3 m/s and flew steadily at higher wind velocity – up to 10–12 m/s. Over 300 flights were videorecorded. Several flies were operated bilaterally. No significant difference was observed between flight performance in intact and operated flies. Only four individuals showed remarkable instability of the operated leg. This small percentage of disabled individuals is safer to ascribe to damage of the operated leg than to damage of the MCP. Most of operated individuals had no difficulties in maintaining their perfect flight posture against the head airstream well above the physiological range of flight speed. There may exist some mechanisms (e.g., enhancement of the muscle activity) which compensate the lack of the presumed lock, but they are questionable.

The function of the MCP is still not proven. In any case, it is hardly believable that the structure, which is an obligatory character of so many families of Brachycera and has passed the route of evolution from a hairy evagination of the articulation membrane to the more elaborated sclerotized instrument, is of no significance to representatives of Brachycera.

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Molecular Diversity of Visual Pigments in the Butterfly *Papilio glaucus*

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Physiological and photochemical studies reveal that some butterflies possess unusually broad sensitivity to light, ranging from the ultraviolet to the red [1, 2]. In the retina of *Papilio* butterflies four (*P. aegeus*) or five (*P. xuthus*) spectral classes of receptors have been previously identified, with peak sensitivities at 360 nm (UV), 390 nm (violet), 460 nm (blue), 530 nm (green), and 600 nm (red) [2–4]. Variation in spectral sensitivity between photoreceptors is primarily conferred by variation in the amino acid sequence of the visual pigments (opsins) expressed within those cells, as only a single retinal chromophore, 3-hydroxyretinal, has been found in *Papilio* butterflies [5]. Until now the molecular variation of the opsins underlying the broad spectral sensitivity in butterflies has been unknown. I report the cloning of six opsin gene fragments from the tiger swallowtail butterfly, *Papilio glaucus.* Their placement within a phylogeny of insect opsins suggests that these butterflies possess at a minimum four long-wavelength opsins and one blue- and one ultraviolet-sensitive opsin.

Opsins are encoded by a multigene G protein coupled receptor family characterized by seven transmembrane helical domains. The six *Papilio* opsin fragments (*Pgl-Rh1*, *Pgl-Rh2*, *Pgl-Rh3*, *Pgl-Rh4*, *Pgl-Rh5*, and *Pgl-Rh6*, Table 1) are alignable with other insect opsins (Fig. 1) and bear several structural features known to be critical to opsin function. In particular, the chromophore-binding lysine residue [6], which is conserved across all known opsins, is also found in all *Papilio* opsins. The putative coding regions of the opsin fragments, starting from a conserved motif in the cytoplasmic loop between transmembrane domains V and VI (QAKKMN), vary in length from 122 (*Pgl-Rh5*) or 123

