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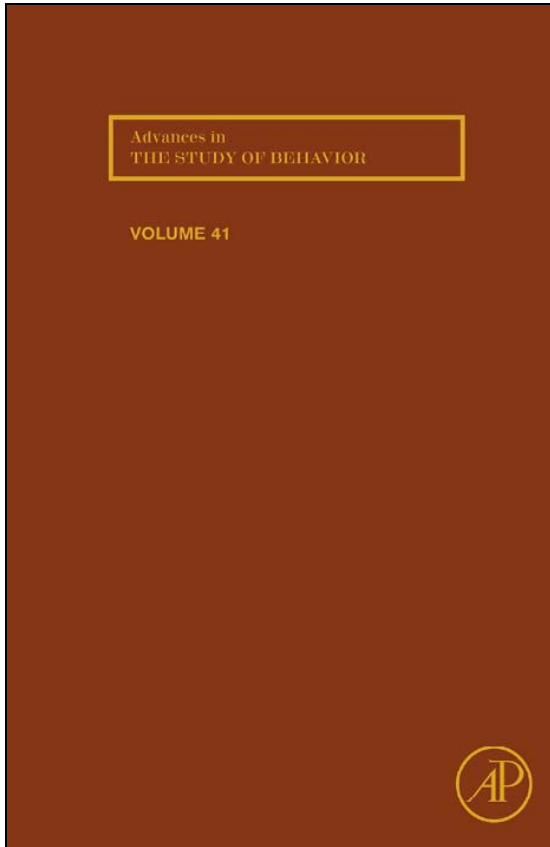
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The Evolution of Mate Preferences, Sensory Biases, and Indicator Traits

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I. INTRODUCTION

A. SEXUAL SELECTION

[Darwin \(1859, 1871\)](#) introduced the theory of sexual selection as a special explanation for the evolution of secondary sexual characters. Secondary sexual characters are traits that differ between the sexes and are neither required for reproduction nor related to sex differences in ecology. Most such traits are expressed more strongly in males than in females and do not develop fully until sexual maturity. These traits require a special explanation precisely because they are useless or even costly for survival. Darwin's solution was that traits that enhance an individual's ability to attract or compete for high-quality mates could be selected for in spite of their survival costs. He proposed that enlarged male sensory organs are adaptations for finding receptive females and that male-specific weaponry and clasping structures are adaptations for competing directly for access to females. His most controversial and interesting suggestion, however, was that sex differences in coloration and other "ornamental" traits are adaptations for attracting and courting the opposite sex.

The idea that male characteristics are shaped by female preferences was viewed with skepticism and remained relatively unstudied until the 1980s. At that time it was still unclear whether females actually discriminate among conspecific males on the basis of secondary sexual characters. Quickly this issue was dispelled as study after study showed that females of many species prefer males with exaggerated (larger, brighter, louder) ornaments and courtship displays ([Andersson, 1994](#)). The focus of sexual

selection research then shifted from explaining the existence of secondary sexual characters to explaining the existence of female mate preferences for such traits.

B. PREFERENCE EVOLUTION THEORY

Some female mate preferences may initially be nothing more than sensory biases that inadvertently enable males with certain trait values to mate more frequently than others (Basolo, 1990; Basolo and Endler, 1995; Christy, 1995; Ryan, 1998; Ryan et al., 1990). That sensory biases should exist is obvious if one considers that sensory systems are under selection in multiple contexts. For example, female water mites (*Neumania papillator*) are blind, sit-and-wait predators that detect copepod prey by their vibrations. When a male of this species detects a female chemically, he vibrates his legs at a frequency that mimics copepods. Females orient to and clutch-trembling males as though they were prey, which puts the males in a good position for presenting spermatophore packets (Proctor, 1991). In this case, the female preference, that is, the response of females to the male leg-trembling display, appears to be nothing more than an unmodified adaptation for ambushing prey (Proctor, 1991, 1992). There are several variations on this hypothesis and a variety of interrelated terms in usage, for example, preexisting bias, receiver bias, sensory exploitation, sensory drive, sensory trap (Basolo and Endler, 1995; Christy, 1995; Endler, 1992; Fuller et al., 2005; Ryan, 1990; Shaw, 1995), but a common thread is that mate preferences need not be adaptive in a mating context.

Under most circumstances, even mate preferences derived from sensory biases would be expected to evolve in response to their costs and benefits in a mating context (Arnqvist, 2006; Fuller, 2009; Sherman and Reeve, 1999; but see Ryan, 1999). Fisher (1930) pointed out that female mate preferences could evolve as a correlated effect of the selection that they impart on male traits. Mathematical models of the “Fisherian” process show that all that is required is for female preferences and male traits to be genetically variable and a positive feedback loop can be established in which females prefer ever larger values of the male trait, until opposing survival selection curtails further evolution of either preference or trait (Kirkpatrick, 1982; Kokko et al., 2006; Lande, 1981). This is sometimes referred to as the “sexy son” hypothesis because preferring attractive males increases the attractiveness of a female’s sons (Kokko et al., 2006). In this sense, preferences that evolve through the Fisherian process are adaptive in a mating context. A pure Fisherian preference has no

influence on a female's own reproduction but increases the mating rate of her sons. Several studies have provided evidence for the requisite genetic correlations, and some theoreticians regard the process as inevitable, but the Fisherian model appears to make no unique, testable evolutionary predictions. Cases in which female preferences and male traits show a correlated pattern of evolution have sometimes been presented as evidence for the Fisherian process, but the same correlated pattern is predicted by most other sexual selection models (Houde and Endler, 1990).

The traditional alternative to the Fisherian model is variously known as the good genes, handicap, or indicator model. The term "good genes" applies to cases in which the preferred trait is an indicator of male genetic quality. The term "handicap" refers to the idea that the honesty of secondary sexual characters as signals of mate quality is maintained by the costs of producing, maintaining, or surviving with these traits (Zahavi, 1975; Zahavi and Zahavi, 1997). The core distinction between Fisherian and good genes models is that the Fisherian model pertains to cases in which genetic variation in the preferred trait affects the attractiveness of male offspring while the good genes model pertains to cases in which genetic variation in the preferred trait affects other components of offspring fitness (Cameron et al., 2003). In both models, the benefits to choosy females are indirect (genetic), and it is possible to combine the two types of benefits into a single mathematical model (Kokko et al., 2002, 2006).

Secondary sexual characters can also be indicators of nongenetic benefits (Andersson, 1994; Grafen, 1990). Most conceivable nongenetic benefits are direct, meaning that they affect the survival or reproduction of females themselves, but some nongenetic benefits are indirect. For example, some female moths obtain alkaloids from male spermatophores and use them to protect their offspring (Conner et al., 2000; Dussourd et al., 1991; Eisner and Meinwald, 1995; Gonzalez et al., 1999; Iyengar and Eisner, 1999). Male traits can also be indicators of both genetic and nongenetic benefits simultaneously. For example, if parasitized males are dull in color, females might both avoid getting parasites and also obtain parasite-resistant genes for their offspring by mating with brightly colored males.

Sexual selection, as a field, has become much broader than just the study of how mate preferences and secondary sexual characters evolve. In particular, the roles of sexual selection in speciation and conservation are topics of considerable current interest (Doherty et al., 2003; Panhuis et al., 2001; Ritchie, 2007; Smith and Grether, 2008). Nevertheless, whether and, if so, how mate preferences are shaped by selection in a mating context are still central unresolved issues (Andersson and Simmons, 2006).

C. EMPIRICAL RESEARCH ON MATE PREFERENCE EVOLUTION

One of the primary aims of sexual selection research over the past 25 years has been to empirically test and distinguish among alternative models of how mate preferences evolve (Andersson, 1994). Strong inference tests (Platt, 1964) have proved elusive, however, and the number of contending models has only grown since this research priority was first articulated (Bradbury and Andersson, 1987). Much of the research in this area amounts to testing the assumptions that specific models make about the nature of variation in male sexual traits, the fitness consequences of female preferences, and the genetic correlation between traits and preferences. This empirical research has resulted in the rejection of specific mathematical models but not in the elimination of any class of models. For example, in early formulations of the Fisherian model, any costs associated with female choice would cause both the preference and male trait to be lost, and thus, for a brief period, empirical demonstrations of such costs were presented as evidence against the model. Theoreticians then rescued the Fisherian model by reformulating it to accommodate mate choice costs (reviewed in Mead and Arnold, 2004). Another source of frustration for empiricists is that the mate preference evolution models are not alternative hypotheses in the strict sense. In principle, multiple processes could contribute to the evolution of any given mate preference, at least at different stages in its evolutionary history (Andersson and Simmons, 2006).

Ultimately, the value of a theory rests on whether it makes accurate predictions. This is particularly true for evolutionary models because showing that the conditions are right for a process to occur in the present (or not) does not prove that the process occurred (or did not occur) in the evolutionary past. A well-known prediction of the sensory bias model is that mate preferences, at least in some rudimentary form, predate the evolution of sexual ornaments. Testing this prediction requires well-defined character states (e.g., presence vs. absence of the ornament), a well-resolved phylogeny, and a protocol for measuring mate preferences even in species that lack the ornament. When these requirements are met, it is possible to make inferences about the evolutionary order of events, and because of this we have good evidence that some mate preferences predate the evolution of ornaments (e.g., Basolo, 1990, 1995; Ryan et al., 1990, but see Fuller et al., 2005). Another successful approach that has been taken to test predictions of sexual selection models is to manipulate the mating system and examine how this affects the evolution of male and female traits over multiple generations. This experimental evolution approach has been taken with fruit flies (*Drosophila melanogaster*) and dung flies (*Scathophaga stercoraria*), and the results show that male seminal products and

female resistance to male seminal products coevolve as predicted by sexual conflict-based models (Holland and Rice, 1999; Hosken et al., 2001; Rice and Holland, 2005). Similar approaches should be taken to clarify whether mate preferences for ornaments evolve in response to changes in their benefits in a mating context.

