



ULTRASTRUCTURAL ANALYSIS OF ANTENNAE AND WING SCALES IN TWO BUTTERFLY SPECIES: INDIAN JEZEBEL (*DELIAS EUCCHARIS*) AND COMMON MORMON (*PAPILIO POLYTES*)

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Abstract: This study investigates ultrastructural variations in the antennae and wing scales of two butterfly species namely the Indian Jezebel (*Delias eucharis*; Lepidoptera: Pieridae) and the Common Mormon (*Papilio polytes*; Lepidoptera: Papilionidae), using scanning electron microscopy (SEM). Antennae function as key sensory organs, while wing scales influence coloration, thermoregulation, and aerodynamics. SEM analysis revealed two major antennal sensilla types in both species: sensilla trichodea and böhm bristles. *D. eucharis* displayed a more uniform distribution of sensilla trichodea with abundant scales and microtrichia, whereas *P. polytes* exhibited a higher density of sensilla and bristles, suggesting enhanced olfactory and tactile capacities. Wing scale morphology also differed markedly. *D. eucharis* showed broader, flattened, less patterned scales, producing bright yet uniform coloration that supports its mimicry-based defense strategy. In contrast, *P. polytes* possessed densely packed, ridged, and overlapping scales, creating iridescence likely linked to mate attraction and predator avoidance. Structural variations included a quasi-honeycomb pattern in *P. polytes* and a reticular lamina in *D. eucharis*, indicating roles in visual signaling and camouflage. These findings highlight interspecific diversity in sensory and structural adaptations, reflecting distinct ecological strategies. The study underscores SEM's value in advancing understanding of lepidopteran morphology and sensory biology.

Keywords: Antennae, Butterfly, Lepidoptera, Papilionidae, Pieridae, SEM, Ultrastructure, Wings.

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INTRODUCTION

The study of sensory structures and wing scales in Lepidoptera has provided valuable insights into the evolutionary adaptations of butterflies and moths to their ecological environments. In particular, the sensory organs, such as the antennae, are crucial for detecting environmental cues like odors and mechanical stimuli, which are essential for behaviors such as mate selection, foraging, and predator avoidance (Braby, 2000). In butterflies, antennae house a variety of sensilla, including sensilla

trichodea, which are specialized for olfactory detection and have been well-studied for their role in pheromone perception (Verma, 2017; Yuvaraj *et al.*, 2018; Limberger, 2021; Talwar, 2021). Wing scales, on the other hand, are complex nanostructures that not only contribute to the striking coloration of many butterfly species but also serve functions related to light manipulation, thermoregulation, and species recognition through structural coloration (Ghiradella, 1998; Vukusic *et al.*, 1999; Wilts *et al.*, 2017; Castro-Gerardino and Llorente-Bousquets, 2019).



Lepidoptera exhibit a wide diversity in the design of their sensory and wing structures, shaped by evolutionary pressures and the specific ecological demands of their environments. For instance, studies on butterfly genus *Patia* have shown the importance of antennal sensilla in pheromone detection and courtship, underscoring the relationship between sensory structures and reproductive strategies (Castro-Gerardino and Llorente-Bousquets, 2019). Similarly, research on the butterfly *Acasia monuste* has revealed the specialization of olfactory receptors in detecting species-specific pheromones, emphasizing the role of olfaction in mate recognition (Limberger, 2021). These findings suggest that the density and distribution of sensory structures are highly adapted to meet the specific ecological needs of each species.

In terms of wing scales, butterflies and moths display significant variability in scale morphology and arrangement, which impacts their coloration patterns. Structural coloration, which arises from the interaction of light with nanoscale ridges and lamellae within the scales, can create iridescent hues that serve functions like camouflage, warning signals, or mating displays (Ghiradella, 1998; Vukusic *et al.*, 1999). For example, Morpho butterflies exhibit brilliant blue iridescence through diffraction and interference in their scales, which functions both as a visual signal and a mechanism for thermoregulation (Vukusic *et al.*, 1999). Likewise, species like *Vanessa* utilize scale nanostructures to enhance cryptic and disruptive coloration, aiding in predator evasion (Ghiradella, 1998).

