BioMed Central

Research article

Direct selection on male attractiveness and female preference fails to produce a response

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Abstract

Background: Theoretical studies suggest that direct and indirect selection have the potential to cause substantial evolutionary change in female mate choice. Similarly, sexual selection is considered a strong force in the evolution of male attractiveness and the exaggeration of secondary sexual traits. Few studies have, however, directly tested how female mate choice and male attractiveness respond to selection. Here we report the results of a selection experiment in which we selected directly on female mating preference for attractive males and, independently, on male attractiveness in the guppy, *Poecilia reticulata*. We measured the direct and correlated responses of female mate choice and male attractiveness to selection and the correlated responses of male ornamental traits, female fecundity and adult male and female survival.

Results: Surprisingly, neither female mate choice nor male attractiveness responded significantly to direct or to indirect selection. Fecundity did differ significantly among lines in a way that suggests a possible sexually-antagonistic cost to male attractiveness.

Conclusions: The opportunity for evolutionary change in female mate choice and male attractiveness may be much smaller than predicted by current theory, and may thus have important consequences for how we understand the evolution of female mate choice and male attractiveness. We discuss a number of factors that may have constrained the response of female choice and male attractiveness to selection, including low heritabilities, low levels of genetic (co)variation in the multivariate direction of selection, sexually-antagonistic constraint on sexual selection and the "environmental covariance hypothesis".

Background

Female mate choice imposes sexual selection on males and is responsible for the evolution of elaborate male ornamentation. Choosy females may benefit adaptively from their choice, both directly (males provide benefits that increase female fecundity) and indirectly (offspring inherit genes that confer superior fitness from the chosen male) [1]. Thus female mate choice and male attractiveness are traits that are causally linked and that mutually influence one another's evolutionary fates. Despite a sophisticated body of theory and substantial empirical research on the mechanics of choice and the benefits that

Table 1: The number of males (M) or females (F) measured (in parentheses) and selected in each generation of selection. The selection treatments include up attractiveness (AT), down attractiveness (UN), up preference for attractive males (PR) and control (CO).

Generation		Block 1 selected (measured)					Block 2 selected (measured)				
		AT	UN	PR	CO	Date	AT	UN	PR	CO	Date
Parental	M	50 (100)	50 (100)	50 (50)	50 (50)	Feb 2000	50 (100)	50 (100)	50 (50)	50 (50)	May 2000
	F	50 (50)	50 (50)	50 (100)	50 (50)		50 (50)	50 (50)	50 (100)	50 (50)	
F1	M	38 (57)	38 (57)	38 (38)	38 (38)	Mar 2001	35 (57)	35 (57)	35 (35)	35 (35)	May 2001
	F	40 (40)	40 (40)	40 (76)	40 (40)		40 (40)	40 (40)	40 (76)	40 (40)	
F2	M	50 (84)	50 (90)	50 (50)	50 (50)	Oct 2001	50 (77)	50 (90)	50 (50)	50 (50)	Jan 2002
	F	50 (50)	50 (50)	50 (95)	50 (50)		50 (50)	50 (50)	50 (114)	50 (50)	

males may provide to females [2-4], there are few empirical measures of how mate choice and attractiveness respond to selection. Here we describe an experiment in which we selected directly on female mate choice and on male attractiveness in guppies (*Poecilia reticulata*), and measured the change in these traits. We also measured the indirect responses to selection of the male ornaments on which females are thought to base their choice, and of life-history traits that may be genetically correlated with attractiveness and thus provide indirect benefits.

Selection acting on female mate choice

Natural selection acts directly on female mate choice due to the direct benefits and costs associated with choosing a mate [2,3]. Examples of direct benefits include increased fertility, fecundity, resource provision, parental provision, breeding territories or a reduction in predation and harassment risks [3]. Natural selection may oppose the evolution of female mate choice if, for example, searching for and evaluating mates results in increased energy expenditure or predation risks [3,5].