The relative importance of the Fisherian and good genes processes may be impossible to resolve, but it should be possible to establish whether most mate preferences have evolved in response to their costs and benefits in a mating context, as opposed to being unmodified sensory biases. Consider, for example, the female preference for long tail feathers in barn swallows (*Hirundo rustica*), which has become a textbook example of the indicator process, and the female preference for long tail fins in green swordtails (*Xiphophorus helleri*), which has become a textbook example of a preexisting sensory bias (e.g., Alcock, 2009; Andersson, 1994; Bradbury and Vehrencamp, 1998; Goodenough et al., 2001; Krebs and Davies, 1993). Excellent evidence has been provided that female barn swallows benefit by mating with long-tailed males (Møller, 1990, 1994) and equally convincing evidence has been provided that the swordtail preference evolved prior to the evolution of the sword itself (Basolo, 1990, 1995, 1998, 2002). But what evidence do we have that the barn swallow preference is more highly modified by selection in a mating context than the swordtail preference? In neither of these cases is the original function of the mate preference known, but in other systems seemingly adaptive mate preferences have been linked to sensory biases that may still have important functions in other contexts (e.g., food detection; Martin and Lopez, 2008; Rodd et al., 2002; Smith et al., 2004). Are such preferences evolutionarily constrained by selection in a nonmating context? Do the underlying sensory biases evolve as a correlated effect of selection in a mating context? Or do adaptive mate preferences usually become genetically decoupled from their original sensory functions? These outcomes are not mutually exclusive but instead represent extremes along a continuum of theoretically possible outcomes (Arnqvist, 2006; Fuller, 2009; Rodriguez and Snedden, 2004; Ryan, 1999; Sherman and Reeve, 1999).

D. OVERVIEW OF THIS CHAPTER

In the first section below, I describe a new approach for testing the indicator models and explain how it can be applied to species with carotenoid-based ornaments. I then show how this approach and others have contributed to our understanding of how a mate preference for carotenoid coloration evolves in Trinidadian guppies (*Poecilia reticulata*). The basic conclusion is that the sensory bias and indicator processes have probably

both played a role in the evolution of this mate preference. I briefly review other examples of adaptive mate preferences that appear to be derived from sensory biases. Next, I turn to the longstanding question of how secondary sexual characters could be evolutionarily stable indicators of mate quality and focus specifically on the problem of how cheating is prevented in species with carotenoid-based ornaments. Guppies again provide an interesting but surprisingly complex case study that illustrates the value of recognizing that color patches are multicomponent traits (Grether et al., 2004b). The two parts of the guppy story intersect because the same environmental gradient appears to have shaped the evolution of the male ornament and the female sensory bias that might have originally caused the ornament to evolve. Throughout the chapter and in a concluding section, I offer suggestions for further research.

II. NEW APPROACH FOR TESTING THE INDICATOR MODELS

A. INDICATOR MODEL PREDICTION

Under the indicator models, mate preferences evolve to exploit indicators of mate quality (Harvey and Bradbury, 1991; Kirkpatrick and Ryan, 1991; Andersson, 1994). If the indicator value of a male trait changes, the payoff function of the female preference for that trait should change and the preference should evolve to a new optimum. For example, in Iwasa and Pomiankowski's (1999) model of the indicator process, male ornament size s is a linear function of quality v ,

$$s = t + t'v,$$

where t is the condition-independent component of ornament size and t' is the degree of condition-dependence or indicator value of ornament size. The strength of the female preference is represented by p . Females with positive (negative) values of p prefer to mate with males with larger (smaller) than average ornaments. Females are assumed to benefit, directly or indirectly, by mating with high-quality males, but the preference also carries a cost b , which increases with p and exponent γ . These costs and benefits balance at the equilibrium female preference,

$$\bar{p} = \left(\frac{\phi \bar{t}}{\gamma b} \right)^{1/(\gamma-1)},$$

where ϕ includes the effects of male quality on the direct and indirect components of female fitness (see Eqn. (14) in Iwasa and Pomiankowski, 1999). Thus, the female preference evolves to a level determined by the

indicator value of the male trait \bar{t} . The indicator value \bar{t} is also allowed to evolve in this model and is shown, at equilibrium, to increase in direct proportion to \bar{p} . Thus, \bar{t} and \bar{p} are mutually reinforcing.

Iwasa and Pomiankowski (1999) do not explicitly model environmentally induced changes in t' , but any change in t' should cause p to evolve in the same direction. The most direct way to test this prediction would be to manipulate the indicator of a trait experimentally and follow the subsequent evolution of the preference over multiple generations. This might be feasible in species with short generation times (e.g., *Drosophila* spp.) by varying an environmental factor that influences the indicator value of a preferred trait in the laboratory. Another approach would be to make use of situations in which populations of a species are distributed along a gradient in such an environmental factor. With some systems, it might be feasible to carry out evolution experiments in the wild by manipulating the local environment or by establishing new populations at sites that differ from the source site in the key environmental factor. A few studies have taken the first step of examining how female preferences vary along environmental gradients that affect the indicator value of male secondary sexual characters (e.g., Grether, 2000; Plath et al., 2006).

B. CAROTENOID-DEPENDENT INDICATORS

One environmental factor that is likely to affect the indicator value of the sexual ornaments of many species is carotenoid availability. Carotenoids produce most of the brilliant orange and yellow colors seen in animals, but animals cannot synthesize these pigments (Fox, 1979; Goodwin, 1984). Carotenoids are present virtually in everything animals consume (Goodwin, 1980, 1984; Hudon, 1994), but species vary in the efficiency with which they absorb ingested carotenoids (Schiedt, 1989), in their ability to convert absorbed carotenoids into usable pigments (Fox, 1979; Goodwin, 1984; Putnam, 1992), and in the rate at which they deposit these pigments in the integument (skin, feathers, etc. Brush and Power, 1976; Hudon, 1991). Thus, even diets rich in total carotenoids can be deficient in the compounds needed by a particular species. This may explain why animals often develop pale coloration in captivity (Brush and Power, 1976; Goodwin, 1984; Hill, 1993; Putnam, 1992; Schiedt, 1989; but see Hudon, 1994). Carotenoid feed supplements are used routinely in the poultry and fish-farming industries to restore normal coloration (Schiedt, 1989; Sommer et al., 1992). The carotenoid requirements of some species are quite high, owing to low assimilation rates. Farmed salmonids, for example, retain only about 5% of the carotenoids they ingest and deposit only a fraction of this in their skin (Hardy et al., 1990; Torrissen et al., 1989). The low assimilation rates do

not appear to be a product of internal regulation, because the amount of pigment deposited increases with the level of carotenoids in the diet (Bjerkeng et al., 1990; Choubert and Storebakken, 1989; Meyers, 1994; Putnam, 1992; Sommer et al., 1992).

In environments where carotenoids needed for pigmenting a particular species are scarce, carotenoid coloration may serve as an indicator of male health and foraging ability that females could use to choose high-quality mates (Endler, 1980; Olson and Owens, 1998). In addition, carotenoid coloration may signal a male's history of disease, current state of health, or potential to respond to future infections, through physiological effects of parasites on carotenoid metabolism, or utilization and oxidation of carotenoids by the immune system (Blount et al., 2003; Camplani et al., 1999; Faivre et al., 2003; Folstad et al., 1994; Lopez et al., 2009; Lozano, 1994; McGraw and Ardia, 2003, 2007; Møller et al., 2000; Olson and Owens, 1998; Peters, 2007; Putnam, 1992; Thompson et al., 1997; von Schantz et al., 1999; Wedekind et al., 1998).

The optimally choosy female would be expected to place relatively more weight on carotenoid coloration in environments where carotenoids are difficult for males to acquire, compared to environments where carotenoids are plentiful (Grether, 2000; Grether et al., 1999). Therefore, in species in which male sexual coloration is limited by carotenoid availability to different degrees in different populations, the indicator model predicts a correlated pattern of variation in female preferences for these traits (Grether, 2000). This is a specific version of the prediction that the equilibrium level of the preference (p) should evolve in response to changes in the indicator value of the preferred trait (t') (Iwasa and Pomiankowski, 1999). I will now describe how this prediction was tested in Trinidadian guppies.

C. DETAILED CASE STUDY: TRINIDADIAN GUPPIES

These small fish have been introduced around the world for mosquito control and are widely used in the aquarium trade (Magurran et al., 2005), so some readers may be surprised to learn that their native range is limited to the coastal river drainages of northeastern South America and a few islands in the Lesser Antilles, including Trinidad and Tobago (Rosen and Bailey, 1963). Wild female guppies are rather drab in coloration but males often have spectacular color patterns (Fig. 1). Virtually the entire skin surface of males is covered with chromatophores (i.e., cells containing light-absorbing pigments or reflective organelles; Grether et al., 2004b). The coloration of males is so variable that most individuals can easily be distinguished in behavioral work (Grether, 2000; Hampton et al., 2009; Houde, 1997; Jirotkul, 1999b). Some components of the color variation

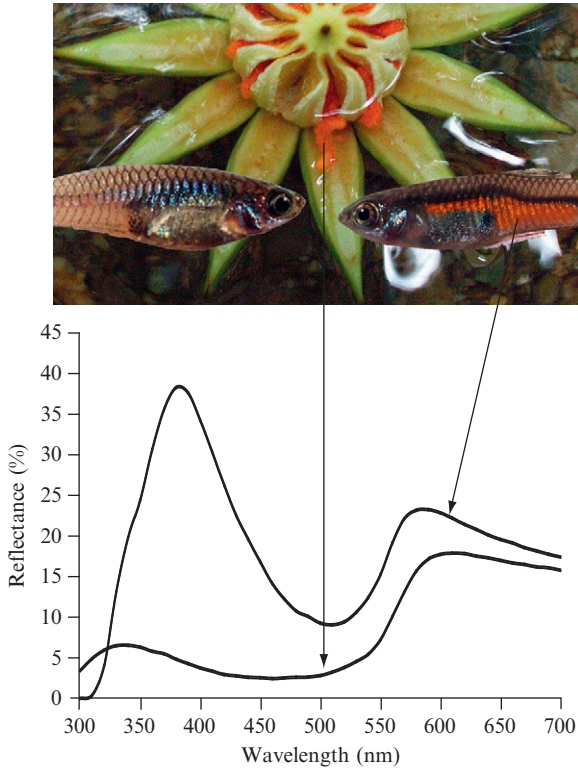


FIG. 1. Guppies (female left, male right) and one of the orange fruits (*Clusia* sp.) that they feed on in Trinidad. The graph below the photographs shows reflectance spectra of the orange aril of the fruit and an orange spot of a male guppy.

are heritable, including the size and position of orange and black (melanin) spots, to the extent that the paternity of male offspring can often be assigned based on their resemblance to potential fathers (Brooks and Endler, 2001a; Houde, 1988, 1992; Hughes et al., 1999, 2005).