In this study, authors focus on the comparative morphology of the antennae and wing scales in Indian jejebel, *Delias eucharis* (Drury, 1773) (Lepidoptera: Pieridae) and Common Mormon, *Papilio polytes* Linnaeus, 1758 (Lepidoptera: Papilionidae) two butterfly species with distinct ecological adaptations. *Delias eucharis* renowned for its bright coloration and visual mimicry of toxic species, which deters predators through Batesian mimicry, while *P. polytes*, as part of a mimicry complex, heavily relies on both olfactory and visual cues for mate selection and predator evasion (Vane-Wright, 1980).

In male *D. eucharis*, the upper side of the wings is white, with forewing veins broadly black, widening into triangular tips and a narrow black costal margin. A broad black postdiscal band runs obliquely from the costa to vein 4, then parallels the termen. Hindwings have narrower black veins for most of their length, a slimmer curved postdiscal band between veins 2 and 6, and pink interspaces beyond it due to the vermilion underside (Moore, 1905; Bingham, 1907).

On the underside, forewings are similar but with broader black vein edges and yellow-tinged upper interspaces beyond the postdiscal band. Hindwings are bright yellow, with broader black veins and a postdiscal band extending from the costa to vein 2. Beyond it, interspaces show large vermilion-red lanceolate spots narrowly edged in white, with interspace 6 basally white (Moore, 1905).

The male *P. polytes* exhibits a single morph while female are polymorphic. Male is a dark-colored swallowtail butterfly. Its upper forewings display a series of white spots that gradually decrease in size toward the apex, while the upper hindwings bear a complete discal band of elongated white spots. Marginal red crescents may or may not be present. Males are typically smaller than females, though this is not always the case. The size of both males and all female forms of *P. polytes* can vary significantly depending on the climatic region (Bingham, 1907).

Wing structure and venation in *D. eucharis* and *P. polytes* are well studied (Patil and Magdum, 2017; Shimajiri and Otaki, 2022). In *D. eucharis* two veins are found to be missing, namely second radial R2 and first cubitus Cu1 from forewing while *P. polytes* had typical vein structure of papilionids (Patil and Magdum, 2017). Wing scales of *D. eucharis* had been studied albeit partially (Sharmila *et al.*, 2023). In *D. eucharis* antennae are black; the head, thorax, and abdomen are white with some black hairs giving a grey-blue sheen, and the palpi tip is black (Moore, 1905). Morphology and chemical characteristics of scales of hind wing of male *P. polytes* had been studied by Thaj and Prasad (2023). While in this research ultrastructure of scales of heart shaped spot of forewing of *D. eucharis* and apical and eye spot region of male *P. polytes* and antennae of both of these butterflies are studied for the first time.

By investigating the antennal sensilla and wing scale structure of these species, the aim is to explore how these features have evolved in response to environmental pressures to optimize sensory perception and coloration strategies. SEM studies are useful in solar energy applications and biomaterial characterisation. Novel material design inspired by biomimicry needs deeper understanding of microscopic image features (Lyons and Shanks, 2025). Additionally, structural adaptations modulate butterfly-environment interactions, facilitating ecological specialization and contributing to their evolutionary success across distinct habitat strata (López *et al.*, 2024).

MATERIALS AND METHODS

Adult male specimens of *Delias eucharis* (Drury, 1773) and *Papilio polytes* (Linnaeus, 1758) were collected

from the gardens of University of Lucknow, Lucknow (26.8467° N, 80.9462° E), (U.P), India. The collection was carried out using manual capture and net sweeping techniques, ensuring minimal stress and handling to preserve the integrity of both the antennae and wing structures.

