# Variation in Response to Artificial Selection for Light Sensitivity in Guppies (*Poecilia reticulata*)

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ABSTRACT: We performed artificial selection on the visual system in guppies (*Poecilia reticulata*), using the optomotor reaction threshold as the selection criterion. Two lines were selected for increased sensitivity to blue light, two were selected for increased sensitivity to red light, and two were unselected controls. There was significant response to selection in all four selected lines and significant heritability for sensitivity. An examination of the spectral sensitivity function showed that the form of the response differed between the red and blue lines and among the red lines. Such divergence is likely because there are many different mechanisms allowing response to selection for spectral sensitivity. Diverse mechanisms allow a divergent response by different populations to the same selective pressures. Such a mechanism can promote diversity in vision and visual signals, and any multicomponent system where different components can respond to the same selective regime.

*Keywords:* artificial selection, genetic variation, selection, sensory drive, vision.

If a population invades a new habitat or its existing habitat changes, then it will experience new environmental conditions during courtship, mate choice, foraging, and predation avoidance. These new conditions can affect the performance of the sensory system, which in turn may induce natural selection on the senses. Changes in the sensory system may cause changes in perception and hence changes in female preferences for male traits and lead to evolutionary changes in male traits and female preferences. Sensory evolution may also induce evolution in microhabitat choice, foraging modes, and many other aspects of a species' biology. These interconnected processes are called "sensory drive," and well-studied components include "sensory exploitation," "sensory traps," and "preexisting biases" (reviewed in Endler and Basolo 1998). A critical assumption of sensory drive is that the sensory system has a genetic basis and can respond to selection. In this study, we tested this assumption by artificial selection for sensitivity to different colors.

Vision is complex because so many processes are involved. Both vertebrate and invertebrate eyes have photoreceptors (cones, rods, or rhabdomes), which are sensitive to different ranges of wavelengths (colors) of light. Visual pigments (and, in some species, colored oil droplets) in the photoreceptors determine which wavelengths are collected by each photoreceptor class. During visual processing, photoreceptor cells communicate with other cells that either combine or take the difference between the outputs of different photoreceptor classes, coding for brightness and color. Still further processing is done in the brain (Lythgoe 1979; Jacobs 1981, 1993; Endler 1990, 1993*b*; Goldsmith 1990; Wandell 1995). The genetics and evolution of visual pigments are just beginning to be understood (Yokoyama 1994, 1997, 2000; Yokoyama and Radlwimmer 1999; Yokoyama and Yokoyama 1996), but with few exceptions (Jacobs et al. 1993, 1996; Jacobs 1996), we do not know the functional or evolutionary implications of genetic variation in visual pigments, nor do we understand the genetics of visual processing. However, in this study, we are primarily interested in whether a response to selection on vision is possible and what form it can take.

We investigated the response of guppies (*Poecilia reticulata*) to artificial selection for sensitivity to red or blue light. The selection criteria was the optomotor response to a particular wavelength band (red or blue). The response to selection will be mediated by variation in genes controlling the visual pigments that determine wavelength specificity of each photoreceptor type (cones and/or rods), those affecting the photoreceptor outputs, those affecting the interactions between the photoreceptor outputs, and those affecting subsequent processing in the retina and the

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brain. A response can result from genetic changes in one or more of these components.

The word "response" has a very different meaning in physiology and evolutionary biology; it means "reaction to a stimulus" in the former and "genetic change as a result of selection" in the latter. Terms like "optomotor response" and "response to selection" are well established in their respective fields. To avoid confusion in this article, we will substitute "reaction" for "response" (e.g., "optomotor reaction") whenever we refer to the physiological meaning.

## Methods

### *The Model System*

Guppies (*Poecilia reticulata*) are small Poeciliid fishes native to streams in Trinidad and adjacent Venezuela. They are ideal for this study because so much is already known about their biology, ecology, genetics, vision, behavior, and sexual selection (Endler 1995; Houde 1997). Guppies have excellent vision; their retinas contain cones with peak absorbances at 359 nm (UV or UVS), 408 nm (SWS or "blue"), 464 nm (MWS or "green"), 533–572 nm (LWS or "red"), and rods at 502 nm (Archer et al. 1987; Archer and Lythgoe 1990; V. N. Rush, personal communication, 1998; the abbreviations "UVS," "SWS," "MWS," and "LWS" stand for ultraviolet, short wavelength, middle wavelength, and long wavelength sensitive, respectively). There is variation in the peak sensitivity of the LWS cones among and within individuals; peaks cluster at 533, 572, and possibly 543 nm (Archer et al. 1987; Archer and Lythgoe 1990). This implies that a female with, say, primarily 533-nm LWS cones might perceive males with more orange spots as more chromatic and males with more reddish spots as less chromatic, whereas another female with primarily 572-nm LWS cones might reverse the ranking. Thus, we have a possible neural mechanism for variation in the female choice criterion (Endler and Houde 1995), even if females were genetically fixed for preferring the "brightest" males. Of course, other visual mechanisms and other sensory systems are also involved in perception and female choice.

### *Measurement of Sensitivity*

We artificially selected guppies for sensitivity to a particular wavelength band (blue or red). We chose sensitivity as a selection criterion because it yields some of the same insights into vision and perception as does visual pigment variation but is not as time consuming as determining photoreceptor pigments. We measured sensitivity by means of the optomotor reaction to particular wavelength bands, which also has the advantage of not harming the fish, allowing a simple selection design. Optomotor chambers take advantage of the fact that fish (and other animals) use objects as directional references by which to orient and to maintain their position (Marler and Hamilton 1968; Nicol 1989); fish stop orienting when they no longer see the objects.

