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**Paysandisia archon: Behavior, Ecology, and Communication**

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### 7.1 Introduction

The order Lepidoptera is separated into two groups: Rhopalocera (butterflies), which includes most of the day-flying Lepidoptera, and Heterocera (moths) containing most of the night-flying Lepidoptera. The period of flying activity is for most of the Lepidoptera correlated with wing pattern: diurnally active adult Lepidoptera usually bear colored and shiny wings that are used primarily for display or mimicry, while nocturnally active usually bear gray or brownish wings for camouflage, since visual communication is less suitable during night-time. Visual cues are known to be very important for day-flying Lepidoptera and several behavioral studies have reported that these cues drive mating behaviors (Hill 1991; Jiggins et al. 2001; Hernandez-Roldan et al. 2014). In moths, visual cues are considered minor stimulation and most of the environmental perceptions rely on odors.

*P. archon* belongs to the Heterocera (moth group) but it is a day-flying lepidopteran. Other day-flying Lepidoptera moths include the Zygaenidae and some Tortricidae that exhibit brightly colored wings, as perceived by human vision, but they also produce a sex pheromone (Zagatti and Renou 1984; Witzgall and Frérot 1989).

A few studies have reported on the cues used to find host plants in day-flying Lepidoptera as well as night-flying moths, although more is known for the latter group, where chemical cues are reported to steer host-plant localization. In all Lepidoptera, physical cues are also known to act during host-plant selection and during probing behavior exhibited by gravid females (Catalayud et al. 2008).

With *P. archon*, we are dealing with an original insect, newly introduced, and developing into an urban biotope that is different from that in the native area. This chapter covers intraspecific behaviors and interactions with host plants, with a focus on chemical and visual cues.
7.2 *P. archon* Reproductive Behavior

In moths, reproductive behavior follows a specific scheme with few exceptions. The adult female releases a sex pheromone produced by an epidermic gland located at the abdominal extremity between the 8th and 9th segments. Pheromone release is associated with a particular behavior characterized by abdomen extension and referred to as “calling behavior.” The pheromone is perceived at long range by the antennae of mature males that fly up to the air stream carrying the pheromone molecules. During calling behavior, the female distends the abdomen and exposes the gland part which is usually invaginated under the 7th segment. The reproductive behavior follows a diel periodicity and is related to the maturity of the insects. The courtship behavior can be very simple (Frérot et al. 2006) or very sophisticated, with a chemical dialogue between the male and females (Baker and Cardé 1979). Little is known about sexual behavior in Castniidae moths. Studies on the reproductive behavior of *P. archon* were predominantly performed to identify a sex pheromone, which could be used to monitor populations.

7.2.1 Diel Periodicity of Mating

The female calling and mating behaviors are periodic events controlled by the diel periodicity. Delle-Vedove et al. (2014) demonstrated that mating occurs under cage conditions during the daytime, for most pairs from 10:00 to 17:00 h. Observations in natura have corroborated these observations, with a mating period from 12:00 h to 15:00 h (Hamidi, personal observation).

The female can mate from the day of adult molting. Female polyandry is infrequent and was reported for only 6% of the studied pairs. After dissection of the bursa copulatrix of females collected in natura, 100% of the mated females bore only one spermatophore (Hamidi, personal observation).

7.2.2 Courtship Behavior

Detailed qualitative and quantitative descriptions of *P. archon* male and female reproductive behavior have been reported in two recent studies showing that the courtship is a sequence of stereotyped behavioral steps (Delle-Vedove et al. 2014; Riolo et al. 2014). A likely perching mate-locating behavior is performed by males; females trigger the courtship sequence by approaching perching males, which then pursue the females (Delle-Vedove et al. 2014; Riolo et al. 2014). Female courtship solicitation has also been observed in Pieridae (Rutowski 1980; Daniels 2007) and Nymphalidae (Bergman et al. 2007) species. This male activation mechanism suggested the lack of a long-range female sex pheromone, which was then confirmed by histological and electrophysiological investigations of the ovipositor (see Section 7.2.3).

A different type of mate-localization behavior was observed by Sarto i Monteys et al. (2012), in which the perching or patrolling male first locates and approaches the female. In some butterflies, one individual can perform both types of behavior or can alternate between the two, depending on ecological factors (Scott 1974; Dennis and Shreeve 1988). However, no data were reported by Sarto i Monteys et al. (2012) on the “perching index”—the proportion of copulations initiated by a sitting male relative to a flying male (Scott 1974).

The courtship sequence of *P. archon* is composed of five main steps: female flight approaching the male (the female approaches the perching male by flight); pair flight
Table 7.1 Behaviors observed in *P. archon* males and females during courtship (from Riolo *et al*. 2014).

