

Receiver-bias implicated in the nonsexual origin of female mate choice in the pentamorphic fish *Poecilia parae* Eigenmann, 1894

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Abstract. Receiver-bias hypotheses of signal evolution posit that male sexually selected traits evolve via prior selection for other functions. We found support for the hypothesis that the origin of female choice of mates is a linked effect of a receiver-bias for carotenoid coloration favored in the context of efficient food detection. Adult pentamorphic livebearing fish or pentas (*P. parae*) nibbled significantly more often at orange, red, and yellow discs than at green, blue, white, and black discs, outside a mating context. This innate attraction to carotenoid colored discs was positively correlated with female preferences for red and yellow *melanzona* males, and for novel red ornaments in fins of an *immaculata* male tested against typically uncolored *immaculata*. Furthermore, preference for carotenoid colored discs was absent in one ancestral taxon, and yet mapped onto a poeciliid phylogeny as ancestral. Overall these results suggest a strong association between a potential trigger of a mate choice preference and a sexually selected trait, thereby corroborating the receiver-bias hypothesis for carotenoid coloration independent and dependent of the assumptions of phylogenetic inference.

Key Words: carotenoid coloration, female mate choice, phylogeny, pleiotropic effect, poeciliid fishes, sensory-bias.

Resumen. Una de las principales hipótesis referentes a la evolución de señales en el contexto de parcialización preferencial por parte del receptor, señala que los caracteres masculinos sexuales evolucionaron anticipadamente para cumplir funciones distintas. En este estudio, nosotros demostramos que el origen de la elección de parejas por parte de las hembras es un efecto ligado a la parcialización preferencial por coloración carotenoidal que es favorecido en el contexto de eficiencia en forrajeo. Los adultos de los peces pentamórficos en *Poecilia parae* tienden a mordisquear en una mayor proporción discos naranjas, rojos y amarillos en comparación a discos verdes, azules, blancos y negros fuera del contexto de apareamiento. Esta atracción innata a colores carotenoidales representados en los discos fue positivamente correlacionado con las preferencias de las hembras por machos que presentan coloración roja y amarilla, y por los adornos rojos raros que están presentes en las aletas de un macho *immaculata* que fue presentado en el experimento con otro macho que típicamente no presenta coloración alguna. Más aun, la preferencia por los discos de colores estuvo ausente en una de los grupos ancestrales, pero que han sido mapeados en la filogenia de poecílidos. Nuestros resultados sugieren una fuerte asociación entre una acción potencial de la preferencia de elección de pareja y un carácter sexualmente selectivo, esto consecuentemente corroborando la hipótesis de parcialización preferencial por colores carotenoidales independientes and dependientes de las presunciones de inferencia filogenética.

Palabras claves: Coloración carotenoidal, elección de pareja, filogenia, efecto pleiotropico, peces poecílidos, parcialización sensorial.

Rezumat. Ipoteza evoluției prin exploatare senzorială spune că trăsăturile sexuale masculine evoluează pe seama selecției lor inițiale pentru alte funcții. Am găsit date care să susțină ipoteza că originea preferinței femelei pentru partener este legată de o tendință de a accepta favorizarea colorației carotenoidice mână în mână cu o eficiență detecție a hranei. Adulții de pești penta (*P. parae*) au ciugulit semnificativ mai mult discurile de culoare portocalie, roșie și galbenă decât pe cele verzi, albastre, albe și negre, înafara unui context sexual. Această atracție înăscută către discurile de culorile amintite a fost pozitiv corelată cu preferința femelelor pentru masculii cu tiparele coloristice *melanzona* roșu și galben, iar în cazul de noi ornamente roșii din înotătoarele masculilor cu tiparul *immaculata* au testat împotriva tipicului mascul *immaculata* necolorat. Mai mult, preferința pentru discurile de culoare carotenoidică au lipsit în cazul unui taxon ancestral, care a fost deja cartat (confirmat) din punct de vedere filogenetic ca fiind ancestral. Per ansamblu, rezultatele sugerează o strânsă asociere între potențialul de atragere a unui partener preferat

și un caracter de selecție sexuală și în consecință, coroborarea ipotezei exploatării senzoriale pentru colorația carotenoidică independentă și dependentă de predicțiile inferenței filogenetice.

Cuvinte cheie: colorație carotenoidică, preferința sexuală pentru mascul a femelei, filogenie, efect pleiotropic, pești poeciliizi, exploatare senzorială.

Introduction. An understanding of the origin and evolution of mating preferences and the cues that trigger these preferences is a major aim of behavioral ecology. One attractive avenue for elucidating the origin and evolution of mating preferences is testing receiver-bias hypotheses of trait evolution which posit that male sexually selected signals evolve through prior selection for other functions; however only the models of Ryan (1990), Ryan & Rand (1990), Endler (1992), and Endler & Basolo (1998) indicated that the origin of a preference and the origin of the sexually dimorphic trait are evolutionarily decoupled. Thus, selection pressures other than a mating response to a novel trait are implicated in the evolution of the receiver's information processing system (Shaw 1995; Schlupp et al 1999; Smith et al 2004). In the context of mate choice, in which females are usually the choosing sex, the receiver-bias or sensory exploitation model predicts that sexually selected traits in males are those that are most conspicuous to the information processing system of females (Smith et al 2004; Garcia & Ramirez 2005). Moreover, Boughman (2002) indicated that receiver-bias models may be important to the process of speciation mediated by female choice, and Kokko et al (2003) suggested that these models are central to understanding the evolution of mate choice.

In addition to investigations in sexual selection which demonstrate that females have preferences for traits that are not exhibited by conspecific males (Basolo 1990; Ryan 1997, 1998), other sources of support for the receiver-bias model come from two empirical studies of foraging behavior (Rodd et al 2002; Smith et al 2004). The sexual preference of female guppies (*Poecilia reticulata*), and three-spined stickle backs (*Gasterosteus aculeatus*) for males with orange spots and red throats respectively, are explained by the idea that orange and red coloration resemble the colors of food (Rodd et al 2002; Kokko et al 2003; Smith et al 2004). Observations of guppies foraging on fruits rich in carotenoids in Trinidad led Rodd et al (2002) to propose, test, and corroborate the hypothesis of a nonsexual origin of the female mate preference. They also showed that the visual system of guppies is tuned to preferentially detect orange food items. Rodd et al (2002) used colored discs to test female and male guppy attraction to colors by recording approaches to and nibbling at the discs. Their study prompted us to test the pleiotropic sensory-bias hypothesis on pentas (*P. parae*), a syntopic (Liley 1966), and close relative of the guppy (Breden et al 1999).

In Guyana, both sexes and juvenile pentas eagerly devoured the red-, yellow- and orange-colored fruits of black sage (*Cordia macrostachya*), Boraginaceae, and bura-bura (*Solanum stramonifolium*), Solanaceae, that fell in their streams and ditches. These made up 45-72% of the fishes diet by volume during fruiting seasons (G. R. Bourne unpubl. data). Black sage and bura-bura shrubs grow on disturbed sites, especially along ditch- and stream-banks (Graham 1963; Myint 1994), and coincide with the distribution of four species of syntopic poeciliids, pentas, guppies, *P. picta*, and *P. vivipara* in coastal Guyana (G. R. Bourne pers. obser.). The black sage drupe and bura-bura berry may be good sources of proteins, sugars, carotenoid and other dietary pigments (McGraw 2005), and were consumed by many species of fishes, birds, and bats (G. R. Bourne pers. obser.). The expression of many ornamental traits depends on carotenoids that animals cannot synthesize *de novo* (Brush 1990; McGraw 2005; Maan et al 2006), and can only be obtained through ingestion (Olson & Owens 1998; McGraw 2005). Moreover, carotenoids are antioxidants and immunostimulants (Britton 1995; Grether et al 2003; McGraw 2005; Maan et al 2006), with a tradeoff between carotenoid allocation for maintaining health versus enhancing ornamentation (Negro 2002; Grether et al 2003; McGraw 2005). Thus, when females exhibit preferences for males with the most intense carotenoid coloration they are choosing mates with strong immune systems (Blount et al 2003; McGraw 2005), and in fact these colorful traits are honest signals (Garcia & Ramirez 2005; McGraw 2005; Maan et al 2006).

