Spectral sensitivity of mollies: comparing surface- and cave-dwelling Atlantic mollies, Poecilia mexicana

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The visual pigments of cones and rods in three species of mollies, Poecilia mexicana, Poecilia latipinna and their asexual hybrid Poecilia formosa, were examined using microspectrophotometry. In P. mexicana, populations from extreme photic habitats were used: one population originated from a clear water habitat, one from a milky water habitat and another from a completely dark cave. Ultraviolet-sensitive cones were found in all species. Differences in the \( \lambda_{\text{max}} \) values of the visual pigments were small between species and among the three P. mexicana populations, but dark-reared cave fishes showed appreciably higher variance. The hybrid species P. formosa showed a highly variable long wavelength cone absorbance, ranging from 528.9 to 598.5 nm, suggesting multiple opsin expression or chromophore mixing.

Key words: Amazon molly; cave fishes; colour vision; microspectrophotometry; UV; video playback.

INTRODUCTION

Visual information is widely used in decision making (Bradbury & Vehrencamp, 1998). Body colour patterns in fishes can play an important role in predator avoidance (Smith, 1997), food selection (Rodd et al., 2002), mate choice (Andersson, 1994) or any combination of these factors. For example, female mating preferences can be based on male ornamentation such as the intensity of certain colour patterns, e.g. guppies Poecilia reticulata (Peters) (Long & Houde, 1989; Kodric-Brown, 1993; Endler & Houde, 1995; McKinnon, 1995; Houde, 1997), cichlids (Hert, 1989), pipefishes (Berglund et al., 1986) and three-spined stickleback Gasterosteus aculeatus L. (McLennan & McPhail, 1990; Milinski & Bakker, 1990; Rowe et al., 2004).

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In poeciliids, visual mating preferences of females have been studied intensively (Farr, 1989). Furthermore, several studies on mate choice of poeciliid females have been carried out using video stimuli (Rosenthal et al., 1996; Rosenthal & Evans, 1998; Körner et al., 1999; Landmann et al., 1999; Nicoletto & Kodric-Brown, 1999; Plath et al., 2003a). This technique can be used to manipulate colour and other signal properties (Rowland et al., 1995), but because video is designed to match the human visual system, colour manipulation without precise knowledge of the physiology of colour vision of the test animal can be problematic (Fleishman et al., 1998; Oliveira et al., 2000). To understand how colour patterns relate to behaviour, the visual pigments in a group of mollies (Poecilia: Mollienesia), the Atlantic molly Poecilia mexicana Steindachner, the sailfin molly Poecilia latipinna (LeSueur) and the Amazon molly Poecilia formosa (Girard) were studied.

Among poeciliids, visual pigments have been studied most intensively in the guppy (Levine & MacNichol, 1979; Archer et al., 1987; Archer & Lythgoe, 1990; Archer & Hirano, 1997), and it seems likely that guppies are capable of colour vision. So far, little is known about the visual pigments in other members of the group. Poecilia mexicana, P. latipinna and P. formosa mainly inhabit shallow freshwater habitats in Mexico and the southern U.S.A. (Miller, 1983; Schlupp et al., 2002). They differ in the degree of use of visual courtship. Sailfin molly males produce a number of visual signals, including colour patterns and courtship in front of the female. The Atlantic molly seems to be less ornamented and no courtship has been described for this species (Parzefall, 2001). These differences appear to be reflected in several aspects of their mating system, e.g. the degree and the effect of sexual harassment (Schlupp et al., 2001; Plath et al., 2003b). This study investigated if such differences might be reflected in the composition of female visual pigments. More specifically, two aspects of molly ecology were used as background for this study: a cave-dwelling population of the Atlantic molly was used to study the fate of visual pigments during cave evolution and a natural hybrid of P. mexicana and P. latipinna, P. formosa, was used to investigate the role of hybridization in this system.

