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Stress-Induced Recombination and the Mechanism of Evolvability*

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Darwin 200' (see Marshall et al. 2010)."

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15 Abstract

16 The concept of evolvability is controversial. To some, it is simply a measure of the standing genetic
17 variation in a population and can be captured by the narrow-sense heritability (h^2). To others,
18 evolvability refers to the capacity to generate heritable phenotypic variation. Many scientists,
19 including Darwin, have argued that environmental variation can generate heritable phenotypic
20 variation. However, their theories have been difficult to test. Recent theory on the evolution of sex
21 and recombination provides a much simpler framework for evaluating evolvability. It shows that
22 modifiers of recombination can increase in prevalence whenever low fitness individuals produce
23 proportionately more recombinant offspring. Because recombination can generate heritable variation,
24 stress-induced recombination might be a plausible mechanism of evolvability if populations exhibit a
25 negative relationship between fitness and recombination. Here we use the fruit fly, *Drosophila*
26 *melanogaster*, to test for this relationship. We exposed females to mating stress, heat shock or cold
27 shock and measured the temporary changes that occurred in reproductive output and the rate of
28 chromosomal recombination. We found that each stress treatment increased the rate of recombination
29 and that heat shock, but not mating stress or cold shock, generated a negative relationship between
30 reproductive output and recombination rate. The negative relationship was absent in the low-stress
31 controls, which suggests that fitness and recombination may only be associated under stressful
32 conditions. Taken together, these findings suggest that stress-induced recombination might be a
33 mechanism of evolvability.

34

35 Keywords: Evolvability, recombination, stress, pangenesis, fitness, mating, heat shock, *Drosophila*

65 In his theory of Pangenesis, Darwin argued that environmental variation could contribute to
66 evolution through the generation of novel heritable variation (Darwin 1868). There are two ways
67 novel environmental conditions and stress can stimulate evolutionary change: by increasing
68 phenotypic variation and/or by increasing genetic variation (reviewed in Hoffmann and Hercus 2000
69 and Badyaev 2005). The former has been termed phenotypic accommodation (related to the “Baldwin
70 effect”, Crispo 2007), which can lead to genetic accommodation, the process by which
71 environmentally induced phenotypic variation becomes constitutively expressed variation after
72 several generations of selection (West-Eberhard 2003). The latter includes adaptive mutation and
73 recombination, which occurs when mutations arise or genetic combinations are generated that allow
74 for rapid adaptation to new conditions. Stress-induced phenotypic and genetic variation can also occur
75 simultaneously. For example, maternal stress can have both genetic and long-term phenotypic
76 consequences for offspring (Badyaev 2005; Priest *et al.* 2007; Priest *et al.* 2008b; Badyaev & Uller
77 2009).

78 There are several empirical studies which support genetic accommodation and adaptive
79 mutation and recombination (Waddington 1953; Rutherford and Lindquist 1998; Cairns *et al.* 1988;
80 Fischer and Schmid-Hempel 2005; for reviews see Rosenburg 2001; Parsons 1988 and Badyaev 2005).
81 The number of studies documenting condition specific mutation and recombination is also growing
82 (Agrawal and Wang 2008; Priest *et al.* 2007). Although these studies are compelling, they are not
83 sufficient to deduce whether stress-induced evolutionary change is common or rare. The technical
84 jargon used in the fields of genetic assimilation and adaptive mutation and recombination may also
85 have contributed to the difficulty of testing the general applicability of these theories (de Jong and
86 Crozier 2003, Braendle and Flatt 2006; Moczek AP 2007).

87 Recent theory on the evolution of recombination (the crossing over of homologous
88 chromosomes during meiosis) provides a much simpler way to evaluate evolvability (Hadany and
89 Beker 2003; Agrawal *et al.* 2005). Recombination has been difficult to explain because its fitness
90 benefits are context-dependent (Feldman 1972; Charlesworth 1976; Barton 1995; Otto and
91 Lenormand 2002). The solution may be that the recombination frequency has evolved to be plastic;

92 frequent in low-fitness individuals, but infrequent in high-fitness individuals. A series of papers by
93 Lilach Hadany and others on Fitness Associated Recombination show that genes which increase
94 recombination in individuals with low fitness are likely to evolve. If plastic modifiers of
95 recombination exist, then they may contribute to evolvability by increasing additive genetic variation.
96 However, there has been no direct test of the relationship between stress, fitness and recombination
97 (though see Tucic *et al.* 1981). We know that abiotic and biotic factors affect the rate of
98 recombination (for example, temperature Plough 1917, Grell 1971, Zhuchenko *et al.* 1986; age,
99 Bridges 1929, Redfield 1966; and nutrition, Neel 1941, Davis and Smith 2001). But, the comparisons
100 have usually only been made between treatment and control, not within treatment. In addition, few
101 studies have examined the consequences of acute stress, defined as a brief and sub-lethal exposure to
102 conditions that reduce fitness. Which individuals in a population show most change in recombination
103 rates from bouts of stress? What types of stresses can accelerate evolution? Could stress-induced
104 recombination be a mechanism of evolvability?

105 Here, we address these questions through two empirical studies of recombination and
106 reproductive output in the fruit fly, *Drosophila melanogaster*. We focused on mating stress, heat
107 shock, and cold shock because they are known to induce stress in fruit flies (Fowler and Partridge
108 1989; Champan *et al.* 1995; Lindquist 1986; Kelty and Lee 1999; Priest *et al.* 2008a).