Each light-adapted fish was placed in an 18.5-cm-diameter cylindrical aquarium (7.5 cm deep). Light adaptation was performed by keeping the fish in natural daylight intensities until just before the experiment and ensured that we were measuring the effects of cones (photopic vision) rather than rods (scotopic vision; Lythgoe 1979). Just outside the cylindrical aquarium was another cylinder 28-cm in (inner) diameter, painted with 18 alternating black and white stripes on the sides and bottom. The outer cylinder rotated at a slow speed (about 6 rpm). Fish orient toward the moving stripes and follow them closely when there is enough light. The fish's behavior was observed by means of a high sensitivity CCD video camera and an infrared light source. The chamber and stripes were illuminated uniformly by a 250-W tungsten-halogen slide projector lamp, lenses, heat and changeable color filters, and a convex mirror to spread the filtered or unfiltered light throughout the chamber. Each fish was initially light adapted in "white" light (no filter in the light path). At the start of each test, a colored filter (Oriel) was placed in the light path to illuminate the stripes with monochromatic light (10-nm bandwidth); ambient light was colored during each test. A rheostat controlled the brightness of the lamp, hence the brightness of the monochromatic light, and the narrow filter bandwidth prevented a shift in color with voltage. Light intensity in visually relevant quanta ($\mu$mol $m^{-2}$ $s^{-1}$; see Endler 1990) was measured in the chamber by a quantum radiometer (LI-COR LI-189) built into the chamber. Light intensity was initially high (40 $\mu$mol $m^{-2}$ $s^{-1}$) to ensure light adaptation (natural guppy populations experience about 10 $\mu$mol $m^{-2}$ $s^{-1}$ during the day; Endler 1991, 1993*a*). Under these conditions, guppies regularly tracked the moving stripes. The light intensity was reduced until the fish no longer tracked the stripes, then increased (to tracking), and decreased again, and increased and decreased a third time if there was any doubt about the minimum tracking intensity. The result is the threshold tracking light intensity $Q$. Lower $Q$ means higher sensitivity. Threshold light intensities were log transformed, $\ln(Q)$, before subsequent analysis because visual systems respond to the log of intensity rather than intensity (details in Vorobyev and Osorio 1998).

Because light intensity is reduced during the measurement of $Q$, the early stages of dark adaptation (shift from cone to rod vision) may take place during the measurements. This was minimized by stopping measurements after 5 min and not including fish that did not respond

during this time ($Q$ was not observed to change over 5 min in pilot experiments). The mean ± SD time to reach $Q$ over all lines and generations was 2.74 ± 1.19 min (blue filter) and 2.89 ± 1.35 min (red filter), and about 1% of fish did not respond in 5 min. Because full dark adaptation in teleosts (which includes exchange between cone and rod positions along the light axis) takes 20–30 min (Wagner et al. 1992), it is likely that dark adaptation (shift to rods) was minimal during measurements of $Q$. This is supported by the lack of a relationship between $Q$ and the time to reach $Q$. If a particular fish took longer to reach threshold, then it would have had more time to dark adapt and therefore its $Q$ should be lower than another fish that reached its threshold more quickly; this predicts a significant negative correlation between $Q$ and the time to reach $Q$. Only 10 of these correlations were significant after the Bonferroni correction (Rice 1989), and the ratio of significantly positive to significantly negative correlations was not significantly different from 1 : 1 with ($\chi^2 = 2.5$) or without ($\chi^2 = 3.37$) the Bonferroni correction to the individual correlation $P$ values. Pooling the data over generations (1–8) results in a significantly positive correlation ($r = 0.15$ B lines measured blue, $r = 0.19$ C lines measured blue; $r = 0.36$ R lines measured red; $r = 0.19$ C lines measured red; all $P < .001$, $N = 1{,}251$–$3{,}221$). This is opposite to the negative correlation predicted if dark adaptation were important and indicates that, on average, fish taking longer to reach $Q$ ended with a higher $Q$. Courtship in guppies normally occurs at light intensities that could allow rod-cone interactions (Endler 1991, 1993*a*), and the initial phases of the transition from photopic (all cone) to mesopic (cone-rod) vision may have occurred during our measurements. In any case, the brief measurement time makes it unlikely that the response is entirely or largely due to rods, which will still be largely shielded by the masking pigments in the pigment epithelium at the back of the retina (Wagner et al. 1992) during the 5-min or less test.

Fish were placed in a very dilute solution of MS-222 (0.4 $\mu$g mL$^{-1}$) a minute before being placed in the chamber to reduce stress. Some fish without this treatment appeared to interpret the rapid reduction in light intensity as the shadow of an approaching predator (Seghers 1974) and would dash rapidly around the measuring chamber. Aside from the elimination of this erratic behavior, the brief and dilute MS-222 treatment before testing did not appear to affect their swimming, orientation, or other behavior.

### *General Design and Traits Selected*

There were three treatments, selected for increased sensitivity to blue light, selected for increased sensitivity to red light, and unselected (controls), with two replicates each. We selected for increased blue or increased red sensitivity. We did not select for high or low blue (or red) sensitivity because selection for decreased sensitivity to one color could result in decreased sensitivity to all wavelengths for reasons independent of vision (such as decreased activity). We measured $Q$ and performed selection under a 420-nm (blue) and a 660-nm (red) filter each generation; controls were measured under both filters. These filters are a compromise between the need to be at the extremes of spectral sensitivity (to minimize the number of cone classes they both stimulate) yet minimize noise caused by insufficient radiant power of the lamp at short wavelengths and low spectral sensitivity of guppies to long wavelengths.

Using the methods of Lythgoe (1979), Endler (1991), Lythgoe and Partridge (1991), and Vorobyev and Osorio (1998), the cone pigment data of Archer and Lythgoe (1990; Archer et al. 1987; J. C. Partridge, personal communication, 2000), the guppy lens transmission data of R. H. Douglas (personal communication, 2000; also Thorpe et al. 1993), the adapting and test lamp spectra, and the transmission spectra of the experimental filters, we can calculate the photon capture functions of each cone under each filter. The $\log_e$(photon captures) of each photoreceptor class under the red treatment (660-nm filter) relative to that of the blue treatment (420-nm filter) are 0.0 (520 rods), 0.0 (359 or UVS cone), 0.0 (408 or SWS cone), 0.0 (464 or MWS cone), 0.79 (533 or $LWS_1$ cone), and 4.46 (672 or $LWS_2$ cone). Note how the 660-nm filter essentially stimulates only the long-wavelength-sensitive (LWS) cones whereas the 420-nm filter stimulates all cones and the rods (as a result of absorption by the $\beta$ and $\gamma$ bands of the visual pigments; see Stavenga et al. 1993), albeit differently (not shown). Note how the 660-nm filter stimulates the 533 cone about 80% as much as the 420-nm filter while it stimulates the 572 cone four and a half times more than the 420-nm filter. This is also seen in the ratio of stimulation of the 572 cones relative to the 533 cones for each filter: 0.51 for the 420-nm filter and 2.89 for the 660-nm filter. Thus, the 660-nm filter has more of an effect on the 572 cones and the 420-nm filter more of an effect on the 533 cones. If there is any mesopic vision the two filters will also have differential effects because the 660-nm filter does not stimulate the rods at all and the 420-nm filter will stimulate the rods 1.6–3.1 times more than the LWS cones (533 and 573, respectively). Together this means that, although the optomotor reaction is usually mediated through the LWS cones (Schaerer and Neumeyer 1996; Anstis et al. 1998), artificial selection under the 420- and 660-nm lights should affect different photoreceptor classes differently, particularly the different LWS cones.

