

1 **Limits to environmental masking of genetic quality in sexual signals**

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20

21 **Abstract**

22

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

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## 42 **1. Introduction**

43

44 Many exaggerated male sexual ornaments are thought to have evolved to provide  
45 information about the genetic quality of the signaller [1,2,3,4,5,6,7]. Yet these traits  
46 typically also respond strongly to environmental variation [8,9,10,11], and it is unclear  
47 what impact this has on their signalling function [12,13]. Does increasing environmental  
48 stress expose the underlying genetic differences in quality or mask them by  
49 overwhelming the genetic signal? Different studies have variously reported that  
50 environmental variation enhances [1,2,14,15] or diminishes [16,17,18] the phenotypic  
51 signal of genetic quality. Others reveal crossover, where genotypes that perform well in  
52 one environment do poorly in another [17,19,20,21]. These contrasting outcomes arise  
53 from a lack of consistency in experimental approach. The main problem is that analysis  
54 has focussed on genetic variation rather than distinct classes of genetic quality, coupled  
55 to a limited rather than wide range of environmental stress. We present a novel  
56 experimental design that addresses both of these deficits, which leads us to propose a  
57 unified understanding of how variation in genetic quality is impacted by environmental  
58 variation. This gives a far clearer understanding of the conditions under which sexual  
59 display traits can function to accurately reveal the genetic quality of signallers [22].

60

61 In this study, we adopt an integrated experimental approach, and for the first time  
62 examine the impact of a similar wide range in both genetic and environmental quality.  
63 We chose to focus specifically on male eyespan variation in the stalk-eyed fly [23,24] as  
64 this trait has been subject to extensive previous work. Male eyespan is highly  
65 exaggerated due to female choice [11,25,26,27,28,29,30,31,32] and also functions as a  
66 signal in male-male antagonistic interactions over mate mating sites [25,33,34]. It is

67 highly condition-dependent relative to other traits in relation to both genetic [24, but see  
68 36] and environmental [9,11,35] stressors, and is responsive to a range of environmental  
69 stress types [9,11,24,35,37,38], while genetically distinct families have also been shown  
70 to respond differently to environmental stress [1].

71

72 Our novel experimental design to study genetic quality-by-environment (G x E)  
73 interactions in signalling traits exploits pre-defined genetic [39,40,41] and environmental  
74 quality classes. To vary genetic quality, crosses were made within or between a set of  
75 parental inbred lines ( $f \sim 0.908$  [42]; Figure 1). This allowed us to compare low genetic  
76 quality, highly homozygous “incross” lines ( $n = 16$ , crosses = 67) with high genetic  
77 quality, highly heterozygous “outcross” lines ( $n = 17$ , crosses = 50). We used incross and  
78 outcross lines not to study the effect of inbreeding *per se*, but because previous work  
79 unambiguously shows they correspond to low and high genetic quality classes  
80 respectively [24]. The large number of independent crosses within or between lines  
81 allows us to capture the contribution of genetic variation in the sexual ornament among  
82 low and high genetic quality classes.

83

84 We likewise generated a wide range of environmental quality variation through  
85 reductions in the amount of food available to developing larvae. This approach is a well-  
86 established method for creating stress in holometabolous insects  
87 [1,11,15,26,30,35,37,40,43,44,45] and has been used extensively in prior stalk-eyed fly  
88 studies [9,11,24,30], where it generates body size variation equivalent to the range found  
89 in natural populations [32,35]. Eggs were collected from each cross, and reared under  
90 conditions of low, high and extreme environmental stress. Intermediate levels of stress  
91 between low and high were omitted due logistical constraints (the study analysed 1185

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94 # Figure 1.

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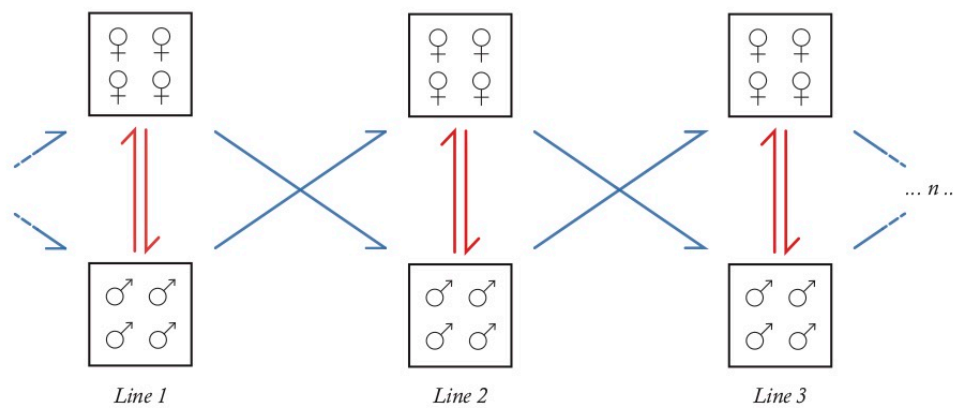
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**Fig. 1.** The crossing protocol used to generate incross and outcross offspring. Each inbred line was crossed with itself to create incross flies (red), or with another inbred line to create outcross flies (blue). Each cross used 4 males and 4 females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

