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Reduced Sexual Attractiveness of Redundant Males in the Maintenance of Guppy Color Polymorphism

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Summary

The Trinidad guppy, *Poecilia reticulata*, is one of the most polymorphic species in existence. Guppies exhibit predominantly Y-linked inheritance of male color patterns, which appear on the body, caudal fin, or dorsal fin and are highly variable between individuals. Little investigation behind the mechanism maintaining polymorphism in guppies has been done. One hypothesis is that guppy polymorphism results from frequency-dependent selection. My study sought to investigate whether reduced sexual attractiveness of "redundant" males, with similar color patterns, may contribute to polymorphism. Experimental groups consisted of two males with similar color patterns (redundant) and two males with distinctly different color patterns (unique). The four males were placed in a tank with four virgin females. I compared sexual responsiveness of female guppies to the redundant vs. the unique males. Females were significantly less likely to show sexual response to the redundant males than they were to the unique males. This suggests that female guppies' preference for unique males contributes to the maintenance of polymorphism in this population.

Introduction

Coloration in animals has evolved for reasons related to defense against predators, sex, and thermoregulation (Guilford 1988). For example, many insects evolved their coloration for defensive purposes. *O. nicaraguensis* displays cryptic coloration, making the beetle inconspicuous to predators as they blend in with the forest floor. Other insects' defense coloration has evolved to mimic species with known hazards to predators. For instance, the *Pseudoxycheila* species mimics the stinging wasp in coloration and therefore protects itself from predators in this way. Additionally, the conspicuous color pattern of *P. tarsalis* serves as an aposematic signal to predators after eating them, as they contain distasteful compounds (Schultz 2001). In birds, the males often display conspicuous coloration in order to attract mates, as a form of sex-based coloration. Male Quetzals have evolved long, exaggerated tails, bright plummage coloration, and facial ornamentation to attract females (Wingquist & Lemon 1994). Some animals use coloration as a thermoregulatory device, like the butterfly *Colias eurytheme*. In cold weather, the butterfly has dark-wings in order to form heat faster in sunlight, which is required for flight. However, it turns a lighter color in warm seasons to minimize overheating (Watt 1969). It is important to study the evolutionary mechanisms which underlie the origins and maintenance of such coloration. In this study, we examined the sexual behavior of guppies (*Poecilia reticulata*) in order to further the understanding of evolution of sex-based coloration in the males.

Wild guppies display a wide range of color pattern variants among the males within a natural population; they exhibit color patterns on their bodies, caudal fins, or dorsal fins that greatly vary in size, shape, position, and color (blue, green, purple, yellow, orange, red, and black). In fact, guppies are one of the most polymorphic species in existence, with few males sharing the same color pattern (Farr 1997; Lank et al. 1995). Inheritance of male color patterns has a genetic basis and is predominantly sex-linked (Winge 1922, 1927). It remains a mystery as to why such extreme polymorphism is seen in guppies.

In many populations, female guppies generally choose to mate with males that display a large amount of orange coloration (Houde 1997). In contrast, Endler (1980) discovered that predators tend to favor less conspicuous male morphs. Later, Olendorf et al. (2006) and colleagues found predators form a search image for the common male morph and therefore indirectly affect selection. Although male color patterns are directionally affected by sexual selection, somehow variation is still powerfully sustained. One of the predominant theories for the maintenance of polymorphism is frequency-dependent selection (Crow & Kimura 1970; Cressman 1988; Roff 1992; Judson 1995).

The theory of frequency-dependent selection involves rare phenotypes being favored over common ones through survival, reproductive or mating success. Since the rare phenotypes are being favored, they will increase in frequency until their advantage disappears. For this reason, many morphs can coexist in a population, and no one phenotype will predominate. In many species, this mechanism has been proven to uphold high levels of polymorphism.

Negative frequency-dependent selection has been studied in various species as a mechanism for the maintenance of polymorphism in a population. This event has been shown to occur in *Drosophila* (Petit 1958; Petit & Ehrman 1969; Ehrman & Spiess 1969), *Tribolium castaneum* (Sinnock 1970), *Nasonia vitripennis* (Grant et al 1974), *Philomachus pugnax* (Lank et al. 1995), and *Escherichia coli* (Elena & Lenski 1997).