Cave Mollies

In Tabasco, Mexico, a population of P. mexicana successfully colonized a limestone cave (Gordon & Rosen, 1962). The cave form of P. mexicana differs from the surface form in some behavioural (Walters & Walters, 1965; Parzefall, 2001; Plath et al., 2003b, 2004) and morphological (Parzefall, 2001) traits. For example, the retina is flattened and the outer part of the cones is reduced in size (Peters et al., 1973). Although the cave form lives in complete darkness, these fish have functional eyes (Plath et al., 2003a, 2004), which are only slightly reduced in size (Peters et al., 1973). Thus, Peters et al. (1973) suggested that this population is phylogenetically young. Theory predicts that eyes will eventually become reduced by regressive evolution. Indeed, eyelessness appears to be a hallmark of cave-dwellers (Wilkens, 1988; Weber et al., 1998). Since the cave population of P. mexicana has evolved some progressive adaptations and regressive modifications during the colonization of its subterranean habitat, it might be expected that the colour vision system of the cave population also
showed signs of regressive evolution. The effects of living in habitats with reduced amounts of, or no, light on the evolution of the visual system in fishes have been studied mostly in deep-sea fishes (Clarke, 1936; Munz, 1958; Bowmaker et al., 1988; Partridge et al., 1988; Bayliss et al., 1996; Collin, 1997; Douglas et al., 1998). Only a few studies examined visual pigments and their genetic basis in cave-dwelling fishes (Yokoyama & Yokoyama, 1990; Langecker et al., 1993; Yokoyama et al., 1995; Jeffery, 2001; Parry et al., 2003), focusing only on the blind Mexican tetra Astyanax fasciatus (Cuvier). To study whether reduced amounts of light in their natural habitats affect the evolution of visual pigments in mollies, the absorption characteristics of the visual pigments of surface-dwelling P. mexicana from a clear water habitat were compared with those of a population inhabiting a milky, sulphurous creek, where light penetrates only the upper few centimetres of the water column. Finally, the visual pigments of the cave form of P. mexicana were examined to test whether the permanent absence of light leads to regressive evolution of the visual pigments.

AMAZON MOLLIES

Another important feature of the mating systems of P. mexicana and P. latipinna is in their ‘sexual parasite’, P. formosa. The Amazon molly is a unisexual, all-female hybrid species (Hubbs & Hubbs, 1932), with P. mexicana being the maternal ancestor and a P. latipinna-like fish being the paternal ancestor (Avise et al., 1991; Schartl et al., 1995). Males of the latter two species serve as sperm donors for the gynogenetic reproduction of P. formosa. It was predicted that the visual system of P. formosa would share characteristics of both parental species.

Specifically, the following questions were asked: Do the visual pigments differ between P. mexicana and the closely related P. latipinna? Do the absorption maxima of the visual pigments differ between P. mexicana from a clear water habitat and that from a milky water habitat? Are there signs of regressive evolution of the visual pigments in the cave form of P. mexicana? For example, do absorption maxima show increased variability because stabilizing selection is absent? Do visual pigments differ between the hybrid species P. formosa and its two parental species?

MATERIALS AND METHODS

Poecilia mexicana from three adjacent but separated populations were collected in South Mexico near Tapijulapa (Tabasco) between 1990 and 1995 (Plath et al., 2003b, 2004). Visibility conditions differ vastly between these habitats. One population inhabits a mostly clear river, the Rio Oxolotan (17°26' N; 92°46' W). Another surface-dwelling population inhabits a milky creek, the Arroyo del Solpho, flowing out of a limestone cave, the Cueva del Azufre. In this habitat, the water is murky due to a high amount of colloidal sulphur. The third population originated from the deepest, isolated cave chamber (chamber XIII according to Gordon & Rosen, 1962). Poecilia latipinna and P. formosa were collected in the San Marcos River in Texas (26°07' N; 97°52' W) in 1996. Poeciliids typically do not migrate far, so it is very likely that they live under the local light conditions throughout their lives. The individuals collected in the field were used as a foundation for laboratory stocks, which provided individuals for this study. The fishes were maintained as randomly outbred large aquarium stocks at 20–22° C. They were fed twice a day ad libitum with commercially available flake food,
supplemented by live food. All fishes were raised indoors with absence of UV light under standard ‘white’ fluorescent light. All surface-dwelling fishes were kept under a 12L:12D cycle. The cave population was divided into two lineages: one was maintained under the normal light and dark cycle and the other in permanent darkness. Up to six adult females from each species, populations or lineages were used for this study.