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Main courtship sequence</strong></td>
<td></td>
</tr>
<tr>
<td>Female flight (FF)</td>
<td>The ♀ approaches the perching ♂ by flight</td>
</tr>
<tr>
<td>Pair flight (PF)</td>
<td>The ♂ chases the ♀ and both fly together</td>
</tr>
<tr>
<td>Alighting close/approaching (A)</td>
<td>The ♂ and ♀ alight facing upwards, or approach each other by walking (&lt;10 cm from each other)</td>
</tr>
<tr>
<td>Copulation attempt (CA)</td>
<td>The ♂ curls its abdomen and shows the claspers, trying to grasp the ♀ copulatory orifice</td>
</tr>
<tr>
<td>Clasping (Cl)</td>
<td>The ♂ clasps the ♀ genitalia</td>
</tr>
<tr>
<td>Copulation (Cp)</td>
<td>The ♂ and ♀ stay motionless in copula, facing upwards</td>
</tr>
<tr>
<td><strong>Other ♂/♀ behaviors</strong></td>
<td></td>
</tr>
<tr>
<td>Contact (C)</td>
<td>The ♂ or ♀ approaches the opposite sex and touches its wings with the antennae or forelegs</td>
</tr>
<tr>
<td>Head dipping (HD)</td>
<td>The ♂ dips its head under the ♀ abdomen or wings</td>
</tr>
<tr>
<td>Immobility (I)</td>
<td>The ♂ or ♀ stay motionless</td>
</tr>
<tr>
<td>Walking (W)</td>
<td>The ♂ or ♀ walk</td>
</tr>
<tr>
<td>Flying (F)</td>
<td>The ♂ or ♀ fly</td>
</tr>
<tr>
<td>Alighting distant (AD)</td>
<td>The ♂ or ♀ alight facing upwards (&gt;50 cm from each other)</td>
</tr>
<tr>
<td>Antenna cleaning (AC)</td>
<td>The ♂ or ♀ brush their antennae once or repeatedly</td>
</tr>
<tr>
<td>Ovipositor extrusion (OE)</td>
<td>The ♀ extrudes the ovipositor once or repeatedly</td>
</tr>
</tbody>
</table>

Multiple repetitions of courtship steps are engaged by both males and females. The average courtship duration was 60.99 ± 18.97 min (mean ± SE), and the longest copulation lasted 94.58 min. On average, adults mated from 0 ± 2 days of age (Riolo *et al*. 2014). Despite the stereotypy of the behavioral sequence, *P. archon* courtship is variable and complex, especially in terms of the number of components and event transitions, which also involve other optional male and female behaviors (Riolo *et al*. 2014).

Mating in *P. archon* depends basically on the success of the pair flight, which appears to be a crucial behavioral step. Sarto i Monteys *et al*. (2012) reported that the *P. archon* male wing appears to distribute a scent over the female antenna during the pair flight. Putative short-range male pheromones have been identified from the proximal halves of male forewings and hindwings. These compounds are released in a passive process associated with the flight phase of the courtship, inducing the female to alight (Sarto i Monteys *et al*. 2012).

However, during *P. archon* pair flight, the colored hindwings might serve to stimulate mate recognition, as it has been reported for several butterfly species belonging to the families Nymphalidae, Papilionidae, and Pieridae (Vane-Wright and Boppré 1993; Robertson and Monteiro 2005; Kemp 2007).
Figure 7.1 Flowchart of behavioral transition probabilities representing successful courtship sequences for *P. archon*. Diamonds (and the square, representing the final step) represent the main courtship sequence; circles represent other male behaviors, and triangles represent other female behaviors. Numbers and corresponding thicknesses of arrows (see legend) are conditional probabilities of a particular transition occurring between two behavioral acts. Transitions of < 0.20 are not included, to enhance the clarity of the figure. Descriptions and abbreviations of behaviors are listed in Table 7.1 (from Riolo et al. 2014).
Alighting at close range after the pair flight appears to represent acceptance of the *P. archon* male by the female, which has been reported as one of the functions of close-range pheromones for both moths and butterflies (Brower, Brower, and Cranston 1965; Grant and Brady 1975).

The other behaviors recorded during the courtship were: contact (the male/female approaches the opposite sex and touches its wings with the antennae or forelegs), head dipping (the male dips its head under the female abdomen or wings), immobility, walking, flying, alighting distant (the male or female alight facing upwards, >50 cm from each other), antenna cleaning (the male or female brush their antennae once or repeatedly), and ovipositor extrusion (the female extrudes the ovipositor once or repeatedly) (Riolo et al. 2014). In particular, a higher antennal cleaning frequency was observed for females. Antennal grooming enhances the sensitivity of the peripheral olfactory system (Böröczky et al. 2013) and may also enhance the female perception of volatiles both for mate and plant-host recognition (Renwick and Chew 1994).

Ovipositor extrusions were observed in *P. archon* females, but this behavior seems not to be related to calling behavior; instead, it might be involved in the female physiological state (i.e. egg load) or in thermoregulatory activity (Riolo et al. 2014). Indeed, no morphological evidence of the presence of a pheromone gland has been found on the ovipositor of *P. archon* (see Section 7.2.3).

