REVIEW

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Biochemistry and biosynthesis of insect pigments

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Abstract. The functional role and commercial importance of insect pigments have been studied for well over a century. They are classified into those synthesized by insects, which include anthraquinones, aphins, pterins, tetrapyrroles, ommochromes, melanins and papiliochromes, and those sequestered from their host plants, the antioxidative carotenoids and water-soluble flavonoids. They can also be categorized into those that are produced by cyclization of linear precursors, e.g. anthraquinones, aphins and tetrapyrroles and those derived from cyclic precursors such as pterins, ommochromes, melanins and anthocyanins. Anthraquinones and aphins are derived by cyclization of linear polyketides via successive condensation of simple carboxylic acid metabolites and occur in two major Superfamilies of Hemiptera, the Coccoidea and Aphidoidae, respectively. Ommochromes, tetrapyrroles and melanins are derived from different amino acid precursors, tryptophan, glycine and tyrosine, respectively. Apart from providing body colouration, ommochromes are visual pigments, melanins act as a protectant against UV and tetrapyrroles facilitate oxygen transport to cells. Papiliochromes are synthesized using both, the essential amino acids tyrosine and tryptophan. Pterins are derived from guanosine triphosphate (GTP) and are also present in ommatidia of eyes. The sequestered pigments, anthocyanins and carotenoids, are synthesized from phenylalanine and by condensation of two isoprene units, respectively, in plants. The biosyntheses of chemochromes in insects are governed by a complex set of enzymes, pathways and genetics. This review provides a comprehensive understanding of the molecules that are not only responsible for the striking colours but also provide functional benefits for insects. The commercially important pigments are also discussed.

INTRODUCTION

Insects are considered to be the most successful arthropods and the largest group of animals, with over 800,000 identified species. New insect species are being identified at a rate of about 5,000 species per year and their predicted total number ranges between 1 and 10 million (Morgan, 2010). Overcoming dramatic changes on earth, insects have dominated the world and according to the pioneers in insect chemical ecology, Meinwald and Eisner (Eisner et al., 1994), the dominant position of insects and other arthropods has been attained due to their ability to synthesize or acquire an extremely diverse array of compounds for defense, offence and communication, which includes a great diversity of secondary metabolites. Though the colours of insects are mainly due to different pigments, the iridescent colours of some Lepidoptera and Coleoptera are due to light interference. The brilliant colours and their variations, displayed by a wide range of insect taxa, have long been an interesting topic of research because of a number of prominent attributes associated with them. Pterins act as a cofactor in ommochrome biosynthesis and is present in combination with the latter in the pigment cells in eyes, which is an example of convergent evolution in both. Lepidoptera have not specialized in a specific pigment molecule, such as pterins, ommochromes, papiliochromes, tetrapyrroles, melanins, carotenoids or anthocyanins, but the different butterfly families are specific in the pigment molecule they synthesize, papiliochromes in Papilionidae, pterins in Pieridae and ommochromes in Nymphalidae. Also, a pigment can perform various biological functions depending upon the ecological factors, for example, anthocyanins help in mate selection in the butterfly, *Polyommatus icarus* (Lepidoptera: Lycaenidae) but in combination with melanin act as a warning colouration in *Parasemia plantaginis* (Lepidoptera: Arctiidae) larvae (Lindstedt et al., 2010). Until recently very little information was available regarding the biosynthesis of insect pigments. The study of pigment chemistry commenced in early 1900 and made remarkable progress due to the development of chromatographic and sensitive mass spectrometric techniques. Chromatographic techniques enabled the separation of components in complex mixtures consisting of similar compounds, and the purification and identification of substances. This review aims to provide more information on the chemistry of diverse pigment molecules synthesized or sequestered by insects and the mode of their biogenesis, which includes the diverse ecological and physiological roles of pigments, such as camouflage, mimicry, warning colouration, mate selection, etc., that provide functional benefits for the insects producing them.

ECONOMIC IMPORTANCE OF INSECT PIGMENTS

In terms of history, the use of the pigment produced by *Kermes ilicius* (Hemiptera: Kermesidae) and *Kermococcus vermilis* (Hemiptera: Kermesidae), is ancient, as kermes-dyed remains have been identified at a prehistoric archaeological site in Provence, France. Kermes dye was very widely used in Asia and Europe for dyeing fabrics. Lac dye

produced by *Kerria lacca* (Hemiptera: Tachardiidae) was commonly used in ancient Chinese and Indian civilizations for dyeing silk and leather and in cosmetics. In verse no. 5 of the 5th volume of Atharva Veda (1500–1200 B.C.) it, is mentioned that lac dye can be used on open wounds or taken orally. The ancient Vinaya Pitaka texts of Buddhism contain details of how to extract and apply lac dye (Dave, 1950). In the mid-800s lac dye was used to add colour to "Enji" paintings and widely used, by traditional artists as a result of cultural exchanges with China and Central Asia (Kutsuna et al., 2012)

The earliest reference to carmine produced by cochineal insects, such as *Dactylopius coccus* (Hemiptera: Dactylopiidae), *D. confuses* (Hemiptera: Dactylopiidae), *Porphyrophora polonica* (Hemiptera: Margarodidae) and *P. hamelii* (Hemiptera: Margarodidae), dates back to 714 B.C. Marcin (1595) of Urzędów records the first scientific study of cochineal in Herbarz polski (Polish Herbal). Historia naturalis cocci radicum tinctorii quod polonicum vulgo audit, the first major treatise on cochineal insects, was written by Johann Philipp Breyne in 1731, this includes an account of the physiology and life cycle of these insects. Also, a monograph, Czerwiec polski (Polish cochineal) written by the Polish biologist Antoni Jakubski in the early1930s describes this insect's biology and gives an historical account of cochineal dye (Baranyovits, 1978).

The use of insect pigments for commercial purposes has a long history. Kermesic, carminic and laccaic acids are three of the best known. Kermesic acid was known as "King's red" and used in paintings since ancient times. It is documented by Pfister that during the ancient Greek period cochineal was used to dye textiles in Egypt and Syria. As recorded in State Papers for 1617–1621, carmine was an important commodity in Persia and this highly prized dyestuff was one of the most valuable exports quoted on the London and Amsterdam Commodity Exchanges on a regular basis. The actual source of carmine was not known until the 18th century (Greenfield, 2005); however, it was used throughout Europe for dyeing fabric, food, body paint and used in a wide range of paints and cosmetics.

Lac dye is the principal red dye used in classical Persian carpets and to dye silk, yielding a range of colours from rose to purple. During the eighteenth century India exported large quantities of lac dye (Hunter, 2000), exporting 901,649 kg in the year 1868–69 which decreased to 51 kg by 1900–01 with the advent of synthetic dyes (Majumdar, 1981). Following the present world-wide ban on azo-dyes, insect dyes have become commercially valuable again and are expected to stage a comeback. These insect pigments are once again being used in medicine, food and cosmetics. Lac dye is now legally registered as a natural food additive in a number of countries; the Chinese National Standard, CNS No. is 08.104; the Korean Food and Drug Administration, KFDA No. is Natural Additives, 13 and the Japanese no. is Natural Additives, 462.

NATURE OF INSECT COLOURATION

Pigments appear coloured because they reflect certain wavelengths of light and absorb and dissipate others as heat. The molecular nature of the compound determines the particular wavelengths absorbed. The absorbed energy causes changes in the energy of the molecules' electrons, leading to transition from a "ground state" to an "excited state" (Streitwieser & Heathcock, 1985). Chromophores of dye molecules often contain unsaturated groups such as C=O and N=N, which are part of a conjugated bonding system, usually involving aromatic rings (Streitwieser & Heathcock, 1985). In the production of colour, the number and arrangement of double bonds, C=C, C=O, C=N and N=N are very important. Specific functional groups are also important, examples are the $-NH_2$ and -Cl radicals, these groups tend to shift the absorptive region of a compound to longer wavelengths. If the electrons in the double bond in a pigment molecule with alternative single and double bonds absorb a photon of visible light, they can enter into an excited state, e.g. β-carotene has ten conjugated C=C bonds. Unlike Humans, insects can perceive short wavelength light (Chapman, 2013).

Insect colouration is mainly due to the presence of various pigment molecules in the cuticle or underlying epidermis or due to the presence of physical structures, but in some, the fat body and haemolymph also provide colour if the cuticle is transparent. It was Goureau (1843), who discovered that the colours produced by the thin, membranous wings of many insects are due to physical structures. Later, the Indian scientist C.V. Raman integrated classical optics with biological iridescence (Raman, 1934, 1935), such as that from shells and bird feathers. Surface structures lead to scattering, interference or diffraction, which result in the white, blue and iridescent colours of some butterfly wings and surfaces of scarab beetles known as schemochromes. Examples are the blue iridescent colour of *Morpho rhetenor* (Lepidoptera: Nymphalidae) and *M. didius* (Lepidoptera: Nymphalidae) (Morgan, 2010). Colours due to chemical pigments are called chemochromes. In many insects, for example, the orange sulphur butterfly *Colias eurytheme* (Lepidoptera: Pieridae) both schemochromes (ultraviolet iridescence) and chemochromes (pterins) contribute to the body colouration (Rutowski et al., 2005). Also, many insects are reported to contain more than one type of chemochromes, e.g. a number of different pigment molecules viz. melanin, carotenes, pterins and biliverdin are present in the haemolymph of migratory locust, *Locusta migratoria* (Orthoptera: Acrididae) (Goodwin & Srisukh, 1948; Fuzeau-Braesch, 1985; Kayser, 1985).