Following collection, the antennae were carefully detached using fine dissecting tools, and debris were removed with soft brushes to maintain structural fidelity. The antennae and wings were then processed for scanning electron microscopy (SEM) in accordance with established protocols (Talwar, 2021). Specimens were fixed in 2.5% glutaraldehyde solution to stabilize tissues and subsequently dehydrated through a graded ethanol series (30%, 50%, 70%, 90%, and 100%) to remove water content.

For SEM preparation, the samples were mounted on bronze stubs and sputter-coated with a thin layer of gold-palladium to enhance conductivity. The morphological analysis was conducted using a JEOL JSM-7610F scanning electron microscope (SEM). Micrographs of the antennae—specifically the club, flagellum, and scape regions—were captured, with particular attention to the sensilla and microtrichia structures. Additionally, wing scales from different regions were examined to document their microstructural characteristics, such as surface ridges and lamellae, to assess any morphological adaptations related to coloration and other functional aspects. This detailed examination of the antennae and wing structure provides insights into the species-specific adaptations of *D. eucharis* and *P. polytes* in relation to sensory perception and visual mimicry.

RESULTS

Ultrastructure of Antennae

The antennae of *Delias eucharis* exhibited a relatively moderate density of sensilla trichodea in comparison to *Papilio polytes*, although these structures were still present in significant numbers (Fig. 1). The flagellum of *D. eucharis* displayed distinct blunt-ended microtrichia with longitudinal grooves, closely associated with the sensilla (Fig. 2). The antennae of *Papilio polytes* were densely covered in scales on the scape and pedicel, with the flagellum lacking scales but featuring an abundance of sensilla trichodea and microtrichia. Two types of sensilla trichodea, designated as type I and type II, were identified based on size and distribution. Type I sensilla were primarily concentrated in the club region of the flagellum. Böhm bristles, a mechanoreceptive structure, were clustered on the scape (Fig. 3-6).

Ultrastructure of Wings

The wing scales of *Delias eucharis* demonstrated a quasi-periodic arrangement, characterized by

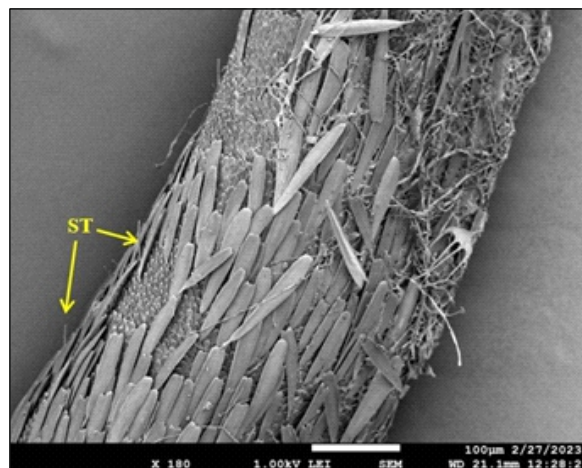


Fig. 1: Ultrastructure of Flagellum region of the antenna of *Delias eucharis* on lower resolution; ST: Sensilla trichodea.

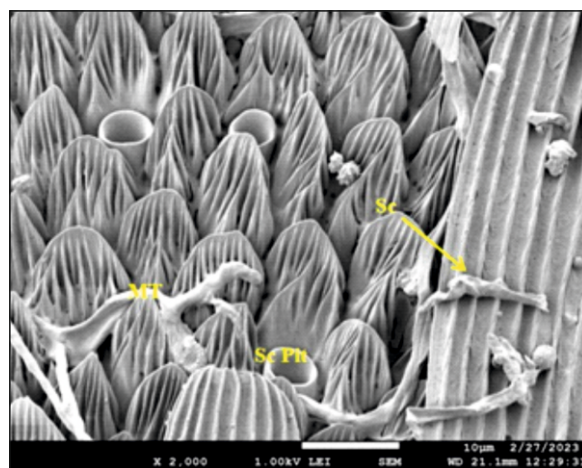


Fig. 2: Ultrastructure of Flagellum region of the antenna of *Delias eucharis* on higher resolution; MT: microtrichia, Sc: scale.

