly the fat body or rudimentary gonads) was also removed. The integuments, gut sacs and

'fat bodies' were washed with tap water and stored at -15°C.

Samples of frass were collected from yellow-green and white final instar poplar hawkmoth larvae and frozen. Also in this year, unfertilised eggs of the poplar hawkmoth were stored for carotenoid analysis.

7.3.2. The Foodplants

In 1981, samples of all the foodplants used in laboratory experiments were collected and frozen at -15°C. These were used two to six months later for developing carotenoid extraction techniques.

In 1982, samples of Salix fragilis, Populus alba, S. viminalis and S. atrocinerea were collected. The main midribs were removed from the leaves and approximately 1.5g wet weight samples were divided into two, one half being analysed fresh the other being frozen for 12 months to investigate the effects of storage on pigment decomposition and/or transformation.

7.3.3. Pigment Analysis

The methods outlined by Britton and Goodwin (1971) were used as a guideline throughout the following analyses. Experimental techniques for extraction, identification and quantification of pigments were developed using frozen foodplant samples which were available in surplus quantities. A summary of the methods used is presented as a flow diagram in FIG. 7.2.

7.3.4. Extraction of Pigments

Samples were cut into small pieces and homogenised at room temperature with acetone. This solvent extracts both

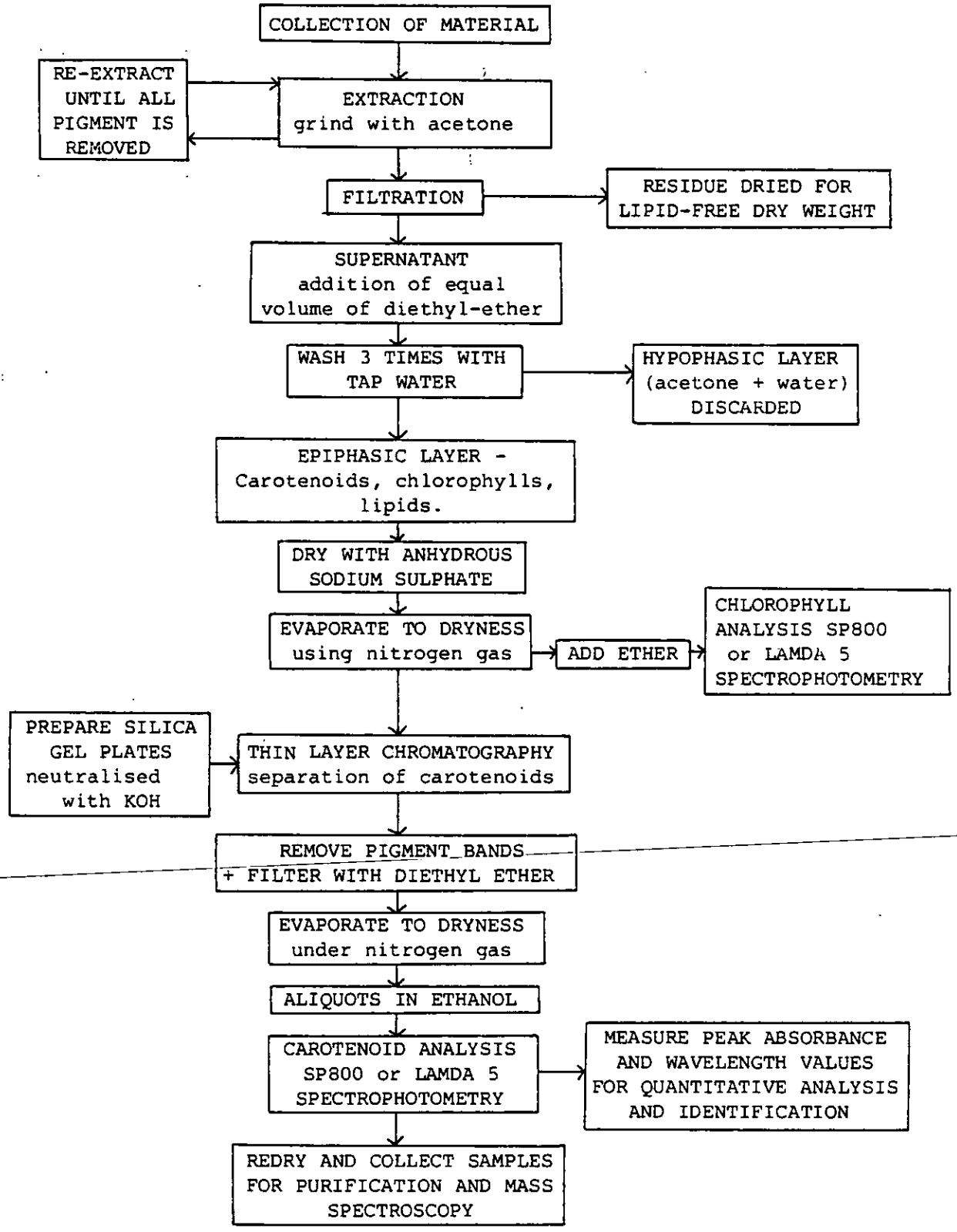


FIG. 7.2. Flow diagram - methods

free and bound carotenoids. For the leaves, a Waring blender was initially employed but later a Ystral high speed homogeniser was found to be more satisfactory since the volume of solvent could be kept small. For the larvae, a pestle and mortar was the most effective way to extract pigments into small volumes (100 ml) of solvent. Samples were reextracted with acetone until no more colour was removed. The combined supernatant was filtered and the residue dried at 110°C to determine the lipid-free dry weights of the tissue. To the acetone solution, an equal volume of diethyl ether was added. This was washed several times with tap water to remove the acetone. Remaining traces of water were removed with anhydrous sodium sulphate. The pigments were finally dried under a stream of nitrogen gas in a warm water bath.

7.3.5. Estimation of chlorophylls a and b

Chlorophyll analysis was made on the total extract dissolved in diethyl ether by measuring absorbance at 660 nm and 642.5 nm, and using the following formulae:

$$\text{Chlorophyll a}(\mu g ml^{-1}) = 9.93(A_{660}) - 0.777(A_{642.5})$$

$$\text{Chlorophyll b}(\mu g ml^{-1}) = 17.6(A_{642.5}) - 2.81(A_{660})$$

7.3.6. Separation of Carotenoids

7.3.6.1. Preparation of Silica Gel Plates

200 mls of distilled water were added to 100 gms of silica gel type G (particle size 10-40 μm) and the mixture was neutralised with 0.5 g of sodium bicarbonate. The mixture was shaken vigorously and spread 0.5 mm thick over ten 20 x 20 cm cleaned glass plates. These were dried overnight at 110°C. However, problems arose from this

method in early extractions due to insufficient neutralisation: during chromatography violaxanthin and neoxanthin were isomerised to auroxanthin and neochrome respectively. The inclusion of one pellet of potassium hydroxide in the aqueous silica slurry was found to be a suitable method of neutralisation.

7.3.6.2. Thin Layer Chromatography (T.L.C.)

The pigments dissolved in 0.5 ml of diethyl ether were applied as a narrow band to an activated silica gel plate. Pigments extracted from larvae were always run in comparison with those from the relevant foodplant. The xanthophylls were separated with diethyl ether as the developing solvent and β-carotene was isolated from the chlorophylls with a 50:50 (v:v) diethyl ether/petroleum mixture. The carotenoid bands were removed from the plate and eluted with diethyl ether the silica being removed by filtration through absorbent cotton wool. The separated pigments were dried in the usual way.

7.3.6.3. Carotenoid Identification

Individual carotenoids were identified by various criteria:- the relative position and colour of the bands during chromatography, the light absorption spectra in ethanol (obtained with the SP800 or Lamda 5 spectrophotometer) and their reaction with acid. The identity of each carotenoid was confirmed either by mass spectrophotometry or by high performance liquid chromatography (H.P.L.C.). The absorption spectra of the carotenoids isolated from foodplants and larvae are presented in FIG. 7.3. Absorption maxima (nm) were compared with those of identified