Measurements of the absorption characteristics of cones and rods were conducted at the Cornell University, Ithaca, NY, U.S.A. The fishes were kept in complete darkness for at least 24 h before sacrifice to avoid bleaching of the visual pigments. The dark-adapted eyes were removed and stored in Puck’s Saline F in darkness at c. 8°C for at least 2 h (normally overnight). Then, the eyes were hemisected and the retinas were removed and placed into Ringer’s solution (pH 7.2) (all under infra-red light, with the aid of an infra-red image converter). Small pieces of retina were transferred to a cover slip and macerated to isolate single cells using razor blades and fine tungsten needles. A second cover slip rimmed with silicone grease was placed over the first, creating a sealed ‘sandwich’. This was placed in a holder and transferred to the stage of a single-beam, computer-controlled microspectrophotometer (Loew, 1994). A 100 W tungsten–halogen lamp together with quartz/fluorite optics allowed for accurate absorbance measurement down to 350 nm, with a rectangular measuring aperture of 1 × 3 μm. The selection criteria used for data inclusion into the \( \lambda_{\text{max}} \) analysis pool were the same as those used by Loew (1994). For those curves meeting the selection criteria, the \( \lambda_{\text{max}} \) of the smoothed, normalized visual pigment absorbance spectrum was obtained using the method of Mansfield (1985) as presented by MacNichol (1986). The templates used were those of Lipetz & Cronin (1988). In the case of the long wavelength-sensitive (LWS) receptor, the curves were often broader than normally encountered in cones containing a single visual pigment, suggesting visual pigment mixing as already described above for \textit{P. reticulata} (Loew, 1995). This could be explained by either A1/A2 chromophore mixing or coexpression of two opsins in the same cell. Both mechanisms would lead to absorbance spectra broader than that for a single visual pigment.

This increased breadth was seen as an increased s.d. around the estimated \( \lambda_{\text{max}} \). Assuming visual pigment mixing, it is technically not correct to report a single \( \lambda_{\text{max}} \) for these receptors. Results for the LWS cones as a single \( \lambda_{\text{max}} \) are reported, however, with the caveat that it might represent absorption by more than one visual pigment.

**RESULTS**

Representative spectra from the clear water population of \textit{P. mexicana} are shown in Fig. 1, along with appropriate template overlays. In each group examined (Table I), four spectral classes of cones, including cones that are most sensitive for UV wavelengths, were detected. In \textit{P. latipinna}, a UV-sensitive single-cone type was found, but its \( \lambda_{\text{max}} \) value could not be determined precisely. Throughout, short-wave receptors (UV and violet) occur as single cones. The two other spectral classes (blue and yellow) occur as double cones (blue plus yellow) and identical (same visual pigment in both members) and non-identical twin cones (two slightly different \( \lambda_{\text{max}} \) values in each member; Loew & Lythgoe, 1978). Generally, the \( \lambda_{\text{max}} \) values are similar between \textit{P. mexicana} and \textit{P. latipinna}. The absorption maxima of the ‘yellow’ long wave receptors, however, are to some extent higher in \textit{P. latipinna}.

The \( \lambda_{\text{max}} \) values of the cones from the \textit{P. mexicana} milky water population were slightly shifted towards shorter wavelengths compared with those from the clear water population, except for the UV-sensitive cone type, where a shift towards maximum absorption at longer wavelength was found (Table I). Only one UV-sensitive cone, however, was found in the milky water population. The
\(\lambda_{\text{max}}\) values of the cave-dwelling population of \(P. \text{mexicana}\) are shifted to both directions, alternately. The four groups of \(P. \text{mexicana}\) differed in the variance of the \(\lambda_{\text{max}}\) values, with the highest variance in the dark-reared lineage of the cave form (Table I). The yellow-absorbing LWS receptor was found to be highly variable in all examined species.