In one of the earliest specific versions of the indicator hypothesis, Endler (1980) suggested that the carotenoid-containing orange spots of male guppies are signals of foraging ability that females could use to choose high-quality mates. The basic assumptions behind this hypothesis have been confirmed. The chroma (color saturation) of the orange spots increases with carotenoid levels in laboratory diets (Grether, 2000; Karino and Haijima, 2004; Kodric-Brown, 1989) and females usually prefer males with larger orange spots and higher orange spot carotenoid concentrations (Brooks and Endler, 2001a,b; Endler and Houde, 1995; Houde, 1987;

Houde and Torio, 1992; Jirotkul, 1999a; Karino and Shinjo, 2004; Kodric-Brown, 1985, 1989; Long and Houde, 1989; but see Hampton et al., 2009; Schwartz and Hendry, 2007). The ability of guppies to find algae is heritable and orange spot chroma correlates positively with algal foraging ability (Karino and Shinjo, 2007; Karino et al., 2005, 2007). It follows that females benefit indirectly by mating with males that have managed to sequester large amounts of carotenoids, and more so in environments where dietary sources of these pigments are scarce. In addition to being affected by carotenoid intake, the chroma of the orange spots is reduced by infection with the ectoparasite *Gyrodactylus turnbulli* (Houde and Torio, 1992) and correlates positively with swimming performance (Nicoletto, 1991). Thus, females probably benefit in multiple ways by preferring high-carotenoid males, including avoidance of sexually transmitted parasites.

The value of the orange spots as indicators of a male's foraging ability should diminish as carotenoid availability increases because the chroma of the orange spots increases asymptotically with carotenoid intake (Fig. 2). Similar diminishing returns functions have been reported for salmonid fishes (Bjerkeng et al., 1990; Choubert and Storebakken, 1989; Meyers, 1994; Putnam, 1992). As the mean rate of carotenoid intake increases, variation around the mean has less influence on chroma, and thus the signal-to-noise ratio must eventually decrease to the point where variation

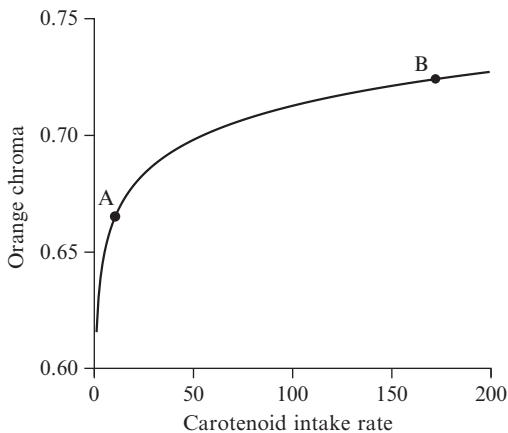


FIG. 2. Diminishing returns relationship between orange spot chroma and carotenoid intake of male guppies. Points A and B represent the mean rates of carotenoid intake in two hypothetical sites. Variation among males in carotenoid intake would have a larger effect on orange spot chroma at site A than at site B (modified from Grether (2000) with permission from Wiley-Blackwell).

in chroma is no longer indicative of variation in foraging ability. Wild populations of guppies occupy different positions on this carotenoid coloration reaction norm (see below), thereby providing an opportunity to test the general prediction that female preferences should evolve in response to changes in the indicator value of male traits.

1. Carotenoid Availability Gradient

As one moves upstream in the Northern Range of Trinidad (Fig. 3), from the lowland rivers to the headwater streams, the fish community becomes progressively less diverse, as larger species are excluded by the combined effects of physical dispersal barriers (waterfalls) and reduced productivity (Endler, 1978; Haskins et al., 1961). Waterfalls also restrict the movement of guppies, and populations in different branches of a river drainage are adapted to the local predation regime in multiple ways (e.g., life history traits, coloration, behavior; reviewed in Endler, 1995). Overlaid on the predation gradient is a parallel gradient in resource availability (Grether

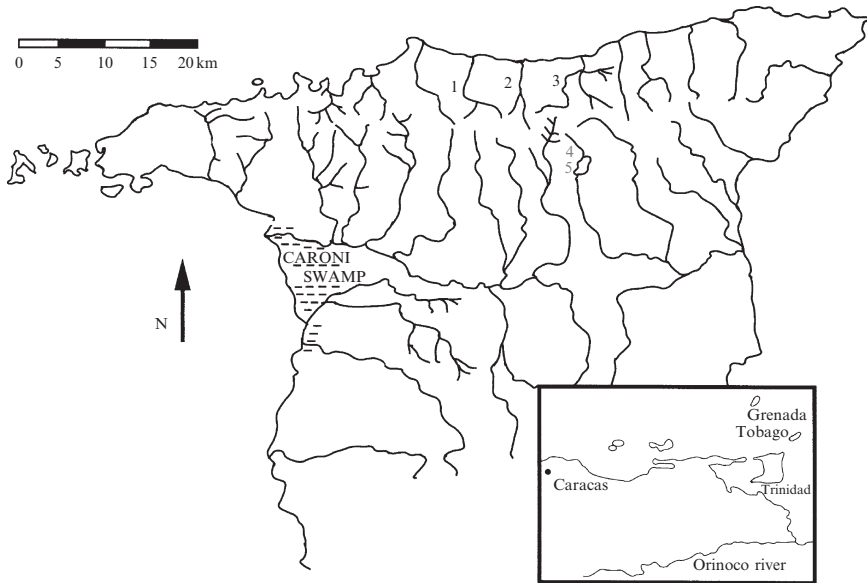


FIG. 3. Map of the Northern Range of Trinidad. The five river drainages on which our research on guppies has focused are numbered as follows: 1, Marianne; 2, Paria; 3, Madamas; 4, Upper Quare; 5, Lower Quare. For details on the study sites and GPS coordinates, see Grether et al. (2001b) and Kolluru et al. (2007). The map is modified from Houde (1997) with permission from A. E. Houde.

et al., 2001b; Reznick et al., 2001). Algal standing crops (periphyton) decrease visibly as one travels upstream. To examine the effects of variation in resource availability without the potentially confounding effects of variation in predation levels, we studied guppies above the barrier waterfalls where they coexist with a single weakly predatory fish, *Rivulus hartii*.

Guppies are omnivorous and their diet consists of approximately equal proportions of benthic invertebrates and unicellular algae (diatoms, green algae, and blue-green algae); terrestrial insects and vascular plant parts make up less than 1% of the diet (Dussault and Kramer, 1981). Most of the carotenoids they consume come from algae, either directly or by consuming algivorous invertebrates. In the upper reaches of Trinidad's watersheds, where the streams are narrow and make relatively small gaps in the rainforest canopy, algae production is strongly influenced by the amount of light available for photosynthesis (Grether et al., 2001b). Small increases in forest canopy openness (2–3%) are associated with substantial increases in algae availability (estimated as periphyton chlorophyll *a* divided by guppy biomass), and this is reflected in the growth rates and sizes at maturity of the fish (Fig. 4; Grether et al., 1999, 2001b).

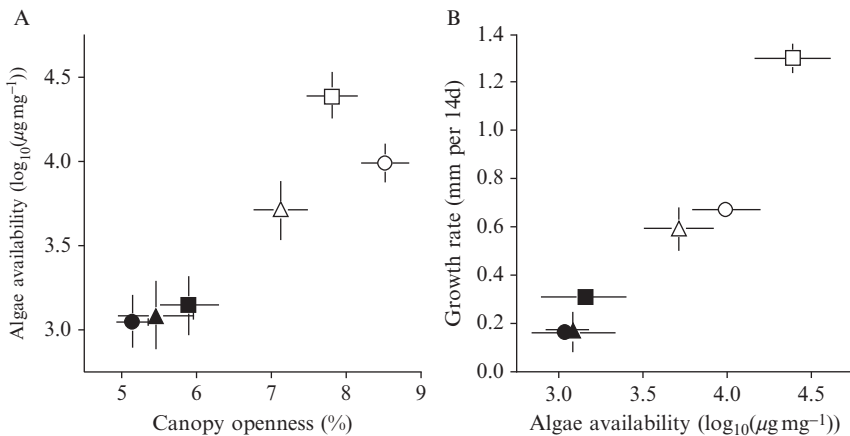


FIG. 4. Relationship across six study sites between (A) algae availability for guppies (μg of chlorophyll *a*/guppy biomass) and canopy openness (%) and (B) guppy growth rates (change in standard length over 2 weeks) and algae availability. Squares, circles, and triangles represent the Marianne, Paria, and Quare drainages, respectively, with filled (unfilled) symbols for sites with relatively closed (open) forest canopies in their respective drainage. Points depict means ± 1 S.E. (modified from Grether et al. (2001b) with permission from the Ecological Society of America).