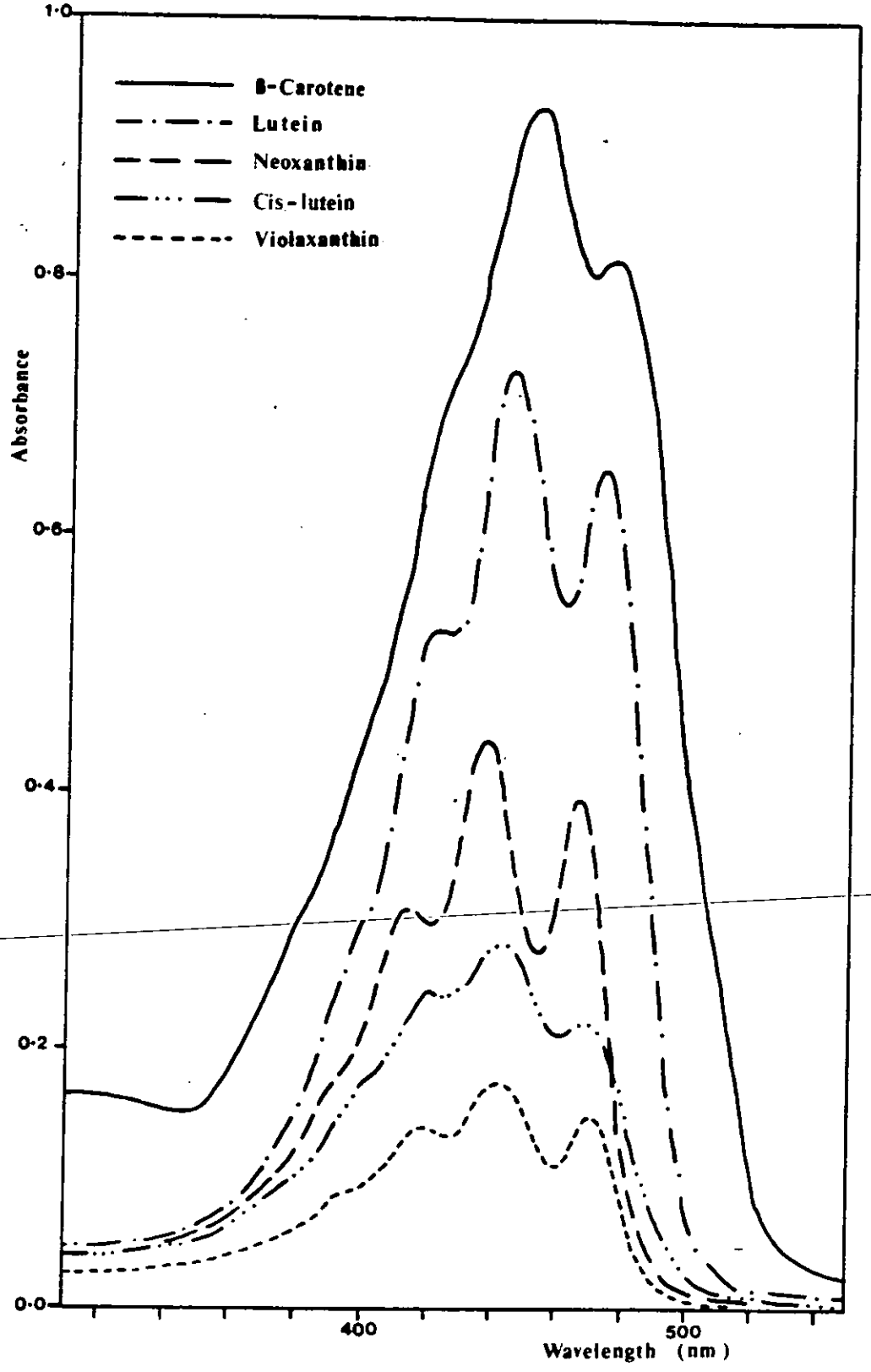


FIG 7.3. Light absorption spectra of the major carotenoids in the foodplant and larval extracts

carotenoids (Davies, 1976).

7.3.6.4. Hcl Test for Carotenoid Epoxides

Carotenoids having epoxy groups were characterised by a modified concentrated Hcl-ether test (Jungawala and Cama, 1962). Violaxanthin and neoxanthin were converted into auroxanthin (a 40 nm spectral shift) and neochrome (a 20 nm spectral shift) respectively by addition of three drops of 0.1 molar hydrochloric acid to diluted samples (peak absorbance less than 0.01).

7.3.6.5. Quantitative Estimation of Carotenoids

Carotenoid concentrations were calculated using the method of Goodwin (1955) which states:-

$$1 \text{ g of tissue contains } \frac{y \times z \times 10^6}{100 \times w \times d \times b} \ \mu g \text{ of pigment}$$

where y = Peak absorbance

z = Volume of solvent (ml)

w = Weight of material (g)

d = Size of cell (1 cm)

$b = \left[\frac{1\%}{1 \text{ cm}}\right]$ value for the pure pigment

Extinction coefficients used for pure pigments dissolved in absolute ethanol are:

β-Carotene	1%	- 2,600
Lutein	1%	- 2,550
Violaxanthin	1%	- 2,550
Neoxanthin	1%	- 2,243 (From Davies, 1976)

7.4. Results and Discussion

7.4.1. The Foodplants

The results presented in TABLE 7.1 show that lutein

(and cis-lutein), β-carotene, violaxanthin and neoxanthin are present in all four fresh foodplants in similar proportions. Lutein and β-carotene are the major carotenoids present in a ratio of 1.4 : 1. The carotenoids comprise approximately 13% of the total pigments, the remainder being the green chlorophylls present in a ratio of chlorophyll a : b of approximately 2.1 : 1.

Analysis of plant material which has been frozen for 12 months shows there to be a differential breakdown of the four carotenoids both within and between different foodplants. Violaxanthin appears to be least stable (only being detected in two of the stored samples) and lutein most stable under the storage conditions. The lutein to β-carotene ratio is increased to 2.7 : 1. The chlorophylls are also affected by storage. In all cases the chlorophyll a to b ratio is decreased after storage suggesting that chlorophyll a is less stable than chlorophyll b in these plants.

These results stress the importance of using fresh material for carotenoid and chlorophyll analysis since there is a differential breakdown of these pigments during storage.

7.4.2. Laothoe populi Larvae

TABLE 7.2. presents the results of initial extractions from whole L. populi larvae but these are unsatisfactory for the following reasons:-

1) 24 hours starvation time is insufficient to clear the gut of plant material.

2) Whole body extractions include pigments from integument,

TABLE 7.1. Major pigments extracted from the principal foodplants (fresh & stored for 12 months at -15°C)

a) Main table

Plant		Carotenoids $\mu g g^{-1}$					% of total				Chlorophylls $\mu g g^{-1}$			Chloro-phyll a : b	carotenoids: chlorophyll
		Lutein + cis-lutein	β-carotene	Violaxanthin	Neoxanthin	Total	Lutein + cis-lutein	β-carotene	Violaxanthin	Neoxanthin	chl. a	chl. b	Total		
Salix fragilis	Fresh	413.0	258.3	83.3	115.2	869.8	47.5	29.7	9.6	13.2	4216	1382	5598	3.05	0.15
	Stored	345.0	102.6	32.4	75.4	555.4	62.1	18.5	5.8	13.6	3104	1338	4442	2.32	0.125
Populus alba	Fresh	449.7	317.1	127.5	113.9	1008.2	44.6	31.5	12.7	11.2	6752	2338	9090	2.89	0.11
	Stored	393.1	65.2	49.7	98.5	606.4	64.8	10.8	8.2	16.2	3580	1868	5448	1.92	0.11
S. atrocinerea	Fresh	555.9	363.5	128.2	149.8	1197.4	46.4	30.4	10.7	12.5	4798	1771	6568	2.71	0.18
	Stored	293.0	196.3	-	60.7	550.0	53.3	35.7	-	11.0	2409	1665	4073	1.45	0.135
S. vimalis	Fresh	614.9	507.7	78.1	156.2	1356.9	45.3	37.4	5.8	11.5	5750	2164	7915	2.66	0.17
	Stored	410.9	177.7	-	59.4	648.0	63.4	27.4	-	9.2	3582	2504	6086	1.43	0.11

TABLE 7.1. b) Relative quantity of lutein and cis-lutein in Salix fragilis and Salix viminalis samples

Plant	Condition	Lutein µgg-1	Cis-lutein µgg-1	Cis-lutein : lutein
S. fragilis	Fresh	371	42	0.11
	Stored	278	67	0.24
S. viminalis	Fresh	570.1	44.8	0.08
	Stored	352.5	58.4	0.16

TABLE 7.2. Pigments extracted from Laothoe populi larvae (starved for 24 hours)

Larval colour	Lutein		'Lutein shadow'		β-carotene		chlorophyll a : b
	$\mu g g^{-1}$ wet-weight	µg/ animal	$\mu g g^{-1}$ wet-weight	µg/ animal	$\mu g g^{-1}$ wet-weight	µg/ animal	
Yellow-green	5.3	5.1	-	-	0.87	0.84	1.34
Yellow-green	3.4	5.6	-	-	0.39	0.64	*
Yellow-green	3.5	6.2	1.1	2.0	*	*	*
Yellow-green	3.2	2.6	-	-	*	*	0.8
Yellow-green	3.9	5.8	-	-	1.1	1.65	1.0
Dull-green	1.2	2.1	*	*	*	*	0.71

* = Trace present on silica plate
- = Lutein and 'lutein shadow' analysed together.

gut sac, fat body haemolymph (and partially digested plant material) thus will not reveal the pigments specifically responsible for larval colouring.

3) Extractions were made using animals stored for two to six months.

4) Dry weights were not obtained so results are difficult to compare.

