



Couto, A., Wainwright, J. B., Morris, B. J., & Montgomery, S. H. (2020). Linking ecological specialisation to adaptations in butterfly brains and sensory systems. *Current opinion in insect science*, 42, 55-60. <https://doi.org/10.1016/j.cois.2020.09.002>

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Linking ecological specialisation to adaptations in butterfly brains and sensory systems

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Abstract:

Butterflies display incredible ecological and behavioural diversity. As such, they have been subject to intense study since the birth of evolutionary biology. However, with some possible exceptions, they are underused models in comparative and functional neurobiology. We highlight a series of areas, spanning sensory ecology to cognition, in which butterflies are particularly promising systems for investigating the neurobiological basis for behavioural or ecological variation. These fields benefit from a history of molecular and quantitative genetics, and basic comparative neuroanatomy, but these strands of research are yet to be widely integrated. We discuss areas for potential growth and argue that new experimental techniques, growing genomic resources, and tools for functional genetics will accelerate the use of butterflies in neurobiology.

Highlights:

- Butterflies are underutilised in evolutionary neurobiology and neuroethology
- They showcase recent ecological radiations and striking behavioural innovations
- Broad range of sensory ecologies reflected in sensory systems
- Variable brain morphologies suggesting diverse adaptations in neural circuitry
- Selected species can become models for functional neuro-genetics and development

Key words:

brain evolution; cognitive ecology; mushroom body; olfaction; sensory ecology; vision

Introduction

There is renewed recognition that studying a greater range of species will benefit our understanding of neurobiology [1]. Butterflies are highly diverse, and have a rich tradition of ecological, evolutionary and genetic research. Although butterflies currently lack the genetic tools of established insect models, these attributes, alongside a history of comparative neuroanatomy and growing genomic resources, provide a solid foundation for investigating the neural basis of ecological and behavioural diversity. Here, focusing on adults, we highlight areas that exemplify the potential of butterflies as a system for integrating ecology and neurobiology.

Sensory ecology and the environment

Butterflies occupy a diverse range of sensory environments, and are excellent systems for investigating how sensory perception evolves. Butterfly eyes are structurally similar to other arthropods, but the spectral sensitivity of photoreceptors (opsins) within each optical unit (an ommatidium) varies greatly across species. Lineage-specific duplications mean opsin number varies from two to nine, expressed in three ommatidia types [2-4]. Variation in opsin repertoire has been associated with light environment [4] and improved colour perception at specific wavelengths [5]. In some butterflies, specialised regions of the eye also detect polarised light [6], the abundance of which varies between habitats. Finally, screening pigments within the ommatidium can modify the spectral sensitivity of expressed opsins, providing a secondary route to enhanced colour discrimination [6].

Butterfly diversity is prime for comparative studies of how visual pathways adapt to contrasting conditions, and how sensory circuits accommodate greater diversity in colour perception. For example, recent work has used *Papilio xuthus* and *Vanessa cardui* to understand how the retina is patterned to produce random arrays of ommatidia types, building on foundational work in *Drosophila* using emerging CRISPR/Cas9 techniques [7].

The structure and size of brain regions (Figure1A,B) that process visual information are also highly variable. Species occupying high-light intensity habitats, such as *Danaus plexippus*, *Papilio xuthus*, and *Heliconius* species, have substantially larger visual neuropils than species that occupy low-light, closed-canopy forests, such as *Godyris zavaletta* [8-11] (Figure1C,G). *Danaus*, *Heliconius* and *Papilio* also share a butterfly-specific optic neuropil, which acts as a relay centre directing visual projection neurons to the mushroom body [11] (Figure1C), that is absent in *Godyris* [8]. In contrast, *Godyris* has converged on a 'moth like' antennal lobe morphology, expanding in size and displaying enlarged, sexually dimorphic sub-units [8] (Figure1E,H). Furthermore, comparisons among closely related, 'incipient' species suggest

such shifts in sensory investment may be critical for local adaptation to new sensory conditions [12].