Pentas exhibit Y-linked sexually selected color polymorphism while another close relative *P. picta* also has discrete but fewer color morphs (Lindholm et al 2004). The guppy, on the other hand, exhibits almost continuous color variation (Haskins et al 1961; Petrescu-Mag & Bourne 2008). Therefore, this clade (*sensu* Breden et al 1999) presents us with opportunities for exploring the role of sexual selection in the maintenance of male color polymorphisms (Lindholm et al 2004). Female guppies prefer males with orange spots as mates, and the origin of this preference was recently determined to result from a pleiotropic effect of selection in a foraging context (Rodd et al 2002; see also Smith et al 2004).

Four criteria are proposed to demonstrate that a male trait evolved because of female receiver-bias: (1) female choice relies on heritable variation in the trait; (2) there is a bias in the psychosensory system that matches the direction of the preferences; (3) the trait must be absent or expressed in a primitive form in ancestors; and (4) preference for the trait is ancestral (Basolo 1990, 1995; Ryan 1997; Endler & Basolo 1998). Our aim was to determine whether pentas have a preexisting receiver-bias for carotenoid coloration by testing the hypothesis that the origin of female preference in pentas is also a linked effect of a receiver-bias for carotenoid coloration that might have arisen in the context of more efficient food detection (Rodd et al 2002). Since we did not have access to the expensive equipment for evaluating the visual system of the penta our tests were based on the assumption that the penta visual system was similar to that of its syntopic close congener for which visual physiological details are known (Rodd et al 2002). Moreover, Alexander & Breden (2004) and F. Breden (pers. comm.) indicated that molecular structure of opsin genes, expressed in the photoreceptor cells in retinas of guppies and pentas are similar, sharing at least one Short Wave-Sensitive, SWS1 (UV sensitive), two SWS2 (blue sensitive), and two RH2 (green sensitive) opsins, while the Long Wave-Sensitive (LWS; carotenoid sensitive) subfamilies, LWS_P180, LWS_S180r and LWS_A180 opsins are also shared. We made this assumption because pentas eagerly ate orange-, red-, and yellow-colored fruit and petals which constituted 45-72% of their diets at certain times of the year (G. R. Bourne unpubl. data). Several experimental approaches were taken to test two predictions generated by the aforementioned hypothesis about the foraging and mating preferences of pentas. First, males and females should nibble more often at carotenoid colored (orange, red, yellow) discs outside a mating context. Second, there is a correlation between the number of nibbles directed at a carotenoid colored disc by a female and the time she spent in front of a male with carotenoid coloration. Third, female preferences for naturally occurring novel carotenoid colored fin trait in the uncolored *immaculata* morph provided additional support for the receiver-bias hypothesis independent of the assumptions of phylogenetic inference (Schlupp et al 1999). Fourth, by mapping the trait of preferences for carotenoid coloration in a feeding context onto the molecular phylogeny of Breden et al (1999), we demonstrated that the trait was ancestral to the ingroup *Poecilia*. Finally, we found that attraction to carotenoid coloration was absent in the outgroup taxon, mosquito fish (*Gambusia affinis*), used by Reznick et al (2002) to root their phylogram of fish in the subgenus *Poeciliopsis*.

Materials and Methods. Study Species: The field and laboratory experiments analyzed here for color and mate preferences were performed on pentas (Cypriniformes: Poeciliidae), omnivorous livebearing fishes with variously colored males, distributed among coastal fresh- and brackish-water streams east of Venezuela, in Guyana, Suriname, French Guiana, and Brazil to the Amazon estuary. It breeds year-round and occurs in mixed-sex schools (Liley 1966). Penta males occur in three distinct morphs—*immaculata*, *melanzona*, and *parae* in the same population (Figure 1). The *immaculata* male is not typically conspicuously colored (Figure 1F, but see 1E), and behaves and looks like a small non-gravid female (Liley 1966). This morph sometimes has black vertical bars that appear during social interactions, whereas the *melanzona* male (Figure 1A, B, C) has two parallel black stripes along the flanks to the tip of the caudal fin enclosing bilateral stripes of one of three colors: blue, red (Liley 1966), or yellow

(Lindholm & Breden 2002). Males and females (Figure 1G) had from zero to six iridescent blue spots on their flanks, and many individuals had a black pectoral spot.

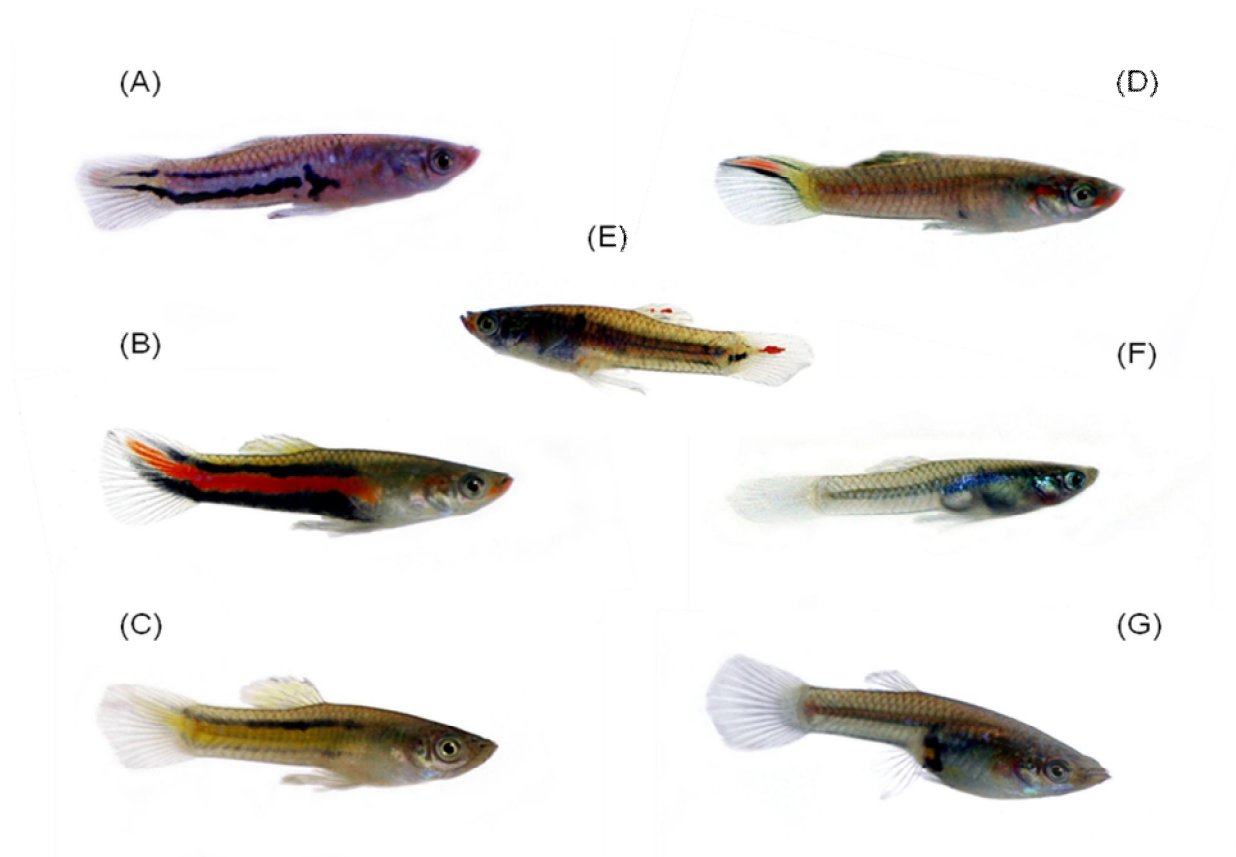


Figure 1. Pentamorphic (penta) livebearing fish (*Poecilia parae*) from a single population in Patientia, Guyana, showing male morphs (A) blue, (B) red and (C) yellow *melanzona*, (D) *parae* male with color pattern in caudal and dorsal fins, (E) the atypical *immaculata* morph collected in June 2004, with novel splotches of red in the dorsal and caudal fins, (F) the usually color pattern-free *immaculata* that resembles a small nongravid female, and (G) larger female.

In addition, *parae* morphs more than other morphs have enlarged dorsal fins with cream and dark splotches and stripes (Figure 1D). The male phenotypes of pentas are so different that *parae* and *melanzona* morphs were thought to be different species (Eigenmann 1912; Regan 1913; Hubbs 1926). The *immaculata* morph was first reported in 1963, and individual females were observed to produce sons of all three phenotypes; thus, it was concluded that the types are a single species, *P. parae* (Liley 1963; Rosen & Bailey 1963). Furthermore, F. Breden (pers. comm.) found that mitochondrial DNA sequences from a *parae* and a *melanzona* of the same population are identical for 500 base pairs of the control region.

