Limits to environmental masking of genetic quality in sexual signals

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Abstract

There is considerable debate over the value of male sexual ornaments as signals of genetic quality. Studies alternately report that environmental variation enhances or diminishes the genetic signal, or leads to crossover where genotypes perform well in one environment but poorly in another. A unified understanding is lacking. We conduct a novel experimental test examining the dual effects of distinct categories of genetic (inbred versus crossed parental lines) and environmental quality (low, through high to extreme larval food stress) on a condition-dependent male ornament. We find that differences in genetic quality signalled by the ornament (male eyespan in *Diasemopsis meigenii* stalk-eyed flies) become visible and are amplified under high stress but are overwhelmed in extreme stress environments. Variance among independent genetic lines increases with environmental stress in both genetic quality classes, but at a slower rate in high quality outcrossed flies. Individual genetic lines generally maintain their ranks across environments, except among high quality lines under low environmental stress, where low genetic variance among lines precludes differentiation between ranks. Our results provide a conceptual advance, demonstrating a unified pattern for how genetic and environmental quality interact. They show when environmental conditions lead to the amplification of differences in signals of genetic quality and thereby enhance the potential indirect genetic benefits gained by female mate choice.

Keywords: condition dependence, Diptera, G x E, good genes, indirect genetic benefits, sexual ornament, sexual selection, stalk-eyed fly

1. Introduction

Many exaggerated male sexual ornaments are thought to provide information about the genetic quality of the signaller (Danielson-Francois et al., 2006; David et al., 2000; Houle, 1992; Jennions & Petrie, 2000; Pomiankowski, 1988; Pomiankowski & Møller, 1995; Taylor et al., 2007). Yet these traits typically also respond strongly to environmental variation (Cotton et al., 2004a; David et al., 1998; Kotiaho, 2000; Zuk et al., 1990), and it is unclear from empirical studies what impact this has on their signalling function (Bussiere et al., 2008; Greenfield & Rodriguez, 2004). One view holds that increasing environmental stress exposes the underlying genetic differences in quality which are otherwise masked by a benign environment (Danielson-Francois et al., 2006; Danielson-Francois et al., 2009; David et al., 2000; Dmitriew & Blanckenhorn, 2014). Alternatively, environmental stress may introduce sufficient randomness to overwhelm the signal of genetic quality (Charmantier & Garant, 2005; Evans et al., 2015; Ingleby et al., 2013) or create crossover, where genotypes that perform well in one environment do poorly in another (Etges et al., 2007; Evans et al., 2015; Jia et al., 2000; Rodríguez & Al-Wathiqui, 2011).

We investigate the hypothesis that these contrasting outcomes likely arise from limitations in previous experimental approaches. A major problem in previous analyses is that they have focussed on genetic variation *per se* rather than comparing distinct categories of genetic quality. Another problem is the limited rather than wide range of environmental stress examined. We present a novel experimental design that addresses both of these deficits. The results lead us to propose a unified understanding of how variation in genetic quality is impacted by environmental variation. This enables a far

clearer understanding of the conditions under which sexual display traits can function to accurately reveal the genetic quality of signallers (Hoffmann & Merila, 1999).

In this study, we adopt an integrated experimental approach. For the first time, we examine the impact of a wide range of both genetic and environmental quality, spanning mild to extreme levels of stress. We focus specifically on male eyespan variation in a stalk-eyed fly (Bellamy et al., 2013; Cotton et al., 2006) as this trait has been subject to extensive previous work. Male eyespan is highly exaggerated and acts in female mate choice as a signal of male genetic quality (Burkhardt & de la Motte, 1988; Cotton et al., 2004a; Cotton et al., 2010; David et al., 2000; Hingle et al., 2001a, 2001b; Wilkinson & Dodson, 1997; Wilkinson et al., 1998; Wilkinson & Reillo, 1994). It is highly condition-dependent relative to other traits in relation to both genetic (Bellamy et al., 2013; but see: Prokop et al., 2010) and environmental (Cotton et al., 2004a; Cotton et al., 2004b; David et al., 1998) stressors, and is responsive to a range of environmental stress types (Bellamy et al., 2013; Bjorksten et al., 2001; Cotton et al., 2004a, 2004b; David et al., 1998; Knell et al., 1999), while genetically distinct families have been shown to respond differently to environmental stress (David et al., 2000).

Our novel experimental design to study genetic quality-by-environment interactions in signalling traits exploits pre-defined classes of genetic (Bellamy et al., 2014; Bonduriansky et al., 2015; Zajitschek & Brooks, 2010) and environmental quality. To vary genetic quality, crosses were made within or between a set of parental inbred lines. This allowed us to compare low genetic quality, highly homozygous "incross" lines with high genetic quality, highly heterozygous "outcross" lines. We used incross and outcross lines not to study the effect of inbreeding *per se*, but because previous work

97 unambiguously shows that they correspond to low and high genetic quality classes
98 (Bellamy et al., 2013), since a suite of traits (thorax, wing and eyespan) in both sexes are
99 reduced among incross lines (Bellamy et al., 2013). The large number of independent
100 crosses within or between lines allows us to capture the contribution of genetic variation
101 in the sexual ornament among low and high genetic quality classes.
102
103 We likewise generated a wide range of environmental quality variation through
104 reductions in the amount of food available to developing larvae. This approach is a well-
105 established method for creating stress in holometabolous insects (Bonduriansky et al.,
106 2015; Burger et al., 2007; Burkhardt et al., 1994; Cotton et al., 2004a, 2004b; Dmitriew
107 & Blanckenhorn, 2014; Hingle et al., 2001a; Knell et al., 1999; Kolss et al., 2009; Sisodia
108 & Singh, 2012) and has been used extensively in prior stalk-eyed fly studies (Cotton et
109 al., 2004a; David et al., 1998; Hingle et al., 2001a), where it generates body size
110 variation equivalent to the range found in natural populations (Cotton et al., 2004b;
111 Cotton et al., 2010). Eggs from each cross were reared under conditions of low, high and
112 extreme environmental stress. Levels of stress between low and high were omitted due to
113 logistical constraints and because those stress levels have been extensively investigated
114 (Cotton et al., 2004a). Extreme stress was defined as the lowest food level where larval
115 viability was not seriously impaired (see below).
116
117 Our use of the terminology of low/high genetic quality and low/high/extreme
118 environmental quality is necessarily arbitrary but justified in terms of the study's
119 motivation and experimental design. We comment further on these definitions in the
120 Discussion. The innovation in our study lies in delivering controlled manipulation of
121 genetic quality *and* environmental stress, over several pre-defined quality levels, thereby

enabling an in-depth exploration of genetic quality-by-environment variation in a male sexual ornament. In order to emphasize this distinction we use G_Q to refer to our genetic quality classes, and G_Q x E for the genetic quality-by-environment interaction. Our approach allows us to investigate the signalling utility of a male ornament, in providing information about indirect genetic benefits to females, in the face of variation in environmental quality.

