Identification of Androgen Induced Pigments in Guppies (*Poecilia reticulata*)

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Introduction

Animals display secondary sexual characteristics including gaudy color patterns to attract their mates. Similarly, female guppies (*Poecilia reticulata)* reported to prefer males with orange spots in their integument for mate selection. These phenomena are considered as having an evolutionary origin (Rodd *et al*., 2002). The common consensus regarding the origin of this orange and red color spots is that the "sexy orange chroma" is mainly formed by the dietary carotenoids, which are not synthesized in the body. Thus the ability to compete in a carotenoids limited environment is thought to be a factor for the evolution of attractive and strong mates that can attract their female counterparts. However, recent evidence suggests that in addition to the carotenoids, drospoterine (red pteridine), which could be synthesized *de novo* is also found in red and orange spots of guppy (Grether *et al*., 2001). But recent evidence suggests (Jayasooriya *et al*., 2002) that the secondary sexual coloration of guppies could also be based on the androgen status rather than the availability of dietary carotenoids or foraging ability of carotenoids rich sources. This idea contradicts some speculations that the female preference for male with red/orange pigments has been evolved through the ability of foraging of rare but carotenoid rich orange colored-fruits, which occasionally fall into streams from rainforest canopy in Trinidad (Rodd *et al*., 2002).

Materials and methods

One-day-old guppy fries obtained from the same brood stock were divided into two cohorts (n=15) and were reared on a 'TEST' feed (Feed added with synthetic analogue of testosterone at a concentration of 250 mg/kg of feed) and a 'CONTROL' feed (Same feed without testosterone analogue). Thus the fries in TEST group were exposed to high concentration of androgen from the day one of their life. Other than that both cohorts were maintained with same conditions. The fries in TEST group started to develop red color by one week. Whereas, those in CONTROL group did not develop any coloration by that age. By the age of three weeks, equal number (n=10) of fish from each group was subjected to extraction of both pigments from their color spots by using methods as previously explained (Grether *et al*., 2001).

Pigments were identified using UV spectrophotometry (GBC Cintra 6, Italy). Absorption spectra were obtained for both extracts from 'TEST' and 'CONTROL' groups. These were compared with the absorption spectra for standard samples of two pigments extracted from natural samples, i.e. extracts of eyes of fruit flies (*Drosophila melanogaster*) for drosopterine and carrots (*Daucus carota*) for carotenoids. Absorption spectra for carotenoids and drosopterine were recorded within following UV absorption wavelengths. For carotenoids the range was 350-550 nm, whereas, for the pteridines, the range was 400-600 nm.

Results

In the case of detection of drosopterine, the sample from CONTROL fish (Figure 1C) did not give an absorption spectrum within 400-600 nm, indicating the fact that the control sample is devoid of drosopterine. Interestingly, extracts from fruit fly eyes and TEST group (Figures 1A and 1B) gave an absorption spectra within that range. In both samples, peak absorption was between 478-495 nm, indicating the presence of drosopterine in the androgen induced red spots in guppy. The standard carotenoid samples extracted from carrot (Figure 1D) showed an absorption spectrum with a peak absorption between 437-446 nm, which is reported to be the standard specification for carotenoids. However, neither the extracts from CONTROL (Figure 1E) nor TEST (Figure 1F) groups gave absorption spectra. This shows that the test and control samples did not contain detectable levels of carotenoid pigments.

Discussion

These results indicate that the androgen could induce the de novo synthesis of red pigments in

guppies and they do not need to depend on dietary carotenoids to express their secondary sexual characteristics. Thus, female's preference for red spots may have been triggered by drosopterine, which could be an indication of male quality. These results warrant the further consideration of role of carotenoids on the determination of sexual coloration and mate preference.

Conclusions

Our data indicate that the 'sexy phenotype' is independent of the diet and may reflect the androgen levels in the body rather than gluttony for carotenoids.

References

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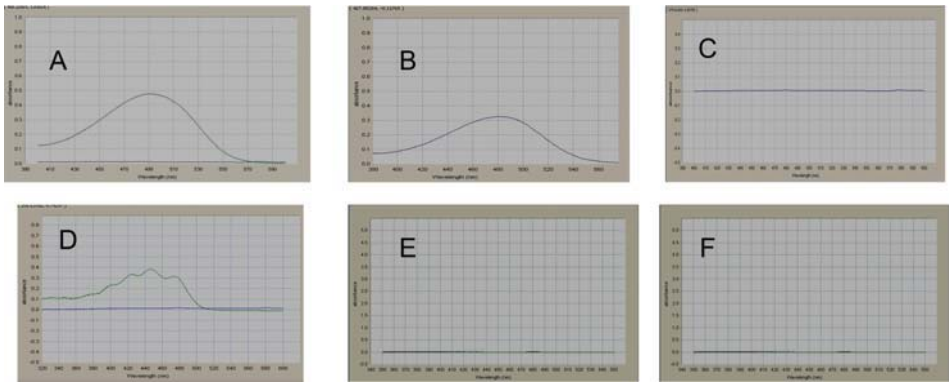

Figure 1. UV spectrograms recorded from the samples extracted for analysis of pteridine (A: Drosophila eyes, B: fries of TEST group, C: fries of CONTROL group) and for analysis of carotenoid ((D: Carrots, E: CONTROL group, F: TEST group)