117 flies from 117 experimental crosses), and because those stress levels have been  
118 extensively investigated previously [11]. The extreme level was defined as the least food  
119 level where larval viability was not seriously impaired (see below)

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121 Our use of the terminology low/high genetic quality and low/high/extreme  
122 environmental quality is necessarily arbitrary but justified in terms of the experimental  
123 design and in the results that follow. We comment further on these definitions in the  
124 Discussion. The innovation in our experimental design lies in delivering controlled  
125 manipulation of genetic quality *and* environmental stress, over several pre-defined  
126 quality levels, thereby enabling an in depth exploration of genetic quality-by-  
127 environment (G x E) variation in a male sexual ornament. We use this to investigate the  
128 signalling utility of the male ornament in providing information about indirect genetic  
129 benefits through female mate choice.

130

## 131 **2. Material and methods**

132

### 133 **(a) Production of experimental flies**

134 A suite of 105 inbred lines were founded from a stock of *Diasemopsis meigenii* [24], an  
135 African stalk-eyed fly, derived from flies collected in South Africa by S. Hilger in 2000.  
136 After 11 generations of full-sib mating, the extant lines were bred in cage culture (~200  
137 flies/cage). For this study, 17 lines that varied between generations F<sub>24</sub>–F<sub>33</sub> were chosen  
138 as parental lines to use in crosses, with flies collected as eggs laid on petri dishes  
139 containing excess puréed sweet corn (10g). At eclosion, flies were placed in large cages  
140 (15l), separated by sex (~2 weeks) and raised until sexual maturity (~10 weeks). Adults  
141 were fed puréed sweet corn with antifungal Nipagin, replenished twice per week. All

142 flies were maintained using our standard protocol at 25°C on a 12:12 hour light:dark  
143 cycle, with fifteen-minute artificial “dawn” and “dusk” periods (reduced illumination) at  
144 the start and end of the light phase throughout the experiment.

145

#### 146 **(b) Variation in genetic and environmental effects**

147 Variation in genetic quality was achieved using previously created inbred lines [24] in a  
148 crossing protocol (Figure 1; modified from [36] and [24]). “Incross” flies were created  
149 from male-female crosses within an inbred line and “outcross” flies were created from  
150 crosses between different inbred lines. In each cross, 4 adult males from line  $x$  and 4  
151 adult females from line  $y$  were allowed to mate in a 1.5l pot ( $x = y$  for incross,  $x \neq y$  for  
152 outcross). Reciprocal male  $x$  - female  $y$  and female  $x$  -male  $y$  pots were set up. Multiple  
153 replicates of each cross (between 1-8) were set up, with higher rates for inbred crosses as  
154 they were less fecund. Eggs were collected twice weekly over 23 days. In all, 142 crosses  
155 were set up, of which 117 generated sufficient offspring across the food treatments: 67  
156 incrosses of 15 inbred lines and 50 outcrosses between 16 pairs of inbred lines. An inbred  
157 line was used in an incross the same number of times as it was used in an outcross, and  
158 as far as possible equal numbers of live adult males and females were collected from each  
159 line, to balance sex chromosomal, cytoplasmic and other male/female parental effects.

160

161 Incross flies have low genetic quality as they are highly homozygous, being derived from  
162 inbred lines created by repeated brother-sister pairings (11 generations), with an expected  
163 inbreeding coefficient of  $f \sim 0.908$  [24]. In contrast, outcross flies have high genetic  
164 quality as they are expected to be heterozygous for most of the alleles fixed in the  
165 parental inbred lines from which they are derived. Although the terms – low and high  
166 genetic quality – are arbitrary, there was evidence of substantial heterosis in a variety of

167 traits when inbred flies were crossed, so the terms reflect the nature of these two genetic  
168 groups [24].

169

170 For each incross or outcross, fertilised eggs were placed in groups of 5 in petri dishes  
171 containing two cotton pads, 15ml water and 5ml of food medium. Three qualities of  
172 food medium were used with “pure” corn diluted with water at ratios of 1:1, 1:10, and  
173 1:20, which we designate as “low”, “high” and “extreme” stress respectively. “Pure”  
174 corn was made by forcing puréed sweet corn kernels through a fine sieve to remove  
175 husks and provide homogeneity. Food qualities were chosen based on a pilot study, with  
176 levels of food stress used that were found to lie within normal rates of egg-adult survival  
177 (Figure 2, see SI.C). Although the terms – low, high and extreme environmental stress –  
178 are again arbitrary, they nonetheless capture particular qualities. The low food level was  
179 similar to the standard media on which larvae are raised. The high food level was used  
180 previously where it was associated with reduced size in a variety of traits [24]. The  
181 extreme food level constitutes the far end of the stress spectrum before differential  
182 survival is evident (Figure 2). In the range used (i.e. 1:1 to 1:20), egg-to-adult survival did  
183 not differ in the pilot, and was at ~50%. We did not go beyond this level, as a serious  
184 loss of adults would have placed greater logistical difficulties in delivering the already  
185 considerable sample size in the experiment. In the main experiment, a census of pupae  
186 was additionally made as a measure of survival for each cross in each environment.