Butterflies therefore provide excellent opportunities to investigate how sensory perception and processing vary across environments. This field can expand to explore how these evolutionary specialisations are developmentally controlled, how sensitive their development is to environmental conditions, and what role they play in facilitating speciation.

Behavioural responses to host-plants

Behavioural responses to plants play a major role in butterfly ecology, but butterflies vary considerably in the number and variety of plants they utilise. Butterflies are generally considered to be visually-orientated foragers, and the physical properties of plants provide important cues [13]. For example, many butterflies show strong biases in colour preference and discrimination during nectar foraging [14-18], and visual recognition of leaf shape can be critically important for host-plant detection [19].

However, unambiguous recognition of a plant resource requires concomitant stimuli to match the “search image” [20]. Foraging is therefore likely to involve multimodal sensory cues, with chemical cues playing an important role alongside vision. Butterflies can navigate towards host-plant odours [21], use odour cues to modify landing rates [15,22], and gustatory receptors expressed in foreleg sensilla are critical for oviposition [23]. Typically, however, butterflies show little evidence of olfactory specialization in gross antennal lobe morphology [24]. Instead, host-plant specialization likely involves adaptations in chemosensory receptor sensitivity or signal integration. Indeed, expression of gustatory and olfactory receptors can be sexually dimorphic, and evolve rapidly [25]. Electrophysiological recordings of odour-evoked activity in the antennal lobe also suggest adaptive changes in odour processing, with increased discrimination of stimuli representation in host-plant specialists, compared to generalists [26].

The multi-modal nature of plant cues also suggests that brain areas where sensory streams converge may be critical for behavioural decision making. In some butterflies, visual and chemosensory information converge through direct inputs in the mushroom body (MB) calyx [11]. The degree of visual projection to the MBs, an established site of visual and olfactory memory [27], varies across butterflies, presumably reflecting the role of different sensory modalities in guiding behaviour. Behavioural experience also affects MB development, and this ‘plasticity’ varies across species, with host-plant generalists having more developmentally plastic MBs than host-plant specialists [28]. How behavioural decisions are affected by these changes in sensory circuitry, and how sensory cues are integrated and weighted, is unknown. While these questions can be explored in established models, the sensory diversity of butterflies

provide opportunities to explore more complex interactions, and to test the generalisation of data from simpler systems.

The temporal and spatial distribution of resource availability also affects behaviours like diapause, seasonal polyphenisms, dispersal and migration. In this context, the most intensely studied butterfly behaviour is the seasonal migration of monarch butterflies, *Danaus plexippus*. A number of molecular and neurophysiological mechanisms have been linked to this behaviour, including concomitant signals from light sensitive magnetosensors in the antenna, and the detection of polarized UV light [29]. Directional visual information are sent to the central complex [30], a brain structure involved in sensory-motor integration and locomotor control in the context of processed internal and external stimuli, and the likely site of integration of both the circadian clock and skylight information, important components of navigational control [30]. Progress in understanding monarch migration demonstrates how careful selection of butterfly study species, based on their natural history, can provide new platforms for discovery in neurobiology.

Intraspecific communication

Butterflies have diverse, and at times stunning, visual and chemical signals that act to attract mates, or repel predators. Signal detection can be directly shaped by the evolution of sensory receptors. For example, in some *Heliconius* butterflies, ultraviolet wing reflections provide mating signals that are undetected by avian predators [31], facilitated by the duplication and shift in absorption profile of a UV-sensitive opsin [5]. In other cases, shifts in the response to intraspecific cues are linked to downstream processing of sensory information. For example, in *Heliconius cydno* and *H. melpomene*, male mating behaviour is governed by divergent preferences for wing colouration. Extensive quantitative trait mapping of the loci underpinning this behaviour identified three genomic regions that together explain 60% of the interspecific difference in mate preference [32]. Further dissection of the major locus, through a combination of population genomics and transcriptomics, has narrowed the list of candidate loci to just five genes, none of which are directly involved in photoreception, suggesting divergent mating behaviours emerge during visual integration or processing [33]. Although a handful of studies have determined the molecular and neural basis of reproductive isolation in *Drosophila*, these are generally mediated by divergence in olfactory reception. *Heliconius* provide a window into visually determined mating preferences. Although the lack of genetic tools means identifying the neural changes involved in mate choice is challenging, the identification of causative loci provides a major step towards this goal.