2. Material and methods

(a) Variation in genetic and environmental quality

A suite of 105 inbred lines were founded from a stock of *Diasemopsis meigenii* (Bellamy et al., 2013), an African stalk-eyed fly, derived from flies collected in South Africa by S. Hilger in 2000. After 11 generations of full-sib mating ($f \sim 0.908$), the extant lines were bred in cage culture (~200 flies/cage). For this study, 17 lines were chosen as parental lines to use in crosses. Eggs were collected from each line and larvae were raised on excess puréed sweet corn. At eclosion, offspring were placed in large cages (15l), separated by sex (~2 weeks), raised until sexual maturity (~10 weeks) and kept under standard conditions (Bellamy et al., 2013).

Variation in genetic quality (G_Q) was achieved using a crossing protocol (Figure 1; modifed from Prokop et al., 2010 and Bellamy et al., 2013). "Incross" flies were created from male-female crosses within an inbred line. "Outcross" flies were created from crosses between different inbred lines. In each cross, 4 adult males from line *x* and 4 adult females from line *y* were allowed to mate in a 1.5l pot ($x = y$ for incross, $x \neq y$ for outcross). Reciprocal male *x* - female *y* and female *x* -male *y* pots were set up. Multiple

147 replicates of each cross (between 1-8) were set up, with higher replication for inbred
148 crosses as they were less fecund. Eggs were collected twice weekly over 23 days. In all,
149 142 crosses were set up, of which 117 generated sufficient offspring across the food
150 treatments: 67 incrosses of 15 inbred lines and 50 outcrosses between 16 pairs of inbred
151 lines. An inbred line was used in an incross the same number of times as it was used in
152 an outcross, and as far as possible equal numbers of live adult males and females were
153 collected from each line, to balance sex chromosomal, cytoplasmic and other
154 male/female parental effects. Throughout this paper, G_L designates the genetic lines
155 within the two genetic quality (G_Q) classes.
156
157 Incross flies have low genetic quality as they are highly homozygous, being derived from
158 inbred lines created by repeated brother-sister pairings (11 generations), with an expected
159 inbreeding coefficient of $f \sim 0.908$ (Bellamy et al., 2013; Falconer & Mackay, 1996). In
160 contrast, outcross flies have high genetic quality as they are expected to be heterozygous
161 for most of the alleles fixed in the parental inbred lines from which they are derived.
162 Although the terms – low and high genetic quality – are arbitrary, there was evidence of
163 substantial heterosis in a variety of traits when inbred flies were crossed, so the terms
164 reflect the nature of these two genetic groups (Bellamy et al., 2013).
165
166 For each incross or outcross, fertilised eggs were placed in groups of 5 in petri dishes
167 containing two cotton pads, 15ml water and 5ml of food medium. Three qualities of
168 food medium were used with "pure" corn diluted with water at ratios of 1:1, 1:10, and
169 1:20, which we designate as "low", "high" and "extreme" stress respectively. "Pure"
170 corn was made by forcing puréed sweet corn kernels through a fine sieve to remove
171 husks and provide homogeneity. Food qualities were chosen based on a pilot study, with

levels of food stress used that were found to lie within normal rates of egg-adult survival (Figure 2, see SI.C). Although the terms – low, high and extreme environmental stress – are again arbitrary, they nonetheless capture particular qualities. The food level for low stress was similar to the standard media on which larvae are raised. The food level for high stress was that used previously where it was associated with reduced size in a variety of traits (Bellamy et al., 2013). The food level for extreme stress constitutes the far end of the stress spectrum before differential survival is evident (Figure 2). In the range used (i.e. 1:1 to 1:20), egg-to-adult survival did not differ in the pilot experiment, and was at ~50%. We did not go beyond this level, as a serious loss of adults would have posed greater logistical difficulties for attaining the large target sample size in the experiment. In the main experiment, a census of pupae was additionally made as a measure of survival for each cross in each environment.

(b) Adult morphology

After eclosion, flies of each cross were collected and frozen at -20°C. All males were measured for eyespan (the distance between the outermost tips of the eyes, Chapman et al., 2017; David et al., 1998) and thorax (the distance between the centre of the most posterior point of the head to the joint between the meta-thoracic legs and the thorax, Meade et al., 2017; Rogers et al., 2008) to a tolerance of 0.01mm, using a video camera mounted on a monocular microscope and ImageJ image capture software v.1.46 (Schneider et al., 2012). The repeatability of these morphological trait measurements is very high at >99% (David et al., 1998). In total 1186 males were phenotyped. All measurements were obtained blind by JMH. In a few cases (n = 9), a measurement was not included in the dataset due to sample damage.