187

### 188 **(c) Adult morphology**

189 After eclosion, flies of each cross were collected and frozen at -20°C. All males were  
190 measured for eyespan (the distance between the outermost tips of the eyes [9,46]) and  
191 thorax (the distance between the centre of the most posterior point of the head to the joint



192 # Figure 2.

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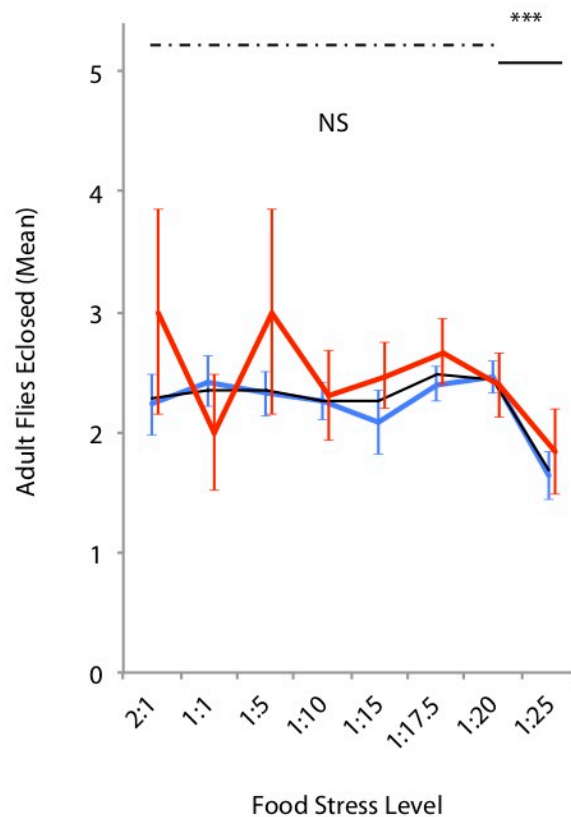
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207 **Fig. 2.** Mean number of adult flies eclosing per petri dish ( $\pm$  SE) given seeding with five

208 eggs, when subject to different larval treatments (ratio of corn:water), for inbred lines

209 (red) and stock (blue), or when pooled (black). Pairwise comparison of adjacent

210 treatments showed a significant drop in survival between the adjacent 1:20 and 1:25

211 treatments (solid line, \*\*\*  $P < 0.001$ ), and no difference between other adjacent levels

212 (dashed line, NS). A similar pattern was observed for inbred and stock considered

213 separately across the adjacent 1:20 and 1:25 treatments (both  $P < 0.001$ ). Inbred and

214 stock populations did not differ at any food level (all  $P > 0.05$ ). Data is based on a pilot

215 experiment (17 crosses, 10 stock, 7 inbred,  $N = 218$  stock, 68 inbred; details SI.C). #

216

217 between the meta-thoracic legs and the thorax [47,48]) to a tolerance of 0.01mm, using a  
218 video camera mounted on a monocular microscope and ImageJ image capture software  
219 v.1.46 [49]. The repeatability of these morphological trait measurements is very high at  
220 >99% [9]. In total 1186 males were phenotyped. All measurements were made blind by  
221 JMH. In a few cases ( $n = 9$ ), a measurement was not included in the dataset due to sample  
222 damage.

223

#### 224 **(d) Statistical analysis**

225 To test for effects of incross/outcross genetic quality (G), environmental (E) and the G x  
226 E interaction on morphological trait variation, several general linear mixed effects  
227 models (GLMMs) were fitted via REML. In each model, G, E and their interaction were  
228 included as fixed effects. Male parental line and female parental line were included as  
229 random effects, as was cross and its interaction with E. Additional random effects of  
230 male line x E, male line x G, female line x E and female line x G explained zero variance  
231 and so were removed in model simplification. GLMMs for male eyespan had thorax  
232 added as a covariate to control for body size. Thorax length accounted for a significant  
233 portion of variance, but its addition did not substantially alter the results (for  
234 completeness, analyses of absolute trait values are given in the SI.A). GLMM models  
235 fitted pairwise to low versus high and high versus extreme environmental stress were  
236 used to further investigate the basis of the observed G x E patterns, as finally were two-  
237 tailed *t*-tests at each level of E to test whether incross male eyespan was larger or smaller  
238 than outcross male eyespan.

239

240 Coefficients of variation (CVs), the ratio of the standard deviation to the mean, were  
241 used to assess how variance in male eyespan responded to genetic quality,

242 environmental and G x E stress. CVs control for changes in variance purely as a function  
243 of size, and are considered to be less biased than heritability estimates in G x E studies  
244 [50]. Least square means for male relative eyespan were extracted from GLMMs for  
245 each cross, for each E and G, to calculate among-cross CVs. Among-cross CVs were  
246 then compared between incross and outcross using modified signed-likelihood ratio tests  
247 (M-SLRT; [51]) in each environment, and also across environments (L-H-X), both  
248 overall and for incross and outcross. Finally, adjacent environment pairs were contrasted  
249 for among-cross CV, low with high (L-H) and high with extreme (H-X), for each genetic  
250 quality. The among-cross contrasts were conducted in the R-package 'cvequality' [52].