Qualitative Assessment of Colors of Male Morphs, Discs and Fruits: We compared newly caught live males ($n = 15$ of each color morph), ripe bura-bura fruit ($n = 15$), and the seven discs matched by human eye (G. R. Bourne) to Smithe's (1975) color swatches in the field, and employed his color nomenclature. This was done to determine whether carotenoid coloration of males, discs, and fruit ingested by pentas were similar, and demonstrate variability in color even when it is known by a single name. Color matching was done on cloud-free-days between 1000-1100 h.

Field Experiments: Field tests were performed on coastal Guyana during the long wet season, May-August 2002, within the village-limits of Patientia (N 06°42.876', W 58°13.107') to assess receiver-bias and the nonsexual origin of female mate choice in pentas independent of phylogenetic inference. At the time of our study, there were no black sage and bura-bura shrubs growing along the ditch edges in this village because of a weed removal campaign by the local government. At this location pentas co-occur with their major piscivorous predators, the pike cichlid (*Crenicichla saxatilis*), patwa (*Cichlasoma portalegreense*), Reitzig's dwarf cichlid (*Apistogramma reitzigi*), and houri (*Hoplias malabaricus*; G. R. Bourne pers. obser.). We modified the protocols of Rodd et al (2002) to measure the attraction of pentas to seven colors. These tests were done in a small, shallow, clear-water ditch (0.86 ± 0.45 m wide and with water 78 ± 42 mm deep). Black, blue, green, orange, red, white, and yellow plastic discs (12.9 ± 0.1 mm diameter and 0.6 mm thick) were fabricated from corrupted 1.44 MB computer diskettes and used to measure penta responsiveness to color. For each trial, a single disc was placed on a submerged leaf collected from the substrate of the ditch (black discs were placed on light-colored leaves, and all other colors were put on dark-colored leaves). Discs were covered by small pieces of slate glued to lightweight monofilament fishing line for easy retrieval. Detritus was allowed to settle and individual pentas to return to the area before the covering slate was removed, and an observation session started. The number of nibbles directed at the disc was recorded on individual tally meters for up to six fishes tracked simultaneously. The duration for testing each color was 5 min.

We defined nibbles as the number of attempts at biting off pieces of a disc, and recorded the sex of adult penta and nibbles delivered to discs. Like Rodd et al (2002) we tallied nibbles because they suggested attempts at ingesting pieces of a disc. Density of fish in a section of ditch probably influence the number of approaches to a particular disc because of social facilitation, potentially increases the noise in the data and makes the statistical analyses conservative (Rodd et al 2002). This bias was reduced by placing discs only in sections of ditches with three to six pentas present during preliminary surveys. More than two pentas visited the discs during most tests. A different location was used for each observation of each color, and colors were presented randomly. Furthermore, note that this was not a choice test as each colored disc was presented singly, so this protocol minimized confounding fish density with color. The nine replicated sites for testing each color were chosen by moving up- or down-ditch to a new section, but far enough to exclude previously tested fish. We tested each of the seven colors two times for a total of 126 tests. Black and white discs were eliminated from statistical analysis because the total number of nibbles at these colors at all sites was two.

Laboratory Experiments: Adult male and female pentas used in the laboratory experiments were collected from a ditch connected to the one in which the field tests were run in Patientia. Fish were transported to the laboratory at CEIBA Biological Center, Guyana (N 06°29.928', W 58°13.111'), and were housed communally in six tubs. Fish were kept on a natural daylight schedule, and fed a brownish powder comprised of a mixture of crushed TetraMin® fish flakes and Kyorin® Cichlid Gold baby pellets every morning. Two groups of three females were kept permanently in the companion chambers of two 38-l pentary mate-choice aquariums (Bourne et al 2003). Pregnant females were screened daily to supply 20 previously untested parturient females for both color disc preferences and mate choice tests (Liley 1966; Bourne et al 2003). These 20 females were presented the seven colored discs simultaneously for 5 min in an unmodified 38-l glass aquarium on a white sand substrate because mud from the substrate from ditches was too easily introduced into the water column by air bubbles used to oxygenate the water. The disc with the highest mean number of nibbles was considered the preferred color. To determine whether the females that nibbled at a specific colored disc preferred the same color in males, a modification of the methods in Bourne et al (2003) was used. Pentary mate preference tests were conducted in the two divided 38-l glass aquariums (Houde 1997; Bourne et al 2003) in the morning and afternoon from 28 July–9 August 2002. Twenty different sets of five males (n = 100 males) were used with the 20 previously used disc color discriminating parturient females

for these mate preference tests. The test female placed in the middle compartment could see all five males matched for size at one end in their five individual chambers and the three companion females at the other end of the test aquarium at the same time. Companion females provide a stimulus of calm fish for the test female (Bourne et al 2003). We observed test females' responses 0.5 m away from the end of the compartment containing the companion females. A mechanical timer was set for a 5 min period, and five digital stop watches, one for each male, were used to record the cumulative times that a test female spent in front of each male against the glass of his compartment. A female was considered to have preferred a particular male when she spent $\geq 55\%$ of her time within a body length of and facing that male (Bourne et al 2003).

During 29 December 2005–4 January 2006 we tested 5 female and 5 male syntopic congeners of the pentas collected from the same ditches in Patientia described above for preferences for colored discs for comparison with pentas. The congeners tested included the South American molly (*P. vivipara*), the guppy, and *P. picta*. Each fish was fasted for 48 h and presented with a random dispersion of five of the seven colored discs simultaneously (blue, green, orange, red and yellow) for 5 min in an unmodified 38-l glass aquarium on a tan pea gravel substrate. White and black discs were not used in this experiment because all pentas tested in our laboratory to date did not nibble at these two colors (G. R. Bourne unpubl. data). The disc with the highest mean number of nibbles was considered the preferred color.

To assess the role of phylogenetic inference (Basolo 1990, 1995; Ryan 1997; Endler & Basolo 1998) on the nonsexual origin of a receiver-bias of female mate choice in pentas, adult least killifish (*Heterandria formosa*) were tested for attraction to carotenoid coloration because this taxon was used as the outgroup by Breden et al (1999) in their molecular phylogeny of fish in the genus *Poecilia* based on sequences of NADH Dehydrogenase Subunit 2 (ND2) mitochondrial gene (1047 base pairs). During 8–23 April 2006, 5 male and 5 female least killifish were individually tested for color disc preferences using the methods described in the paragraph immediately above. Likewise we tested attraction to carotenoid coloration by 5 male and 5 female mosquito fish (*Gambusia affinis*). This taxon was employed by Reznick et al (2002) to polarize their maximum likelihood phylogram of the fish genus *Poeciliopsis* that also included the least killifish as an additional outgroup taxon. This phylogeny was based on mitochondrial gene sequences to examine the multiple times that placentas have evolved in sister taxa that either lack or have intermediate stages of placentas (Reznick et al 2002).

Data Analysis: To determine whether adult pentas, guppies, *P. vivipara*, *P. picta*, and least killifish preferred carotenoid coloration in discs, we square root transformed ($\sqrt{x + 3/8}$; Zar 1996) the dependent variable, total number of nibbles at discs, to facilitate the use of a 1-way ANOVA model with Tukey-Kramer's multiple comparisons test to identify variation among means (Sokal & Rohlf 1995) for both field and laboratory generated data sets. This modified square root transformation produced residuals that were normally distributed and homoscedastic (Sokal & Rohlf 1995). To examine the effect of sex on attraction to color discs, number of nibbles at discs was square root transformed as above and subjected to unpaired t-tests (Sokal & Rohlf 1995).

Female mate preference data (time spent in front of males in seconds) were analyzed by a Kruskal-Wallis ANOVA model with Dunn's multiple comparisons test to identify variation among means, because these data violated parametric assumptions before and after appropriate transformation (Sokal & Rohlf 1995). Furthermore, to demonstrate a linear relationship between the time a female penta spent with a male, and the highest number of nibbles she previously directed to a particular colored disc we used Spearman correlation. Statistical tests were performed on SYSTAT 7.0 program (Wilkinson 1997) for Windows.