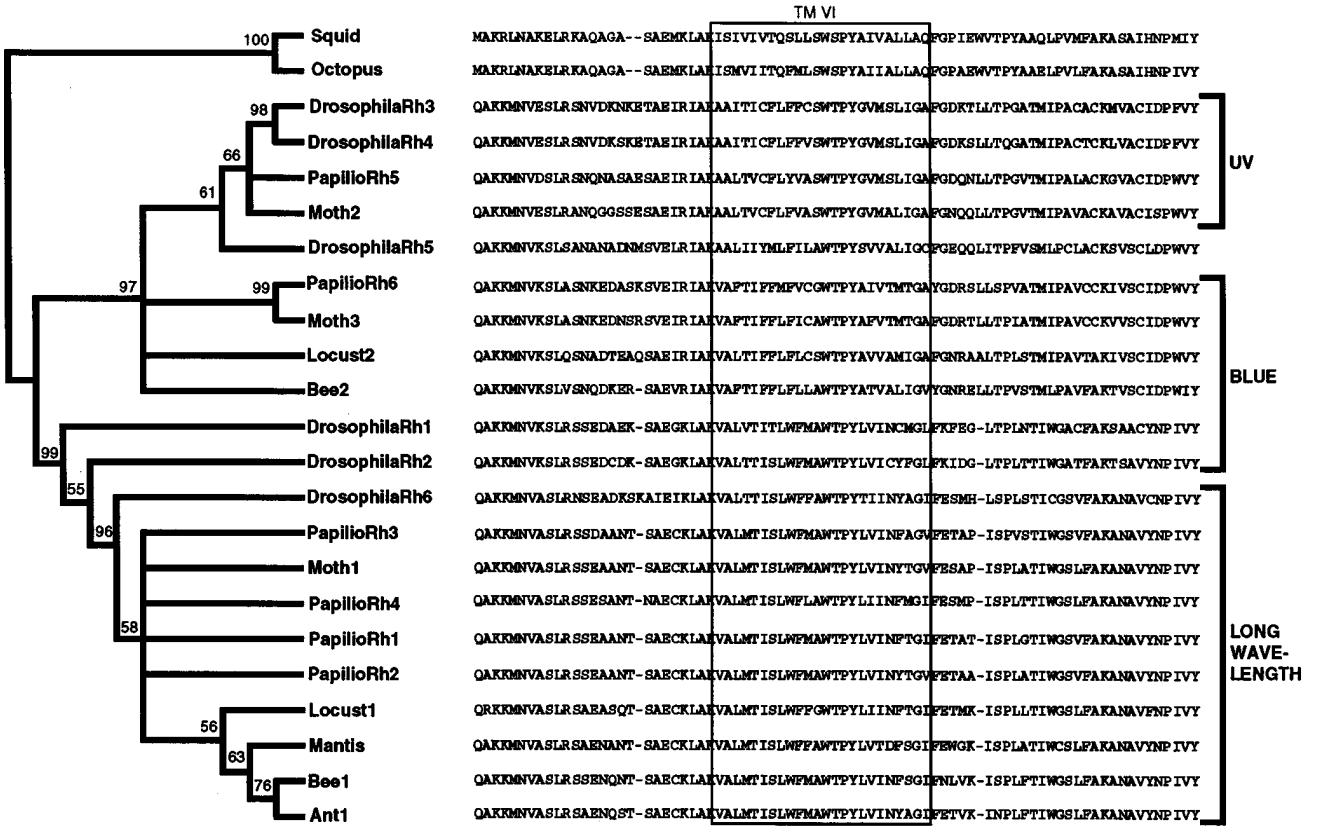

Fig. 1. The alignment of opsin sequences used for phylogenetic analysis. *Dashes*, gaps; *box*, transmembrane domain VI. Amino acid sites aligned by eye. Heuristic bootstrap 50% majority-rule consensus tree (bootstrap replicates = 1000, random addition replicates = 2). *Numbers above nodes*, bootstrap values supporting the node. Maximum parsimony analysis was conducted using test version 4.0d57 of PAUP*, written by David L. Swofford. GenBank accession numbers: Squid, Z49108; Octopus, X07797; DrosophilaRh3, M17718; DrosophilaRh4, M17730; PapilioRh5, AF030156; DrosophilaRh5, U67905; PapilioRh6, AF030157; Bee2, U70841; DrosophilaRh1, K02315; DrosophilaRh2, M12896; PapilioRh3, AF030158; PapilioRh4, AF030159; PapilioRh1, AF030160; PapilioRh2, AF030161; Locust2, X80072; Ant1, U32501; Bee1, U26026; Mantid, X71665; Locust1, X80071. EMBL accession number: DrosophilaRh6, Z86118. GSDB database accession number: Moth1 (MANOP1), 76082; Moth2 (MANOP2), 109852; Moth3 (MANOP3), 1249561

(*Pgl-Rh1, Pgl-Rh2, Pgl-Rh3, Pgl-Rh4*) to 127 (*Pgl-Rh6*) amino acids.

All pairwise comparisons of *Papilio* opsins at synonymous sites approach or exceed saturation (Table 1), suggesting that these sequences are anciently diverged. In comparison, the most similar pair of *Papilio* opsins is 20 times more divergent at synonymous sites than intron 4 of the marmoset (*Calithrix jacchus)* long-wavelength opsin alleles [7]. The higher than expected degree of saturation between some pairs of genes, which affects this estimate of divergence, is due to differences in preferred codon usage between genes (data not shown).

Previous phylogenetic analyses of invertebrate opsins have shown that opsins fall into distinct functional clades according to absorption spectrum [8–11]. Phylogenetic methods may therefore be useful in identifying the putative absorption spectrum of new opsin sequences. The placement of *Pgl-Rh5* within the UV clade suggests that it corresponds to an ultraviolet opsin (Fig. 1) [12]. Likewise, *Pgl-Rh6* may correspond to a blue-sensitive opsin [9, 13]. *Pgl-Rh1*, *Pgl-Rh2*, *Pgl-Rh3*, and *Pgl-Rh4* all fall within the "long-wavelength" clade. The exact spectral properties of these visual pigments cannot be determined without measuring the absorption spectrum of a chromophore-reconstituted opsin that is expressed in vitro, or by combining electrophysiology with in situ expression studies. Recently it has been proposed that the peak sensitivities of the violet and red receptors in *P. xuthus* are affected by the spectral properties of accessory pigments [14, 15]. Kitamoto et al. [16] have reported primary structures of three opsins and their in situ hybridization in the *P. xuthus* retina.

The possibility that one or more of the opsins identified in this paper are expressed in either the ocelli or in the brain cannot be eliminated, given the methods used to isolate these sequences (Table 1). Some *Papilio* butterflies (*P. troilus* and *P. polyxenes)* are known to possess ocelli [17]. An invertebrate rhodopsin that is ocellar-specific is known from *Drosophila* [18], and extraocular photoreceptors have been found in the brain of the beetle *Epilachna varivestis* [19]. These findings suggest that there may be more spectral classes of receptors

Table 1. Proportion of nonsynonymous and synonymous substitutions

	Papilio opsin					
	Pgl-Rh1	*Pgl-Rh2*	*Pgl-Rh3*	*Pgl-Rh4*	*Pgl-Rh5*	*Pgl-Rh6*
Pgl-Rh1	–	0.05	0.05	0.11	0.44	0.45
Pgl-Rh2	0.94	–	0.05	0.10	0.46	0.46
Pgl-Rh3	0.81	0.77	–	0.12	0.45	0.45
Pgl-Rh4	0.77	0.71	0.76	–	0.44	0.46
Pgl-Rh5	0.88	0.65	0.71	0.71	–	0.30
Pgl-Rh6	0.76	0.74	0.83	0.76	0.89	–

Proportion of nonsynonymous substitutions are given above the diagonal, and proportion of synonymous substitutions are given below the diagonal. Gaps are not included in the comparisons of 110 amino acid sites. Nucleotide and amino acid sequences used in this analysis are available from GenBank (See Fig. 1 for accession numbers). Proportions calculated using MEGA 1.01. Methods: mRNA was isolated from the head of a single female *P. glaucus*, and amplified by RT-PCR using a primer designed to a conserved region in the cytosolic loop spanning transmembrane domains V and VI (5′GANCARGCNAARA ARATGA) and 3′RACE strategy. PCR products were cloned (TA cloning kit, Invitrogen), and multiple clones were sequenced in both directions (Applied BioSystems)

in *Papilio* than are currently known. Isolation of these sequences provides excellent starting material for tissue-specific hybridization studies in combination with electrophysiology.

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