251

252 To explore the consistency of genetic lines across environments, another key aspect of G  
253 x E interactions, genetic correlations ( $r_g$ ) across adjacent environments were calculated.  
254 GLMMs were fitted with cross as a random effect and the variance component for cross  
255 was extracted for each environment. GLMMs were then carried out between pairs of  
256 adjacent environments (L-H, H-X), with the cross x E interaction included as a random  
257 effect, and the interaction variance component extracted. As before, thorax length was  
258 added as a fixed covariate to control for body size. An estimate of  $r_g$  was then calculated  
259 as:

260

$$261 \quad r_g = \sigma_{1,2} / \sqrt{\sigma_{1,1}^2 \sigma_{2,2}^2},$$

262

263 where  $\sigma_{1,1}^2$  and  $\sigma_{2,2}^2$  are the genetic variances in environments 1 and 2 respectively, and  
264  $\sigma_{1,2}$  is the genetic covariance between the two environments [53]. Broad bounds of the  $r_g$   
265 values were tested via model simplification and likelihood ratio tests (details in SI.A)

266

## 267 (e) Statistical software used

268 All statistical analyses were conducted in JMP v.12.0.1 (SAS Institute 1989-2015) and R  
269 v.3.4.2 [54]. GLMM tables, effect coefficients and extended methods are shown in SI.A.

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## 271 3. Results

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### 273 (a) Response in mean trait

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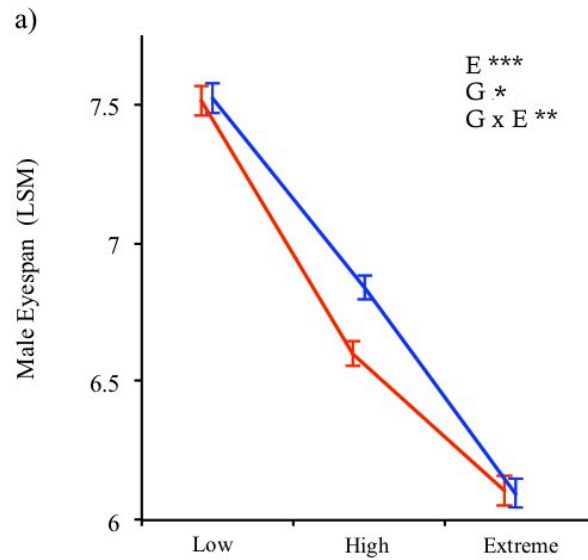
275 As expected, male eyespan ( $F_{2,45.47} = 693.4, P < 0.001$ ) and thorax ( $F_{2,41.80} = 343.4 P <$   
276  $0.001$ ) were smaller under higher environmental stress. The same was the case under  
277 genetic stress for eyespan ( $F_{1,22.94} = 4.783, P = 0.028$ ) but not for thorax, though its  
278 response was in the same direction ( $F_{1,13.78} = 3.222, P = 0.095$ ). After controlling for body  
279 size variation, the same direction of change was observed in male eyespan for  
280 environmental ( $F_{2,54.66} = 258.1, P < 0.001$ ) and genetic stress ( $F_{1,7.421} = 6.203, P = 0.039$ ).  
281 All following comparisons report relative trait values.

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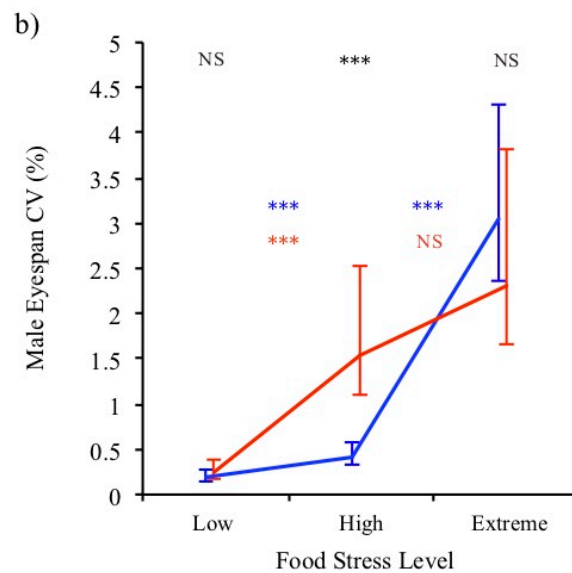
283 In addition, there was a genetic quality-by-environment interaction ( $F_{2,39.33} = 5.379, P =$   
284  $0.009$ , Figure 3a). The nature of the G x E was evident from comparison of adjacent  
285 environments. The difference in male eyespan between incross flies with low genetic  
286 quality and outcross flies with high genetic quality increased from low to high  
287 environmental stress (i.e. scale variance G x E,  $F_{1,18.35} = 6.352, P = 0.021$ ). But there was  
288 convergence between genetic quality classes after a further increase from high to extreme  
289 environmental stress (i.e. inverse scale variance G x E,  $F_{1,15.64} = 8.664, P = 0.010$ ). This  
290 pattern was confirmed by looking at environments separately. The difference between  
291 incross and outcross male eyespan was evident at high ( $t = 8.65, df = 19.81, P < 0.001$ ),