BIOCHEMISTRY AND BIOSYNTHESIS OF INSECT PIGMENTS

Insect pigments are mainly anthraquinones, aphins, pterins, tetrapyrroles, ommochromes, melanins, carotenoids and flavonoids. These pigments may be water or lipid-soluble; water-soluble insect pigments include papiliochromes, anthocyanins and flavonoids. These are also soluble in organic solvents and in strong acids and bases (Kayser, 1985; Umebachi, 1975; Burghardt et al., 2001) Pterins are poorly soluble in water, insoluble in non-polar organic solvents but soluble in strong acid or alkali (Blau & Thöny, 2008;

Hevener et al., 2010) and among ommochromes, except for rhodommatin, which is water-soluble (Nijhout, 1997), others are soluble in acidic methanol (Linzen, 1974; Nijhout & Koch, 1991). Carotenoids are an important lipid soluble pigment and the most widely distributed of all natural pigments. Anthraquinonoid pigments are poorly soluble in water but soluble in hot organic solvents, and among aphins, which are derived from perylene, protoaphins are water-soluble whereas xanthoaphins are lipid-soluble. Melanins are insoluble in both water and lipid solvents. Table 1 provides a comprehensive overview of the nature, distribution, genes and their products and biological function of different classes of insect pigments.

This great variety of pigment molecules gives rise to the strikingly beautiful insect colouration; the advent of technology resulted in remarkable progress in understanding the chemistry of pigments, their physical properties, biosynthetic pathways and their biological and ecological functions. Unfortunately, there has been little progress in determining the function and biogenesis of these compounds and the information on these topics is still fragmentary. The insect pigments described here are highly diverse with regard to structure and colour, which reflects the diversity of insects and their metabolic pathways.

Anthraquinones

Anthraquinones, the pigment of coccids (Morgan, 2010), constitute a large class of dyes and pigments, and are structurally built from an anthracene ring (tricyclic aromatic) with a keto group on carbon atoms nine and ten. They belong to the family of polycyclic aromatic hydrocarbons and, upon oxidation with potassium dichromate/sulphuric acid, produce anthraquinone.

Polyketide anthraquinones occur in Coccoidea. The artist's colour, Venetian red, is the pigment kermesic acid produced by *Kermococcus ilicius*, which feeds on the oak, *Quercus coccifera.* The food colouring cochineal, is obtained from dried females of *D. coccus*, a bug feeding on *Opuntia* cactus (prickly pear). The pigment, carminic acid is a glucosylated pigment of coccids, kermesic acid with an attached C-glucoside. Deep red coloured carmine is thought to be a chemical weapon against predation as it deters ants (Eisner et al., 1980). The lac insect of *Kerria* species also produces pigment; their body is crimson in colour due to the presence of a complex of water-soluble

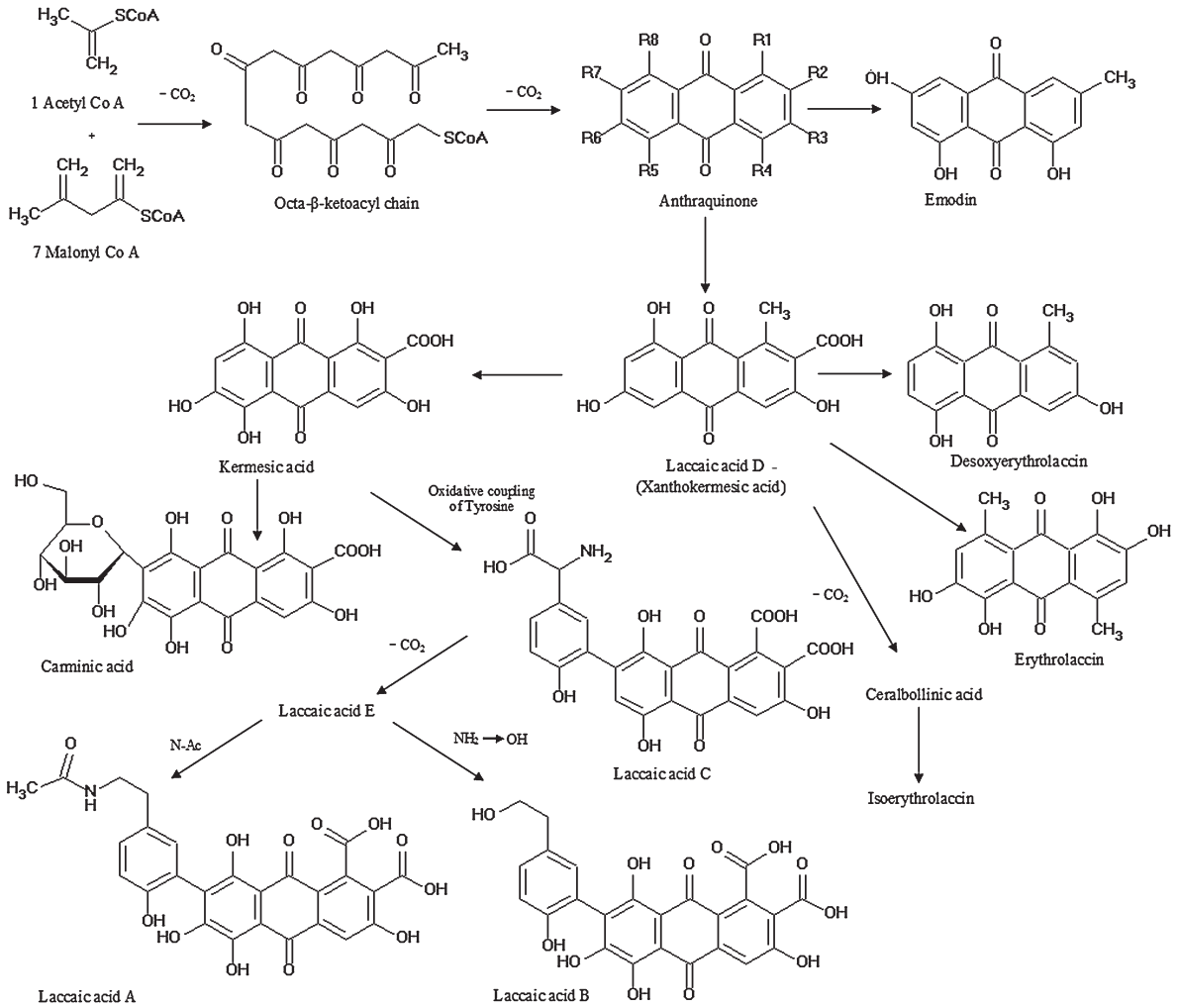

Fig. 1. Suggested polyketide pathway for anthraquinone biosynthesis in scale insects; the cyclized octaketide chain gives rise to laccaic acid D and emodin, as recorded in lower organisms. Kermesic acid is produced by *Kermococcus ilicius*, carminic acid, the food colouring cochineal by *Dactylopius coccus* and laccaic acid by *Kerria lacca.* (Modified from Venkataraman & Ramarao, 1972.)

TABLE 1. An overview of the properties of different classes of insect pigments.