Results. Qualitative Assessment of Colors Associated with Pentas, Discs, and Fruit: The conspicuous male morphs of pentas (Figure 1A, B, C) include 'blue' *melanzona* in which the dominant color was iridescent Venetian blue, sky blue, or light sky blue.

'Red' *melanzona* in which the colors ranged from ruby, flame scarlet, and chrome orange to spectrum orange, and 'yellow' *melanzona* in which the colors were orange yellow, spectrum yellow, trogon yellow, and sulfur yellow. The *parae* morph (Figure 1D) had colored, bilateral caudal stripes, similar to the range of colors exhibited by *melanzona* morphs; usually with 'blue,' but also with either 'red' or 'yellow,' and many with dark vertical bars similar to those exhibited by *immaculata* males. A few *parae* morphs were buff-yellow along the distal two-thirds of their bodies. The *immaculata* morph (Figure 1F) usually has no conspicuous coloration and resemble a slim female. However, one *immaculata* collected in 2004 had ruby splotches in the dorsal and caudal fins (Figure 1E). Although there was much variation in color within morph types, in this paper we used the single color designations for convenience.

Disc colors were actually Smithe's (1975) ruby, burnt orange, spectrum yellow, Shamrock green, smalt blue, jet-black, and white. Bura-bura berries were ruby, spectrum red, geranium, spectrum orange, orange yellow, and burnt orange. Ripe black sage drupes were spectrum red, geranium, geranium pink, scarlet, flame scarlet, and chrome orange.

Male and Female Penta Attraction to Colored Discs and Female Preferences for Carotenoid Colored Males: During the attraction to color field tests, black and white discs did not receive enough nibbles to warrant statistical analysis (see methods). Male and female pentas differed in their attraction to the other five colored discs and exhibited a significant difference in nibbling among the five colored discs with the highest number directed at yellow discs (Figure 2A; $F_{9, 313} = 7.14$, $P < 0.0001$). However, Tukey HSD multiple comparisons identified only nine of the 45 pairs responsible for the significant differences (Figure 2A). For the sexes combined pentas nibbled more often at yellow discs (Figure 2B; $F_{4, 318} = 10.75$, $P < 0.0001$). The rank order of color preference was yellow, orange, red, blue, and green. There was significant effect of sex on attraction to orange discs with females expressing greater preferences than males ($t_8 = 3.68$, $P = 0.03$).

There is a correlation between the number of nibbles directed at discs with carotenoid coloration and the time spent by the female in front of a carotenoid colored male. Females preferred carotenoid coloration (red and yellow *melanzona*) in males (Figure 3A), and the novel *immaculata* male with the carotenoid colored fins tested against typical *immaculata* males (Figure 3B).

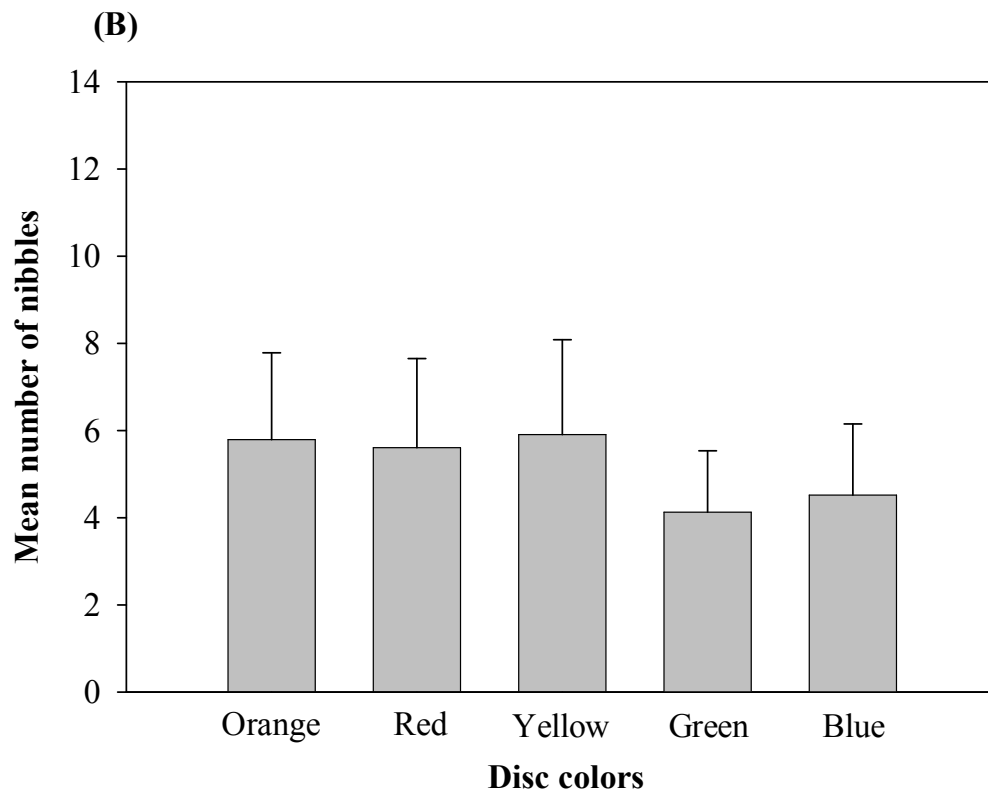
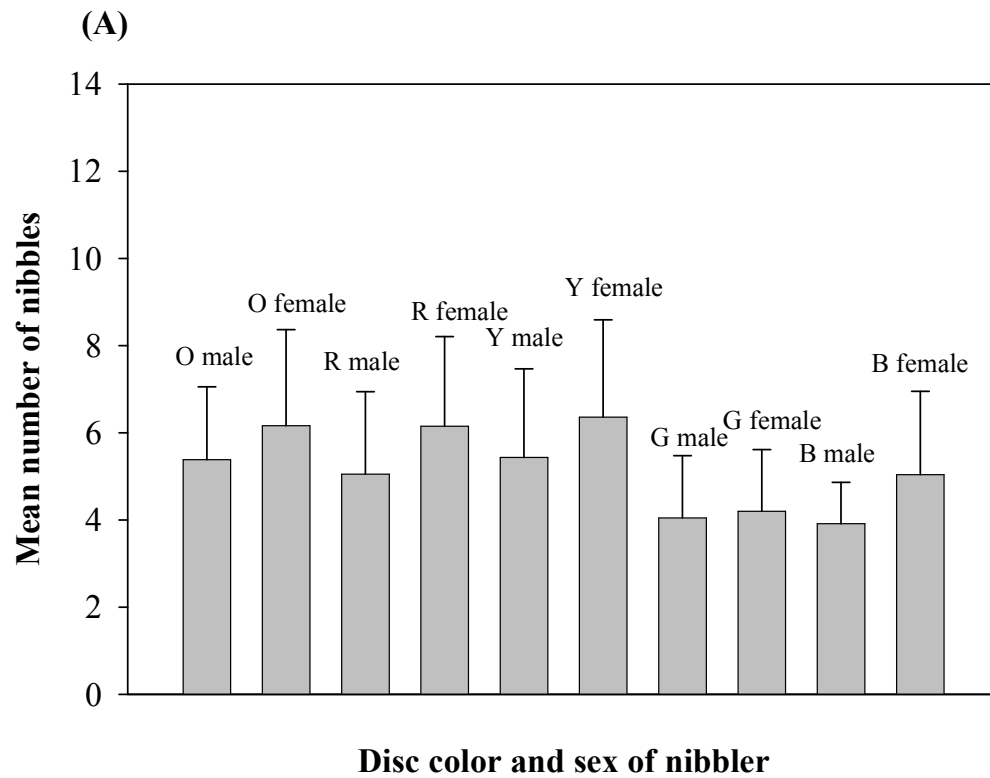


Figure 2. Graphs comparing nibbling behaviors (A) of male and female pentas under field conditions in a high piscivore predation ditch at Patientia, Guyana, showing significant sexual differences ($F_{9, 313} = 7.14$, $P < 0.0001$). Disc colors were O = orange, R = red, Y = yellow, G = green, B = blue and (B) combined for the sexes ($F_{4, 318} = 10.75$, $P < 0.0001$).