197 **(c) Statistical analysis**

198 To test for effects of incross/outcross genetic quality (G_Q), environmental (E) and the G_Q
199 x E interaction on morphological trait variation, several general linear mixed effects
200 models (GLMMs) were fitted via REML. In each model, G_Q, E and their interaction
201 were included as fixed effects. Male parental line and female parental line were included
202 as random effects, as was cross and its interaction with E. Additional random effects of
203 male line x E, male line x G_Q, female line x E and female line x G_Q explained zero
204 variance and so were removed in model simplification. GLMMs for male eyespan had
205 thorax added as a covariate to control for body size. Thorax length accounted for a
206 significant portion of variance, but its addition did not substantially alter the results (for
207 completeness, analyses of absolute trait values are given in the SI.A). GLMM models
208 fitted pairwise to low versus high and high versus extreme environmental stress were
209 used to further investigate the basis of the observed G_Q x E patterns. Two-tailed *t*-tests at
210 each level of E were used to test whether incross male eyespan was larger or smaller than
211 outcross male eyespan.
212
213 Coefficients of variation (CVs), the ratio of the standard deviation to the mean, were
214 used to assess how variance in male eyespan responded to genetic quality,
215 environmental stress and the G_Q x E interaction. CVs control for changes in variance
216 purely as a function of size, and are considered to be less biased than heritability
217 estimates in genotype-by-environment studies (Rowinski & Rogell, 2017). Least square
218 means for male relative eyespan were extracted from GLMMs for each cross, for each E
219 and G_Q, to calculate among-cross CVs. Among-cross CVs were then compared between
220 incross and outcross using modified signed-likelihood ratio tests (M-SLRT,
221 Krishnamoorthy & Lee, 2014) in each environment, and also across environments (L-H-

X), both overall and for incross and outcross. Finally, adjacent environment pairs were contrasted for among-cross CV, low with high (L-H) and high with extreme (H-X), for each genetic quality. The among-cross contrasts were conducted in the R-package 'cvequality' (Marwick & Krishnamoorthy, 2016.).

To explore the consistency of genetic lines (G_L) within each genetic quality class across environments, genetic correlations (r_g) across adjacent environments were calculated. GLMMs were fitted with cross as a random effect and the variance component for cross was extracted for each environment. GLMMs were then carried out between pairs of adjacent environments (L-H, H-X), with the cross x E interaction included as a random effect, and the interaction variance component extracted. As before, thorax length was added as a fixed covariate to control for body size. An estimate of r_g was then calculated as:

$$r_g = \sigma^2_{1,2} \Big/ \sqrt{\sigma^2_{1,1}\sigma^2_{2,2}},$$

where $\sigma^2_{1,1}$ and $\sigma^2_{2,2}$ are the genetic variances in environments 1 and 2 respectively, and $\sigma_{1,2}$ is the genetic covariance between the two environments (Roff & Wilson, 2014). Broad bounds of the r_g values were tested via model simplification and likelihood ratio tests (details in SI.A). A test for G_L x E was also made for each environment pair, following the same protocol used to test G_Q x E, but with an explicit test of line (SI.A).

(d) Statistical software used

All statistical analyses were conducted in JMP v.12.0.1 (SAS Institute 1989-2015) and R v.3.4.2 (R Core Development Team, 2017). GLMM tables, effect coefficients and extended methods are shown in SI.A.

3. Results

(a) Response in mean trait

As expected, male eyespan ($F_{2,45.47}$ = 693.4, P < 0.001) and thorax ($F_{2,41.80}$ = 343.4 P < 0.001) were smaller under higher environmental stress. The same was the case under genetic stress for eyespan ($F_{1,22.94}$ = 4.783, P = 0.028) but not for thorax ($F_{1,13.78}$ = 3.222, P = 0.095). After controlling for body size variation, the same direction of change was observed in male eyespan for environmental ($F_{2,54.66}$ = 258.1, P < 0.001) and genetic stress ($F_{1,7.421}$ = 6.203, P = 0.039). All following comparisons report relative trait values (i.e. with body size as a covariate).

In addition, there was a genetic quality-by-environment interaction ($F_{2,39.33}$ = 5.379, P = 0.009, Figure 3a). The nature of the G_Q x E was evident from comparison of adjacent environments. The difference in male eyespan between incross flies with low genetic quality and outcross flies with high genetic quality increased from low to high environmental stress (i.e. scale variance G_Q x E, $F_{1,18.35}$ = 6.352, P = 0.021). But there was convergence between genetic quality classes after a further increase from high to extreme environmental stress (i.e. inverse scale variance G_Q x E, $F_{1,15.64}$ = 8.664, P = 0.010). This pattern was confirmed by looking at environments separately. The difference between incross and outcross male eyespan was evident at high ($t_{19.81}$ = 8.65, P < 0.001), but

absent at low ($t_{19.79}$ = 1.98, P = 0.073) and extreme levels of environmental stress ($t_{21.87}$ = -1.01, P = 0.298).

When comparisons were limited to incross lines, there were environmental (χ_1^2 = 276.7, P < 0.001) and genetic line differences (χ_1^2 = 11.08, P < 0.001) but no G_Q x E interaction (χ_1^2 = 4.281, P = 0.509). A similar pattern was found in outcross lines, where there were environmental (χ_1^2 = 243.4, P < 0.001) and genetic line differences (χ_1^2 = 5.14, P = 0.023) but no G_Q x E interaction (χ_1^2 = 7.71, P = 0.173). These results indicate that G_Q x E interactions were only apparent in the comparison of genetic quality (i.e. incross vs. outcross), and not in the comparison of genetic lines within low or high genetic quality groups.

(b) Response in trait variance

The genetic quality G_Q x E pattern was further examined by looking at the among-cross variance in the response to stress. Coefficients of variation (CV) were used to control for the positive scaling in variance due to changes in mean trait size. Male eyespan among-cross CV (Figure 3b) was larger with greater environmental stress overall (R_M = 26.55, P < 0.001), and separately for incross (incross R_M = 40.00, P < 0.001) and outcross lines (R_M = 130.35, P < 0.001). But the extent of increase in CV from low to high environmental stress was considerably more marked among incross males with low genetic quality (1.30% increase, R_M = 28.95, P < 0.001) than outcross males with high genetic quality (0.23% increase, R_M = 11.95, P < 0.001). Differences among outcross lines were revealed to a much greater extent once the level of environmental stress increased even further, in the transition from high to extreme environmental stress (2.63% increase, R_M = 57.34, P < 0.001). This pattern contrasted again with males from incross

lines, where CV did not differ between high and extreme environmental stress levels (0.78% increase, $R_M = 1.848$, $P = 0.174$, Figure 3b). The difference between incross and outcross CV was seen only under high environmental stress (low stress $R_M = 0.814$, $P = 0.367$, high stress $R_M = 24.32$, $P < 0.001$, extreme stress $R_M = 1.148$, $P = 0.284$; Figure 3b), similar to the patterns for mean eyespan.