109 Materials and Methods

110 In Experiment 1, we reanalyzed data from a study on the consequences of mating on the rate
111 of recombination in female fruit flies (Priest *et al.* 2007). Our reanalysis was limited to only one of
112 the three independent marker sets used in the study, Kruppel (Kr) and Black cells (Bc), because it had
113 sufficient linkage distance between markers and large enough sample size to be able to assess the
114 relationship between offspring production and stress-induced recombination. In Experiment 2, we
115 used a longitudinal approach to measure age-specific changes in female offspring production and
116 recombination rate that occurred as a consequence of temperature stress.

117 Strains and Recombination

118 We used coupled phenotypic markers to assess recombination frequency in female *D.*
119 *melanogaster*. For Experiment 1, we obtained lines that expressed 2nd chromosome dominant
120 phenotypic markers, Kruppel (Kr^{lf-1}) and Black cell (Bc¹), from the *Drosophila* stock center in
121 Bloomington, Indiana. The Dahomey line was generously provided by Prof. Linda Partridge
122 (Partridge and Andrews 1985). We backcrossed each of the markers into the Dahomey line for eight
123 generations to homogenize the background. For Experiment 2, we obtained the Oregon-R line and a
124 line that expressed recessive phenotypic markers for the 2nd chromosome, nub (nub¹), black (b¹), and
125 purple (pr¹), from the *Drosophila* stock center in Bloomington, Indiana. To homogenize the
126 background for the recessive marker lines we backcrossed them into the Oregon-R line for two
127 generations, generated F1s to reconstitute the marker lines and repeated this process two additional
128 times. The tight linkage of both of the marker sets minimised the probability of undetected double
129 recombination events. Each of the marker lines was cultured at 50 eggs/vial for three generations
130 before the start of the experiment to limit environmental variation that arises from differences in larval
131 density. The flies were maintained at 25°C, 50% relative humidity in a 12:12 light cycle.

132 We focused our analysis on recombination in females because crossing over between paired
133 chromosomes does not occur in male *D. melanogaster*. We assessed the rate of recombination of a
134 female at a particular age interval by determining the proportion of recombinant offspring produced
135 by that female during the interval. In Experiment 1, we scored the proportion of wild type and Bc Kr /
136 + + recombinant offspring produced by Bc + / + Kr heterozygous females. In Experiment 2, we
137 scored the proportion of nub, nub b, b pr, pr, nub pr (double recombinant) and b (double recombinant)
138 offspring produced by nub b pr / + + + heterozygous females that were originally mated with nub b pr
139 / nub b pr males. We focused our assessment of recombination rate on the entire nub-pr interval,
140 rather than for each pair of markers, because double recombinants were extremely rare (4 double
141 recombinants for every 10,000 samples). Estimates of recombination using these methods may
142 underestimate genome wide consequences, because they employ markers in heteromeric regions,
143 while centromeric regions appear to be much more sensitive to environment-induced recombination
144 (Neel 1941).

145 Stress experiments

146 The methodological details of Experiment 1 have been previously reported (Priest *et al.*
147 2007). Briefly, we collected four-hour-old virgin Bc + / + Kr heterozygous females. When the
148 females were two-days-old they were placed in individual vials with three wild-type (Dahomey)
149 virgin males. At female age 3-day, males were discarded and the females were randomly assigned one
150 of three treatments: High, Medium, or Low mating. Low mating females did not receive any
151 additional exposure to males. The Medium mating females were additionally exposed to three new
152 virgin males for 24 hours at age 5-days. The High mating females were exposed to virgin males every
153 day for eight days. We collected the eggs that were deposited by each female in the bottom of the
154 vials over a 48 hour window, from female age 6-day to 8-day. The females were discarded after their
155 eggs were collected. After the adult offspring had emerged from the vials, they were flash frozen and
156 the phenotypes of all of the offspring were scored. The timing of the mating treatments and egg
157 collection intervals were such that females were prevented from additional exposure to males for 96,
158 24 or zero hours (Low, Medium and High mating, respectively) before the 48 hour egg collection
159 interval.

160 In Experiment 2, we collected four-hour-old virgin nub b pr / + + + heterozygous females. At
161 day 2, they were placed in individual vials with three nub b pr tester males. At day 3, the males were
162 discarded. On day 4, 6 and 8, all of the females were transferred to fresh media vials. At day 10 the
163 females were transferred to empty shell vials (with cotton balls pushed close the bottom of the vial to
164 restrict movement) and were randomly assigned one of three treatments: Heat shock, Cold shock, or
165 sham (control) treatment. Heat shock was applied by placing the vials into a 37°C water bath for 20
166 minutes. Cold shock was applied by putting the females into a freezer that shifted temperature from
167 18°C to 3°C over a 2.5 hour interval. The sham treatment involved holding the females in shell vials
168 at 25°C for 2.5 hours. After the treatment, the females were placed in media vials, which were
169 randomly distributed and given blind labels. The vials were visually inspected two days later to assess
170 egg production. The females were given an additional day in their vials, to increase the sample of eggs,
171 before they were transferred to fresh media vials on day 13. On day 16, they were discarded. We

172 scored all of the vials, except for a set of day 6 vials that were accidentally washed before they had
173 been scored. The heat shock treatment we used is fairly standard for experiments with live *D.*
174 *melanogaster* (Lindquist 1986). Our cold shock treatment involved cooling flies slowly (at 0.1°C/
175 minute) to induce physiologically relevant cold hardening mechanisms (Kelty and Lee 1999).