292 # **Figure 3.**

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309 **Fig. 3.** a) Male eyespan (least-squares mean  $\pm$  SE) and b) coefficient of variation (CV  $\pm$   
310 95% CI) across environmental stress (low, high and extreme) and genetic class, incross  
311 (red) and outcross (blue). The red and blue lines are shown for illustrative purposes and  
312 clarity. Asterisks denote significance: NS non-significant, \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P <$   
313 0.001. For CVs, the significance of incross versus outcross contrasts are displayed above  
314 each food level category (black asterisk at the top). The significance of within incross  
315 (red asterisks) and outcross (blue asterisks) contrasts are shown between pairs of adjacent  
316 food levels. Incross and outcross lines are jittered for clarity. #

317 but absent at low ( $t = 1.98$ ,  $df = 19.79$ ,  $P = 0.073$ ) and extreme levels of environmental  
318 stress ( $t = -1.01$ ,  $df = 21.87$ ,  $P = 0.298$ ).

319

320 When comparisons were limited to incross lines, there were environmental ( $\chi_1^2 = 276.7$ ,  
321  $P < 0.001$ ) and genetic line differences ( $\chi_1^2 = 11.08$ ,  $P < 0.001$ ) but no G x E interaction  
322 ( $\chi_1^2 = 4.281$ ,  $P = 0.509$ ). A similar pattern was found in outcross lines, where there were  
323 environmental ( $\chi_1^2 = 243.4$ ,  $P < 0.001$ ) and genetic line differences ( $\chi_1^2 = 5.14$ ,  $P = 0.023$ )  
324 but no G x E interaction ( $\chi_1^2 = 7.71$ ,  $P = 0.173$ ). These results indicate that G x E  
325 interactions were only apparent in the comparison of genetic quality (i.e. incross vs.  
326 outcross), and not in the comparison of genetic lines within low or high genetic quality  
327 groups.

328

### 329 **(b) Response in trait variance**

330

331 The genetic quality G x E pattern was further examined by looking at the among-cross  
332 variance in the response to stress. Coefficients of variation (CV) were used to control for  
333 the positive scaling in variance due to changes in mean trait size. Male eyespan among-  
334 cross CV (Figure 3b) was larger with greater environmental stress overall ( $R_M = 26.55$ ,  $P$   
335  $< 0.001$ ), and separately for incross (incross  $R_M = 40.00$ ,  $p < 0.001$ ) and outcross lines  
336 ( $R_M = 130.35$ ,  $P < 0.001$ ). But the extent of increase in CV from low to high  
337 environmental stress was considerably more marked among incross males with low  
338 genetic quality (1.30% increase,  $R_M = 28.95$ ,  $P < 0.001$ ) than outcross males with high  
339 genetic quality (0.23% increase,  $R_M = 11.95$ ,  $P < 0.001$ ). Differences among outcross lines  
340 were revealed to a much greater extent once the level of environmental stress increased  
341 even further, in the transition from high to extreme environmental stress (2.63%

342 increase,  $R_M = 57.34$ ,  $P < 0.001$ ). This pattern contrasted again with males from incross  
343 lines, where CV did not differ between high and extreme environmental stress levels  
344 (0.78% increase,  $R_M = 1.848$ ,  $P = 0.174$ , Figure 3b). As for mean eyespan, the difference  
345 between incross and outcross CV was seen only under high environmental stress (low  
346 stress  $R_M = 0.814$ ,  $P = 0.367$ , high stress  $R_M = 24.32$ ,  $P < 0.001$ , extreme stress  $R_M =$   
347 1.148,  $P = 0.284$ ; Figure 3b).

348

### 349 (c) Across environment genetic correlations

350

351 To further evaluate the role of male eyespan as a signal of genetic quality, we examined  
352 whether genetic lines performing well in one environment performed well across all  
353 environments (Figure 4), a critical part of the G x E pattern. When pooling all lines,  
354 there was a positive genetic correlation ( $r_g$ ) between low and high ( $r_g = 0.563$ ,  $\chi_1^2 = 11.54$ ,  
355  $P < 0.001$ ), and high and extreme environmental stress ( $r_g = 0.360$ ,  $\chi_1^2 = 22.94$ ,  $P <$   
356 0.001). There was also a genetic correlation-by-environment interaction between low and  
357 high stress ( $\chi_1^2 = 15.27$ ,  $P < 0.001$ ) in which the genetic lines fanned out under higher  
358 environmental stress.

359

360 Analysing the two genetic quality classes separately, for low quality incross lines, genetic  
361 correlations ( $r_g$ ) were positive between low and high ( $r_g = 0.267$ ,  $\chi_1^2 = 4.184$ ,  $P = 0.041$ ),  
362 as well as between high and extreme stress environments ( $r_g = 0.082$ ,  $\chi_1^2 = 11.11$ ,  $P <$   
363 0.001). For the high quality outcross lines, there was no genetic correlation between low  
364 and high stress environments ( $\chi_1^2 = 0.221$ ,  $P = 0.469$ ), but  $r_g$  was positive between high  
365 and extreme stress environments ( $r_g = 0.171$ ,  $\chi_1^2 = 5.189$ ,  $P = 0.023$ ). The lack of  $r_g$  was  
366 due to severely reduced variation among outcross lines in the low ( $CV_{\text{low}} = 0.188$ ,  $CV_{\text{high}}$

367 # Figure 4.