Pigment class	Colour	Chemical family	Solubility	Precursor molecule	Localization	Genes	Enzymes involved	Taxonomic distribution	Functions
Anthraquinones	Crimson Red Yellow	Polycyclic aromatic hydrocarbon	Poorly soluble in water	Linear polyketides	Body pigment	–	Polyketide synthase Glycosyl-transferase	Hemiptera: Coccoidea	Chemical defence against predators, deters ants Antibacterial and antifungal properties
Aphins	Black Brown Red Green	Dimeric naphthaquinone	Some are water-soluble & some are lipid-soluble	Linear polyketides	Haemolymph	–	–	Hemiptera: Aphididae	Ornamental & warning colouration
Pterins	Red Yellow Orange Colourless	Pteridines: nitrogen containing cyclic compounds	Sparingly soluble in water	Guanosine triphosphate	Body pigments Scales Wings	*GTP-CH I* *purple* *SPR*	GTP cyclo-hydrolase 6-pyruvoyl-tetrahydropterin synthase Sepiapterin reductase	Many insect orders, e.g., Lepidoptera, Hymenoptera, Hemiptera	Cofactors of enzymes Growth factor Warning colouration Circadian rhythm regulation Eye pigmentation
Ommochromes	Yellow Red Brown Black	Tricyclic compounds	Poorly soluble in water	Tryptophan	Ommatidia of compound eyes Epidermis	*vermilion* *kf* *cinnabar* *white* *scarlet*	Tryptophan oxidase Kynurenine formamidase Kynurenine 3-hydroxylase	Many insect orders, e.g., Lepidoptera, Odonata	Eye pigmentation Removal of excess tryptophan to avoid toxicity
Tetrapyrroles	Green Blue Yellow	Four pyrrole rings	Water-soluble	Glycine	Wings Fat bodies Haemolymph	*BBP*	Bilin-binding protein	Most insect orders (in small quantity); Diptera, Phasmida, Mantodea, Orthoptera, Lepidoptera	Body colouration Facilitates oxygen transport to cells
Melanin	Black Brown Yellow Colourless	Nitrogen containing compounds	Insoluble in both water & lipid	Tyrosine	Cuticles	*TH* *DDC* *ebony* *tan* *yellow* *laccase2*	Tyrosine hydroxylase DOPA decarboxylase NBAD synthase NBADH DCE Phenol oxidase	Many insect orders	Wound-healing Encapsulates invaders Antibiotic property UV protectant
Papiliochromes	White Yellow Red	A peptide with two aromatic rings	Soluble in water, organic solvents & in strong acids and bases	Tyrosine and tryptophan	Wings	–	–	Lepidoptera: Papilionidae	Reduces wing iridescence in papilionid butterflies
Carotenoids	Yellow Green Blue-green Blue Red	Tertraterpenes	Lipid-soluble	Two isoprene units	Integument Haemolymph	*carB* *carRA* *YRG*	Phytoene dehydrogenase Lycopene cyclase/Phytoene synthase Yellow-related gene	Lepidoptera, Orthoptera, Hemiptera	Photoreception Antioxidant Ornamental colouration Photo induced electron transfer in aphids
Anthocyanins and flavones	Cream or yellow	Flavonoid	Soluble in water, organic solvents & in strong acids and bases	Phenylalanine	Wings	*PAL* *CHS* *CHI* *F3H* *DFR* *ANS* *LDOX*	Phenylalanine ammonialyase Chalcone synthase Chalcone isomerase Flavanone 3'-hydroxylase Dihydroflavonol 4-reductase Anthocyanidin synthase Leucoanthocyanidin dioxygenase	Lepidoptera: Papilionidae, Satyride, Lycaenidae	Determines mating preferences

Note: When no information is available, it is represented by a dash (–).

pigments collectively called lac dye. These pigments are polyhydroxy-anthraquinones, which are very similar in chemical structure. There are at least six different components in the body coloration of lac insects, of which the major components are laccaic acids A and B (Pandhare et al., 1966, 1967, 1969; Burwood et al., 1967; Bhide et al., 1969; Oka et al., 1998a, b) and minor components are laccaic acid C, D, E and F (Mehandale et al., 1968; Rama Rao et al., 1968; Hu et al., 2011). The protective resinous covering of the lac insect, from which commercial shellac is derived, appears yellowish. The colour of the resin is due to certain alcohol-soluble anthraquinone pigments related to laccaic acids, such as desoxyerythrolaccin, erythrolaccin and isoerythrolaccin, which also share a common biochemical pathway. Emodin is an example of a very widely distributed anthraquinone, occurring in species of the Australian scale insect *Eriococcus*, and in the roots of rhubarb. The structure of the anthraquinone pigments of scale insects are depicted in Fig. 1.

There are several reviews of the major routes for the biosynthesis of anthraquinones (Leistner, 1985; Inouye & Leistner, 1988; Van Den Berg & Labadie, 1989; Han et al., 2001). The established literature indicates that the polyketide pathway is the main biosynthetic pathway leading to anthraquinones in insects. The polyketide pathway, also known as the acetate or malonate pathway is characterized by the formation of anthraquinones by the folding of a polyketide chain with both rings hydroxylated e.g. 1, 8-dihydroxylated anthraquinones (Chrysophanol type anthraquinones) (Van Den Berg & Labadie, 1989; Han et al., 2001). This is reported in a wide range of organisms and the suggested key enzyme is polyketide synthase. This anthraquinone biosynthetic pathway begins with the formation of a polyketide chain from one acetyl-CoA and seven malonyl-CoA units resulting in an octaketide chain by decarboxylation of each malonyl unit at every elongation step (Fig. 1). The elongated chain subsequently cyclises to an anthrone, which is further oxidized to anthraquinone. At some instance, Post-PKS enzymes, like glycosyltransferases, transfer the sugar moieties to anthraquinones resulting in the formation of glycosides.

Aphins

Aphids vary in colour and may be green, red, brown or black. The systematic chemical analysis of this unique series of natural pigments in aphids (not found in any other insects) called aphins, was done by Lord Todd and his collaborators in the 1950s and 1960s followed by Donald W. Cameron. Their findings can be traced back to a series of publications entitled "Colouring matters of Aphididae". Aphins are dimeric naphthoquinones and the two most important aphins are protoaphin-*fb* and protoaphin-*sl* (first isolated from the common bean aphid, *Aphis fabae* (Hemiptera: Aphididae) and the brown willow aphid, *Tuberolachnus salignus* (Hemiptera: Aphididae), respectively, both protoaphins differ only at one chiral centre. The biosynthesis of aphins using a presumed polyketide precursor is depicted in Fig. 2. The protoaphins are water soluble pigments that occur in the haemolymph; in solutions of pH

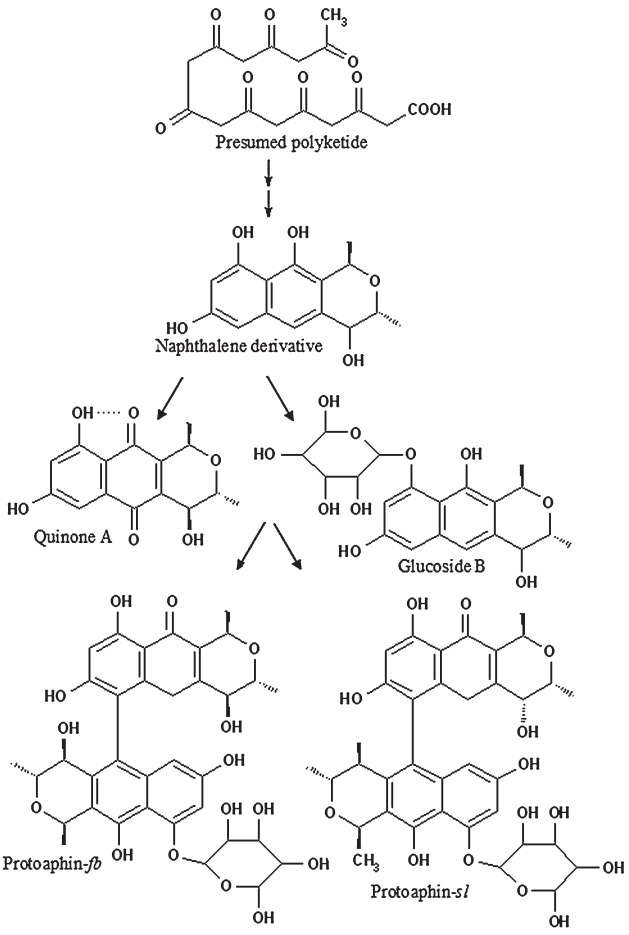

Fig. 2. Biosynthesis of aphins, the pigment of aphids (Morgan, 2010). Reproduced by permission of The Royal Society of Chemistry.

greater than 5.5, it has a deep purple colour, which changes reversibly to brownish-yellow in a more acidic solution. Corresponding to these three colours there are three distinct substances, which can be isolated by fractional crystallization; the yellow xanthoaphin, orange chrysoaphin and red erythroaphin. Unlike protoaphin, they all fluoresce intensely, especially in ultraviolet light. Furanaphin and 6-hydroxymusizin have been isolated from *Aphis spiraecola* (Hemiptera: Aphididae) and a new series of aphins, the red pigments uroleuconaphins, were isolated from *Uroleucon nigrotuberculatum* (Hemiptera: Aphididae) (Horikawa et al., 2004, 2006, 2008). Some species, e.g. *Macrosiphium rosae* (Hemiptera: Aphididae), contain only a green pigment aphinin. The aphid, *Aphis nerii* (Hemiptera: Aphididae) is bright orange, containing glucoside B and a number of naphthalene derivatives related to it, which in this case might serve as warning colouration (Morgan, 2010).