Female mate choice may also be subject to indirect selection when there are genetic correlations between mate choice and other fitness components exposed to selection [1,6,7]. Indirect selection requires that there is additive genetic variation underlying female mate choice and overall fitness, and that the male displays and ornaments indicate this variation in fitness [7,8]. Thus, females benefit indirectly from being choosy because of the superior genes that chosen males pass on to the mutual offspring. Such a process favours the spread of "choosiness" genes leading to the evolution of an adaptive preference for male traits that indicate increased genetic quality (i.e. breeding value for total fitness) [7,8].

Despite the many studies on the direct and indirect benefits of female mate choice, the potential of the two processes to effect the evolution of mate choice has yet to be directly demonstrated [4]. It is particularly important that empiricists attempt such tests – logistically demanding as

they may be – because recent theoretical treatments have differed as to the likely strength and relative importance of direct and indirect selection on mate choice [9-11].

Selection acting on male attractiveness

In many species, females can only differentiate between potential mates based on the secondary sexual traits of the individual males. If phenotypic variation in male displays or ornaments exists, then any female biases toward mating with males of certain phenotypes will lead to differential male mating success [3,4]. If any of this phenotypic variation is heritable, differential mating success will, in turn, lead to the exaggeration of the ornament by sexual selection [1,3,12]. Several studies have demonstrated sexual selection operating on male ornamental and display traits (see [3] for an extensive review), the presence of substantial additive genetic variation in these traits [13,14] and in male attractiveness itself [15].

Artificial selection experiments are useful for understanding how traits respond directly to selection and indirectly to selection on correlated traits. There have been several recent demonstrations that attractive male display traits respond, in relatively few generations, to direct artificial selection [16-19]. Despite this wealth of evidence, there are no direct demonstrations that sexual selection on male attractiveness causes measurable evolutionary change in attractiveness and the ornamental and display traits that underpin it.

Here we present the results of an experiment in which we selected directly on male attractiveness and on female preference for attractive males in the guppy, *Poecilia reticulata*. Female guppies actively choose males based on highly distinctive and polymorphic ornamentation patterns, and both males and females mate multiply [20]. Accordingly, there is the strong potential for mate choice evolution and sexual selection. Importantly, the guppy has already proved to be a highly suitable organism for understanding the evolution of mate choice by indirect selection due to its non-resource based mating system

Table 2: Realised heritabilities of directly selected traits. Selection intensity is the cumulative intensity over the three generations, in units of phenotypic standard deviation. The response to selection is the divergence in the selection trait from the control line means, in control phenotypic standard deviations. Means and standard errors (SE) are included for the responses to selection and realized heritabilities. Selection intensities and responses in the up direction will have a positive sign, in the down direction a negative sign.

Selection treatment	Selection intensities		Selection response				Realized heritabilities			
	Block 1	Block 2	Block 1	Block 2	Mean	SE	Block 1	Block 2	Mean	SE
Up attractiveness (AT)	1.000	0.904	0.009	-0.044	-0.017	0.019	0.009	-0.048	-0.020	0.029
Down attractiveness (UN)	-1.030	-0.927	0.003	0.055	0.0288	0.018	-0.003	-0.059	-0.031	0.028
Up preference for attractive males (PR)	1.178	0.994	0.072	-0.191	-0.060	0.093	0.061	-0.192	-0.066	0.127

[15,16,19,21-23]. There is also substantial demonstrated additive genetic variation in male attractiveness and mating success [15,24] and in the ornamental traits on which attractiveness is based [15,25].

We directly selected the most attractive males (up attractiveness treatment), the least attractive males (down attractiveness treatment) and the females showing the strongest preference for attractive males (up preference treatment) and compared changes in these lines with control lines that experienced no selection. Instead of selecting on specific male traits and female preference functions, we selected individuals based on the results of behavioural mate choice trials. We thus imitated the actual processes involved in sexual selection and preference evolution in natural populations. We attempt to answer three important and related questions. First, how does female mate choice evolve under direct selection and as a correlated response to selection on male attractiveness? Second, how does male attractiveness evolve under direct selection and a correlated response to selection on female preferences? Third, how do other traits, especially those thought to determine attractiveness and those associated with other fitness components, evolve as a correlated response?