176 Statistical analysis

177 We calculated a standardized rate of recombination in cM/Mb by dividing each of the
178 recombination values we had measured for each female and age by the estimated physical distance of
179 the marker intervals in mega base pairs. We obtained physical marker distances from FlyBase (Kr-Bc:
180 7.3 Mb; nub-pr: 7.4 Mb). We calculated 95% confidence intervals for each estimate of female
181 recombination rate using the Wilson score interval (see online supplementary figures). To test for the
182 effects of the treatments on recombination, in both Experiment 1 and 2, we constructed logistic
183 regression models with mating and temperature treatment as fixed effects and the proportion of
184 recombinant offspring as the response variable. We used logistic regression because it weights
185 estimates of recombination according to the sampling intensity. To exclude the possibility that the
186 effects of treatments on recombination rates were driven by a few influential observations or
187 observations derived from females with low fecundity, we repeated the analysis after removing
188 females with low reproductive output. We also repeated the analysis after removing females that were
189 identified as statistically significant outliers using regression deletion diagnostics function
190 “influence.measures” in the *R* statistical software. The results of the logistic regression models
191 without females of low reproductive output or without females that were statistical outliers are
192 qualitatively similar to those using the entire data set (data not shown). In Experiment 2, we also used
193 a repeated measures analysis, which treats female as the unit of replication, to examine the possibility
194 that stress-induced changes in recombination were driven by mortality-induced changes in cohort
195 composition, not physiological changes within females. To test for the effects of the treatments on
196 offspring production, we used analysis of variance (ANOVA), with mating and temperature treatment
197 as fixed effects and the number of offspring as the response variable. For Experiment 1, ANOVA was

198 performed on square transformed data. For Experiment 2, analysis was conducted on untransformed
199 data.

200 To examine the relationship between recombination and offspring production, we first
201 constructed ANCOVA models which included treatment as a fixed effect, offspring production as a
202 covariate, offspring production x treatment interaction and the proportion of recombinant offspring as
203 the response variable. We estimated the fitted slopes for each level of treatment in the model to assess
204 the relationship between recombination and offspring production. We used the interaction coefficients
205 of the ANCOVA model to assess differences between the slopes of treatment levels. We repeated this
206 analysis using logistic regression to account for differences in sampling intensity. For Experiment 2,
207 ANCOVA was conducted on natural log transformed data. Each analysis conformed to statistical
208 model assumptions. All statistical analyses were performed using version 2.10.1 of the *R* statistical
209 software (R Development Core Team 2009).

210 Results

211 Mating stress

212 Similar to what was reported previously with the data (Priest *et al.* 2007), there was a
213 significant effect of the mating treatment on the rate of recombination ($\chi^2 = 18.7$, $df=2,123$, $p < 0.001$;
214 Figure 1). On average, bouts of mating that occurred 0-3 days before the end of the assessment
215 resulted in a 29.3% increase in recombination rate, relative to bouts of mating that occurred 6-days
216 before the end of the assessment (3.22 ± 0.2 cM/Mb for High, 2.62 ± 0.1 cM/Mb for Medium, and
217 2.49 ± 0.1 cM/Mb for Low mating). There was a significant effect of mating treatment on offspring
218 production ($F_{2,123} = 3.06$, $p = 0.05$). High mating treatment females produced more offspring than
219 either Medium or Low mating treatment females, although the only significant difference was
220 between High and Medium mating treatment (Tukey's test: High-Medium, $t = 2.42$, $p = 0.05$; High-
221 Low, $t = 1.66$, $p = 0.23$; Medium-Low, $t = 0.74$, $p = 0.74$).

222 The effect of mating on the relationship between offspring production and recombination was
223 complex. In the ANCOVA, there was a significant negative relationship (i.e., a negative slope)

224 between offspring production and recombination within the High mating treatment ($t=4.3$, $p < 0.001$;
225 Figure 2). This indicates that females with lower offspring production in the two day post-mating egg
226 collection interval have a higher rate of recombination than females that produced many offspring
227 over the same post-mating period. The slopes of the relationship between offspring production and
228 recombination did not differ significantly from zero in the Medium and Low mating treatments
229 (Medium: $t = 0.24$, $p = 0.81$; Low: $t = 1.58$, $p = 0.12$). Overall, ANCOVA found that the rate of
230 recombination in females was significantly influenced by mating ($F_{2,120} = 10.8$, $p < 0.001$), offspring
231 production ($F_{1,120} = 13.3$, $p < 0.001$) and mating \times offspring production interaction ($F_{2,120} = 4.0$, $p =$
232 0.02). However, in the logistic regression analysis, which weights recombination rate estimates
233 according to sample size, the slope of the relationship between offspring production and
234 recombination did not differ significantly from zero for any level of mating treatment (High: $z = 0.55$,
235 $p = 0.46$; Medium: $z = 0.001$, $p = 0.97$; Low: $z = 1.99$, $p = 0.16$). Taken all together, logistic regression
236 only found a significant effect of mating ($\chi^2 = 18.7$, $df = 2, 123$, $p < 0.001$), while offspring production
237 ($\chi^2 = 1.50$, $df = 2, 122$, $p = 0.22$) and mating \times offspring production interaction ($\chi^2 = 1.04$, $df = 2, 120$, p
238 $= 0.59$) were not significant.