Olfactory cues also serve a variety of functions in mating discrimination [34,35]. While butterflies widely lack the specialised olfactory ‘macroglomeruli’ used by male moths to detect long-distance female pheromone trails [24] (Figure1D), some butterflies nevertheless utilise

long-range pheromones to attract mates and repel rivals. In at least one case, this is accompanied by independent specialisation in the main olfactory neuropil [8,36]. Ithomiine butterflies have distinct, but phylogenetically variable, sub-clusters of enlarged, sexually-dimorphic olfactory glomeruli (Figure 1E), a potential neuroanatomical correlate for enhanced pheromonal processing [8,36]. This peculiar case of a re-emergence of a trait lost in a lineage's evolutionary past provides a potential case study in neuroanatomical convergence.

In many butterflies, both vision and olfaction contribute to mating decisions [37,38]. What remains unclear is how these parallel strands of information are integrated, and whether their independent evolution affects distinct aspects of courtship. More generally, the recent speciation events evident in many butterfly radiations provide opportunities to use forward genetics to identify molecular mechanisms underpinning behavioural divergence. This can be a first step towards understanding how divergent behavioural preferences are produced by changes in the nervous system, and whether there are biases in the relative contribution of sensory reception and processing that might reflect constraints on behavioural evolution.

Cognitive neuroecology

By associating rewarding or aversive experiences with sensory cues, animals can adopt flexible behavioural responses in different conditions. Butterflies learn associations in several contexts including nectar foraging [14,39], host-plant seeking [19,40], and mate choice [41,42]. This has important ecological effects, providing mechanisms to adapt to changes in resource availability [19], facilitating range expansion [12], and optimising reproductive behaviours [42].

Identifying the neural basis of cognitive evolution is an enticing goal, and butterfly neurobiology is well placed to progress this field. A likely site of neural adaptations underpinning cognitive evolution are the MBs, which have established roles in associative memory [27]. In butterflies, MBs are extremely variable at a volumetric level, ranging from ~4% to 40% of central brain volume, with some species rivalling bees, established models of insect intelligence, in absolute size [9] (Figure 1A,B,G,H). There is also evidence of substantial structural plasticity in butterfly MBs, plausibly linked to host-plant ecology [28], that can be manipulated experimentally [9,43]. This experience-dependent volumetric expansion is likely to be the result of internal changes in MB neural morphology during behavioural maturation [44]. However, the cellular and molecular basis of this plasticity, and its behavioural relevance are yet to be determined.

MB size can also vary dramatically across relatively short phylogenetic timescales. For example, *Heliconius* butterflies (Figure 1A,G,H) are thought to have undergone a 4-fold expansion in MB size during their origin, ~12-18mya [9]. For comparison, a similar expansion event in Hymenoptera is at least 10 times older [45]. Substantial MB variation across closely related clades permits comparisons of cognitive performance and MB anatomy in species that

are ecologically and phylogenetically similar, minimizing noise and simplifying inferences. Our in-depth knowledge of butterfly ecology also provides plausible adaptive explanations for this variation, in the case of *Heliconius* highlighting a derived dietary adaptation, pollen-feeding, which is associated with spatially faithful ‘trap-line’ foraging [9]. While it is not yet known whether other butterfly lineages show similar shifts in MB investment, future work in this system has the potential to illuminate the links between the cellular basis of MB expansion, cognitive and behavioural specialization, and associated energetic costs; which together determine the fitness landscape of neural elaboration.