The periphyton in Trinidadian streams contains at least nine different carotenoids, but based on the carotenoids found in the skin of guppies and the known biochemical pathways, only three of these algal carotenoids are converted into skin pigments (lutein, β -carotene, and zeaxanthin; Grether et al., 1999; Hudon et al., 2003). The availability of these assimilable carotenoids is strongly correlated with overall algae availability (Fig. 5). As carotenoid availability increases, the amount of assimilable carotenoids in the foreguts and skin of guppies both increase but the skin:foregut carotenoid ratio decreases (Fig. 6; Grether et al., 1999), just as we would expect if guppy populations occupy different positions on the asymptotic carotenoid coloration reaction norm (Fig. 2). The relationship between orange spot area and the concentration of carotenoids in the orange spots provides additional evidence for carotenoid limitation. In most guppy populations, orange spot area is highly heritable and not affected by carotenoid intake. If dietary carotenoids are scarce, large orange spots should be

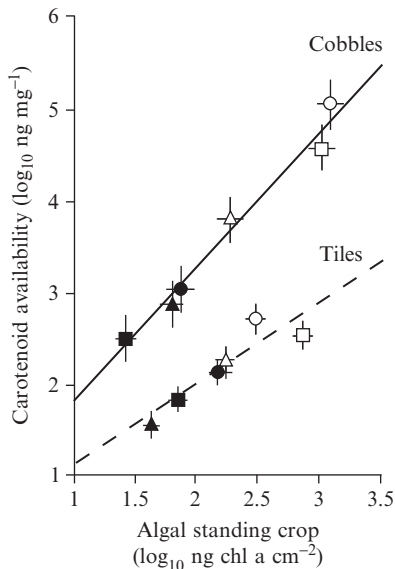


FIG. 5. Relationship across six study sites between carotenoid availability for guppies (ng of assimilable carotenoids per mg of guppy) and algal standing crops (ng of chlorophyll per cm of substrate). Solid lines are for the natural cobble substrate (points are displaced vertically by +1 in *a* and +2 in *b*), and dashed lines are for unglazed clay tiles that were placed in the stream and harvested after 45 days (no displacement). Symbols follow Fig. 4. Points represent means \pm 1 S.E. and lines are from least-squares regressions (modified, with symbol corrections, from Grether et al. (1999) with permission from Royal Society Publishing).

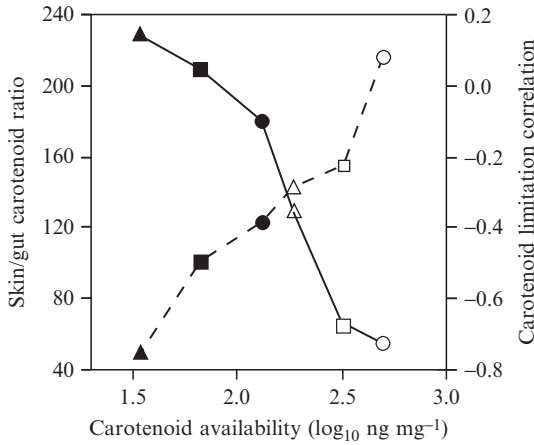


FIG. 6. Key evidence for carotenoid limitation of guppy coloration in nature. As carotenoid availability increases, the mean ratio of skin carotenoid content to foregut carotenoid content declines (points connected by solid line) while the within-site correlation between orange spot carotenoid concentration and total orange area increases (points connected by dashed line). Symbols follow Fig. 4 (modified from Grether et al. (1999) with permission from Royal Society Publishing).

more difficult to fill with pigment than small orange spots, resulting in a negative within-site correlation between total orange spot area and the concentration of carotenoids in the spots. As shown in Fig. 6, the magnitude of this negative correlation was greater, hence carotenoid limitation was greater, at sites with lower carotenoid availability. This implies that carotenoid deposition is closer to saturating at high-carotenoid-availability sites, and thus that carotenoid coloration should be a better indicator of male foraging ability in the low-carotenoid-availability sites.

2. Testing Predictions of the Carotenoid-Dependent Indicator Hypothesis

If the female preference for carotenoid coloration is free to evolve through the indicator process, the strength of this preference should be greater in low-carotenoid-availability sites than in high-carotenoid-availability sites. There are two possible routes to this outcome, corresponding to two alternative predictions. First, mean preference strength could shift genetically, such that populations exposed to different average environments diverge in preference strength. Second, a reaction norm could evolve such that the development of the preference within individual females depends on the same environmental factors that affect the indicator value of the trait or closely correlated factors. That is, variation in carotenoid availability within streams

(among pools or seasonally) could potentially select for conditional expression of female mate preferences, instead of genetically fixed population differences. If so, the strength of a female's preference for carotenoid coloration may depend on her assessment of carotenoid availability, as some animals can detect and respond behaviorally to ingested carotenoids (Carroll et al., 1997). Alternatively, females might use overall food availability as an indirect measure of carotenoid availability, since these variables are positively correlated in the field (Fig. 5).

To test the genetic divergence prediction, we measured the strength of the female preference for carotenoid coloration in lab-reared fish from paired high- and low-carotenoid-availability sites in five different river drainages (Fig. 3; Grether, 2000; Grether et al., 2005b). This sampling design helps to control for phylogenetic effects to the extent that guppy populations within one drainage are closer to each other genetically than populations in different drainages, as would be expected from the dispersal mode of these fish, and as suggested by genetic surveys (Crispo et al., 2006; Suk and Neff, 2009).

The experiment involved raising the offspring or grandoffspring of wild-caught fish in aquaria and feeding the males three different dietary levels of carotenoids (trace, low, or high; for detailed diet information, see Grether, 2000; Grether et al., 2005a). The experimental diets were designed to contain the same three assimilable carotenoids as periphyton. Based on comparisons of the mean skin carotenoid content of lab-reared and wild-caught fish, the low- and high-carotenoid diets are near the lower and upper ends of the natural range of carotenoid availability, while the trace-carotenoid diet is below the natural range (Kolluru et al., 2006). In the mate choice tests, we used an open-aquarium design in which three unrelated virgin females were allowed to interact freely with three males raised on different carotenoid levels. Each group of males was tested, in separate sessions, with females from their own population and with females from the other population in the same drainage. Male guppies perform discrete courtship displays called sigmoid displays and females give stereotyped responses that correlate with their probability of mating (Houde, 1997). We used the response of females to a male's sigmoid displays as a measure of his attractiveness to females in the group. The slope of the regression of male attractiveness on the level of carotenoids in the male diet provides a measure of strength of the preference for carotenoid coloration (cf. Houde and Endler, 1990). For all 10 populations, male attractiveness increased with dietary carotenoid levels, but the strength of this preference differed significantly between populations in the predicted direction in only two of five river drainages (Fig. 7). Thus, while female preference for carotenoid coloration has clearly diverged genetically among populations, the direction of divergence is not predicted by carotenoid availability in the wild.

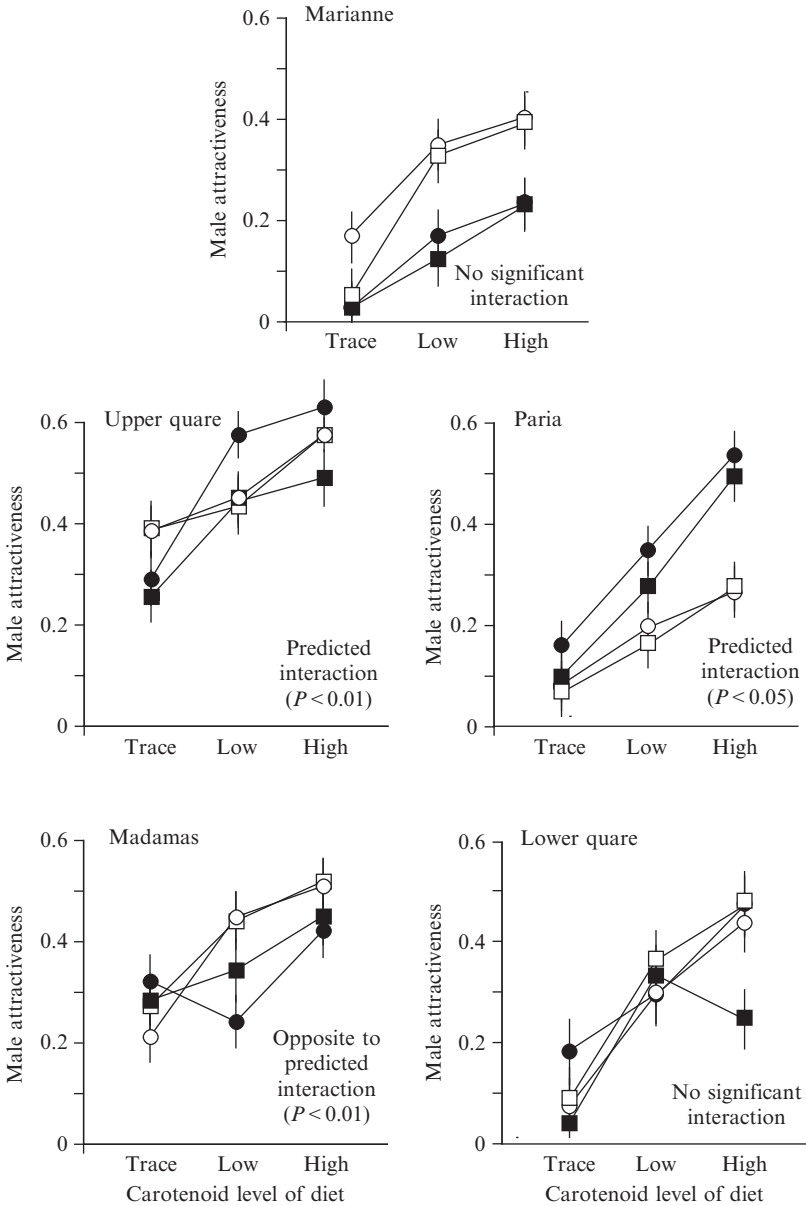


FIG. 7. Female preference functions for male carotenoid coloration in guppy populations from two sites differing in carotenoid availability in each of five river drainages (drainage names are shown in the figure). Within each drainage, filled circles represent the attractiveness of males from the low-carotenoid-availability sites to females from the

To test the phenotypic plasticity prediction, we raised females from birth on one of two food levels (low vs. high) and one of two dietary carotenoid concentrations (trace vs. high) and examined the effects of diet on the expression of the preference for carotenoid coloration. Based on the size at maturity of males raised on the same food levels, the low food level is near the low end of food availability in the wild while the high food level represents intermediate food availability in the wild (Kolluru et al., 2006). As predicted, females in the low-food, trace-carotenoid diet group developed stronger preferences for carotenoid coloration than females in the other three diet groups (Fig. 8). Although the strength of the female preference for carotenoid coloration varied significantly among populations, the sensitivity of the preference to female carotenoid intake did not.