However these preliminary extractions provided a guideline for later analyses. The three carotenoids found present in the larvae were lutein, β-carotene and an unidentified pigment which appears below lutein during chromatography as a relatively broad band and has a spectrum similar to lutein but shifted approximately 3 nm to the left. This pigment was referred to as "lutein shadow" in early samples.

Chlorophylls a and b were found to be present in similar proportions in these early extractions.

The results presented in TABLE 7.3 show the pigments extracted from the integuments and body tissues of caterpillars which had been starved for 48 hours prior to freezing. Two pigments were isolated from the integuments of most samples, lutein and "lutein shadow". Mass specrophotometry showed the latter to be identical to lutein. Further, during H.P.L.C., lutein and "lutein shadow" behaved in similar ways, having the same retention and separation times. It was concluded from these results that "lutein shadow" is a cis-lutein or a mixture of cis-isomers of lutein.

Integuments from yellow-green larvae fed on S. fragilis

TABLE 7.3. Pigments in the integument and body of 48 hour starved Laothoe populi larvae

Larval colour	Larval foodplant	Integumental pigments ($\mu g g^{-1}$) Lutein	Cis-Lutein	β-carotene	Cis-lutein : lutein ratio	Body pigments ($\mu g g^{-1}$) Lutein	Cis-lutein	β-carotene	chloro-phyll a	chloro-phyll b	a : b
YG	Populus alba	29.4	8.7	*	0.30	17.6	*	*	18.9	4.0	4.7
YG	P. alba	32.3	2.2	*	0.07	40.5	*	5.8	110.4	24.7	4.5
YG	P. alba	37.8	4.0	*	0.11	10.0	*	*	*	*	-
YG	Salix fragilis	72.7	5.7	*	0.08	43.0	7.6	*	363.0	122.7	3.0
YG	S. fragilis	146.4	9.7	*	0.07						
DG	S. fragilis	5.0	1.6	*	0.32	17.6	*	*	9.7	11.0	0.9
DG	S. fragilis	12.5	*	*	-	46.5	*	37.0	*	*	-
DG	S. atrocinerea	27.4	9.8	*	0.36	103.9	11.4	41.4	4.2	4.0	1.04
W	Populus alba	0.9	-	-	-	13.6	*	*	7.6	4.4	1.7

YG - Yellow-green
DG - Dull-green
W - White

* - trace present on silica plate

contained two to three times more carotenoid than similar coloured larvae fed on P. alba and 12 times as much as dull-green larvae fed on S. fragilis. The white larval integument analysed contained only 0.9 $\mu g g^{-1}$ of lutein which is only 2% of the quantity found in dull-green morphs.

The pigments in the internal body tissues were very variable between specimens probably due to the presence of different amounts of plant material in the guts. Measureable quantities of chlorophyll a and b were present in six of the eight samples. The presence of chlorophyll in earlier extractions from whole larvae (see TABLE 7.2) was probably due to residual plant material inside the gut.

The results presented in TABLE 7.4 confirm that the yellow pigments present in the integuments of L. populi larvae are lutein and cis-lutein. β-carotene was barely detectable on the T.L.C. plates and corresponding spectra showed the β-carotene isolated to have been largely broken down. The cis-lutein : lutein ratio is approximately 0.08 : 1, but varies between 0.05 : 1 and 0.16 : 1. During thin layer chromatography, the cis-lutein ran as a broad band immediately below lutein and it was not always possible to draw a line between the two pigments. This probably accounts for the variation in the quantity of cis-lutein in relation to lutein shown in TABLES 7.3 and 7.4.

Cis-lutein is also present in the foodplants but only in S. fragilis and S. viminalis samples was this pigment analysed separately. TABLE 7.1b shows that the cis-lutein : lutein ratio is 0.11 to 1 in the former and 0.08 to 1 in the latter plant. Thus, this ratio is similar in both

TABLE 7.4. Pigments in the integument of Laothoe populi larvae#

Larval Colour	Foodplant	Lutein $\mu g g^{-1}$	Cis-lutein $\mu g g^{-1}$	β-carotene	Cis-lutein : lutein	Chlorophyll a : b
Yellow-green	Salix fragilis	75.4	–	*	–	0.89
"	"	46.9	7.7	*	0.16	0.68
"	"	80.0	6.1	*	0.08	0.67
"	"	82.2	4.1	*	0.05	0.70
"	S. atrocinerea	70.9	3.27	*	0.08	0.98
"	S. fragilis	57.4	4.35	*		
Dull-green	S. fragilis	21.1	1.3	*	0.06	1.15
"	"	13.2	1.4	*	0.11	0.57
"	"	21.6	1.2	*	0.06	0.42
"	"	27.8	1.6	*	0.06	0.73
"	"	37.7	–	*	–	0.83
White	Populus alba	2.77	n.d.	n.d.		0.15
"	"	3.43	n.d.	n.d.		0.68
"	S. fragilis	4.13	n.d.	n.d.		1.71

– = Lutein and cis-lutein analysed together * = Trace present on T.L.C. plate

Larvae starved for 48 hours, killed using ethyl acetate and integument dissected off and washed prior to freezing.

larval integument and foodplant.

In the case of both foodplants, storage increased the relative amount of cis-lutein (perhaps indicating that this is a breakdown product of lutein). This again emphasises the importance of extracting from fresh material and may also account for some of the variation in the relative amount of cis-lutein between larval samples.

There is substantially more carotenoid present in the integument of yellow-green larvae compared with dull-green larvae and even smaller quantities in white larvae. The ratios of carotenoids in these three morphs are 21 : 7.1 : 1 respectively. FIG. 7.4 illustrates this difference.

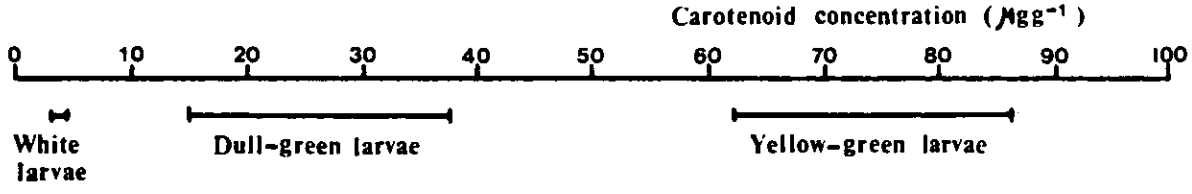


FIG. 7.4. Comparison of carotenoid concentration in the integuments of white, dull-green and yellow-green larval morphs of Laothoe populi

The chlorophyll a : b ratios in larval integuments (TABLE 7.4) are variable. However, since these pigments appeared to be present in extremely low quantities these results are likely to be unreliable. Further, it may have been another pigment other than chlorophyll which gave a peak absorption at 670 nm. The nature of the blue/green pigment needs further research.

Thus, there is a variation in content between the material ingested and that deposited within the integument in poplar hawkmoth larvae. The caterpillars are selectively storing lutein and cis-lutein. Yellow-green morphs are storing up to six times as much lutein as dull-green larvae, whilst white larvae exclude the majority of carotenoid from deposition within the cuticle.

7.4.3. The Gut Sacs

TABLE 7.5 shows that lutein, cis-lutein and β-carotene are taken up into the gut lining by all three colour morphs of Laothoe populi larvae, whereas TABLES 7.3 and 7.4 show that only the former two carotenoids are sequestered in measureable quantities in the integument of this species. The fate of the absorbed β-carotene is not known and this area will need to be researched further. It is possible that the β-carotene is being converted into vitamin A for visual functions in this species. Much work has been carried out on the synthesis of vitamin A (Goodman and Olson, 1969; Krinsky, 1971) and β-carotene has been recognised as a precursor of this vitamin (Goodwin, 1963).

Yellow-green and dull-green morphs absorb approximately five times as much lutein as white morphs which suggests that, in the latter, lutein uptake is being inhibited at the gut wall. In dull-green larvae, however, it appears that the inhibition is occurring between the gut lining and the integument. Again, this is an area which requires further research.

Approximately twice as much β-carotene was found in the gut-walls of dull-green larvae compared with yellow-

TABLE 7.5. Pigments in the gut sacs of Laothoe populi larvae (five specimens per extraction)

Larval Colour	Carotenoids $\mu g g^{-1}$					Chlorophylls $\mu g g^{-1}$			Chlorophyll a : b	Carotenoids: Chlorophylls
	Lutein	Cis-lutein	β-carotene	Auro-xanthin	Total	Chl.a.	Chl.b.	Total		
Yellow-green	25.9	2.5	16.2	2.9	47.5	6.5	4.1	10.6	1.6	4.5
Dull-green	24.7	7.8	34.0	✓	66.5	23.2	28.8	52.0	0.8	1.3
White	5.1	✓	5.0	0	10.1	✓	✓	-	-	-

✓ - Trace present on T.L.C. plate.

green larvae and an even smaller quantity of this pigment was found in the guts of white larvae.