239 Temperature stress

240 Before the stress treatment was imposed, there was a decline in the rate of recombination with
241 age in the four egg collection intervals (Figure 3). Significant increases in recombination frequency
242 were detected in the three day interval after the stress treatment was imposed ($\chi^2 = 49.5$, $df = 2, 23$, $p <$
243 0.001), but not in the subsequent three day interval ($\chi^2 = 1.06$, $df = 2, 22$, $p = 0.59$; Figure 3). This
244 indicates that the consequences of temperature stress are immediate and short-lived. On average, Heat
245 Shock and Cold Shock increased recombination rate between the nub-pr markers of chromosome 2 by
246 more than ten and five times, respectively, relative to control temperature (Tukey's test: Heat-Control,
247 $z = 5.56$, $p < 0.001$; Cold-Control, $z = 5.16$, $p < 0.001$; 4.75 ± 1.0 cM/Mb for Heat Shock, 2.67 ± 0.5
248 cM/Mb for Cold Shock, 0.43 ± 0.2 cM/Mb for Control). There was no significant difference between
249 Heat Shock and Cold Shock treatments (Tukey's test: $z = 0.82$, $p = 0.69$; Figure 3). A repeated
250 measures analysis, consisting of the interval before and two intervals after the temperature stress

251 treatment was imposed, revealed a significant positive effect of treatment on recombination (Log
252 Likelihood Ratio test: $\chi^2 = 13.55$, $df = 2$, $p < 0.01$). This indicates that the stress-induced changes were
253 driven by changes within females, not mortality-induced changes in cohort composition. There were
254 no significant effects of the temperature stress treatment on offspring production ($F_{2,23} = 2.26$, $p =$
255 0.12).

256 Similar to the mating treatment in Experiment 1, the temperature stress treatment also altered
257 the relationship between offspring production and recombination. In the ANCOVA, there was a
258 significant negative relationship between offspring production and recombination within the Heat
259 Shock treatment ($t=5.2$, $p < 0.001$), but not within the Cold Shock ($t = 0.93$, $p = 0.36$) or Control
260 treatments ($t = 0.13$, $p = 0.90$; Figure 4). This indicates the heat stressed females with low fecundity
261 have greater rates of recombination than highly fecund females. The result also shows that heat shock
262 and cold shock have different consequences on the relationship between offspring production and
263 recombination. Overall, ANCOVA found that the rate of recombination was significantly influenced
264 by temperature ($F_{2,20} = 39.8$, $p < 0.001$), offspring production ($F_{1,20} = 10.5$, $p = 0.004$) and temperature
265 x offspring production interaction ($F_{2,20} = 8.8$, $p = 0.002$). The weighted, more conservative, logistic
266 regression analysis revealed similar findings. The slope of the relationship between offspring
267 production and recombination was significantly negative in the Heat Shock treatment ($z = 2.36$, $p =$
268 0.018), and did not differ significantly from zero for either Cold Shock or Control (Cold Shock: $z =$
269 0.79 , $p = 0.43$; Control: $z = 1.28$, $p = 0.17$). Overall, logistic regression showed that the temperature
270 treatment ($\chi^2 = 49.5$, $df = 2,23$, $p < 0.001$) and temperature x offspring production ($\chi^2 = 6.05$, $df = 2$, p
271 $= 0.049$) were significant, while the main effect of offspring production was not significant ($\chi^2 = 2.6$,
272 $df = 1$, $p = 0.11$).

273

274 Discussion

275 Pangenes is perhaps Darwin's most puzzling intellectual contribution. In contrast to his
276 other ideas, which were usually well supported by data, pangenes is more similar to a conjecture

277 than a formal theory. He speculated that organisms could accelerate adaptation to novel environments
278 by secreting cell-specific factors (gemmules) that accumulate in the germline and contribute to
279 heritable trait expression in offspring (Darwin 1868). Although Darwin himself admitted that there
280 was little evidence to support it, he stuck by his theory because he was convinced that it would one
281 day find empirical support: “[My] much despised child, ‘pangenesis,’ who I think will some day,
282 under some better nurse, turn out a fine stripling (Darwin 1887, p. 120).” Darwin expressed his
283 optimism about the eventual acceptance of pangenesis to many of his colleagues, including Huxley,
284 Hooker, Gray, Hildebrand, Müller, Ogle, Carus and Weir (Stanford 2006). Even when his cousin,
285 Francis Galton, completed an extensive set of experiments in rabbits that failed to support it, Charles
286 Darwin did not refute pangenesis (Clark 1984).

287 So, why, in the absence of any concrete empirical support, was Darwin such an advocate for
288 the hypothesis? One explanation is that pangenesis might have reflected Darwin’s intuition about the
289 evolutionary process. Darwin’s insight was that the material basis of inheritance itself can be open to
290 direct influences of the environment, independent of its effect on mediating natural selection. Though
291 pangenesis had the wrong mechanism of inheritance and incorporated molecular details which, in
292 hindsight, seem fanciful (Charlesworth and Charlesworth 2009), we now know that there are many
293 phenomena that appear to represent cases of evolvability (for example, Waddington 1953; Rutherford
294 and Lindquist 1998; Cairns *et al.* 1998; Hoffmann and Hercus 2000; Rosenburg 2001; West-Eberhard
295 2003, 2008; Schlichting 2004; Badyaev 2005; Pigliucci *et al.* 2006; Lucht *et al.* 2002; Lui 2008). The
296 problem, however, remains that we do not know whether an organism’s capacity to generate heritable
297 variation is a common or rare contributor to phenotypic evolution.