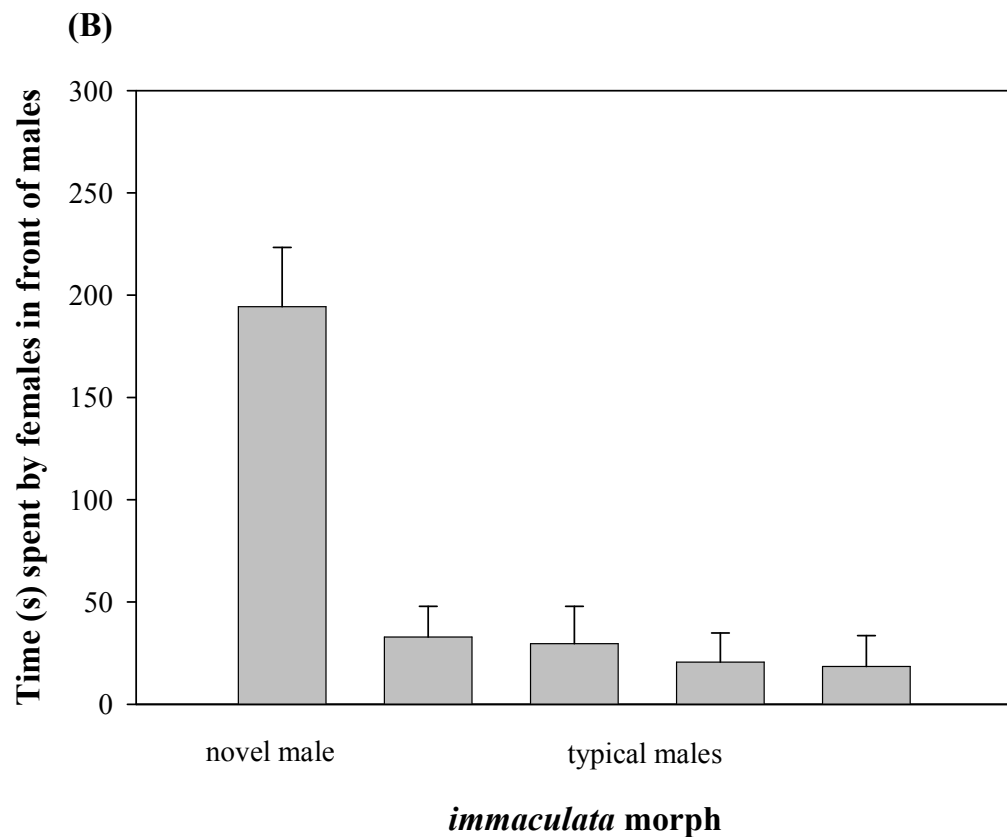
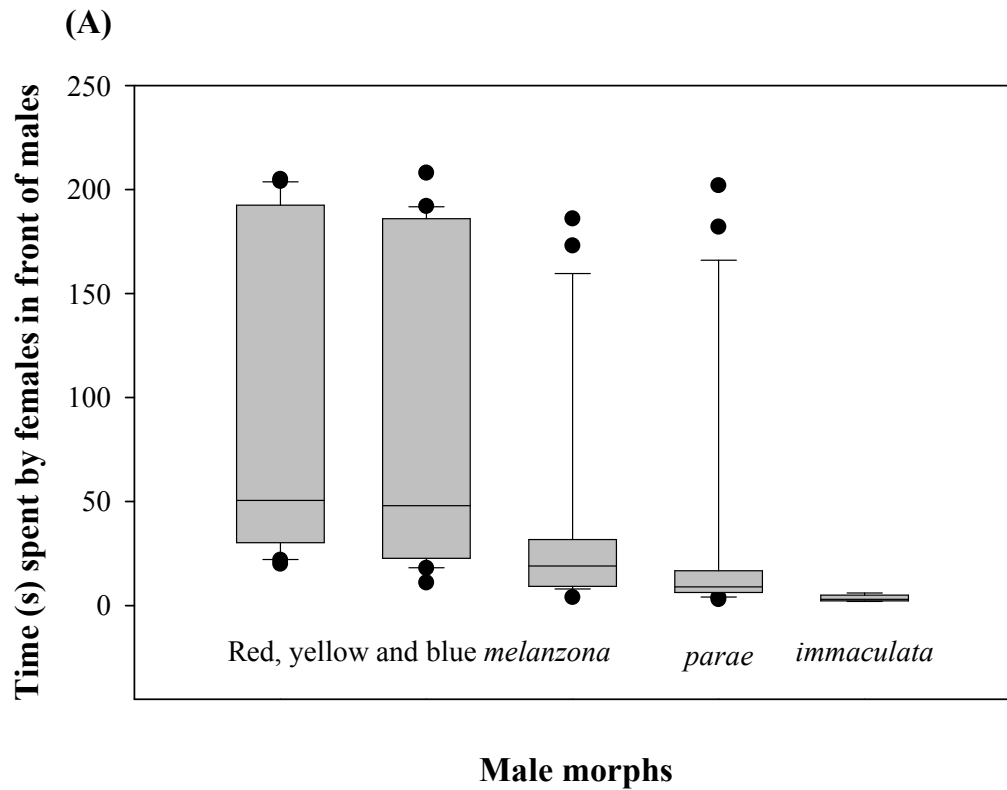


Figure 3. Box plots comparing (A) times spent by parturient female pentas in front of simultaneously presented red, yellow and blue *melanzona*, *parae*, and *immaculata* male morphs matched for size; note the strength of female mate preference for carotenoid coloration (red and yellow *melanzona*) in males; the box height gives the interquartile range; horizontal lines indicate medians; the whiskers show the extent of the non-outliers; and closed circles indicate mild and extreme outliers and (B) preferences for mean times spent by females in front of the novel *immaculata* male with red in its fins tested against typical uncolored *immaculata* morphs.

Furthermore, there was a highly significant positive correlation between female mate preferences and female nibbling at discs (Figure 4A; $r_s = 0.90$, 2-tailed $P < 0.0001$, $n = 20$), and between female preferences for the novel *immaculata* male and nibbles at carotenoid colored discs (Figure 4B; $r_s = 0.95$, 2-tailed $P = 0.0001$, $n = 10$).