In the guppy, extensive research into this mechanism has been conducted. Most of these studies have examined mating success of males with color patterns that are unfamiliar to females. Some studies suggest that females may be likely to choose mates that have a rare color pattern (Farr 1977, 1980), while others suggest that novel color patterns are attractive to females (Hughes et al. 1999; Eakley & Houde 2004). Potentially, choosing novel males could increase the fitness of a female's offspring. Mating with a novel male would introduce genetic diversity into the population and decrease inbreeding depression. This mechanism could explain the maintenance of color polymorphism in guppies.

Although frequency-dependent sexual selection is hypothesized to be a major factor contributing to polymorphism in guppies, non-sexual selection may also play a role. In a study by Olendorf

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*This article was written as a Senior thesis under the direction of Dr. Anne Houde et al. (2006), predation was examined as a possible mechanism for the maintenance of polymorphism. Their study manipulated frequencies of males with different color patterns in three natural populations in Trinidad and estimated the survival of rare and common phenotypes. The results concluded that rare phenotypes survive possibly because guppy predators develop a search image for common male morphs.

The first studies on frequency-dependent sexual selection in guppies were conducted by Farr (1977, 1980). He examined female responses to "rare" male courtship displays and their reproductive success. Farr noted that female guppies rarely respond to male displays, in general. But when Farr placed a new male into the tank, females readily responded and showed preference for the male. Farr also showed that rare morphs had a higher mating success.

Hughes et al. (1999) also examined frequency-dependent selection. In this study, Hughes and colleagues exposed females to a particular male color pattern and then introduced a new male type in equal numbers. As in the Farr study, Hughes et al. concluded that females familiar with a certain group of males (having similar phenotype to one another) had a significantly lower probability of mating with those males after exposure to novel males. Researchers found that familiar males also produced significantly fewer offspring. These findings suggest a frequency-dependent mechanism of selection, in that females prefer to mate with unfamiliar males. This relates to the rare male mating advantage, in that rare males are likely to be unfamiliar.

Eakley and Houde (2004) later studied how a previous mate affects the choice of a subsequent mate. They found that females avoid males they mated with previously and males similar to them. Our study expanded on these studies since we showed females discriminate against males with similar color patterns (redundant) relative to males with unique color patterns. This is the first study of frequency-dependence to look at female sexual responses to males that vary in frequency of color pattern types.

Two redundant males and two males with unique color patterns were observed interacting with four virgin females, during which time female sexual response scores were taken for each male. Unlike other frequency-dependent studies, where virgin females are first familiarized with a particular male phenotype and subsequently introduced to a new male phenotype, this study presents all male morphs at the same time. An advantage of our study is that we observe a variety of morphs. Other frequency-dependent studies used a limited number of male morphs, however, we observed three different morphs in each trial. Every trial presents a new selection of morphs as well, and in this way we are able to study the effects of a wide-range of phenotypes. This enables us to investigate frequency-dependent sexual selection as it may occur in the wild. That is, in natural populations, females are presented with many different male phenotypes from which to choose. We predict that unique males will have higher female response scores because females will discriminate against redundant males.

One reason for possible discrimination against redundant males may be females' lack of interest in re-mating with a male similar to their previous mate as Eakley and Houde showed. Alternatively, it may be that females will simply have a preference for less-common phenotypes *per se,* as in Farr's studies.

Polymorphism in Guppies

Guppies are extremely polymorphic organisms in respect to male color patterns; they exhibit a wide range of colors in multifarious combinations and patterns. The species is highly sexually dimorphic, with females being nearly twice the size of males and displaying a uniform grayish-brown color. Although females may contain the genes for male color pattern, they are not expressed visibly as in the case of males (Winge & Ditlevsen 1947). Normally, guppies have typical X-Y sex determination, however, XX males and XY females have been observed (Winge & Ditlevsen 1947). These findings indicate the X and Y chromosomes are not sole determinants of sex but rather that the accumulation of male/female-determining autosomes may contribute more significantly towards sex determination (Winge & Ditlevsen 1947). At the beginning of this century, Ojvind Winge affirmed the concept of "one-sided masculine inheritance," making the guppy the first organism in which a Y-linked inheritance was demonstrated (Winge 1922). In the beginnings of his research, Winge believed the female did not influence the pigmentation of her sons. A year later, he discovered a case of "crossing over" between the X and Y chromosome and determined the X chromosome contained a gene responsible for an elongated caudal fin characteristic (Winge 1923). This led to Winge's thorough investigation of the frequency and types of "cross-over" events in guppies.