Pterins

These nitrogen containing cyclic compounds belonging to a class called pteridines are pigments of butterflies and were first described by Wieland and Schöpf in 1925 (Brown, 2009) and reviewed by Albert (Albert, 1953; Albert et al., 1954) in a series of publications and Ziegler-

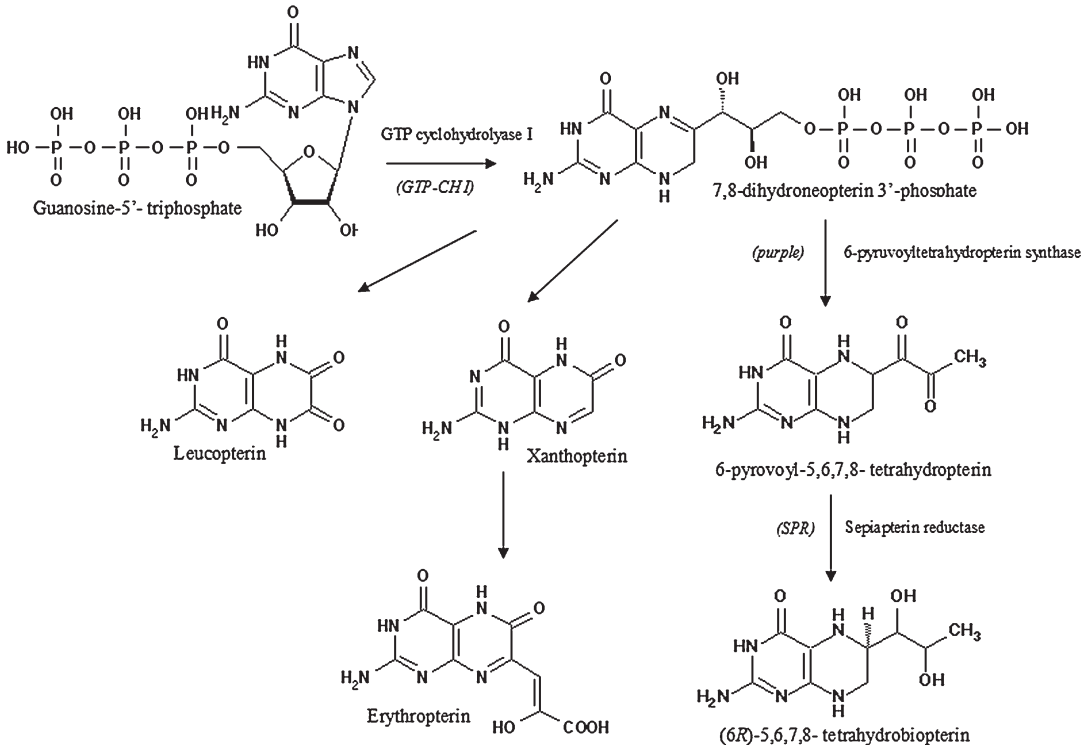

Fig. 3. Pathway depicting biosynthesis of pterins from the precursor molecule GTP (modified from Morgan, 2010; Futahashi et al., 2010). Reproduced by permission of The Royal Society of Chemistry.

Gunder (1956, 1958) from a chemical and biological point of view, respectively. The first structures elucidated were the white pigment, leucopterin [from *Pieris brassicae* and *P. rapae* (Lepidoptera: Pieridae)] and yellow pigment, xanthopterin [from *Gonepteryx rhamni* (Lepidoptera: Pieridae)]. Pterins have the same basic structure, but differ in the radicals attached to the nucleus. Not all pterins appear coloured; some are important metabolically as cofactors of enzymes concerned in growth and differentiation and may act as controlling agents in these processes, e.g. tetrahydrofolic acid and flavin. The compound biopterin occurs in every animal cell or tissue as a cofactor of some enzymatic reactions, e.g. the hydroxylation of phenylalanine to tyrosine and tyrosine to dihydroxyphenylalanine (DOPA). In some insects, biopterins are thought to act as a growth factor.

Pterins are also the body pigments of Lepidoptera and Hymenoptera. They are important pigments in lepidopteran scales, where they are concentrated in pigment granules located on the crossribs of wings (Chapman, 2013). Xanthopterin, a yellow coloured pigment is found in animals, including many insects, for example, in common wasps, *Vespa vulgaris* and *V. crabro* (Hymenoptera: Vespidae). Partial opacity of black melanin and partial transparency of the cuticle means it is possible to see the underlying pterin, which provides the black and orange warning colouration of the milk weed bug *Oncopeltus fasciatus* (Hemiptera: Lygaeidae) (Forrest et al., 1966). Erythropterin is the most abundant of the five pterins found in the fire bug *Pyrrhocoris apterus* (Hemiptera: Pyrrhocoridae) (Bel et al., 1997). Red erythropterin is also present in the orange-tip butterfly, *Anthocharis* (Lepidoptera: Pieridae). The yellow colour of the brimstone butterfly, *Gonepteryx*, is due to chrysopterin and the males are a brighter yellow than females due to their having a higher concentration of this pigment. A high concentration of pterin granules in the epidermis also accounts for the yellow colouration of Hymenoptera (Chapman, 2013).

Pterins are often found in combination with ommochromes and are cofactors of the enzymatic catalysis involved in ommochrome biosynthesis. Pterins along with ommochromes are found in the screening pigment cells of ommatidia. The accumulation of pterins (the products of purine degradation) in the eyes of higher Diptera indicates the age of these insects; they are also supposed to be involved in the regulation of circadian rhythms (Chapman, 2013).

There is little information on pterin biosynthesis in insects, which is probably similar to that available for mammals. In mammals, the biosynthesis begins with guanosine triphosphate (GTP). As shown in Fig. 3 the imidazole ring of guanine loses one atom of carbon as formic acid, resulting in the formation of a ribose triphosphate derivative of diaminopyrimidine. Upon the ring opening the ribose sugar gives rise to an open chain keto sugar, which cyclizes with the free amine group to form dihydroneopterin triphosphate. GTP cyclohydrolase I (encoded by *GTP CH I* gene), 6-pyruvoyltetrahydropterin synthase (*purple*) and sepiapterin reductase (*SPR*) are some of the enzymes responsible for catalyzing the different steps in the biosynthesis of pterin.

Ommochromes

Ommochrome pigments in insect eyes function as screening pigments, which cut out stray light. They are

also capable of changing colour, which is redox dependent and reversible, e.g., the epidermal ommochrome pigments in dragonflies change from yellow (oxidixed form) to red (reduced form) (Futahashi et al., 2012) (Fig. 4). Ommochromes can be extracted from ommatidia of compound eyes and epidermis. They are derived from tryptophan and produce a wide range of colours, from yellow, red, brown and black. Becker determined the metabolic origin and distribution of ommochromes. Butenandt and his collaborators in 1950 studied the chemical nature of ommochromes following the study of eye colour mutants of *Drosophila melanogaster* (Diptera: Drosophilidae), reviewed by Karlson.

Ommochromes usually occur as granules in conjugation with proteins, which also contain calcium. Examples of ommochrome colouration in insects are the pink coloured immature adults of *Schistocerca* (Orthoptera: Acrididae), the red colour in Odonata and the red and brown colour in nymphalid butterflies. The blue colour in blue Odonata is due to the presence of dark brown ommochrome (Chapman, 2013). Ommochromes can be divided into ommatins and ommins. Ommatins have low molecular mass, alkalilabile and are responsible for lighter colours, whereas, ommins have high molecular mass and are stable in alkali. Dark colours are a resultant of mixture of ommatin and ommins (Casas & Théry, 2009).

The biosynthetic pathway for the production of ommatins starts from tryptophan, which is converted to formylkynurenine by the action of tryptophan oxydase. Formylkynurenine is then converted to kynurenine by kynurenine formamidase and kynurenine 3-hydroxylase is then responsible for 3-hydroxykynurenine formation, which enters into pigment granules via ABC transporters and then by oxidative dimerization ommochromes are synthesized (Butenandt & Schafer, 1962; Reed & Nagy, 2005; Ferguson & Jiggins 2009; Osanai-Futahashi et al., 2012). Examples of ommochromes are red dihydroxanthommatin and yellow xanthommatin; xanthommatin, which is widely distributed in insects, and is a screening pigment in the accessory cells of their eyes, usually in association with pterins. They are also present in retinula cells. By using labelled xanthommatin it is possible to show that it is converted into rhodommatin (Fig. 4).

Study of eye colour mutants of *Drosophila melanogaster* has contributed much to the current understanding of ommatins. These 10–15 pigments range from brown-yellow to purple in colour. Ommins are insoluble compounds, which are probably formed from linear polymers of hydroxykynurenine. Sulphur present in some ommins is derived from cysteine or methionine.