(c) Across environment genetic line correlations

To further evaluate the role of male eyespan as a signal of genetic quality, we examined the performance of individual genetic lines (G_L) within each genetic quality class (incross and outcross) across environments, for evidence that a line which performed well in one environment performed well across all environments or exhibited a crossover pattern in which different lines performed well in different environments (Figure 4). For low quality incross lines, genetic correlations (r_g) were positive between low and high ($r_g = 0.267$, $\chi_1^2 = 4.184$, $P = 0.041$), as well as between high and extreme stress environments ($r_g = 0.082$, $\chi_1^2 = 11.11$, $P < 0.001$). There was no evidence of G_L x E overall or for any pair of environments for low quality lines (all $P > 0.05$, see SI A.3). For the high quality outcross lines, there was no genetic correlation between low and high stress environments ($\chi_1^2 = 0.221$, $P = 0.469$), but r_g was positive between high and extreme stress environments ($r_g = 0.171$, $\chi_1^2 = 5.189$, $P = 0.023$). The lack of r_g was due to severely reduced variation among outcross lines in the low ($CV_{low} = 0.188$, $CV_{high} = 0.416$, $CV_{extreme} = 3.05$) compared to high ($R_M = 57.34$, $P < 0.001$) or extreme stress environments ($R_M = 88.17$, $P < 0.001$; Figure 4). There was no evidence of G_L x E overall or for any pair of environments for high quality lines (all $P > 0.05$, see SI).

(d) Survival across environmental stress

Larval survival was measured through a census of pupae. There was a survival effect of E ($F_{1,64.36} = 64.36$, $P < 0.001$) but not of G_Q ($F_{1,20.04} = 0.852$, $P = 0.367$) or G_Q x E ($F_{2,64.28} = 0.976$, $P = 0.382$). The effect was a reduction in survival at extreme environmental stress (pupae counts: LSM ± SE low = 2.17 ± 0.10, high = 2.29 ± 0.09, extreme = 1.55 ± 0.09). A Tukey's HSD test confirmed that survival was lower under extreme relative to either low or high environmental stress level ($P < 0.05$). Survival did not differ between incross and outcross in any of these comparisons (all $P > 0.05$, see SI.A).

4. Discussion

In this study we explicitly test whether environmental stress amplifies or obscures the signal of genetic quality in male sexual ornaments. We do so in a novel way by direct manipulation of *both* genetic and environmental quality, the latter over multiple levels. The results enable us to put forward a unified explanation of how genetic and environmental quality interact, advancing our understanding of the genetic benefits of mate choice, with the potential to explain the diverse responses seen in other systems.

The response of male eyespan – the primary sexual ornament in *D. meigenii* – accords with previous studies in stalk-eyed flies, showing that this male ornament is a sensitive signal of both environmental (Cotton et al., 2004a, 2004b; David et al., 1998) and genetic stress (Bellamy et al., 2013; David et al., 2000). Of greater interest, the new data captures a full range of genetic quality-by-environment (G_Q x E) interactions. The difference between low and high genetic quality, in both eyespan mean and variance (coefficient of variation), increases with the transition from low to high environmental

stress (Figure 3). This is an example of "scale variance" gene-by-environment interaction in which higher environmental stress amplifies genetic differences. It has been observed across a range of species, for example in structural wing pigmentation (UV angular visibility) in the butterfly *Colias eurytheme* (Kemp & Rutowski, 2007), male song attractiveness in the lesser waxmoth, *Achroia grisella* (Danielson-Francois et al., 2006), and attractiveness traits in the black scavenger fly, *Sepsis punctum* (Dmitriew & Blanckenhorn, 2014), all examples of traits associated with sexual success. In contrast, the transition from high to extreme environmental stress decreases the difference between our low and high genetic quality classes, in both eyespan mean and variance (Figure 3). This reversed pattern is an example of "inverse scale variance" gene-by-environment interaction in which stress denudes genetic differences. It again has been observed across a range of species, for example, iridescent and orange area in the guppy *Poecilia reticulata* (Evans et al., 2015), cuticular hydrocarbon blend in *Drosophila simulans* (Ingleby et al., 2013), and to a more limited extent, UV brightness in the alfalfa butterfly *C. eurytheme* (Kemp & Rutowski, 2007).

Our results are novel and striking because we see *both* scale variance and inverse scale variance in the same trait in a single species. This leads us to propose a unified hypothesis for gene-by-environment interactions in signals of genetic quality (G_Q x E). Moderate to large increases in environmental stress lead to amplification of the phenotypic expression of genetic quality, whereas as environmental stress becomes extreme, increases in phenotypic variation overwhelm the underlying genetic differences in quality. We note that in some previous studies, separate traits respond differently to environmental stress, suggesting variation in the threshold at which amplification transitions to restriction (e.g. Danielson-Francois et al., 2006; Kemp & Rutowski, 2007).

369 Future studies will be needed to identify which characteristics are associated with
370 sensitivity levels in different traits, and whether these relate to costs of trait expression.
371
372 Yet, some evidence from other studies of sexual ornaments seems to contradict the
373 unified hypothesis. They report no interaction between genetic quality and
374 environmental stress, for example in morphological traits and cuticular hydrocarbons in
375 *D. melanogaster* (Bonduriansky et al., 2015) and several sexual traits in *P. reticulata*
376 guppies (Zajitschek & Brooks, 2010). Both of these experiments examined groups that
377 differ predictably in genetic quality (hemiclonal lines and inbred versus outbred lines,
378 respectively). But the lack of response likely reflects the application of insufficiently
379 intrusive environmental stress. For example, the "stressful" environment in guppies was
380 a moderate density (Zajitschek & Brooks, 2010), while that in *D. melanogaster* was a
381 minor reduction to 70% of the normal diet (Bonduriansky et al., 2015). A previous study
382 in stalk-eyed flies likewise found little impact of food reduction of this order (Cotton et
383 al., 2004a). For comparison, our dilution for extreme stress was a restriction to just 5% of
384 the standard diet. Moreover, as each of these studies used just two levels of
385 environmental stress, analysis of complex genetic quality-by-environment interactions
386 was precluded. This is not a criticism of either study, which had different goals to ours,
387 but highlights that neither would provide an adequate test of our hypothesis.
388
389 Another commonly reported pattern across diverse species is "crossover" G x E in which
390 different genotypes are superior in different environments. For example, this has been
391 reported for male signal rate in the lesser waxmoth (Jia et al., 2000) and song traits of
392 *Enchenopia* treehoppers (Rodríguez & Al-Wathiqui, 2011). We found no evidence of
393 crossover in the genetic quality classes (Figure 3). This is perhaps unsurprising as our

