Genet. Res., Camb. (1998), **72**, *pp.* 13–18. *With 2 figures.* Printed in the United Kingdom © 1998 Cambridge University Press

Effects of stress combinations on the expression of additive genetic variation for fecundity in *Drosophila melanogaster*

CARLA M. SGRÒ[1] AND ARY A. HOFFMANN*
School of Genetics and Human Variation, La Trobe University, Bundoora, Victoria 3083, Australia

(*Received 28 November 1997 and in revised form 4 March 1998*)

Summary

To test whether stressful conditions altered levels of heritable variation in fecundity in *Drosophila melanogaster*, parent–offspring comparisons were undertaken across three generations for flies reared in a combined stress (ethanol, cold shock, low nutrition) environment or a control environment. The stressful conditions did not directly influence fecundity but did lead to a reduced fecundity in the offspring generations, perhaps reflecting cross-generation maternal effects. Both the heritability and evolvability estimates were higher in the combined stress treatment, reflecting an apparent increase in the additive genetic variance under stress. In contrast, there were no consistent changes in the environmental variance across environments.

1. Introduction

It is well known that levels of genetic variability for traits in populations can change with environmental conditions. This can be detected by changes in the narrow-sense or broad-sense heritabilities of traits as well as the more recently proposed 'evolvability' measure (Houle, 1992). A common explanation for heritability changes is that levels of environmental variability are altered. The narrow-sense heritability (defined as $V_A/(V_G+V_E)$) may decrease with greater environmental stress, because the environmental variance (V_E) increases whereas the additive genetic variance (V_A) and overall genetic variance (V_G) remain constant. This hypothesis is widely used to explain decreasing heritabilities under stressful conditions in plants (Blum, 1988). As a consequence, selection in stressful conditions is considered to be less effective than selection in optimal conditions when the same genes contribute to the selection response in both environments.

However, there are other explanations that predict different patterns of changes in heritability and its components with environmental conditions. It has been proposed that some environmental conditions are likely to result in the expression of genetic variability, which can then be selected. This follows early work by Waddington and others (1961) who argued that exposure to stressful environmental conditions can result in phenotypic changes due to the expression of new genetic variation, whose expression eventually remains 'switched on' in the absence of any stress. While Waddington considered the evolution of developmental abnormalities, there is also evidence that heritable variation in quantitative traits can be increased by stressful conditions (Parsons, 1987; Hoffmann & Parsons, 1991).

A related explanation is that genetic variation under some conditions may be increased because these conditions are novel for an organism. Because such conditions are not normally encountered, there is no history of selection on a trait decreasing levels of genetic variability by selecting against extreme phenotypes. This 'selection history' hypothesis has been advocated by a number of workers (Jinks *et al.*, 1973; Holloway *et al.*, 1990; Kawecki, 1995; Pigliucci *et al.*, 1995) to explain changes in genetic variance in novel and often stressful environments.

In *Drosophila*, numerous experiments have provided heritability estimates for a range of traits, but few experiments have considered estimates under different conditions, particularly those resulting in stress. Heritability estimates for size-related traits are often similar across laboratory environments (e.g. Robertson, 1964; Sgrò & Hoffmann, 1998*a*), although genetic variance for these traits may be increased

* Corresponding author.
[1] Present address: Galton Laboratory, Department of Biology, University College London, 4 Stephenson Way, London NW1 2HE, UK.

https://doi.org/10.1017/S0016672398003310 Published online by Cambridge University Press

under some conditions (Moed *et al*., 1997). Heritable variation for life history traits under stress has rarely been considered, although some laboratory conditions may increase the expression of genetic variance for development time (Gebhardt & Stearns, 1992; Neyfakh & Hartl, 1993; Sgrò & Hoffmann, 1998*a*).