At generation 9, we used 420-, 460-, 500-, 540-, 580-, 620-, 640-, and 660-nm filters to obtain the spectral sensitivity curve for each fish. This method yields results sim-

ilar to that obtained by flicker ERG, another nondestructive method (see fig. 2 of Endler 1991).

### *Artificial Selection Protocol*

Guppies were collected from the headwaters of the Paria River (Trinidad; Paria 4, National Grid PS 896 886). This population was chosen because we know much about the sexual selection, genetics, ecology and vision of this population (Archer et al. 1987; Archer and Lythgoe 1990; Endler 1991, 1995; Houde 1991, 1997; Endler and Houde 1995). Two hundred of the wild-caught fish were used to initiate the selection lines within a month of capture. One generation later (March 1991), six groups of 10–20 males and 10–20 females were taken at random and used to set up each of the six experimental lines: two control lines (C1, C2), two lines to be selected for increased sensitivity to blue light (B1, B2), and two lines to be selected for increased sensitivity to red light (R1, R2). This was generation 0.

All lines in all subsequent generations were handled as follows: the guppies taken as parents were placed together in 91 × 47 × 56-cm aquaria for normal mating under a mixture of natural light from north-facing windows and fluorescent lighting. These aquaria are the "home population tanks." After 3–4 wk of mating, males were photographed to record their color patterns, and females were then placed singly in 7-L tanks with gravel and *Fontinalis* moss to produce young. After each female produced young, she was removed, and her young were allowed to grow large enough for spectral sensitivity measurements. We kept sibling records of each fish from generation 5 onward, but it was impractical to relate the spectral sensitivity of siblings to that of their parents because parents were put into the home tanks for mating after measurement, females chose mates of their own accord, mothers had broods after being taken out of the home tanks, and mothers had no distinguishing traits. Sensitivity was measured on two to six offspring of each mother. In each replicate, we measured approximately 50 males and 50 females, roughly equally represented from as many families as possible (usually near 50). This maximizes the effective population size ($N_e$), reducing the effects of genetic drift and making artificial selection more efficient (Falconer and Mackay 1996). More than two fish per family were measured because 1% of the fish do not track, and we wanted at least 100 fish per line measured before selection. This yielded the distribution of threshold light sensitivities for that line: blue for the blue lines, red for the red lines, and both measurements for each fish in the controls. The total time taken to handle, to measure, and to record each fish at one wavelength (15–20 min) precluded measuring each fish at more than one wavelength (except for the controls) each generation without causing significant delay and time phase differences between lines; consequently, we only have two filter measures for controls each generation and all filters measured only at generation 9.

The mass selection was performed as follows. In the selected lines (B1, B2, R1, R2), we calculated the threshold required to allow the 40 most sensitive fish (20 males and 20 females with the lowest $Q$) to be parents of the next generation. Measuring at least 100 fish before selection and selecting the most sensitive 40 represents a compromise between maximizing the sample size while minimizing time spent in measurement and selection and between maximizing the selection differential while minimizing genetic drift and inbreeding, which can result from very high selection differentials (Falconer and Mackay 1996). The sex of immature fish was determined by noting the presence or absence of pigment near the anus in females, and once gender was determined, males and females were kept separate so that only virgins were used in selection to make up the next generation. We selected both sexes, even though colors are found only in males. Selection on both sexes is better than on only one because it is more efficient (Falconer and Mackay 1996) and there is no significant difference among the sexes in the distribution of the polymorphic red (LWS) cone pigments (Archer et al. 1987) or spectral sensitivity (V. N. Rush and K. D. Long, unpublished data). There are also no significant differences between juveniles and adults. The control lines (C1, C2) were treated exactly the same way as the experimental lines (B1, B2, R1, R2) except for selection; in the controls, 20 males and 20 females were selected at random after measurement of their sensitivity. The net effect is that 20 males and 20 females were used as the parents of the next generation in both experimentals and controls.

The virgin selected fish were allowed to mature, females in their home population tank and males in a 38-L tank. When all individuals were mature, selected males were placed into the home tank with the selected females for 3–4 wk, initiating the next generation. We waited until all males were mature before allowing mating in order to prevent an early maturing male advantage. We were able to obtain two to three generations per year.

A mycobacterial infection invaded the lines after generation 7, starting with one of the controls. Some fish died as a result of mycobacteria in the home tanks during the 3–4-wk mass mating of the parents of generations 8 and 9. In both these generations, selection was carried out, but sick or dead fish were replaced with unselected fish from the same lines in the same generation during the 3–4-wk mating period, based on daily checks. Up to half (generation 8) or two-thirds (generation 9) of fish were replaced, depending on replicate. To make this clearer, for a given line, if $a$ is the number of selected fish (their $Q$ is

below the critical $Q$ that results in 40% selected) and $b$ is the number of unselected fish (above the critical $Q$; $a/[a + b] = 0.4$), if a number $n = sa$ became ill and/or died before the mating period was over ($s$ is the fraction sick), $n$ fish were taken at random from the $b$ unselected fish and added to the mating tank. This reduces the selection differentials by an unknown amount because many of the selected fish mated before becoming ill and being replaced during the mating period. This makes estimation of the actual selection differential in generations 8 and 9 impossible; it could be as high as in previous generations, 0, or even negative if the unselected ($b$) fish happened to produce more offspring than the selected ($a$) offspring. Consequently, we used only generations 0–7 for the realized $h^2$ estimates. Only healthy fish were used in the spectral sensitivity measurements. In generation 9, this resulted in smaller sample sizes for the controls and R1. Note that this relaxed selection means that the differences between experimentals and controls in generation 9 will be an underestimate of the total genetic change as of generation 7; this is a classical observation in artificial selection experiments with relaxed selection (Falconer and Mackay 1996). Our tests of genetic divergence in spectral sensitivity are therefore conservative.

### *Realized* $h^2$ *Estimates*

Realized $h^2$, standard errors, and confidence limits were calculated using both classical regression methods (Falconer and Mackay 1996; Lynch and Walsh 1998) and a regression method developed by Hill (1972) and extended by Walsh and Lynch (1995). Hill's (1972) method has the advantage over a regular regression of cumulative response on cumulative differential in that it corrects for genetic drift (by using data from controls) and unequal sample sizes during selection and yields a better estimate of the standard error of heritability.

### *Final Measurements*

In generation 9, the complete spectral sensitivity function was taken for each fish by measuring each fish at 420, 460, 500, 580, 620, and 660 nm, instead of just the selection wavelengths of 420 and 660. The wavelength of maximum sensitivity (lowest threshold) was calculated by fitting a quadratic regression to the thresholds for a given fish and finding its minimum. We will call the wavelength of greatest sensitivity the best wavelength.