Winge found the X and Y chromosomes in guppies are so similar that in fact they are completely homologous, with the exception of a single gene—the male-determining gene (Winge 1927). Because of the homology of the X and Y chromosomes, "crossing-over" is a frequent occurrence. In his research, Winge (1947) discovered 20 color genes, eighteen being X or Y-linked and 2 being autosomal. He states that the Y chromosome always contains at least one of the absolute Y-linked alleles—Ma, Pa, Ir, or Ar, which code for whole color patterns (Fig 1). However, Y-linked genes not located near the male-determining gene can cross over onto the X chromosome, and X-linked genes can, alternatively, cross over onto the half of the Y chromosome that is not male-determining. Winge (1947) found the maximum cross over percentage to be ten percent. A more recent review lists 16 Y-linked traits, 24 that recombine between the X and Y chromosome, two that are X-linked, and two that are autosomal (Lindholm & Breden 2002).

Winge's and other studies are based on laboratory varieties of guppies descended from individual males from the wild, and may not represent the full range of color pattern variation. In order to evaluate the full representation of male color patterns, we set up a breeding experiment. In conjugation with my behavioral study, I conducted a genetics study to further investigate sex linked traits in a laboratory population derived from wild guppies. Twenty-three males in the study were bred with three females each, and offspring of each female were examined. Orange body color patterns, spots, and tail patterns of the dads and sons were analyzed. This was accomplished by first identifying each of these traits in the entire population of parents and offspring in the study. Then the traits were numbered, and every male fish was analyzed by marking all of their individual traits. Looking at all the traits represented in this study, I have

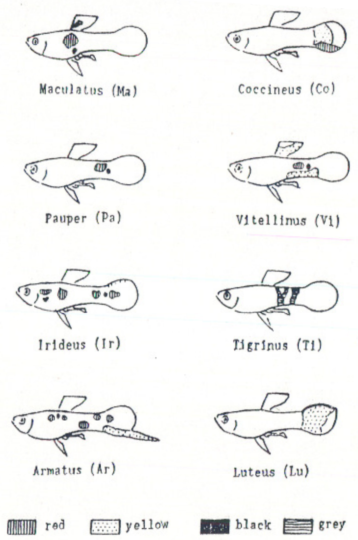

FIG. 1.—The effects of some colour genes in males of *Lebistes reticulatus*; at the left are seen some of the "absolutely Y-linked" genes; at the right some genes that may occur in X as well as in Y.

(Winge 1947)

been able to identify traits inherited directly by the fathers and ones influenced by the mothers. Preliminary data also shows, as in Winge's studies, that fathers, sons, and brothers tend to look strikingly similar. Some families were more similar in color patterns than others, suggesting that certain traits are more likely directly inherited from the father.

Results

In initial analyses, the mean female response in the 28 trials was significantly higher for unique males than redundant males (fig 2, t=-7.25, df=27, P<0.001). That is, females were much more likely to respond to a unique male and continue in the courtship sequence. Although few actual copulations were observed, unique males, on average, scored higher on the scale than did redundant males. The fraction of responses with scores of 2 and higher was significantly greater in unique males than for redundant males (Fig 3, t=7.22, df=27, P<0.001). Redundant males tended to display slightly more frequently than unique males. However, this difference was not significant (Fig 4, t=1.73, df=27, P=0.10).

For each individual male, area of orange was measured. Males in the first 12 trials were selected differently than those in the last 16 trials. Due to possible confounding effects, redundant males in the last 16 trials were more orange than those in the previous trials (Table 1), and these redundant males were significantly more orange than unique males in the last 16 trials. Overall, redundant males were slightly more orange than unique males.

