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Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*)

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Abstract

Male guppies (*Poecilia reticulata*) exhibit extreme phenotypic and genetic variability for several traits that are important to male fitness, and several lines of evidence suggest that resource level affects phenotypic expression of these traits in nature. We tested the hypothesis that genetic variation for male secondary sex traits could be maintained by genotype-specific effects of variable resource levels (genotype–environment interaction). To do this, we measured genetic variation and covariation under two environmental conditions – relatively low and relatively high food availability. We found high levels of genetic variation for most traits, but we only found a significant G × E interaction across food levels for one trait (body size) for one population. The across-environment correlations for size were large and positive, indicating that the reaction norms for size did not cross. We also found that male colour pattern elements had nearly an order of magnitude more genetic variation than did male size. Heritability estimates indicated that Y-linked genes are responsible for some of the genetic variation in male size and colour traits. We discuss implications of these results for theories of the maintenance of genetic variation in male secondary sexual traits in guppies.

Introduction

A central question in evolutionary biology is: what maintains genetic variation in natural populations? This question is of particular interest when traits are closely tied to fitness because these traits experience strong selection. If selection is purely directional, variation will be determined by a balance between the input of new variation (by mutation and gene flow) and its elimination by selection and genetic drift. However, several forms of 'balancing' selection can maintain genetic variation above the mutation/selection/drift equilibrium, and the degree to which balancing selection contributes to maintenance of variation is a continuing debate within evolutionary biology (cf. Houle, 1998; Charlesworth & Hughes, 2000).

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Male guppies (*Poecilia reticulata*) exhibit high levels of phenotypic and genetic variability for several secondary sexual traits that are important to male fitness. For example, male guppies have highly variable colour patterns, including variation in colour, number, size and position of spots (Winge, 1922; Winge & Ditlevsen, 1947; Haskins *et al.*, 1961). This variation is known to have a substantial genetic component (Winge, 1922; Winge & Ditlevsen, 1947; Haskins *et al.*, 1961; Brooks & Endler, 2001; Karino & Haijima, 2001).

Guppies are sexually dimorphic for adult size and males are highly variable in size at maturity (Reznick & Endler, 1982; Reznick, 1982). Discrete genetic polymorphism for male size is characteristic of some species in the family Poeciliidae (Kallman, 1989). However, size variation in male guppies is a quantitative trait, approximately normally distributed within populations, and characterized by high heritability (Reznick *et al.*, 1997).

In addition to being highly variable, size and colour have measurable effects on male fitness. For example, colour affects male mating success (Farr, 1980a; Endler,

1983; Houde, 1988), behavioral correlates of mating success (Kodric-Brown, 1985; Breden & Stoner, 1987; Houde, 1987, 1997; Stoner & Breden, 1988; Brooks & Caithness, 1995; Endler & Houde, 1995), and predation risk (Endler, 1978, 1980, 1983; Godin & McDonough, 2003). In general, males having more orange coloration are preferred by females, while males with brighter or more conspicuous coloration are at greater risk of predation. Male size has also been implicated as a factor in female choice (Endler & Houde, 1995), in male mating success (Reynolds & Gross, 1992; Reynolds 1993), and in susceptibility to predation (Seghers, 1973; Liley & Seghers, 1975; Mattingly & Butler, 1994; Reznick *et al.*, 1996). Larger males generally have a reproductive advantage, while predator-mediated selection on size depends on the predation regime.

These studies provide evidence for directional selection on male size and colour. However, purely directional selection erodes genetic variation if not counterbalanced by some other evolutionary process. The striking levels of variation for male secondary sexual traits in guppies suggest that they are subject to some form of balancing selection that contributes to the maintenance of variation.

There have been few direct tests of mechanisms maintaining colour and size variation, and almost all of these have been tests of one particular mechanism: frequency-dependent selection via mate choice (Farr, 1980a,b; Hughes *et al.*, 1999). Genotype-by-environment interaction ($G \times E$) is one process that can contribute to the maintenance of genetic variation under some conditions (Hedrick *et al.*, 1976; Hedrick, 1986; Gillespie & Turelli, 1989). The most fundamental of these conditions is that alleles affecting the trait of interest must have different fitness effects under different environmental conditions. In particular, the rank order of genotypic fitnesses must change across environments. One measure of this effect that has been applied in other organisms is the cross-environment genetic correlation, r_C (cf. Fry *et al.*, 1996).

One difficulty with tests of the $G \times E$ model is that it is clearly impossible for a single experiment to test all the potential environmental variables that can affect fitness. It is only possible to test specific hypotheses based on environmental variation that has the potential to be a strong selective force. We therefore chose to study an environmental variable that seemed particularly likely to affect fitness via interactions with male secondary sex traits: resource availability. Productivity is known to fluctuate between and within natural guppy populations (Reznick, 1989; Reznick *et al.*, 1990, 2001; Grether *et al.*, 2001), lending support to the notion that fluctuating resource levels could be a ubiquitous and important selective force in this species. Food availability and quality also have demonstrable effects on male size and colour. Reznick (1990) showed that low food levels cause males to mature at a later age and smaller size, with consequent effects on expected fitness. Aspects of colour are also affected by food availability. Kodric-Brown (1989) and Grether *et al.* (1999) showed that carotenoid availability had a direct effect on the brightness of orange spots in adult males.