### *Genetic Correlation between Red and Blue Responses*

In generation 9, all lines were measured in both blue (420) and red (660) light. This allows an estimate of the genetic correlation $r_A$ between the reactions to red and to blue light from

$$r_A^2 = (CR_x/R_x)(CR_y/R_y), \quad (1)$$

where $R$ is the response to direct selection in a selection line, CR is the correlated response in the same line, line $x$ is selected in red light and line $y$, in blue light or vice versa (Falconer and Mackay 1996, eq. [19.7]). We used equation (1) to estimate $r_A$, using all four combinations of red and blue lines for $x$ and $y$. We cannot estimate genetic correlations between wavelengths other than 420 and 660 because only 420 and 660 were measured in previous generations (no data on $R_y$ other than for 420 and 660).

## Results

### *Response to Selection and Heritability Estimates*

Figure 1 shows the progress of selection relative to the control means through generation 9. All selected lines responded significantly to artificial selection. Note the greater heterogeneity of the red lines compared to the blue lines; most blue replicate standard errors overlap each generation but few red replicates do. There was a rebound in generation 8 after the halting of artificial selection in the parents of generation 8.

Figure 2 shows the progress of selection plotted as cumulative response to selection (relative to controls) versus cumulative selection differential for generations 1–7. Progress was greater and more rapid in the red than the blue lines. Realized heritabilities are 0.2–0.4 and all highly significantly different from 0 (table 1). Heritabilities in the red lines are 1.2–1.8 times that of the blue lines, although twice their SE overlap.

### *Spectral Sensitivity Curves*

Figure 3 shows the spectral sensitivity functions for generation 9, and statistical tests are summarized in tables 2–5. Statistical tests for differences in sensitivity were done on the individual wavelengths, wavelengths pooled, and on the best wavelength (wavelength of maximum sensitivity) for each fish. The pooled-wavelength tests were done because each photoreceptor responds over a large range of wavelengths (Stavenga et al. 1993). For those tests, we pooled data at the shorter (420–500) and longer (580–660) wavelengths. There was insufficient wavelength resolution (filters too widely spaced) for us to pool wavelengths to test explicitly for differential changes in the 533- and 572-nm LWS cones.

An ANOVA of the experimental versus control treat-

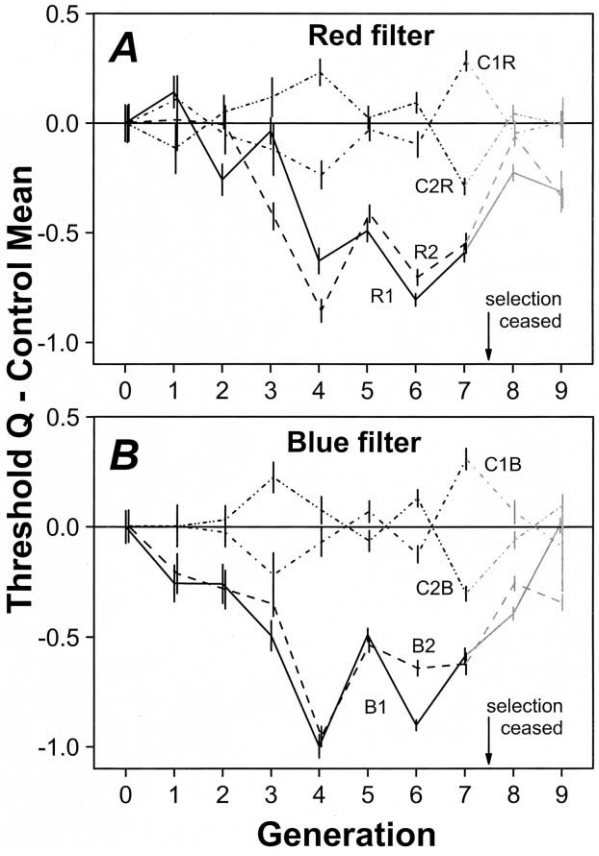


**Figure 1:** Course of the selection experiment. *A*, Measurements using the red filter (660 nm). *B*, Measurements using the blue filter (420 nm). All means and standard errors (*vertical bars*) adjusted by subtracting the mean of both controls each generation for that filter. Data in ln(*Q*) units. R1, R2 = red-selected lines; B1, B2 = blue-selected lines; C1R, C2R = control lines measured in red light; C1B, C1B = control lines measured in blue light. Arrows mark the appearance of the mycobacteria infection and the cessation of artificial selection (see text).

ments in generation 9 shows significant treatment effects for some wavelengths and significant heterogeneity among lines within treatments at other wavelengths (table 2). Except for blue at 580, 620, and 580–660 pooled, there is either a significant treatment effect or a significant within-treatment heterogeneity. The within-treatment heterogeneity is most marked in the red treatment (table 2; fig. 3).

Table 3 shows that all of the within-treatment heterogeneity is found in the blue and red lines; the controls are remarkably homogeneous even though they were the lines most affected by small $N_e$ and small sample sizes in generations 8 and 9 (table 3; fig. 3). The homogeneity of the controls is probably not an artifact of the reduced statistical power of small sample sizes (minimum detectable difference among controls 8.4% compared to 7.5% in blues and 3.3% in reds); the mean and SD differences between replicates among filters were 1.8% ± 0.9% (range 0.4–3.1) for controls, 2.6% ± 2.6% (range 0.3–7.3) for blues, and 4.5% ± 2.7% (range 0.5–9.3) for reds. The blue lines and especially the red lines are very much more heterogeneous than the controls (fig. 3).

Because the controls were homogeneous while the treatments were heterogeneous (table 3), we asked whether each selected line diverged from the pooled controls and if so how (table 4). The blue lines diverged from the controls at shorter and longest wavelengths whereas the red lines diverged from the controls either at long wavelengths (R1) or at most wavelengths (R2; see table 4; fig. 3). The pooled wavelengths are similar with relatively more blue shift in B1 (also shown by a significant change in best wavelength), a shift in both segments in B2 and R2, but no change in R1. In general, the response to selection was either to increase overall spectral sensitivity or to shift it in the direction of the selected wavelength (table 4; fig.