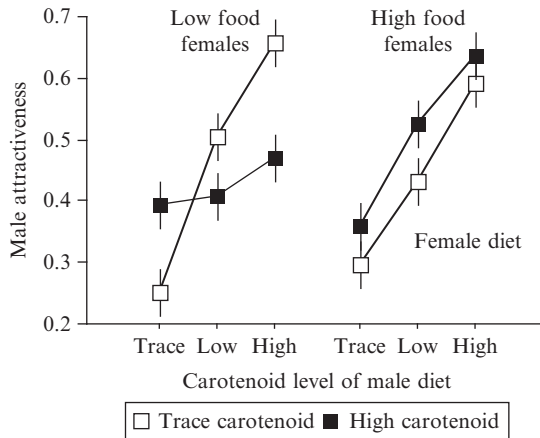


FIG. 8. Effects of diet on the strength of the female mate preference for carotenoid coloration. Points represent least-squares means \pm 1 S.E. (modified from Grether et al. (2005b) with permission from Royal Society Publishing).

low-carotenoid-availability sites, and unfilled circles represent the attractiveness of males from the low-carotenoid-availability sites to females from the high-carotenoid-availability sites. Conversely, filled squares represent the attractiveness of males from the high-carotenoid-availability sites to females from the low-carotenoid-availability sites, and unfilled squares represent the attractiveness of males from the high-carotenoid-availability sites to females from the high-carotenoid-availability sites. Points represent least-squares means \pm 1 S.E. The predicted pattern was for females from low-carotenoid-availability sites to express stronger preferences than females from high-carotenoid-availability sites, as reflected by the interaction between male carotenoid diet and female site. (Graphs on left modified from Grether, 2000 with permission from Wiley-Blackwell; graphs on right based on unpublished data and on statistical results presented in Grether et al., 2005b.)

Food intake had a significant negative effect on the sensitivity of the female preference to carotenoid intake, to the extent that the effect of carotenoid diet was only apparent in low-food females (Fig. 8), but food level, *per se*, had no overall effect on the strength of the female preference. In the wild, this reaction norm presumably causes females to base mating decisions more on the color of a male's orange spots, and less on other factors, when carotenoids are scarce in the diet (Grether et al., 2005b). In light of this plastic response to carotenoid intake, it makes sense that the preference has not diverged genetically in response to average differences among sites in carotenoid availability.

Before these results can be accepted as providing evidence for the indicator process, it is important to consider whether the sensitivity of the mate preference to carotenoid intake might instead have evolved as a foraging adaptation.

3. Orange Attraction Sensory Bias

In what began as a casual side project, Helen Rodd and Kim Hughes discovered that foraging guppies of both sexes are attracted to orange objects. After observing guppies eating orange fruit of the cabrehash tree (*Sloanea laurifolia*), which occasionally fall into Trinidadian streams, Rodd and Hughes tested the responsiveness of guppies to coins painted five different colors (red, orange, yellow, green, and black). At seven of eight sites, guppies approached and pecked the orange coins significantly more often than the other colors except red (Rodd et al., 2002). To determine whether this response develops in fish that have never been exposed to orange fruit, we tested lab-reared guppies from 10 different wild populations by presenting them with arrays of eight colored disks (red, orange, yellow, green, blue purple, black, white). The lab-reared fish were also preferentially attracted to orange and red disks (Fig. 9). Moreover, the average level of orange attraction for a particular population was strongly predictive of the average strength of the female preference for carotenoid coloration in the same population (Fig. 10), to the extent that orange attraction accounted for over 85% of the variation among populations in the strength of the female preference (Grether et al., 2005b; Rodd et al., 2002). The source of this interpopulation variation is unknown but might be related to the distribution of orange fruit. This hypothesis has yet to be tested.

The fleshy aril of *S. laurifolia* fruits contain high levels of the carotenoid zeaxanthin, which is one of the carotenoids that guppies can assimilate. Thus, orange attraction could serve to help guppies obtain carotenoids and other nutrients when algae are scarce. To determine whether orange attraction shows the same responsiveness to carotenoid intake as the mate

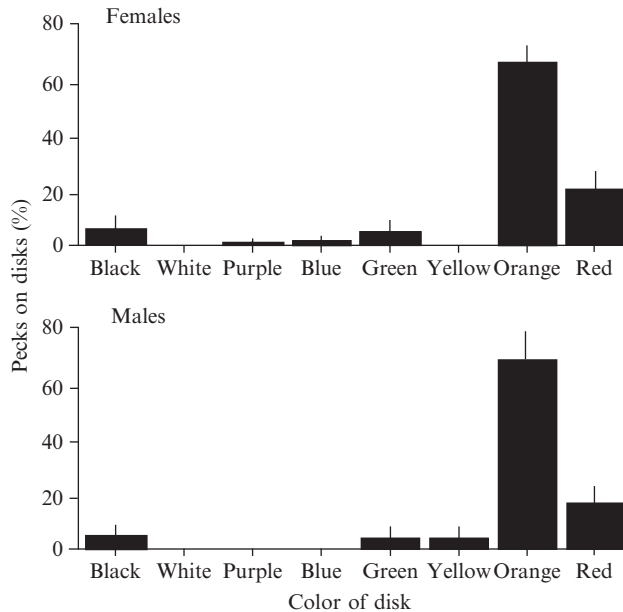


Fig. 9. Responses of lab-reared guppies to colored disks of eight colors (percentage of pecks). Based on previously unpublished data.

preference, we tested guppies of both sexes that had been raised on different diets. Females raised on a trace-carotenoid diet were more strongly attracted to orange disks than females raised on a high-carotenoid diet, but diet had no significant effect on orange attraction in males (Fig. 11; Grether et al., 2005b). We have since repeated this experiment on four additional populations of guppies using three dietary carotenoid levels (trace, low, and high) and obtained similar results (G. F. Grether et al., unpublished data). The largest difference between diet groups was between the high-carotenoid diet and the low-carotenoid diet, which shows that enhanced attraction to orange is not an artifact of raising guppies on an abnormally low (trace) carotenoid level. A separate experiment ruled out the possibility that the color of the food, as opposed to the carotenoid content, is responsible for the changes in orange attraction (F. H. Rodd et al., unpublished data).

In combination, these results strongly suggest that orange attraction and the carotenoid coloration mate preference are different expressions of the same underlying neural mechanisms. If orange attraction is a foraging adaptation, this might be a preexisting bias that led to the evolution of orange coloration through the sensory exploitation process (Ryan et al.,

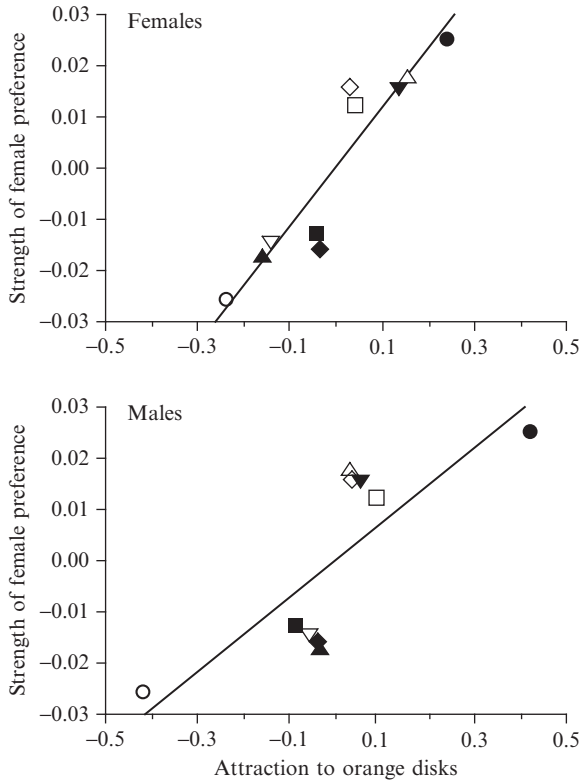


FIG. 10. The strength of the female mate preference for carotenoid coloration plotted against the mean level of orange attraction (pecks on orange disks) exhibited by females ($r^2 = 0.86$, $P < 0.0001$) and males ($r^2 = 0.63$, $P = 0.004$) from the same ten populations. The plotted values are residuals from river drainage means, to control for genetic differentiation among drainages. River drainage is indicated by the shape of the symbol (circle, Paria; square, Marianne; upward pointing triangle, Lower Quare; downward pointing triangle, Upper Quare; diamond, Madamas). Sites with relatively open (closed) forest canopies in their respective drainages are indicated by unfilled (filled) symbols (modified from Grether et al. (2005b) with permission from Royal Society Publishing).

1990; Shaw, 1995). Some other species of poeciliid fishes are also attracted to orange objects (F. H. Rodd, personal communication), but whether this sensory bias evolved prior to orange coloration has not yet been resolved.