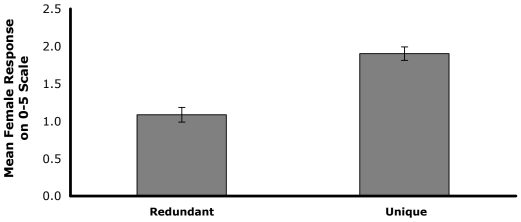

Figure 2. Mean female response to redundant and unique males. Female response was rated on a 0-5 scale. Error bars represent standard errors.

In order to control for possible effects of males' area of orange and variation between groups on female response rates and male display rates, general linear models were used to test for an effect of unique vs. redundant males. These analyses confirmed that females rate of response was significantly higher for unique males than for redundant males (Table 2). Due to the discrepancy in orange between the two data sets, analyses were performed separately, representing the first 12 trials for set 1 and the last 16 trials for set 2. Unique males elicited a much higher response rate in both sets. Display rates for unique and redundant males were very similar in set 1, and in set 2 redundant males displayed more. These analyses demonstrated that the area of orange coloration did not affect the female response rate or the male display rate (Table 3). Paired t tests with corrections for orange area also had similar results, showing that response rates were significantly higher for unique than for redundant males (set 1: t=3.3, d.f.=11, P=0.007; set 2: t=3.4, d.f.=15, P=0.004). Paired tests for male display rates showed significant difference between unique and redundant males (set 1: t=0.07, d.f.=11, P=0.95; set 2: t=1.54, d.f.=15, P=0.14).

Discussion

Our results showed female guppies were significantly more responsive to unique males than to the redundant males. This result is consistent with the idea that patterns of mate choice may lead to frequency dependent selection. Previous studies of guppies have

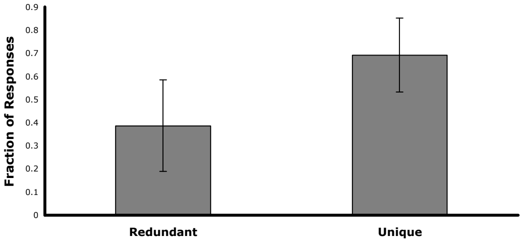

Figure 3. Fraction of female responses to redundant and unique males with scores of two and higher. Error bars represent standard errors.

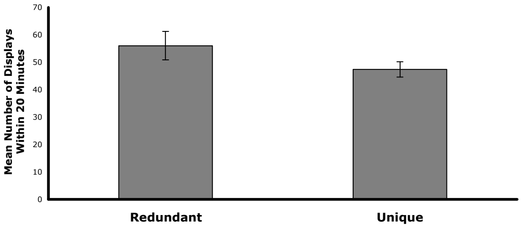

Figure 4. Mean number of displays between redundant and unique males with a 20- minute observation session. Error bars represent standard error.

Table 1. Fraction of Orange (Orange/Body) in Redundant and Unique Males for two sets of trials.

Set	Redundant	Unique	T	DF	P
1	0.12 (0.01)	0.10 (0.01)	1.77	23	0.09
2	0.21 (0.01)	0.14 (0.01)	-5.36	31	<0.001

Set 1 represents the first 12 trials and set 2 the last 16 trials. Standard errors are given for redundant and unique groups.

suggested that "rare" males may have increased mating success (Farr 1977, 1980) and that females prefer unfamiliar over familiar males (Hughes et al. 1999, Eakley & Houde 2004). However, no previous study has examined the sexual response behavior of female guppies when presented with a range of naturally occurring male color pattern morphs that differ in frequency. Our study shows that females prefer unique males and discriminate against redundant males when presented simultaneously to females. This behavior could potentially lead to a mating success advantage to unique males, which in turn could help maintain color pattern variation in the population through negative frequency dependent selection.

The fraction of male displays eliciting female responses was significantly higher for unique males than redundant males. There are a number of possible behavioral mechanisms that could account for this result. For instance, there were two redundant males with a single color pattern phenotype but only one of each of the unique male phenotypes. Therefore, females were more likely to meet by chance a redundant male than they were one of the unique males. Hypothetically, if a female was courted by one of the redundant males and rejected him, and then was later courted by the other redundant male, she could extend her rejection to a similar male. Additionally, she could discriminate against the first male as well. Females may thus become less responsive to redundant males. A second possibility is that female guppies may discriminate against males they have already mated with, given the females are more likely to have mated previously with a redundant male than either unique male (Eakley & Houde 2004). Finally, in nature, it could be possible that females can calculate morph frequencies and discriminate against common male morphs, favoring rare morphs within the population.