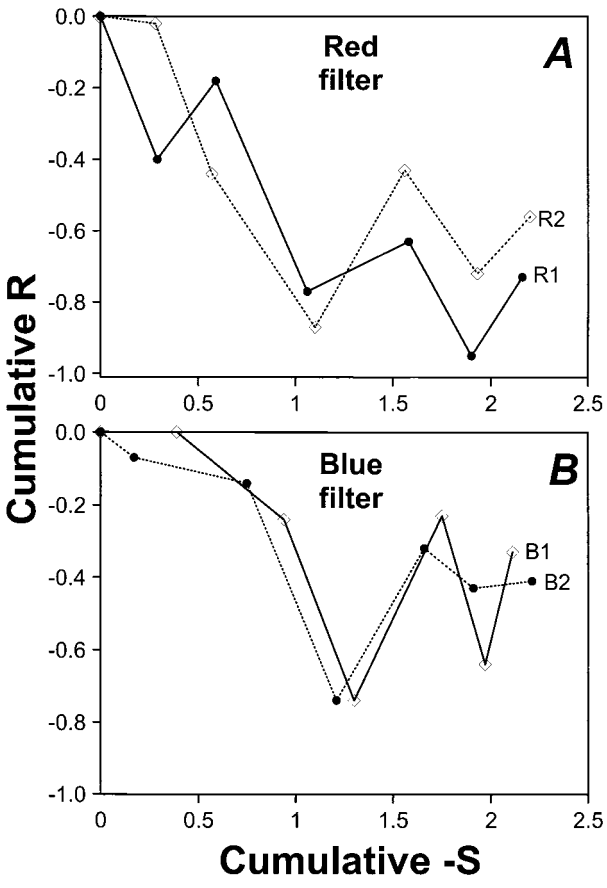


**Figure 2:** Course of the selection experiment plotted as cumulative response to selection (*R*) versus cumulative selection differential *S* (−1 × *S* because *S* is always negative); data through generation 7. *A*, Measurements using the red filter (660 nm). *B*, Measurements using the blue filter (420 nm).

3). If we ignore the wavelengths where the lines within treatments responded heterogeneously, there was significant increase in sensitivity at 460, 500, and 660 nm in the blue lines and at 660 nm in the red lines (table 2); the direction expected from the spectral sensitivity curves of the 533 and 572 cones.

Because some of the response to artificial selection involved many wavelengths in some lines (B2, R2; table 4), it is important to ask whether the response to selection involved changes in spectral sensitivity (shapes of the curves in fig. 3) or simply increased overall sensitivity. We tested this in two related ways, comparing pooled controls with each of the four selected lines (table 5): First, a two-way ANOVA was performed using treatment (controls vs. experimental line) and filter (420–660 nm) as factors. Changes in spectral sensitivity would show up as a significant treatment × filter interaction and changes in total sensitivity would show up as a significant treatment effect. The filter effect should always be significant because spectral sensitivity is not flat (fig. 3). Second, vision physiologists frequently normalize sensitivity curves to a common value. In order to do this, we excluded all fish with five or fewer filters measured (about 1% of all fish), extrapolated missing values if present (another 1%), and then calculated the mean of each fish's ln($Q$), or $q_m$. This is a measure of the fish's overall sensitivity. We then calculated the mean of $q_m$ for all fish and then rescaled each fish's spectral sensitivity curve so that all fish in all lines had the same $q_m$. These normalized spectral sensitivity curves were then used in the same kind of two-way analysis as the raw data. The results of both analyses are shown in table 5. As expected, there was always a highly significant filter effect. Except for R1, there was a significant treatment effect in the raw data, which indicates that three of the four lines increased in overall sensitivity. As expected from the normalization, the treatment effect disappeared after normalization. In all cases, for both the raw and normalized data, there was a significant interaction between treatment and filter, which indicates significant changes in the shapes of the spectral sensitivity curves. In R1, there was no change in overall sensitivity, but there was a change in shape. When the normalized data were tested separately for each filter (as in table 4), after the sequential Bonferroni correction, there were significant changes in shape at the 500- and 540-nm filters in both B1 and B2, in the 580-

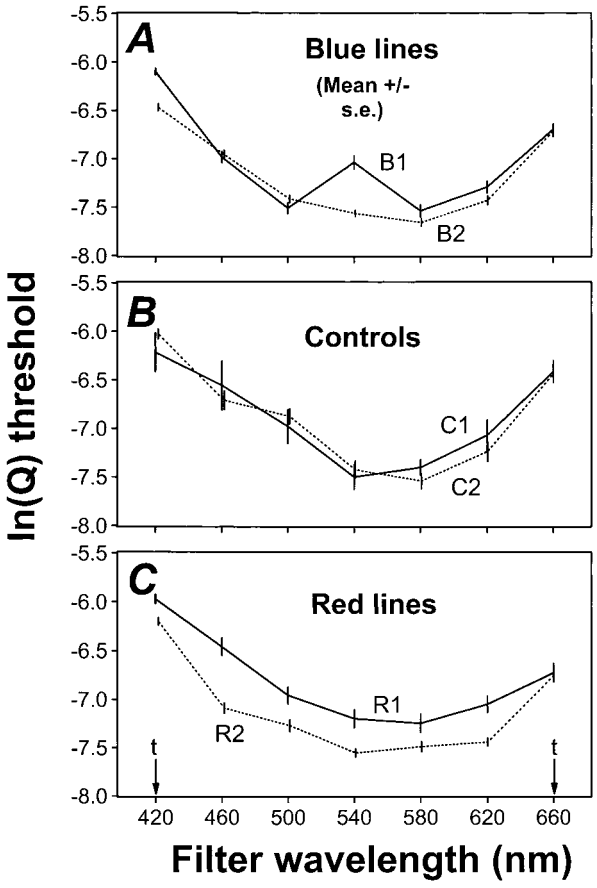


**Figure 3:** Spectral sensitivity curves. *A*, Blue lines *B1*, *B2*. *B*, Control lines *C1*, *C2*. *C*, Red lines *R1*, *R2*. Solid and dashed lines are for within-treatment replicates 1 and 2, respectively. Arrows marked *t* indicate the targets of selection, the filters used for selection in the blue (420 nm) and red (660 nm) lines. For statistical tests of differences among these six lines, see tables 2–5.

**Table 1:** Estimates of heritability of spectral sensitivity and its standard error, generations 1–7

| Selection line | R1 | R2 | B1 | B2 | C1 | C2 |
|---|---|---|---|---|---|---|
| $h_r^2$ | .44 ± .06 | .36 ± .07 | .25 ± .06 | .26 ± .06 | … | … |
| $h_T^2$ | .34 ± .09 | .27 ± .09 | .16 ± .07 | .19 ± .07 | … | … |
| $h_c^2$ | .39 ± .09 | .30 ± .08 | .24 ± .07 | .22 ± .07 | … | … |
| Average $M$, $N$ | 95.7, 43.6 | 95.1, 41.5 | 108.3, 42.3 | 104.0, 44.6 | 96.4, 40.0 | 101.9, 40.0 |

Note: Calculations: $h_r^2$ from regression of cumulative response on cumulative selection differential (Falconer and Mackay 1996); all four regressions are significantly different from 0 (df = 5, $P < .02$ or .01). $h_T^2$ from ratio of final cumulative response to final cumulative differential (Lynch and Walsh 1998). $h_c^2$ from Hill's method for unidirectional selection (Walsh and Lynch 1995). $M$ = numbers measured; $N$ = numbers after selection.