Results
Neither male attractiveness nor female preference for attractive males responded significantly to direct selection (Table 2). The realized heritabilities in both attractiveness and preference are inconsistent in magnitude and direction among lines such that the mean heritability estimates are small, and not significantly different from zero (Table 2).

More generally, there were no significant differences among treatments in any of the male attractiveness, female choosiness or female preference function measures (Table 3). Two of the three male ornaments most strongly associated with male attractiveness in the Alligator creek guppy population (tail area and the area of orange colour-

ation [15]) also did not differ significantly between selection treatments, but the third (area of iridescence) did (Table 3). Furthermore, the total number of spots differed significantly among treatments (Table 3). Post-hoc comparisons revealed that iridescence was significantly lower in the down attractiveness treatment (compared with all other treatments) and that spot numbers were significantly lower in the down attractiveness line than in the up preference line. Taken together these results indicate that although preference and attractiveness did not respond to direct or indirect selection, some ornamental traits did. Moreover, it seems that these changes were largely due to a decrease in attractive ornaments (iridescence and spot number – an index of overall pattern complexity) in the down attractiveness line.

Survival did not differ significantly between the selection treatments (one-way ANOVA $F_{3,4} = 0.98$, P = 0.492), and does not appear to have responded indirectly to selection on either male attractiveness or female preference for attractive males. Selection did, however, result in correlated changes in at least one fitness component: female fecundity in the down attractiveness treatment was significantly higher than all other treatments, and in the up preference line it was significantly lower than all other treatments ($F_{3,4}= 69.00$, P = 0.000, and Tukey's post-hoc comparisons).

Discussion
Neither female mate choice nor male attractiveness responded significantly to the selection that we imposed, in contrast to the predictions of theoretical models of sexual selection and mate choice evolution. Direct and indirect selection on mate choice are expected to cause substantial evolutionary change [4,9-11,26]. Similarly, sexual selection resulting from female choice of attractive males is widely understood to cause the evolution of male attractiveness and the exaggeration of secondary sexual traits [3,12]. We spend the remainder of this paper considering why we failed to detect significant responses to three generations of selection on male attractiveness and female

Table 3: Nested analysis of variance comparing traits between selection treatments and between blocks nested within treatment. The means used in the analysis have been standardised for the effect of block.

Measured character	Selection treatment			Block within treatment		
	df	F	P	Df	F	P
PARTITIONED-AQUARIA BEHAVIOUR TRIALS						
Male attractiveness	3, 4	0.31	0.820	4, 444	0.21	0.935
Female preference for attractive males	3, 4	0.93	0.505	4, 444	0.31	0.869
Female responsiveness	3, 4	2.32	0.217	4, 444	1.30	0.268
Female discrimination	3, 4	0.22	0.881	4, 444	1.11	0.351
OPEN-AQUARIA BEHAVIOUR TRIALS						
Male attractiveness	3, 4	2.85	0.169	4, 376	0.47	0.760
Female responsiveness	3, 4	0.51	0.694	4, 184	0.91	0.459
MALE ORNAMENTATION						
Body size	3, 4	1.09	0.451	4, 444	6.49	0.000
Tail size	3, 4	1.67	0.309	4, 444	2.29	0.059
Black	3, 4	0.70	0.598	4, 444	1.52	0.194
Fuzzy black	3, 4	1.55	0.332	4, 444	0.82	0.513
Orange	3, 4	1.14	0.433	4, 444	3.11	0.015
Iridescence	3, 4	7.31	0.042	4, 444	0.49	0.746
Yellow	3, 4	4.52	0.090	4, 444	0.76	0.553
Tail colour	3, 4	0.23	0.874	4, 444	11.29	0.000
Spot number	3, 4	11.22	0.020	4, 444	4.70	0.001

preferences, despite the widespread expectation that such a response should occur.

Lack of response in female preference
Statistical power
As the true level of replication in this kind of artificial selection experiment is the line, even a very large, long-term study such as ours may have low statistical power. This is even more the case when the measures involved are subject to large environmental variances via measurement error as is likely to be the case with our behavioural estimates of female preferences and attractiveness. Such environmental variance would result in low measured heritability of female preferences and male attractiveness and modest response to selection.