We predicted that if resource variability contributes to the maintenance of variation in male size or colour, then we would detect significant genotype-by-food level interaction and that cross-environment genetic correlations would be substantially <1. While we know that increased food generally causes increased adult size and decreased age at maturity, support for our prediction would come in the form of differences among genotypes in the pattern of this response. To test these predictions, we used a full-sib breeding design in which sets of brothers were raised on two different feeding regimes. We evaluated genetic and environmental variance components, broad-sense heritabilities, genetic correlations, and $G \times E$ for two measures of male size and several aspects of male coloration.

Methods

Experimental fish

To test for $G \times E$, it is necessary to raise individuals of known relatedness in at least two different environments. To control food availability, individual guppies must be raised in isolation from one another. Because of the logistic requirements of raising many related fish in individual aquaria, we chose to replicate a full-sib breeding design at two levels of food availability, in two different populations. A half-sib design would have allowed us to partition genetic variation into additive and nonadditive (plus maternal) components (Lynch & Walsh, 1998), but would have severely restricted the number of families that could be measured. Potential biases introduced by our choice of design are discussed below.

The progenitors of experimental fish were raised under controlled and uniform laboratory conditions, without inbreeding, for three generations prior to the experiment, as in Reznick (1982, 1983). Maternal environmental variation was thus partially controlled. Fish were derived from two different natural populations in Northern Trinidad. One population inhabited the downstream El Cedro River (EC), and the other the Guanapo River (GP). The El Cedro is actually a tributary of the Guanapo River, but the collection sites were separated by approximately 1 km and a series of rapids/waterfalls. The El Cedro is also a smaller stream with a less diverse fish community (Reznick *et al.*, 2001). Both populations are from sites that have been classified in previous studies as 'high predation' localities, where guppies co-occur with a diversity of potential predators (Reznick *et al.*, 2001). Some of these predators, such as the pike cichlid *Crenicichla alta*, prey preferentially on mature size classes

of guppies (Mattingly & Butler, 1994; Reznick *et al.*, 1996).

Breeding design

Eight pairs of unrelated EC males and females, and eight pairs of unrelated GP males and females were each mated in standard 8-L aquaria. Male and female fry from these 16 families were separated at approximately 4 weeks of age, before sexual maturity and before males had begun to express colour patterns. Male fry were isolated in individual 8-L aquaria until mature.

Four male fry were randomly chosen from each family for the experiment. Two of these males were raised on 'low' food levels, and two were raised on 'high' food levels. Food availability was quantified by feeding the fish volumetrically (to the nearest 0.1 μL) with a Hamilton micropipette, as in Reznick (1990). Fish were given measured amounts of liver paste (Gordon, 1950) in the morning, and of newly hatched brine shrimp in the evening. High food fish received a graduated schedule of feeding: 5 μL for the first 2 weeks, then 10 μL for the remainder of the experiment. Low food fish received 2.5 μL for the first 2 weeks, then 5 μL for the remainder of the experiment. For most families, additional males were raised on both food levels to provide replacements for experimental males that died before the end of the experiment. Positions of aquaria housing experimental males were randomized within the laboratory. All fish were maintained on a 12 h light/dark cycle, at 25–26 °C.

Measuring male size and colour

We measured all morphological traits on lightly anaesthetized, sexually mature males. Standard length and mass were measured using electronic calipers and an electronic balance. We measured male colour patterns using images digitized from photographs or videotape. We recorded the number, area, and location of coloured spots. Areas were measured using the public domain NIH Image Analysis program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image). Colours were classified as orange or black pigment colours, or as blue, white, green, or purple structural colours. Position was scored using a modification of Endler's (1978) partitioning of the guppy body plan (see Fig. 1). Each spot was thus labelled as being a particular colour (O, B, or S for orange, black or 'structural', respectively), and as being in a particular segment (1, 2, or 3 for anterior, posterior, caudal, respectively). Dependent variables for statistical analyses were the 'colour pattern elements' defined by the total area of each colour in each position. For example, the total area of orange spots in position 2 was the dependent variable O-2. Before analysis, total spot area was standardized by total body area (measured from the two-dimensional image of the body in a photograph) to adjust for differences in male size.

Fig. 1 Schematic of male guppy, showing the divisions that were used to name the colour pattern elements.

Statistical analysis and genetic interpretation

Genetic variance and heritability

Body mass was log-transformed to improve the fit of the data to assumptions of parametric analysis (residuals distributed normally and variances were homoscedastic). We used untransformed standard length values because this variable met the assumptions of ANOVA. Angular transformation was used to improved the fit of the colour data to the assumptions of ANOVA. SAS Proc Mixed (Littell *et al.*, 2002) was used to fit a mixed linear model and to estimate variance components.

Because males from each full-sib family were raised on two different food levels, the data were analysed as a factorial mixed linear model with family, food-level and family-by-food-level interaction terms included in the model. Family and family-by-food interaction were treated as random effects; food level was a fixed effect. The two populations were analysed separately.

Restricted maximum likelihood variances for family and family-by-food interaction were obtained from Proc Mixed. Some colour traits had residuals that deviated substantially from normality even after transformation (Shapiro-Wilk Statistic <0.9). For these traits (B-1, B-2, and S-1 in the EC population; B-2, S-1, and S-2 in the GP population), we obtained *P* values from a distribution generated by 1000 random permutations of the data. For traits that met the assumptions of the parametric analysis, the permutation *P*-values were very close to the parametric *P* values derived from the model, and we report only the parametric *P* values.