**Table 2:** Overall comparisons among treatments in generation 9

| Wavelength (nm) | Blue vs. control lines | | | | Red vs. control lines | | | |
|---|---|---|---|---|---|---|---|---|
| | Between treatments | | Replicates within treatments | | Between treatments | | Replicates within treatments | |
| | df | *P* | df | *P* | df | *P* | df | *P* |
| 420 | 1, 2.14 | .58 | 2, 245 | .0001* | 1, 2.16 | .84 | 2, 144 | .002* |
| 460 | 1, 20.4 | .0006* | 2, 237 | .66 | 1, 2.07 | .75 | 2, 148 | .0001* |
| 500 | 1, 7.45 | .0006* | 2, 238 | .32 | 1, 2.30 | .44 | 2, 147 | .011* |
| 540 | 1, 2.17 | .69 | 2, 241 | .0001* | 1, 2.17 | .73 | 2, 144 | .001* |
| 580 | 1, 5.31 | .30 | 2, 242 | .18 | 1, 2.41 | .58 | 2, 151 | .030* |
| 620 | 1, 4.25 | .16 | 2, 229 | .14 | 1, 2.13 | .74 | 2, 143 | .003* |
| 660 | 1, 207 | .0001* | 2, 239 | .96 | 1, 51.4 | .0001* | 2, 172 | .92 |
| Segments and best wavelength: | | | | | | | | |
| 420–500 | 1, 5.73 | .0052* | 2, 728 | .23 | 1, 2.08 | .69 | 2, 447 | .0001* |
| 580–660 | 1, 4.06 | .062 | 2, 718 | .10 | 1, 2.27 | .52 | 2, 474 | .0073* |
| Best λ | 1, 2.14 | .61 | 2, 164 | .0065 | 1, 2.00 | .62 | 2, 120 | .037 |

Note: All tests used Type III SS. Noninteger between-treatment degrees of freedom arise from the Satterthwaite approximation for combined df and SS in the mixed-model nested ANOVA (SAS Institute 1987, Proc. GLM, p. 610); treatments are fixed effects, replicates within treatments are random effects. Tests are for control lines measured with the same filter. Each row consists of two nested ANOVAS for the given filter, set of filters, or best wavelength, one for the blue lines and one for the red lines.

* Significant with the sequential Bonferroni correction at $\alpha = 0.05$, independent test method, $P \leq 1 - (1 - \alpha)^{1/(1+k-i)}$ (Rice 1989). Bonferroni correction done separately for single filters 420–660 ($k = 7$), segments ($k = 2$), and columns; it is not relevant for best λ.

nm filter for R2, and in the 660-nm filter for R1. The changes in shape were in the direction of selection. In summary, the response to selection consisted of both an increase in overall sensitivity and a change in the shape of the spectral sensitivity curves in the direction of artificial selection (*t*; fig. 3).

### *Genetic Correlation between Red and Blue Responses*

The $r_A$ estimates are shown in table 6. The four possible combinations of the red and blue lines in equation (1) yield four estimates of $r_A$, which yields an overall estimate of $r_A = 0.85 \pm 0.14$ (table 6). The SD of 0.14 may be inflated by the differences between red and blue line mean effects. We can eliminate these effects by treating the four estimates of $r_A$ as making up the cells of a two-way ANOVA (treatment A = blue lines 1 and 2; treatment B = red lines 1 and 2) with no replication and no interaction and use the error MS as an estimate of the variance of $r_A$. This yields a SD estimate of 0.02.

Following Falconer and Mackay (1996, eq. [19.9]), we can use the realized heritabilities (table 1) and this estimate of $r_A$ to predict what the CR/*R* ratios should be:

$$\mathrm{CR}/R = r_A(i_y/i_x)(h_y/h_x), \quad (2)$$

where *i* is the selection intensity (standardized selection differential) and *h* is the square root of the realized heritability for a given line. Assuming $i_x = i_y$ (part of the experimental design), this yields CR/*R* ratios of about 0.7 for the red lines and about 1.0 for the blue lines, similar to the observed values (table 6). This suggests that more of the response in blue lines was due to indirect rather than direct selection.

## Discussion

There was a significant response to selection (figs. 1, 2). There were significant heritabilities at both ends of the spectral sensitivity function as shown by the response to selection at 420 and 660 nm (table 1). This suggests that guppy populations can respond evolutionarily to changes in the light environment or changes in the context of visual behavior, which may occur as a result of either habitat changes or invasion of new habitats. Response to selection on visual behavior has also been found in quail (color preferences; Kovach 1980; Kovach and Wilson 1988) and in deer mice (photoperiod response; Desjardins et al. 1986). There is wide variation in vision among other animals (Lythgoe 1979; Jacobs 1981, 1993; Goldsmith 1990; McDonald and Hawryshyn 1995). Among-species variation in vision in fishes seems to be related to light environment (Levene and MacNichol 1979; Lythgoe 1979). These observations and the results of our study suggest that vertebrate sensory systems can evolve in response to natural selection, just as do other kinds of traits.

**Table 3:** Variation within treatments in generation 9

| Wavelength (nm) | Between control lines | | Between blue lines | | Between red lines | |
|---|---|---|---|---|---|---|
| | df | *P* | df | *P* | df | *P* |
| 420 | 1, 48 | .18 | 1, 197 | .001* | 1, 96 | .0003* |
| 460 | 1, 49 | .50 | 1, 188 | .70 | 1, 99 | .0001* |
| 500 | 1, 46 | .55 | 1, 192 | .17 | 1, 101 | .0038* |
| 540 | 1, 46 | .68 | 1, 195 | .0001* | 1, 98 | .0001* |
| 580 | 1, 46 | .37 | 1, 196 | .10 | 1, 105 | .015* |
| 620 | 1, 45 | .41 | 1, 184 | .067 | 1, 98 | .0001* |
| 660 | 1, 51 | .85 | 1, 188 | .81 | 1, 121 | .72 |
| Segments and best wavelength: | | | | | | |
| 420–500 | 1, 147 | .77 | 1, 581 | .10 | 1, 300 | .0001* |
| 580–660 | 1, 146 | .45 | 1, 572 | .045 | 1, 328 | .0013* |
| Best λ | 1, 29 | .18 | 1, 135 | .0027 | 1, 91 | .029 |

Note: All tests used Type III SS.