In this study we tested whether the expression of additive genetic variance in early fecundity in *D. melanogaster* is increased under stressful conditions. In laboratory experiments, narrow-sense heritability estimates for fecundity can be extremely variable. For instance, Tantawy & El Helw (1970) reported an estimate of $0{\cdot}02 \pm 0{\cdot}035$, compared with mean estimates of $0{\cdot}70 \pm 0{\cdot}175$ obtained by Service & Rose (1985). Some of the variability may be associated with maternal age (Rose & Charlesworth, 1981), but it could also be related to environmental effects on the expression of genetic variation (Sgrò & Hoffmann, 1998*a*), particularly as the heritability of this trait in field flies can change markedly between collections (Sgrò & Hoffmann, 1998*b*).

We estimated the additive and environmental genetic variances by considering three generations reared under either stressful or favourable conditions. The stressful environment consisted of a combination of three stresses that substantially reduced fitness, as evidenced by a large reduction in egg-to-adult viability. Combined stresses are more likely to be encountered in the field than single stresses. The stresses (cold, ethanol, poor nutrition) are relevant to field conditions. They are therefore unlikely to be novel, so that a history of selection is unlikely to account for environmental effects on the genetic variance. We monitored environmental effects on the means of traits across three generations as well as on variability in the traits.

2. Materials and methods

(i) *Stocks and conditions*

Flies were obtained from a mass bred laboratory population of *Drosophila melanogaster* initiated in March 1995 from 100 field-inseminated females from nature. The field females were collected by pooting adults directly off a pile of rotting fruit in an orchard in Wandin North, approximately 80 km northeast of Melbourne. The population was maintained in the laboratory at 25 °C under continuous light in 12×50 ml bottles on a dead yeast–sucrose–agar medium. Approximately 150–200 flies were set up in each bottle to ensure a census population size of 2500–3000 flies. The stock population had been cultured in the laboratory for approximately 20 generations before experiments were initiated at a generation time of around 3 weeks.

We used a combination of stressful conditions involving food quality, ethanol and cold – stresses likely to be relevant to populations in nature. Adult *D. melanogaster* utilize 3 % ethanol vapour as a resource, while 12 % ethanol is stressful and reduces longevity (Parsons, 1982). A range of ethanol concentrations can be found in nature (Gibson *et al*., 1981; McKechnie & Morgan, 1982). Food quality and food availability are likely to be variable in nature as reflected by variability in the size of flies emerging from the same resource. Extreme temperatures play a role in influencing the distribution *Drosophila* species and short exposures to extreme cold or heat may be particularly important (Parsons, 1982). Because all developmental stages can encounter extremes, we exposed eggs, larvae and adults to the stressful environments.

Pilot experiments were performed to determine the levels of stress that would provide conditions leading to moderately high mortality levels (i.e. around 80–90 %). Stressful growth conditions for the eggs and larvae involved 8·5 % ethanol added to a sucrose, agar and yeast medium. The amount of yeast was reduced from 6 % (v/v) to 3 % (v/v). In addition, eggs and first instar larvae were each exposed to a cold stress involving 1 °C for 45 min by placing vials with medium into a refrigerated bath. A probe placed in the medium showed that the medium reached 1 °C within 10–12 min of being placed in the bath. The adult stresses involved exposure in empty vials to a cold shock of −1 °C for 30 min. Adults were stressed after their fecundity had been measured, and before their eggs were collected for the next generation.

(ii) *Experimental design*

There were three generations (Fig. 1). For the stressed treatment, egg, larval and adult stages were exposed to different combinations of stresses. In the grandoffspring generation, unstressed as well as stressed grandoffspring were set up from stressed F_1s to test for cross-generation effects induced by the stresses.

Eggs for the parental generation were collected by placing spoons filled with 2 ml of medium into bottles with adults from the mass bred population. The medium was dyed green with food colour to allow eggs to be discerned more easily. A live yeast paste (1 g yeast to 10 ml water) was spread across the surface of the medium to stimulate oviposition. Spoons were left in the bottles for 3 h in darkness at 25 °C to stimulate egg laying. Eggs were transferred to 30 ml vials with 10 ml of medium; 20 eggs were placed in each vial for the treatment where eggs and larvae were not stressed, and 30 eggs in each vial for the treatment where eggs and larvae were stressed.