Low heritability
An obvious possible explanation for the lack of a significant selection response is that the heritabilities of male attractiveness and female mate choice may be low. Low heritabilities can be attributed either to a lack of additive genetic variation or to high levels of environmental variation [27]. If heritabilities are low, then the response to selection may be smaller than the minimal detectable difference for this experiment. Our finding that female preference for attractive males did not respond significantly to

direct selection is consistent with estimates of zero heritability of this trait in this population under similar lab conditions [23]. In the study by Brooks and Endler [23], the heritability estimates of all components of mate choice (including responsiveness, discrimination and preferences for attractive males and various univariate male traits) were low and, in all cases other than responsiveness, not significantly greater than zero.

Both our result and the heritability estimates of Brooks and Endler [23] suggest that there might be a lack of additive genetic variation and / or abundant environmental variation, including measurement error, in mate choice (but see[16,19]).

Lack of response in male attractiveness
Low heritability
Although the same caveats about statistical power apply to male attractiveness, the possibility that male attractiveness failed to respond to direct selection due to a lack of additive genetic variation is less likely. By combining the selection intensity that we imposed with previous (significant) estimates of the heritability of male attractiveness in this guppy population ($h^2_{sire} = 0.596$, $h^2_{sire\&dam\ combined} = 0.348$, [24]) in the breeders' equation [27], we expected a change of 0.575 – 0.336 standard deviations in the

direction of selection on attractiveness. The small responses that did occur were inconsistent in direction and more than an order of magnitude smaller than the predicted responses. The observed responses were also at least three standard errors different from the predicted responses, indicating that we have the power to conclude that the predicted changes did not occur. The imposed selection intensities are comparable to those of other selection experiments on guppies. In the study by Houde [16], for example, a selection intensity of 0.386 per generation resulted in significant changes in male colouration. The selection intensities we imposed (mean 0.33 per generation) are also above the mean values documented in the wild (0.16, [28,29]).

Univariate accounts of phenotypic evolution are often inadequate. The evolution of a trait is influenced not only by its heritability and the intensity of selection operating directly upon it, but also by indirect selection when natural selection operates on genetically correlated traits [30]. Two recent studies have documented traits that fail to respond to selection as predicted by the univariate breeders' equation [31,32], and suggested that multivariate understanding of genetic variation and the operation of selection is necessary. We will now evaluate three possible multivariate explanations for why male attractiveness failed to respond as predicted to selection: complexity in the relationship between male attractiveness and the ornaments that underpin it, genetic constraint due to the patterns of genetic covariation among traits, and the "environmental covariance hypothesis".

Complex fitness surface

Blows, Brooks and Kraft [33] showed that in this population of guppies, the multivariate linear and nonlinear sexual selection fitness surface has at least three local fitness peaks corresponding roughly with large areas of orange colouration, of iridescence, and large tails. Consequently, there appear to be at least three ways in which male guppies can maximise attractiveness. Thus sexual selection on attractiveness and the ornaments that underpin it may not necessarily proceed in one direction. This is consistent with our observation that changes in ornamentation among lines and among treatments in our experiment occurred in different multivariate directions. Selecting on attractiveness, therefore, may have resulted in complex and inconsistent changes in ornamentation if different lines evolved toward different local optima in the fitness surface.

Genetic variance and covariance

There are two similar processes that may constrain the evolution of female preferences and male attractiveness. First, the genetic variation in and covariation among male ornaments that determine attractiveness are likely to influence the direction and rate of evolutionary change in male attractiveness. Second, trade-offs between attractiveness and other fitness components, including sexually antagonistic effects of genes influencing attractiveness may prevent any net change by sexual selection.

By selecting on male attractiveness, we imitated the direction of sexual selection in this population. The small responses of ornamental traits to selection on male attractiveness suggest that we may have selected in a direction that contains little multivariate genetic variation. Although there is abundant genetic variation in most ornamental traits in this guppy population, Brooks and Endler [15] demonstrated that most of this variation is not in the direction of sexual selection. By combining the genetic variance-covariance matrix, **G**, with the vector of estimated selection gradients, β, in the multivariate breeders equation [30,34], they predicted that one generation of sexual selection would result in changes of only 1–6% of one trait standard deviation. Thus the components of selection operating directly on ornamental traits are largely opposed by indirect selection on correlated traits, and multivariate genetic constraint on male ornamentation is a plausible explanation for the lack of response in attractiveness itself.