Ommochrome production helps in the removal of excess tryptophan to avoid toxicity. During moulting or starvation there is an excess of toxic tryptophan in locusts possibly due to the break-down of protein either during structural rearrangement or energy production. Locusts rid themselves of the toxic tryptophan by converting it into ommochromes, which cause the faecal pellets to turn red (due

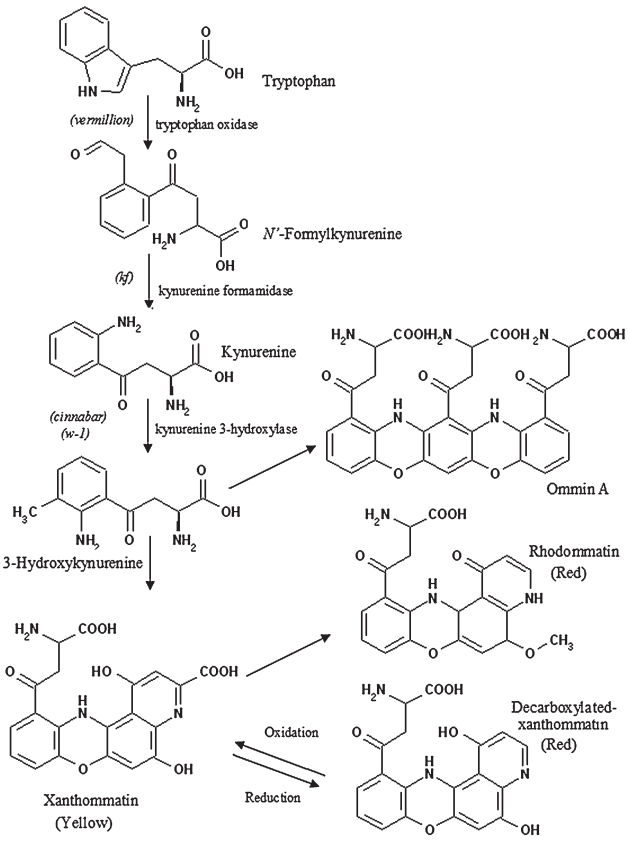

Fig. 4. Representation of the formation of ommochromes and ommins using amino acid tryptophan as the precursor molecule (Ferguson & Jiggins, 2009; Hines et al., 2012; Osanai-Futahashi et al., 2012).

to the presence of ommochromes in faeces) (Chapman, 2013).

Tetrapyrroles

Tetrapyrroles consist of four pyrrole rings, connected to each other by one-carbon (methine or methylene) bridges, in either a linear or cyclic manner. Bilirubin and phycobilin are linear tetrapyrroles (bilanes) with three one-carbon bridges and porphyrins and chlorins are cyclic tetrapyrroles with four one-carbon bridges. Because of their ability to form metal complexes, these compounds are particularly important in biological systems. The tetrapyrroles, the key to the nature of green pigments, were discovered by Przibram & Lederer in 1933. They have shown for several locusts that the green colour was due to a mixture of a yellow and a blue chromoprotein; the prosthetic group of the blue one was a bile pigment, which they isolated in a crystalline state from *Carausius morosus* (Phasmatodea: Diapheromeridae) (Cromartie, 1959). The name "insectoverdins" was proposed for such mixtures in 1941 by Junge. The tetrapyrroles have been identified in major groups of animals. In mammals, they are known as bile pigments. In insects, they are found in Phasmida, Mantodea, Orthoptera and Lepidoptera (Morgan, 2010). Biliverdin is responsible for the green colour of many grasshoppers and lepidopteran larvae.

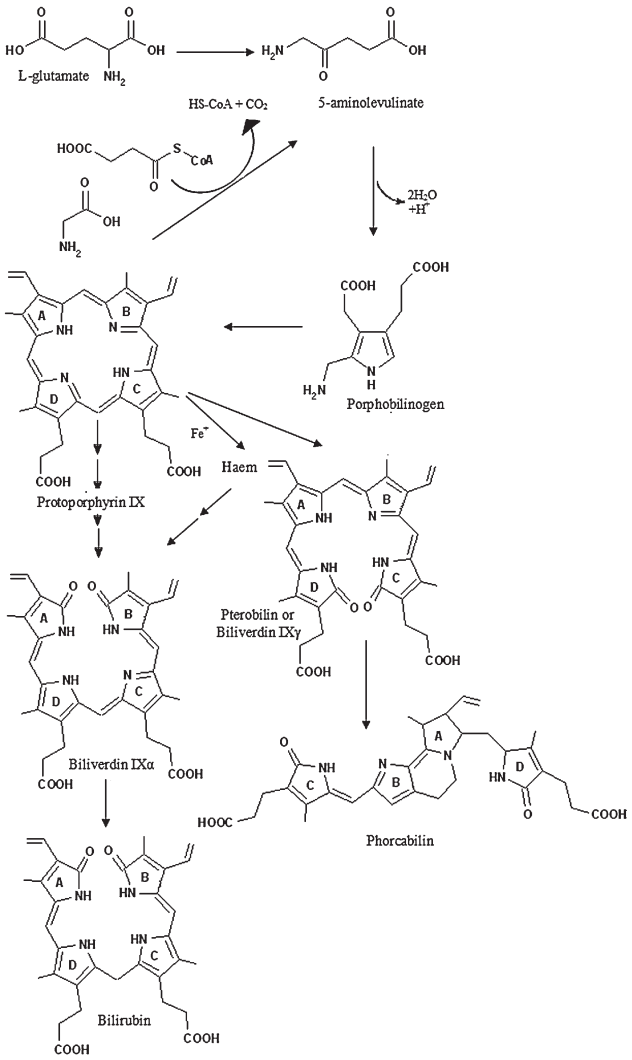

Fig. 5. Biosynthetic pathway showing formation of insect bilins, using 5-aminolaevulinic acid as the starting unit (Morgan, 2010). Reproduced by permission of The Royal Society of Chemistry.

The biosynthesis begins with a Claisen condensation between glycine attached to pyridoxal phosphate and succinyl CoA to yield 5-aminolaevulinic acid. Two molecules of 5-aminolaevulinic acid condense to give porphobilinogen. These molecules couple up to give dimer, trimer and tetramer, which cyclize to yield a variety of tetrapyrroles, e.g., protoporphyrin IX (Fig. 5). Porphyrin biosynthesis occurs in the wings of adult *P. brassicae* (Rilk-van Gessel & Keysera, 2007). Cytochromes containing a haem group are ubiquitous but present in small quantities in all the insects and therefore do not contribute to insect colouration. Only a few insects living at low oxygen pressures produce haemoglobin, such as *Chironomus* larvae (Diptera: Chironomidae), the botfly *Gasterophilus* (Diptera: Oestridae), and *Anisops* and *Buenoa* (Hemiptera: Notonectidae), which live in stagnant water. The blue-green pigment biliverdin is produced by the oxidative ring opening at the α-meso position of protoporphyrin IX (between rings A and D) and reduction of the central –CH= gives orange coloured bilirubin (Fig. 5). Both of these pigments are found in the fat bodies of *Chironomus* larvae. Pterobilin, found in the majority of Lepidoptera, is formed by the cleavage of protoporphyrin IX between rings C and D (Fig. 5).

Melanin

Melanins in insect cuticle are discussed by Wigglesworth (1952) and Dennell (1957). These nitrogen containing tyrosine derivatives occur in the cuticles of Blattodea, Diptera, Coleoptera and adults and some larval forms of Lepidoptera (Chapman, 2013). Melanins are of two types, black eumelanin, a polymer of dihydroxyindole carboxylic acid and its reduced forms; and a cysteine containing red-brown phaeomelanin, a polymer of benzothiazine units. Insect melanin can be either a polymer of dopamine or DOPA, depending upon its purpose, for example, the polymers of DOPA are used in wound-healing and for encapsulating invading micro-organisms. Moreover, the reactive quinone intermediates in the melanin biosynthetic pathway exhibit antibiotic properties. Cuticular melanin can be present either in the form of dense granules (formed from DOPA) or diffusely spread (polymer of dopamine). Melanin granules help to strengthen the cuticle and also protect against UV damage.

Remarkably little is known about the structure of melanins, despite their abundance in a wide range of taxa. Since, melanins are amorphous, insoluble and not amenable to either solution or crystallographic structural studies, it is not possible to derive a definitive chemical structure for them using the current biochemical and biophysical techniques (Nosanchuk & Casadevall, 2006). Therefore, the understanding of the structure of melanin is based on spectroscopic analyses of their structure and analyses of their degradation products (Wakamatsu & Ito, 2002).

The negatively charged, high molecular weight, hydrophobic melanin pigments (White, 1958; Nosanchuk & Casadevall, 1997; Nosanchuk et al., 1999; Jacobson, 2000) are formed from phenols and indoles (Wakamatsu & Ito, 2002). Biosynthesis of melanin begins with the conversion of tyrosine to DOPA and then to dopamine under the action of tyrosine hydroxylase (encoded by gene *TH*) and dopa decarboxylase (*DDC*), respectively. Dopamine is the primary precursor of insect melanin (Hiruma & Riddiford, 1984, 2009; Hiruma et al., 1985; Futahashi & Fujiwara, 2005; Gibert et al., 2007; Arakane et al., 2009). The dopamine is incorporated into cuticular premelanin granules that contain dopachrome conversion enzyme (DCE) and diphenoloxidase (*yellow*, *laccase2*), where, finally, the pigment precursor molecule is converted into melanin. It is believed that DOPA or dopamine is further oxidized to dopaquinone or dopaminequinone and then to dopachrome or dopaminechrome, which is re-aromatized, and then oxidized to indole-5,6-quinone. The indole-5,6-quinone upon oxydative dehydrogenation, causes the aromatic units to link to form a polymer as shown in Fig. 6 (Arakane et al., 2009; Futahashi et al., 2010; Morgan, 2010). The extended conjugated system of double bonds absorbs the entire range of wavelengths of the visible spectrum giving the polymer its black appearance (Morgan, 2010).