394 experimental design deliberately created distinct classes (incross and outcross), which
395 differ in quality (highly homozygous vs. highly heterozygous). We did not expect to
396 observe performance reversal of genetic quality classes in different environments. But we
397 also did not find evidence for crossover of the multiple genetic lines within each quality
398 classes. Genetic lines showed strong evidence of positive correlation of performance
399 across environments (Figure 4). The lack of crossover probably reflects our
400 environmental treatment which simply reduces the amount of food, and like the genetic
401 quality classes, generates a strong association of treatment categories with quality. Other
402 types of environmental variation which have weaker associations with quality, for
403 example the composition of the diet, may produce less consistent genetic responses. In
404 addition, it is worth noting that "crossover" G x E is not really a distinct category, and
405 can co-occur with "scale" or "inverse scale" G x E patterns (Ingleby et al., 2010). For
406 instance, crossover has been observed embedded within scale variance in the lesser
407 waxmoth (Danielson-Francois et al., 2006) and within inverse scale variance in the
408 guppy (Evans et al., 2015). Where crossover features in the G x E pattern, we suspect it
409 reflects a lack of strong quality differences in either or both genetic and environmental
410 parameters, allowing different genotypes to be specialised in their performance in
411 different environments.
412
413 The experiments here emphasize the importance of examining a range of environments
414 from low through to an extreme form of stress, alongside similar dimensions of genetic
415 quality variation. We created distinct classes of environmental quality variation by
416 applying food restriction to developing larvae. Our approach differs from previous
417 studies (Bonduriansky et al., 2015; Burger et al., 2007; Cotton et al., 2004a; David et al.,
418 2000; Dmitriew & Blanckenhorn, 2014; Hingle et al., 2001a) by including food dilution

419 taken to an "extreme", identified in a pilot experiment as the point before larval survival
420 showed a clear-cut decline (Figure 2). The reason for choosing this point was in part
421 logistical, in order to easily collect similar sample sizes across the different stress levels.
422 We also wanted to avoid the possibility that differential survival causes changes in trait
423 mean and variation across the different genetic quality and environmental stresses.
424 Despite this precaution, there was a moderate effect of the extreme environmental stress
425 on larval survival in our study. We suspect that this effect was of minor importance
426 because the survival deficit was equal for incross and outcross flies while for survivors
427 the mean eyespan was lowest and the CV of eyespan highest in the extreme environment
428 (Fig. 3). Hence our conclusions appear to be robust. We used food quantity as an
429 environmental stress because of its ease of manipulation and its use in many previous
430 studies. Competition for food is likely to be a factor in many species and so we suspect
431 that the results we report here are general stress responses. This needs to be established
432 through comparison with other stresses, such as fluctuations in temperature, pH or food
433 quality, that are part of the normal range of environmental stress in the wild (Hoffmann
434 & Parsons, 1993).
435
436 In the pilot experiment (Fig. 2), as well as the main experiment, there was no difference
437 in egg-to-adult survival between flies in the incross and outcross genetic quality
438 treatments. The lack of a viability difference suggests that there was a strong purging of
439 deleterious alleles during the creation of the inbred lines, as expected and observed in
440 other studies (Crnokrak & Barrett, 2002; Crow & Kimura, 1970; Garcia-Dorado, 2012).
441 Our objective was not to study inbreeding *per se*, as this is unlikely to be the object of
442 female mate preference in this species.
443

444 We used inbreeding status as an investigative tool, in order to uncover the nature of
445 genetic quality-by-environment interactions on variation in signal trait size. We suspect
446 that many previous G x E studies have failed to use a sufficient range of variation in
447 genetic quality. A typical approach is to use distinct genetic lines, like brother-sister
448 families (David et al., 2000; Rodríguez & Al-Wathiqui, 2011) or inbred lines (Danielson-
449 Francois et al., 2006). Independent lines provide information about genetic variation but
450 may differ only slightly, and unpredictably, in genetic quality, and then only with
451 differences established *post hoc*. In our study, we distinguish between variation in *genetic*
452 *quality* in the comparison of incross and outcross flies (G_Q), and genetic variation *between*
453 *lines* within these quality categories (G_L). In accordance with a prior study of stalk-eyed
454 flies (David et al., 2000), our results show differences in performance between lines (G_L).
455 Crucially, there was no genetic line-by-environment interaction (G_L x E) once analysis
456 was limited to a particular genetic quality class (either for incross or outcross). The set of
457 lines in each genetic quality class appear to be sufficiently similar in quality that they
458 respond in an equivalent manner when challenged with our wide range of environmental
459 stress levels (Figure 4). Only the comparison between incross and outcross flies revealed
460 a strong gene-by-environment interaction.
461
462 Taking the results together allows us to comment on sexual selection on males and the
463 potential indirect genetic benefits that arise from female mate choice. We expect sexual
464 selection to be severely attenuated under benign and extreme environmental stress, but
465 strong in high stress environments which amplify genetic quality differences. If some
466 degree of stress is typical of ecological conditions in nature, sexual selection could often
467 be stronger than currently estimated from laboratory experiments – usually carried out
468 under low stress conditions of *ad libitum* food, constant temperature, and without