Selection acting on a trait in members of one sex may be constrained if there is antagonistic pleiotropy between the trait and fitness components in the opposite sex [35]. This is now known as intragenomic sexual conflict [36,37]. The continued exaggeration of male attractiveness, for example, may have been constrained by some costs to the expression of the female preference for attractive males [38] or negative genetic covariance between male attractiveness and important offspring fitness components [24,39].

In our study, female fecundity was significantly different between the four selection treatments. The most fecund treatment was the one in which we selected the least attractive males (UN), while the least fecund treatment was the one in which we selected on female preference for attractive males (PR). Furthermore, the eight line means of fecundity were significantly negatively correlated with line mean attractiveness (r = -0.69, P < 0.05), and with iridescence (r = -0.93, P < 0.005) and orange colouration (r = -0.68, P < 0.05) which are both key components of male attractiveness [15,33]. These results indicate that there may be a fecundity cost associated with male attractiveness and female preference for attractive males. The lack of response to selection may, therefore, be due to the fecundity selection that occurred within each selection line if females within the tank contributed unequally to the next generation. For example, although we selected for increased attractiveness, the least attractive males among

those selected may have benefited by having more fecund daughters than the most attractive males. Such a phenomenon would reduce the effective intensity of selection on attractiveness, causing any differences between treatments to fall below that the power our analyses could detect. The fact that we were able to detect the fecundity differences, yet did not observe any response to the selection is, however, paradoxical. The results do suggest that antagonistic evolution may be responsible in part for the lack of evolutionary change in female mate choice.

Other, similar, forms of natural selection within each line may also have constrained the response of male attractiveness to selection. Male attractiveness is negatively genetically correlated with the survival of juveniles and adult males [24], although we found that adult survival did not differ significantly between the selection treatments.

Environmental covariance hypothesis
Traits that are heritable and environmentally, but not genetically, correlated with fitness should not respond to selection – even though phenotypic selection analyses might predict such a response [31,40-42]. This "environmental covariance hypothesis" has been invoked to explain why antler size in the red deer, *Cervus elaphus*, did not respond to measured directional selection despite being highly heritable [31]. In this example, nutrition had independent positive effects on both fitness and antler size. Thus although fitness and antler size were positively correlated, this correlation was environmental rather than genetic (as would have been expected if large antlers caused higher fitness). A lack of evolutionary response is therefore attributed to a misidentified target of selection.

Like antler size, male attractiveness in the guppy is highly reliant on dietary condition. Orange colouration, for example, is a major component of attractiveness and partly derived from carotenoids in the diet of the guppy. Studies have also shown that increasing the carotenoid content of food will result in increased attractiveness [43,44]. It is conceivable, under the environmental covariance hypothesis that the commonly-documented relationship between orange colouration and attractiveness is due to an environmental correlation, and that sexual selection might thus have no net effect on the area orange colouration. This is unlikely, however, for two reasons. First, in the specific case of orange colouration, manipulative studies have shown that the relationship between levels of orange and attractiveness is causal: females prefer to mate with males because they directly assess the amount of orange in their colour pattern [45,46]. Second, male attractiveness and several components of male colouration are genetically correlated with one another and with mating success [15,24]. It appears unlikely, therefore, that

the environmental covariance hypothesis can explain the lack of response by male attractiveness to selection.

Implications for the evolution of female mate choice
Our findings that neither male attractiveness nor female preference for attractive males responded significantly to three generations of selection (direct or indirect) have important implications for the evolution of mate choice by indirect selection. A long standing topic of controversy (the lek paradox) is whether and how sufficient additive genetic variation in male fitness and attractive ornamentation can be maintained in the face of selection to provide an indirect fitness benefit to choosy females [2,3]. Recent work has demonstrated both that considerable genetic variation in display traits exists [14] and suggested several possible processes that might maintain it [47-50].