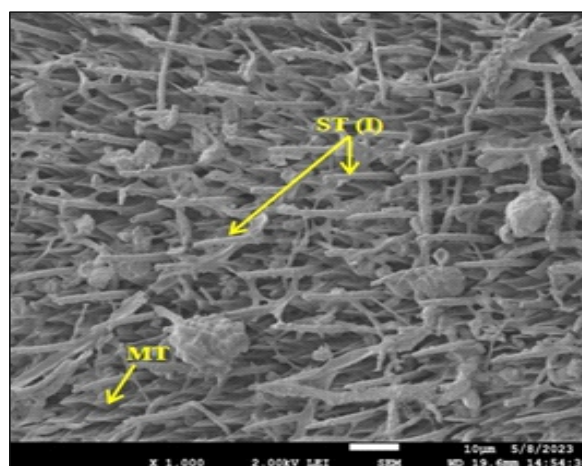


Fig. 3: Ultrastructure of the Antennal club region of the antenna of *Papilio polytes*; ST (I): Sensilla trichodea (Type I), MT: Microtrichia.

intricate microstructures consisting of longitudinal pillars interconnected by cross-ribs. The scales on the wings of *D. eucharis* were categorized based on their apical termini, including bipartite, tripartite, tetrapartite, and multipartite forms (Fig. 7-8).

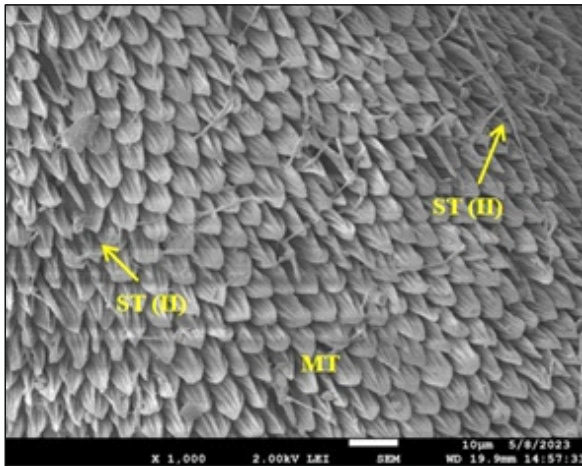


Fig. 4: Ultrastructure of the Flagellum region of the antenna of *Papilio polytes*; ST (I): Sensilla Trichodea (Type I), ST (II): Sensilla Trichodea. (Type II), MT: microtrichia.

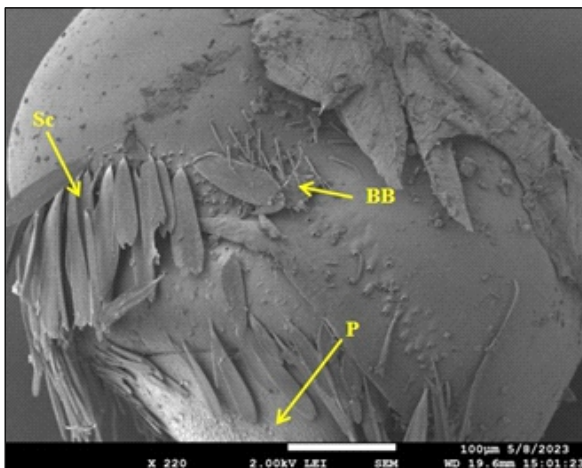


Fig. 5: Ultrastructure of Basal scape and pedicel regions of the antenna of *Papilio polytes*; BB: Böhm bristles, Sc: Scale, P: Pedicel.