469 predators, parasites, or ecological competitors. We note that our experimentation used
470 stress from a unimodal environment variable (food availability), controlling all other
471 physical and biotic factors, and that we used a simple measure of male signalling, leaving
472 aside other, more subtle aspects of male behaviour used in female evaluation of their
473 partners (Chapman et al., 2017). Thus the majority of environments probably lie
474 between the low and high stress regimes, which is consistent with the considerable range
475 in eyespan observed among wild caught stalk-eyed flies (Cotton et al., 2010). Benign
476 environmental conditions, equivalent to low stress in our experiment (in which larvae
477 have excess food and little competition), are unlikely to be common in nature. Stressful
478 environmental conditions, like those studied here (i.e. our high and extreme regimes),
479 may well be more common. Our analysis shows that sexual selection will be largely
480 ineffective at distinguishing genetic quality when the extreme environment imposes a
481 large randomisation. Even harsher environments that additionally impose severe effects
482 on survival are likely to have even less of an effect because they impose demographic
483 limits on the maintenance of a viable population (e.g. at a range margin, Bridle & Vines,
484 2007), and this is likely to be more important in restricting the effect of sexual selection.
485 Further investigation is needed to place these deductions on a sounder footing, both
486 using laboratory experiments and observations from natural settings.
487
488 The outcome in nature for female choice will depend on the distribution of
489 environmental stress, its spatial and temporal variability, and hence its consequence for
490 the pool of available mates in a given population (Greenfield & Rodriguez, 2004). If
491 conditions can be categorised as low, high or extreme, then the indirect benefits of mate
492 choice will be greatest in high stress environments, as these bring out genetic differences
493 to the greatest extent. As genetic line correlations across environments were positive

494 (with the exception of outcross lines between low and high stress, where a lack of
495 variation precluded reliable calculation), genetic differences will be evident to some
496 extent in all environments. Where environmental conditions in a population are a
497 mixture of low, high and extreme, individuals with the most exaggerated sexual
498 ornaments will be an assortment of those with high genetic quality from a range of
499 environments diluted by those less well genetically endowed but who experienced lower
500 environmental stress during development. This will reduce but not abolish the indirect
501 genetic benefits that accrue from female mate choice. To conclude, while environmental
502 variation places contingencies on signalling, sometimes amplifying and sometimes
503 muting its value, genetic variation in quality between individuals will always to some
504 extent be evident in the sexual ornament and be transmitted to their offspring.
505
506

Data accessibility. Data are made available at the Dryad Digital Repository doi:10.5061/dryad.6p150kf

Author contributions. JMH, KF and AP conceptualised the study and methodology, and wrote, reviewed and edited the paper. The formal analysis was carried out by JMH, who with HACD carried out the experiments. Stalk-eyed fly resources were provided by AP and KF, who secured funding and supervised the project.

Competing interests. The authors declare no competing interests.

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714
715

716 **FIGURE LEGENDS**

717

718 **Figure 1**. The crossing protocol used to generate incross and outcross offspring. Each
719 inbred line was crossed with itself to create incross flies (red), or with another inbred line
720 to create outcross flies (blue). Each cross used 4 males and 4 females and was repeated to
721 generate two families per cross.

722

723 **Figure 2.** Mean number of adult flies eclosing per petri dish (± SE) given seeding with
724 five eggs, when subject to different larval treatments (ratio of corn:water), for inbred lines
725 (red) and stock (blue), or when pooled (black). Pairwise comparison of adjacent pooled
726 treatments showed a significant drop in survival between the adjacent 1:20 and 1:25
727 treatments (solid line, *** $P < 0.001$), and no difference between other adjacent levels
728 (dashed line, NS). A similar pattern was observed for inbred and stock considered
729 separately across the adjacent 1:20 and 1:25 treatments (both $P < 0.001$). Inbred and
730 stock populations did not differ at any food level (all $P > 0.05$). Data is based on a pilot
731 experiment (17 crosses, 10 stock, 7 inbred, $N = 218$ stock, 68 inbred; details SI.C).

732

733 **Figure 3**. a) Male eyespan (least-squares mean ± SE, GLMM after control of thorax
734 size) and b) coefficient of variation (CV ± 95% CI) across genetic quality (incross (red)
735 and outcross (blue)) and environmental stress (low, high and extreme). The red and blue
736 lines are shown for illustrative purposes and clarity. Asterisks denote significance: NS
737 non-significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. For CVs, the significance of
738 incross versus outcross contrasts are displayed above each food level category (black
739 asterisk at the top). The significance of within incross (red asterisks) and outcross (blue

asterisks) contrasts are shown between pairs of adjacent food levels. Incross and outcross lines are jittered (x-axis) for clarity.

Figure 4. Genetic line (G_L) mean male eyespan (least-squares mean, GLMM after control of thorax) at each environmental stress for each genetic quality class a) incross lines (red) and b) outcross lines (blue). Asterisks denote significance of the effect of cross, NS non-significant, * $P < 0.05$, *** $P < 0.001$. An alternative representation is shown as the absolute deviation of each line from the c) incross and d) outcross population mean. Error bars are excluded for clarity.

SUPPLEMENTAL INFORMATION

Supplemental information includes all details of statistical effect size estimates for the tests of mean effects, and additional method details.