Similarly, for the chlorophylls found in these samples the concentration was highest in dull-green larvae and least in white larvae.

7.4.4. The Frass

TABLE 7.6 shows that the frass of white larvae contains approximately three times as much chlorophyll as that of yellow-green larvae when fed on the same diet. The chlorophyll a : b ratio is higher than in both foodplant and caterpillar integument.

White morphs also excluded slightly more β-carotene, lutein and cis-lutein. However, replicate extractions would be necessary for these differences to be significant.

Neochrome and auroxanthin were present in both samples, these being breakdown products of neoxanthin and violaxanthin respectively.

Unfortunately, no sample of frass from dull-green larvae was collected.

7.4.5. The Fat Body

Attempts to extract carotenoids from the fat bodies of caterpillars were unsuccessful since pigments remained in the aqueous layer during extraction. The small ether-soluble fraction of pigment was analysed in the normal way, and gave maximum absorbtion peaks at 420 nm, 442 nm and 472 nm. The spectrum did not shift with addition of acid. These characteristics suggest that the pigment is cis-lutein.

TABLE 7.6. Pigments in the frass of yellow-green and white final instar Laothoe populi larvae (all fed on Populus alba)

Colour of larvae	Chlorophylls $\mu g g^{-1}$			Carotenoid $\mu g g^{-1}$							
	Chloro-phyll a	Chloro-phyll b	Total chloro-phylls	β-carotene	Lutein	Cis-Lutein	Neo-Chrome	Auro-xanthin	Total carotenoids	chl.a: chl.b	carotenoids: chlorophylls
Yellow-green	2299	197	2496	86	209.6	36.4	48.6	25.7	406.3	11.67	0.16
White	6494	552	7046	100	237.4	55.3	20.6	23.7	437	11.76	0.06

chl. - chlorophyll

7.4.6. The Eggs

The eggs of the poplar hawkmoth were shown to contain lutein only, at a concentration of 16.6 $\mu g g^{-1}$.

7.4.7. Smerinthus ocellata Larvae

Unfortunately, very few larvae of this species were available for pigment analyses due to poor survival in the laboratory experiments. The results in TABLE 7.7 show that lutein is the major yellow pigment being sequestered in the integument of this species, with cis-lutein also being present. An unidentified pigment with maximum absorbtion peaks at 416 nm, 440 nm and 468 nm was also isolated from one individual.

Although data are sparse, the results do suggest that green eyed hawkmoth caterpillars contain more integumental lutein than grey caterpillars and much more than the white-green caterpillars from white muslin sleeves (Experiment 4, Chapter 5).

7.5. Summary of Results

Analysis of foodplant and caterpillar integument shows a variation in content between the material ingested and that deposited within the integument. The pigments identified in all four foodplants were chlorophylls a and b, lutein, cis-lutein, β-carotene, neoxanthin and violaxanthin. The pigments found to be present in the integuments of final instar poplar and eyed hawkmoth caterpillars were lutein and cis-lutein. Although poplar hawkmoth larvae are absorbing β-carotene, chlorophylls a and b, lutein and cis-lutein into the gut wall only the latter two

TABLE 7.7. Pigments in the integuments and other body tissues of Smerinthus ocellata larvae (starved for 48 hours)

Larval colour	Foodplant *Salix* spp	Extraction	Pigments ($\mu g g^{-1}$)					Chl.a: Chl.b	Cis-lutein :lutein
			Lutein	Cis-lutein	Chl.a	Chl.b	Others		
Grey	*atrocinerea*	Integument	42.9	*	19.5	31.5	-	0.62	*
Grey	*atrocinerea*	a) Integument	54.5	4.1	-	-	-	-	0.07
		b) Other tissues	38.0	4.9	5.5	57.0	-	0.10	0.13
White -green	*viminalis*	a) Integument	19.2	1.3	19.5	5.0	-	3.90	0.07
		b) Other tissues	33.7	5.1	206.4	63.6	β-carotene 23.4 neoxanthin 9.8 auroxanthin 4.5	3.24	0.15
White -green	*fragilis*	Integument	27.6	trace	8.2	9.1	β-carotene (trace)	0.90	*
Green	*fragilis*	Integument (3rd instar)	69.2	*	-	-	-	-	*

* Cis-lutein analysed with lutein

pigments are being selectively stored within the integument.

There is substantially more carotenoid present in the integuments of yellow-green poplar hawkmoth larvae compared with dull-green larvae and even smaller quantities in white morphs. The chlorophyll content of larval integuments is too low to be significant. Clearly, although all larvae are being fed on similar foodstuffs, dull-green and white caterpillars are excluding the majority of carotenoids from deposition within the cuticle.

Similarly for the eyed hawkmoth, green caterpillars contain more lutein than grey and much more than white-green caterpillars.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1. Environmental Polymorphism in Lepidopterous Larvae

Although environmental polymorphism in pupae is a well researched subject (section 1.3), very little attention has been given to environmentally controlled polymorphisms in larvae. It is not that environmental polymorphism in caterpillars is unheard of. As early as 1892, Poulton reported that a variety of lepidopterous larvae become darker coloured when reared in dark surroundings and more recently, Owen (1980) has shown that colour in larvae of the European brimstone moth (Opisthograptis luteolata L.) is not genetically determined. One possible reason for the emphasis of research on pupal colour is that pupae tend to be dimorphic having discrete green and brown morphs (intermediates being rare or absent) whereas larvae tend to display a more continuous variation in colour i.e. they are not strictly polymorphic. This makes larval colour a more difficult parameter to classify and quantify than pupal colour. For Laothoe populi larvae, although three discrete morphs were recognised in the field, green "intermediates" were common in some laboratory reared broods. For Smerinthus ocellata two colour categories, green and grey, were defined but there was some variation within these two groups in both the field and in laboratory reared caterpillars.

A more likely reason for an emphasis of research on pupal polymorphism is that the critical time at which an

insect is sensitive to environmental stimuli occurs immediately prior to pupation when the larva has ceased to feed. Thus, controlled experiments can be carried out over relatively short periods of time (about 22 hours for Papilio spp. (Smith, 1978), 15 hours for Pieris spp. (Smith, 1980)) and independently of food. For larvae however, the stimulus for colour change needs to be present over a prolonged period of time. In the present work, although many L. populi larvae on Populus alba became white after 13 days, some did not change colour until several weeks later. Colour change in these larvae is reversible (three white larvae became green when transferred from P. alba to Salix fragilis - see section 4.2.3). This suggests that larvae are sensitive to critical cues throughout their life, the limiting factor being the time it takes to respond to a stimulus. A 'sensitive period' as such was not identified and controlled conditions were therefore maintained over the entire larval lifespan (three to eight weeks). This presented problems, particularly when experimenting with different backgrounds (sections 4.5 and 4.6) since daily handling of larvae and long periods without food resulted in high mortalities and very few larvae survived to the final instar. Caterpillars were very active in the 'coloured' boxes (presumably because they were searching for a suitable resting site) and this was probably the reason for relatively slow development of larvae in these experiments.

8.2. Environmental Polymorphism in Laothoe populi Larvae

In the field surveys (Chapter 2) it was found that larvae on P. alba were white (or slightly greeny-white) whilst those found on S. fragilis were yellow-green in colour. Thus, on both foodplants, poplar hawkmoth caterpillars are well camouflaged (as judged by the human eye). The laboratory experiments (Chapter 4) have shown that white and yellow-green larval morphs of this hawkmoth are determined environmentally. The critical stimulus appears to be the intensity of light reflected from the larval substrate. Caterpillars reared on S. fragilis at night but on a white background during the day tend to become white whilst those reared on a grey, green or black background tend to become green (see PLATE 7). However, incident light may also be important since the light reflected from a substrate is dependent on the intensity of light falling on it. Sumner (1929) showed that the colour in flatfish is not simply determined by the absolute amount of light reflected from the substrate but that the degree of pigmentation is directly proportional to incident light intensity and inversely proportional to the intensity of reflected light. Pigmentation in the stick insect Carausius morosus is also dependent on the contrast between incident and reflected light intensities (Dustmann, 1964, cited in Angersbach, 1975). More recently, Angersbach (1975) has shown that melanization in Pieris brassicae pupae is enhanced not only by a strong contrast in overhead and background light, but also by a contrast between lateral light intensities.

In the present experiments on L. populi larvae, although the incident light was similar for all coloured cages, this parameter was not kept constant. It would be interesting to investigate the effect of different intensities of incident radiation to determine whether this insect is responding to a brightness contrast.

Although the ocelli are probably the main light receptors in caterpillars, in P. brassicae there is evidence of a second site of photoreception on the dorsal area of the head capsule (Angersbach, 1975). The larval photoreceptors of L. populi were not investigated in the present study. Angersbach (1975) investigated the roles of the different ocelli (and of the extraocular site) in receiving critical light stimuli by blinding the eyes of P. brassicae prepupae with black varnish. Painting of lateral ocelli of L. populi larvae to assess their relative importance in photoreception would be impractical, since repainting would be necessary after each moult.