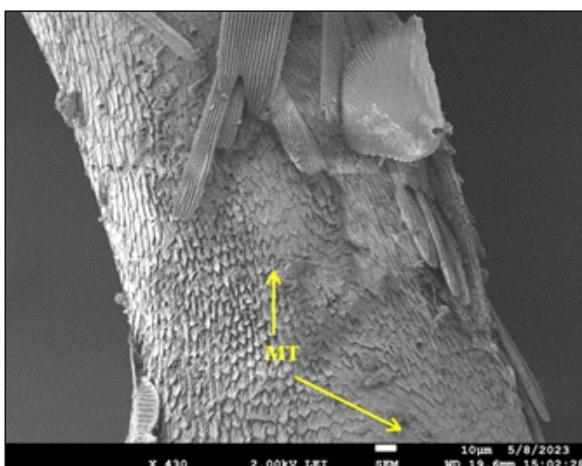


Fig. 6: Ultrastructure of proximal part of the flagellum region of the antenna of *Papilio polytes*; MT: microtrichia.

The scales of *Papilio polytes* displayed a quasi-honeycomb arrangement, with the scales themselves exhibiting a range of apical structures. Scales were categorized as bipartite, tripartite, tetrapartite, and multipartite. Notably, scales in the dark brown regions

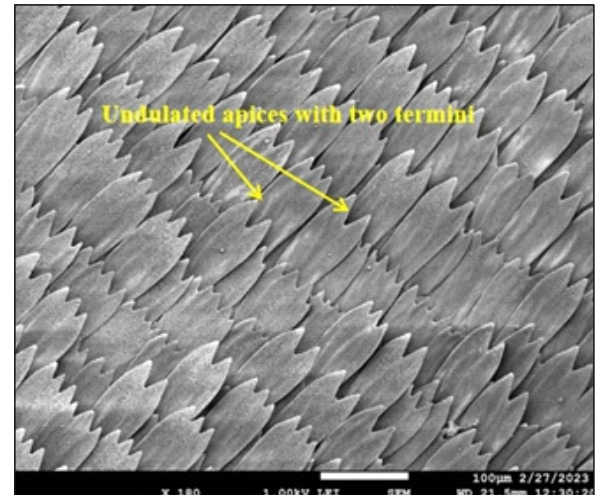


Fig. 7: *Delias eucharis*: wing's heart shaped pattern region; Ultrastructure on lower resolution.

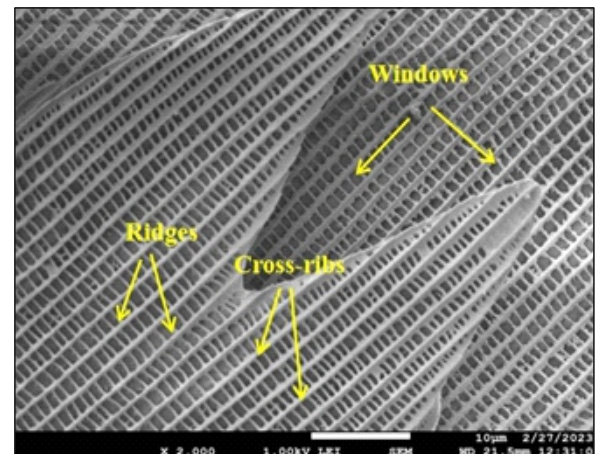


Fig. 8: *Delias eucharis*: wing's heart shaped pattern region; Ultrastructure on higher resolution; quasi-periodic structure.

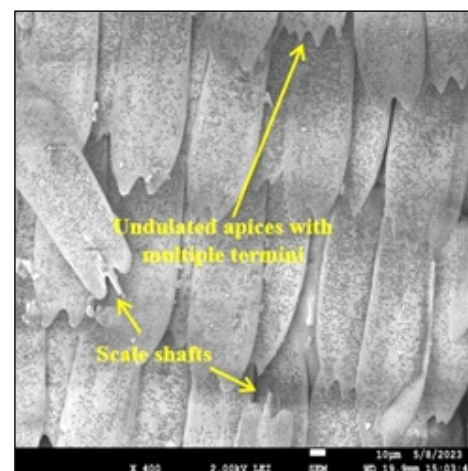


Fig. 9: *Papilio polytes* wing's apical region; Ultrastructure on lower resolution.

of the wings possessed pointed apices, while those in the eye spot regions showed blunt termini (Fig. 9-11).