Figure 1

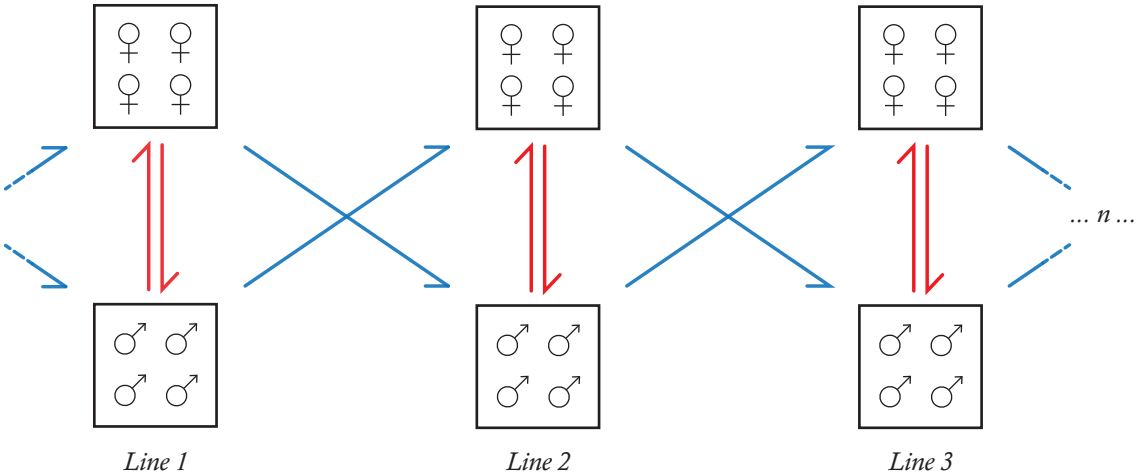


Figure 2

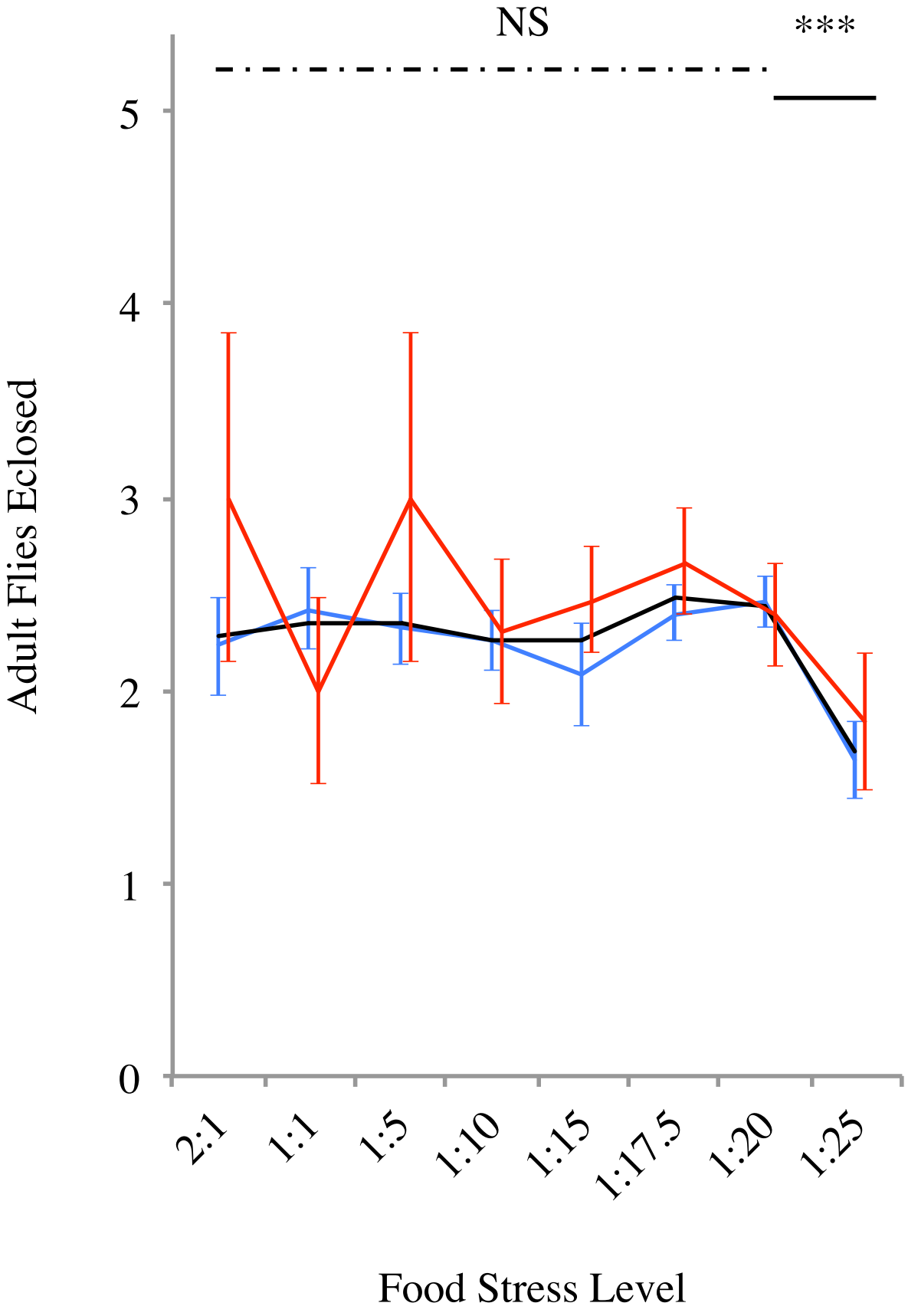

NS
***
5
4
3
2
1
0
Adult Flies Eclosed
2:1
1:1
1:5
1:10
1:15
1:17.5
1:20
1:25
Food Stress Level