8.3. Environmental Polymorphism in Smerinthus ocellata Larvae

For the eyed hawkmoth, larval colour also appears to be environmentally determined by some stimulus associated with light. The results of rearing experiments in the field (section 5.4) suggest that overhead light is important in determining caterpillar coloration in this species. However, the factors which cause a caterpillar to become green were not established. All larvae reared in the laboratory conditions became grey or slightly greeny-grey

in colour irrespective of their foodplant, the lighting conditions or larval colour of their parents. It may be some quality of direct sunlight which maintains the green colour in eyed hawkmoth larvae. This would account for the lack of this morph in the artificially illuminated 'laboratory'.

The variation in colour observed amongst larvae in the present field surveys may be due to a difference in resting position on the various foodplants due to their different structures. On Salix atrocinerea, fourth and fifth instars tend to rest on the stems and petioles of the plant, presumably because the leaves are too small. In this position they are exposed to bright overhead radiation and background radiation from either stems (green) or leaves. Most larvae on this foodplant were green. On S. alba, however, larvae tend to rest on the underside of leaves (usually in the shade) with their heads downwards, even during their final instar. All larvae observed on this foodplant were grey. On S. viminalis larvae rest on either stems or the underside of leaves (PLATES 1 and 2). On this foodplant approximately equal proportions of green and grey morphs were observed. Unfortunately, these observations on resting behaviour of larvae on the different foodplants were not quantified.

Poulton (1886a) observed a correlation between size of leaf and larval colour of S. ocellata. Yellow-green larvae were found on small leafed S. viminalis whereas grey-green larvae were found on large leafed varieties of this plant. These differences in larval colour may have been due to

differences in resting positions and hence light conditions experienced by the larvae on the two types of osier.

8.4. Genetic Polymorphism in L. populi and S. ocellata

8.4.1. Dull-green coloration in L. populi Larvae

In the field surveys dull-green poplar hawkmoth caterpillars were occasionally observed both on willow and white poplar bushes (APPENDIX 2). Although well camouflaged on green leafed plants (see PLATE 4) these morphs are conspicuous (as judged by the human eye) against the white undersurface of P. alba leaves. In the 'laboratory' larvae which received no light stimulus (i.e. were reared in darkness) tended to become dull-green or intermediate-green in colour but this does not explain the occurrence of dull-green morphs in field conditions. Analysis of the data for broods reared on S. fragilis indicated that dull-green and intermediate-green morphs are probably genetically determined (section 4.3.2.1). It is possible that the dull morphs need a higher threshold of light intensity or contrast to respond with the appropriate colour. Alternatively, they may be physiologically incapable of either receiving or responding to visual stimuli.

Experiments by Allen and Clarke (1968) and Allen (1972, 1975, 1976) have demonstrated that polymorphisms can be actively maintained when predators which hunt by sight, form a searching image for the commoner prey morph. This phenomenon, known as apostatic selection may be responsible for maintaining the yellow-green/dull-green dimorphism observed on willows and white/dull-green dimorphism on

white poplar. Thus, the relatively rare dull-green morphs may actually be at a selective advantage over the commoner yellow-green and white morphs but this remains speculative since it is not known whether the predators of poplar hawkmoth caterpillars hunt by searching image.

8.4.2. Red Spots on L. populi and S. ocellata Larvae

The occurrence of red spots on poplar hawkmoth caterpillars is another aspect of colour which appears to be inherited. In the field there was no correlation between the frequency of larvae having red spots and foodplant and in the 'laboratory' higher proportions of red spotted morphs occurred in offspring from parents which had spots as larvae than from "non-spotted" parents.

Red spots are also found on eyed hawkmoth larvae. In the field there appears to be a correlation between foodplant and frequency of caterpillars with red spots. Significantly more red spotted larvae were found on Salix atrocinerea than on S. viminalis and significantly fewer on S. alba than on either of the former two species. These differences may be due to differences in environmental conditions (e.g. light or diet) or red spots may be an inherited character (as in L. populi larvae) the morph frequencies being determined by selective predation. Alternatively, the larvae on the three willow species at Lytham St. Annes may be from genetically different populations. This could arise either if female moths selectively lay eggs on the foodplants on which they fed as larvae, or if the distances between the different willows are greater than the normal distance an eyed hawkmoth flies. At Lytham

St. Annes the three Salix species (S. atrocinerea, S. viminalis and S. alba) are several hundred metres apart. However, it is not known over what range a female eyed hawkmoth flies during egg laying. Unfortunately, since very few larvae survived in the breeding programme, experiments to determine the environmental and/or genetic basis of red spots in this species were not carried out.

The significance of the red spots is unclear. To the human eye their presence makes these otherwise cyptic larvae quite conspicuous. Hinton (1976) hypothesized that red spots in the crab spider Misumena vatia (Clerck) function as a warning coloration for birds and other vertebrates which hunt by sight. Neck (1978) suggested that red coloration in the crab spider Peucetia viridens (Henz) serves to warn predators of its painful bite. Hawkmoths are generally believed to be acceptable as food for bird predators (Jones, 1932; Lane, 1964). However, Bisset et al. (1960) have demonstrated that pupae of the poplar hawkmoth contain histamine at a level approximating 100 $\mu g g^{-1}$ freeze dried tissue (cited in Rothschild et al. 1970). In the field predation experiments (Chapter 6) the high mortality observed amongst larvae of this species suggests that these caterpillars are very palatable and thus it is unlikely that the red spots serve any warning function. A more likely explanation is that red spots serve to make larvae less conspicuous on leaves which have spots themselves (see PLATE 6 and PLATE 1). Also, the presence of red spots in different numbers on different

larvae increases the variety of morphs and may be of advantage to the species since predators are less likely to form a searching image.

8.5. Plant Defences

Survival of eyed hawkmoth larvae in the laboratory was unexpectedly poor, particularly in the final year of the study. This may have been due to successive years of inbreeding, although a number of caterpillars from wild populations were collected each year. It was curious that larvae would simultaneously die after reaching a certain size (usually second to fourth instar) in apparently good health. This phenomenon not only occurred in broods fed on willow cuttings but also in batches fed on rooted plants. For example, in one brood reared on rooted Salix repens most larvae survived perfectly well for the first week, but on day seven, all caterpillars died. One possible explanation of this sudden mortality of larvae is that the plants themselves have a defence system against over-grazing. Van Someren (1937) suggested that a change in the chemical composition of foliage after grazing may retard larval growth and eventually cause death. More recently Edwards and Wratten (1982) have demonstrated an induced anti-herbivore defence reaction in birch. They showed that grazing reduced not only the palatability of damaged leaves themselves but also that of nearby leaves. Smith (1983) demonstrated that mortality in Urania larvae and pupae is higher when larvae feed on Omphalea plants which have previously been grazed by three or more generations. He

suggested that repeated grazing results in an increase in chemicals which are toxic to Urania but he did not isolate any such toxin. An anti-herbivore defence system has also been demonstrated in lupin leaves, which show a rapid increase of quinolizidine alkaloid accumulation within a few hours after wounding (Wink, 1983). Willow species may similarly produce toxins when overgrazed, which would account for high mortality in laboratory reared S. ocellata larvae. Bryant et al. (1985) demonstrated that severe winter browsing of the adult-form of the Alaska feltleaf willow (Salix alaxensis) by snowshoe hares causes it to revert to juvenile-form stump sprouts. In this condition the twigs are unpalatable and of lower nutritional quality to the hares which, Bryant et al. suggested, may be related to increased lignin and phenolic content of the twigs. The possibility of a chemical defence system in the Salix species used in this work remains to be studied.

Alternatively, the sudden mortality occurring in some broods may have been due to infection by micro-organisms although this was not investigated. In future experiments on eyed hawkmoths, rearing larvae at very low densities with frequent (i.e. daily) renewal of the foodplant may help to reduce mortality.

8.6. Survival Value of Crypsis:- Predation on Larvae

Colour crypsis (or homochromy) has probably evolved as a consequence of selective predation by animals which hunt by colour vision. It therefore seems reasonable to predict that cryptic caterpillars will be at a selective advantage

over conspicuous ones. The field predation experiments, designed to demonstrate this hypothesis, indicated that crypsis is of survival value to L. populi larvae on P. alba. However, on S. fragilis, although the average survival time of a green larva was longer than that of a white morph, the difference was not significant. Further experiments would be necessary for the results to be conclusive.

The potential avian predators of L. populi larvae in Cuerden Valley park are listed in APPENDIX 12, but none of these was actually observed feeding on the released caterpillars. Since L. populi caterpillars normally rest on the underside of leaves (section 4.8) it is probable that birds hunt either from amongst the foliage or from the ground. Alternatively, it has been suggested that birds may be initially attracted to partially eaten leaves and then seek out the caterpillar responsible for the damage (Thurston and Prachuabmoh, 1971).