In *P. archon* males, a particular “scratching behavior” was observed that could be related to the emission of short-range pheromone compounds from male mid-legs (Hamidi, personal observation; Frérot et al. 2013). However, male scratching was also observed when males were alone in cages, suggesting that the role of male scent is not restricted to courtship, and may be involved in searching and locating conspecific males (Delle-Vedove et al. 2014).

To investigate the role of antennal olfaction and visual stimuli in mate recognition, Riolo et al. (2014) carried out bioassays with antennectomized adults and dummies. When normal females were tested with antennectomized males, successful courtship was observed, but without copulation. However, when normal males were tested with antennectomized females, the entire courtship sequence was performed, and copulation did occur. Moreover, the males repeatedly approached (by walking) the dummy females, touched the dummy wings with the forelegs, and one copulation attempt was observed. Females were observed flying toward the male dummy and alighting close several times and touching the male dummies with the forelegs. These observations suggest that antennal olfactory stimuli have more important roles in the *P. archon* male than female during courtship, and that visual stimuli are involved in mate communication. Olfactory and visual cues might operate synergistically and help the female in her decision to accept or reject a specific male, as has been reported for different Pieridae species (Rutowski 1978; Silberglied and Taylor 1978). However, the presence of a single correct stimulus in males can be sufficient to lead females to copulation (Riolo et al. 2014).

### 7.2.3 Chemical Cues

*P. archon* has a telescopic ovipositor, which consists of the last three abdominal segments: the 8th uromere forms the ovipositor base, and the 9th and 10th uromeres are fused together, forming the true ovipositor. The 9th and 10th uromeres are connected to the base by an intersegmental membrane that is about as long as the two uromeres together. In the resting position, the ovipositor lies completely retracted beneath the abdomen. SEM images of the outer surface of the intersegmental membrane show parallel series of longitudinal grooves, and a smooth surface that is devoid of any
apertures or projections (Fig. 7.2). Histological investigations revealed that the epidermal cells of the intersegmental membrane do not have the typical features of glandular cells, as have been observed for many pheromone glands (Fig. 7.2) (Riolo et al. 2014).

These histological observations were further supported by electrophysiological analysis. A total of 24 compounds were identified from solvent extractions of virgin female ovipositors: 5 aromatic compounds, 4 alcohols, 4 aliphatic aldehydes, 4 terpenes, 4 hydrocarbons, 2 acids, and 1 ketone. None of the identified compounds elicited any significant gas chromatography-electroantennography detection (GC-EAD) responses on male antennae (Riolo et al. 2014).

The Castniidae males possess diverse and often spectacular androconia (Le Cerf 1936), which in *P. archon* are located on the extremity of the mid-legs (Frérot et al. 2013). The androconia release huge quantities of the pheromone-like compound E2,Z13-16:OH (Frérot et al. 2013). This compound was first identified by Sarto i Monteys et al. (2012) but from wing extracts, probably because of passive adsorption on the wing cuticle. This compound was previously identified in other Sesioidea, confirming the classification of *P. archon* and, by extension, the Castniid family in the Sesioidea, in this systematic group. Recently, Quero et al. (2016) confirmed the presence of large quantities of E2,Z13-16:OH in extracts from midlegs. They also reported that males *P. archon* produce a variety of other pheromone like components. Analysis of the wing extracts showed the presence of two isomers of farnesal and three different acetates were identified from the male genitalia extract, although the biological activity of all these compounds is still unknown.

### 7.3 Host-Finding and Chemical Cues

*P. archon* developed as a specialist insect on its original host plants, the palms. The real biotope from which it comes is not well defined. The species was described from Paysandú in Uruguay. The conditions in this region differ significantly from those in regions populated by the moth in Europe. The latter landscape, including new fauna and flora and associated odors, is completely different from that encountered in the native biotope. In Europe, *P. archon* is an urban and commercial nurseries lepidopteran.

#### 7.3.1 Behavior

All gravid females observed under controlled conditions or in the wild are attracted by palms and land on the upper part of the crown, never on the leaves (Fig. 7.3). There, they exhibit probing behavior that includes antennation and probing with the ovipositor. Observations using palms covered with a dark sheet to eliminate visual cues confirmed that mated females are attracted by palm chemical cues (Hamidi and Frérot, 2016).

