VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 179-199 (2024)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Butterfly Linguistics: Decoding the Biology of Visual and Olfactory Communication

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Received: 3rd June, 2024; **Accepted:** 18th July, 2024; **Published online:** 21st July, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.019

Abstract: The order Lepidoptera, comprising butterflies and moths, is a significant group within class Insecta by the virtue of its ecological and economical role. As arthropods, butterflies possess an advanced neural and sensory system among invertebrates. This review delves into the variety of sophisticated visual and olfactory communication strategies that have been evolved by butterflies.

Visual communication in butterflies is mediated by their compound eyes, which are highly complex and sensitive to a wide range of colours. Due to diversity in photoreceptor classes and variations in spectral sensitivities within a single eye, butterflies possess colour vision and can even differentiate between ultraviolet and visible light spectrum. They utilise rhodopsins as their visual pigments and undergo a phototransduction process comparable to that of humans.

Butterflies also make use of scent glands and recipient organs on antennae for olfactory communication. Olfaction is facilitated by releasing and receiving pheromones. These sex pheromones offer invaluable insights about mate selection and suitability in many butterfly families. While butterflies may rely more on visual cues for flower selection and oviposition, they equally utilise visual and olfactory signals for mate selection.

Keywords: Lepidoptera, Butterflies, Ommatidia, Photoreceptor, Rhodopsin, Sex-Pheromones, Androconia

Citation: Bhatt Divyanshu, Borah Ankit, Jahan Farhat and Singh Vandana Kumari: Butterfly linguistics: Decoding the biology of visual and olfactory communication. Intern. J. Zool. Invest. 10(2): 179-199, 2024.

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Introduction

Arthropods constitute the largest taxa among terrestrial animals and a significant proportion of them inhabit tropical rainforests (Basset *et al*., 2012). The largest group under phylum Arthropoda, class Insecta accounts for more than half of all species discovered and are found in diverse ecosystems (New *et al*., 1995; Ruppert *et al*., 2003). Within class Insecta lies an important taxon, order Lepidoptera which houses butterflies and moths. Butterflies in particular, are essential indicators of a healthy ecosystem. They account for a multitude of ecological processes such as

pollination, inducing genetic variation in flowering plants via their pollination and migration, and maintenance of the food web (acting as a food source). Butterflies are also highly responsive to habitat loss and climate change, their phenology varies with shifts in temperature, thus acting as indicators of environmental quality (Settele, *et al*., 2010; Kharouba *et al*., 2013; Ghazanfar, 2016).

Their habitat patterns, vegetation requirements, ecological interactions with other species give valuable insights into the functioning and conservation status of the entire ecosystem. Butterflies are well known with regards to their ecology, biology and taxonomy, given the fact that they have been at the centre of extensive studies (Bonebrake *et al*., 2010).

The taxonomic classification of Lepidoptera (butterflies and moths) as per Ruppert *et al.* (2003) is given below:

Kingdom	Animalia
Phylum	Arthropoda
Infraphylum	Tracheata
Superclass	Hexapoda
Class	Insecta
Subclass	Pterygota
Superorder	Holometabola
Order	Lepidoptera

Despite their brilliance and ecological role, butterfly populations have experienced a sharp decline over the past four decades (New *et al*., 1995; Forister *et al*., 2021). As a matter of fact, despite conservation efforts, insect population has been on a significant decline, in part owing to the fact that most large-scale conservation projects focus on restoration of plant and vertebrate species (New *et al*., 1995)

Diversity, Distribution and Classification:

The order Lepidoptera is estimated to encompass more than 157,338 extant species (Stork, 2018). while some accounts even claim around 500,000 extant species of Lepidoptera (Kristensen *et al*., 2007). Among these, approximately 20,000 species of butterflies have been identified worldwide (Kristensen *et al*., 2007). The earliest documented fossil evidence of Lepidoptera, the insect order that comprises butterflies and moths, can be traced back to a minimum of 190 million years ago (Grimaldi and Engel, 2005) and 90 million years later, butterflies and moths are said to have diverged from the ancestral lepidopteran and became further evolved for diurnal and nocturnal rhythms, respectively (Braby *et al*., 2006).

In the order Lepidoptera, there are 42 superfamilies and 131 families. Out of these, superfamily Calliduloidea has a single family Callidulidae (49 species) which includes Old World butterfly-moths, abundant in the Oriental region (south and south-east Asia). Superfamily Papilionoidea has 7 families, five of which, consist of true butterflies: Papilionidae, Pieridae, Riodinidae, Lycaenidae, Nymphalidae while family Hesperiidae has skippers and family Hedylidae has moth-butterflies (Van Nieukerken *et al*., 2011; Heikkilä *et al*., 2011; Goldstein, 2017). Figure 1 depicts a graphical illustration on the species diversity of the seven butterfly families within superfamily Papilionoidea.

The smallest family Hedylidae, having around 36 species, is found abundantly in the South American, Neotropical realm. On the contrary, Hesperiidae is a large family with about 4113 discovered species and is almost cosmopolitan in distribution (Goldstein, 2017). Among the true butterfly families, Papilionidae houses around 570 species of swallowtail butterflies whose characteristic feature is modified hind wings and are especially abundant in the North temperate mountains and the tropical regions. Under this family, comes the tribe Troidini which includes the largest butterflies and inhabit South East Asia and the Indian subcontinent (Fig. 2). Family Pieridae includes 1,164 species of white or yellow- or orange-coloured butterflies with black spots and found in northern areas of North America and

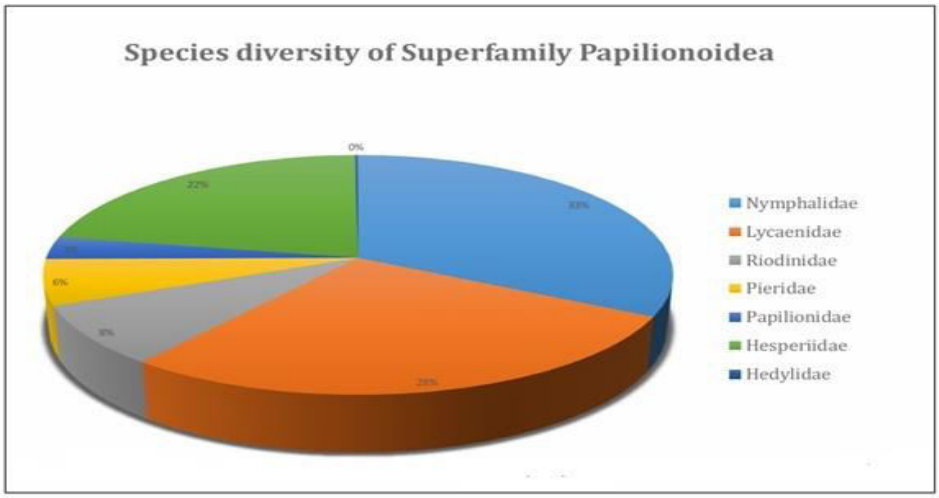

Fig. 1: Graphical representation of the relative number of species (in %) across the seven butterfly families. Data sourced from Goldstein (2017).