Figure 3

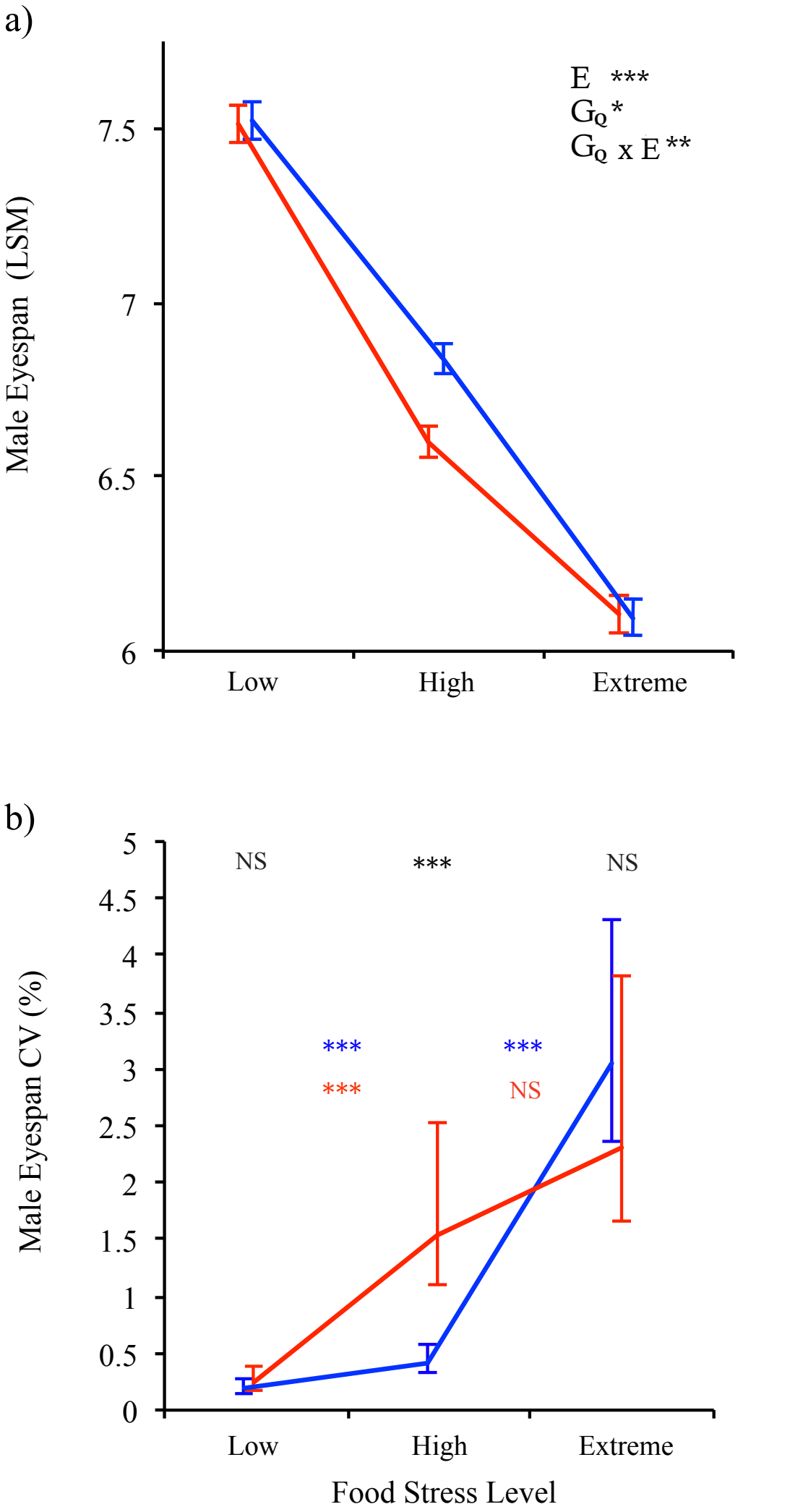
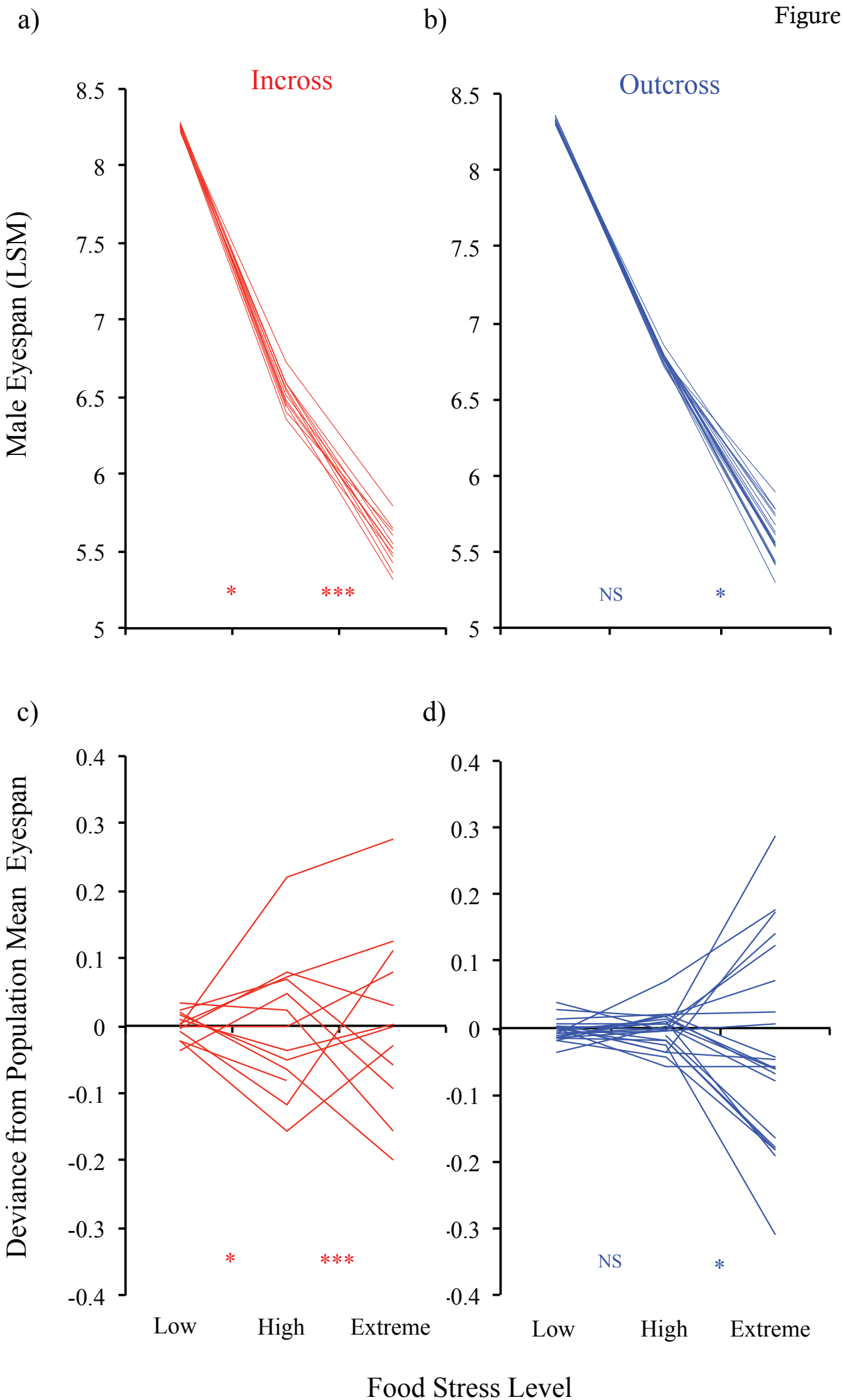

a)
E ***
G_Q *
G_Q x E **
Male Eyespan (LSM)
7.5
7
6.5
6
Low
High
Extreme
b)
NS
***
NS
***
***
***
NS
Male Eyespan CV (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Low
High
Extreme
Food Stress Level

Figure 4


a)
b)
Incross
Outcross
Male Eyespan (LSM)
8.5
8
7.5
7
6.5
6
5.5
5
*
***
NS
*
c)
d)
Deviance from Population Mean Eyespan
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
*
***
NS
*
Low
High
Extreme
Low
High
Extreme
Food Stress Level