Biological interpretation of genetic variance components from mixed-model analyses must be made with caution (Fry, 1992). For full-sib families, assuming autosomal additive gene effects, the average additive genetic variance within environments is $2(\sigma^2_G + \sigma^2_I)$, where σ^2_G is the among-family variance component, and σ^2_I is the family-by-food interaction variance component (Lynch & Walsh, 1998, p. 669). In a full-sib design, σ^2_G includes variance from maternal and paternal effects, in addition to nonadditive genetic effects. Maternal effects due to common environment should have been reduced by rearing fish in the laboratory for three generations prior to the assays, and by raising experimental fish in individual aquaria. However, these effects

might not have been completely eliminated and σ^2_G should be interpreted as an upper bound.

For autosomal inheritance, the broad-sense heritability (H^2) is $2(\sigma^2_G + \sigma^2_I)/(\sigma^2_G + \sigma^2_I + \sigma^2_E)$, where σ^2_E is the residual variance. This is the heritability expected if the population were confined to one of the two environments. An alternate estimate of heritability is more appropriate if one is interested in potential response to selection of a population in which individuals are randomly distributed across environments. In such a population, the interaction variance does not contribute to the resemblance between relatives and $H^2 = 2(\sigma^2_G)/(\sigma^2_G + \sigma^2_I + \sigma^2_E)$ (Lynch & Walsh, 1998, p. 669). As we were primarily interested in evaluating G × E, and not in predicting response to selection in an environment that varies only between our particular two food levels, we will report the first of these two estimates (the expected within-environment heritability). Because nonzero genetic variance implies nonzero heritability, we considered H^2 values to be significant if the corresponding σ^2_G value was significant.

The above formulae for H^2 assume autosomal inheritance as brothers share, on average, half their alleles at autosomal loci. In the extreme, if all variation were due to Y-linked loci, the above equations would yield H^2 estimates near 2.0, as brothers share all alleles on the Y chromosome. Therefore, H^2 values significantly >1 support partial Y-linkage, although values <1 are also consistent with Y-linkage plus substantial environmental variation. To evaluate the evidence for Y-linkage, we report approximate standard errors for H^2, calculated from the formula given by Becker (1984, p. 54).

Cross-environment correlations

For an experiment conducted in two environments, the among-family variance is the covariance of family mean values across environments. This covariance can be used to calculate the genetic correlation of trait expression in the two different environments, r_C. This is the parameter of interest for determining whether environmental variation can maintain genetic variation (Via & Lande, 1985, 1987; Gillespie & Turelli, 1989).

The cross-environment genetic covariance and correlation were calculated by the method described by Fry (1992). This method ('SAS method') is robust to violations of the assumption that among-family and error variances are the same in the two environments. In this formulation, r_C is calculated as σ^2_G divided by the square root of the product of environment-specific variance components [$\sigma^2_G/\sqrt{(\sigma^2_{G1} \times \sigma^2_{G2})}$, equation 1 in Fry, 1992]. Using the SAS method, σ^2_G can take on negative values because it is equivalent to the covariance among family mean values in the two environments. The covariance is significantly different from zero if a two-tailed F test of the corresponding ANOVA is significant. We calculated r_C values for each trait for which σ^2_G was significant by this criterion. Although Fry's method is unbiased and allows straightforward calculation of significance, it cannot be used if σ^2_{G1} or σ^2_{G2} is near zero. In that case, we calculated r_C as $(\sigma^2_G/\sigma^2_G + \sigma^2_I)$, where σ^2_I is the variance due to family-by-environment interaction. If among-family variances differ in the two environments, the latter method will tend to underestimate $|r_C|$ (Fry, 1992). As both methods are based on ratios of variance component estimates, values outside the range of ±1 are possible.

Trait correlations

We estimated genotypic correlations (r_G) among pairs of morphological traits (x, y) by the formula $r_G = \mathrm{Cov}_G(x, y)/\sqrt{(\sigma^2_x \times \sigma^2_y)}$, where the genetic covariance $\mathrm{Cov}_G(x, y)$ was calculated as $(\sigma^2_{x+y} - \sigma^2_x - \sigma^2_y)/2$ (Hughes, 1995; Falconer & Mackay, 1996). As standard length and body mass were highly correlated in both populations (EC: $\rho = 0.95$, $P = 0.01$; GP: $\rho = 0.99$, $P < 0.0001$), we calculated correlations only for standard length. Statistical significance of these estimates was determined from distributions generated by 1000 random permutations of the data.

Results

Male size

As expected, males on the high food regime were generally larger than their brothers raised on low food. Mean values and standard deviations for mass and standard length for all families from both populations are shown in Fig. 2. Both mass and standard length showed highly significant family and food-level effects in both populations (Table 1). H^2 values ranged from 1.29 to 1.57 suggesting some Y-linkage for alleles affecting these traits.

In the GP, but not the EC population, family-by-food interaction effects were highly significant for both size traits (accounting for 18 and 16% of the phenotypic variation, respectively). The r_C values were significantly or marginally significantly positive in both populations and ranged from 0.89 to 0.96 (Table 1). Thus, although the effect of food availability did differ among families (in the GP population), families that were relatively large on high food were also relatively large on low food (Fig. 1). Consequently, there is little support for the reversals in the rank order of genotypes that are expected if resource-based G × E were maintaining genetic variation for male size.