Europe, tropical regions of Asia and Africa as well as subtropical and temperate regions (except New Zealand). On the other hand, families Lycaenidae (coppers, blues, hairstreaks) with 5,201 species and Riodinidae (metalmarks) with 1,532 species are closely related and constitute 50% of the total butterfly population. Riodinidae is exclusively found in the Neotropical region, whereas Lycaenidae has a nearly worldwide distribution, spanning across major continents such as Africa, Australia, Asia, and North America as well as tropical, temperate and Oriental regions. Family Nymphalidae is the largest family with 6,152 species and at least 12 subfamilies, occupying various habitats around the globe. These subfamilies are found in diverse regions such as tropical regions, temperate regions, the Neotropics, the Indo-Australian region, the Himalayas, Asia, and Africa. Additionally, some subfamilies have a cosmopolitan distribution. This diverse family includes migrating butterflies such as the red admiral *Vanessa atalanta* and monarch butterfly *Danaus plexippus* (Levin *et al.*, 2000; Goldstein, 2017). Figure 2 depicts a biogeographic map showcasing the global distribution of the seven butterfly families.

Table 1 gives an overview of the classification of butterflies along with their species diversity and characteristic features.

Sensory Reception in Arthropods:

A sense organ, also called a sensory organ, is a specialised structure in living organisms that is responsible for receiving and detecting specific types of sensory inputs from the environment. Animals are known for their variety of sensory organs and responses to different environmental stimuli.

Arthropods show a progressively advanced neural and sensory system among invertebrates, for instance butterfly eyes can even perceive ultraviolet light due to their increased number of photoreceptor classes. Sensory mechanisms in invertebrates, particularly arthropods, encompass five basic types, namely vision, chemoreception (including olfactory reception), tactile reception, mechanoreception and thermoreception.

Chemical communication plays a vital role in shaping diverse behaviours of insects, including mating, foraging, host selection, and oviposition. Insects utilise various chemosensory receptors to interact with their chemical environment. These receptors consist of olfactory receptors (ORs), gustatory receptors (GRs), ionotropic receptors (IRs), transient receptor potential (TRP) channels, and pickpocket (Ppk) channels. TRP channels, which are a type of ion channels, hold particular significance in transmitting signals across

Fig. 2: Biogeographic map depicting global richness and distribution of butterflies. The various butterflies included in the illustration (from the top left) are: Family Papilionidae (1. *Parnassius clodius*, 2. *Zerynthia polyxena*, 6. *Papilio thoas*, 9. *Baronia brevicornis*, 29. *Graphium angolanus*, 35. *Iphiclides feisthamelii*, 37. *Papilio machaon*, 44. *Papilio bianor*, 45. *Parnassius actius*, 52. *Bhutanitis lidderdalii*, 55. *Lamproptera meges*, 56. *Trogonoptera brookiana*, 57. *Graphium xenocles*, 58. *Cressida cressida*, 59. *Protographium leosthenes*, 60. *Pachliopta hector*); Family Nymphalidae (3. *Limenitis arthemis astyanax*, 8. *Anaea aidea*, 15. *Haetera piera*, 18. *Danaus erippus*, 19. *Argyrophorus argenteus*, 21a. *Ortilia ithra*, 22. *Boloria polaris*, 26. *Vanessa atalanta*, 31. *Precis ceryne*, 32. *Hypolimnas anthedon*, 33. *Stygionympha wichgrafi*, 34. *Junonia rhadama*, 40. *Boloria chariclea*, 41. *Aglais io*, 49. *Neope niphonica*, 53. *Athyma nefte*); Family Pieridae (4. *Anthocharis stella*, 7. *Pieris rapae*, 11. *Phoebis avellaneda*, 12. *Dismorphia altis*, 14. *Dismorphia theucharila*, 21b. *Colias hecla*, 24. *Anthocharis belia*, 25. *Euchloe charlonia*, 27. *Pseudopontia paradoxa*, 28. *Mylothris jacksoni*, 36. *Anthocharis damon*, 54. *Eurema brigitta*); Family Riodinidae (5. *Calephelis muticum*, 48. *Dodona ouida*, 51. *Stiboges nymphidia*); Family Lycaenidae (10. *Lycaena helloides*, 13. *Lamasina ganimedes*, 20. *Chlorostrymon simaethis*, 23. *Lycaena phlaeas*, 30. *Aslauga purpurascens*, 39. *Zizeeria knysna*, 42. *Agriades optilete*, 46. *Plebejus argus*, 47. *Lycaena helle*, 50. *Glaucopsyche lycormas*); Family Hesperiidae (16. *Hylephila phyleus*, 38. *Muschampia lavatherae*, 43. *Carterocephalus argyrostigma*); Family Hedylidae (17. *Macrosoma albida*). Data for butterfly distribution sourced from (Encyclopedia of Biodiversity, 2000), (Kullberg *et al.*, 2018), (SBBT, 2017), (Global Biodiversity Information Facility, GBIF, n.d.), (Contributors to Wikimedia projects, 2023).

different sensory systems. A TRP channel was recently discovered as a potential target for feeding inhibitors, indicating its potential application in insecticide development (Nesterov *et al*., 2015). Further, in the fruit fly *Drosophila*, PPK, belonging to the degenerin/epithelial Na^+ (DEG/ENaC) channel family, plays a vital role in governing pheromones-associated sexual behaviours (Joseph and Carlson, 2015).

Sensory communication in Lepidoptera:

The Lepidoptera order, which includes butterflies and moths, is known for its remarkable species diversity and extensive research on chemical communication. Among them, nocturnal moths have been extensively studied, particularly due to their significance as pest insects. Given their night-flying behaviour, chemical communication plays a vital role for these insects. As a result, the sex pheromones of numerous moth species have been identified. These pheromones typically attract males to females over significant distances, making them valuable tools for agricultural pest control. Moth sex pheromones predominantly belong to the Type I class, characterised by long-chain aliphatic compounds with 8 to 20 carbons, specific double bond positions and geometries, and terminal alcohol, aldehyde, or acetate groups (Ando and Yamamoto, 2020). The Type II class encompasses polyunsaturated hydrocarbons

Table 1: Diversity, distribution and description of the seven butterfly families in superfamily Papilionoidea

Family	Identifying characteristic	Number of genera	Number of species	References
Nymphalidae	Four walking legs (greatly reduced forelegs); Caterpillar may have spines on body	559	6000-6500	Encyclopedia of Biodiversity (2000); Powell (2009); Goldstein (2017)
Lycaenidae	Three pair of walking legs in both sexes; Short secondary setae on body of caterpillars	416	5000-6000	Encyclopedia of Biodiversity (2000); Powell (2009); Goldstein (2017)
Riodinidae	Males have atrophied forelegs, Longer secondary setae	146	1350-1550	Encyclopedia of Biodiversity (2000); Hall (2006); Powell (2009); Goldstein (2017); Cong *et al.* (2017)
Pieridae	Three pair of walking legs with distinctly bifid claws; Granulations in smooth caterpillar body	91	1000-1200	Encyclopedia of Biodiversity (2000); Powell (2009); Goldstein (2017)
Papilionidae	Three pair of walking legs with nonbifid tarsal claws; Caterpillar bears an osmeterium for defence	32	550-650	Encyclopedia of Biodiversity (2000); Goldstein (2017); Yu *et al.* (2023)
Hesperiidae	Antenna are hooked and far apart from each other	570	35-40	Powell (2009); Goldstein (2017)
Hedylidae	Resting posture with elevated mid-legs; Reduced forelegs in males	1	3500-4200	Powell (2009); Goldstein (2017)

derived from linolenic or related polyunsaturated fatty acids, as well as epoxide derivatives. The remaining compounds fall into the third group. The structures of moth pheromones have been extensively reviewed, and a freely accessible database called Pherobase exists, offering a resource to explore pheromone structures (Ando and Yamakawa, 2011, 2015).