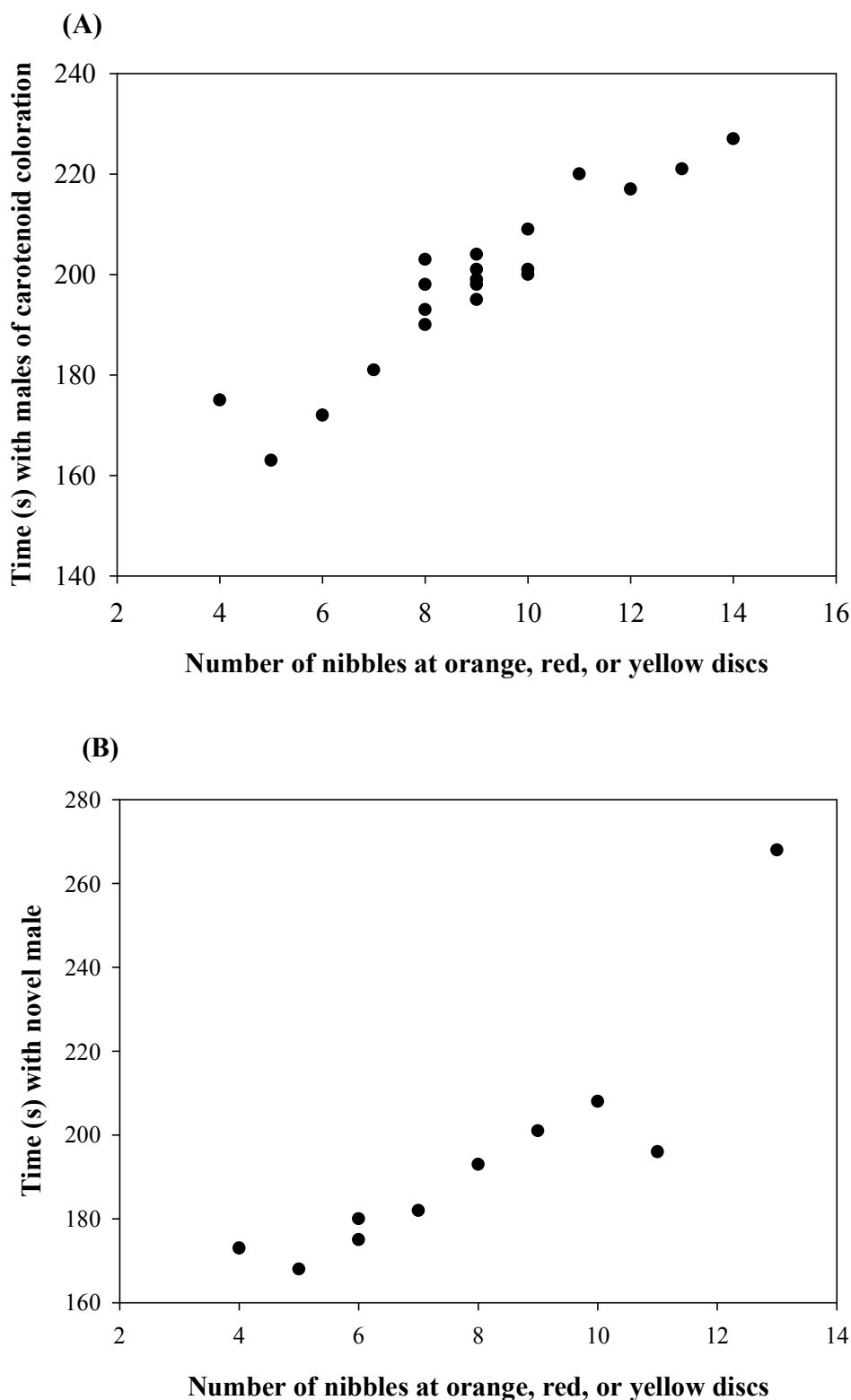


Figure 4. Correlational relationships for (A) the 20 parturient female pentas tested in the laboratory for nibbling behavior on carotenoid colored discs (scored as the highest number of nibbles directed at an orange, red, or yellow disc) and their preferences for red and yellow *melanzona* male morphs (each female spent most time in front of one of the carotenoid colored male morphs) and (B) the 10 parturient females nibbling at discs and time spent in association with the novel *immaculata* male.

Syntopic Congener Attraction to Carotenoid Coloration: The South American molly showed significant differences in nibbling among the five colored discs with the highest number directed at yellow discs (Figure 5A; $F_{4, 45} = 37.08$, $P < 0.0001$). The rank order of color preference was yellow, orange, red, green, and blue, and with a significant effect of sex on nibbling by females being more attracted to yellow discs than males ($t_8 = 2.82$, $P = 0.02$). *Poecilia picta* exhibited significant differences in nibbling among the five colored discs with the highest number directed at yellow discs (Figure 5B; $F_{4, 45} = 15.49$, $P < 0.0001$). The rank order of color preference was yellow, orange, red, green, and blue but without an effect of sex on attraction to discs. There was a significant difference in nibbling among the five colored discs by the guppy with the highest number directed at orange discs (Figure 5C; $F_{4, 45} = 39.26$, $P < 0.0001$). The rank order of color preference was orange, red, yellow, green, and blue, and no effect of sex on attraction to color.

Outgroup Attraction to Carotenoid Colored Discs and Phylogeny: The least killifish, the outgroup taxon in the molecular phylogeny of Breden et al (1999) nibbled most often at the yellow disc (Figure 6A; $F_{4, 45} = 41.06$, $P < 0.0001$). Although the rank order of color preference was yellow, orange, red, green, and blue, there was no effect of sex on attraction to color. Moreover, the mosquito fish, the outgroup taxon in a maximum likelihood phylogram for the genus *Poeciliopsis* that includes the least killifish as an additional outgroup (Reznick et al 2002), nibbled most often at blue discs (Figure 6B; $F_{4, 45} = 11.06$, $P < 0.0001$). The rank order of color preference was blue, green, yellow, red, and orange. There was also a significant effect of sex on nibbling with females being more attracted to green ($t_8 = 2.36$, $P = 0.05$) and blue ($t_8 = 3.63$, $P = 0.007$) discs than males.

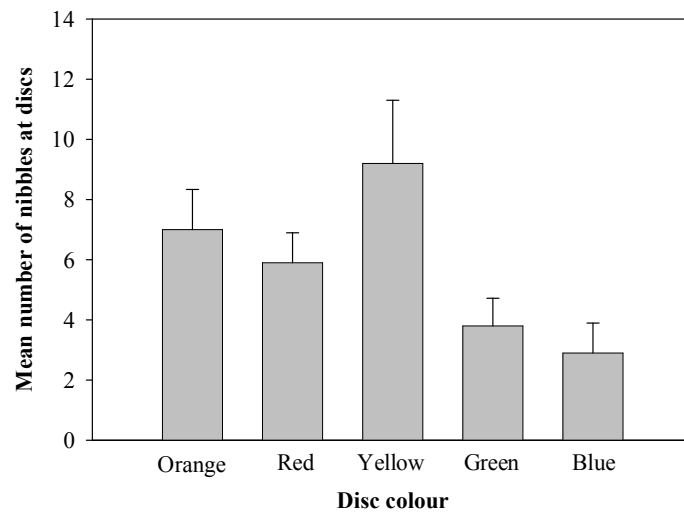
Attraction to carotenoid coloration mapped (black bar; Figure 7) onto a poeciliid phylogeny (Breden et al 1999) in a position ancestral to the *Poecilia* taxa.

Discussion. Basolo (1990, 1995), Ryan (1997), and Endler & Basolo (1998) stipulated four criteria for demonstrating that a male trait evolved because of female sensory-bias. First, female choice relies on heritable variation in the trait. Thus, if the trait is present, there is a preference for it and the trait is used in mate choice. Second, there is a bias in the psychosensory system that matches the direction of the preferences, that is, it predicts the direction of the preferences. Third, the trait must be absent or in a primitive form in ancestors, that is, the trait is derived. Finally, preference for the trait is ancestral. How well did our findings match these four criteria?

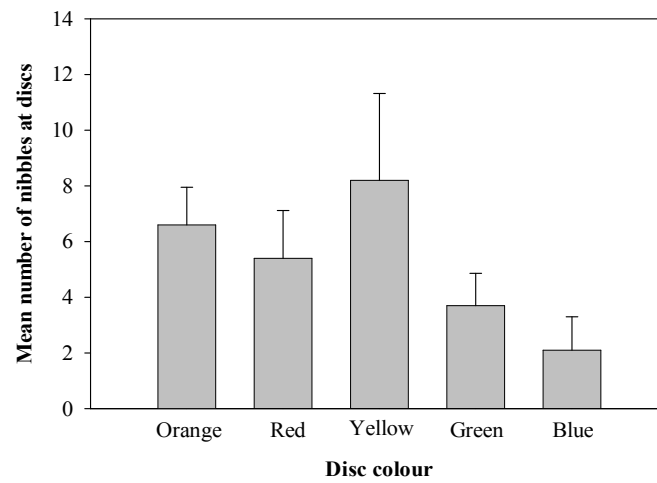
This study and two others provide strong evidence satisfying criterion one. Bourne et al (2003) and Lindholm et al (2004) demonstrated that female pentas from different populations base their choice of mates on variation in the colors, red and yellow. That these colors in fish are carotenoid pigments was clearly demonstrated by Maan et al (2006) in their study of cichlid fish. Additionally, the fact that both female guppies and female pentas exhibit preferences for males with carotenoid coloration suggests that our assumption that the visual system of pentas is similar to that of guppies (*sensu* Rodd et al 2002), thus allowing efficient detection of carotenoid-colored foods is valid. However, note that Endler & Houde (1995) found choice based on variation in area covered by orange coloration for guppies (also see Kokko et al 2003).

We have also presented evidence satisfying the second criterion. It can be concluded that pentas and their syntopic congeners (South American molly, *P. picta*, and the guppy) tested for their responses to inanimate colored discs in a feeding context exhibited a bias in their psychosensory system that matched the direction of the preference, because both sexes were attracted to orange, red, and yellow discs beyond the context of reproduction. Likewise both sexes of guppies are attracted to carotenoid coloration, orange and red objects (Rodd et al 2002; this study). In addition, recent experimental evidence from three-spined sticklebacks indicates that color perception of both sexes is quite similar (Boulcott & Braithwaite 2007). Taken as a whole, once carotenoid colors are present in male fishes and are used as a cue by females in choosing specific individuals as mates (Garcia & Ramirez 2005), then the color perceptions of both sexes will be similar (Boulcott & Braithwaite 2007).

