Stress-Induced Recombination and the Mechanism of Evolvability*

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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*

"The tissues of the body, according to the doctrine of pangenesis, are directly affected by the new conditions, and consequently throw off modified gemmules, which are transmitted with their newly acquired peculiarities to the offspring….

[I]n the cases in which the organisation has been modified by changed conditions…the gemmules cast off from the modified units of the body will be themselves modified, and, when sufficiently multiplied, will be developed into new and changed structures."

Charles Darwin 1868 (pp. 394-397)

As we pass the bicentennial of the birth of Charles Darwin, it seems appropriate to consider not only his ideas that are highly regarded, but also those that are "less fashionable" (Marshall *et al.* 2010, this issue). Darwin's "Provisional Hypothesis of Pangenesis" is a fitting topic in this light (Darwin 1868). Pangenesis was Darwin's solution to the problem of inheritance. It is usually treated as being synonymous with blending inheritance. Though modern genetics has led to its rejection, a key component of the theory may have been overlooked (West-Eberhard 2008). At its core, pangenesis is a theory about evolvability. In modern biology, evolvability has two distinct yet interrelated meanings (Radman *et al.* 1999, Pigliucci 2008; Brookfield 2009). Some define evolvability as a static measure of the capacity of populations to respond to selection, which is captured by narrow-sense heritability or the additive genetic coefficient of variation (Fisher 1930; Houle 1992; for historical reference see Edwards 2010, this issue). Others define evolvability as a dynamic process that generates heritable variation (Wagner and Altenberg 1996; Kirschner and Gerhart 1998). As is evident in pangenesis, Darwin embraced dynamic processes in evolution (Darwin 1868).

In this paper we take a multi-pronged approach to evaluate evolvability as a dynamic process. We discuss historical aspects, with a particular focus on pangenesis. We review the accumulating evidence that the environment has a role in generating heritable variation. We argue that the capacity to evolve might depend on the relationship between physiological and genomic responses to stress. And, we present two empirical studies on stress-induced chromosomal recombination in *Drosophila* to illustrate this idea. Our results suggest that the genome might have a dynamic role in facilitating evolutionary change.

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

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

Recent theory on the evolution of recombination (the crossing over of homologous chromosomes during meiosis) provides a much simpler way to evaluate evolvability (Hadany and Beker 2003; Agrawal *et al.* 2005). Recombination has been difficult to explain because its fitness benefits are context-dependent (Feldman 1972; Charlesworth 1976; Barton 1995; Otto and 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 Tucíc *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

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

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

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

Statistical analysis

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

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

Temperature stress

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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

What types of molecules can generate heritable variation?

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

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

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

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

How can we mathematically characterize evolvability?

One of the reasons the concept of evolvability is controversial is that models of evolvability usually invoke levels of selection above the individual (Pigliucci 2008). Because natural selection 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

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

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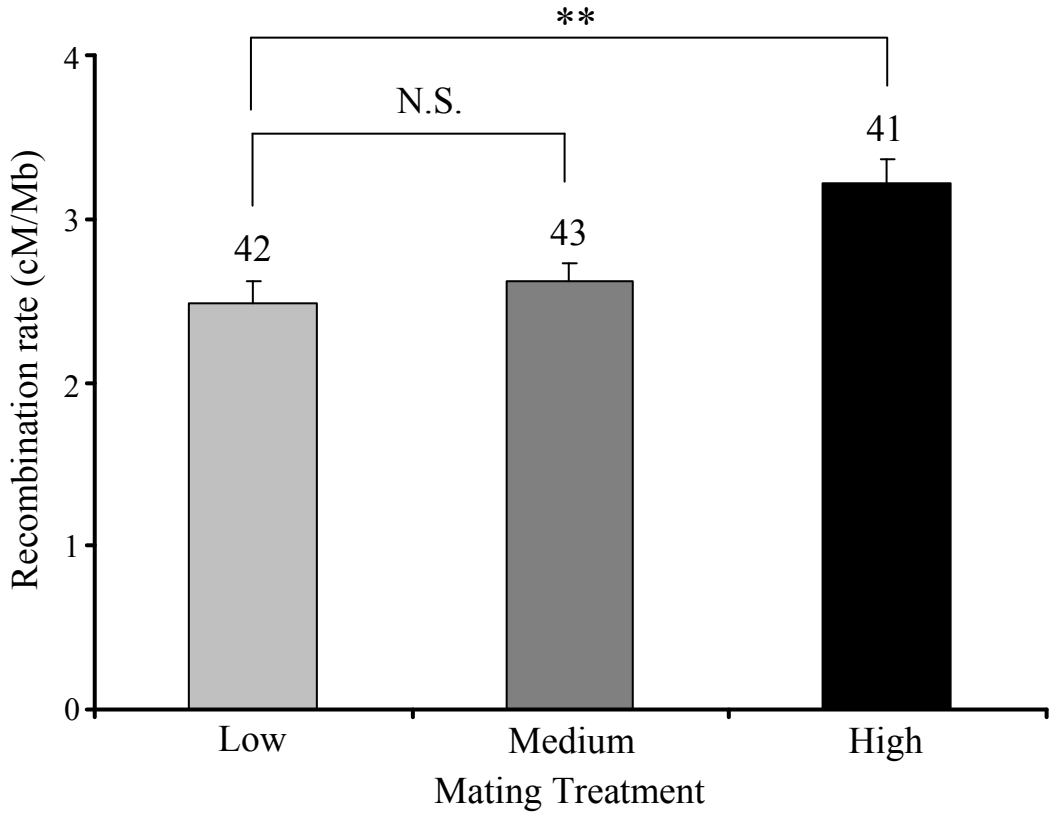