While our study suggests that females may discriminate against redundant males, a number of other studies have also suggested that mate choice may result in frequency dependence. These include studies suggesting a "rare male mating advantage," in which females may favor uncommon morphs (Farr 1977, 1980), and studies of familiarity or novelty (Hughes 1999; Eakley & Houde 2004).

As in our study, Farr (1977) introduced females to two male phenotypes which were presented in different frequencies. However, Farr did not conduct behavioral observations in his study; instead, he simply recorded the reproductive success of the males. He did note that when a novel male was placed into a group of males and females, the novel male's presence elicited sexual responses from otherwise unresponsive females. In order to assess the reproductive success of the "rare" males, Farr formed groups consisting of males with two different color patterns in a 9:1 ratio. The rare males sired a disproportionate number of offspring (> 30%). One limitation in Farr's study, which was corrected for in our research, was the fact that Farr used a limited number of male morphs. Our observations were conducted with a different set of male morphs for each of the 28 trials, representing the full range of variation in a natural population.

Other studies on the effects of morph frequency on male mating success have been conducted with *Drosophila*. Ehrman (1966) found females mated equally with males of different genotypes when present in equal ratios. However, when introduced at different frequencies, rare males had higher mating success. Experiments done on the "rare-male effect" have posed problems because their experimental designs contained biases (Partridge & Hill 1984; Partridge 1988). Some biases could have existed because individual females had fixed preferences for different male types and male-male competition occurred between males of similar color patterns.

Hughes et al. (1999) overcame these biases by using a "familiarization" period. They tested the hypothesis that females may prefer to mate with unfamiliar (or novel) males. In their study, they sought to determine whether female preference for males with a particular color pattern would be affected by prior experience with those males. The results showed females were less likely to mate with familiar than unfamiliar males and this could provide a mating success advantage to rare types that are more likely to be unfamiliar.

Eakley and Houde (2004) also found evidence that male guppies discriminate in favor of novel males. Their study examined the sexual responses of female guppies one day after mating with an initial male. They found that females were more responsive sexually to a novel male than to either the original mate or to a male with a color pattern similar to the original mate's. Eakley and Houde examined females' preference for a second mate and concluded that females greatly preferred what they termed 'novel' males.

Since female guppies generally prefer males with a larger area of orange coloration, we measured the area of orange on every fish. Our results showed that the redundant males, on average, actually had a slightly higher area of orange coloration than did the unique males. This may be because very similar males with higher amounts of orange were more readily available for choice as redundant male sets. Also, half of the trial groups came from a genetics study in which fish may have been more orange and attractive, due to chooser bias. The redundant males from the second

Table 2. Least-square means (standard errors), on the untransformed scale, and significance tests for redundancy calculated on transformed variables.

Trait	Set	Unique	Redundant	F	DF	P
Response	1	0.71 (0.05)	0.40 (0.06)	11.1	1, 11	0.007
Response	2	0.65 (0.04)	0.38 (0.05)	14.3	1, 15	0.002
Display	1	20.4 (1.65)	20.6 (3.72)	0.01	1, 11	0.99
Display	2	26.3 (2.18)	33.8 (3.22)	2.44	1, 15	0.14

Set 1 represents the first 12 trials and set 2 the last 16 trials.

Table 3. Intercepts and slopes (standard errors) and significance tests for effects of orange area on male response and display rates.