Eclosing adults were removed every 6 h to provide virgins for the fecundity measurements. Virgin females and males were held in 30 ml vials with 10 ml of

https://doi.org/10.1017/S0016672398003310 Published online by Cambridge University Press

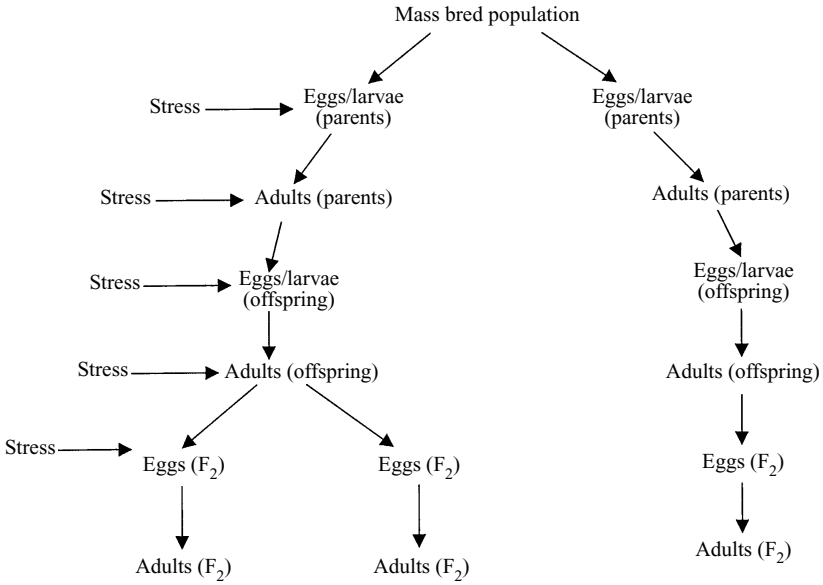

Fig. 1. Outline of experimental design. Life history stages exposed to stress and generations are shown.

medium and then mated in pairs. For the stressed treatments, flies for the fecundity measurements were taken from the peak period of eclosion, since stress extended the development time and we wanted to avoid introducing variability due to differences in female age (Parsons, 1963).

Fecundity was scored over the first 5 d after eclosion. A female was placed with a male from the same treatment into an empty 30 ml vial containing a spoon filled with 2 ml of medium (as above). Pairs were left for 24 h before spoons were replaced with fresh ones. The spoons from the previous 24 h were frozen at −15 °C for a day and then stored at 4 °C until eggs could be counted. This was repeated for 5 d. After this period, adults to be stressed were placed as pairs in an empty 30 ml vial and immersed in a water bath set to −1 °C for 30 min. Each pair of adults was then placed in a vial containing medium and left at 25 °C for 24 h to recover from the cold shock before eggs were obtained for the next generation.

We set up 150 pairs of flies for the parental generation of the stressed treatment, and 120 pairs for the control treatment. Offspring were mated to males from a different family and the same number of pairs were set up for each respective treatment to produce grandoffspring. Comparisons are based on around 100–120 families because not all pairs produced offspring for both treatments, particularly the stressed treatment. Heritabilities were calculated from single parent regressions involving the regression of family means onto parental means. In addition to estimating heritabilities, we calculated the evolvability of fecundity given as $I_A = V_A/\bar{x}^2$ following Houle (1992). Both measures were computed from comparisons between parents and offspring, and between the F_1 and F_2 individuals.

3. Results

(i) *Stress effects on means*

Means from the stressed and unstressed treatments of the same generation were compared because these were tested at the same time and minor environmental conditions between generations are likely to influence fecundity. Because egg counts were normally distributed, untransformed data were compared with *t*-tests. Means (Fig. 2) indicate that exposure to stressful experimental conditions did not influence the fecundity of the parental generation ($t = 0{\cdot}54$, 253 d.f., $P = 0{\cdot}591$). However, there was a significant difference in the fecundity of daughters from the stressed and unstressed treatments ($t = 6{\cdot}30$, 457 d.f., $P < 0{\cdot}001$). Fecundity was lower for the stressed flies (Fig. 2) and there was a reduction in the fecundity of this