298 This study was designed to examine the relationships between stress, offspring production
299 and recombination. We did not find the negative between offspring production and recombination in
300 control conditions, which is the pattern predicted by the theory of Fitness Associated Recombination
301 (Hadany and Beker 2003; Agrawal *et al.* 2005). Instead, we found that this relationship is only
302 apparent under particular forms of acute stress.

303 The consequences of acute stress can be immediate and quite short lived. Previous analysis of
304 Experiment 1 revealed that the bouts of mating only elevate recombination rate for short periods
305 (Priest *et al.* 2007). We found a similar pattern in Experiment 2. Recombination rate was elevated 0-3
306 days after temperature stress had occurred, but not 3-6 days after it had occurred. Interestingly, though
307 it increased recombination rate, the consequences of cold shock were not associated with offspring
308 production. Taken together, the results indicate that if these conditions frequently occur in nature, then
309 it is possible that heat shock and perhaps also mating stress, but not cold shock, might have a general
310 role in accelerating evolution.

311 At the outset, the mating stress experiment seemed to be ideal for testing Fitness Associated
312 Recombination because there was a significant effect of the mating treatment on both recombination
313 rate and offspring production. However, tests of that relationship are complicated by the nature of
314 acute stress. Frequent mating has negative long term consequences for offspring production and
315 survival (Fowler and Partridge 1989; Chapman *et al.* 1995; Priest *et al.* 2008a), but acute bouts of
316 mating can also have short term benefits (Wolfner 1997; Priest *et al.* 2008b; Long *et al.* 2010). In
317 Experiment 1, we found that exposure to males actually increased offspring production. Though this
318 did not prevent us from testing for a negative relationship between offspring production and
319 recombination, it may have limited our ability to detect such a pattern. Similarly, though heat stress is
320 thought to have long term fitness costs for fruit flies (Sayeed & Benzer 1996), individual bouts of heat
321 stress generally do not have fitness consequences (Krebs & Loeschcke 1994). In Experiment 2, there
322 was no evidence that heat shock or cold shock affected offspring production. The discordance
323 between acute and chronic stress in both experiments highlights the difficulties in empirically
324 assessing the relationship between fitness and recombination.

325 Experiment 1 and 2 could have been greatly influenced by a small collection of females with
326 low fecundity, which is a problem because recombination rate estimates are less accurate with small
327 sample sizes (see online supplementary figures). This potential problem was addressed by analyzing
328 the results with logistic regression, which weights observations by sample size. The robustness of the
329 results were further checked by repeating the analysis after removing observations based on small

330 sample size and repeating the analysis after removing observations that were deemed statistically
331 influential. Overall, with only one exception (analyses with logistic regression that involved the High
332 mating treatment), the weighted and non-weighted regressions produced similar findings.

333 The choice of statistical analysis can influence our ability to detect negative relationships
334 between fitness and recombination. All weighted regression techniques, including logistic regression
335 adopted in this study, necessarily penalize observations that are based on small sample sizes. But,
336 these are precisely the individuals that are expected to be most affected by the treatments if stress-
337 induced recombination is a general mechanism of evolvability. Another statistical issue is that, as we
338 expected, the stress treatments shifted the range of data points in recombination frequency and
339 offspring production. This is problematic because it is inappropriate to test for differences in
340 relationships between treatments when there are non-overlapping data ranges. To compensate for both
341 of these issues, in future work we will need to increase our sample size and identify ecologically
342 relevant stresses with large effect sizes.

343 There are many other possible confounding factors that influence our ability to generalize the
344 evolutionary significance of these results. The ones we are most concerned about are body size,
345 genetic variation between *Drosophila* lines, age, culture conditions, larval interactions during
346 development, and even, potentially, the barometric pressure in the laboratory at the time heat stress
347 experiments are conducted (B. Mackowiak and N. K. Priest, personal communication). Though we
348 attempted to distribute some unaccounted for sources of variation through randomization, we simply
349 need more studies of this sort, with substantially larger sample sizes, to be confident of the
350 relationship between stress-induced fitness and heritable variation.

351 What types of molecules can generate heritable variation?

352 In his theory of pangenesis, Darwin proposed that an organism's capacity to adapt to novel
353 conditions depends on its ability to produce gemmules, factors secreted by cells that accumulate in the
354 germline and contribute to heritable trait expression in offspring (Darwin 1868). We now know that
355 gemmules do not exist (Charlesworth and Charlesworth 2009). Nevertheless, it is possible that

356 lineages have evolved to express or respond to specific kinds molecules which accelerate adaptation
357 to novel conditions. Though it should only be considered as speculation, it is useful to consider what
358 molecules might have a role in evolvability.

359 One such candidate molecule is the heat-shock protein, Hsp90, which is thought to capacitate
360 evolutionary change (Rutherford and Lindquist 1998). Other, potential evolvability molecules could
361 be stress hormones or, for that matter, any agent that induces stress or activates a stress-induced
362 cascade. For example, it is well known that bacteria such as *Escherichia coli* can enter a hyper-
363 mutable state via stress-induced genetic pathways in response to stimuli such as nutrient starvation
364 (reviewed in Tenailon *et al.* 2004).