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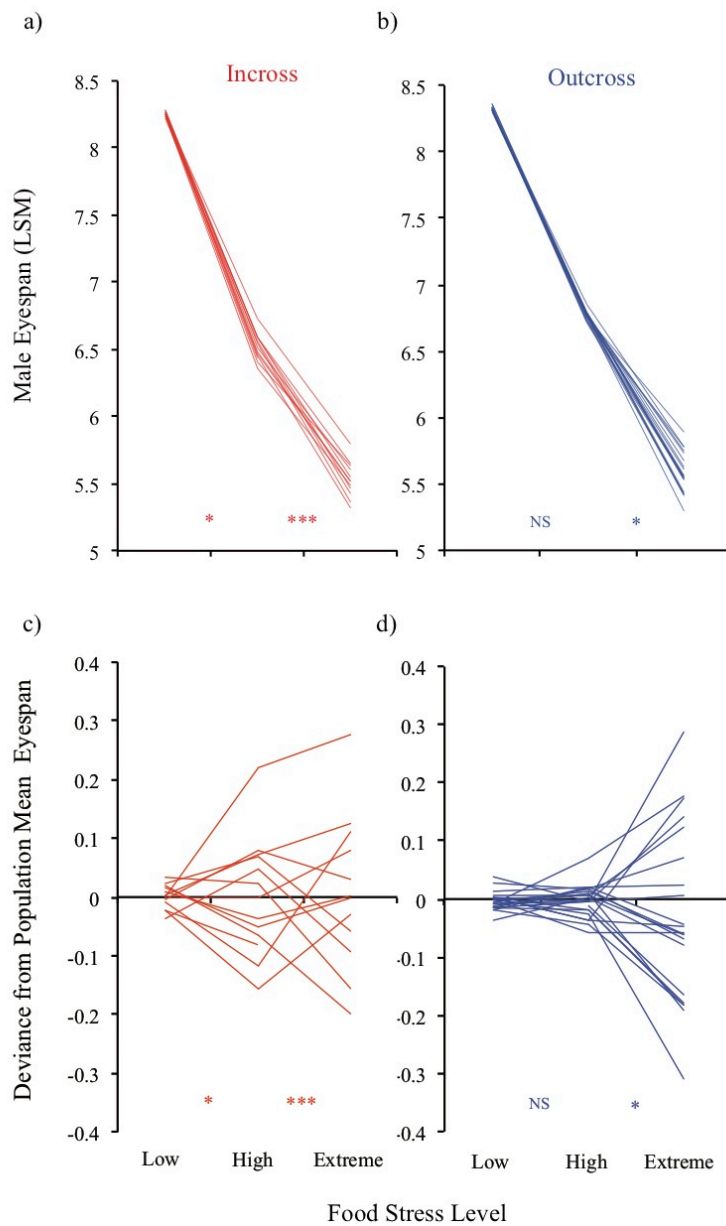
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386 **Fig. 4.** Mean male eyespan (least-squares mean relative values) at each environmental

387 stress for each cross a) incross (red) and b) outcross lines (blue). Asterisks denote

388 significance of the effect of cross, NS non-significant, \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

389 An alternative representation is shown as the absolute deviation of each line from the c)

390 incross and d) outcross population mean. Error bars are excluded for clarity. #

391



392 = 0.416,  $CV_{\text{extreme}} = 3.05$ ) compared to high ( $R_M = 57.34$ ,  $P < 0.001$ ) or extreme stress  
393 environments ( $R_M = 88.17$ ,  $P < 0.001$ ; Figure 4).

394

#### 395 **(d) Survival across G and E stress**

396 Larval survival was measured through a census of pupae. There was a survival effect of  
397 E ( $F_{1,64.36} = 64.36$ ,  $P < 0.001$ ) but not of G ( $F_{1,20.04} = 0.852$ ,  $P = 0.367$ ) or G x E ( $F_{2,64.28} =$   
398  $0.976$ ,  $P = 0.382$ ). The effect was a reduction in survival at extreme environmental stress  
399 (pupae counts:  $LSM \pm SE$  low =  $2.17 \pm 0.10$ , high =  $2.29 \pm 0.09$ , extreme =  $1.55 \pm 0.09$ ).  
400 A Tukey's HSD test confirmed that survival was lower under extreme relative to either  
401 low or high environmental stress level ( $P > 0.05$ ). Survival did not differ between incross  
402 and outcross in any of these comparisons (all  $P > 0.05$ , see SI.A).