#### 7.3.2 Antenna Morphology

The role of host-plant volatiles involved in host-plant location by *P. archon* has been reported (Ruschioni et al. 2015). The role of olfaction in *P. archon* is crucial, and is mediated mainly by antennal olfactory sensilla. Sarto i Monteys et al. (2012) and Ruschioni et al. (2015) investigated the structure of the antennae of *P. archon*, which are characterized by a basal scape, a short pedicel, and an elongated flagellum, typically swollen in its distal part and ending in the apiculus (Fig. 7.4). The absence of sexual dimorphism combined with the above-mentioned features make the antennae of *P. archon* much more similar to those of butterflies than moths (Myers 1968; Odendaal 1985; Carlsson
Figure 7.2  *P. archon* ovipositor. (a) Light microscopic dorsal view showing 8th uromere, intersegmental membrane (IM), 9th and 10th uromeres, and apodemes (Ap). (b) SEM detail of intersegmental membrane outer surface. (c, d) Cross-section of 9th and 10th uromeres at positions A and B (Fig. 7.2a). (e, f) Cross-section of intersegmental membrane at positions C and D (Fig. 7.2a). In, integument; Pr, proctodeum; Mu, muscle; Tr, trachea. Scale bar: 1 mm (a), 2 μm (b), 200 μm (c–f) (from Riolo et al. 2014).
7.3 Host-Finding and Chemical Cues

Figure 7.3 Percentage of virgin and mated female landings observed on either palm leaves or crown under wind-tunnel conditions ($n = 29$ and $n = 61$, respectively) (Hamidi and Frérot, 2016).

Figure 7.4 Schematic drawing (a) and SEM overall view (b) of an antenna of a $P. archon$ male. SC, scape; PE, pedicel; FL, flagellum; CL, club; AP, apiculus. Scale bars: 1 mm (from Ruschioni et al. 2015).

While most of the antennal surface is covered with enlarged and distally dentate scales, sensilla are located over about 20% of the total antennal surface, termed the “sensillar area.” The sensillum types occurring in this area are:

- **sensilla trichoidea**, characterized by an elongated cuticular shaft that decreases in diameter toward the apex. These sensilla show numerous dorsally located pores and are inserted into the antennal wall through an inflexible socket (Fig. 7.5a, b). Transmission electron microscopy (TEM) revealed a thick-walled sensory cuticle with two or three unbranched sensory neurons (Fig. 7.5c, d). These are the most abundant type of sensilla, occurring in relatively higher numbers in $P. archon$ males;
- **sensilla basiconica**, showing an evenly perforated sensory cuticle organized in a long, thin shaft (Fig. 7.5e, f). In this case, the 2–3 sensory neurons develop into several
Figure 7.5 Representative SEM (a, b, e, i, j) and TEM (c, d, g, k, l) images of the most abundant sensillae on the antennae of *P. archon*. (a–d) Sensilla trichoidea, showing low-magnification details (a), herringbone grooves and pores (P) (b), and cross-sections of the shaft with thick-walled cuticle pierced by pores (P) and outer dendritic segments (ODS) with three sensory neurons (c), and of the base with three sensory neurons enclosed in a common dendritic sheath (ODS) (d). (e–h) Sensilla basiconica, showing low-magnification details (e), the numerous pores (P) (f), cross-section of the shaft with the thin-walled cuticle with pores (P), and dendritic branches (DB) (g), and an oblique section of the base at the level of the ciliary constriction (CC) (h). (i–l) Sensilla auricilica, showing low-magnification details (i), the numerous pores (P) (j), cross-section of the shaft with the thin-walled cuticle with pores (P), and dendritic branches (DB) (k), and an oblique section of the base, with two sensory neurons enclosed in a common dendritic sheath (ODS) (l). Scale bars: 10 μm (a); 2 μm (d, e, h, l); 1 μm (b, g, i, k); 500 nm (c); 200 nm (f, j) (Ruschioni et al. 2015).
dendritic branches inside the shaft lumen (Fig. 7.5g, h). These sensilla are more abundant in females than males;

- sensilla auricilica have a laterally flattened cuticular shaft and the sensory cuticle is also perforated by numerous minute pores over its surface (Fig. 7.5i, j). Two sensory neurons innervate this sensillum, producing dendritic branches (Fig. 7.5k, l). Sensilla auricilica are present in both sexes, but more in female than in males;

- sensilla chaetica are very long and present in both sexes in relatively low numbers. The sensory cuticle is not perforated except for the presence of an apical pore (Fig. 7.6a). There are five sensory neurons associated with this sensillum, four of which enter the peg while the fifth ends at the articulated socket in a tubular body;

- sensilla coeloconica, present in low numbers in both sexes with no differences. They appear as grooved pegs hidden inside a cuticular cavity (Fig. 7.6b). The peg is perforated by numerous pores, and each sensillum has three sensory neurons;

- sensilla ampullacea, present in limited numbers in both sexes, with an aporous peg almost completely embedded inside the antennal cuticular wall (Fig. 7.6c).