On the other hand, butterflies, known for their visually striking signals, generally do not rely on pheromones. However, male butterflies have evolved various scent-dispersing structures across many families. These structures emit a diverse range of compounds, which are structurally more varied than those found in moths. The compounds used by butterflies can be produced through different biosynthetic pathways within their own bodies or acquired from plants and modified. The functions of these compounds can vary, serving purposes such as attraction, repellence, or defence. In the case of butterflies, pheromones are often utilised in short-range communication during courtship (Vane-Wright and Boppre, 1993).

Vision in Insects:

Vision is one of the most important sensory perceptions for animals and eyes are the highly adaptable organs for collecting visual information and passing it on to the neural circuit to ensure an

organism's survival and reproduction (Cronin *et al*., 2014).

Insects possess colour vision wherein they rely on spectral constituents of light (wavelengths) irrespective of light's brightness, for distinguishing visual stimuli. Colour vision requires retinal receptors to detect different wavelengths of light. Insects occupy diverse habitats and may have diurnal or nocturnal rhythms. Light's intensity and spectral composition vary in different habitats (Kinoshita *et al*., 1999; Briscoe and Chittka, 2001; De Kok *et al*., 2021).

There exists a vast diversity in colour receptors among the many species of insects; further, there are variations in the photosensitivity to wavelengths of different regions of eye in a single species of insects (Stavenga, 1992; Briscoe and Chittka, 2001).

The class Insecta demonstrates three kinds of eyes:

(i) *Stemmata*: Simple eyes with only a single lens and less receptors, found in holometabolous larvae only (Gilbert, 1994; Bitsch and Bitsch, 2005; Koenig and Gross, 2020).

(ii) *Ocelli*: Simple eyes that may have a less-developed retina but spatial resolution is low, found in hemimetabolous and holometabolous insects (Koenig and Gross, 2020; De Kok *et al*., 2021).

(iii) *Compound eyes*: Main organ of sight in adult insects consisting of numerous units called ommatidia (up to thousands in each eye) containing photoreceptors that form a mosaic image (De Kok *et al*., 2021).

Vision in Butterflies:

Butterflies also use colour vision during the day for foraging, distinguishing flowers, recognising potential mates, and locating sites suitable for laying eggs (Stavenga and Arikawa, 2006; Perr *et al*., 2016; Morell, 2016; Arikawa, 2017). They too have compound eyes. Ommatidia are the numerous units constituting each compound eye and each ommatidium consists of a cornea, a crystalline cone and many photoreceptor cells. At the central axis is a cylindrical rhabdom formed by the fusion of rhabdomeres which acts as an optical waveguide. Rhabdomeres are specialised organelles formed by folding of the membrane of photoreceptor cells into a great many tubular microvilli. Visual pigments of photoreceptor cells are present in their rhabdomeres (Zelhof *et al*., 2006; Matsushita *et al*., 2012; Arikawa, 2017; De Kok, 2021).

In butterflies and bees, the same visual axis is shared by all rhabdomeres within an ommatidium due to the fusion of rhabdomeres of all photoreceptors (Zelhof *et al*., 2006, Briscoe, 2008, Arikawa *et al*., 2009; Wernet *et al*., 2015).

The eyes of honey bees and butterflies are anatomically similar. Honey bees, like most insects, have a trichromatic colour vision due to the three types of spectral photoreceptors in their eyes, corresponding to sensitivities in UV, green and blue light wavelengths (De Ibarra *et al*., 2014; Arikawa, 2017).

Despite the similarities, butterfly retina is more complex, e.g. in the eyes of *Papilio xuthus* or the Japanese Yellow Swallowtail butterfly, at least six different classes of receptors have been identified with sensitivities peaking in the following spectral regions: UV, blue, violet, red, green, board-band (Koshitaka *et al*., 2008). Arrangement of these receptors within an ommatidia results in three types of ommatidia and these ommatidia are distributed randomly in its compound eyes (Qiu *et al*., 2002; Wakakuwa *et al*., 2007; Perr *et al*., 2016; Kelber, 2016).

There is also great diversity in the number of photoreceptor classes and their corresponding spectral sensitivities across various species of butterflies. We define spectral sensitivity as the part of incident light absorbed by the visual pigments in the butterfly ommatidia that eventually generate an electric signal (Akashi *et al*., 2018). In butterfly *Vanessa cardui*, there are only three classes of photoreceptors present

Table 2: Types of ommatidia in the butterfly *Papilio xuthus*

Type of ommatidium	Reflectance Spectrum Peak	Pigment clusters Arrangement, Position	Fluorescence under UV/Violet Illumination	Receptor classes present	Ratio	Reference
Type I ommatidia	635 nm	Trapezoidal, Adjacent to the rhabdom	No	Four (UV, Narrow-Band Blue, Double-peaked Green, Red)	50%	Qiu *et al.* (2002); Koshitaka *et al*. (2008)
Type II ommatidia	675 nm	Square, Adjacent to the rhabdom	Yes	Three (Violet, Single-peaked Green, Broad-Band)	25%	Qiu *et al*. (2002); Koshitaka *et al*. (2008); Kinoshita and Stewart (2020)
Type III ommatidia	635 nm	Rectangular, Adjacent to the rhabdom	No	Two (Wide-Band Blue, Double-peaked Green)	25%	Qiu *et al.* (2002); Koshitaka *et al*. (2008)

reminiscent of the ancestral holometabolous insect (Briscoe *et al*., 2003; McCulloch *et al*., 2016) while in *Heliconius erato*, a nymphalid butterfly, there are five classes of photoreceptors (Mc Culloch *et al*., 2016). The number of classes of photoreceptors increase to six in *Pieris rapae* (Marshall and Arikawa, 2014), nine in both *Colias erate*, the eastern pale clouded yellow butterfly (Pirih *et al*., 2010) and in *Troidesaeacus*, the golden birdwing butterfly (Chen *et al*., 2013). *Graphium sarpedon* or the common bluebottle butterfly possesses as many as 15 classes of photoreceptors in its compound eyes (Morell, 2016; McCulloch *et al*., 2022).