365 To identify further candidate molecules, we first need to determine the types of stress
366 organisms typically encounter. J.B.S. Haldane and W. D. Hamilton, in particular, believed that disease
367 has a profound role in evolution (Haldane 1949; Hamilton 2001). Pathogens could be a key source of
368 environmental stress that helps to drive the evolution of recombination (see Fischer and Schmid-
369 Hempel 2005). A pair of exciting plant studies recently showed that fungal and viral infections can
370 trigger a systemic stress response in the plants, which include defence signal transduction as well as
371 increased recombination in uninfected tissues (Lucht *et al.* 2002; Kovalchuk *et al.* 2003). In addition,
372 since it is quite likely that many small ectotherms such as the fruit fly experience considerable thermal
373 stress upon exposure to direct sunlight (Heinrich 1993), molecules involved in the temperature stress
374 pathway, perhaps even heat shock proteins could also be potential candidates. Lastly, as toxic
375 compounds in male seminal fluid are responsible for mating stress and can stimulate maternal effects
376 which increase the fitness of daughters (Chapman *et al.* 1995; Priest *et al.* 2008b), they may also
377 stimulate recombination (Priest *et al.* 2007).

378 How can we mathematically characterize evolvability?

379 One of the reasons the concept of evolvability is controversial is that models of evolvability
380 usually invoke levels of selection above the individual (Pigliucci 2008). Because natural selection
381 lacks foresight and tends to fix alleles that maximise current fitness regardless of the consequences for

382 future evolutionary potential of the population, evolvability is generally not expected to be selected at
383 the level of individuals. The evolution of sexual reproduction and recombination are particularly
384 difficult to explain because neither is likely to provide immediate fitness benefits to the individual
385 expressing it and may even be deleterious for the offspring of the individual if recombination breaks
386 apart existing beneficial allele combinations. Therefore, it is thought that some form of group or even
387 higher levels of selection such as species and clade selection might be necessary for the evolution of
388 evolvability (van Valen 1973; Stanley 1975; Williams 1992; Pigliucci 2008).

389 In contrast, models of Fitness Associated Recombination (FAR) do not require higher levels
390 of selection. In models of FAR recombination evolves by the spread of modifier alleles which have no
391 effect on the fitness of the individuals that bear them (Hadany and Beker 2003, Agrawal *et al.* 2005).
392 Instead, the modifiers form associations with loci under positive selection and increase in frequencies
393 via hitchhiking on the selective sweeps of beneficial alleles in a population. By demonstrating that
394 rare mutant plastic recombination modifiers can invade populations of uniform recombination
395 modifiers, FAR models show that short-sighted selection at the level of the gene can favour alleles
396 which increase levels of genetic variation and thereby enhance population-level evolvability.

397 FAR can easily evolve in haploid models (Hadany and Beker 2003), but the relationship is
398 more complex in diploid models (Agrawal *et al.* 2005). Under normal genetic assumptions, plastic
399 modifiers of recombination do not evolve because during meiosis the modifier is just as likely to
400 segregate with low fitness-encoding haplotypes as high fitness-encoding haplotypes (Agrawal *et al.*
401 2005). However, the modifier may evolve if it is encoded by gene expression in mothers (Agrawal *et*
402 *al.* 2005). It seems likely that stress-induced recombination could have a central role in models of
403 recombination in fluctuating environments (spatial heterogeneity and Red-Queen dynamic) or if
404 fitness and recombination are also associated with negative fitness interaction between loci e.g.
405 negative epistasis (Otto and Michalakis 1998; Otto and Lenormand 2002; Hadany and Comeron 2008).

406 There are other possible mechanisms of stress-induced evolvability that need further
407 mathematical treatment. Stress-induced mutation might be a potent force for generating heritable

408 phenotypic variation (Taddei *et al.* 1997; Rosenburg 2001; Agrawal and Wang 2008). Stress might
409 also contribute to evolvability through phenotypic accommodation, the first step in the process of
410 genetic accommodation (West-Eberhard 2003). According to the theory of phenotypic
411 accommodation, previously hidden genetic variation can become expressed after environmental
412 stresses overcome the normally canalized developmental process. If novel phenotypic variation is
413 beneficial in the stressful environment, then selection will favour alleles underlying the selected
414 phenotype. This results in genetic accommodation which stabilizes the expression of the phenotype,
415 i.e. constitutive expression independent of stress exposure (West-Eberhard 2003; Moczek 2007).
416 Though we have emphasized the role of stress, mathematical models of evolvability do not have to
417 invoke stress. Computational models have been used to describe how genetic modularity contributes
418 to evolvability (Wagner and Altenberg 1996). Quantitative genetic models of maternal effects and
419 other indirect genetic effects might also be considered models of evolvability because they allow for
420 the additive genetic variation in one individual to be positively influenced by trait expression in
421 another (Kirkpatrick and Lande 1989; Wolf 2003).

422 Regardless of the mechanism, it is clear that we will only be able to resolve the general
423 significance of evolvability by testing mathematical models with empirical evidence. This study is, to
424 the best of our knowledge, the first attempt at empirically elucidating the relationships between fitness
425 and stress-induced variation for ecologically relevant stresses. Our results did not provide evidence
426 for a general relationship between offspring productions and recombination as predicted by FAR.
427 Instead, the expected negative relationship was only found in the heat shock treatment. These results
428 suggest that certain types stress might have the capacity to stimulate evolutionary change. Thus, while
429 future studies utilising larger sample sizes are clearly needed, it is possible that part of the intuition
430 behind Darwin's theory of pangenesis is correct.