(A)



(B)



(C)

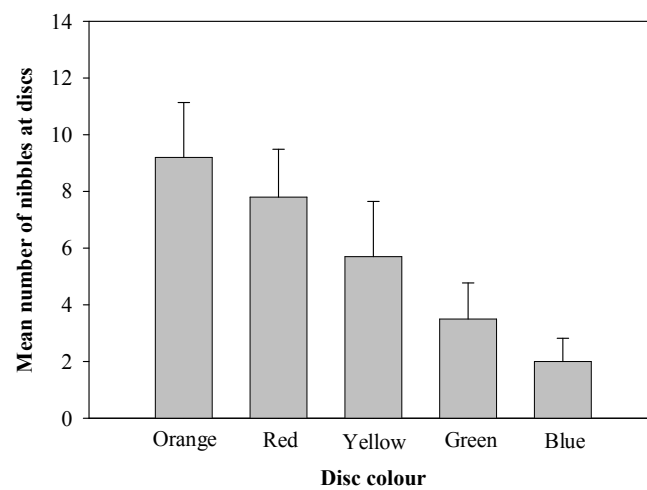
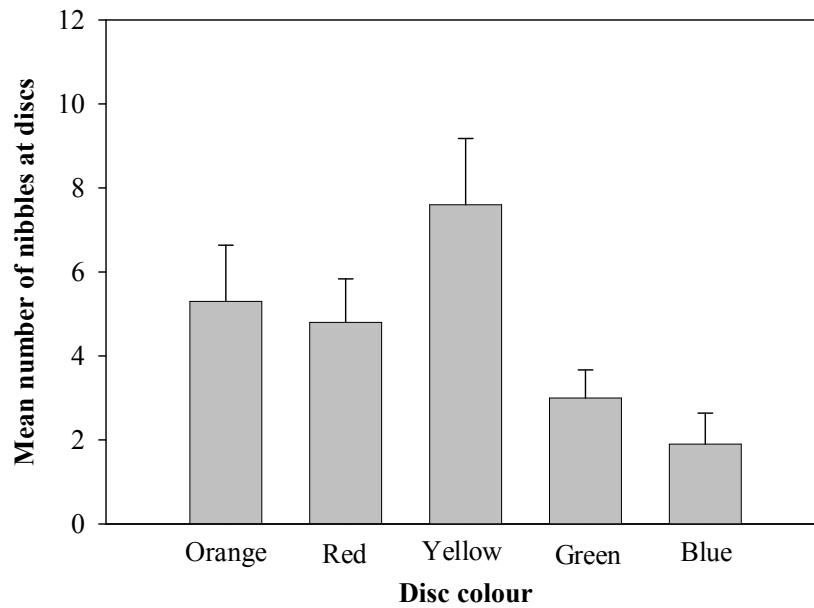


Figure 5. Attraction to colored discs, recorded as the mean number (± 1 SD) of nibbles delivered to five discs by syntopic congeners of pentas from Patientia, Guyana. All figures indicated a preference for carotenoid colored discs (A) South American molly (*Poecilia vivipara*) preferred yellow discs the color of the dorsal fin in males, (B) *Poecilia picta* also preferred yellow discs the basic ground color of most males in the sampled population and (C) guppy (*Poecilia reticulata*) showed a preference for orange discs the dominant color splotches in males.

(A)



(B)

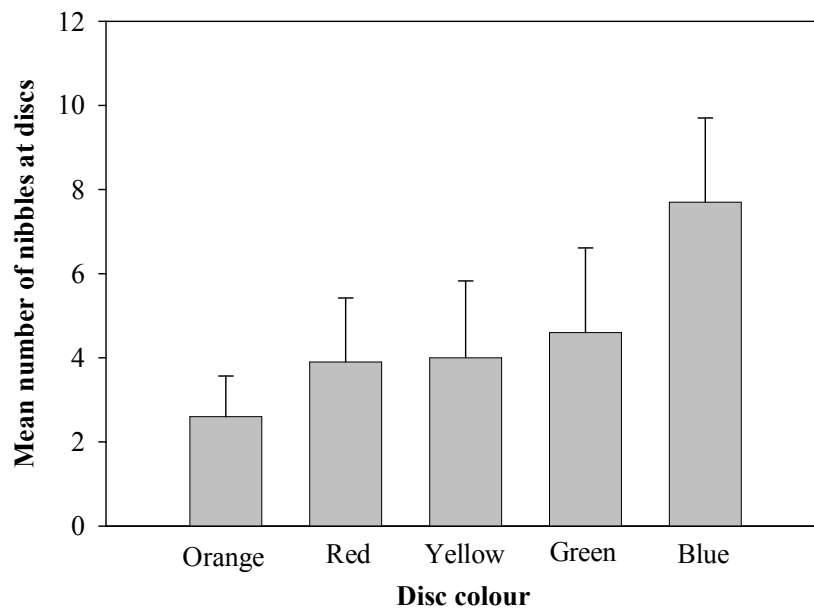


Figure 6. Mean attraction to colored discs, measured as the number of nibbles over 5 min for outgroup taxa (A) Least killifish (*Heterandria formosa*) whose ancestors came from Savannah River, South Carolina, U.S.A., the outgroup taxon in Figure 7. Male and female killifish clearly preferred yellow discs and (B) mosquito fish (*Gambusia affinis*) which is ancestral to the least killifish according to the phylogram of Reznick et al (2002) clearly preferred blue discs, thus, the trait for attraction to carotenoid coloration in a feeding context is absent in at least one ancestor of the genus *Poecilia*.