What does the sensitivity of both the mate preference and orange attraction to carotenoid intake tell us about their evolutionary history? If this reaction norm can satisfactorily be explained as a foraging adaptation, then we would have no evidence that the mate preference has evolved through

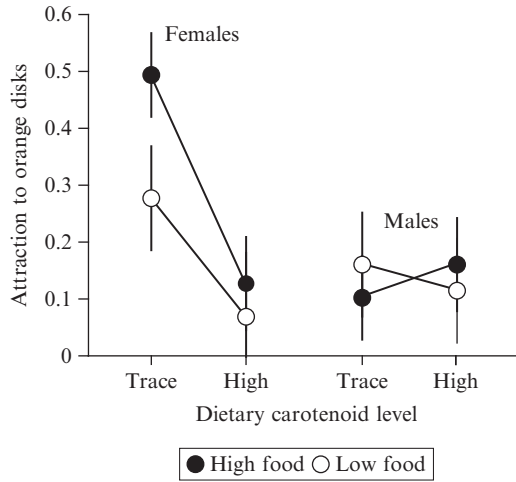


FIG. 11. Effects of diet on the rate at which guppies of both sexes pecked orange disks. Points represent least-squares means \pm 1 S.E. (modified from Grether et al. (2005b) with permission from Royal Society Publishing).

the indicator process. The sex-specificity of the reaction norm, however, suggests otherwise. If this is an adaptation for seeking out carotenoid-rich fruits when other sources of carotenoids are scarce, why is orange attraction only affected by carotenoid intake in females? Males benefit from consuming carotenoids in at least two ways that females do not. First, carotenoids increase their attractiveness to females (Grether, 2000; Kodric-Brown, 1989). Second, carotenoid consumption enhances the ability of males to reject foreign tissue (scale allografts). This immunological benefit was not found in females, perhaps because females do not face a trade-off between allocating carotenoids to immune function versus sexual coloration (Grether et al., 2004a). Female-specific benefits of carotenoid consumption have been reported in birds. Carotenoids transferred from female birds to their eggs have been shown to increase hatching rates and several components of offspring quality, including growth rates, survival to fledging, and the coloration of mature sons (Berthouly et al., 2008; Cucco et al., 2008; McGraw et al., 2005). Female guppies also deposit carotenoids in their eggs, and allocation to eggs versus maternal tissue increases with the level of carotenoids in the diet (Grether et al., 2008). Nevertheless, we found no effects of maternal carotenoid diet on female fecundity, offspring size or condition at birth, juvenile offspring growth rates, or the size, condition, or coloration of mature male offspring (Grether et al., 2008). Other possible

benefits to females of carotenoid consumption have not been ruled out, but the scope for females to benefit more than males do from consuming these pigments is remote at best. Therefore, the sensitivity of the mate preference and orange attraction to carotenoid intake is not likely to have evolved in a foraging context. Instead, this appears to be a case of a sensory bias being modified by the indicator process.

4. *Other Examples of Mate Preferences for Indicator Traits that may be Derived from Foraging Responses*

Three-spined stickleback (*Gasterosteus aculeatus*) and nine-spined stickleback (*Pungitius pungitius*) of both sexes are attracted to red objects (plastic strips) in a foraging context but only three-spined stickleback display red (carotenoid-based) breeding coloration (Smith et al., 2004). In the clade that includes both of these species, red breeding coloration is a derived condition unique to three-spined stickleback. Nine-spined stickleback and some populations of three-spined stickleback have black nuptial coloration, which is ancestral for the clade. In most populations of three-spined stickleback, females prefer males with red coloration, and this is a condition-dependent trait that reflects variation among males in parental care and parasite resistance (reviewed in Smith et al., 2004). There is no direct evidence, as yet, that the mate preference for red males and the foraging preference for red prey are linked, but the strength of the mate preference has been correlated with variation among populations in sensitivity to red light (Boughman, 2001). The sensitivity of female three-spined stickleback to red light varies seasonally and peaks during the spawning season when males express maximal red coloration (Cronly-Dillon and Sharma, 1968). Male three-spined stickleback are also sensitive to red light and preferentially attracted to red food items, but males do not show seasonal variation in sensitivity to red. This sex difference may be a product of the indicator process, although whether females benefit in other ways from exhibiting seasonal variation in sensitivity to red light has not been investigated. Another piece of evidence that the mate preference for red coloration in three-spined stickleback has been shaped by the indicator process is that the strength of this preference correlates positively with the degree of condition-dependence of red coloration among populations of three-spined stickleback in the lakes of British Columbia (Boughman, 2007). The direction of causality is unclear, however, because directional sexual selection may cause secondary sexual characters to become condition-dependent (Iwasa and Pomiankowski, 1999; Kodric-Brown and Brown, 1984). If it could be shown that the variation in condition-dependence was

caused by an environmental factor (e.g., carotenoid availability), then the correlation between condition-dependence and the mate preference for red coloration would constitute strong support for the indicator hypothesis.

The mate preference for another carotenoid-based trait, the terminal yellow band (TYB) of some Goodeinae fishes, also appears to be derived from a foraging adaptation, but has become partially decoupled from foraging behavior in species that possess a TYB (Garcia and Ramirez, 2005). Species without TYBs, which may represent the ancestral condition, respond to TYBs as though they were food items, while such responses are reduced in species in which males have evolved TYBs (Garcia and Ramirez, 2005).

The pheromones that male lizards use to attract females contain chemical compounds that are also found in prey and evoke feeding responses in the lizards (reviewed in Martin and Lopez, 2008). In Iberian rock lizards (*Iberolacerta cyreni*), the provitamin D content of a male's pheromones reflects the quality of his diet, and females show stronger sexual responses to the secretions of males with higher provitamin D content. Experimentally, increasing female hunger levels through food deprivation increases the rate at which females tongue-flick (a feeding response) to cotton swabs scented with mealworm prey, male pheromones, and provitamin A, but not to unscented control swabs (Martin and Lopez, 2008). Provitamin D has health benefits in lizards, and thus the provitamin D content of a male's pheromones may be an indicator of his health and quality as a mate. If so, this may be another example of an indicator trait that evolved to exploit a sensory bias that originally evolved as a foraging adaptation and which now serves the dual function of enabling females to find high-quality food and high-quality mates.

III. EVOLUTION OF INDICATOR TRAITS

A. THE THEORY OF HONEST SIGNALING

The general question of how secondary sexual characters could be stable indicators of mate quality has received considerable attention. The specific problem of how genetic variation in male quality is maintained in the presence of directional selection caused by female preferences is known as the "lek paradox." Many theoretical solutions to the lek paradox have been proposed (e.g., Fromhage et al., 2009; Kokko and Heubel, 2008; Miller and Moore, 2007; Neff and Pitcher, 2008; Rowe and Houle, 1996; Tomkins et al., 2004; Wolf et al., 2008) and some proposed solutions have received specific empirical support (e.g., Birkhead et al., 2006; Kotiaho et al., 2001;

Neff and Pitcher, 2009), but this remains a controversial topic (Andersson and Simmons, 2006). Indicators of direct benefits do not suffer from the same paradox because environmental variation in mate quality cannot be depleted by selection. Nevertheless, how the honesty of such traits is maintained is still an important issue. Zahavi's (1975) famous solution is that for a trait to be a stable indicator (i.e., handicap), it must have costs that are greater for low-quality individuals than for high-quality individuals, such that the optimal trait value (where costs and benefits balance) increases with phenotypic quality. Zahavi's logic leads to the conclusion that all evolutionarily stable signals must be costly in this way (Zahavi and Zahavi, 1997). A large number of empirical studies have provided evidence that sexually selected traits are costly, with the usual inference being they must, therefore, be honest indicators of something (mate quality, fighting ability, etc.). There is an element of circularity here, as the costs of secondary sexual characters are what originally drew Darwin's attention. The interesting question of how cheating is prevented is rarely investigated empirically.

B. THE CAROTENOID COLORATION PARADOX

When it comes to carotenoid-based coloration, the usual focus is on whether carotenoids are scarce in the diet and/or whether a trade-off exists between displaying carotenoids in the integument versus using them for other biological functions (Lozano, 1994; Olson and Owens, 1998). A substantial literature is devoted to enumerating the health benefits of carotenoids in species with carotenoid coloration (Clotfelter et al., 2007; McGraw and Ardia, 2003, 2004, 2007; McGraw and Klasing, 2004, 2006; Peters, 2007; Smith et al., 2007). But if carotenoids are scarce and have other important biological functions, why do animals rely on these pigments for attracting mates? The Zahavian answer would be that carotenoids attract mates precisely because they are scarce and biologically valuable. But this does not explain how cheating is prevented if alternatives to carotenoids exist. Whether or not females benefit from the indicator value of male carotenoid coloration, it would be advantageous for males to be optimally pigmented, regardless of their quality or the availability of carotenoids in the environment. Carotenoid-limited populations would therefore seem to be vulnerable to invasion by mutants displaying synthesized pigments or structural layers that mimic the spectral properties of carotenoids.

Yellow, orange, and red pteridine pigments are found in numerous vertebrate and invertebrate taxa (Bagnara and Hadley, 1973; Fox, 1976; Henze et al., 1977; Oliphant and Hudon, 1993). Animals synthesize

pteridines *de novo* from carbohydrates and proteins (Hurst, 1980). In some species, carotenoids and pteridines are found together in individual pigment cells (Goodrich et al., 1941) or in the same cells types at different stages of development (Bagnara and Hadley, 1973; Hama, 1970). How has the honesty of carotenoid coloration been maintained, over evolutionary time, in the presence of these synthesizable alternatives? What prevents males from enhancing their attractiveness, and “deceiving” females with pteridines?

C. DETAILED CASE STUDY: TRINIDADIAN GUPPIES

The orange spots of male guppies contain red pteridines (drosopterin) in addition to carotenoids, and both types of pigments contribute positively to the chroma of the orange spots (Grether et al., 2001a). In view of the evidence that female guppies prefer males with higher orange spot chroma (Grether, 2000; Houde and Torio, 1992; Karino and Shinjo, 2004; Kodric-Brown, 1989), what prevents males in carotenoid-poor environments from increasing their attractiveness to females by producing drosopterin? If males did use drosopterin to compensate for carotenoid scarcity, we would expect a negative correlation between the drosopterin and carotenoid content of the orange spots across sites differing in carotenoid availability. Instead, we found the opposite pattern. Orange spot drosopterin content correlates positively across sites with orange spot carotenoid content and also with carotenoid ingestion (Fig. 12; Grether et al., 2001a). Clearly drosopterin do not serve to compensate for carotenoid scarcity, but why not? And what accounts for the unexpected positive correlation between drosopterin production and carotenoid availability? I will present the three kinds of explanations that have been offered to account for this pattern and then the results of experiments designed to distinguish among them.