Other potential predators of the caterpillars are rodents (and other small mammals) but these may hunt prey during the night in which case crypsis would offer no defence. Although no rodents were observed, small burrows were present near to the roots of some of the experimental bushes.

Although L. populi and S. ocellata larvae are cryptic in the visible part of the spectrum these insects may be conspicuous to nocturnal predators such as owls which hunt by infra red vision. Cott (1940), however, did show that S. ocellata larvae reflect infra red in a similar way to

leaves and will therefore be inconspicuous to owls. It is not known if the same is true for L. populi larvae.

8.7. Pigment Uptake by L. populi and S. ocellata Larvae

The biochemical analyses (Chapter 7) have shown that lutein and a cis-isomer of lutein are present in the integuments of both L. populi and S. ocellata final instar larvae. These pigments are also present in the four larval foodplants analysed, lutein being the principal carotenoid in each case. Why these caterpillars use lutein in preference to any other dietary carotenoid for body coloration is not clear from this study. It is possible that a lutein specific binding protein or lipoprotein is present in the integument or haemolymph (E. H. Evans, pers. comm.), but this would need to be researched.

Very little research appears to have been carried out on integumental carotenoids in other lepidopterous larvae. Most workers have investigated the carotenoids present in the whole bodies of starved caterpillars e.g. Rothschild, 1974; Mummery et al., 1976. Although Dahlman (1969) investigated the cuticular pigments of Manduca sexta larvae, he did not identify the carotenoids present and simply referred to them as xanthophylls. Clarke (1971), however, showed that the normal development of colour in Hyalophora cecropia larvae is dependent on the presence of lutein in the diet and Kayser (1974) showed that lutein is the predominant pigment in the larval integument of P. brassicae.

Analysis of the gut lining of L. populi larvae has

shown that β-carotene is also absorbed by this species in similar proportions to lutein. The fate of this pigment was not investigated but it is probably important in photoreception. The most important degredation product of β-carotene is retinal which serves as the light absorbing prosthetic group of insect visual pigments (White, 1978, cited in Kayser, 1982).

In a recent review, Kayser (1982) classified Lepidoptera into two groups on the basis of their carotenoid composition. Firstly those which absorb β-carotene and lutein in equal amounts for example Pieris brassicae and those which absorb lutein in preference to β-carotene for example Aglais urticae L. Although L. populi absorbs these two carotenoids into the gut in similar proportions, in terms of the whole animal, lutein is the principal carotenoid (TABLE 7.2).

Lutein was identified in the ova of the poplar hawkmoth. Feltwell and Rothschild (1974) have suggested that carotenoid accumulation in the eggs of the large white butterfly (P. brassicae) may possibly be a protective mechanism against the harmful effects of solar radiation. It is unlikely that this is the case for L. populi ova since these tend to be laid on the undersides of leaves, in the shade.

This work has shown that white L. populi caterpillars, although feeding on the same dietary carotenoids as yellow-green larvae, contain virtually no integumental carotenoid. It is the light cue received by a larva which determines whether or not dietary lutein is taken up and

deposited within the integument. Thus, caterpillars which "see" green (grey or black) take up and store lutein and this pigment accounts for their yellowness. (The blue component of colour is probably due to bile pigments as in many other green insects - see 1.6). Caterpillars which "see" white, exclude lutein and all other dietary carotenoid from the integument and therefore appear white. Further experiments should be carried out to investigate the mechanisms by which the visual cues are translated into differential uptake of lutein.

APPENDIX 1. Eyed hawkmoth field data, 1978 to 1983

Foodplant	Year	Number of caterpillars of each colour		Total no. of caterpillars	Number with red spots
		Green	Grey		
Salix alba	1978	1	4	5	0
	1979	0	15	15	0
	1980	0	2	2	0
	1981	1	4	5	0
	1982	0	4	4	0
	1983	0	1	1	0
	Totals	2	30	32	0
S. atrocinerea	1978	41	3	44	18
	1979	7	2	9	0
	1980	9	1	10	4
	1981	30	1	31	26
	1982	3	0	3	0
	1983	6	0	6	1
	Totals	96	7	103	49
S. viminalis	1978	7	7	14	3
	1979	10	11	21	3
	1980	5	5	10	2
	1981	28	18	44	12
	1982	2	2	4	0
	1983	0	0	0	0
	Totals	50	43	93	20
S. repens	1978	5	9	14	6
	1979	5	20	25	0
	1980	0	0	0	0
	1981	0	0	0	0
	1982	0	3	3	0
	1983	0	0	0	0
	Totals	10	32	42	6
S. fragilis	1981	12	0	12	3
S. caprea	1982	9	2	11	0
	1983	3	2	5	1
	Totals	12	4	16	1

APPENDIX 2. Poplar hawkmoth field data for 1980 to 1983

Foodplant	Year	Number of caterpillars of each colour				Total number found	Number with red spots
		YG	DG	WG	W		
Populus alba	1980			2	7	9	6
	1981		1	4	13	18	15
	1982			3	21	24	19
	1983			2	1	3	2
	Totals		1	11	42	54	42
Salix fragilis	1980	27				27	18
	1981	2				2	0
	1982	1				1	0
	1983	1				1	0
	Totals	31				31	18
S. atrocinerea	1980	1	1			2	1
	1981	1	0			1	1
	Totals	2	1			3	2
S. caprea	1981	3				3	2
	1983	3				3	3
	Totals	6				6	5
S. viminalis	1980		1			1	1
	1982	1				1	
	Totals	1	1			2	1
S. alba	1980		1			1	1
	1981	1	2	1		4	3
	Totals	1	3	1		5	4
S. daphnoides	1981	2	3			5	3
P. nigra	1981	2				2	0

YG - Yellow-green
DG - Dull-green
W - White
WG - White-grey

APPENDIX 3. Head capsule width (H.C.W.) measurements for poplar hawkmoth larvae

3a) First instar H.C.W. measurements (independent of foodplant)

H.C.W. (mm)	Frequency of occurrence
1.10	2
1.12	1
1.14	9
1.16	13
1.18	17
1.20	33
1.22	8
1.24	5
1.26	1
1.28	2

APPENDIX 3. continued

3b) Second to final instar H.C.W. measurements of larvae reared on Salix fragilis

H.C.W. (mm)	Frequency of occurrence
1.7	4
1.8	12
1.9	33
2.0	24
2.3	7
2.4	4
2.5	13
2.6	9
2.7	22
2.8	15
2.9	15
3.0	4
3.1	1
3.2	1
4.1	1
4.2	1
4.3	1
4.4	2
4.5	2
4.6	3
4.7	2
4.8	1
4.9	1

APPENDIX 3. continued

3c) Second to final instar H.C.W. measurements of larvae reared on Populus alba

H.C.W. (mm)	Frequency of occurrence
1.4	6
1.5	19
1.6	17
1.7	18
1.8	21
1.9	15
2.0	6
2.1	5
2.2	4
2.3	4
3.1	2
3.2	2
3.3	1
3.4	1
4.2	2
4.3	2
4.4	1
4.5	4
4.6	3
4.7	2
4.8	2

APPENDIX 4. Head capsule width (H.C.W.) measurements of eyed hawkmoth larvae reared on various foodplants

H.C.W. (mm)	Frequency of occurrence
0.90	2
0.92	7
0.94	4
0.96	3
0.98	2
1.00	3
1.3	6
1.4	29
1.5	32
1.6	8
1.8	1
1.9	1
2.0	5
2.1	2
2.8	4
2.9	3
3.0	3

APPENDIX 5. Survival of sibling poplar hawkmoth larvae reared on Salix fragilis and Populus alba

5a) Brood 1

Time from hatching (days)	Larvae fed on S. fragilis				Larvae fed on P. alba			
	no. of larvae	% survival (excluding pupae)	no. of larvae +pupae	% total survival (larvae + pupae)	no. of larvae	% survival (excluding pupae)	no. of larvae +pupae	% total survival (larvae + pupae)
0	65	100			65	100		
7	48	73.8			42	64.6		
10	44	67.7			32	49.2		
13	44	67.7			29	44.6		
15	44	67.7			25	38.5		
17	44	67.7			24	36.9		
19	44	67.7			22	33.8		
21	43	66.2			22	33.8		
24	43	66.2			22	33.8		
26	41	63.1			22	33.8		
28	36	55.4			21	32.3		
31	21	32.3	36	55.4	21	32.3		
32	16	24.6	36	55.4	20	30.8		
34	14	21.5	35	53.8	20	30.8		
35	13	20.0	35	53.8	20	30.8		
37	12	18.5	35	53.8	20	30.8		
39	10	15.4	35	53.8	20	30.8		
41	8	12.3	35	53.8	19	29.2		
43	7	10.8	35	53.8	17	26.2	19	29.2
45	5	7.7	35	53.8	14	21.5	19	29.2
46	5	7.7	35	53.8	12	18.5	19	29.2
47	4	6.2	34	52.3	10	15.4	19	29.2
49	3	4.6	34	52.3	8	12.3	19	29.2
51	3	4.6	34	52.3			19	29.2
53	0	0	34	52.3	5	7.7	17	26.2
56					1	1.5	15	23.1
58					0	0	14	21.5