DISCUSSION

The structural differences in the antennae and wings

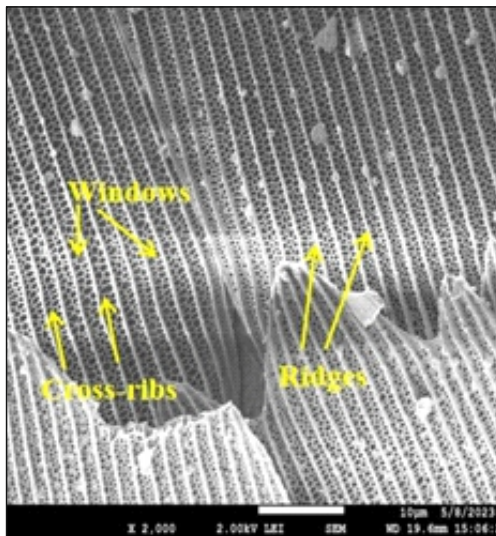


Fig. 10: *Papilio polytes* wing's apical region; Ultrastructure on higher resolution: quasi-honeycomb-like structure.

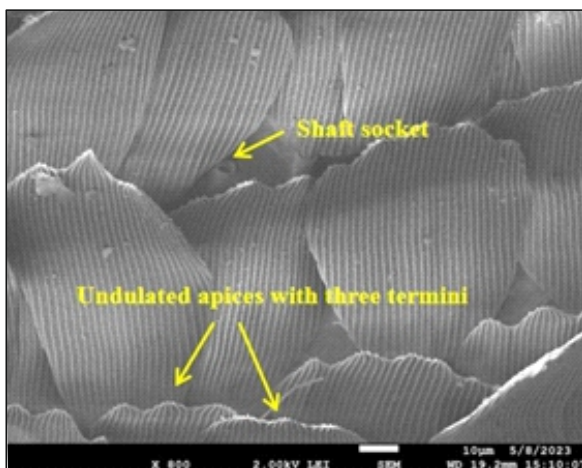


Fig. 11: *Papilio polytes* wing's eye spot region; Ultrastructure on lower resolution.

of *Delias eucharis* and *Papilio polytes* reflected unique adaptations to their respective ecological niches, particularly in terms of sensory perception and wing scale architecture. These variations provided insights into their distinct lifestyles and contributed to a broader understanding of lepidopteran evolution by offering a comparative framework with other butterfly and moth species.

The antennae of both *Delias eucharis* and *Papilio polytes* were equipped with sensilla trichodea and microtrichia, sensory structures that were essential for olfaction and mechanoreception. However, notable variations in their density and distribution suggested varied sensory capabilities. *Delias eucharis* had a relatively lower density of sensilla trichodea compared to *P. polytes*, aligning with its ecological strategy focused more on visual mimicry rather than olfactory communication. The blunt-ended microtrichia on the flagellum of *D. eucharis* further

supported a more visually oriented sensory strategy, with olfaction playing a secondary role in mate recognition and predator avoidance.

In contrast, *P. polytes* exhibited a higher density of sensilla trichodea, especially in the club region of the antennae, indicating a greater reliance on olfaction. These sensilla were crucial for detecting chemical signals, such as sex pheromones, which were vital for reproductive success (Zheng *et al.*, 2014; Li *et al.*, 2018a; Xu *et al.*, 2021). The abundance of these sensilla in *P. polytes* was likely an adaptation to its involvement in mimicry complexes, where the ability to recognize conspecifics was essential (Palmer *et al.*, 2018). BÖhm bristles found at the scape of antenna of *P. polytes* resembled like those identified in various other species of butterflies like *Papilio demoleus*, *Junonia lemonias*, *Danaus chrysippus* and *Pontia daplidice* (Talwar, 2021); also in some moth species as well e.g., *Dioryctria rubella* (Xu *et al.*, 2021). Studies show that these sensilla function as mechanoreceptors and also help in perceiving antennal movement and position (Li *et al.*, 2018b; Xu *et al.*, 2021).