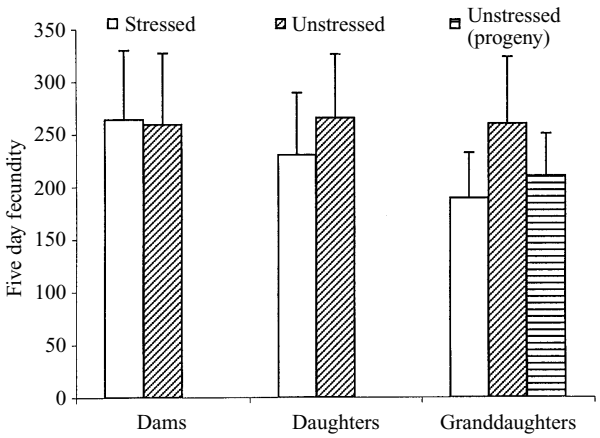

Fig. 2. Mean fecundity over 5 d for dams and their daughters and granddaughters. Error bars are standard deviations. Means and standard deviations are based on 118–235 individuals.

https://doi.org/10.1017/S0016672398003310 Published online by Cambridge University Press

Table 1. *Narrow-sense heritability* (h^2) *and evolvability* (I_A) *for 5-day fecundity*

Comparison	n	$h^2 \pm$ SE	P	I_A
Dams–daughters				
Stressed	70	$0{\cdot}552 \pm 0{\cdot}147$	$< 0{\cdot}001$	0·0245
Unstressed	121	$0{\cdot}127 \pm 0{\cdot}146$	0·385	0·0057
Daughters–granddaughters				
Stressed	160	$0{\cdot}427 \pm 0{\cdot}107$	$< 0{\cdot}001$	0·0223
Unstressed	234	$0{\cdot}180 \pm 0{\cdot}140$	0·198	0·0066

Probabilities are for one-tailed tests to determine whether estimates are significantly greater than zero. The number of families is given by n.

generation compared with the adult stressed generation. This difference was even more marked in the grandoffspring generation, when stressed and unstressed treatments also differed significantly ($t = 13{\cdot}29$, 430 d.f., $P < 0{\cdot}001$). The decrease in fecundity of the stressed flies over the three generations suggests that there are cumulative deleterious effects arising from the stressful conditions. Stress in the parental generation seems to lead to reduced fecundity in the offspring generation. This was further evident from the unstressed offspring of the stressed F_1 parents; these flies had a lower fecundity than females from unstressed parents ($t = 9{\cdot}49$, 428 d.f., $P < 0{\cdot}001$) although fecundity was higher than for stressed flies from the same generation. These effects of stress cannot be related to body size because size was decreased in the stress treatments by a similar amount in all generations (R. Woods, unpublished data).

Variance ratios indicated no differences in the variability of fecundity for comparisons undertaken on data from the parental ($F_{(137, 118)} = 1{\cdot}05$, $P = 0{\cdot}386$) and offspring ($F_{(236, 207)} = 1{\cdot}06$, $P = 0{\cdot}323$) generations. However, the unstressed treatment differed significantly from the stressed treatment in the grandoffspring generation ($F_{(235, 197)} = 2{\cdot}49$, $P < 0{\cdot}001$). In addition, the unstressed treatment differed significantly for variability in fecundity when compared with unstressed F_2 individuals from stressed F_1 generations ($F_{(235, 195)} = 2{\cdot}21$, $P < 0{\cdot}001$). These differences persist when coefficients of variation are computed for the unstressed (CV $= 24{\cdot}5 \pm 1{\cdot}20$) and stressed (CV $= 19{\cdot}2 \pm 1{\cdot}01$) treatments as well as the unstressed progeny from stressed F_1 generations (CV $= 22{\cdot}7 \pm 1{\cdot}20$). Stressful conditions therefore increase variability in fecundity when cumulative effects are sufficient to cause a marked decrease in fecundity.