- The antennae of the palm borer moth (PBM) are equipped with at least three different types of olfactory sensilla (i.e. sensilla trichoidea, sensilla basiconica, and sensilla auricilica). Using electrophysiological recordings, it has been shown that P. archon antennae respond positively to host-plant volatiles, but single sensillum recordings are required to determine the precise role of the different sensilla. Although P. archon belongs to a moth family, their antennae show obvious homologies with those of day-flying butterflies (e.g. no sexual dimorphism, clubbed structure, reduced sensillar area where the different sensilla are concentrated). This is of great importance in terms of the evolution of different communication and selection strategies between the two main Lepidoptera groups (i.e. butterflies (diurnal, based on visual cues) and moths (nocturnal, based on pheromone production/detection)). Identifying and understanding the mechanisms involved in the perception of host-derived stimuli would be of great benefit in the implementation of monitoring and management techniques for P. archon.
7.3.3 Chemical Cues

Locating a host plant is a crucial step in *P. archon*. In this host-location process, *P. archon* can combine semiochemical cues and physical information, such as plant color, shape, and texture. Electrophysiological studies on moths have suggested a role for plant volatiles in host-plant recognition (Ruschioni *et al.* 2015). Indeed, plant volatiles are often complex mixtures of chemicals, some of which are detected by the highly sensitive olfactory system of phytophagous insects to locate suitable host plants. As a consequence, to characterize the biologically active compounds in these mixtures, techniques such as high-resolution GC coupled with EAG are required (see Chapter 5, this volume). EAG bioassays carried out to determine the role of *P. archon* antennae in perceiving plant volatiles showed that they respond to ester and terpene compounds previously identified as volatiles of damaged and/or fermenting palm tissues. In particular, *P. archon* antennae showed responses to linalool, ethyl acetate, ethyl propionate, ethyl butyrate, ethyl isobutyrate, and ethyl lactate (Ruschioni *et al.* 2015). Linalool is a terpene alcohol found in many flowers and spice plants and one of the major compounds of palm volatiles (Knudsen, Tollsten, and Bergström 1993; Caissard *et al.* 2004). Esters, also produced by damaged and fermenting palm-tissue (Vacas *et al.* 2014), can attract other insect pests of palms, such as some palm weevil species (see Chapter 5, this volume). *P. archon* males and females both responded to these potential host-plant volatiles, but females showed higher sensitivity. In both cases, *P. archon* antennae showed dose-dependent EAG responses, with the highest ethyl isobutyrate dose of 1000 μg tested for both sexes (Ruschioni *et al.* 2015). These findings, combined with morphological and ultrastructural studies on the *P. archon* olfactory system (Sartoi Montey *et al.* 2012; Ruschioni *et al.* 2015), suggest that these potential host-plant volatile compounds can mediate the host-location behavior of *P. archon*. However, information about the chemical cues exploited by *P. archon* during the host-location process is still lacking; moreover, the chemicals tested in the laboratory are non-specific compounds that are mainly produced by fermenting tissues of infested palms. Therefore, further studies are needed to determine the roles of volatiles from infested and/or healthy palms in the field. The appropriate combination of physical traits (e.g. color, shape, and size of the host plant), and chemical sensory inputs involved in recognition of a plant as a host by *P. archon* to locate suitable oviposition sites, also need to be identified.

7.3.4 Conclusion

Results from the various analyses leave no doubt that gravid females localize and choose the host palm on the basis of olfactory cues through olfactory sensilla, especially sensilla basiconica. Plant volatile organic compounds elicit EAG responses and moths behave in wind-tunnel trials and in the field with a characteristic attraction flight. Day-flying butterflies also rely on olfactory cues to navigate toward their host plants, but other sensory modalities are involved in the final decision process.

7.4 Visual Cues: Their Roles in Mate and Host Location

The main visual organ of insects, the compound eye, is an assembly of several hundred to several thousand ommatidia. In each ommatidium, the dioptric apparatus, composed
of the corneal facet lens and the crystalline cone, directs light to the underlying rhabdom, which contains microvillar parts of photoreceptor cells with the light-sensitive rhodopsin molecules. In a single ommatidium, there are 6–12 photoreceptors that may belong to different classes of spectral sensitivity. Insect photoreceptors are often sensitive to light polarization, due to the orientation of the rhodopsin molecules in the microvilli (Wehner and Labhart 2006; Land and Nilsson 2012).

In the apposition eye, the ommatidia are optically isolated by the longitudinal sheaths of pigments. In the superposition eye, the dioptic apparatuses are separated from the photoreceptive parts by a clear zone. Each dioptic apparatus then serves adjacent ommatidia. Conversely, up to a few hundred adjacent dioptic apparatuses focus the light coming from a particular direction to a single rhabdom unit, increasing the light-catching of the photoreceptors (Stavenga 2006; Belušič, Pirih, and Stavenga 2013).

The apposition eye is suitable for diurnal activity, but it may also be used by some crepuscular and nocturnal insects (bees: Greiner, Ribi, and Warrant 2004; butterflies: Frederiksen 2008). The superposition design, on the other hand, is used by some diurnally active insects, for example the hummingbird hawk-moth *Macroglossum stellatarum* (Warrant, Bartsch, and Günther 1999), the Ascalaphus owlfly *Libelloides macaronius* (Belušič, Pirih, and Stavenga 2013), and notably the skipper butterflies of the family Hesperiidae (Horridge, Giddings, and Stange, 1972). Because the most studied families of moths (Bombycidae, Sphingidae, Noctuidae, Arctiidae) have superposition eyes and all common butterfly families (Nymphalidae, Pieridae, Papilionidae, Lycaenidae) have apposition eyes (Nilson, Land, and Howard 1988), an unfortunate false implication leads to an untested credo that, among Lepidoptera, only butterflies have apposition eyes.