Table 2 illustrates an overview of the three distinct ommatidia types observed in butterflies, specifically focusing on *Papilio xuthus.* The ommatidia can be differentiated based on the receptor class present in them, position and colour of pigment clusters around the rhabdom. In *Papilio xuthus*, type I, type II, type III ommatidia occur in the relative percentage of 50%, 25%, 25% and have four, three, two receptor classes, respectively in fixed arrangement. The bandwidth of reflectance spectra for all 3 types is between 40 to 50 nm. The colour of pigments around the rhabdom is either red or yellow, these pigments function as spectral filters (Briscoe and Chittka, 2001; Qiu *et al*., 2002; Takemura *et al*., 2005; Koshitaka *et al*., 2008). Strong fluorescence is shown only by Type II ommatidia when illuminated with UV or violet light possibly due to the presence of 3-hydroxy retinol in the distal part of ommatidia where it acts as a filter and absorbs UV light (Qiu *et al*., 2002; Perr *et al*., 2016).

Typically, butterflies have nine photoreceptors in their ommatidium with eight elongated cells (R1 to R8) and an additional small basal photoreceptor cell (R9) beneath which is the basement membrane (Stavenga and Arikawa, 2006; Frentiu *et al*., 2007; Briscoe, 2008).

This increased number of photoreceptors per ommatidium as compared to Drosophila which contains only eight photoreceptors per

ommatidium and may contribute to the richness of spectral colour vision of butterflies (Katz *et al.,* 2009;Yamaguchi *et al*., 2010; Arikawa, 2017).

A characteristic feature of almost all butterflies (except papilionidae) is that their eyes have a tapetum at the base of ommatidium which acts as a reflector. Tapetum is a folded tracheole consisting of alternating layers of air and cytoplasm. Incident light enters the cornea, crystalline cone and passes on to the rhabdom, however, some of the light does not get absorbed by the rhabdom but is instead reflected by the tapetum, this reflected light goes out of the eye as eye glow or eye shine. The eye shine differs among neighbouring ommatidia supporting the idea of spectral heterogeneity of different ommatidia in the same eye (Stavenga 2002; Takemura *et al.*, 2007).

Certain butterfly species display sexual dimorphism in their compound eyes meaning that there are noticeable differences between the eyes of males and females with regards to eye size, types of photoreceptors and their spectral sensitivities (Chen *et al*., 2016; McCulloch *et al*., 2017; Bergman *et al*., 2021; McCulloch *et al*., 2022). Figures 3 a, b demonstrates the structure of a butterfly ommatidia; and the difference in spectral sensitivities of photoreceptors in males and females of a species, *Pieris rapae*, respectively.

Butterfly eyes are often differentiated into two regions: Ventral eye and Dorsal eye with ventral region being more sensitive for identifying flowers (Kitamoto *et al*., 1998; Awata *et al*., 2010).

Arrangement of photoreceptor cells in the male of butterfly *Pieris rapaecrucivora* is such that R5 to R8 occupy the lower 1/3rd of rhabdom while R1 to R4 occupy the distal 2/3rd of rhabdom (Briscoe and Chittka, 2001).

Visual Pigments:

Usually, each photoreceptor cell contains only one visual pigment with certain exceptions (Sison-Mangus *et al*., 2006). In insects, visual pigments are located in the rhabdomere or rhabdom (in bees and butterflies) where they absorb the light received by the ommatidia (Stavenga and Arikawa, 2006). Rhodopsins are the visual pigments found in butterflies consisting of a chromophore which is light sensitive and a protein component, opsin (Pepe, 1999; Briscoe and Chittka, 2001; Wernet *et al*., 2015).

In human beings, the chromophore is 11-cis-retinal which isomerises to its trans- configuration after energy transfer from photon (Palczewski, 2012) but in insects, it can either be 11-cis retinal, a derivative of vitamin A1 that is found in early insects or the (3S) and (3R)-enantiomers of 11-cis 3-hydroxyretinal, a derivative of vitamin A3 that evolved near the end of cretaceous period (Briscoe and Chittka, 2001; Stavenga and Arikawa, 2006). Figure 4 depicts the chemical structures of both types of chromophores and rhodopsin.

Opsins, on the other hand, are proteins with seven transmembrane helical domains belonging to a family of G-protein coupled receptors. Within the transmembrane region of each opsin protein, is a binding pocket for its ligand typically chromophore. Within this binding pocket, certain amino acid side groups interact with the chromophore. These interactions play a role in shifting the sensitivity of the chromophore from absorbing short-wavelength light (around 377 to 400 nm) to absorbing longer wavelengths of light. The maximum absorption (λ_{max}) of the visual pigment is determined by both the opsin protein's amino acid sequence and the properties of the chromophore. Thus, spectral sensitivity depends upon the energy level of π-electron orbitals in the chromophore (Briscoe and Chittka, 2001; Frentiu *et al*., 2007; Wernet *et al*., 2015).

After the absorption of photon by the visual pigment, rhodopsin, the chromophore gets isomerised by a photochemical reaction, resulting in the formation of a thermostable metarhodopsin (M). This metarhodopsin can be photoconverted to its original state, rhodopsin (R)(Stavenga, 2002; Vanhoutte, 2003; Stavenga and Arikawa, 2006).

As has been mentioned earlier, butterflies

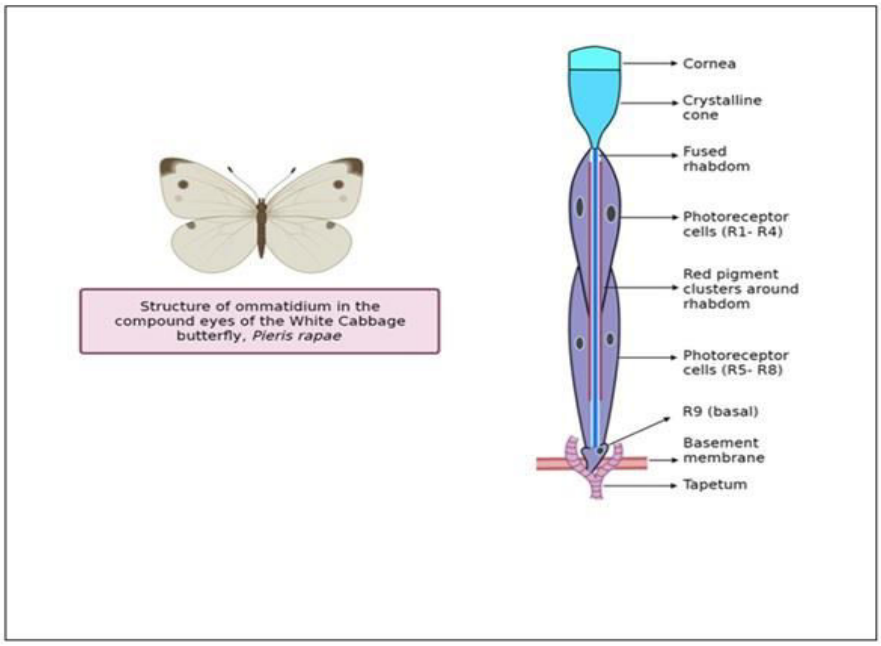

Fig. 3a: Schematic diagram showing structure of ommatidium of butterfly, *Pieris rapae*. Each unit or ommatidium consists of nine photoreceptor cells with R1 to R4 distal, R5 to R8 proximal and R9 basal in location. Their rhabdomeres, containing visual pigment are fused into a common rhabdom which is surrounded by four clusters of visual pigment in the proximal photoreceptors (shown by the red markings). The shape of arrangement of these clusters determines the type of ommatidia. Created on Biorenders. Data derived from Qiu *et al*. (2002) and Van Der Kooi *et al*. (2021).