Male colour

Mean values and standard deviations of relative colour area for each sib group are given in Appendix 1. Because any given spot will be one of three colours (O, B, and S) in one of three positions (1, 2, and 3), there are nine colour-position combinations. However, there were no

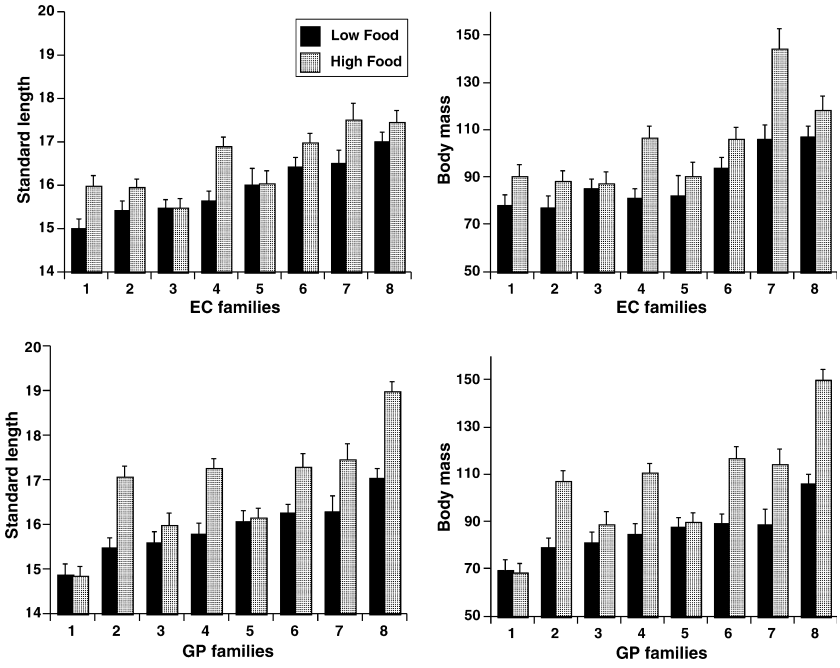

Fig. 2 Size of adult males from full sib families raised on two different food levels. Solid bars represent low-food treatment; shaded bars represent high-food treatment. Error bars show standard errors. EC: El Cedro population; GP: Guanapo population.

Table 1 Effects of family and environment on adult male size.

Trait	Source	d.f.	P	σ^2_X	H^2	r_C
El Cedro population						
Length	Family	7	0.016	0.391	1.44 (0.27)	0.92*
	Food level	1	<0.01			
	Family × food	7	0.15	0.042		
	Error	28		0.167		
Mass	Family	7	0.038	0.014	1.29 (0.32)	0.89***
	Food level	1	0.01			
	Family × food	7	0.15	0.002		
	Error	28		0.009		
Guanapo population						
Length	Family	7	0.02	0.701	1.57 (0.22)	0.94* (0.42)
	Food level	1	0.01			
	Family × food	7	0.001	0.215		
	Error	49		0.250		
Mass	Family	7	0.02	0.026	1.55 (0.23)	0.96* (0.43)
	Food level	1	<0.01			
	Family × food	7	0.003	0.007		
	Error	49		0.010		

σ^2_X is the variance component associated with a given random effect; H^2 is the expected within-environment heritability with standard error in parentheses; r_C is the cross-environment correlation for family effects.
*$P < 0.05$; ***$P < 0.10$.

structural colours in area 3 (the caudal fin), so we had eight colour-position combinations (colour elements) that occurred in our data.

For the EC population, four of eight colour elements had significant full-sib family effects and H^2 estimates >1 (Table 2), consistent with strong genetic determination and some Y-linkage. There was highly significant σ^2_G for orange coloration in all three body regions, and for black in the caudal fin (B-3). There was also marginally significant σ^2_G for structural colour in posterior body. For the colour traits with significant among-family variance, all the H^2 estimates were >1 (ranging from 1.07 to 1.79), indicating some Y-linkage for these traits (Table 2).

Compared with the EC population, the GP population had significant σ^2_G for fewer colour elements. Only O-1 and O-2 showed significant family effects and these had H^2 values of 1.17 and 0.55, respectively (Table 3). None of the black and structural colour elements had significant among-family variances. The lower estimates of heritability in this population suggest less Y-linkage and more nongenetic variation in colour patterns.

In both populations, the Food and Family-by-Food effects on colour patterns were nonsignificant (Table 2 and 3). All cross-environment correlations (r_C) that were estimable were large and >0.9. All r_C estimates but one were significantly or marginally significantly >0.

For each size and colour trait, Table 4 gives the genetic coefficient of variation ($CV_G = 100\sqrt{\sigma^2_G}/\bar{x}$), a standardized estimate of genetic variation that is useful for comparing traits measured on different scales (Houle, 1992). Population-specific mean CV_G values for size and for colour are also shown; these means include the zero values for traits without significant genetic variance. Colour had a mean CV_G that was an order of magnitude greater than that for size (mean CV_G for colour for both populations combined is 40.2%; that for size is 3.8%). These results are similar to those reported by Brooks & Endler (2001), where the mean additive genetic coefficient of variation (CV_A) for colour-element area was

Table 2 Effects of family and environment on relative area of colour spots in three regions of the body in the El Cedro population.