403

404

## 405 **4. Discussion**

406

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

413

414 The response of male eyespan – the primary sexual ornament in *D. meigenii* – accords  
415 with previous studies in stalk-eyed flies, showing that this male ornament is a sensitive  
416 signal of both environmental [9,11,35] and genetic stress [1,24]. Of greater interest, the

417 new data captures a full range of G x E interactions. The difference between low and  
418 high genetic quality, in both eyespan mean and variance (coefficient of variation),  
419 increases with the transition from low to high environmental food stress (Figure 3). This  
420 is an example of “scale variance” G x E in which higher environmental stress amplifies  
421 genetic differences. It has been observed across a range of species, for example in  
422 structural wing pigmentation (UV angular visibility) in the butterfly *Colias eurytheme* [55],  
423 male song attractiveness in the lesser waxmoth, *Achroia grisella* [2], and attractiveness  
424 traits in the black scavenger fly, *Sepsis punctum* [15], all examples of traits associated with  
425 sexual success. In contrast, the difference between our low and high genetic quality  
426 classes, in both eyespan mean and variance, decreases with the transition from high to  
427 extreme environmental food stress (Figure 3). This reversed pattern is an example of  
428 “inverse scale variance” G x E in which stress denudes genetic differences. It again has  
429 been observed across a range of species, for example, iridescent and orange area in the  
430 guppy *Poecilia reticulata* [17], cuticular hydrocarbon blend in *Drosophila simulans* [16], and  
431 to a more limited extent, UV brightness in *C. eurytheme* [55].

432

433 Our results are novel and striking because we see *both* scale variance and inverse scale  
434 variance in the same trait in a single species. This leads us to propose a unified  
435 hypothesis for G x E interactions in signals of quality. Moderate to large increases in  
436 environmental stress lead to amplification of the phenotypic expression of genetic  
437 quality, whereas as environmental stress becomes extreme, increases in phenotypic  
438 variation overwhelm the underlying genetic differences in quality. We note that in some  
439 previous studies, separate traits respond differently to environmental stress, suggesting  
440 variation in the threshold at which amplification transitions to restriction (e.g. [2, 55]).

441 Future studies will be needed to identify which characteristics are associated with  
442 sensitivity levels in different traits, and whether these relate to costs of trait expression.  
443  
444 Yet, some evidence from other studies of sexual ornaments seems to contradict the  
445 unified hypothesis which report no interaction between genetic and environmental stress,  
446 for example in morphological traits and cuticular hydrocarbons in *D. melanogaster* [40]  
447 and several sexual traits in *P. reticulata* guppies [39]. Both of these experiments examined  
448 groups that differ predictably in genetic quality (hemiclinal lines and inbred versus  
449 outbred lines, respectively). But the lack of response likely reflects the application of  
450 insufficiently intrusive environmental stress. For example, the “stressful” environment in  
451 guppies was a moderate density [39], while that in *D. melanogaster* was a minor reduction  
452 to 70% of the normal diet [40]. A previous study in stalk-eyed flies likewise found little  
453 impact of food reduction of this order [11]. For comparison, our dilution for extreme  
454 stress was a restriction to just 5% of the standard diet. Moreover, as each of these studies  
455 used just two levels of environmental stress, analysis of complex G x E was precluded.  
456 This is not a criticism of either study, which had different goals to ours, but highlights  
457 that neither would provide an adequate test of our hypothesis. Another commonly  
458 reported pattern across diverse species, also potentially at odds with our interpretation, is  
459 “crossover” G x E in which different genetic lines are superior in different environments.  
460 This has been shown for male signal rate in the lesser waxmoth [19] and song traits of  
461 *Enchenopia* treehoppers [20]. However, “crossover” G x E is not really a distinct  
462 category, and can co-occur with “scale” or “inverse scale” G x E patterns [56]. For  
463 instance, crossover embedded within G x E scale variance patterns in the lesser  
464 waxmoth [2] and inverse scale variance in the guppy [17] has been observed. Once  
465 again, the interpretation that crossover dominates the G x E pattern requires

466 investigation of sufficient levels of environmental stress relative to the traits in question.  
467 Without this, crossover should only be seen as part of G x E response, exerting  
468 ambiguous limits on the signalling function of the sexual ornament.  
469  
470 It is vitally important to examine a range of environments from low through to an  
471 extreme form of stress, alongside similar dimensions of genetic quality variation. Distinct  
472 classes of environmental quality variation were created in a standard manner through  
473 food restriction applied to developing larvae [1,11,15,30,40,45]. These treatments  
474 differed from previous studies in the use of food dilution to an “extreme”, defined as the  
475 point before larval survival showed a clear-cut decline (Figure 2). The reason for  
476 choosing this point was in part logistical, in order to easily collect similar sample size  
477 across the different stress levels. We also wanted to avoid the possibility that differential  
478 survival causes changes in trait mean and variation across the different genetic quality  
479 and environmental stresses. Despite this precaution, there was a moderate effect of the  
480 extreme environmental stress on larval survival. This could have contributed to the trait  
481 patterns observed if there was a lower level cut-off in the eyespan of survivors. We  
482 suspect this effect was minor as the mean was lowest and the CV highest in the extreme  
483 environment (Fig. 3), and more importantly, the survival deficit was equal across incross  
484 and outcross flies. Our conclusions appear to be robust. Our use of food quantity as an  
485 environmental stress was for its ease of manipulation and its use in many previous  
486 studies. Competition for food is likely to be a factor in many species and so we suspect  
487 that the results we report here are general stress responses. This needs to be established  
488 through comparison with other stresses, such as fluctuations in temperature, pH or food  
489 quality, that are part of the normal range of environmental stress in the wild [57].  
490