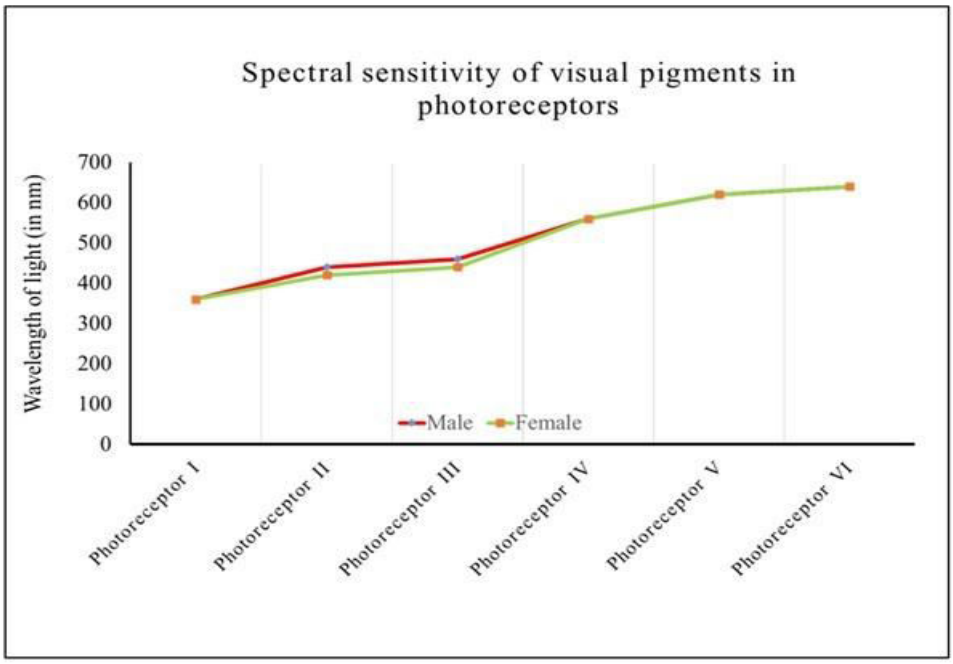

Fig. 3b: Spectral sensitivities of the six classes of photoreceptors in the eyes of males and females of the white cabbage butterfly *Pieris rapae*. Data for spectral sensitivity sourced from Van Der Kooi *et al*. (2021).

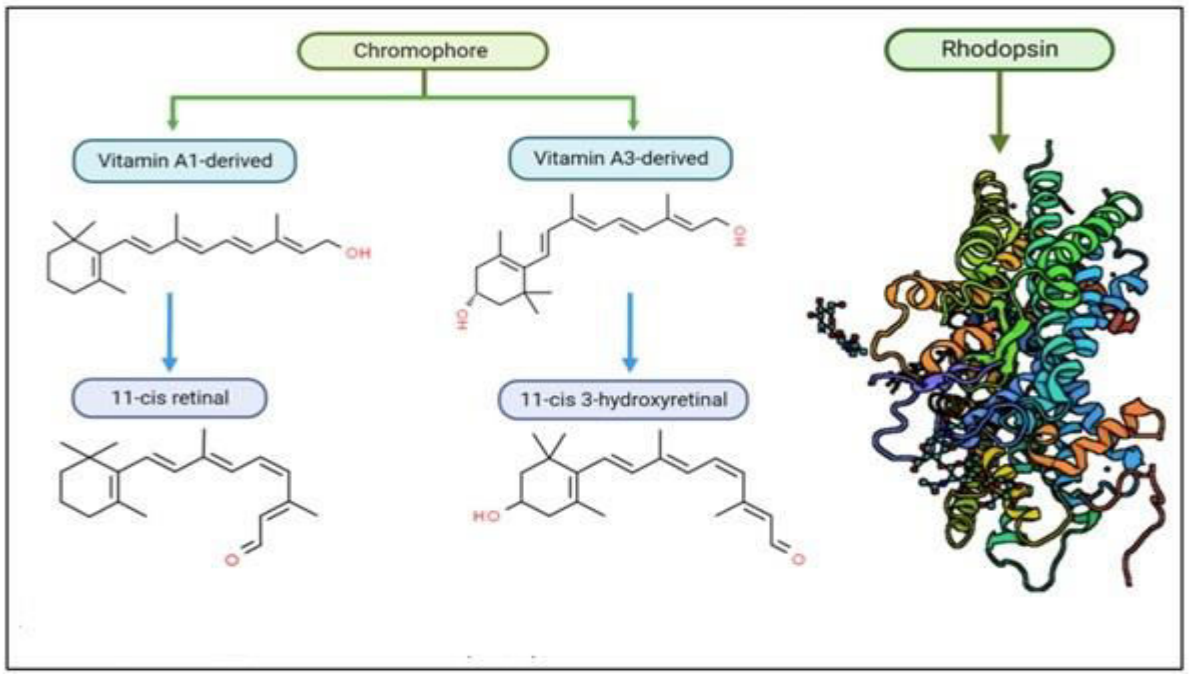

Fig. 4: Chromophores in class Insecta can either be derived from vitamin A1 in which case, it is 11-cis retinal, or it can be derived from vitamin A3, where it will be the (3S) and (3R)-enantiomers of 11-cis 3-hydroxyretinal. On the right, is the crystal structure of rhodopsin, a protein belonging to G coupled receptors family. (Chemical structures have been sourced from Chemspider, Crystal structure of protein from Biorender's protein database).

possess a united rhabdom where rhabdomeres of individual photoreceptor cells are fused together. In such a fused rhabdom, visual pigments in the rhabdomeres function as spectral filters for each other. As a result, the sensitivity spectra of photoreceptors are not the same as the sensitivity spectrum of their individual visual pigment. This variation in sensitivity is determined by how the rhabdomeres are arranged within the rhabdom (Briscoe and Chittka, 2001; Stavenga and Arikawa, 2006). For instance, in the cabbage white butterfly, *Pieris rapae,* due to the presence of four clusters of red pigments in R5–8 photoreceptor cells around the rhabdom, the spectral sensitivity of the photoreceptor changes resulting in the three known ommatidial types with their characteristic absorption spectra (Qiu *et al*., 2001).

In nymphalid butterflies, all the three types of ommatidia (type I, type II, type III) share a long-wavelength absorbing visual pigment (sensitive to green light) in six out of the nine photoreceptors present in each ommatidia. The distinction exists between the three is due to variation in the receptors that absorb short-wavelength light: one type of ommatidium contains two receptors, one for UV and one for blue light, another type has two receptors for blue light while the third type contains two receptors for UV light (Stavenga and Arikawa, 2006; Pirih *et al*., 2022).