Fig 2.

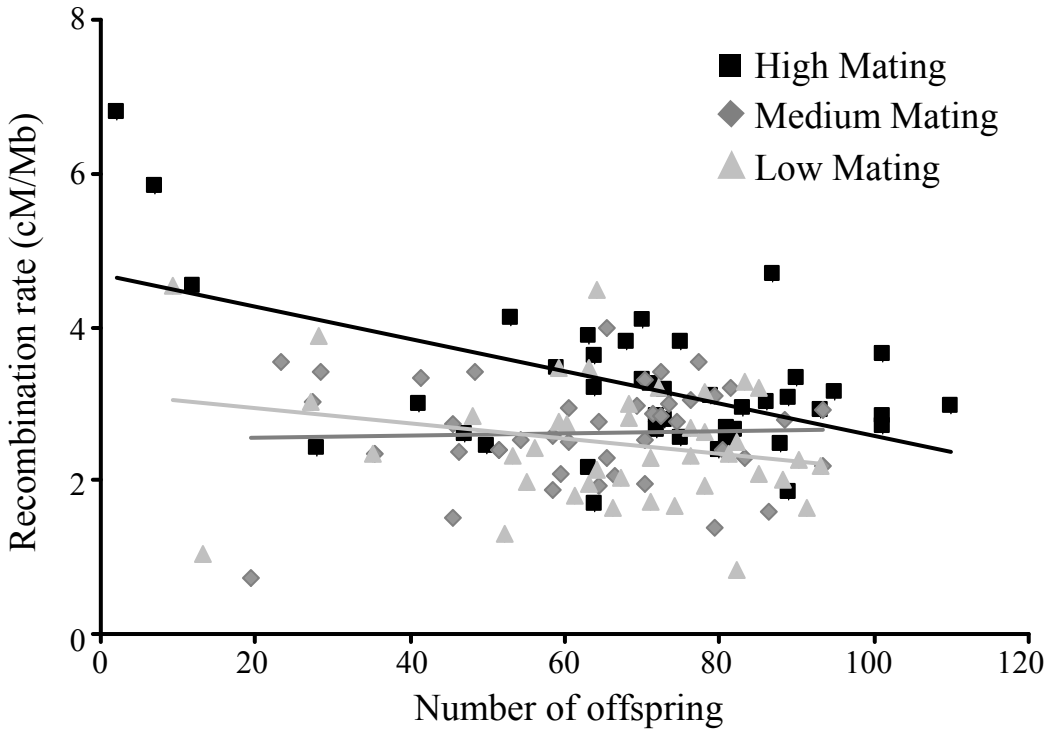

Recombination rate (cM/Mb)
Number of offspring
High Mating
Medium Mating
Low Mating
0
2
4
6
8
0
20
40
60
80
100
120

Fig 3.