* Significant after the sequential Bonferroni correction as in table 2.

The results of the quantitative genetic analysis are consistent with what is known of the optomotor reaction mechanism. The optomotor reaction is thought to be mediated most strongly by the long wavelength sensitive (LWS or red) cones (Schaerer and Neumeyer 1996; Anstis et al. 1998). Our spectral sensitivity data (fig. 3) show single minima of 540–580 nm (except for line B1), which is consistent with primary input from the LWS cones (peak sensitivity at 533–572 nm) and little or no input from the other cones or rods. This is a standard interpretation for this kind of data (see Endler 1991 for discussion relative to guppies).

The LWS cones are polymorphic with modes at 533 and 572 nm, and on average the 533 cones make up about a third of the LWS cones in any individual (Archer et al. 1987; Archer and Lythgoe 1990). If the LWS cones are most important in the optomotor reaction, we expect artificial selection for the optomotor reaction to be different or more effective at 660 than 420 nm. There are two reasons. First, selection using red (660-nm) light will be more effective at stimulating the LWS cones than selection using blue (420-nm) light, at least for the 572-nm cones (see "Methods"), and the 572-nm cones are more common in the retina. If so, then the red lines experienced direct selection on the LWS cones, and the blue lines experienced weak or indirect selection on the 572-nm cones. The opposite will occur for the rarer 533-nm cones. Direct selection is usually more effective than indirect selection (Falconer and Mackay 1996; Lynch and Walsh 1998). Second, because the 533-nm cones are comparatively rarer and respond more strongly to the blue than in the red treatment (conversely for the 572-nm cones), more LWS cones will respond to the red than the blue treatment. Thus, even if the optomotor response results from the sum of all LWS (533 and 572) outputs, the optomotor output will be stronger under the red than the blue treatment because more LWS cones will be stimulated under the red treatment. This will reduce environmental variance ($V_E$) because of photoreceptor noise and measurement error in the red compared to the blue treatments, which makes selection more effective in red light. Lower $V_E$ is also consistent with greater realized $h^2$ in the red than the blue lines. All of these mechanisms could produce the observed differences in the response to selection; stronger and faster response in the red lines (figs. 1, 2) and a higher ratio of correlated to direct response in the blue than in the red lines (table 6).

The high genetic correlation between the reaction to red and blue lights (table 6) is also what one would expect if these are two aspects of the same trait—a reaction mediated through the LWS cones. The observed ratio of the correlated response to the direct response (CR/*R*) is higher in B1 than the red lines (table 6). This is expected if a reaction is primarily mediated through the LWS cones, with the blue reaction being carried by the comparatively insensitive blue tail of the LWS cone spectral sensitivity curve (for the 570 cones or for the LWS mean of around 560 nm) or weak optomotor interactions between all of the cones (LWS, MWS, SWS, UVS). The predicted CR/*R* are also in the direction expected for LWS-cone major effects; the predicted relative correlated red reaction in the blue lines is around 1 while the predicted relative correlated blue response in the red lines is only around 0.8 (table 6). Such asymmetry in genetic correlations is expected when one of a set of traits is being selected and the others are being dragged along by genetic correlations (Lynch and Walsh 1998). All of these data suggest that the effect of selection is stronger when the LWS cones are more

**Table 4:** ANOVA for single selected lines versus pooled controls

| Wavelength (nm) | Blue 1 vs. controls | | Blue 2 vs. controls | | Red 1 vs. controls | | Red 2 vs. controls | |
|---|---|---|---|---|---|---|---|---|
| | df | *P* | df | *P* | df | *P* | df | *P* |
| 420 | 1, 148 | .65 | 1, 147 | .0001* | 1, 85 | .22 | 1, 109 | .056 |
| 460 | 1, 146 | .0032* | 1, 142 | .0028* | 1, 88 | .10 | 1, 111 | .0001* |
| 500 | 1, 142 | .0001* | 1, 144 | .0001* | 1, 84 | .58 | 1, 111 | .0001* |
| 540 | 1, 146 | .0002* | 1, 143 | .063 | 1, 81 | .041 | 1, 111 | .15 |
| 580 | 1, 145 | .80 | 1, 145 | .036 | 1, 83 | .016 | 1, 116 | .78 |
| 620 | 1, 137 | .36 | 1, 139 | .0081* | 1, 80 | .23 | 1, 110 | .0062* |
| 660 | 1, 150 | .0011* | 1, 142 | .0011* | 1, 93 | .0044* | 1, 132 | .0001* |
| Segments and best wavelength: | | | | | | | | |
| 420–500 | 1, 440 | .0001* | 1, 437 | .0001* | 1, 261 | .33 | 1, 335 | .0001* |
| 580–660 | 1, 436 | .030 | 1, 430 | .0001* | 1, 260 | .70 | 1, 362 | .0047* |
| Best λ | 1, 75 | .034 | 1, 120 | .15 | 1, 53 | .14 | 1, 98 | .19 |

Note: All tests used Type III SS.

* Significant after the sequential Bonferroni correction as in table 2.

strongly stimulated, which leads to a stronger direct response in the red lines and a stronger correlated response in the blue lines. Whether or not this is actually true, these observations suggest that selection of different parts of the same sensory system function may not always result in the same response to selection.

Given the calculated differences in effects of artificial selection on the 533 and 572 cones in the red and blue lines, we should have seen differences in both the shape and the best wavelength among lines. Except for B1, we saw only differences in the shapes of the curves (tables 4, 5). If selection actually had changed the proportion and/or interactions between the 572 and 533 cones, it would have taken more wavelength resolution (more closely spaced filters) in spectral sensitivity measurements than we had (fig. 3) to detect a change in best wavelength among the lines, although we would have detected it had the other cones or rods been affected by artificial selection. However, the significant differences in spectral shape (table 5; fig. 3) suggests that some changes in cone proportions or interactions may have taken place. It is interesting that, after correcting for changes in intensity, changes in shape were observed at 500 and 540 nm in both blue lines and 580 or 660 nm in the red lines. This is consistent with differential response by the 572 cones in the *R* lines and either a differential response by the 533 cones or also the 502 rods or both in the B lines. The spectral resolution of our data is not high enough to test these specific hypotheses. However, we can conclude that the response to selection was heterogeneous between replicates within selection lines (tables 2–5).