491 To create distinct classes of genetic quality, a set of highly homozygous inbred lines  
492 (incross) were compared against crosses between lines (outcross) which are predicted to  
493 be highly heterozygous for the mutational load carried by incross lines. In the pilot  
494 experiment (Fig. 2), as well as the actual experiment, there was no difference in egg-to-  
495 adult survival between flies in the incross and outcross genetic quality treatments. The  
496 lack of a viability difference suggests that there was a strong purging of deleterious alleles  
497 during the creation of the inbred lines, as is expected and observed in other studies [58-  
498 60]. Our objective was not to study the inbreeding *per se*, as this is unlikely to be the  
499 object of female mate preference in this species. Rather we use inbreeding status as an  
500 investigative tool, in order to uncover the full nature of genetic quality-by-environment  
501 interactions on variation in signal trait size. In particular, previous G x E studies have  
502 failed to use a sufficient range of variation in genetic quality. A typical approach is to use  
503 distinct genetic lines, like brother-sister families [1,20] or inbred lines [2]. But groups that  
504 differ predictably in genetic quality have not been examined properly against a wide  
505 range of environmental stress [39-40]. Independent lines provide information about  
506 genetic variation but may differ only slightly, and unpredictably, in genetic quality, and  
507 then only with differences established *post hoc*. In our study, we distinguish between  
508 variation in genetic *quality* in the comparison of incross and outcross flies, and genetic  
509 variation between lines within these quality categories. In accordance with prior studies  
510 [1], our results show differences in performance between lines. Crucially, there was no  
511 among-line G x E once analysis was limited to a particular genetic quality class, both for  
512 incross and outcross. The set of lines in each genetic quality class appear to have been  
513 sufficiently similar in quality that they responded in an equivalent manner when  
514 challenged with our wide range of environmental stress levels (Figure 4). Only the  
515 comparison between incross and outcross flies revealed a strong G x E interaction, in

516 which high quality (outcross) line resisted the effect of high but not extreme  
517 environmental stress.

518

519 Taking the results together allows us to comment on sexual selection on males and the  
520 potential indirect genetic benefits that arise from female mate choice. We expect sexual  
521 selection to be severely attenuated under benign and extreme environmental stress, but  
522 strong in high stress environments which amplify genetic quality differences. As stress is  
523 likely to be the norm under common ecological conditions in nature, sexual selection  
524 could often be stronger than currently estimated from laboratory experiments – typically  
525 carried out under low stress conditions of *ad libitum* food, constant temperature, no  
526 predators and parasites, and no ecological competitors. We note that our  
527 experimentation used stress from a unimodal environment variable (food availability),  
528 controlling all other physical and biotic factors, and that we used a simple measure of  
529 male signalling, leaving aside other, more subtle aspects of male behaviour used in  
530 female evaluation of their partners [46]. This implies that benign environmental  
531 conditions, equivalent to low stress in our experiment (i.e. in which larvae have excess  
532 food and little competition), are rare. Extreme environments are likewise also likely to be  
533 rare as they are not those that maintain viable populations. The majority of  
534 environments probably lie between the low and high regimes, which is consistent with  
535 the considerable range in eyespan observed among wild caught stalk-eyed flies [32].

536

537 The outcome in nature for female choice will depend on the distribution of  
538 environmental stress, its spatial and temporal variability, and hence its consequence for  
539 the pool of available mates in a given population [12]. If conditions can be categorised as  
540 low, high or extreme, then the indirect benefits of mate choice will be greatest in high

541 stress environments, as these bring out genetic differences to the greatest extent. As  
542 genetic line correlations across environments were positive (with the exception of  
543 outcross lines between low and high food stress, where a lack of variation precluded  
544 reliable calculation), genetic differences will be evident to some extent in all  
545 environments. Where environmental conditions in a population are a mixture of low,  
546 high and extreme, individuals with the most exaggerated sexual ornaments will be an  
547 assortment of those with high genetic quality from a range of environments diluted by  
548 those less well genetically endowed but who experienced lower environmental stress  
549 during development. This cuts at the indirect genetic benefits but nonetheless there will  
550 be advantage to female mate choice. To conclude, while environmental variation places  
551 contingencies on signalling, sometimes amplifying and sometimes muting its value,  
552 genetic variation in quality between individuals will always to some extent be evident in  
553 the sexual ornament and feed through to their offspring.  
554  
555

556 **Data accessibility.** Data are made available at the Dryad Digital Repository [TO ADD].

557

558 **Author contributions.** JMH, KF and AP conceptualised the study and methodology,

559 and wrote, reviewed and edited the paper. The formal analysis was carried out by JMH,

560 who with HACD carried out the experiments. Stalk-eyed fly resources were provided by

561 AP and KF, who secured funding and supervised the project.

562

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564

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573

574

## 575 **SUPPLEMENTAL INFORMATION**

576 Supplemental information includes all details of statistical effect size estimates for the

577 tests of mean effects, and additional method details. [Available after formal publication.]

578

579



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