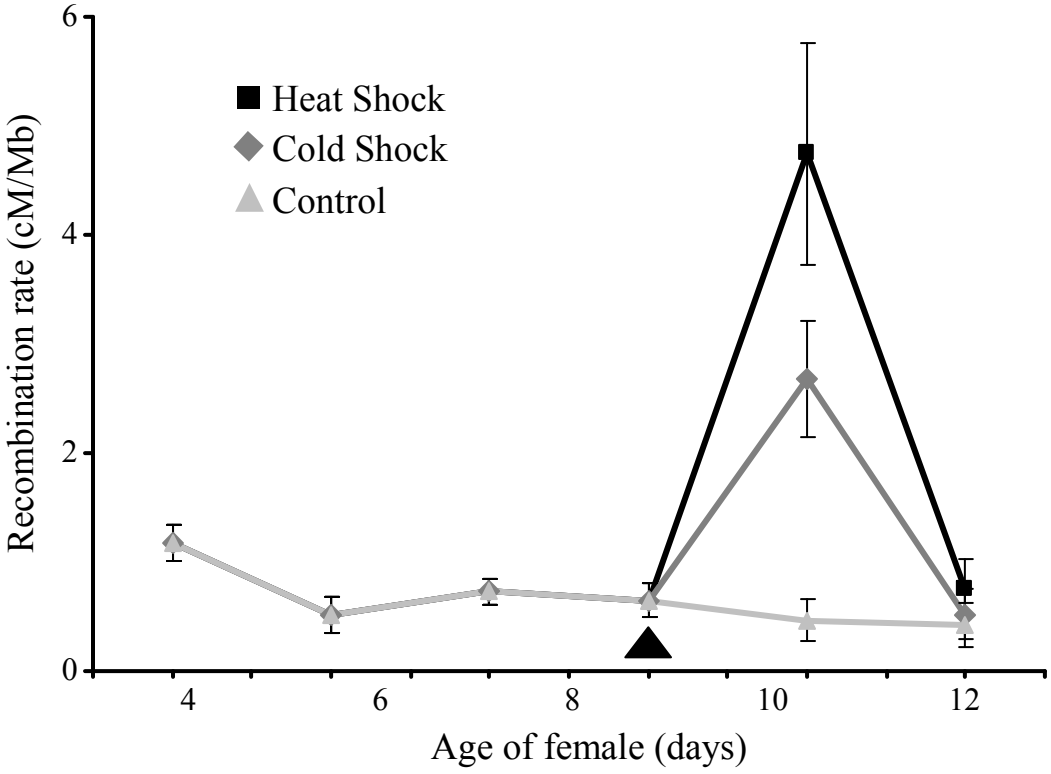
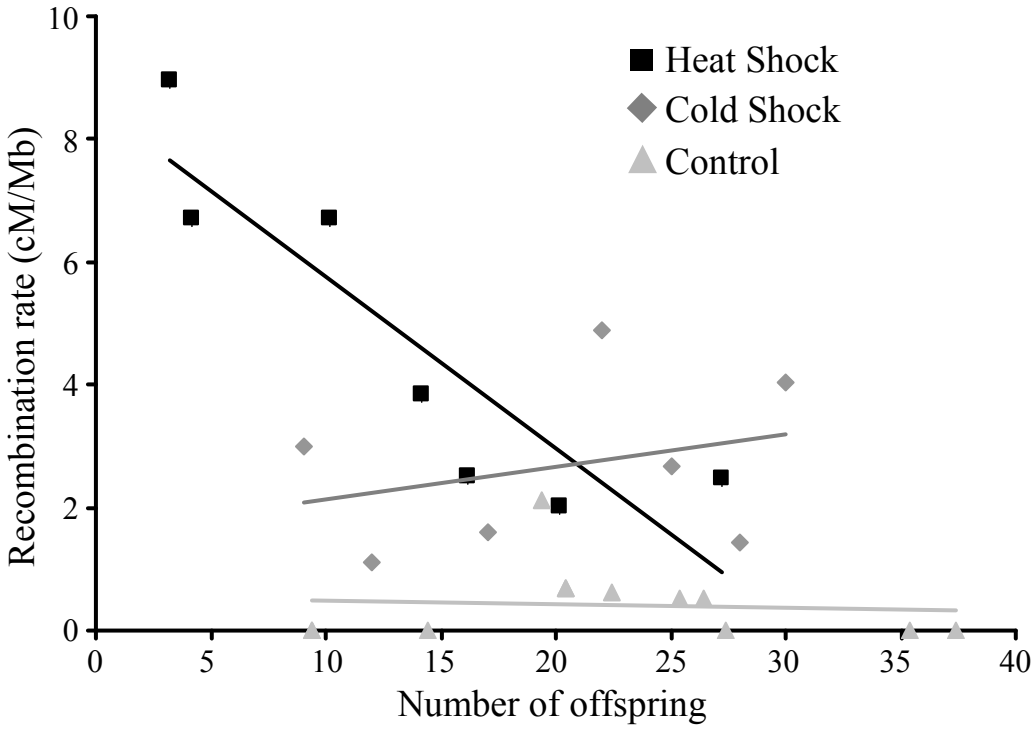

Heat Shock
Cold Shock
Control
Recombination rate (cM/Mb)
0
2
4
6
4
6
8
10
12
Age of female (days)

Fig 4.


Heat Shock
Cold Shock
Control
Recombination rate (cM/Mb)
Number of offspring
0
2
4
6
8
10
0
5
10
15
20
25
30
35
40