The honeybee’s eye, with photoreceptors that are sensitive to ultraviolet (UV), blue and green wavelengths, is the classical model for insect eyes, but there are many variations on this scheme. A single photoreceptor may express more than a single opsin gene. A compound eye might be composed of several types of ommatidia with different photoreceptor-class allocations. The distribution of ommatidial types may be different in the dorsal and ventral parts of the eye. Butterflies with up to 10 photoreceptor classes with sexual dimorphism have been reported (Arikawa 2003; Wakakuwa, Stavenga, and Arikawa 2007; Ogawa 2013).

Apart from the compound eyes, most flying insects possess an ocellar system. This system is commonly composed of three external ocelli—simple (camera) eyes with lenses (e.g., in flies, bees, and locusts). The spectral sensitivity of the ocelli is usually dichromatic, with UV and green photoreceptors. The ocellar lenses are under-focused, so the ocelli have low spatial resolution. The ocellar system is used to estimate the head’s orientation relative to the horizon (pitch and roll angles) for the purpose of flight stabilization, exploiting the property of the visual world below the horizon, which reflects a very small amount of UV and thus creates a large contrast with the UV-enriched sky hemisphere, whereas the contrast in the green part of the spectrum is much smaller (Mizunami 1994; Taylor and Krapp 2007; Krapp 2009).

In Lepidoptera, external ocelli have been found in Noctuidae (Dow and Eaton 1976) and Sphingidae (Dickens and Eaton 1973), for example. Lense-less, internal ocelli have also been described (e.g. in the nocturnal moth *Manduca sexta*). Internal ocelli serve for circadian clocking rather than for flight stabilization (Eaton 1971).
7.4.1 Optical Design of *P. archon’s* Retina

The compound eye of the *P. archon* female measures about 3 mm laterally by 5 mm sagit-tally and contains around 10,000 facets (Fig. 7.7). The dimensions of the male eye, which has about 7000 facets, are about a fifth smaller. The diameter of the facet lenses in both sexes is approximately 25 μm. Each compound eye covers an almost hemispherical visual field; the two visual fields overlap frontally, with a dorso-frontal acute zone, and there is a posterior dead angle. The interommatidial angles vary between 1.2° and 1.5° and are possibly somewhat smaller (1.0°) in the acute zone.

Macrophotography of the *P. archon* compound eye reveals a pseudopupil pattern (Fig. 7.7a), which is a telltale sign of the apposition eye design and is otherwise encountered in bees, locusts, and some butterfly families (Stavenga 1979). The rhabdons start immediately below the crystalline cone and the retina lacks any clear zone (Fig. 7.7b). The compound eye structure of *P. archon* is typical for an apposition eye.

Although the name PBM may suggest otherwise, *P. archon* actually has eyes that are very similar in design to those of the common diurnal butterfly families. Even though this may be a surprise from a phylogenetic standpoint (Regier et al. 2013; Kawahara and Breinholt 2014), it fits well with the exclusive diurnal activity of *Paysandisia*.

![Figure 7.7](image)

**Figure 7.7** Visual system of *P. archon*. (a) The compound eye (RE, retina) and ocellus (OC) of a female moth, immobilized with beeswax (yellow mass). The compound eye has multiple pronounced pseudopupils (dark spots). (b) Semi-thin cross-section of the distal part of the retina, with (distal to proximal): cornea (C), crystalline cones (CC), primary pigment cells (PPC) and photoreceptor cells (R) showing dark stripes of perirhabdomal pigment granules. The rhabdons of the photoreceptor cells (adjacent to the perirhabdomal pigments) connect to the tip of the CC. Scale bar: 1 μm (a), 20 μm (b). *(See color plate section for the color representation of this figure.)*
7.4.2 Spectral Sensitivity of the Ocelli

*P. archon* has two ocelli with lenses that are approximately 0.5 mm in diameter. The spectral sensitivity of the ocelli, measured by electroretinography (ERG), exhibits two peaks in the UV and green parts of the spectrum (Fig. 7.8a).