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Figure Legends:

Figure 1. The effect of mating frequency on the average recombination rate in females within the *Kr-Bc* marker interval of chromosome 2 (Data from Priest *et al.* 2007). Sample sizes are displayed above the standard error bars. Mean total offspring production used in calculation of recombination rate were 70.3 (High), 65.2 (Medium) and 62.5 (Low). Differences between treatments were evaluated using Tukey’s multiple comparison tests. Significance codes “N.S.” ($p>0.5$) and “***” ($p<0.01$).

Figure 2. The effect of mating frequency on the relationships between offspring production and recombination rate within the *Kr-Bc* marker interval of chromosome 2 (Data from Priest *et al.* 2007). Each data point represents the estimated value of recombination for a single female over a single three-day (day-13) egg laying period. Sample sizes: 41 (High mating, black square), 43 (Medium mating, grey diamond) and 42 (Low mating, light grey triangle). Fitted lines are linear regressions on untransformed data.

Figure 3. The effect of brief and extreme changes in temperature on average recombination rate in female fruit flies within the *nub-pr* marker interval of chromosome 2. The flies that survived to day 10 were either exposed to heat shock, cold shock, or sham treatment. The black arrow indicates when the stress treatment was applied. Differences between treatments were evaluated using Tukey’s multiple comparison tests on log-transformed data. Significance is indicated by letters above the standard error bars, with different letters indicating a significant differences at $p = 0.05$.

Figure 4. The effect of brief and extreme changes in temperature on the relationships between female recombination rate and female offspring production within the *nub-pr* marker interval of chromosome 2. Each data point represents the estimated value of recombination for a single female over a single three-day (day-13) egg laying period. Sample sizes: 7 (Heat shock, black square), 7 (Cold shock, grey diamond) and 10 (Control, light grey triangle). Fitted lines are linear regressions on untransformed data.