Trait	Set	O Int (se)	O Slope (se)	F	DF	P
Response	1	0.70 (0.22)	-0.90 (0.58)	3.79	1, 34	0.06
Response	2	0.24 (0.15)	0.34 (0.31)	1.07	1, 46	0.31
Display	1	19.3 (9.06)	4.02 (23.3)	0.13	1, 34	0.72
Display	2	34.0 (10.1)	-0.43 (20.6)	0.03	1, 46	0.87

Set 1 represents the first 12 trials and set 2 the last 16 trials. O is amount of orange coloration.

half of the trials were more orange than in the first half of the trials. Since the unique males did not have a larger area of orange, the difference in female preference could not have been due to a preference for more orange males. Further analysis of the data showed no effect of area of orange on attractiveness to females. Females' response to redundant vs. unique males appears to have significantly overridden the effect of orange found in previous studies.

Male guppies tend to inherit most of their color patterns from their fathers and often look identical or at least similar to their fathers and brothers (Winge 1922, 1927; Houde 1992; Hampton unpublished data), making the maintenance of color pattern polymorphism especially difficult to explain. Our study presents a possible mechanism for sexual frequency-dependent selection, in which female guppies discriminate against males that are similar to other males in the group. Our data strongly support female preference for unique males, however, it will be important to obtain data on mating success of redundant and unique males. Genetic variation may be maintained if females mate with rare males. Mating preference for rare males may benefit females, because males introduced to a population from outside the local pool may provide heterozygosity and mating with these males could lead to higher fitness of offspring and a reduction of inbreeding effects. Furthermore, females who mate with a second different male would likely have offspring with greater variation, and this would reduce the risk of a single incompatible mating.

Our study provides a possible mechanism for frequency-dependent sexual selection. However, the frequency-dependent survival found by Olendorf et al. (2006) may also promote polymorphism in guppy populations. In conclusion, the behavior of both predators and females may be responsible for maintaining polymophism, but it will be important to show that both lead to direct fitness effects.

Materials and Methods

Fish used in this study were descendents of wild guppies from the Paria River population in Trinidad. They were housed in 40 liter aquaria, which contained filtered and conditioned tap water, gravel, and moss. Each aquarium was exposed to a 12-h light: 12-h dark illumination cycle by fluorescent lights, and a temperature of about 22-27ºC was constantly maintained. Virgin females used in each trial were raised in isolation from males in 40 liter aquaria separated into 4 compartments with 6 females in each; they were used in experiments after reaching sexual maturity at about 12 weeks of age. All fish were fed twice daily with Tetramin flake food in the morning and brine shrimp in the afternoon.

Experimental groups were set up consisting of two "redundant" males with similar color patterns and two unique males (selected at random), all reared in the same community aquarium and all of sexually mature age. Redundant males from the first 12 trials were collected from an aquarium by examining all the males caught and selecting 2 males whose color patterns were similar (redundant) and 2 whose were not similar (unique). In the second set of 16 trials, redundant males were collected from a breeding experiment in which full sibs were housed in the same 4 liter tanks; twins were all of sexually mature age. Unique males in this set of data were collected from the same 40 liter tanks as in the previous trials. Two 40 liter aquaria containing 4 compartments each were used to house the groups of males for two to eight days, until needed for observations. Four nonvirgin, sexually mature females were added to each compartment with the males.

Fish were placed in an observation tank the day before observations were conducted, allowing sufficient interaction time between all individual fish. Experimental groups consisted of one group of males (2 redundant males and 2 unique males) and 4 virgin females (matched for size). Observations were conducted in a dark room at 8:30 am, 30 minutes after the fish were fed flakes and the aquarium light came on. The 40 L observation tank was illuminated by a fluorescent light on a 12-h light: 12-h dark cycle and contained bottom gravel and filtered water only.

We observed the sexual behavior of virgin females the morning after they were introduced to a group of males. The virgin females were placed in the tank with the males a day before actually conducting observations, in order to allow them to complete initial matings. Virgin females reared apart from males mate, possibly indiscriminately, within minutes of first encountering males and then enter a refractory period during which little sexual behavior occurs for an hour or more (Houde 1997). The next morning, females can still be reliably observed showing sexual responses to males.

Each male was observed for 10 minutes at a time and then observed again for another 10 minutes, at least 10 minutes after the first observation ended. The observation order of the males was selected at random by using cards marked with the males' identification numbers. A total of 28 groups of males were observed.