From an evolutionary perspective, visual pigments found in invertebrates can be broadly categorised into two group based on the wavelength of light they absorb, ones that absorb long-wavelength light e.g. Green (G) absorbing and ones that absorb short wavelength e.g. Ultraviolet (UV) and Blue (B) absorbing. It is believed that the ancestral insect had these three clades: G, UV and B which resulted in the basic trichromatic vision found in insect orders like hymenoptera (Briscoe and Chittka 2001; Arikawa *et al*., 2005; Sison-Mangus *et al.*, 2006).

How did this ancestral trichromatic vision diversify into the many polychromatic vision systems we know today? Numerous studies attribute this to gene duplication, particularly in Lepidopteran families (Frentiu *et al*., 2007;

Briscoe, 2008).

For instance, in family Pieridae, the visual pigment that absorbs blue light diversified into Violet and Blue absorbing pigments (Arikawa *et al*., 2005) while in family Lycaenidae, the blue light absorbing pigment divided into blue-green and blue visual pigments (Sison-Mangus *et al.*, 2006). Likewise, red and green light absorbing rhodopsins diversified from green light absorbing pigments in family Papilionidae (Briscoe, 2001).

Few studies analysed the genetics behind vision in three species of butterfly: *Vanessa cardui*, *Danaus plexippus*, and *Heliconius erato* and discovered that visual pigments in these species were encoded by: one L opsin gene (for green) expressed by R3 to R9 photoreceptor cells and two S opsin genes (for Ultra Violet, Blue) expressed by R1-R2 photoreceptor cells (Briscoe *et al*., 2003; Saumane*t al*., 2005; Frentiu *et al*., 2007).

Vertebrates possess ciliary opsins in the photoreceptor cells of the retina of their eyes. Mammals, in particular, eutherians have a dichromatic colour vision primarily due to the presence of two cone pigments encoded by opsin genes in photoreceptor cells in retina: short wavelength-sensitive (S) and long wavelength-sensitive (L). Certain primates evolved further and developed a trichromatic vision due to the emergence of a middle-wavelength sensitive (M) cone pigment, corresponding to colour vision from green to red regions of the spectrum (Frentiu *et al.*, 2007).

Insects possess rhabdomeric opsin present in photoreceptor cells of ommatidia and certain butterflies too possess red-green colour vision as we saw earlier in the case of family Papilionidae as a result of gene duplication and spectral filtration. In herbivorous primates, trichromatic vision perhaps developed to better discriminate among leaves (Frentiu *et al.,* 2007), whereas in butterflies, the divergence to yield red-sensitive photoreceptors probably developed to find suitable foliage for oviposition (Frentiu *et al.*, 2007; Morehouse and Rutowski, 2010; Kinoshita *et al*., 2017; De Kok, 2021).

Further, in butterflies, flower choice is guided by both olfactory and visual sensory inputs which are processed in mushroom bodies of their brain (Kinoshita *et al*., 2014; Vogt *et al.*, 2016; Stöckl *et al*., 2016)

Butterflies also rely on visual, tactile and olfactory signals for identification during courtship rituals (Chengzhe *et al*., 2017). A majority of butterflies have apposition eyes, where each ommatidium in the eye functions as a separate photoreceptor, collecting light from a small portion of the visual field which is then processed by the ommatidium's rhabdom. The individual ommatidia are oriented in such a way that their visual axes point in slightly different directions, providing a wide field of view. There is also a screening pigment sheath between adjacent ommatidia to block the light entering from neighbouring ommatidia ensuring that each ommatidium receives light only from its corresponding lens and direction (Yack *et al.*, 2007; Warrant and Dacke, 2016). This is in stark contrast to moths which have superposition eyes where light passes through multiple neighbouring lenses to reach a common photoreceptor cell shared by several ommatidia. Thus, the photoreceptor cell in superposition eyes is capable of integrating the light inputs from multiple ommatidia which share a common visual field (Yack *et al*., 2007; Briscoe, 2008; Song *et al*., 2013; Warrant and Dacke, 2016).

There is another astonishing linkage between visual ecology of butterflies and the expression of visual pigments found in different regions of their eyes for sexual signals. For instance, in butterfly, *Lycaena heteronea*, wings are blue in colour and so, blue receptors are abundant in its ventral eye while in butterfly, *Lycaena rubidus,* wings reflect in red and UV only and so, ventral eye does not have blue receptors (Briscoe and Chittka, 2001). Thus, it can be deduced that wing colour and colour vision may have co-evolved in butterflies (Arikawa *et al.*, 2005).

While one may assume that colour vision in an organism requires only the occurrence and expression of two or more genes encoding visual pigments, this assumption is not entirely true. Colour vision only requires different spectral sensitivities of photoreceptors in the eye and these differing sensitivities can be due to different spectral filters too, not just due to more than two visual pigments (Arikawa, 2017).

Olfactory reception:

Olfactory communication, which involves the sense of smell, is a prevalent form of communication found in many living organisms, despite the fact that most studies, have explored vision and hearing as primary forms of communication (Wyatt, 2003). In olfactory communication, the transmission of messages typically involves combinations of various chemical components. The intricate nature of these chemical combinations offers the potential to convey intricate information about individuals. This has been observed and documented in eusocial invertebrates (Guerrieri *et al*., 2009 and Thom *et al*., 2007) as well as vertebrate species (Osada *et al*., 2003; Boule *et al*., 2009), highlighting the capacity for sophisticated communication through olfaction. Olfactory signals convey diverse information about potential mates, including identity, quality, genetic compatibility, and reproductive traits, guiding mate selection (Nieberding *et al*., 2012).

Thus, olfaction is important to many butterfly species since it is their primary sense for finding food, locating mates, and avoiding predators. Along with these activities, butterflies must find food for themselves and suitable partners for reproduction. For these goals, different insect groups use different senses. Olfaction, which is essential for survival and reproductive success in most creatures, is likely to be equally important for butterflies. However, when compared to moths and other insects, research on the olfactory abilities of butterflies within this subgroup has been restricted (Carlsson *et al*., 2013). One probable explanation is that butterflies rely less on olfaction and more on visual signals, which distinguishes them from their nocturnal relatives in the Lepidoptera family (Hambäck *et al*., 2007; Brückmann *et al*., 2010). However, new research has revealed behavioural responses in butterflies to floral fragrances and odours (Andersson and Dobson, 2003; Ômura and Honda, 2009) as well as responses generated due to the odour linked with their host plants (Ikeura *et al*., 2010).

The use of olfactory communication is expected to be essential in mate choice and sexual selection processes as is indicated by numerous studies surrounding female moths and their sex pheromones for communication. These pheromones released by females attract the males from far away and their unique makeup is also used for species identification. Males of some species of moths and butterflies have been seen to produce sex pheromones but in comparison to female sex pheromones in moths, our knowledge of male sex pheromones (MSP) in sexual communication is considerably less extensive. Male sex pheromones (MSP) are commonly used in close proximity throughout the courtship phase and could be used as signals to notify females of the social standing, genetic similarity, or immunocompetence of the male (Johansson *et al*., 2007; Nieberding *et al*., 2012). The precise characteristics of the male chemical signals that are important for sexual selection, meanwhile, are still mostly unknown. To accurately forecast females' mate preferences and acquire a greater knowledge of how sexual selection has impacted the evolution of these pheromone signals, it is essential to identify the precise information that females extract from sexual signals (Andersson and Simmons, 2006; Nieberding *et al*., 2012).