Trait	Source	d.f.	*P*	σ^2_X	H^2	r_C
O-1	Family	5	0.001	0.43	1.24 (0.35)	1.73**
	Food level	1	0.27			
	Family × food	5	0.17	0.21		
	Error	16		0.40		
O-2	Family	5	0.001	1.02	1.79 (0.13)	0.99**
	Food level	1	0.64			
	Family × food	5	0.86	0.02		
	Error	16		0.12		
O-3	Family	7	0.004	0.51	1.07 (0.39)	1.00**
	Food level	1	0.06			
	Family × food	7	0.48	0.04		
	Error	21		0.48		
B-1	Family	5	0.12	0.04	0.33 (0.39)	1.44
	Food level	1	0.61			
	Family × food	5	0.58	−0.01		
	Error	16		0.14		
B-2	Family	5	0.17	0.08	–	–
	Food level	1	0.37			
	Family × food	5	0.19	−0.21		
	Error	16		0.64		
B-3	Family	7	0.004	0.05	1.35 (0.32)	1.17**
	Food level	1	0.52			
	Family × food	5	0.32	0.00		
	Error	16		0.02		
S-1	Family	7	0.61	0.0	0.0 (0.30)	–
	Food level	1	0.45			
	Family × food	7	0.64	0.0		
	Error	21		0.05		
S-2	Family	5	0.07	0.16	0.44 (0.41)	
	Food level	1	0.43			
	Family × food	5	0.89	−0.08		
	Error	15		0.27		

σ^2_X is the variance component ($\times 10^3$) associated with a given random effect; other columns labelled as in Table 1. Numbers in parentheses are standard errors. '–' Indicates that r_C value was not estimable by either method. An entry of 0 in the H^2 column indicates that the estimate was zero or negative.
**$P < 0.01$.

Table 3 Effects of family and environment on relative spot area in three regions of the body in the Guanapo population.

Trait	Source	d.f.	*P*	σ^2_X	H^2	r_C
O-1	Family	7	0.007	8.49	1.17 (0.35)	1.20*
	Food level	1	0.27			
	Family × food	7	0.75	−0.35		
	Error	21		5.72		
O-2	Family	7	0.03	4.97	0.55 (0.40)	*1.42*
	Food level	1	0.25			
	Family × food	7	0.34	−1.53		
	Error	21		9.19		
O-3	Family	7	0.75	−0.14	−0.08 (0.26)	
	Food level	1	0.58			
	Family × food	7	1.00	0.06		
	Error	21		2.21		
B-1	Family	7	0.40	1.74	0.14 (0.33)	
	Food level	1	0.59			
	Family × food	7	0.65	−0.88		
	Error	21		11.19		
B-2	Family	7	0.51	0.00	−0.39 (0.11)	
	Food level	1	0.79			
	Family × food	5	0.76	−0.66		
	Error	21		4.02		
B-3	Family	7	0.65	−0.31	0.33 (0.37)	
	Food level	1	0.81			
	Family × food	5	0.40	−0.91		
	Error	21		3.05		
S-1	Family	7	0.08	3.61	1.47 (0.27)	
	Food level	1	0.13			
	Family × food	7	0.93	−0.82		
	Error	21		1.01		
S-2	Family	5	0.30	0.55	−0.51 (0.04)	
	Food level	1	0.17			
	Family × food	5	0.92	−2.47		
	Error	15		9.32		

Labels as in Table 2. Numbers in italics represent r_C values calculated using the formula as $[\sigma^2_G/\sigma^2_G + \sigma^2_I]$ (see text). *$P < 0.05$.

53.2% and that for size (body and tail area) was 12.8%. This correspondence between independent studies suggests that our estimates are not strongly inflated by nonadditive genetic variance.

Genotypic correlations among traits

Table 5 shows genotypic correlations among the traits exhibiting significant genetic variance. The EC population had significantly negative correlations between O-1 and O-2 and between O-1 and body size; there was a marginally significant negative correlation between O-1 and B-3. The only significant positive correlation was between O-2 and body size. GP fish also demonstrated a significant negative correlation between O-1 and O-2. None of the other correlations in GP fish were significant.

Discussion

Maintenance of size variation

Our results show that both genotype and resource availability have substantial effects on adult male size and that there was significant G × E for length and mass in one population. Cross-environment correlations for size traits were always large and positive, and thus do not support the hypothesis that crossing reaction norms maintain genetic variation in size. However, if different size phenotypes are favoured in different environments, spatial or temporal environmental variation could maintain genetic variation in male size even with strongly positive r_C. For example, small males (with fast maturation rates) might be favoured in some habitats and not others. With sufficient gene flow between habitats, polymorphism might be maintained.

This hypothesis does not seem to have been tested directly, but several studies support the notion that large

Table 4 Coefficients of genetic variation $CV_G = 100\sqrt{\sigma_G^2}/$(trait mean) for secondary sex traits in both populations.