APPENDIX 5. continued

5b) Brood 2

Time from hatching (days)	Larvae fed on S. fragilis				Larvae fed on P. alba			
	no. of larvae	% survival (excluding pupae)	no. of larvae +pupae	% total survival (larvae + pupae)	no. of larvae	% survival (excluding pupae)	no. of larvae +pupae	% total survival (larvae + pupae)
0	48	100			42	100		
4	42	87.5			32	76.2		
5	-	-			30	71.4		
6	40	83.3			-	-		
7	37	77.1			20	47.6		
9	28	58.3			15	35.7		
11	22	45.8			10	23.8		
12	21	43.7			-	-		
13	21	43.7			9	21.4		
15	21	43.7			9	21.4		
19	20	41.7			9	21.4		
21	20	41.7			9	21.4		
22	20	41.7			9	21.4		
25	16	33.3	19	39.6	9	21.4		
26	12	25.0	19	39.6	9	21.4		
27	11	22.9	19	39.6	9	21.4		
29	10	20.8	19	39.6	9	21.4		
31	6	12.5	19	39.6	9	21.4		
32	5	10.4	19	39.6	9	21.4		
33	4	8.3	19	39.6	9	21.4		
35	3	6.25	19	39.6	9	21.4		
36	1	2.1	18	37.5	9	21.4		
40	0	0	18	37.5	8	19.0		
43					6	14.3	7	16.7
45					4	9.5	6	14.3
47					2	4.8	6	14.3
49					0	0	6	14.3

APPENDIX 5. continued

5c) Brood 3

Time from hatching (days)	Larvae fed on S. fragilis				Larvae fed on P. alba			
	no. of larvae	% survival (excluding pupae)	no. of larvae +pupae	% total survival (larvae + pupae)	no. of larvae	% survival (excluding pupae)	no. of larvae +pupae	% total survival (larvae + pupae)
0	48	100			51	100		
3					41	80.4		
5	42	87.5			30	58.8		
7	37	77.1			13	25.5		
10					13	25.5		
11					8	15.7		
14	29	60.4			8	15.7		
16					8	15.7		
17					7	13.7		
18	27	56.25			7	13.7		
21	25	52.1			7	13.7		
22	17	35.4	24	50.0	7	13.7		
23	15	31.25	24	50.0				
24	13	27.1	24	50.0				
25	11	22.9	24	50.0	5	9.8		
27	6	12.5	24	50.0				
29	5	10.4	24	50.0				
31	3	6.25	23	47.9	4	7.8		
36	0	0			4	7.8		
37								
39					3	5.9	4	7.84
41					2	3.9	4	7.84
43					0	0	4	7.84

APPENDIX 6. Larval development rate of poplar hawkmoth siblings reared on Salix fragilis and on Populus alba

6a) Brood 1

Number of days spent as larvae reared on	
S. fragilis	P. alba
31	43
31	43
31	45
31	45
31	45
31	46
31	46
31	47
31	47
31	49
31	49
31	51
31	56
31	58
31	
32	
32	
32	
32	
32	
34	
35	
37	
39	
39	
41	
41	
43	
45	

6a) continued

Number of days spent as larvae reared on	
S. fragilis	P. alba
	45
	49
	53
	53
	53

APPENDIX 6. continued

6b) Brood 2

Number of days spent as larvae reared on	
S. fragilis	P. alba
25	43
25	45
25	47
26	47
26	49
26	49
26	
27	
29	
31	
31	
31	
31	
32	
33	
35	
36	
40	

6c) Brood 3

Number of days spent as larvae reared on	
S. fragilis	P. alba
22	39
22	41
22	43
22	43
22	
22	
22	
23	
23	
24	
24	
25	
25	
27	
27	
27	
27	
27	
29	
31	

APPENDIX 7. Pupal weight, wingspan and egg production of sibling poplar hawkmoths, reared on either Salix fragilis or Populus alba

7a) Brood 1

Larvae fed on S. fragilis			Larvae fed on P. alba		
Pupal weight(g)	Adult wingspan (mm)	Number of eggs produced	Pupal weight(g)	Adult wingspan (mm)	Number of eggs produced
1.319	3.38	68	2.312	Cr.	97
1.699	3.27	93	2.450	4.10	157
1.728	3.33	92	2.501	3.84	n.a.
1.828	3.50	118	2.525	3.98	111
1.873	3.56	188	2.542	4.15	152
1.897	3.70	111	2.614	3.88	140
1.911	3.72	79	2.666	4.05	191
1.920	3.28	86	2.692	3.95	50
1.955	3.65	91	2.770	4.10	184
1.963	3.49	108	2.855	3.96	176
1.990	3.42	126	2.966	3.96	48
2.002	3.37	137	2.995	4.14	214
2.010	3.55	116	3.053	4.23	121
2.024	3.52	48			
2.080	3.59	137			
2.080	3.74	136			
2.105	3.47	16			
2.126	3.19	51			
2.131	3.74	125			
2.137	3.57	135			
2.154	3.63	123			
2.158	3.61	83			
2.169	3.50	134			
2.246	3.70	131			
2.390	3.40	83			
2.535	3.90	198			
2.560	3.95	124			
*1.740	*3.07				
*1.832	*3.26				

APPENDIX 7. Continued

7b) Brood 2

Larvae fed on S. fragilis			Larvae fed on P. alba		
Pupal weight(g)	Adult wingspan (mm)	Number of eggs produced	Pupal weight(g)	Adult wingspan (mm)	Number of eggs produced
1.874	3.45	98	2.647	3.96	106
1.892	3.46	93			
1.934	cr.	n.a.	2.753	4.05	152
1.961	3.44	93	2.966	4.19	134
1.992	3.47	108	*2.112	*3.60	
2.045	cr.	n.a.	*2.302	*3.83	

7c) Brood 3

Larvae fed on S. fragilis			Larvae fed on P. alba		
Pupal weight(g)	Adult wingspan (mm)	Number of eggs produced	Pupal weight(g)	Adult wingspan (mm)	Number of eggs produced
1.860	3.20	96	2.457	3.72	195
1.909	3.40	73	2.472	3.64	214
2.091	3.48	133	2.720	3.75	98
2.116	3.55	52			
2.122	3.77	n.a.			
2.131	3.63	123			
2.214	3.69	83			
2.259	3.84	182			
2.284	3.86	142			
2.526	4.12	163			
*1.680	* cr.				
*1.784	* cr.				
*1.804	*3.06				
*1.825	*3.04				
*1.906	*3.40				
*1.954	*3.33				
*1.958	*3.50				

* asterisked numbers show data for male hawkmoths.
n.a. = data not available cr. = wings of adults crumpled

APPENDIX 8. Resting positions of Laothoe populi larvae on a) Salix fragilis and b) Populus alba

a) S. fragilis

Larval instar	Resting position of caterpillars: Leaf (upper surface)	Leaf (under surface)	Stalks
i	5	62	1
i	11	38	2
i	7	39	2
ii	3	30	0
ii	5	32	0
ii	5	35	0
ii	11	32	2
iii	8	70	0
iii	7	31	0
iii	3	38	0
iv	4	33	0
iv	5	31	0
iv	3	49	0
iv	5	42	0

APPENDIX 8. Continued

b) P. alba

Larval instar	Position of caterpillars			
	Leaf upper surface	Leaf under surface	Side of leaf	Stalks
i	0	36	3	2
	1	41	1	2
	1	9	0	0
	1	9	0	0
	*15	50	1	2*
	**27	24	0	2**
	9	31	0	2
	2	26	0	2
	***16	30	2	3***
ii	4	23	6	0
	0	9	0	0
	1	19	0	0
	7	54	0	1
	2	12	0	4
	4	18	0	2
	2	18	0	0
ii/iii	4	24	0	1
	1	9	0	0
	2	26	0	0
	1	8	0	1
	11	25	0	1
iii	0	10	0	0
	6	32	0	2
	2	24	0	2
	2	32	0	2
	2	45	0	2
	1	45	0	2
	3	7	0	0
iv and v	1	9	0	0
	2	23	0	0
	4	23	0	2
	0	9	0	1
	4	16	0	2
	2	6	0	2
	0	9	0	1

APPENDIX 9. Survival of green Laothoe populi larvae on various foodplants in the field (Cuerden Valley Park)

a) Populus alba

	Number of larvae surviving on				
Replicate	30.6.82	6.7.82	13.7.82	20.7.82	27.7.82
1	10	5	4	0	0
2	10	10	8	0	0
3	10	5	3	0	0
4	10	9	6	2	1
5	10	8	8	8	4
6	10	9	8	7	7
7	10	9	8	8	5
8	10	7	6	6	6
TOTAL	80	62	51	30	23