The long-lived African butterfly species *Bicyclus anynana* has been the subject of recent research, which has shed important light on the intricacy of male olfactory cues and their function in sexual selection. This species offers a unique paradigm for researching the information carried by male olfactory signals during sexual selection since females rely on a three-component male sex

pheromone (MSP) for mate selection (Costanzo and Monteiro, 2007; Nieberding *et al*., 2008).

The MSP's structure enables females to discern the age differences between courting males. Additionally, the pheromonal signature that each male has served as the foundation for inter-individual recognition and a parameter of its quality and identity does not alter with age. Further, a female's mate choice is guided by male olfactory signals and even subtle variations in its composition can be recognised by females (Nieberding *et al*., 2012)

In summary, the research on *Bicyclus anynana* has shed light on the intricate nature of male olfactory signals, their role in mate choice, and the potential implications for the evolutionary dynamics of olfactory communication in the context of sexual selection.

In the field of butterfly olfactory research, significant progress has been made in understanding the response of butterfly species to various volatile compounds. Moreover, recent studies utilising calcium imaging have recorded odour-evoked activity in the antennal lobe (AL), which is the primary central olfactory neuropil, in two butterfly species (Carlsson *et al*., 2011). Recently, two butterfly genomes were sequenced that were associated with olfactory function (Zhan *et al*., 2011).

Notably, the analysis of these genomes uncovered a remarkable resemblance, both in number and quality, of olfactory receptor genes of butterflies with their nocturnal counterparts, the moths. This observed genetic similarity in the olfactory receptors is noteworthy, particularly considering the divergent lifestyles, rhythms and behaviours of moths and butterflies, as well as the considerable evolutionary time that has passed since their divergence from a common Lepidopteran ancestor.

These advancements in genomic research and physiological studies have provided valuable insights into the olfactory mechanisms of butterflies and their relationship to moths. They contribute to our understanding of the evolutionary processes and adaptations that have shaped the olfactory systems in these diverse insect groups.

In insects, the main olfactory organ is the antenna, which contains numerous sensilla that harbour the olfactory sensory neurons (OSNs). The morphology of butterfly antennae has been examined in a few studies (Carlsson *et al*., 2013). One distinctive characteristic of butterflies is their club-shaped antenna, where the basal segments are thin and the distal segments of the flagellum (known as the bulb) are significantly thicker. Another notable feature in butterflies is that they do not exhibit sexual dimorphism in antennae, probably due to absence of long-distance pheromone communication in females, unlike moths (Andersson *et al*., 2007).

While butterflies primarily utilise vision and not pheromones, nevertheless numerous butterfly families have evolved unique structures and compounds for olfactory communication. These pheromones differ from family to family and even species to species.

The subfamily Danainae of the Nymphalidae family includes the Danaini butterflies, also referred to as milkweed butterflies. Two kinds of scent glands are found in the males of this tribe's butterflies. One of these is a hairpencil (HP), which is an expanding brush located on the abdomen, employed to deliver courting pheromones to the female antennae (Mann *et al*., 2020; Ehlers and Schulz, 2023). In certain species, a special type of dust or pheromone transmission particles is created by hairpencil glands to transfer chemicals. Androconia, the second type of scent gland found in male butterflies, is located on the forewings. The presence of biosynthetic enzymes in androconia might be due to the fact that butterflies must establish contact between androconia and hairpencil in order to generate sex pheromones (Ehlers *et al*., 2023).

In the queen butterfly, *Danaus chrysippus*, three major dihydropyrrolizines constitute the

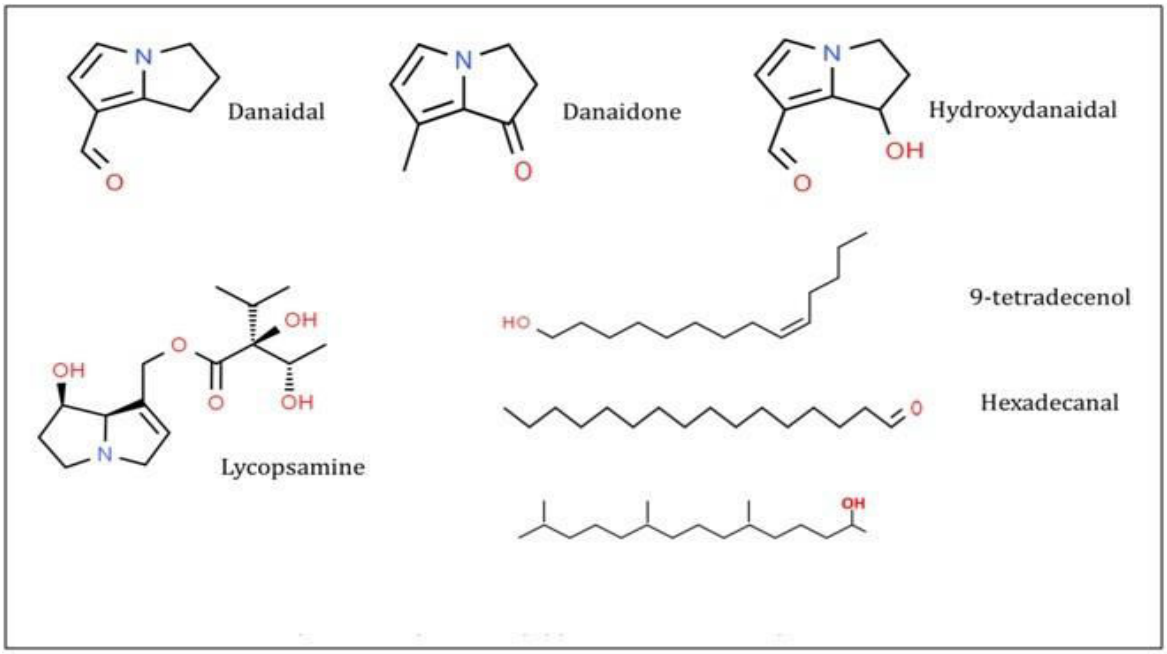

Fig. 5: Chemical structures of various pheromones found in different butterfly taxa from top left: Danaidal, Danaidone and Hydroxydanaidal in Danainae; Lycopsamine in ithimiines; 9-tetradecenol, Hexadecanal, 2,6,10-trimethylpentadecan-2-ol, and in Bicyclus anynana. (Chemical structures have been sourced from Chemspider).

majority of courtship pheromones, the most abundant of which is Danaidone followed by Hydroxydanaidal and Danaidal. These compounds are biosynthesized from pyrrolizidine alkaloids and subsequently transferred to the female during copulation for egg protection (Blomquist, 2003). They are found in other tribes such as ithomiines as well as in arctiid moths, *Creatonotos transiens* where they serve the same function. Hydroxydanaidal is also abundantly found in the genera *Parantica*, *Tirumala*, *Danaus* and *Euploea*, arctiid moths (in R configuration, stereo-chemically). The chemical structures of these three-courtship pheromone- associated compounds are shown in Figure 5 (Eisner and Meinwald, 2003; Honda *et al*., 2005; Ehlers *et al*., 2023).