The shapes of the spectral sensitivity curves of the blue and red lines differ, the replicates within selection lines also differ, but the control replicates do not (tables 2–5; fig. 3). This may be at least partly due to differing responses to the same selection by different processes in the visual system among lines. Speaking generally, some cells in the retina collect the signals from several or all cone classes and send the resulting brightness or luminance signal to the brain (Endler 1990; Lythgoe and Partridge 1991): $T = Uw_u + w_sS + w_mM + w_lL$, where *U*, *S*, *M*, and *L* are the outputs from the UV, short, medium, and long wavelength cones, respectively, and $w_u$, $w_s$, $w_m$, and $w_l$ are their weighting factors. (If only the LWS cones were responding, as in this experiment, then $T_l = w_3L_3 + w_7L_7$, where "3" indicates the 533 cone and "7" the 572 cone.) Other cells take the differences between photoreceptor cell classes and send these color signals (hue and chroma in human terms) to the brain (Endler 1990; Lythgoe and Partridge 1991); for example, the blue chromatic signal from the blue-"on" ganglion cells can be approximated by $C_b = (Sk_s - Mk_m)/(Sk_s + Mk_m)$, where $k_s$ and $k_m$ are weighting coefficients. For the purposes of discussion, the details are not important; what is important is that selection can affect any stage or process of visual processing (*T*, *C*, *U*, *S*, *M*, *L*, *R*, *w*, *k*), as well as how the signals are processed in the brain. Which process or coefficients happens to respond to selection in a given population will determine how visual signals are processed and the visual system and perception evolves. Divergences among lines (fig. 3) may have resulted from selection happening to affect different parameters among the lines. Possible responses to selection include changes in overall sensitivity (*T* or $T_l$), changes in difference functions ($C_i$), or their components ($w_j$) between pairs or among groups of LWS, MWS, SWS, or UVS cones, between one or more of these cones and the rods (mesopic vision), or between the LWS polymorphs (533, 572, optomotor response), changes in the relative abun-

dance of these photoreceptor classes, or changes in signal processing in the brain. Although we do not know which processes have been altered in this experiment, the observed divergence indicates how the visual system can evolve in a number of different ways to the same selection regime.

The diversity of responses to the same selection (fig. 3) has an important implication for evolution. It suggests that, although we might be able to predict the response if we knew the "target" wavelength(s) of selection, we cannot necessarily predict the correlated responses at other wavelengths (see also Falconer and Mackay 1996; Lynch and Walsh 1998). This is very interesting because it provides a mechanism for the generation of diversity in female choice and sexually selected male traits: different reactions by different visual processing systems to the same selection can have extremely different effects on the perception of color patterns. Changes in perception are likely to result in changes in preference (Endler 1992, 1993*b*). Because of the way sensory systems work (Lythgoe and Partridge 1989; Endler 1990, 1991), the relationship between the preference function and the properties of the sensory system is likely to be nonlinear; small changes in the sensory system could give rise to large changes in the intensity and form of the preference function. Changes in preferences affect the evolution of male traits (Lande 1981; Andersson 1994; Iwasa and Pomiankowski 1994; Kirkpatrick 1996; Kirkpatrick and Barton 1997). Separate populations responding to the same selection could therefore diverge, favoring completely different male color patterns and female preferences. Given that different populations are likely to live in different conditions, there are even stronger possibilities for divergence in visual systems, as has actually been observed in sticklebacks (McDonald and Hawryshyn 1995). Such divergence could result in divergence in female preferences and male traits, as has been found in guppies (Endler and Houde 1995), and could lead to speciation.

These conclusions also apply to sensory modes other than vision. In all sensory systems, different loci are likely to contribute to parameters analogous to *T*, *C*, *U*, *S*, *M*, *L*, *R*, *w*, and *k*. For example, in sound and chemoreception, respectively, *T* is a loudness or concentration parameter, and *C* is a parameter indicating pitch or a particular smell/taste independent of intensity. Different loci are likely to affect different sensory parameters, and, as in vision, selection for the same trait value can lead to multiple responses depending on which loci happen to respond to selection.

The diversity of responses to the same selection is a fundamental property of any trait that is controlled by multiple loci. Quantitative traits are a classical example where selection for, say, increasing the trait value results in increase of the mean in different populations, but each population has an increased mean as a result of allele frequency changes at different loci (Falconer and Mackay 1996). Diverse responses to identical natural selection are therefore likely in all sensory modes. Considering that no two populations ever occupy identical selective environments, the potential for diversification in sensory properties, perceptual abilities, and signal structure is profound. It may well be asked, What prevents rampant diversification? The answer may lie in a combination of gene flow among populations and constraints arising from multiple

**Table 6:** Generation 9 estimates of genetic correlation between spectral sensitivity to red and blue light and the resulting predicted CR/*R*

| Line combination | $r_A$ | Line | Observed CR/*R* | Realized[a] $h^2$ | Predicted[b] CR/*R* |
|---|---|---|---|---|---|
| R1, B1 | .87 | R1 | .60 | .39 | .66 |
| R2, B2 | .82 | R2 | .86 | .30 | .76 |
| R1, B2 | .69 | B1 | 1.25 | .24 | 1.02 |
| R2, B1 | 1.04 | B2 | .79 | .22 | 1.07 |
| Mean | .85 | | | | |
| SD | .14 | | | | |

Note: Estimates of genetic correlation use equation (1). CR/*R* = ratio of correlated response to response to selection within a line.

[a] From table 1.

[b] Using equation (2) and assuming equal direct selection intensities on red and blue lines (the experimental design).

**Table 5:** Results of two-way ANOVA of spectral sensitivity for raw and normalized data

| | | B1 vs. controls | | B2 vs. controls | | R1 vs. controls | | R2 vs. controls | |
|---|---|---|---|---|---|---|---|---|---|
| Source | df | Raw | Normalized | Raw | Normalized | Raw | Normalized | Raw | Normalized |
| Treatment | 1 | .0002 | .97 | .0001 | .97 | .086 | .94 | .0001 | .74 |
| Filter | 6 | .0001 | .0001 | .0001 | .0001 | .0001 | .0001 | .0001 | .0001 |
| Interaction | 6 | .0001 | .0001 | .005 | .0001 | .0022 | .0005 | .018 | .0004 |
| Error df | | 999 | | 995 | | 577 | | 766 | |

Note: Values in the table are the *P* values for each *F*-test. All tests used Type III SS. Raw: original data, ln(Q). Normalized: data normalized to the common mean ln(*Q*). Treatment: C versus B or R. Filter: 420, 460, 500, 540, 580, 620, 660 nm. Error df: same for "raw" and "normalized" but different for each line. Unlike in tables 2–4, fish with five or fewer filters measured were excluded in order to make the mean normalization valid; these fish were excluded for both the raw and normalized calculations.

sensory functions and genetic correlations among the parameters within a sensory mode.

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