APPENDIX 9. continued

b) Salix fragilis

	Number of larvae surviving on				
Replicate	30.6.82	6.7.82	13.7.82	20.7.82	27.7.82
1	10	1	0		
2	10	0	0		
3	10	2	0		
4	10	1	0		
5	10	0	0		
6	10	0	0		
7	10	10	10	9	5
8	10	10	8	8	4
TOTAL	80	24	18	17	9

c) Salix caprea

	Number of larvae surviving on				
Replicate	30.6.82	6.7.82	13.7.82	20.7.82	27.7.82
1	10	8	6	0	0
2	10	3	1	0	0
3	10	6	0	0	0
4	10	7	3	3	2
5	10	7	5	2	1
TOTAL	50	31	15	5	3

APPENDIX 10. Survival of white and green Laothoe populi caterpillars on Populus alba and on Salix fragilis bushes in Cuerden Valley Park

a) P. alba

Replicate	Number of green : white larvae surviving after 0	2	4	6	8 days
1	3 : 3	3 : 3	1 : 1	0 : 0	-
2	3 : 3	2 : 2	0 : 0	-	-
3	3 : 3	1 : 3	1 : 3	0 : 1	0 : 0
4	3 : 3	1 : 1	0 : 1	0 : 0	-
5	3 : 3	3 : 3	0 : 0	-	-
6	3 : 3	2 : 3	0 : 0	-	-
7	3 : 3	0 : 0	-	-	-
8	3 : 3	0 : 1	0 : 0	-	-
9	3 : 3	2 : 3	0 : 0	-	-
10	3 : 3	0 : 0	-	-	-
11	3 : 3	1 : 1	0 : 1	0 : 0	-
12	3 : 3	1 : 3	0 : 0	-	-
TOTALS	36 : 36	16 : 23	2 : 6	0 : 1	0 : 0

APPENDIX 10. continued

b) S. fragilis

Replicate	Number of green : white larvae surviving after 0	2	4	6	8	10 days
1	3 : 3	1 : 0	0 : 0	-	-	-
2	3 : 3	3 : 2	3 : 2	0 : 2	0 : 1	0 : 0
3	3 : 3	0 : 1	0 : 1	0 : 0	-	-
4	3 : 3	1 : 0	1 : 0	0 : 0	-	-
5	3 : 3	2 : 3	1 : 3	0 : 0	-	-
6	3 : 3	0 : 3	0 : 1	0 : 0	-	-
7	3 : 3	3 : 2	2 : 2	0 : 1	0 : 0	-
8	3 : 3	3 : 1	3 : 0	1 : 0	0 : 0	-
9	3 : 3	3 : 2	3 : 1	2 : 0	1 : 0	0 : 0
10	3 : 3	2 : 3	1 : 0	1 : 0	0 : 0	-
11	3 : 3	0 : 1	0 : 0	-	-	-
12	3 : 3	0 : 0	-	-	-	-
TOTALS	36 : 36	18 : 18	14 : 10	4 : 3	1 : 1	0 : 0

APPENDIX 11. Survival of green Laothoe populi larvae on Salix fragilis bushes in Cuerden Valley Park

Replicate	Number of larvae surviving after							
	0	2	4	6	8	10	12	14 days
1	6	4	1	0	-	-	-	-
2	6	4	0	-	-	-	-	-
3	6	6	6	6	5	4	1	0
4	6	0	-	-	-	-	-	-
5	6	2	1	0	-	-	-	-
6	6	4	1	0	-	-	-	-
7	6	2	0	-	-	-	-	-
8	6	3	0	-	-	-	-	-
9	6	3	0	-	-	-	-	-
10	6	6	6	6	6	0	-	-
TOTALS	60	34	15	12	11	4	1	0

APPENDIX 12. List of birds recorded at Cuerden Valley Park. Asterisk denotes a potential predator of Laothoe populi larvae.

English Name	Scientific Name	
Dabchick	Tachybaptus ruficollis	A
Great crested grebe	Podiceps cristatus	A
Heron	Ardea cinerea	A
Mute Swan	Cygnus olor	A
Wigeon	Anas penelope	A
Teal	Anas crecca	A
Mallard	Anas platyrhynchos	A
Pochard	Aythya ferina	A
Tufted Duck	Aythya fuligula	A
Goldeneye	Bucephala clangula	A
Sparrowhawk	Accipiter nisus	
Kestrel	Falco tinnunculus	
Partridge	Perdix perdix	
Moorhen	Gallinula chloropus	A
Coot	Fulica atra	A
Snipe	Gallinago gallinago	
Woodcock	Scolopax rusticola	
Redshank	Tringa totanus	A
Common sandpiper	Tringa hypoleucos	A
Black-headed gull	Larus ridibundus	A
Herring Gull	Larus argentatus	A
Lesser black-backed gull	Larus fuscus	A
Great black-backed gull	Larus marinus	A
Common tern	Sterna hirundo	A
Woodpigeon	Columba palumbus	

	English Name	Scientific Name	
	Tawny Owl	Strix aluco	
	Kingfisher	Alcedo atthis	A
★	Great spotted woodpecker	Dendrocopos major	
	Sand martin	Riparia riparia	A
	Swallow	Hirundo rustica	A
	House martin	Delichon urbica	A
★	Grey wagtail	Motacilla cinerea	
★	Pied wagtail	Motacilla alba	
	Dipper	Cinclus cinclus	
	Wren	Troglodytes troglodytes	
★	Dunnock	Prunella modularis	
★	Robin	Erithacus rubecula	
★	Blackbird	Turdus merula	
★	Song thrush	Turdus philomelos	
	Redwing	Turdus iliacus	
★	Mistle thrush	Turdus viscivorus	
★	Whitethroat	Sylvia communis	
★	Chiffchaff	Phylloscopus collybita	
★	Willow warbler	Phylloscopus trochilus	
★	Goldcrest	Regulus regulus	
★	Long-tailed tit	Aegithalos caudatus	
★	Marsh tit	Parus palustris	
★	Coal tit	Parus ater	
★	Blue tit	Parus caeruleus	
★	Great tit	Parus major	
	Tree creeper	Certhia familiaris	
★	Jay	Garrulus glandarius	
★	Magpie	Pica pica	

	English Name	Scientific Name
★	Jackdaw	Corvus monedula
★	Carrion crow	Corvus corone
★	Starling	Sturnus vulgaris
★	House sparrow	Passer domesticus
★	Chaffinch	Fringilla coelebs
	Brambling	Fringilla montifringilla
★	Greenfinch	Carduelis chloris
	Goldfinch	Carduelis carduelis
	Siskin	Carduelis spinus
	Linnet	Acanthis cannabina
	Redpoll	Acanthis flammea
★	Bullfinch	Pyrrhula pyrrhula
	Reed Bunting	Emberiza schoeniclus

A = feeding or breeding by or on the lake.

(From Edmunds, J., Edmunds, M. and Thom, M. (1982)).

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J. GRAYSON

POLYMORPHISM IN HAWKMOTH CATERPILLARS - AN ECOLOGICAL AND BIOCHEMICAL STUDY OF CRYPSIS IN SMERINTHUS OCELLATA (L.) AND LAOTHOE POPULI (L.)

Extensive field surveys in Lancashire and Merseyside have shown that for both Smerinthus ocellata (the eyed hawkmoth) and Laothoe populi (the poplar hawkmoth) there is a correlation between larval and foodplant coloration.

For L. populi, experiments have shown that foodplant determines the colour of full grown caterpillars. Siblings reared on Salix fragilis L. (which has green leaves) became yellow-green, intermediate-green or dull-green whereas those reared on Populus alba L. became either white or green. Further experiments have shown that reflected light intensity may be the vital cue which determines whether a caterpillar becomes white or green. Thus for this species the polymorphism is environmentally rather than genetically determined. However, the genetic background appears to have some effect on the proportion of dull-green and intermediate-green morphs in broods.

For S. ocellata, both laboratory and field experiments have shown that the coloration of caterpillars is also determined by some environmental factors related to light. There is no simple genetic or nutritional control of larval colour in this species.

Pigment extraction and analysis have shown that the proportions of different carotenoids in the main foodplants are very similar, as are the chlorophyll a to b ratios and carotenoid-to-chlorophyll ratios. The principal carotenoid in the food of both species of caterpillar, lutein, is sequestered by the insects in the integument and contributes to the animal's coloration. Cis-lutein is also present in small quantities in the integuments of both L. populi and S. ocellata caterpillars. Yellow-green L. populi larvae contain more lutein in the integument than dull-green morphs and in white caterpillars this carotenoid is barely detectable.

Field predation experiments indicated that white caterpillars of L. populi are at a selective advantage compared with yellow-green morphs on the white undersurface of P. alba leaves. However, both white and green morphs suffered similar predation on S. fragilis bushes.