Female guppies are thought to benefit from mating with attractive males because attractiveness itself is highly heritable and thus choosy females' sons are more likely to be attractive than are the sons of indiscriminate females [24]. The fact that in our study there was no measurable increment in male attractiveness (or in any measure of female choice) when we selected directly on male attractiveness indicates that the "attractive sons" benefit to choosy females may be smaller than one might infer from the univariate heritability of male attractiveness. This may be due to the fact that male attractiveness appears to be negatively genetically correlated with other fitness components, including male survival [24] and female fecundity (this study). It may also be due to the fact that male attractiveness is influenced by a suite of male traits, and that there is little multivariate genetic variation in the direction of selection despite the presence of additive genetic variation in each one of the male traits.

The indirect (genetic) benefits of mate choice can only arise if preferred males have higher breeding values for total fitness than non-preferred males [7]. It becomes clear from the issues that we have raised in this discussion that demonstrating heritable variation in one or a handful of attractive display traits is not sufficient evidence to dismiss the lek paradox. There is only one demonstration in any species that female preference is genetically correlated with offspring fitness [39], and no compelling demonstration that substantial genetic variation in offspring total fitness is correlated with variation in a trait that females use to choose mates. Tradeoffs and sexual antagonism between fitness components, and the possibility that little multivariate genetic variation in suites of ornaments is in the direction of sexual selection both raise the possibility of a new multivariate form of the lek paradox.

Methods

We collected guppies as juveniles from Alligator Creek, 30 km southwest of Townsville, Queensland. The use of wild-caught individuals to begin the selection lines ensures that naturally occurring genetic (co)variation is present. We raised the fish for the first selected (parental) generation in 100 litre single sex stock tanks.

We imposed three generations of selection on three selection treatments by selecting (1) directly up and (2) down on male attractiveness and (3) up on female preference for attractive males. We also conducted an unselected control. We use the following two letter abbreviations for the four types of line in the remainder of the manuscript: AT – Up attractiveness; UN – Down attractiveness; PR – Up preference; CO – Control. There were two replicates of each of the four types of line, but due to logistic constraints on the number of fish that we could maintain and measure, the replicate lines were conducted in two blocks. Each block contained one line from each of the four types. We performed the same experimental measures on each block but staggered the blocks by two months.

The dates on which we performed selection in each generation, and the number of individuals that we measured and selected are given in Table 1. In each generation, selected males and females from a line were placed into a 300 litre tank together to mate and produce offspring. This allows for sexual and other forms of natural selection to operate within lines at this stage, and has both advantages and disadvantages. An advantage over designs in which males and females are randomly paired and mated is that if mate choice is possible, linkage disequilibrium between male attractiveness and female preferences [1,6] may be maintained [51,52]. This disequilibrium is a crucial element of the genetic architecture of choice and attractiveness, and should be carefully considered when designing selection experiments. The disadvantages of our approach lie in the interpretation of any response (direct or correlated). Gray and Cade [53] point out that within-line sexual selection may cause an overestimate of the genetic correlation between preference and trait. This is not a problem in our study as we found no evidence of direct or indirect responses in these traits (see results). However, any selection on survival or fecundity within lines may either amplify or attenuate any response to the selection imposed by the researcher, a possibility we address in the discussion.

Offspring were collected daily and reared at initial densities of ten fry per six-litre tank. At approximately 40 days old, the fry were sexed based on the presence of female egg spots, and separated into single sex tanks. Tanks were covered on three sides with brown paper, and contained both floating and sessile plastic plants to provide refuge from
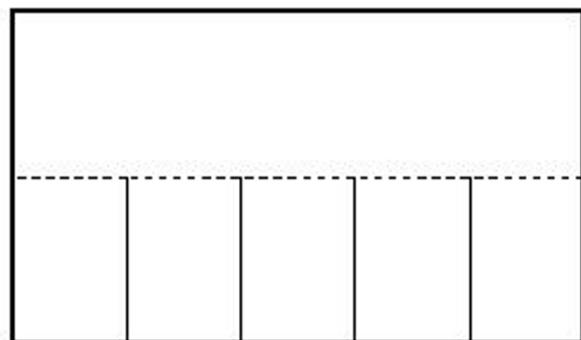