EC population		GP population	
Trait	CV_G	Trait	CV_G
Size			
Mass	**2.6 (0.7)**	Mass	**3.6 (1.5)**
Length	**3.8 (1.3)**	Length	**5.1 (1.9)**
Mean (size)	3.2 (0.6)	Mean (size)	4.4 (0.8)
Colour			
O-1	**65.4 (26.6)**	O-1	**94.1 (15.2)**
O-2	**66.6 (8.6)**	O-2	**73.4 (24.9)**
O-3	**43.9 (16.5)**	O-3	0.0*
B-1	12.5 (9.6)	B-1	28.5 (21.1)
B-2	20.9 (16.9)	B-2	0.0*
B-3	**10.3 (2.7)**	B-3	0.0*
S-1	0.0*	S-1	62.5 (35.1)
S-2	**137 (66.3)**	S-2	49.6 (17.2)
Mean (colour)	41.8 (15.6)	Mean (colour)	38.5 (13.1)

Values shown in bold are those where among-family variance was significant at $P < 0.05$. Numbers in parentheses are standard errors. *CV_G estimate was zero or negative.

Table 5 Genotypic correlations between male traits in the El Cedro and Guanapo populations.

	El Cedro population				Guanapo population	
	O-2	O-3	B-3	Size	O-2	Size
O-1	**−0.51***	0.45***	**−0.34*****	0.12	**−1.32****	−0.17
O-2		−0.27	**0.37***	**−1.07****		0.02
O-3			0.24	−0.28		
B-3				−0.01		

Bold values indicate significant correlations. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

males are favoured in some populations and small males in others. For example, a series of studies by Reznick and colleagues have shown that male maturation rate and male size differ genetically between sites characterized by high and low predation rates, and that size evolves rapidly when predation pressures change (Reznick, 1982; Reznick & Bryga, 1987, 1996; Reznick *et al.*, 1997). In high predation sites, characterized by *C. alta* predators, males mature at a faster rate and at a smaller size than they do in sites without these predators. Movement of males between sites with different predator communities could thus help to maintain variation. Haskins *et al.* (1961) provided direct evidence for such movement by using visible genetic markers to document long-distance down-stream gene flow (i.e. from low- to high-predation sites). Downstream movement of alleles from low- to high-predation populations was also observed following introduction of guppies to a previously uninhabited low-predation site (Shaw *et al.*, 1992; Becher & Magurran, 2000). Even within high predation sites, *C. alta* is patchily distributed (they are not found in pools with little cover). Thus, some guppies might live their entire lives in a pool without a cichlid while others might live in a pool constantly inhabited by cichlids. In sites with *C. alta*, small body size is favoured because of rapid maturation time, but nearby sites without *C. alta*, could favour large male size due to female preference (Reynolds & Gross, 1992; Endler & Houde, 1995).

Other types of environmental variation could also result in variable selection on male size. Two studies have suggested that social structure can affect male maturation rate and size at maturity (Rodd *et al.*, 1997; Evans & Magurran, 1999). For example, male guppies from some populations adjust their rate of development and size at maturity to the density of male conspecifics; males from other populations do not (Rodd *et al.*, 1997). Male density varies considerably in natural populations because both the sex ratio and density of adult guppies vary across sites and across time (Reznick & Endler, 1982; Rodd & Reznick, 1997). Variation in density and in the plastic response to density could lead to variable selection on male size. Demographic variation is thus a promising place to search for G × E that can maintain size variation.

Maintenance of colour variation

Although the extreme variability of male colour patterns has often been noted, it has rarely been quantified. The colour-element CV_G values reported here and in Brooks & Endler (2001) are among the highest ever reported for morphological traits (Houle, 1992; Pomiankowski & Moller, 1995). While dietary carotenoids can affect the brightness of orange spots, environmental factors do not appear to influence the features of colour patterns that demonstrate high genetic variability: size, colour and position of colour (especially orange) elements (Kodric-Brown, 1989; Grether, 2000). Therefore, it is likely that some form of balancing selection is involved in the maintenance of this variation, unless colour-pattern genes are highly mutable, but G × E based on resource variability does not appear to be involved.

If G × E does not maintain variation in colour pattern, what does? At least three other mechanisms have been proposed. One is gene flow between populations with differing selection regimes. Endler (1980) found that guppy populations maintained in the presence of *C. alta*, evolved changes in spot size to match the grain size of the gravel substrate, but that populations reared only in the presence of the small gape-limited predator *Rivulus hartii*, did not. The difference was attributed to selection for crypsis in the high-predation populations. As discussed above, gene flow between high- and low-predation sites would be required in order for predation regime to maintain genetic variation in colour patterns. While some gene flow seems likely, to our knowledge the

amount of gene flow required to maintain the observed levels of variability has not been calculated.

Brooks (2000) found support for another mechanism (antagonistic pleiotropy) when he reported a negative genetic correlation between male survival and male attractiveness using a half-sib breeding design. Antagonistic pleiotropy between fitness traits is potentially capable of maintaining genetic variation; however, as pointed out by Brooks (2000), this is only true under rather restrictive conditions (i.e. with restricted dominance effects and selection coefficients) (Rose, 1982; Curtsinger *et al.*, 1994; Hedrick, 1999). Further tests of this mechanism are badly needed.

A third mechanism, which has received the strongest support, is negative frequency-dependent selection. Three different studies have shown that males with rare or novel colour patterns have greater than expected reproductive success, suggesting that female preference for rare colour patterns maintains variation (Farr, 1977, 1980b; Hughes *et al.*, 1999). In theory, this mechanism is capable of maintaining large amounts of genetic variation within populations (Crow & Kimura, 1970), with few conditions or restrictions.