**DISCUSSION**

Colour vision can serve a number of adaptive functions, \textit{e.g.} food detection, species recognition, sex recognition and perception of courtship displays. The presence of more than one spectral class of photoreceptor, usually cones containing different visual pigments, is a prerequisite for colour vision. Four cone types were found in all the three species of mollies. Three of those respond to
### Table I. Mean ± s.d. absorption maxima (λ_max) of the rods and cones of three species of the genus *Poecilia*. The number (n) of measured cells is given in parentheses

<table>
<thead>
<tr>
<th>λ_max</th>
<th><em>Poecilia mexicana</em> (clear water)</th>
<th><em>Poecilia mexicana</em> (milky water)</th>
<th><em>Poecilia mexicana</em> (cave, light)</th>
<th><em>Poecilia mexicana</em> (cave, dark)</th>
<th><em>Poecilia formosa</em></th>
<th><em>Poecilia latipinna</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod Green</td>
<td>503.9 ± 2.8 (11)</td>
<td>502.8 ± 4.0 (11)</td>
<td>504.3 ± 2.0 (11)</td>
<td>503.5 ± 4.4 (31)</td>
<td>503.8 ± 4.1 (13)</td>
<td>502.6 ± 2.5 (28)</td>
</tr>
<tr>
<td>Cone UV</td>
<td>349.5 ± 2.6 (8)</td>
<td>366.3 (1)</td>
<td>—</td>
<td>373.7 (1)</td>
<td>352.4 ± 1.2 (4)</td>
<td>*</td>
</tr>
<tr>
<td>Violet</td>
<td>402.7 ± 4.0 (14)</td>
<td>400.1 ± 2.7 (5)</td>
<td>400.8 ± 1.3 (6)</td>
<td>404.2 ± 7.6 (3)</td>
<td>404.2 ± 4.7 (36)</td>
<td>403.0 ± 2.4 (3)</td>
</tr>
<tr>
<td>Blue</td>
<td>461.4 ± 3.3 (27)</td>
<td>458.3 ± 2.7 (6)</td>
<td>463.6 ± 3.1 (14)</td>
<td>455.9 ± 6.4 (3)</td>
<td>462.8 ± 4.5 (49)</td>
<td>463.3 ± 2.7 (10)</td>
</tr>
<tr>
<td>Yellow 1</td>
<td>536.6 ± 4.7 (35)</td>
<td>529.8 ± 4.3 (12)</td>
<td>534.9 ± 7.6 (24)</td>
<td>532.3 ± 5.7 (11)</td>
<td>560.3 ± 16.1 (125)</td>
<td>551.2 ± 5.9 (6)</td>
</tr>
<tr>
<td>Yellow 2</td>
<td>563.0 ± 3.0 (1)</td>
<td>—</td>
<td>—</td>
<td>572.2 ± 5.0 (5)</td>
<td>—</td>
<td>**</td>
</tr>
</tbody>
</table>

—, cell type could not be found; *, cell type was found but its λ_max value could not be determined precisely; **, in *P. formosa*, the long wavelength receptor could not be divided in two classes, its continuous distribution ranges from 528.9 to 598.5 nm.
wavelengths in the spectrum visible to the human eye, and one lies in the UV region of the spectrum. Ultraviolet vision is not uncommon in fish species (Carleton et al., 2000), especially in marine species (Losey et al., 1999). Ultraviolet light can be present down to 100 m in oceanic waters (McFarland, 1986) and is present in reasonable amounts in shallow freshwater habitats (Bowmaker, 1990; Loew & McFarland, 1990). Ultraviolet vision apparently plays a role in mate choice in guppies (Kodric-Brown & Johnson, 2002; Smith et al., 2002; White et al., 2003), but a similar role in P. mexicana was not found (M. Waschulewski, K. Lunau, P. E. Nelson, J. Zamzow, J. Parzefall & I. Schlupp, unpubl. data). In another group of poeciliids, UV signals are important in predator–prey interactions and in sexual signalling (Cummings et al., 2003). This is particularly interesting because here UV provides a private channel for Xiphophorus nigrensis Rosen. Since typical video playback uses only three colours to create colour images, the presence of four cones indicates that colour on the screen will be misrepresented for mollies as UV is absent. Because of this, the use of video playback to study colour is not recommended for mollies (D’Eath, 1998; Fleishman et al., 1998; Oliveira et al., 2000). In general, interspecific differences (P. latipinna v. P. mexicana) in the absorption characteristics of cones and rods and differences among populations are small. Small differences like this might not have a significant effect on quantum capture at a specific wavelength (Dartnall, 1965).