Conclusion

The combination of rich ecological and behavioural information means there are likely to be multiple butterfly lineages that can be developed as case studies in behavioural innovation and specialisation. To date, their proximate basis has most successfully been approached using molecular genetics and comparative anatomy. Integrating these approaches to understand the effects of candidate loci in nervous system development and function, and how they affect circuit properties, remains challenging. However, basic techniques such as immunohistochemistry and neural tracing, provide an initial route in, and tools are emerging that will widen the scope of experimentation. Indeed, some butterflies stand up well against suggested blue-prints for developing ‘model organisms’ [46]. For example, work in *Danaus* has shown the potential of behavioural experiments using flight simulators [47], facilitating *in vivo* neurophysiological experiments. Rich genomic data already exist for several butterfly clades, and both expressed transcripts and non-coding regulatory regions are being profiled (e.g. [48]). Techniques also exist to disrupt gene function. Although RNAi is difficult to achieve in Lepidoptera [49], it has been used successfully in some species [e.g. 23]. Efficacy appears tissue dependent, but brain tissue is potentially relatively sensitive [49]. More recently, CRISPR/Cas9 gene-editing has been successfully deployed to study eye development in butterflies [7], and the behavioural effects of disrupting antennal lobe development in moths (e.g. [50]). Growing a community of collaboratively minded research groups that share tool development, and the engagement of expertise from traditional insect models, will be key to further developing this field. Regardless, as insect neurobiology turns towards developing more diverse study organisms, we are confident that butterflies will provide ample inspiration for aspiring neuroethologists.

Declarations of interest:

None.

Acknowledgements:

We are grateful for funding from ERC Starter Grant (758508) and NERC IRF (NE/N014936/1) to SHM, and a NERC GW4+ DTP to JBW, to Sinead English and Richard Merrill for comments on a draft manuscript, and to Basil el Jundi and Antoine Wystrach for inviting us to contribute.

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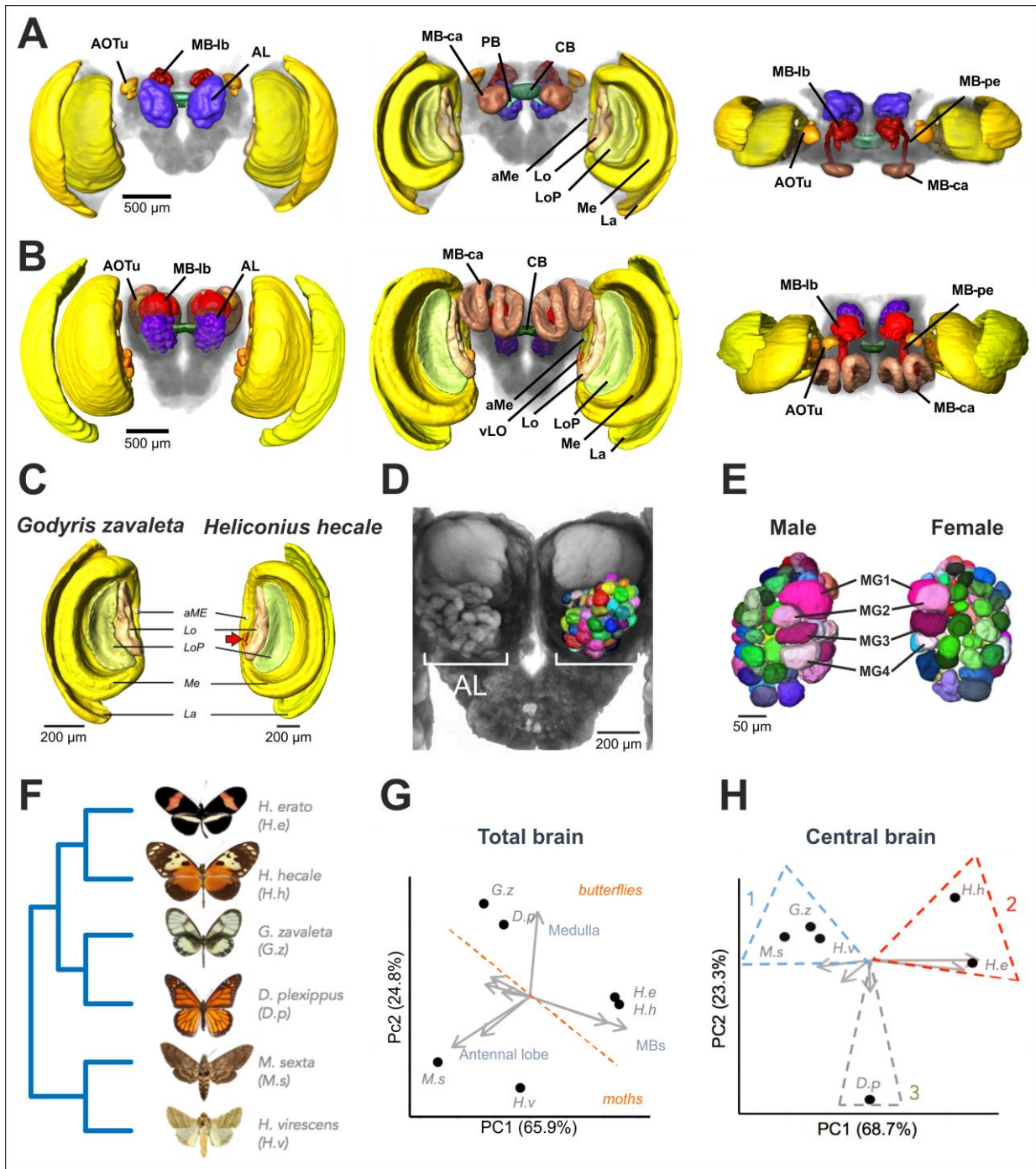


