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# Heat-shock tolerance and inbreeding in *Drosophila buzzatii*

JESPER DAHLGAARD*, ROBERT A. KREBS & VOLKER LOESCHCKE
*Department of Genetics and Ecology, University of Aarhus, Ny Munkegade Bldg. 540, 8000 Aarhus C, Denmark*

The effect of inbreeding on survival after a short-term heat shock was tested for two age groups of the cactophilic fruit fly, *Drosophila buzzatii*, reared under nonstress conditions. Four inbreeding levels ($F = 0$, $F = 0.25$, $F = 0.375$, $F = 0.5$) were generated by outcrossing or full-sib mating. All flies were conditioned at 36.5°C for 75 min prior to exposure to stress, to activate the synthesis of heat-shock proteins. These proteins are known to protect cells against stress damage. The younger group of flies were exposed to a thermal stress of 40.7°C for 88 min, 103 min, or 118 min and the older flies to the same temperature only for 88 min or 103 min, as the survival of older flies after heat stress was much lower than that of the younger flies. Survival after heat shock declined with increased inbreeding in both age groups. For the younger flies, the slope of the regression line, $F$, on survival was lower at higher stress levels. For the older flies, inbreeding effects were similar at both stress levels. Mortality without stress also differed significantly among inbreeding groups, mainly because of a large difference between the $F = 0.5$ group and all others.

**Keywords:** *Drosophila buzzatii*, heat shock, inbreeding, interactions, stress tolerance, survival.

## Introduction

Inbreeding may reduce the performance of individuals in fluctuating or stressful environments due to a larger environmental sensitivity of inbred individuals to both abiotic and biotic stress factors (Maynard Smith, 1956; Pederson, 1968; Hoffmann & Parsons, 1991; Parsons, 1992). Additionally, the adaptive potential of populations (Beardmore, 1983) and the potential to evolve stress resistance may be limited where increased inbreeding causes a loss of genetic variation.

The environment influences the magnitude of inbreeding depression (Langridge, 1962; Barlow, 1981; Komaki, 1982; Levin, 1984; Ruban *et al.*, 1988; Dudash, 1990). Inbreeding depression and its counterpart, heterosis, have been shown generally to be larger in harsh environments than in more benign ones for a variety of traits: reproductive output, viability, viability to first reproduction and growth rate (Parsons, 1959; Griffing & Langridge, 1963; Pederson, 1968; Ruban et al., 1988; Dudash, 1990). The majority of stress types that have been studied, however, were not severe ones.

Developmental acclimatization for tolerance against high temperature stress in dry air was much greater for outbred than for inbred adults of *Drosophila subobscura* (Maynard Smith, 1956). Inbreeding similarly affected cold-shock tolerance in *D. melanogaster* (Ehiobu *et al.*, 1989). Interactions between stress and inbreeding at a range near the physiological limit of an organism, however, have not been analysed.

Here we report on the potential interaction between the degree of inbreeding and the severity of thermal stress on survival in two age groups of adult *D. buzzatii*. For this stress treatment, genetic variation for survival is present (Loeschcke *et al.*, 1994; Krebs & Loeschcke, 1994a). In all experiments, flies were first conditioned by a short exposure to a nonlethal thermal stress to activate the genes coding for heat-shock proteins (DiDomenico *et al.*, 1982a,b) before the flies were exposed to a potentially lethal heat shock. These heat-shock proteins provide some protection to cells from stress damage and increase the proportion of individuals that survives exposure to high temperatures (Lindquist, 1986).

In nature *D. buzzatii* feeds and breeds in association with rotting cladodes of several *Opuntia* species (Barker & Mulley, 1976). Temperatures in and around these rots may greatly exceed ambient (Krebs & Loeschcke, 1994a), possibly subjecting individuals to short-term exposures of thermal stress. The cactus niche provides an ephemeral patch structure as a result of desiccation of the rotting cladodes after one or two

*Correspondence.

generations (Prout & Barker, 1989). Often only a small number of females contribute offspring to each rot (Thomas & Barker, 1990). Therefore, periods of local mating between relatives may occur followed by dispersal and mixed mating when flies migrate to new feeding and breeding sites (Prout & Barker, 1993).

## Materials and methods

The *D. buzzatii* population used here originated from seven isofemale lines that were collected in March 1991 in Argentina, near El Chañar, in the Tucuman province (Lat. 26°48′S). Initially, lines were maintained in the laboratory of J.S.F Barker in four vials, five pairs per vial, on a cactus-supplemented medium from April 1991. A mass population was derived by combining equal numbers of males and females from each line in September 1992, and this population was maintained for five further generations at 25°C under continuous light (with 10 bottles per generation, 20–30 pairs per bottle, using instant *Drosophila* medium from Carolina Biological Supply).

Lines of four inbreeding levels, $F=0$, $F=0.25$, $F=0.375$ and $F=0.5$, were prepared following the scheme presented in Fig. 1. For each of the 18 lines within each inbreeding level, four pairs of offspring were placed separately in vials, with offspring of only one of these four vials contributing to subsequent generations. For the final cross, two males were placed with each female in vials, and these flies were transferred every 2 days to fresh medium to provide sufficient numbers of flies for the experiments.