A similar system with two ocelli and UV–green dichromatic sensitivity has been described for the noctuid moth *Trichoplusia ni* (Dow and Eaton 1976; Eaton 1976). We

![Graph](image)

**Figure 7.8** Spectral sensitivity of *P. archon* ocelli (a) and compound eyes (b, c) and representative spectra of the relevant environmental cues. (a) Spectral sensitivity of ocellus, measured by ERG. Data are fitted with double nomogram function with peak sensitivities at 350 and 550 nm. (b) Distribution of spectral sensitivity peaks of impaled photoreceptors, grouped in 10 nm bins. The 40 cells comprise three clearly distinguishable classes (UV peaking at 355 nm, blue peaking at 454 nm, long wavelength, LW). The peaks of the LW photoreceptors are widely dispersed across a 50 nm interval (550–600 nm) with bimodal distribution, forming two distinct classes (green-sensitive peaking at 550 nm, orange-sensitive peaking at 570 nm). (c) Spectral sensitivity of a compound eye obtained with ERG. Data are smoothed by adjacent averaging. Black curve shows sensitivity of dark-adapted retina. Pink, blue, and green curves correspond to retina adapted with UV, blue, and green light at 395, 445, and 550 nm, respectively. Chromatic adaptation reveals selective suppression of sensitivity in the three parts of the spectrum, corresponding to at least three classes of photoreceptors with peak sensitivities at 350, 450, and 550 nm. (d) Reflectance spectra of relevant visual cues. Dotted bars show average sensitivities of four classes of retinal photoreceptors. Orange curve shows the reflectance spectrum of orange scales on the inner wings. Reflectance rises monotonically above 500 nm with the steepest slope between 550 and 600 nm, coinciding with the peaks of the two LW photoreceptor classes. In addition, there is a smaller reflectance peak in the UV. The green curve shows reflectance of the host-plant leaves (*Washingtonia filifera*). The leaves have a reflectance peak in the green part (550 nm) and in the near infrared part of the spectrum (>700 nm). The gray curve shows that the reflectance spectrum of the trunk, which appears silvery-brown to us, has a flat reflectance spectrum that rises slightly toward the LW part. (See color plate section for the color representation of this figure.)
note that, although the most common form of the ocellar system has three external ocelli, a system with two external ocelli provides enough information for the estimation of flight angles. We therefore assume that the ocelli in *P. archon* are used for flight control. This may be important for the design of behavioral experiments.

### 7.4.3 Spectral and Polarization Sensitivity of the Retina

Spectral sensitivity of the compound eye, measured extracellularly via ERG, shows broadband sensitivity. Chromatic adaptation of the retina with monochromatic light at different wavelengths selectively suppresses the sensitivity in the UV, blue, and green parts of the spectrum, respectively (Fig. 7.8c). The extracellular measurements suggest that the retina of *P. archon* is equipped with at least three different classes of photoreceptors.

Intracellular measurements of photoreceptor spectral sensitivities with a sharp micro-electrode revealed that their maxima could be grouped into four classes with peaks at about 360, 460, 550, and 570 nm, respectively (Fig. 7.8b). All photoreceptor classes were sensitive to the direction of polarization of the light. The ratio of sensitivity to linearly polarized light in the preferred and non-preferred direction was about 1:2 in all spectral classes.

In *P. archon*, the basic trichromatics scheme (UV−blue−green) seems to be expanded by an additional long-wavelength-sensitive photoreceptor class. The 20 nm difference in the peaks of the two long-wavelength photoreceptor classes may seem small, but it is well above the measurement and template-fitting error. Cases of closely peaking receptor classes have been reported from several species of butterfly, for example *Pieris rapae* (Wakakuwa, Stavenga, and Arikawa 2007) and *Colias erate* (Pirih, Arikawa, and Stavenga 2010; Ogawa et al. 2013). The sensitivity difference of the two long-wavelength classes could be due to the presence of two opsin genes or to optical screening mechanisms.

### 7.4.4 Tuning of Vision to Visual Cues

The most important visual cues are located on conspecifics and host plants. The body of *P. archon* is mostly covered by brown scales, which mimic the brown color of the palm trunk and allow the animal to camouflage itself on the host plant. The inner wings have black, white, and orange scales. The wing colors are diffuse and not polarized. The reflectance of black and white scales is spectrally flat. The reflectance of the orange scales rises steadily above the minimum at 460 nm and has a smaller peak in the UV region (Fig. 7.8d). The orange spots probably play an important signaling role during courtship behavior, which consists of pronounced, rhythmical displays of the inner wing upon landing.

We may, with some caution, think of *P. archon*’s three color channels as analogous to the situation in humans, plus an additional UV channel. The peaks of human S, M, and L rod receptors are 420, 534, and 564 nm, respectively (note that the peak difference of the latter two is only 30 nm). The wavelength-discrimination ability of humans in the green−red part of the spectrum, which is predominantly based on the M and L receptor classes, increases steadily from 530 nm toward 600 nm (Zhaoping, Geisler, and May 2011).

In *P. archon*, the two long-wavelength photoreceptor classes peak at 550 and 570 nm, respectively. In this part of the spectrum, the reflectance of the orange scales rises sharply. Using the analogy with human vision, we may assume that these two
7.4 Visual Cues: Their Roles in Mate and Host Location

Photoreceptor classes are well tuned for detecting the hue of the orange wing patch. The excitation of blue-sensitive photoreceptors by the orange patch would be low, while the UV photoreceptor class might be able to detect the smaller UV reflectance peak, which is nevertheless brighter than the trunk background (Fig. 7.8d). The compound eye of \textit{P. archon} thus seems well equipped for detection of the orange patch and for determination of the orange hue.