Female response to the males' courtship displays was marked on a 0-5 scale described by Houde (1997). On this scale, a score of zero indicates no female response to the male's sigmoid display, and a score of 1 is recorded when the female simply turns her head toward the displaying male but subsequently either turns away or does not progress in the courtship sequence. A response score of 2 and above was considered to be a sexual response. This was recorded when a receptive female glided towards the displaying male (Liley 1966; Houde 1997). Scores of 3, 4, and 5 represented advanced stages in the courtship sequence after stage 2 had already occurred. The fraction of displays eliciting a sexual response was calculated using Microsoft Excel as a measure of each male's attractiveness.

After an 80 minute observation session, the males were removed from the tank and photographed on both sides. Later, body area and orange coloration measurements were made for each male in the study using NIH Image J Software (http://rsb.info.nih.gov/ij). Measurements were done by tracing each orange spot on an individual fish's body and adding the total areas of orange. The body of the fish, excluding the tail, was also traced to measure the fish's body area. Calculations were then made to assess the fraction of orange relative to body area.

An initial set of statistical analyses was performed using paired t-tests on the response scores, with fraction of responses and display rates averaged for the unique and redundant males within each group. Averaged data for redundant and unique males were used because the possibility of non-independence within groups required that this analysis be done with trial (group) as the experimental unit. A more complex analysis, taking into account orange coloration and possible between-group variation, was performed by Kimberly Hughes (University of Illinois, Champaign-Urbana). She created a general linear model for each set of data separately; set 1 was the first 12 trials and set 2 was the last 16 trials. See Appendix 1 for full details of statistical analyses provided by Dr. Hughes. She looked to see how the dependent variables (fraction responses or displays) were predicted by category (redundant or unique), amount of orange, group, and group-by-category interaction. The interaction of category and orange coloration was not significant in either set for either trait, so it was not included in the final model. In addition, Dr. Hughes conducted paired t-tests to compare the response rates and display rates between the unique and redundant males, in which the dependent variables were corrected for the effect of amount of orange coloration.

Appendix

Details of statistical methods and results of analyses carried out by Dr. Kim Hughes

(This text written by Dr. Hughes)
The two variables that were measured as proportions (response rate and the amount of orange per male) were transformed by taking arc sine square root, after which both the variables themselves and the residuals from all analyses were normally distributed. For the count variable (displays) we used a square root transformation, after which the variable and the residuals were normally distributed. Because of the differences in sampling procedures between the two sets, we conducted separate statistical analyses within sets. We tested for differences between unique and redundant males within sets using general linear models of the form $y = \mu+R+O+G+G*R+e$, where y is the dependent variable (fraction responses or displays), R is the category (redundant or unique), O is the amount of orange, G is the group, and G*R is the group-by-category interaction. The interaction of category and orange coloration was not significant in either set for either trait, so we did not include it in the final model. We treated R and O as fixed effects, G and G*R as random effects, and assumed that the errors within groups were correlated (using the SUBJECT and GROUP options within SAS Proc Mixed). This model accounts for the non-independence of males tested at the same time and for additional non-independence of males in the same color category within group, and it adjusts the degrees of freedom accordingly. We also conducted paired t tests within sets on the difference between unique and redundant males, by first calculating the mean trait value for the redundant and unique males within each group and then treating mean scores as paired observations within groups. In this analysis, we first removed effects of orange coloration by taking the residuals from a regression of the dependent variable on the amount orange.

Results

The general linear model analysis indicated that Unique males elicited significantly higher rates of response from females than did Redundant males in both sets of the experiment (Table 1). Within a category response rates were very similar across sets, with Unique males eliciting 1.8 times higher response rate in Set 1 and 1.7 times higher in Set 2. In contrast, display rates for Unique and Redundant males were nearly identical in Set 1, with a non-significant trend for Redundant males to display more in Set 2.

The results of paired t tests were concordant with those from the linear models: Response rates significantly higher for Unique than for Redundant males in both sets (set 1: t=3.3, d.f.=11, P=0.007; set 2: t=3.4, d.f.=15, P=0.004). Paired tests for male display rates were not significant (set 1: t=0.07, d.f.=11, P=0.95; set 2: t=1.54, d.f.=15, P=0.14).

There was no significant effect of orange coloration on either female response rate or male display rate (Table 2).

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