In the hairpencils of *Amauris niavius*, lactones are present. The secretion primarily consists of cyclic sesquiterpenes like niaviolide and epoxyniaviolide. The use of lactones in olfaction is probably due to the ease of conversion of lactones into forms of less and more volatility. Lactones are also present in the striped crow butterfly, *Euploea mulciber (*Stritzke *et al.*, 2003; Honda and Ômura, 2010).

The ithomiines, which are the sister subfamily to the danaines, are commonly referred to as glass-wing butterflies due to their occasional transparent wings. They are found in Central and South America and encompass a wide variety of species. The ithomiines have erectable hairs on their wings that are utilized to disperse volatile substances during courtship but abdominal scent brushes are not found in them (Ehlers and Schulz, 2023).

Males of this subfamily consume pyrrolizidine alkaloids (PA) for body functioning and for conversion sex pheromones. While the main PA in this subfamily is lycopsamine (Fig. 5), which is produced from a variety of PAs with variable side chain stereochemistry, especially in *Mechanitis polymnia* species. In their androconia, ithomiines also make use of derivatives like danaidal and hydroxydanaidal, which are also found in several

different genera (Schulz and Hötling, 2015; Ehlers and Schulz, 2023).

Additionally, seven genera contain methyl hydroxydanaidoate, a significant chemical that is peculiar to ithomiines (Schulz and Hötling, 2015). Secretion of androconia mainly contains hydroxydanaidal and methyl hydroxydanaidoate with lactones in trace amounts (Ehlers and Schulz, 2023).

A large amount of the necessary alkaloid, lycopsamine, which may then be converted into different dihydropyrrolizine or lactone compounds, is made available by controlling the PA content in male butterflies (Ehlers and Schulz, 2023).

The Nymphalidae family contains the Satyrinae subfamily, usually referred to as the browns. These butterflies are distinct from other butterflies because they lack the characteristic warning colour (aposematism), due to their diet which chiefly consists of plants that lack secondary chemicals (Ehlers and Schulz, 2023). Most of the research on olfactory communication in sexual interactions in this subfamily has been focussed on the African butterfly genus, *Bicyclus*, which is a member of the Elymniini tribe (Bacque *et al*., 2015).

The quantity and placement of male smell glands (androconia), which are found on the hind- and forewings of the *Bicyclus* genus, as well as modified hair-like scales covering these glands (probably for scent dispersal), are used to distinguish males from females (Brakefield *et al*., 2009; Wang *et al*., 2014). Sex pheromones are released by the male androconia and received by sensory organs on the antennae of females and are responsible for mate-selection and identification (Costanzo and Monteiro, 2007).

Pheromone transmission is thought to happen when a particular activity, such as thrust or fluttering or thrust, causes the androconial hairs to stretch out and become visibly noticeable Brakefield *et al*., 2009). A male sex pheromone mixture is produced by the butterfly species *Bicyclus anynana* in the androconia. Three chemical compounds chiefly make up the mixture: hexadecanal, 2,6,10-trimethylpentadecan-2-ol, and 9-tetradecenol, the structures of which are given in Figure 5. The biosynthesis of these compounds takes place in the androconia and begins once the butterfly attains adulthood (Nieberding *et al*., 2008).

The Heliconiini butterflies belong to the subfamily Heliconiinae within the Nymphalidae family. Within this butterfly tribe, male individuals possess two distinct scent glands known as abdominal clasper scent glands (CSG) and androconia. The primary function of the abdominal clasper scent glands is to transfer antiaphrodisiacs (AA) and a complex blend of compounds to the female's abdominal pouch during copulation. This reduces the attractiveness of female butterflies to other male butterflies which in turn, ensures that the egg-laying process is uninterrupted (Schulz *et al*., 2007; Klein and De Araújo, 2010; De Oliveira Borges *et al*., 2020).

The androconia, specialized brush-like scales found in the overlapping region of the fore and hind wings, play a crucial role in Heliconius mating behavior. In species that are monoandrous, males exhibit pupal-mating, where they search for immature individuals, guard them, and mate with the emerging female once she is ready (Jiggins and Lamas 2016). Males utilise volatile monoterpenes released in latter stages of pupa to determine the maturity status of the female (Estrada *et al*., 2009). The androconial chemicals emitted by the hind-wing androconia are chemically different from those released by abdominal clasper scent glands; they influence mating behaviour and can differ among species. These chemical compounds mainly include fatty acid-derived compounds like aldehydes, alcohols, acetates, esters, and alkanes with C18 and C20 chains (Darragh *et al*., 2017; Mann *et al*., 2017).

The chemical composition of male butterfly pheromones exhibits remarkable diversity, showcasing a wide array of compounds with

intriguing origins. These may be lactones, terpenes, pyrrolizidine alkaloids, alcohols, aldehydes, fatty acids and numerous other hydrocarbons. These compounds are derived through two main mechanisms: either the butterflies synthesise them within their own bodies, or they acquire precursor molecules from the plants they interact with and feed on.

Conclusion

Butterflies are one of the most advanced invertebrates when it comes to visual prowess. Their compound eyes, composed of ommatidia, possess nine photoreceptors, and the types of photoreceptors in a compound eye can be anywhere from three to as high as fifteen. Butterflies also differ from other insects in having a fused rhabdom where visual pigment is located and a basal tapetum in each ommatidia, responsible for their characteristic eye shine. The visual chemistry behind vision in insects involves photoisomerization of rhodopsin, similar to vertebrates. The sensitivity of a photoreceptor is influenced by the presence of screening pigments and the resultant variable sensitivities to different wavelengths of light, give rise to their unique colour vision. The wing colour of butterflies of a species is also influenced by the presence of visual pigments encoded in their eyes.

Butterflies also rely on olfactory signals especially during mate selection. Butterflies respond to floral scents and other odours. Males usually have scent glands located on the abdomen or androconia located on wings which release pheromones that are received by the female's antenna. Different butterfly families use different chemical compounds (alcohols, fatty acids, alkaloids, aldehydes, lactones and other hydrocarbons) and have different modifications in males. Pheromones are also said to provide information about the quality and identification of male butterflies as well as the maturity status of female butterflies in some families. Currently, olfaction in butterflies has not been explored as well as other means of sensory communication.

Butterflies are a critical part of most ecosystems, being efficient pollinators and forerunners of inducing floral variations, however, the alarming decline in butterfly populations is not just a cause of concern but also a wake-up call. Long-term effects of falling populations of these fascinating insects may result in ecosystem instability and biodiversity collapse. Stern measures must be undertaken to reduce the usage of chemical pesticides and to preserve insect-biodiversity hotspots alongside vertebrate-conservation zones.

Acknowledgements

We would like to express our heartfelt gratitude to all those who have contributed to this research paper. We thank Prof. (Dr.) Rama, Principal, Hansraj College, University of Delhi for providing us with this magnificent opportunity and a platform to voice our opinions.

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