1. *Metabolic Cost Hypotheses*

Since unicellular algae are the primary food source as well as the primary carotenoid source for guppies, food availability and carotenoid availability are closely linked. Guppies grow faster and mature at a larger size at sites with higher algae availability (Fig. 4) and these population differences disappear when the fish are raised from birth in a common laboratory environment (Grether et al., 2001b). If drosopterin production is metabolically costly, then the optimum level of production may decrease as algae availability decreases. In theory, this could have two possible outcomes, either of which could explain the observed geographic pattern (Fig. 12), but

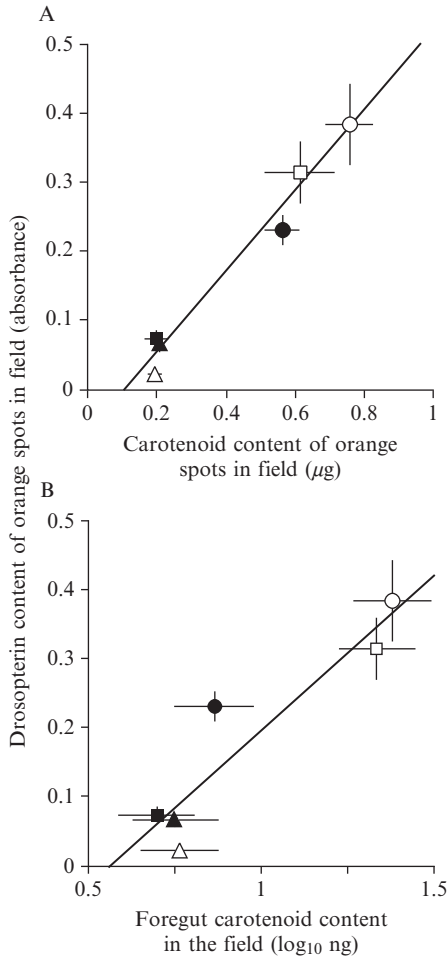


FIG. 12. Geographic covariation between (A) the drosopterins and carotenoid content of the orange spots of male guppies ($r = 0.99$, $n = 6$, $P < 0.0001$), and (B) drosopterins content of the orange spots and carotenoid ingestion, as inferred from foregut contents ($r = 0.93$, $n = 6$, $P = 0.0048$). Symbols follow Fig. 4. Points represent stream means \pm S.E. and lines are from least-squares regressions. Drosopterins are shown in absorbance units because the extinction coefficient is unknown (absorbance is linearly related to pigment content; modified from Grether et al. (2001a) with permission from Royal Society Publishing).

with different theoretical consequences. If drosopterin production is linked to food intake within individual males, drosopterins and carotenoids might be parallel indicator systems—both types of pigments would improve the value of the orange spots as indicators of foraging ability and health.

If, instead, fixed population differences in drosopterin production have evolved, drosopterins might be an expensive substitute for carotenoids that dilute the indicator value of the orange spots.

2. *Chroma-Brightness Trade-Off Hypothesis*

Another possible explanation for the geographic pattern (Fig. 12) is that the optimal balance of orange spot chroma and brightness depends on ambient lighting. Chroma increases as carotenoid and drosopterin content increase, but brightness (e.g., as estimated by total reflectance) decreases. If brighter coloration is favored in darker sites, as reported for forest birds (Marchetti, 1993), this could select for lower drosopterin production in darker sites, resulting in a positive relationship between drosopterin production and carotenoid availability.

3. *Hue Preference Hypotheses*

Another way to explain the geographic pattern is that female guppies may prefer a particular hue of orange that males can only reach by combining carotenoids and drosopterins in a particular ratio. The carotenoids in guppy skin are mostly esters of tunaxanthin, a yellow carotenoid. Shifts in the carotenoid:drosopterin ratio alter the shape of the reflectance spectrum and the perceived hue of the orange spots (Fig. 13). If female guppies prefer a particular orange hue, males would be selected to match actual or expected carotenoid deposition with a complementary amount of drosopterins. A hue preference might merely be a property of the orange attraction sensory bias with no function in a mating context. A more intriguing possibility is that a hue preference might be a female counteradaptation to males “cheating” with drosopterins in a carotenoid-indicator system. Imagine that males in an ancestral population only deposited carotenoids in their orange spots and that females simply preferred males with higher chroma. A mutation for depositing drosopterins in the orange spots would be advantageous for males, but as the mutation spread the indicator value of the orange spots would decrease because their chroma would no longer be a pure signal of carotenoid content. Selection might then have favored females who discriminated among males on the basis of hue (pigment ratio) as well as chroma (total pigment content). Carotenoids that appear yellow at low concentrations shift to orange at high concentrations (Hudon et al., 2003), and thus a preference for orange, as opposed to yellow or red hues, might be optimal for identifying high-carotenoid males.

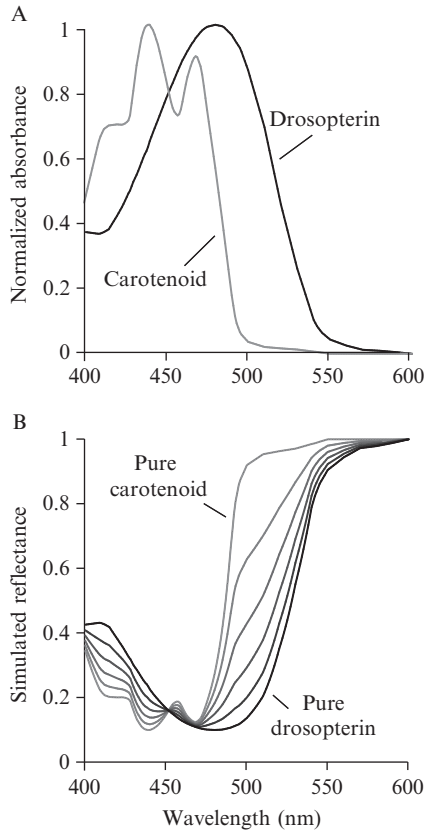


FIG. 13. (A) Absorbance spectra for carotenoids and drosoperins extracted from the orange spots of male guppies. (B) Simulated reflectance spectra for different carotenoid:drosoperin ratios. The simulation indicates that the orange spots would appear yellower (to human eyes) as the proportion of carotenoids in the spots increases, and redder as the proportion of drosoperins increases (modified from Grether et al. (2001a) with permission from Royal Society Publishing).

4. Unraveling the Geographic Pattern

To determine whether the geographic variation in drosoperin production in the field is genetic or environmentally induced, we analyzed the biochemical composition of the orange spots of second generation, laboratory-born males. To examine whether carotenoid intake directly affects drosoperin production, the fish were raised from birth on three different dietary concentrations of carotenoids. The results showed clearly that the population differences in drosoperin production are mostly genetic.

Fish born and raised in the lab developed roughly the same drosopterin levels as wild-caught fish from the same populations (Grether et al., 2005a). Full-sib analyses showed that the drosopterin content of the orange spots is heritable within some populations. The level of carotenoids in the diet had a weak negative effect on orange spot drosopterin content, but this plastic response is opposite in direction to the geographic pattern and only explained a trivial amount of variation in drosopterin content of the lab-reared fish. In a separate experiment, males were raised from birth on two different food levels to determine whether drosopterin production is constrained by food intake. Males in the high-food treatment were significantly larger at maturity than males raised on the low-food level, and the low-food males were small even in comparison to males in low-algae-availability sites in the field (Kolluru et al., 2006). Nevertheless, food level had no effect on the concentration of drosopterins in the orange spots (G. F. Grether et al., in preparation).

5. *Distinguishing among Hypotheses*

Even though the population differences in drosopterin production are mostly genetic, the results of the food-level experiment are difficult to reconcile with the metabolic cost hypotheses. If the cost of drosopterin production were high enough to override attractiveness benefits, food limitation ought to have some measurable effect on drosopterin production in individual fish, but this was not the case. The chroma-brightness hypothesis can also be rejected because neither orange spot brightness nor orange spot drosopterin content correlate with ambient light levels (or canopy openness) in the field (Grether et al., 2001a).