Figure 1
The choice tank used in measuring male attractiveness and female preference. Brown paper covered the side and back walls (bold line). Brown river sand covered the floor of tank. Scored glass separated (solid line) the five small compartments. Transparent glass (dotted line) separated the large and small compartments. Tank dimensions: 30 by 20 cm.

harassment and (in the case of fry) cannibalism. Water was aerated and filtered using air-driven filters under a layer of light brown gravel. The temperature was kept constant at 26°C. A mixture of fluorescent and daylight lighting was used to illuminate the tanks. Throughout the experiment, we fed the fish five times a week on one-day old brine shrimp and twice a week on commercial flake food for tropical fish.

Selecting on attractiveness and preference

Our measures of male attractiveness and female preference were designed to capture these traits for the block as a whole so that preference-display runaways within selection lines do not obscure changes in the treatments relative to other lines within the block. Thus each male was seen by females from every treatment and likewise each female saw males from every treatment.

We measured male attractiveness and female mate choice in behavioural trials in partitioned-aquaria (Figure 1). We placed one male into each of the five small compartments, and a naive virgin focal female into the large compartment from where she could observe the five males. In the first generation of selection we randomly assigned one sixth of all males to the PR line, one sixth to the CO line, and one third to each of the AT and UN lines. Selection was applied (if at all) only to the individuals that had been assigned to the appropriate line. In the second and third generations, each choice tank contained one male from each line plus either an extra AT or UN male.

Up to twenty choice tanks were used per day during behaviour trials. Tanks were arranged over four rows, orientated towards an observer seated one meter away. During the behavioural trials, two daylight incandescent globes, placed behind the observer, provided lighting. All tanks experienced similar lighting intensities at the water surface (range 1.0–1.9 $\mu mol.m^{-2}.sec^{-1}$).

On the evening before a trial, we placed the five males and one female into each choice tank. Observations commenced the following morning between 0700 and 0800 hours and involved scanning all tanks consecutively fifty times. If a female was within one body length of and directly facing the compartment containing a male, we scored his compartment number. A male's attractiveness to a given female was the total number of such "visits" she paid him (maximum possible = 50). Similar partitioned-aquarium measures have been used extensively in studies of guppy mate choice, and attractiveness scores have been shown to significantly predict mating success [15,20]. We repeated the behavioural trial over five consecutive days, using a new focal female each day. On two of the five days the female was from the PR line, and the female was from each of the other three lines on one day each.

A male's attractiveness may be influenced by three factors: his actual attractiveness to the females that saw him, the choice tank he was in, and his location within the tank. It is important to control for the latter two factors. Typically, with the choice tank used in this experiment the outer two positions have elevated scores, followed by the next two positions (middle positions) and finally the centre position. To correct for the effect of position, we multiplied the scores of each male by a correction factor. We calculated the correction factor for each week of observations based on the average score recorded at each position in all tanks on all five days in that week.

A preference function is how a female ranks prospective mates based on a specific male trait [54]. A female's "preference for attractive males" indicates the extent to which a female's choices are consistent with those of her peers [23]. We estimated a female's preference for attractive males as the slope coefficient of the least-squares regression of how she rated the five males on the mean scores that those males received from the other four females who saw them. A positive slope indicates that a female rated the males in the same way as the other four females, and thus presumably the majority of the population. Furthermore, the larger the positive slope the more strongly the female of interest responded to attractive males. The use of linear regression gradients as estimates of the strengths of selection is valid irrespective of whether the assumptions of linear regression significance tests (e.g. normality) are met [30]. This method is, however, prone to error

because slopes were estimated from only five data points. Furthermore, although there is no autocorrelation in the estimated slopes, the use of the mean of four females' scores as the independent variable (attractiveness) means that there is some nonindependence to the preference estimates within a trial. This nonindependence did not, however, result in significant resemblance between the preference measures taken within a tank in a given week (ANOVA $F_{36,395} = 1.184$, $P = 0.221$).