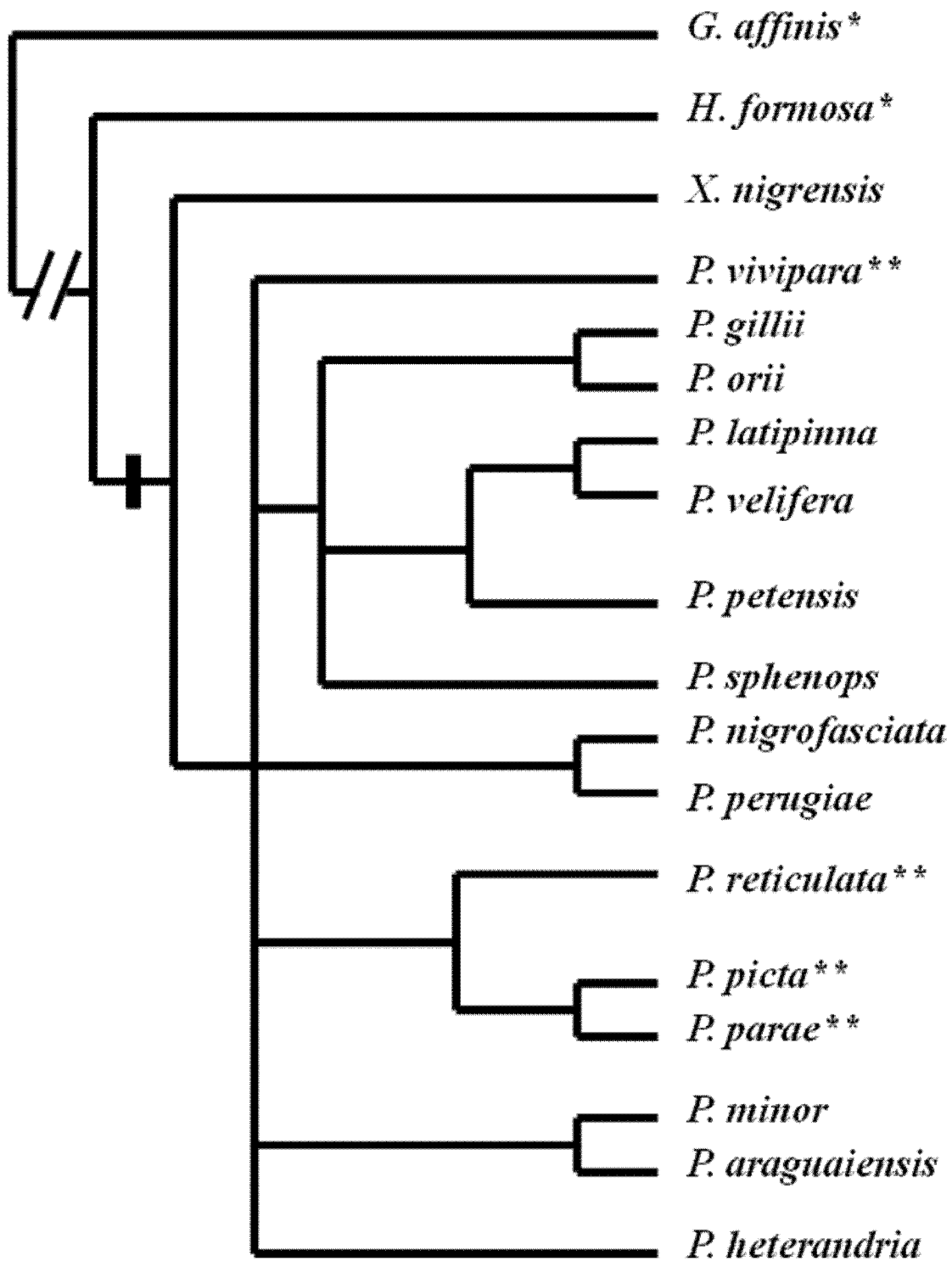


Figure 7. Modified (the two populations of *P. reticulata*, were combined) strict consensus tree of Breden et al (1999: 101, Figure 3) of 18 species in the genera, *Heterandria* (outgroup), *Xiphophorus*, and *Poecilia*. The black bar indicates where interest in carotenoid colored foods occurred as the lineage diversified. Since this trait was exhibited by the outgroup taxon, *H. formosa*, we have assumed that preference for carotenoid colored foods is shared with the common ancestor of members of the subfamily Poeciliini. *Gambusia affinis* an additional outgroup taxon from Reznick's phylogeny (2002) placed into Breden's phylogeny exhibited no preference for carotenoid colored discs. Males of the species in Breden's phylogeny have carotenoid coloration on some part of their body and females, where their reproductive behaviors have been studied, are known to use this color cue for mate selection. *Outgroup taxa and **sympatric taxa from Guyana tested for innate attraction to carotenoid colored disks in this study.

To satisfy the aforementioned third and fourth criteria (Basolo 1990, 1995; Ryan 1997; Endler & Basolo 1998), we demonstrated that male carotenoid coloration was derived in the ancestral lineage since male and female least killifish were attracted to carotenoid coloration in discs. This was the outgroup taxon of the poeciliid phylogeny (Breden et al 1999) on which we mapped attraction to carotenoid coloration in a feeding context. Furthermore, the mosquito fish, which was used to root a maximum likelihood phylogram for the genus *Poeciliopsis* that also included the least killifish as an additional outgroup (Reznick et al 2002), did not exhibit attraction to carotenoid coloration in a feeding context. The rank order of color preference for the mosquito fish was blue, green, yellow, red, and orange. Thus, the mosquito fish lineage, ancestral to the least killifish lineage does not have the trait for attraction to carotenoid coloration in a feeding context, thereby satisfying assumptions of phylogenetic inference and criterion three of Basolo (1990, 1995), Ryan 1997, and Endler & Basolo (1998).

However, at this time we do not know whether congeneric females of the mosquito fish clade prefer the trait even when absent in conspecific males. Yet, there is a growing body of evidence that a mate preference for carotenoid coloration exists even in poeciliids that do not normally have carotenoid coloration. The congeners *Poecilia latipinna* and *P. mexicana*, prefer conspecifics with a novel, orange-colored spot on the dorsal fin (see Schlupp et al 1999). Bourne et al (2003), Lindholm et al (2004), and this study found that female pentas preferred carotenoid coloration in red and yellow *melanzona* morphs tested against blue *melanzona*, *parae*, and *immaculata* morphs. On the contrary, Breden & Bertrand (1999) demonstrated that *P. picta* females from Trinidad did not choose males on the basis of carotenoid coloration. However, in this study we found that both male and female *P. picta* were attracted to carotenoid coloration in discs in a feeding context, and G. R. Bourne & A. Braser (unpubl. data) found female preference for males based on carotenoid coloration from populations in Georgetown, Mahaicony, and Patientia, Guyana. In any case, findings suggest that a sensory-bias favoring carotenoid coloration exists in the *Poecilia* but may sometimes be lost, probably when ecological conditions such as fruit availability (Rodd et al 2002), and we would add, when piscivore predation does not favor its maintenance (Godin & McDonough 2003).

In addition to the four criteria previously discussed, Rodd et al (2002) considered a fifth criterion for the pleiotropic mate preferences with an ecologically driven origin — one would expect those preferences to evolve where the relevant ecological conditions, such as when a preferred food item is rare. Thus, the consumption of pigment in the occasional fruit suggests a possible evolutionary mechanism for the development of carotenoid-dependent ornaments in pentas and their syntopic congeners. These excess carotenoids may diffuse passively to the skin (Negro et al 2002), and the yellow and red coloration then used as an honest indicator signal in mating displays (Hill 1991; Blount et al 2003; McGraw 2005). This smoking-gun evidence was presented by Garcia & Ramirez (2005) when they indicated that a communication system driven by receiver-biases can evolve into honest signaling system. The important point here is that this fifth criterion incorporates the ecological and geographical elements suggested earlier by Endler & Basolo (1998).

We have presented evidence that adult pentas were innately attracted to orange, red, and yellow objects, but less so to green and blue discs, and not to either black or white ones. Attraction of pentas to orange, red, and yellow colors could have evolved for detecting rare nutritious food resources, which in the case of pentas and their syntopic congeners included the red-, yellow- and orange-colored fruits of black sage, and burabura (G. R. Bourne pers. obser.). Female mate preference for carotenoid coloration could have arisen as a pleiotropic effect (*sensu* Williams 1966) of foraging behavior not only for the pentas (this study) but also for syntopic South American mollies, guppies, and *P. picta* (G. R. Bourne, A. Braser, J. Masonoff (unpubl. data), and also for the guppy (Rodd et al 2002). The alternative explanation, preference for carotenoid colored objects arising as an effect solely of female mate choice for carotenoid coloration, is less likely because male pentas and male syntopic congeners also exhibited preferences for orange, red, and yellow objects. Similar evidence was presented for the guppy where both sexes preferred orange and red objects (Rodd et al 2002). This is an especially attractive explanation

since guppies and pentas are closely related (Breden et al 1999), and many aspects of the reproductive behavior of pentas and guppies are similar (Liley 1966; Houde 1997; Bourne et al 2003; Lindholm et al 2004).

The non-significant attraction to green objects may be more difficult to explain because we know of no instances of green coloration in male pentas. However, our unpublished observations suggested that attraction to green objects also probably evolved in a feeding context. We found that when small pieces of green periphyton mats separated from the substrate and floated to the surface they were voraciously ripped apart and devoured by poeciliids and characins (tetras), presumably for carotenes (Grether et al 2001) and other nutrients (G. R. Bourne & L. C. Watson unpubl. data). Attraction to blue objects is even more difficult to explain when similar logic is employed; we know of only one dark blue fruit, a small berry known locally as quackoo (*Marlieria obtusa*), Myrtaceae, that sometimes ends up in streams and ditches occupied by pentas. Blue fruits contain flavonoids that are important antioxidants that contribute blue color and structural elements for UV hues found in some animals (McGraw 2005). However, fishes in Guyana did not enthusiastically eat quackoo (G. R. Bourne pers. obser.).

Conclusions. Our findings implied that the mate preference (= mate choice; Bourne et al 2003) of female pentas for carotenoid coloration on males originated as a result of a sensory-bias for orange- red- and yellow-colored objects, maybe rare foods, carotenoid-containing fruit. However, the correlational relationship between response to carotenoid objects and female mating preference suggests experiments that should be conducted to elucidate causal relationships. Moreover, additional study is needed to clarify to what extent geographical variation in female preference for carotenoid coloration is a result of natural selection on foraging behavior, and to what extent mate preference may have been co-opted by sexual selection mechanisms (Rodd et al 2002). We presented evidence for innate attraction to carotenoid colored discs and positive correlation with female preferences for red and yellow *melanzona* males, and for novel red ornaments in fins of an *immaculata* male tested against typically uncolored *immaculata*. In addition, evidence was presented that preference for carotenoid colored discs was absent in one ancestral taxon, and yet mapped onto a poeciliid phylogeny as ancestral. Overall these results suggest a strong association between a potential trigger of a mate choice preference and a sexually selected trait, thereby corroborating the receiver-bias hypothesis for carotenoid coloration independent and dependent of the assumptions of phylogenetic inference (Hillis et al 1996; Ryan 1996; Payne & Pagel 2001). This is the third study to show both an association between a potential trigger of a mate-choice preference and a sexually selected trait, thereby corroborating the sensory-bias hypothesis for the evolution of female mating choice in pentas (see Rodd et al 2002; Smith et al 2004).

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