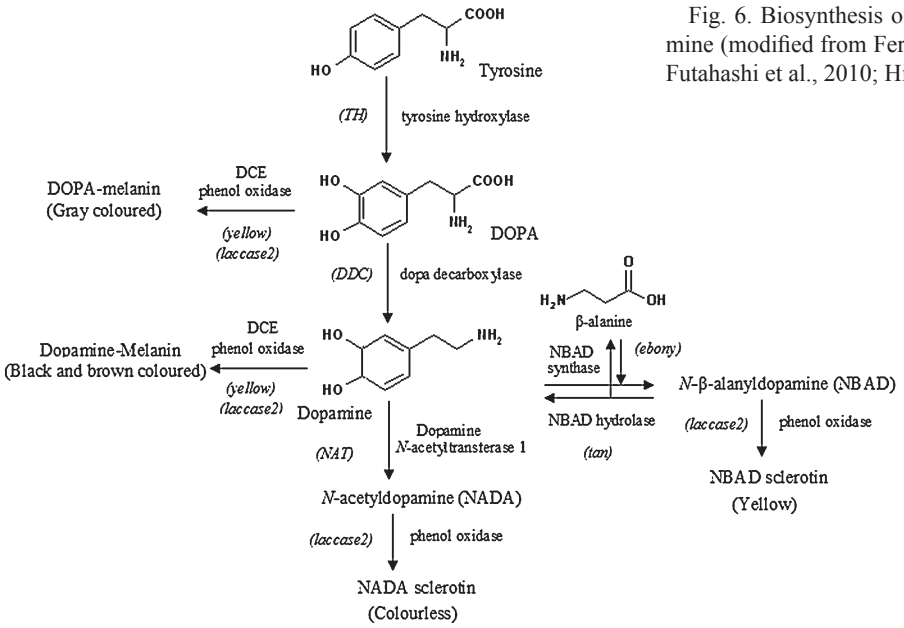

Fig. 6. Biosynthesis of insect melanin from DOPA and dopamine (modified from Ferguson et al., 2011; Arakane et al., 2009; Futahashi et al., 2010; Hines et al., 2012).

Following a different route, dopamine in conjugation with α-alanine gives rise to N-beta-alanyldopamine (NBAD) (a product of the gene *ebony*), which is a precursor of yellowish pigments (Wright, 1987; Koch et al., 2000); phenol oxidase then catalyzes the synthesis of the NBAD pigment (Arakane et al., 2009). Dopamine-*N*-acetyl transferases (NATs) converts dopamine to N-acetyl dopamine, a precursor for colourless sclerotin (Wright, 1987; Wittkopp et al., 2003; Futahashi et al., 2010; Hines et al., 2012). A recent study on *Drosophila melanogaster* has shown that the silver nanoparticles (AgNPs) present in insect food, affects cuticular melanization (flies have a paler body colour), reduces fertility and the ability to move vertically (Armstrong et al., 2013).

Papiliochromes

The study of wing pigments of butterflies by Nijhout (1991) has shown that the different butterfly families specialize in different classes of pigment: papiliochromes in Papilionidae, pterins in Pieridae and ommochromes in Nymphalidae. Papiliochromes are slightly analogous to the ommochromes in providing white, yellow and red colouring to the wings of some butterflies (Umebachi, 1975). These pigments are derived from tyrosine as well as from tryptophan, through well known pathways of melanin and ommochrome biosynthesis and occur only in swallowtail butterflies, Papilionidae. Papiliochrome II, a wing pigment of the swallowtail butterfly *Papilio xuthus* (Lepidoptera: Papilionidae), is formed from one molecule of L-kynurenine, derived from tryptophan, and one molecule of β-alanyl-dopamine.

Papiliochrome II, a white pigment, is a peptide in which two aromatic rings are linked by a bridge between the aromatic amino group of kynurenine and the catecholamine side chain of norepinephrine derived from quinone (Thompson & Suarez, 2003). Its biosynthesis involves the non-enzymatic condensation of N-β-alanyl-dopamine quinone methide with L-kynurenine to produce a mixture of two diastereoisomers of Papiliochrome II.

Carotenoids

Carotenoids are an integral component of animal biochemistry. C_{40} isoprenoids are classified as tertraterpenes and characterized by long hydrocarbon chains and loops, and are used in photoreception, antioxidation and ornamental colouration. Carotenes and xanthophylls (oxidized derivatives of carotenes) together constitute carotenoids. In nature there are over 800 carotenoid compounds. Carotenoids are a major group of pigments that are lipid soluble and contain no nitrogen. They absorb a variable range of wavelengths of blue and green light (essential for vision and colouration in animals). In insect integument and haemolymph, carotenes are coupled with proteins to give green, blue-green, blue and red colours. Carotenes are highly unsaturated and very unstable in air; nevertheless they are found in many taxa. Vitamin A aldehyde or retinal, an important part of the visual pigment of insects, is a cleavage product of β-carotene. Some of the carotene derivatives found in insects are, lycopene, β-carotene, zeaxanthin, violaxanthin, astaxanthin, xanthophylls and β-carotene monoepoxide. Xanthophylls or leutin occurs in almost all Lepidoptera examined.

Almost all insect carotenoids contain 22 carbon atoms in a central chain with nine double bonds and nine carbon atoms in the end groups. The colour produced by carotenoids depends mainly on the linear or cyclic form of the terminal groups and their degree of unsaturation. Insects sequester carotenoids from their diet and their post-ingestive modification is not uncommon. Orthopteroids are known to prefer carotenes, while Lepidopterans selectively absorb xanthophylls. The carotenes in the aphid, *Macrosiphum liriodendra* (Hemiptera: Aphididae), exists in two colour variants, green and pink. The green variant contains only cyclized carotenes whereas the pink form contains two partly cyclized and two uncyclized carotenes (Morgan, 2010).

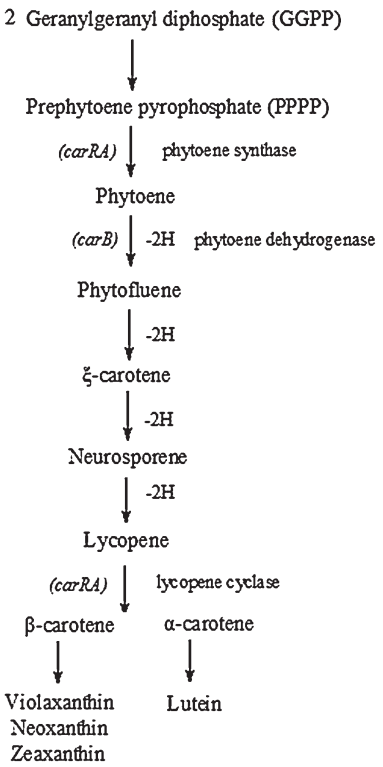

Fig. 7. Formation of carotenoids via phytoene by the condensation of two molecules of GGPP (Rodríguez-Sáiz et al., 2004).

Many other insects, particularly aphids, contain carotenes along with other pigments. Another aphid that takes advantage of carotenoid coloration is the two colour morphs of pea aphid, *Acyrthosiphon pisum* (Hemiptera: Aphididae). The green aphid morph has large quantities of the greenish-yellow carotenoids: *alpha*-, *beta*- and *gamma*-carotene, whereas the red morph has decreased amounts of these carotenoids and large amounts of the red carotenoid, torulene (Moran & Jarvik, 2010). More recently, *Acyrthosiphon pisum* has been reported harbouring genes required for carotenoid synthesis as in plants, algae and fungi, possibly by lateral transfer during the course of evolution (Moran & Jarvik, 2010). The presence of carotenoid biosynthetic machinery in aphids is suggestive of them being involved in some major physiological role other than their antioxidative property. It is reported that the capture of light energy results in the photo induced electron transfer from excited chromophores to acceptor molecules in aphids and appears to be an archaic photosynthetic system consisting of photo-emitted electrons that end in the synthesis of ATP molecules (Valmalette et al., 2012).

The biosynthesis of carotenoids that occurs in lower organisms (Rodríguez-Sáiz et al., 2004), starts with a condensation reaction in which two geranylgeranyl diphosphate molecules are joined end-to-end to form an intermediate cyclopropane ring, prephytoene pyrophosphate (PPPP). With the formation of a 15-15' double bond and loss of a proton, prephyteone is converted into phyteone. Formation of phyteone is catalyzed by phytoene synthase. Reduction of phyteone by phytoene dehydrogenase (*carB* gene) leads to phytofluene, which is further reduced to ξ-carotene and still further to neurosporene. Neurosporene, upon reduction, gives rise to lycopene, which is an acyclic carotene that does not have a ring at the end of the chain. Cyclization of lycopene by lycopene cyclase (*carRA*) (Rodríguez-Sáiz et al., 2004) may occur by adding five or six carbon rings to one or both ends; an example is β-carotene, in which there are 6-carbon ring at both ends (Fig. 7).