603 Fig 1.

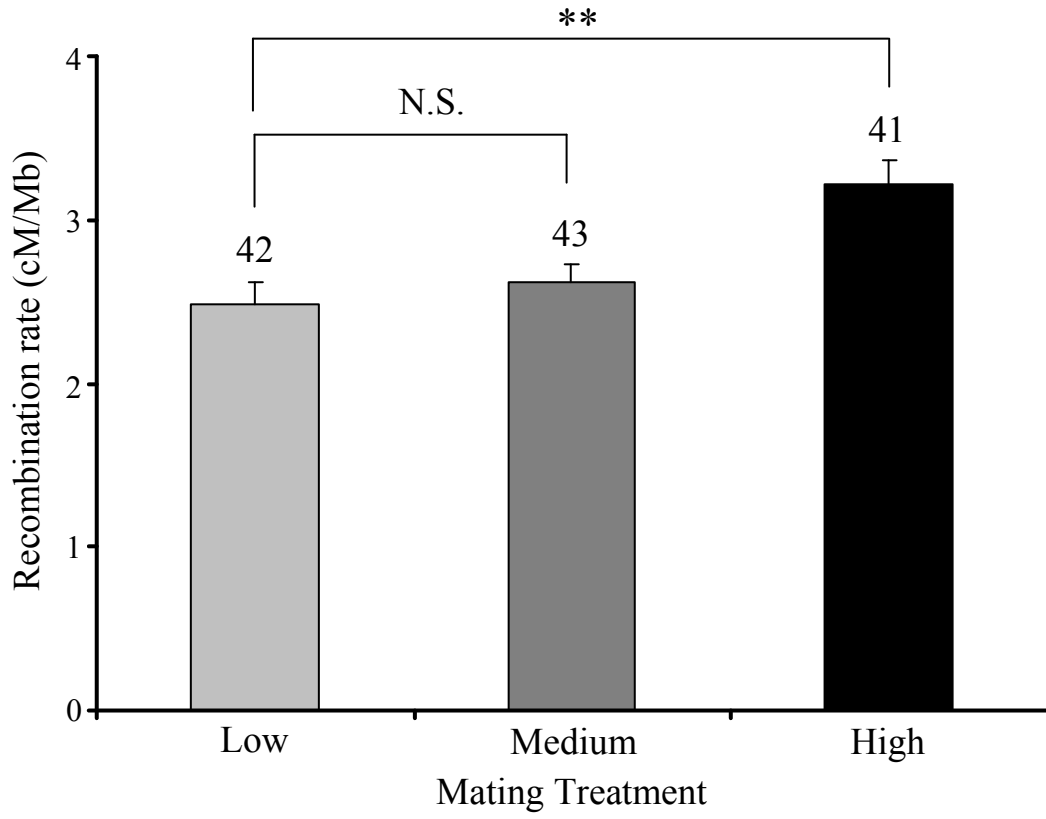
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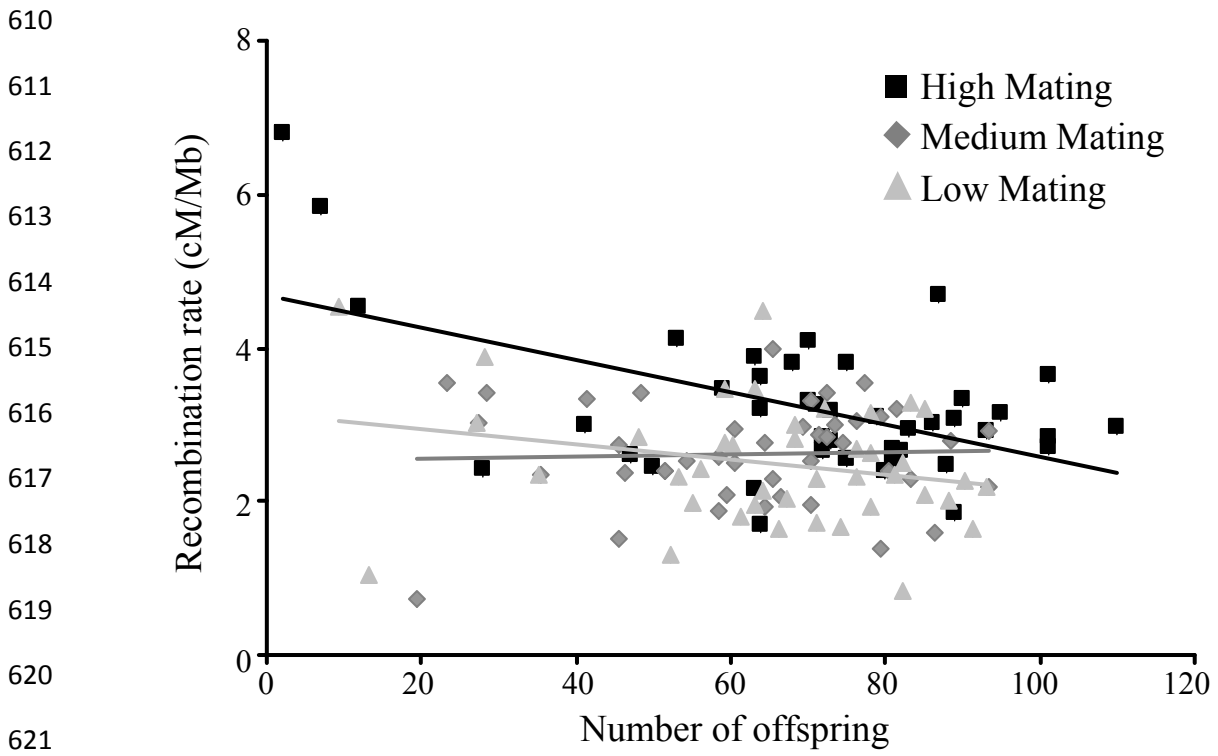
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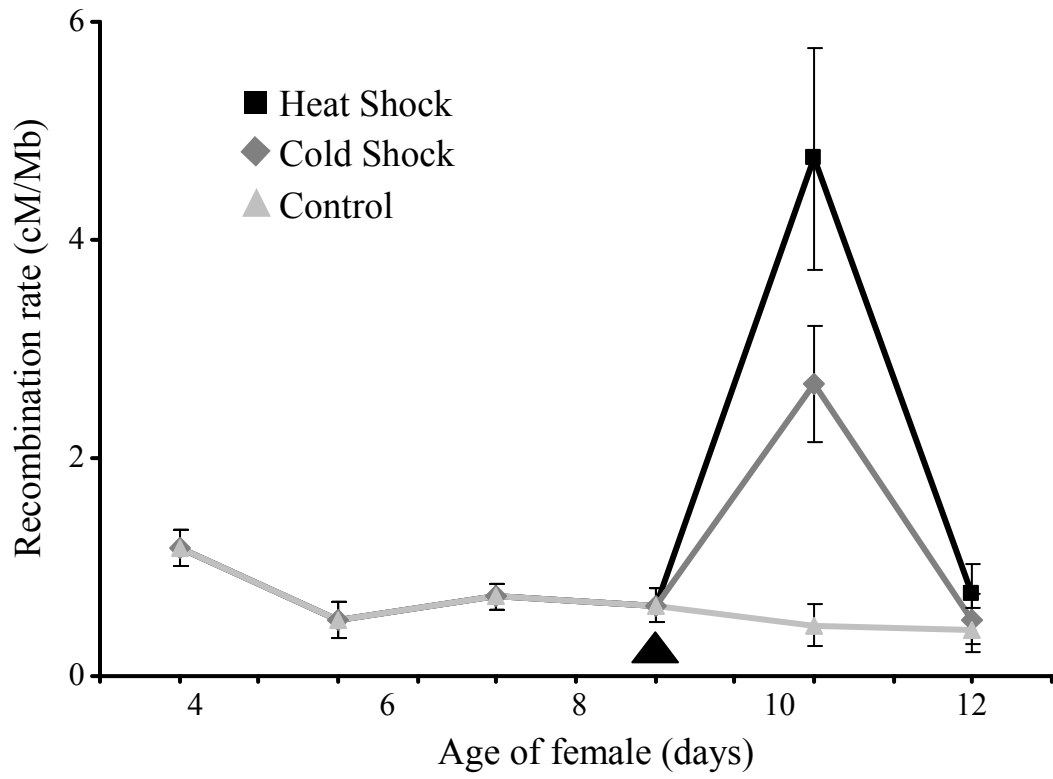
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609 Fig 2.



637 Fig 3.



665 Fig 4.

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