Terminal measures
In the F3 generation, we measured a suite of traits to estimate the direct response of each selected trait and any correlated responses in other potentially correlated traits. We measured male attractiveness, female mate choice, male ornamentation, survival and fecundity. A total of 57 virgin males and 107 virgin females from each selection line per block were used for these terminal measures.

We used two different types of behavioural trials to measure female mate choice and male attractiveness. First, we conducted partitioned-aquaria behavioural trials (as in the selection process) which allow for individual identification of each focal female without direct interactions between males and females. We then used open-aquarium behavioural trials, in which males and females can interact freely and the full range of male courtship and female response behaviours can occur [20]. In the partitioned-aquaria trials, male attractiveness and female preference for attractive males were measured as described above for selection. We estimated a female's responsiveness as the number of times she was seen with any of the males and discrimination as the coefficient of variation in her number of visits to the five males in the tank.

The open-aquarium behavioural trials were conducted in 100 L aquaria under the same lighting conditions as in the partitioned-aquaria trials. On the night before observations, we placed eight males and eight females into the behavioural tank. Each set of eight males contained two males from each selection treatment. The females on any given day were all from the same selection treatment. We used eight new males and eight new females on each day. Males were individually identified by the observer from their unique colour patterns.

Observations began between 0700 and 0800 hours. We watched each male for a five-minute period in random order, and then for a second five-minute period each in a different random order. Finally, we spent ten minutes scanning the tank, shifting from one male to the other approximately every 30 seconds, to ensure that we observed a total of at least five displays per male. We followed the standard procedures of Houde [20,21] in scoring a male's attractiveness as the proportion of his

sigmoid displays that elicited at least a "glide" response from a female. We measured female responsiveness as the mean response of females to all males in the trial. Only a single measure of responsiveness could be obtained for each trial, as females cannot be individually distinguished.

We photographed the right side of each male against a white background with a Nikon Coolpix 990 digital camera, including a ruler with millimetre graduations in the picture for calibration. Each male was anaesthetised beforehand with iced water and illuminated dorsally and anteriorly (30° angle of incidence) with low intensity halogen light (Fostec ACE 150 watt light source). We then traced the area of the body, the tail and each colour spot using Measure Master (Version 3.4) digital imaging analysis software. From the tracings, we calculated body area and tail area, and the proportion of the body covered by black, fuzzy black, orange, yellow and iridescent spots. We also counted the of coloured spots on his body.

We conducted short-term adult survival and fecundity trials by placing 50 males from one line (used in the previous attractiveness measures) and 50 naive virgin females from the same line into a 250 litre mating tank. Each day for the next 60 days we collected and counted the number of offspring produced. At the end of the 60-day trial we recorded the number of adults of each sex remaining.

Statistical analysis

We standardised the measures of traits by block means and standard deviations in order to control for environmental variation among blocks. We then assessed differences between the treatments using nested analysis of variance and one-way analysis of variance.

We estimated realized heritabilities of directly selected traits by applying the standardized form of the breeder's equation (Equation 11.3, ref [27]). We estimated the response to selection as the difference between the line means for each trait of interest in the **F3** generation and the corresponding block's control line means. We then standardised the response to be in units of the control line phenotypic standard deviation for the trait of interest. By standardising the response, we are able to use the intensity of selection, i (Equation 11.5, ref [27]), instead of the selection differential, s, which was considerably more difficult to calculate in this experiment. We calculated i based on the proportion selected and properties of normal distributions (Appendix A, ref [27]) and modified our estimate based on the ratio of the selected sex to the other sex (Equation 11.6b, [27]). We then calculated realized heritabilities from the standardized breeders' equation. Each replicate selection line provides one estimate of the

realized heritability, allowing a mean and standard error to be directly estimated for each selection treatment.

Author's contributions

RB conceived and initiated the study. All authors measured behaviour, conducted selection and contributed to the design of the terminal measures. MH undertook the terminal measures, analysed the data and wrote a first draft of the manuscript. All authors contributed to the final analyses and writing of the manuscript.

Acknowledgements

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