Carotenoids provide protection to cells from damage due to photo-oxidation, but this property of carotenoids is still unknown in insects. Apart from imparting red and yellow colouration to many insects, carotenoids in combination with a blue pigment (often a bilin) produce a green pigment known as insectoverdin.

Anthocyanins and flavones

Anthocyanins and flavones are odourless and nearly flavourless water-soluble flower pigments producing pH dependent red, purple or blue colours. Belonging to a parent class of molecules called flavonoids their biosynthesis follows a phenyl-propanoid pathway. Insects are probably the only animals that sequester flavonoids from plants. These flavonoids mainly occur in butterflies and are common in Papilionidae, Satyride and Lycaenidae as cream or yellow pigments. Post-ingestive modifications of flavonoids may occur in insects either due to the action of insects or of their gut flora.

The sequestration and metabolism of anthocyanins do not follow a simple pattern. For example, the leaves of *Oxalis corniculata* contain three closely related C-glycosylflavones (iso-orientin, isovitexin and swertisin). The pale grass blue butterfly, *Pseudozizeeria maha* (Lepidoptera: Lycaenidae), sequesters only isovitexin in its wings, but its larvae convert it to saponarin, which is converted back to isovitexin at the pupal stage. The sequestration of these compounds is reported to occur more frequently in female butterflies (Mizokami et al., 2008; Mizokami & Yoshitama, 2009). The larvae of *P. brassicae* can selectively metabolize flavonoids. When reared on a particular variety of cabbage (*Brassica oleracea* var. *costata*), which contains a range of 20 flavonoids, it was shown that the main component in the butterfly larvae was a minor component in the plant and only two other significant flavonoids in the plants were present in the larvae (Ferreres et al., 2007). The females of the common blue butterfly, *Polyommatus icarus* (Lepidoptera: Lycaenidae), sequester more flavonoids and males appear to prefer females with more pigment (Burghardt et al., 2001). They accumulate flavonoids from their larval food and store the pigments in their wings as part of their colour.

Biosynthesis of anthocyanin pigments may start with the shikimate pathway to produce the amino acid phenylalanine or with the production of 3 molecules of malonyl-CoA, a C3 unit from a C2 unit (acetyl-CoA). Due to the activity of chalcone synthase (*CHS*), an intermediate chalcone-like compound is formed via a polyketide folding mechanism. The chalcone is isomerized by chalcone isomerase (CHI) to the prototype pigment naringenin, which is subsequently oxidized by enzymes such as flavanone hydroxylase (*FHT* or *F3H*), flavonoid 3' hydroxylase and flavonoid 3' 5'-hy-

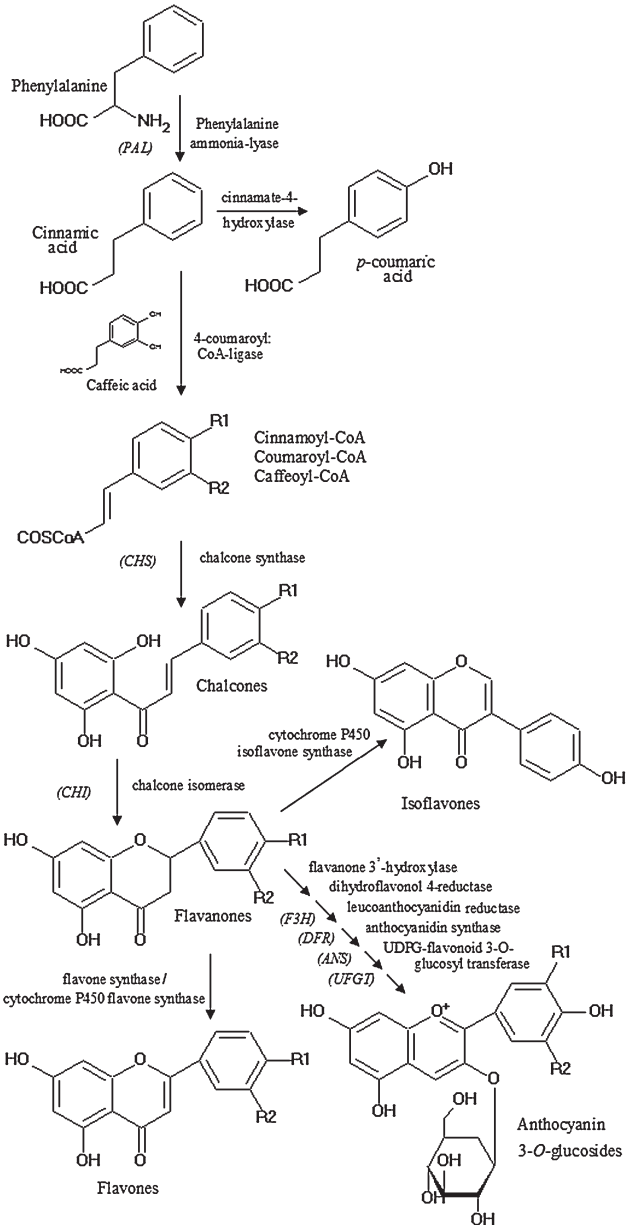

Fig. 8. A summary of flavonoid biosynthesis, starting with phenylalanine and resulting in the production of chalcones, isoflavones, flavones and anthocyanins (Boss et al., 1996; Ramazzotti et al., 2008; Kovinich et al., 2010).

droxylase. The oxidation products of these enzymatic reactions are further reduced by dihydroflavonol 4-reductase (*DFR*) to the colourless compounds leucoanthocyanidins (Nakajima et al., 2001). The products of leucoanthocyanidin reductase (*LAR*), flavan-3-ols, are the true substrates of anthocyanidin synthase (*ANS*) or leucoanthocyanidin dioxygenase (*LDOX*). The resulting unstable anthocyanidins upon glycosylation by UDP-3-O-glucosyltransferase finally yield relatively stable anthocyanins (Boss et al., 1996; Ramazzotti et al., 2008; Kovinich et al., 2010) (Fig. 8).

MOLECULAR BIOLOGY OF INSECT PIGMENTATION

Insect body colour pigments are generally synthesized in the epidermal cells or are found in modified epidermal cells (Nijhout, 1997), but in most of the cases the pigments are incorporated into the exoskeleton by means of a sclerotization process (Hopkins & Kramer, 1992). Exceptions are the eye pigments, ommochromes, which are localized in a specific type of cell.

Development of pigmentation

As indicated by Wittkopp & Beldade (2009) the development of insect pigmentation is most often studied in *D. melanogaster*. Based on this understanding the pigmentation process is divided into two stages, first the spatiotemporal positioning of pigments determined by "patterning" genes, and second the biosynthesis of pigments determined by "effector" genes. By directly or indirectly activating the expression of effector genes that encode the enzymes and co-factors required for pigment biosynthesis, patterning genes help in the regulation of the distribution of this pigment.

The biochemical pathway leading to the production of pteridines includes the *GTP-CH I*, *purple* and *SPR* genes (Morehouse et al., 2007); another enzyme, Xanthine dehydrogenase (XDH) coded by *rosy* locus is required for the production of drosopterin, a *Drosophila* eye pigment (Chovnick et al., 1990; Wootton et al., 1991; Hille & Nishino, 1995; Pitts & Zwiebel, 2001).

Similarly, biosynthesis of ommochromes (red, brown and yellow pigments) via tryptophan and controlled by *cinnabar*, *vermilion* and *white* genes, occurs widely in insects. A number of other genes involved in this process have also been identified; these include *scarlet*, *deep orange*, *garnet*, *light*, *carmine*, *carnation*, *lightoid*, *claret* and *pink*. Recently, *Red Egg* (*re*) gene, a novel transporter family gene was identified in *Bombyx* and *Tribolium* (Coleoptera: Tenebrionidae), which is responsible for egg/eye pigmentation involving ommochromes (Osanai-Futahashi et al., 2012). Ommochromes are not only responsible for eye colour in *Drosophila* but also play an important role in wing and body pigmentation in other species (Nijhout, 1997; Reed & Nagy, 2005). The wing patterns of *Heliconius* (Lepidoptera: Nymphalidae) butterflies are well studied and a number of regulatory genes have been identified, which provides a glimpse of how the development of wing patterns in butterflies are controlled and have evolved (Reed et al., 2008; Ferguson & Jiggins, 2009; Ferguson et al., 2011; Hines et al., 2012). Reed et al. (2008) report the presence of the genes *cinnabar* and *vermilion* in *Heliconius erato* and two new ommochrome pathway genes, *karmoisin* and *kynurenine formamidase* (*kf*) responsible for the wing patterns of *Heliconius* (Ferguson & Jiggins, 2009). *Karmoisin* (*kar*) is responsible for incorporating tryptophan from haemolymph into developing wing scale cells (Dow, 2001) and *vermillion*, *kf*, and *cinnabar* convert it into the yellow pigment 3-hydroxykynurenine (3-OHK). *Scarlet* (*st*)/ *white* (*w*) heterodimer facilitates the transport of 3-OHK into pigment granules, where it is converted into xanthommatin or dihydroxanthommatin by further spontaneous redox reactions. Eye colour due to xanthommatin in *Triatoma infestans* (Hemiptera: Reduviidae) is genetically controlled by a single autosomal locus in such a way that

black eyes are produced by the dominant gene (wild) and red eyes by the homozygous recessive gene (mutant) (Wygodzinsky & Briones,1954; Dujardin & Bermúdez, 1986).