(ii) *Genetic variability*

The three generations allow two estimates of heritable variation using parent–offspring regressions (i.e. parent–offspring and F_1–F_2). Heritabilities were computed from parent–offspring comparisons by doubling the regression coefficients, because fecundity was scored on only one sex. Results of these analyses (Table 1) suggest that stressful environmental conditions influenced the expression of heritable variation for fecundity. In both cases, only the comparison for the stressed environment is significant. Heritabilities in the stressful conditions were around 50%, compared with less than 20% in the unstressed conditions. However, standard errors were fairly large and it is therefore not immediately clear whether there are significant differences between the regression coefficients. To test this, we followed the procedure in Sokal & Rohlf (1995, p. 495) for comparing coefficients. For the parent–offspring comparison, the F value comparing the variance among and within regression coefficients was marginally non-significant ($F_{(1, 187)} = 3{\cdot}66$, $P = 0{\cdot}057$), while for the F_1–F_2 comparison it was not significant ($F_{(1, 387)} = 1{\cdot}53$, $P = 0{\cdot}219$). When probabilities are combined following Sokal & Rohlf (1995, p. 794), differences among the coefficients are marginally non-significant ($\chi^2 = 8{\cdot}79$, 4 d.f., $P = 0{\cdot}066$). Hence there is suggestive evidence for a difference in the coefficients between environments.

Estimates of evolvabilities are similar in the two comparisons (Table 1) and show similar patterns to the heritabilities. This is not surprising; because the phenotypic variances and means did not change much, heritability differences reflect changes in the additive genetic variance and this is directly reflected in the evolvabilities. Estimates of V_A were 1755 and 1249 respectively for the dam–daughter and F_1–F_2 comparisons in the stressed environment. Equivalent figures for the non-stressed environment were 397 and 478. Stressful conditions may therefore increase the expression of genetic variation, although large experimental designs are needed to demonstrate this effect.

Comparisons of the stressed F_1 flies with the unstressed F_2 flies indicate that at least some of the genetic effects are common across both environments. The regression coefficient ($0{\cdot}131 \pm 0{\cdot}066$) based on 153 families was significantly different from 0 ($P < 0{\cdot}05$)

https://doi.org/10.1017/S0016672398003310 Published online by Cambridge University Press

and suggests a 'cross-environment' heritability of around 25%.

4. Discussion

Previous studies have indicated that the heritability for life history traits may depend on environmental conditions (e.g. Service & Rose, 1985; Van Noordwijk *et al*., 1988; Etges, 1993; Holloway *et al*., 1990; Larrson *et al*., 1997; Sgrò & Hoffmann, 1997). The data presented here suggest that stress combinations may increase the heritability for fecundity. Our highest heritability estimates are similar to those reported by Rose & Charlesworth (1981) over 5 d at 25 °C, and by Sgrò & Hoffmann (1998*a*) at 14 °C over 10 d. In contrast, our lowest estimates are consistent with other studies suggesting that fecundity may often have a low heritability (Roff & Mousseau, 1987), and highlight the difficulty in making generalizations about the heritability of this trait.

Estimates of the variance components in our experiment suggest that the heritability changes were not associated with altered levels of environmental variability. Instead, the results suggest that V_A has increased under the combined stress treatments. This increase may reflect stress effects on the expression of genetic variation or a past history of selection. Because flies from the base population had not been reared for many generations under optimal laboratory conditions, it is difficult to determine whether the unstressed or stressed treatments were relatively more novel for the flies, particularly as flies would have encountered the types of stresses we considered under natural conditions.

Regardless of whether differences are due to past selection or direct stress effects, our results and those described previously (Sgrò & Hoffmann, 1998*a*) indicate that evolutionary changes in fecundity will depend on environmental conditions. Evolvability estimates for fecundity under stress are similar to those estimated by Houle (1992), but somewhat lower than those estimated by Sgrò & Hoffmann (1997) for fecundity at 14 °C and 28 °C. Culture and/or adult conditions may therefore have a marked influence on the extent to which traits will change under natural selection.