Figure 1: Examples of variation in butterfly brain anatomy

Surface models of a *Godyrys zavaleta* (A) and *Heliconius hecale* (B) brain, from [8, 9]. The main sensory neuropils are colour coded whereas the rest of the central brain including the lateral horn and the subesophageal ganglion are shown in transparency. Respectively, from left to right, the brains are presented from anterior, posterior, and dorsal view. Brain composition is generally similar between the two species, whereas the size of individual neuropils, like the mushroom bodies (MB), can vary greatly. (C) Surface reconstruction of the optic lobe neuropils in *Godyrys zavaleta* and *Heliconius hecale* presented from a posterior view. Although different in size, the same neuropils are found in both species with exception of the ventral lobe of the lobula (vLO,

red arrow), which is absent in *Godyrus zavaleta*. (D) 3D reconstruction of an antennal lobe (AL) glomeruli superimposed on a volume rendering of the anterior surface of the central brain in *Heliconius hecale* showing homogeneity in the size of glomeruli. (E) Surface reconstructions, viewed from anterior, of the AL glomeruli in male and female *Godyrus zavaleta*. Note the presence of a subset of four glomeruli that form the sexually dimorphic macro-glomerular complex (MGC1-4). (F) phylogenetic relationships of selected Lepidoptera for which directly comparable data (used in G,H) are available. Branches are not drawn proportional to divergence dates. (G,H) Principal component analysis of segmented neuropil volumes, including the entire brain (G) or the central brain only (excluding optic lobes, H), showing major axes of variation, corrected for allometric scaling with the unsegmented central brain [9]. Species data points are indicated by the first letter of their genus and species name, as followed: D.p *Danaus plexippus*; H.e *Heliconius erato*; H.h *Heliconius hecale*; G.z *Godyrus zavaleta*; M.s *Manduca sexta*; H.y *Heliothis virescens*. Abbreviations: antennal lobe (AL); accessory medulla (aME); anterior optic tubercle (AOTU); central body (CB); lamina (La); lobula (Lo); lobula plate (LoP); mushroom body (MB); mushroom body calyx (MB-ca); mushroom body lobe (MB-lb); mushroom body peduncle (MB-pe); medulla (ME); protocerebral bridge (PB); ventral lobe of the lobula (vLO).