Two genes are associated with the green colouration of *Papilio xuthus* larvae, *bilin-binding protein* (*BBP*) and *yellow-related gene* (*YRG*) (Futahashi & Fujiwara 2008a, b) and a recent investigation by Shirataki et al. (2010) indicate that *BBP* and *YRG* control the blue pigmentation (either green or blue) and yellow pigmentation of larvae (either green or yellow), respectively. Melanins, which are synthesized by a branched biochemical pathway, involve the polymerization of modified molecules, such as dopa, dopamine, *N*-β-alanyl-dopamine and N-acetyl-dopamine, into grey, black-brown and yellow pigments. NADA-sclerotin is often colourless or a very pale straw colour (Andersen, 2012). Spatiotemporally regulated expression of the *TH*, *DDC*, *tan*, *ebony*, *yellow* and *laccase2* genes determines the location and relative abundance of these pigments (Prud'homme et al., 2006; Jeong et al., 2008).

In lower organisms, *carB* and *carRA* genes are reported to be involved in carotene biosynthesis, in which *carB* codes for phytoene dehydrogenase and the *carRA* gene has two domains, of which R is responsible for lycopene cyclase activity and A encodes phytoene synthase (Rodríguez-Sáiz et al., 2004). The genes *PAL, CHS, CHI, F3H, DFR, ANS, LDOX* and *UFGT* are reported to be involved in the biosynthesis of flavonoids in plants (Boss et al., 1996; Ramazzotti et al., 2008).

Pigmentation regulation and hormones

The hormones responsible for the development of discrete alternatively pigmented phenotypes are ecdysone, JH and the neurosecretory hormone corazonin. Particular patterns of gene expression are determined by developmental hormones. If a specific relevant hormone is absent or below a threshold concentration, it results into one pattern of gene expression, whereas if it is above threshold a different pattern of gene expression can be stimulated. The developmental pathways and resultant phenotypes diverge due to the alternative patterns of gene expression. Several different mechanisms are known to control pigment synthesis. Possibly, there could be a de novo expression of enzymes at the beginning of pigment synthesis. An alternative to this is the presence of inactive pro-enzymes throughout the integument, which are activated at the start of pigment synthesis. Alternatively, active enzymes can be present in the integument but the timing of pigment synthesis is controlled by transporters, which import the necessary precursors.

In insects, change in colour or pattern can occur only at a moult, which is therefore generally fixed for the duration of the instar. The overall epidermal colour of the stick insect, *Carausius morosus*, is darker at night regardless of its cuticle being completely transparent and colourless (Bückmann & Dustmann, 1962; Bückmann, 1977). This colour change is controlled by a circardian clock involving a neurosecretory hormone (Raabe, 1966), which continues to cycle even if the animals are kept in constant darkness (Bückmann, 1977). In caterpillars of *Cerura vinula* and *M. sexta*, the synthesis of epidermal ommochromes is stimulated by ecdysone produced at the end of larval life. The transformation in colour and pattern of many hemipteran larvae and swallowtail butterfly larvae that occurs when they metamorphose into adults is controlled by juvenile hormone (JH) (Wigglesworth, 1959; Willis et al., 1982; Futahashi & Fujiwara, 2008 a) and the expression of cuticular pigmentation genes in *Papilio xuthus* larvae is regulated by 20-hydroxyecdysone (Futahashi & Fujiwara, 2007). Phenotypic plasticity of cuticular pigmentation is more widespread than that in epidermal pigmentation (Nijhout, 2010).

CONCLUSION

Insects can be amazingly colourful, displaying exquisitely fine colour patterns and this has led to many interesting studies including the examination of few fossil insects, which have retained their culticular metallic colour, to determine whether the original colour was preserved (Stankiewicz et al., 1997; Parker & McKenzie, 2003; McNamara et al., 2011a, b). The pigment molecules may benefit insects by providing them with a colour signature, for example, extremely diverse butterfly wing patterns can be used to distinguish most of the approximately 18,000 species of butterflies (Kronforst et al., 2012). The physiological and ecological roles of the many varieties of pigments are well studied. The development of colour patterns, role of developmental hormones as mediators of environmentally induced pigment synthesis and regulation of pigment biosynthesis are now well understood. Colours may be involved in species recognition, mating or camouflage, or as warning colours in aposematic species or play an important role in an insect's physiology. Examples are the wing patterns of butterflies, which are important in thermoregulation, crypsis, warning, mimicry and mate choice (Nijhout, 1991). Field observations and laboratory studies indicate that pigmentation has important roles in thermoregulation (Gilbert et al., 1996, 1998; Munjal et al., 1997), camouflage (Spieth, 1974; Bock, 1980), resistance to desiccation, ultraviolet radiation (Hollocher et al., 2000) and (indirectly) parasitic infection. Papiliochrome II pigment reduces the wing iridescence in four papilionid butterflies of the *nireus* group (Wilts et al., 2011). Pigments are also known to be useful for determining the age of insects, for example, the pterins deposited in the eyes of higher Diptera and melanins in the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae); a red pigment in the veins of wings, darkens with age and finally becomes brown/ black. Timmons et al. (2010) consider these pigments to be phaeomelanin and eumelanin, respectively. Phaeomelanin, however, is not reported in any other insect. Another functional benefit of pigment is that of the sex-specific retinal pigments in the eastern pale clouded yellow butterfly, *Colias erate* (Lepidoptera: Pieridae), which are assumed to be associated with sexual behaviour such as courtship and oviposition (Ogawa et al., 2013). A recent study on the aphid *Acyrthosiphon pisum* revealed that the cartenoids are not only anti-oxidants but also involved in

light-induced electron transfer and ATP synthesis. Insects with genes controlling carotenoid biosynthetsis in their genome are likely to have acquired these genes by lateral transfer during evolution, which provides a new dimension to the study of sequestered insect pigments (Valmalette et al., 2012). The butterfly *Heliconius* expresses recently duplicated ultraviolet (UV) opsins and its yellow wing colours exhibit higher UV reflectance (Briscoe et al., 2010). Bybee et al. (2012) show that the colour that reflects UV is the pigment 3-hydroxy-L-kynurenine (3-OHK). This co-occurrence of enhanced UV vision and UV-reflecting wing pigment allows *Heliconius* to discriminate con-specific individuals in the presence of mimics (Bybee et al., 2012).

Understanding phenotypic evolution and developmental biology has been greatly helped by studies on insect pigmentation, for example, *Drosophila* wing patterns and butterfly eyespots. In depth investigations into the evolutionary genetics of the diversity of pigmentation in *Drosophila* indicates that mechanisms of pigmentation development vary among and within species (Wittkopp & Beldade, 2009). During the course of evolution, pigment patterns of insects and their different aspects have evolved independently of each other. The biosynthetic pathways for producing pigments and the enzymes involved are highly conserved (Kayser, 1985). The differences in the deployment of similar sets of regulatory and structural genes might account for the great diversity of pigment patterns. Different regulatory genes might control the expression of the same set of pigments and structural genes in different regions of the body. The identification of genes involved in pigment biosynthesis may help to resolve the genetic changes in development that occur in populations because pigmentation varies within species and can respond rapidly to selection. The genes that are involved in the development and evolution of pigmentation may also help resolve classic evolutionary problems such as industrial melanism, Batesian and Mullerian mimicry and phenotypic convergence. There are frequent divergent and convergent changes involved in the evolution of pigment patterns. The convergent pigment patterns recorded in species of *Drosophila* provide an excellent opportunity to address whether the same genes are responsible for similar phenotypes in different evolutionary lineages (Wittkoop et al., 2003).

Despite the great progress in our understanding of the genetic, development and physiological mechanisms underlying colour pigment biosynthesis in insects, there are, however, still many exciting opportunities for original research. Insect pigmentation helps link variation in genes to variation in development and phenotypes, which is necessary for a complete understanding of evolutionary diversification. Detailed genetic analysis of pigment pathways will help us understand how epidermal cells select a specific pigment biosynthesis pathway, or how they switch between alternative pathways, which may lead to a comprehensive picture of the evolution of pigmentation.

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