Our results also indicate that the rearing stresses did not directly influence fecundity; parental flies had the same mean fecundity regardless of whether they were stressed. However, there were cumulative effects on fecundity evident in the offspring and grandoffspring generations. These effects could be the result of two factors, namely selection and environmental effects that carry over across a generation. Selection could have occurred in the stressful treatment because a small proportion of the eggs placed in the vials survived the stressful conditions, inadvertently selecting for altered fecundity if there is a genetic correlation between stress resistance and fecundity. Previous studies suggest that a correlated decrease in fecundity due to selection is possible. There is evidence that increased stress resistance can decrease early fecundity (Hoffmann & Parsons, 1989; Hoffmann & Parsons, 1991). Genes affecting resistance to the stresses used here may have had this effect, particularly as genes increasing cold resistance in *D. melanogaster* are associated with a reduction in early fecundity (Watson & Hoffmann, 1996).

Carry-over effects have not been described in previous work on *D. melanogaster* considering the effects of culture temperatures on fecundity. Sgrò & Hoffmann (1998*a*) found no evidence for carry-over effects in a laboratory-adapted population of *D. melanogaster* exposed to 14 °C and 28 °C. Similarly, Huey *et al*. (1995) found little evidence for parental effects on fecundity in the laboratory in response to temperatures of 18 °C and 25 °C. Finally, Sgrò & Hoffmann (1998*b*) found no evidence for parental effects in response to extreme culture temperatures in *D. melanogaster* collected from a natural population, and their F_1 and F_2 generations reared in the laboratory. However, carry-over effects for fecundity may be induced by short exposures to more intense stresses. Watson & Hoffmann (1996) found that exposure of adults to −2 °C for a period sufficient to cause mortality resulted in a reduced fecundity in the offspring generation in both *D. melanogaster* and *D. simulans*.

In summary, our results suggest that exposure to combinations of environmental stresses may increase the expression of additive genetic variability for fecundity. This pattern is different from the one often seen for morphological traits in *Drosophila*, which either shows little change in heritability or a change largely due to effects on V_E (Robertson, 1964; Prout & Barker, 1989; Sgrò & Hoffmann, 1998*a* but see Moed *et al*., 1997). An additional test of these results and predictions would be to carry out a selection experiment under different environmental conditions. If the expression of additive variance is increased by the stressful conditions as suggested by these results, the response to selection will be greater under some combinations of extreme conditions.

This work was partially supported by a grant from the Australian Research Council. We thank Miriam Hercus and Richard Woods for assistance with the experiments.

References

Blum, A. (1988). *Plant Breeding for Stress Environments*. Boca Raton: CRC Press.

Etges, W. (1993). Genetics of host–cactus response and life history evolution among ancestral and derived populations of cactophilic *Drosophila mojavensis*. *Evolution* **47**, 750–767.

https://doi.org/10.1017/S0016672398003310 Published online by Cambridge University Press

Gebhardt, M. D. & Stearns, S. C. (1992). Phenotypic plasticity for life-history traits in *Drosophila melanogaster*. III. Effect of the environment on genetic parameters. *Genetical Research* **60**, 87–101.

Gibson, J. B., May, T. W. & Wilks, A. V. (1981). Genetic variation at the alcohol dehydrogenase locus in *Drosophila melanogaster* in relation to environmental variation: ethanol levels in breeding sites and allozyme frequencies. *Oecologia* **51**, 191–198.

Hoffmann, A. A. & Parsons, P. A. (1989). An integrated approach to environmental stress tolerance and life history variation: desiccation tolerance in *Drosophila*. *Biological Journal of the Linnean Society* **37**, 117–136.

Hoffmann, A. A. & Parsons, P. A. (1991). *Evolutionary Genetics and Enviromental Stress*. Oxford: Oxford University Press.

Holloway, G. J., Povey, S. R. & Sibly, R. M. (1990). The effect of new environment on adapted genetic architecture. *Heredity* **64**, 323–330.

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Huey, R. B., Wakefield, T., Crill, W. D. & Gilchrist, W. (1995). Within- and between-generation effects of temperature on early fecundity of *Drosophila melanogaster*. *Heredity* **74**, 216–223.