The hue preference hypothesis predicts that the hue of the orange spots is conserved across sites differing in carotenoid availability. Testing this prediction is complicated by the absence of information on how nonhuman animals perceive hue. Hue, chroma, and brightness technically refer to how a color stimulus is perceived, not to physical properties of the reflectance spectrum, although this distinction is often blurred in practice (Endler, 1990). Hue refers to what we perceive as different categories of color. Reflectance spectra with the same overall shape can vary in brightness and chroma without changing in hue. For example, humans classify reflectance spectra with unimodal peaks at 480 and 530 nm as blue and green, respectively. Variation in the height of a peak relative to the baseline is perceived as variation in chroma (pale vs. saturated), while variation in the height of the whole spectrum is perceived as variation in brightness (bright vs. dark). Using a spectrometer, we examined the physical shape of orange spot reflectance spectra (see Fig. 1) and also calculated photoreceptor cone

excitation estimates based on the known spectral sensitivity functions of guppy cones. Guppies have four classes of cones, each maximally sensitive to a different wavelength of light (U, 389 nm; S, 410 nm; M, 465 nm; and L, 533–572 nm; Archer and Lythgoe, 1990). Contrasts between the M and S cones (i.e., excitation differences) are especially effective for detecting changes in the carotenoid:drosopterin ratio (see Grether et al., 2005a). We also used a nonspecies specific method (segment classification; Endler, 1990) and a color vision model based on guppy cone sensitivities (Grether et al., 2004b) to calculate estimates of hue (angles in color space). By all measures, the spectral shape or hue of the orange spots was conserved among populations in the field, relative to fish from the same populations reared on different carotenoid levels in the laboratory (Fig. 14; Grether et al., 2005a). Thus, genetic variation among populations in drosopterin production compensates for environmental variation in carotenoid availability, resulting in a roughly constant carotenoid:drosopterin ratio, which minimizes variation in the spectral shape or hue of the orange spots. If this countergradient pattern was caused by selection for a constant

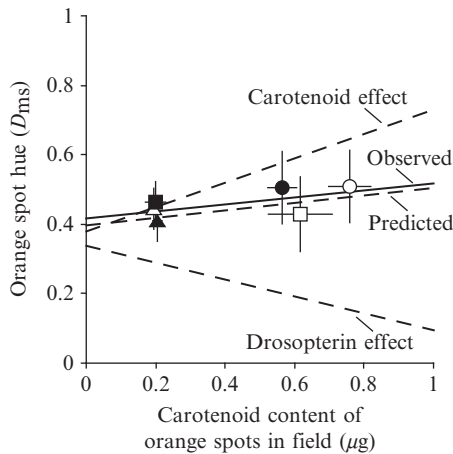


FIG. 14. Diagram showing that guppy populations in the field vary little in a photoreceptor cone-based measure of orange spot hue (D_{ms}) because of the counterbalancing effects of drosopterin and carotenoids. Plotted points represent population means (± 1 S.E.). The solid line represents the least-squares regression through the observed population means; the upper and lower dashed lines represent the effect of each pigment separately on D_{ms} ; the middle dashed line represents the predicted values of D_{ms} , given the observed orange spot pigment content means (modified from Grether et al. (2005a) with permission from Wiley-Blackwell).

orange hue across sites differing in carotenoid availability, it would be a rare example of genetic compensation in a secondary sexual character (Conover et al., 2009; Grether, 2005; Price, 2006).

To determine whether female guppies prefer males with a specific orange hue, we crossed low- and high-drosopterin populations in the lab and performed full-sib crosses in the F_1 generation to generate an F_2 generation with high levels of genetic variation in drosopterin production against a randomized genetic background. Mate choice tests with F_2 males raised on a low-carotenoid diet indicate that females prefer males with intermediate drosopterin levels, as predicted by the hue preference hypothesis and not by the prevailing notion that females always prefer higher orange chroma (K. Deere et al., in preparation).

6. *Optimal Pigment Combinations*

The evidence that female guppies have an orange hue preference raises another interesting signal evolution question. Why do males combine red drosopterins with yellow tunaxanthins to produce orange coloration instead of using orange pigments? Many animals convert ingested yellow carotenoids into orange 4-keto-carotenoids (e.g., astaxanthin, canthaxanthin). Although no wild poeciliids have been reported to use 4-keto-carotenoids, they have been found in *Xiphophorus* hybrids (Rempeters et al., 1981). Thus, the absence of 4-keto-carotenoids in guppies seems unlikely to reflect a deep phylogenetic constraint. One possible adaptive explanation for the use of tunaxanthin is that this pigment also serves a utilitarian function, such as crypsis, protection from ultraviolet light, or immune system enhancement (Fox, 1976; Goodwin, 1984; Møller et al., 2000; Needham, 1974; Rothchild et al., 1975). Consistent with this hypothesis, tunaxanthin is found in lower concentrations in the skin outside the orange spots and also in the drab skin of females (Hudon et al., 2003). However, given that animals can selectively deposit different types of carotenoids in different parts of their integument (Hudon, 1991; Hudon and Brush, 1989), it is not clear why male guppies would be constrained to also use tunaxanthins for attracting females.

The key to solving the puzzle of why male guppies use drosopterins and tunaxanthin may be to simultaneously consider all three axes of color perception that are likely to influence a male's attractiveness to females (i.e., hue, chroma, and brightness). First, a single type of carotenoid would not permit males to achieve a consistent hue across different levels of carotenoid availability because the shape of a pigment's absorbance spectrum changes with its concentration (Hudon et al., 2003). Second, while similar hues could be obtained by combining drosopterins with a 4-keto-carotenoid such as

astaxanthin, computer simulations show that, holding total pigment content constant, higher chroma, and brightness can be achieved by combining drosopterins with tunaxanthin (Hudon et al., 2003). Thus, the dual pigment system may enable male guppies to achieve the preferred hue while maximizing chroma and brightness.

7. *Conclusions and Unanswered Questions about Drosopterins*

Male guppies appear to use drosopterins to enhance their attractiveness to females but in a manner that does not eliminate the value of carotenoids as indicators of male health and foraging ability. Drosopterins may enable males to achieve the orange hue preferred by females more efficiently than the alternative route of converting ingested carotenoids into orange carotenoids. Whether drosopterins actually replaced the use of orange carotenoids in an ancestral species might be possible to evaluate by examining whether the appearance of drosopterins coincides with the disappearance of 4-keto-carotenoids in the cyprinodont phylogeny (Hudon et al., 2003). The presence of orange carotenoids in *Xiphophorus* hybrids (Rempeters et al., 1981) could mean that the metabolic pathway for conversion of ingested carotenoids into orange 4-keto-carotenoids is vestigial in poeciliids (i.e., retained from ancestor that used orange carotenoids).

Since most of the interpopulation variation in drosoprotein content of the orange spots is genetic and drosoprotein content is highly heritable in some populations, it would be logical to infer that drosoprotein production is relatively insensitive to environmental influences. We have recently discovered, however, that the drosoprotein and carotenoid contents of the orange spots both decrease rapidly after males are infected with the ectoparasite *Gyrodactylus turbulli* (G. F. Grether and G. R. Kolluru, unpublished data). This raises the possibility that drosopterins actually enhance the value of the orange spots as indicators of a male's immunological competence or disease history. Like carotenoids, pteridines are antioxidants, and some pteridines are known to play a direct role in the immune system (reviewed in McGraw, 2005). Thus, it is plausible that drosopterins are mobilized by the immune system in response to infections. Alternatively, *Gyrodactylus* might interfere somehow with drosoprotein synthesis.

Another reason for thinking that drosoprotein content of the orange spots might be an indicator of mate quality is that drosoprotein expression appears to be testosterone-dependent (Devasurendra et al., 2007; Jayasooriya et al., 2002). Testosterone is immunosuppressive, and thus it has been hypothesized that testosterone-dependent secondary sexual characters are indicators of immune system strength or parasite loads (Folstad and Karter, 1992; Mougeot et al., 2009; Peters, 2007). The evidence that drosoprotein

production is testosterone-dependent in guppies is that testosterone-treated food causes red coloration to develop in guppies of both sexes by 1 week of age, several weeks before male coloration is normally expressed. Pigment extractions at 3 weeks revealed that the red patches of testosterone-treated fish contained drospterins but no carotenoids (Devasurendra et al., 2007; Jayasooriya et al., 2002). Whether drospterin levels in adult male guppies reflect variation in testosterone titers remains to be determined.

IV. ADDITIONAL SUGGESTIONS FOR FURTHER RESEARCH

If the only way to truly make progress in science is to eliminate hypotheses (Platt, 1964), then the study of mate preference evolution has not been very fruitful. The research agenda of “testing the alternatives” that emerged from a Dahlem conference in 1986 (Bradbury and Andersson, 1987), and which shaped the direction of research in sexual selection for several years has produced only a handful of simple evolutionary stories. In guppies and most other well-studied systems, the evolutionary stories that are emerging are complicated and incomplete. Apparently nature is more complex than we would like it to be.

In their review of the current state of sexual selection research, Andersson and Simmons (2006) suggested that a possible way to move forward is take a bottom-up approach and use molecular tools to study the genes and gene products that influence the expression of secondary sexual characters and mate preferences. No doubt genomic research will deepen our understanding of how mate preferences and secondary sexual traits develop and evolve. I think the prospects for progress will be greatest, however, if these methods are combined with well-designed comparative and experimental evolution studies.

The general approach that I have outlined above for testing indicator models is to exploit or create situations in which the indicator value of secondary sexual traits varies among populations and then examine whether the mate preference evolves as predicted. To avoid causal ambiguity, it is critical for the variation in the indicator value of the trait to be caused by an external environmental factor (e.g., carotenoid availability) or to be under experimental control. I hope this review encourages researchers to find ways to test this prediction in other study systems, but I will restrict my specific research suggestions to extensions of our work on guppies.

Our inference that the carotenoid-dependence of the orange attraction sensory bias is a byproduct of the indicator process, as opposed to a foraging adaptation, is based entirely on the sex-specificity of this reaction norm (Grether et al., 2005b, 2008). Though logical, this inference is shaky because

the sex difference might have evolved for some other unknown reason. One possible way to resolve this would be to examine the effects of dietary carotenoid levels on orange attraction in other poeciliid species. If orange attraction is affected by carotenoid intake in species in which males lack orange coloration, and especially if a phylogenetic analysis indicates that this is the ancestral condition, this would eliminate all evidence for the indicator process and support the alternative hypothesis that the mate preference is an unmodified sensory bias. Alternatively, if only species with orange coloration have carotenoid-dependent sensory biases, this would clinch support for the indicator process.

Our research on guppies has raised far more questions about proximate mechanisms than it has answered. Are orange attraction and the mate preference for carotenoid coloration genetically correlated within populations? How exactly are they linked at the neurobiological level? How do female guppies detect carotenoid intake and how does this influence the development of orange attraction and the mate preference for carotenoid coloration? We are currently investigating the possibility that shifts in opsin gene expression are involved, but other possible mechanisms have not been explored. Are the effects of carotenoid intake on the behavior of females reversible within individual fish if carotenoid availability changes, or is this a fixed developmental response? Answers to these questions might have a bearing on evolutionary mechanisms as well.

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