CAVE MOLLIES

The visual pigments of the P. mexicana populations from habitats with different visual conditions (clear water, milky water and cave) did not differ strongly from each other. In the milky water population, only a marginal shift towards absorption at shorter wavelengths was found in rods and in most cones, while the UV receptor was shifted towards absorption at considerably longer wavelengths.

The similarity of the $\lambda_{\text{max}}$ values of the two cave lineages, raised in light or darkness, indicates genetic fixedness of the absorption characteristics of the visual pigments. Higher variance in the dark-raised lineage, however, suggests an ontogenetic effect on the development of the visual system. The functional- ity of the cave molly’s eyes including the visual pigments may indicate young phylogenetic age. Alternately, the visual system may be maintained because it is linked to a trait still under sexual selection (Plath et al., 2003a). Crandall & Hillis (1997) found genes coding for rhodopsin to be still functional in eye- less, cave-dwelling crayfishes. These findings indicate a physiological function of rhodopsin besides absorption of photons. The ‘yellow I’ receptors, however, show considerably higher variance in light-reared, cave-dwelling P. mexicana than in surface-dwelling ones. This finding possibly points to an onset of regressive evolution of this receptor type, whereby the variance of a trait may increase due to the absence of stabilizing selection (Wilkens, 1988).

The long wavelength yellow cones generally showed relatively high variance in all species examined, suggesting mixtures of more than one visual pigment. High $\lambda_{\text{max}}$ variance of the long wavelength cone was also found in the guppy (Archer et al., 1987; Archer & Lythgoe, 1990) and was explained by assuming
coexpression of two different opsins yielding mixtures of two visual pigments with $\lambda_{\text{max}}$ values at 533 and 572 nm. Like the guppy, *P. mexicana* males show yellow–orange ornaments. Colouration in *P. mexicana*, however, is not as variable as in guppy males (Houde, 1997).

**AMAZON MOLLIES**

*Poecilia formosa* is a hybrid species, with *P. mexicana* as maternal and *P. latipinna* as paternal ancestor. Not surprisingly, the yellow pigments show intermediate absorption characteristics in *P. formosa*, ranging from the $\lambda_{\text{max}}$ value of the shorter yellow pigment of *P. mexicana* to the longer yellow pigment of *P. latipinna*. *Poecilia formosa* lives in syntopy with either *P. mexicana* or *P. latipinna* and copulates with males of both species to obtain sperm for their gynogenetic reproduction. The *P. formosa* yellow 1 pigment ranges from 529 to 599 nm and has a very high S.D. It seems possible that these fish have a shorter wavelength yellow 1 pigment and an additional pigment, with a peak at c. 560 nm, potentially representing a mixture of this and a longer wavelength yellow 2 pigment. This, however, warrants further study. The widened range of the absorption of the yellow-absorbing pigments between *P. formosa* and its two paternal species predicts differences in the ability to discriminate among yellow ornaments. For example, the female preference for an orange ornament in *P. latipinna* males differed between *P. latipinna* and *P. formosa* females (Schlupp et al., 1999). In this study, females of *P. latipinna* significantly preferred a male with an orange ornament, whereas females of *P. formosa* showed no preference. This had been discussed in the context of a pre-existing bias, but an alternative explanation would be to assume that differences in the visual pigments are responsible for the difference in responses. In another experiment, Schlupp et al. (1999) found significant but opposite responses to a red ornament: *P. mexicana* females showed a significant preference for the red ornament, but *P. formosa* significantly preferred males without an ornament. In this case, both *P. mexicana* and *P. formosa* were able to detect the red ornament. Although the differences in the colour vision system among the species are small, they may lead to different mating preferences or different accuracy of mating decisions among the species.

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