One caveat that applies to all previous tests of mechanisms maintaining variation is that they have been conducted in laboratory environments. Testing these hypotheses in natural settings will be critical to distinguishing among the different models. Such tests will be logistically difficult, but guppies are quite amenable to field experimentation (cf. Reznick *et al.*, 1990, 1996, 1997), and provide a rare opportunity to examine these processes in natural populations.

Heritability and genetic correlation

Estimates of H^2 that substantially exceed 1.0 indicate that some loci responsible for variation are Y-linked. In this study, H^2 estimates for size traits ranged from 1.29 to 1.57. In the GP population these estimates exceeded 1.0 by more than twice the standard error, providing strong support for partial Y-linkage. Some species in the same family as guppies have Y-linked polymorphism at the P (Pituitary) locus that influences male size. In *Xiphorus nigrensis*, three alleles at this locus appear to largely determine adult male size (Kallman, 1989). Our results suggest that the P locus might also be involved in size variation in guppies.

Our study also provides evidence for partial Y-linkage of colour pattern elements. In the EC population, four of eight colour elements (including all the carotenoid elements) had H^2 estimates >1.0. The estimate for O-2 exceeded 1.0 by more than six times the standard error, providing strong support for substantial Y-linkage. Other quantitative-genetic studies have supported partial Y-linkage for colour patterns, as did the early breeding experiments of Winge (Winge, 1922; Winge & Ditlevsen, 1947; Houde, 1992, 1997; Brooks & Endler, 2001).

Both our results and those of Brooks & Endler (2001) indicate that carotenoid colour area has demonstrates higher genetic variance and higher H^2 than black or structural area. Black coloration is under partial neuronal control and is influenced by anaesthetic (Houde, 1997). This effect could explain the relatively large component of nongenetic variation for black area. Structural colours are thought to be largely genetic (cf. Endler, 1980) but the appearance of these colours to the human eye is dependent upon light exposure and angle. Again, much of the nongenetic variation of structural area is likely due to measurement error.

There is other evidence that Y-linkage of colour elements is far from complete. Two recent studies reported inbreeding depression for colour elements in guppies (Sheridan & Pomiankowski, 1997; Van Oosterhout *et al.*, 2003). Genes present in only one copy (such as those on the nonrecombining portion of the Y chromosome) cannot contribute to inbreeding depression. Therefore, these studies suggest either that some genes determining colour pattern are autosomal or that colour elements are condition-dependent and respond to inbreeding at other loci. Our results support the first interpretation, as condition (determined by food level) did not affect colour elements.

Haskins *et al.* (1961) suggested that the degree of Y-linkage for male colour patterns varies among populations. Our data support this hypothesis, as the GP population had generally lower H^2 values for colour elements, and only one element had an H^2 estimate that exceeded 1. Variation in Y-linkage among populations, and the evolutionary forces that could lead to such variation, is a topic worthy of further study.

Patterns of genetic correlation among colour and size traits suggest that that pleiotropy or linkage disequilibrium affect these characters. Both populations in this study showed significant negative genetic correlations between orange elements in the anterior and posterior body. In EC, there was also a strongly negative genetic correlation between anterior orange and body size. Brooks & Endler (2001) also reported nonsignificant trends for negative correlations between colour elements and between body size and orange area.

A negative relationship between orange in the anterior and posterior body could be explained as a simple result of geometry. If the presence and size of a colour element is genetically determined, but its exact position can vary, then an orange spot might occur in region 1, or in region 2, but not in both, or the spot might overlap the two regions, thereby producing a negative correlation. However, the negative association between body size and orange, and that between O-1 and B-3 cannot be explained by a similar mechanism.

Pleiotropy of individual genes, or linkage disequilibrium between loci, are therefore implicated by the negative correlations between colour and size. It has been argued that a Y-linked 'supergene' is responsible for

some colour variation in guppies (Yamamoto, 1975). If a major size-determining locus also occurs on the nonrecombining portion of the Y chromosome, then linkage disequilibrium between size and colour alleles could contribute to negative correlations among traits. Combined with the extreme variation in secondary sexual traits, this apparent involvement of sex chromosomes in the maintenance of variation makes this species an appealing one for molecular evolutionary studies of sexually selected traits.

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Appendix 1 Least-square mean values and standard errors for colour pattern elements. Units: (mm^2).