Studies of other butterflies, such as of genus *Patia*, showed similarly high densities of sensilla trichodea, reinforcing the role of these structures in pheromone detection and mate recognition (Castro-Gerardino and Llorente-Bousquets, 2019). Additionally, skipper butterflies like *Parnara guttata* and *Pelopsidas mathias* demonstrated extreme specialization in their antennal sensilla for the pheromone detection, further underscoring the evolutionary significance of these structures in Lepidoptera (Xiangqun *et al.*, 2014).

The differences in wing scale architecture between *Delias eucharis* and *P. polytes* provided valuable insights into their respective adaptations for coloration, thermoregulation, and light manipulation. *Delias eucharis* featured a quasi-periodic arrangement of wing scales, characterized by intricate microstructural designs formed by longitudinal pillars connected by cross-ribs. This structural arrangement facilitated bright, uniform coloration, which was crucial for the species' Batesian mimicry strategy, wherein it visually mimicked toxic species like *Danaus chrysippus* to deter predators (Vane-Wright, 1980). The blunt-ended scales of *D. eucharis* further enhanced its visual deception, reflecting its reliance on visual cues for predator avoidance and survival in predator-rich environments. The scales of heart shape marginal spots of *D. eucharis* in our study were different from those of white reason of wings and beads along the ridges were also found to be absent in marginal spots in-concurrent with that of white reason of the wing (Sharmila *et al.*, 2023).

On the other hand, *P. polytes* exhibited a quasi-honeycomb pattern in its wing scale architecture, with scales ranging from bipartite to multipartite forms. This complex arrangement aided in structural coloration, where light interaction with the nanoscale features of the scales produced iridescent hues. Such structural coloration, seen in butterflies like *Morpho rhetenor* and *Papilio blumei*, played a role not only in display but also in species recognition, camouflage, and thermoregulation (Vukusic *et al.*, 1999). The pointed apices of scales in the dark brown wing regions of *P. polytes* likely contributed to heat absorption, an important adaptation for thermally sensitive ectotherms like butterflies.

Comparative studies of other butterfly species, such as *Vanessa cardui* and *Pararge aegeria*, revealed similar variations in wing scale structures that correlated with their ecological functions. For example, the multi-layered scale arrangement in *Vanessa* species enhanced iridescence and camouflage, while simpler scale structures in *Pararge* supported cryptic coloration (Ghiradella, 1998). The quasi-honeycomb design of *P. polytes* scales, therefore, reflected a multifaceted adaptation for survival, including mimicry, camouflage strategies, thermoregulation, and predator evasion.

The differences observed in the antennae and wing scale structures of *D. eucharis* and *P. polytes* were likely the result of distinct ecological pressures shaping their evolutionary trajectories. *Papilio polytes*, as part of a mimicry complex, relied on both olfactory and visual cues for mate selection and predator avoidance. The higher density of olfactory sensilla suggested a greater dependence on chemical communication in environments where closely related species coexisted, necessitating precise mate recognition. The complex wing scale architecture further supported its ability to signal conspecifics and deter predators through iridescent coloration.

Conversely, *D. eucharis* demonstrated a sensory and structural configuration more suited to visual mimicry. Its quasi-periodic wing scale pattern facilitated bright, uniform coloration essential for mimicking toxic species and reducing predation risk. The reduced reliance on olfactory structures in *D. eucharis* likely reflected an adaptation to environments where visual deception was a more effective survival strategy. Adaptive modifications in butterfly wing nanostructures along the forest canopy gradient reveal strategies to cope with varying thermal and aerodynamic conditions, raising further questions on eco-evolutionary flight and thermoregulation (López *et al.*, 2024).

CONCLUSION

The comparative morphological analysis of *Delias eucharis* and *Papilio polytes* highlighted the evolutionary plasticity of sensory and structural adaptations in Lepidoptera. The observed differences in sensilla density, wing scale architecture, and their associated functional implications underscored how ecological pressures drove the diversification of morphological traits, optimizing survival and reproductive success in different environmental contexts.

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