The palm trees do not bear any specific color that can be immediately distinguishable from the surrounding vegetation. The spectral signature of leaves is mostly due to chlorophyll, with the scattering peak in the green part of the spectrum coinciding with the 550 nm photoreceptor class. The high reflectance in the near-infrared part of the spectrum is beyond the detection range of \textit{P. archon} vision (Fig. 7.8d).

The color of palm trunks is either brown or silver. Brown trunks have a monotonically rising reflectance from the short toward the long-wavelength part of the spectrum, while a silver trunk has a flat reflectance spectrum, potentially creating a motif reflecting in the UV range.

In Fig. 7.9, we give a simple simulation of the visual acuity and polarization sensitivity of \textit{P. archon}'s compound eye. An urban scene with several palm trees extends

![Figure 7.9](image_url)

\textbf{Figure 7.9} Simulation of an urban visual scene containing palm trees using the visual acuity of \textit{P. archon}. The scene extends about 90° × 60°. (a) RGB picture taken with the polarizer set horizontally to minimize sky irradiance. (b) RGB picture taken with the polarizer set vertically and down-sampled to match the optical acuity of \textit{P. archon}. (c) Polarization and intensity contrast in the red channel. (d) Polarization and intensity contrast in the blue channel. Non-polarized pixels are shown in gray; magenta and green tints indicate vertical and horizontal polarizations, respectively. Down-sampled facets span approximately 1.5°. (See color plate section for the color representation of this figure.)
about $90^\circ \times 60^\circ$ (Fig. 7.9a). The nearest palm tree is about 10 m away. The scene has been down-sampled to simulate *P. archon*’s visual acuity (Fig. 7.9b). When one looks at the picture from afar, it becomes clear that, despite the down-sampling, there is more than enough visual information to recognize palm trees with rather high certainty. In Fig. 7.9c and d, the potential polarization clues are shown. Non-polarized pixels are gray, whereas blue and magenta tints indicate polarization in the vertical and horizontal direction, respectively. While the leaves and trunks are approximately as bright as the sky background in the red channel, they stand out as being non-polarized (Fig. 7.9c). In the blue channel, the trees form a dark contrast against the sky (Fig. 7.9d). We note that the tree–sky contrast would be even stronger in the UV channel (Belušič, Pirih, and Stavenga 2013). The dry leaves form a bright stripe in the long-wavelength channel. Under direct sun illumination, the cuticular wax present on the leaves of some palm trees may additionally create spectrally neutral (white) specular reflections, which are highly polarized at grazing incidence angles (not shown). The polarization angle of specular reflections is similar to that of the sky background in that direction.

It is possible that *P. archon* uses a combination of visual clues (e.g. the trunk silhouette, the characteristic pattern of the foliage, the polarization, and the spectral signatures) to detect the palm trees. The use of true color vision (Kelber, Vorobyev, and Osorio 2003) has been shown for foraging behavior in the butterfly *Papilio xuthus* (Kinoshita, Shimada, and Arikawa 1999), which uses four out of eight photoreceptor types for this behavior (Koshitaka et al. 2008). However, the same tetrachromatic system is not necessarily used for all behavioral tasks: importantly, motion vision in insects is normally color-insensitive and based on the output from the main green–blue photoreceptor class (Kelber, Vorobyev, and Osorio 2003). In the case of *P. archon*, the neural system for detecting mates might, for instance, be based only on the blue, green, and yellow channels, while the neural system for detecting palm trees could be based on the UV and yellow channels.

### 7.4.5 Hints for Designing Visual Traps and Laboratory Experiments

If one wishes to design visual targets resembling *P. archon* wing coloration and shape, it is—for now—best to assume that all color channels are potentially used for color discrimination. If orange color is to be exploited for the construction of visual traps and lures, it should mimic well the reflectance of the orange scales. We have constructed lures by using a set of four monochromatic LED lights. In the greenhouse, the animals did not react to the presentation of the flashing orange lights, whereas they could be attracted to dead specimens glued to a wooden pole and, to some extent, to dummies printed on paper. The most straightforward explanation for the failure of the LED lure is that the wing coloration works as a proximity-recognition signal by virtue of both its color and its pattern, and not as a distance signal, based only on color. Many nocturnal insects are attracted to bright, UV-rich light. Unfortunately, *P. archon* has not been observed to fly at night, and using black light as a lure during the day does not seem feasible, even under overcast conditions.

The well-developed ocellar system in *P. archon* suggests that behavioral experiments in the laboratory should be performed in sufficiently bright ambient light, with attention to the upper visual hemisphere being rich in UV light. Given the animal’s size and preference for high temperature, we recommend that behavioral experiments be performed in warm and spacious environments. We also suggest that the experiments be performed at normal relative humidity (50–70%) to avoid desiccation shock in the animals.
References