Family	Low food	High food	Low food	High food
El Cedro population				
	B-1		O-1	
232	0.87 (0.82)	0.29 (0.58)	3.04 (1.04)	4.09 (0.74)
205	2.43 (0.58)	1.70 (0.47)	1.59 (0.74)	1.87 (0.60)
216	1.27 (0.82)	1.72 (0.47)	1.13 (1.04)	0.94 (0.60)
230	1.19 (0.41)	1.10 (0.47)	2.81 (0.52)	0.00 (0.60)
240	1.57 (0.47)	1.83 (0.47)	0.29 (0.60)	0.00 (0.60)
245	0.65 (0.82)	1.61 (0.58)	0.00 (1.04)	0.00 (0.74)
	B-2		O-2	
232	0.00 (1.36)	1.44 (0.96)	3.07 (1.11)	1.66 (0.79)
205	1.41 (0.96)	2.34 (0.78)	0.00 (0.79)	0.00 (0.64)
216	1.14 (1.36)	1.58 (0.78)	0.00 (1.11)	0.73 (0.64)
230	1.40 (0.68)	1.28 (0.78)	3.10 (0.56)	4.36 (0.64)
240	1.07 (0.78)	2.82 (0.78)	3.71 (0.64)	5.38 (0.64)
245	1.64 (1.36)	1.85 (0.96)	3.70 (1.11)	4.19 (0.79)
	B-3		O-3	
232	3.70 (0.83)	2.47 (0.59)	4.63 (1.44)	1.80 (1.02)
205	3.98 (0.59)	3.56 (0.48)	3.31 (1.02)	3.08 (0.83)
216	3.10 (0.83)	4.18 (0.48)	2.20 (1.44)	2.60 (0.83)
230	3.18 (0.42)	2.21 (0.48)	3.51 (0.72)	3.19 (0.83)
240	1.76 (0.48)	2.98 (0.48)	0.63 (0.83)	0.00 (0.83)
245	4.91 (0.83)	6.62 (0.59)	4.82 (1.44)	2.36 (1.02)
	S-1		S-2	
232	0.00 (0.04)	0.00 (0.03)	0.00 (0.05)	0.00 (0.03)
205	0.00 (0.03)	0.00 (0.02)	0.00 (0.03)	0.00 (0.03)
216	0.00 (0.04)	0.00 (0.02)	0.00 (0.05)	0.00 (0.03)
230	0.00 (0.02)	0.00 (0.02)	0.00 (0.02)	0.00 (0.03)
240	0.06 (0.02)	0.00 (0.02)	0.04 (0.03)	0.10 (0.03)
245	0.00 (0.04)	0.00 (0.03)	0.07 (0.05)	0.07 (0.03)
Guanapo population				
	B-1		O-1	
GP1	1.65 (0.99)	1.43 (1.21)	0.00 (0.60)	0.00 (0.73)
GP2	4.62 (1.21)	1.74 (1.21)	0.00 (0.73)	0.00 (0.73)
GP3	2.17 (1.21)	3.08 (1.21)	0.00 (0.73)	0.00 (0.73)
GP4	1.88 (0.99)	2.44 (0.85)	1.56 (0.60)	2.08 (0.52)
GP5	3.02 (1.21)	2.86 (1.71)	2.77 (0.73)	3.16 (1.03)
GP6	0.00 (1.71)	2.25 (0.85)	0.00 (1.03)	0.54 (0.52)
GP7	6.30 (1.71)	6.06 (1.21)	0.00 (1.03)	0.00 (0.73)
GP8	3.13 (0.99)	6.04 (0.99)	0.65 (0.60)	1.88 (0.60)
	B-2		O-2	
GP1	2.56 (0.83)	2.73 (1.02)	4.62 (0.79)	4.07 (0.96)
GP2	2.09 (1.02)	1.94 (1.02)	0.00 (0.96)	2.37 (0.96)
GP3	2.78 (1.02)	2.84 (1.02)	0.00 (0.96)	1.06 (0.96)
GP4	2.44 (0.83)	3.59 (0.72)	1.20 (0.79)	1.19 (0.68)
GP5	2.64 (1.02)	2.58 (1.44)	0.00 (0.96)	0.00 (1.36)
GP6	2.13 (1.44)	2.98 (0.72)	0.94 (1.36)	1.86 (0.68)
GP7	2.64 (1.44)	2.46 (1.02)	0.00 (1.36)	0.00 (0.96)
GP8	1.81 (0.83)	3.50 (0.83)	0.53 (0.79)	1.19 (0.79)
	B-3		O-3	
GP1	2.42 (0.55)	2.09 (0.68)	0.00 (0.26)	0.00 (0.32)
GP2	2.05 (0.68)	1.47 (0.68)	0.00 (0.32)	1.14 (0.32)
GP3	0.40 (0.68)	1.58 (0.68)	0.87 (0.32)	0.00 (0.32)
GP4	1.92 (0.55)	1.54 (0.48)	0.00 (0.26)	0.00 (0.23)
GP5	0.72 (0.68)	3.60 (0.96)	0.00 (0.32)	0.00 (0.45)
GP6	2.57 (0.96)	2.16 (0.48)	0.00 (0.45)	0.47 (0.23)
GP7	2.68 (0.96)	1.11 (0.68)	0.00 (0.45)	0.00 (0.32)
GP8	3.04 (0.55)	2.21 (0.55)	0.00 (0.26)	0.00 (0.26)
	S-1		S-2	
	0.54 (0.37)	0.00 (0.46)	0.60 (0.80)	0.00 (0.98)
GP2	0.00 (0.46)	0.00 (0.46)	0.00 (0.98)	0.00 (0.98)
GP3	0.00 (0.46)	0.00 (0.46)	0.00 (0.98)	0.00 (0.98)
GP4	0.00 (0.37)	0.59 (0.32)	0.93 (0.80)	1.64 (0.69)
GP5	0.67 (0.46)	1.58 (0.64)	0.16 (0.98)	3.01 (1.39)
GP6	0.00 (0.64)	0.21 (0.32)	0.00 (1.39)	0.21 (0.69)
GP7	0.00 (0.64)	0.11 (0.46)	0.00 (1.39)	0.20 (0.98)
GP8	0.00 (0.37)	0.53 (0.37)	0.63 (0.80)	1.77 (0.80)