Jinks, J. L., Perkins, J. M. & Pooni, H. S. (1973). The incidence of epistasis in normal and extreme environments. *Heredity* **31**, 263–269.

Kawecki, T. J. (1995). Expression of genetic and environmental variation for life history characters on the usual and novel hosts in *Callosobruchus maculatus* (Coleoptera, Bruchidae). *Heredity* **75**, 70–76.

Larrson, K., Rattiste, K. & Lilleleht, V. (1997). Heritability of headsize in the common gull *Larus canus* in relation to environmental conditions during offspring growth. *Heredity* **79**, 201–207.

McKechnie, S. W. & Morgan, P. (1982). Alcohol dehydrogenase polymorphism in *Drosophila melanogaster*: aspects of alcohol and temperature variation in the larval environment. *Australian Journal of Biological Sciences* **35**, 85–93.

Moed, G. H., De Jong, G. & Scharloo, W. (1997). Environmental effects on body size variation in *Drosophila melanogaster* and its cellular basis. *Genetical Research* **70**, 35–43.

Neyfakh, A. A. & Hartl, D. L. (1993). Genetic control of the rate of embryonic development: selection for faster development at elevated temperatures. *Evolution* **47**, 1625–1631.

Parsons, P. A. (1963). Parental age and the offspring. *Biological Reviews* **39**, 258–275.

Parsons, P. A. (1982). Evolutionary ecology of Australian *Drosophila*: a species analysis. *Evolutionary Biology* **14**, 297–350.

Parsons, P. A. (1987). Evolutionary rates under environmental stress. *Evolutionary Biology* **21**, 311–347.

Pigliucci, M., Schlichting, C. D. & Whitton, J. (1995). Reaction norms of *Arabidopsis*. 2. Response to stress and unordered environmental variation. *Functional Ecology* **9**, 537–547.

Prout, T. & Barker, J. S. F. (1989). Ecological aspects of the heritability of body size in *Drosophila buzzatii*. *Genetics* **123**, 803–813.

Robertson, F. W. (1964). The ecological genetics of growth in *Drosophila*. *Genetical Research* **5**, 107–126.

Roff, D. A. & Mousseau, T. A. (1987). Quantitative genetics and fitness: lessions from *Drosophila*. *Heredity* **58**, 103–118.

Rose, M. R. & Charlesworth, B. (1981). Genetics of life history in *Drosophila melanogaster*. I. Sib analysis of adult females. *Genetics* **97**, 173–186.

Service, P. M. & Rose, M. R. (1985). Genetic covariation among life-history components: the effects of novel environment. *Evolution* **39**, 943–945.

Sgrò, C. M. & Hoffmann, A. A. (1998*a*). Effects of temperature extremes on genetic variances for life history traits in *Drosophila melanogaster* as determined from parent–offspring regression. *Journal of Evolutionary Biology* **11**, 1–20.

Sgrò, C. M. & Hoffmann, A. A. (1998*b*). Heritable variation for fecundity in field-collected *D. melanogaster* and their offspring reared under different environmental temperatures. *Evolution* **52**, 134–143.

Sokal, R. R. & Rohlf, F. J. (1995). *Biometry*, 3rd edn. New York: Freeman.

Tantawy, A. O. & El-Helw, M. R. (1970). Studies on natural populations of *Drosophila*. IX. Some fitness components and their heritabilities in natural and mutant populations of *Drosophila melanogaster*. *Genetics* **64**, 79–91.

Van Noordwijk, A. J., Van Balen, J. H. & Scharloo, W. (1988). Heritability of body size in a natural population of Great Tit (*Parsus major*) and its relation to age and environmental conditions during growth. *Genetical Research* **51**, 149–162.

Waddington, C. H. (1961). Genetic assimilation. *Advances in Genetics* **10**, 257–293.

Watson, M. J. O. & Hoffmann, A. A. (1996). Acclimation, cross-generation effects, and the response to selection for increased cold resistance in *Drosophila*. *Evolution* **50**, 1182–1192.

https://doi.org/10.1017/S0016672398003310 Published online by Cambridge University Press