Upon emergence, virgin flies of each inbreeding group were collected daily, mixed among all vials, separated by sex under light ether anaesthesia, and placed in groups of about 20 individuals per vial on a yeast-sucrose-agar medium with added live yeast. Flies were transferred to fresh vials every 3 days, again prior to conditioning and prior to exposure to the heat stress. From *Drosophila melanogaster*, it is known that inbreeding does not affect size (Kidwell & Kidwell, 1966). However, because larval density differences due to inbreeding depression potentially could occur and give rise to size differences between outbred and inbred flies, and because density is known to affect heat-shock tolerance (Loeschcke *et al.*, 1994), a sample of flies was weighed. Differences in size among inbreeding groups were small and not significant. In the

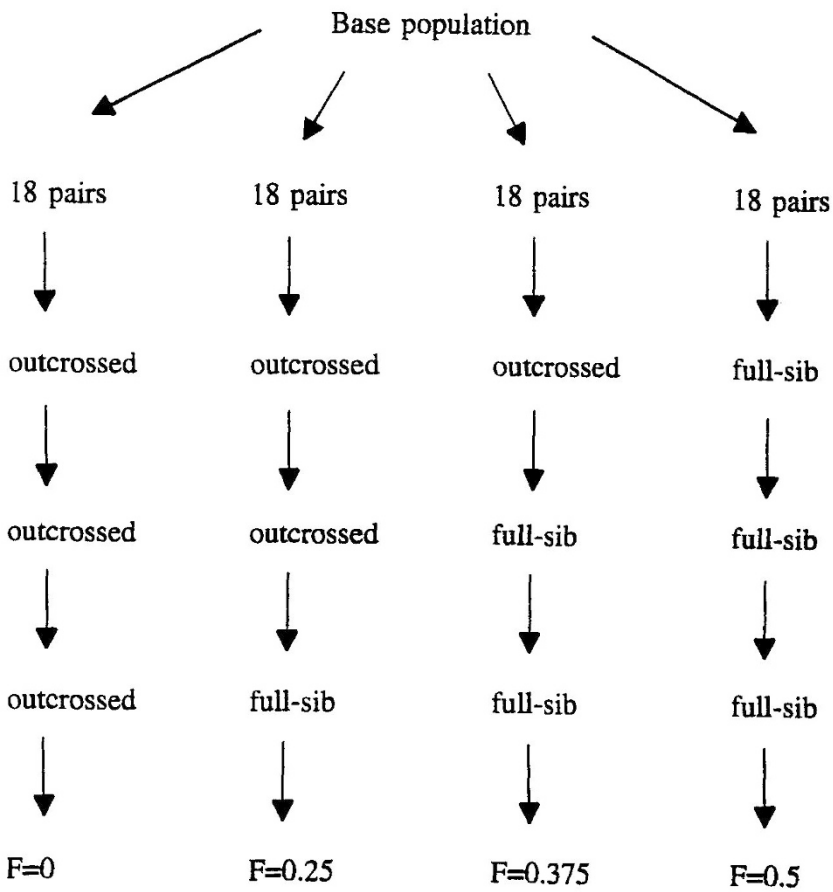


**Fig. 1** Scheme for the production of flies at the four inbreeding levels. All inbreeding groups were derived from the same number of pairs from the same base population. Females either were paired to a male from the base population (outcrossed) or were crossed with a brother (full-sib).

second experiment, mortality was recorded under nonstress conditions (25°C) as flies were transferred to new vials: days 3, 6, 9, 12 and 15.

The conditioning treatment was exposure to 36.5°C (within vials) for 75 min, which was performed 24 h before flies were exposed to a potentially lethal heat stress at 40.7°C (within vials) for varying periods of time. Both conditioning and exposure to the heat stress were performed in inverted food vials with stoppers moistened and inserted fully to obtain nearly saturated humidity (treatment details described in Krebs & Loeschcke, 1994b). After treatment, flies were returned immediately to 25°C. Flies were exposed to heat stress for time periods 88, 103 and 118 min for the younger set of flies (aged 7–8 days old), whereas only the two shorter time periods were used for the older flies (aged 16 days old). For both age groups of flies, the experiment was carried out in three blocks each 2 days apart. For the younger flies there were eight replicates of each inbreeding level and sex within each block, and for the older flies there were six.

The proportion of surviving flies was scored 22 h after exposure to heat stress to allow survivors time to recover mobility. Flies were considered alive if they were able to walk following a slight touch with a brush. Each vial was assigned a reference number that after scoring (blind) could be cross-referenced to determine the inbreeding group. Mortality during conditioning was less than 1 per cent of younger flies and less than 4 per cent of older flies. Differences in mortality among inbreeding groups during conditioning were not significant and those that died at this stage were scored as nonsurvivors to heat stress. All survival data were arcsine-square-root transformed for analysis of variance, with sex and stress treated as fixed factors and inbreeding as a continuous variable (SAS Institute, 1989, general linear models procedure).

**Table 1** ANOVA for survival of *Drosophila buzzatii* males and females after heat shock, at two ages, across either two (old flies) or three stress levels (young flies), with four inbreeding groups ($F = 0$, $F = 0.25$, $F = 0.375$, $F = 0.5$)

| | 7–8 days old | | 16 days old | |
|---|---|---|---|---|
| Source | d.f. | MS | d.f. | MS |
| Block (stress) | 6 | 0.70*** | 4 | 0.20* |
| Stress (St) | 2 | 10.15*** | 1 | 6.24*** |
| Inbreeding ($F$) | 1 | 0.64** | 1 | 0.61** |
| Sex | 1 | 0.03 NS | 1 | 0.03 NS |
| St × F | 2 | 0.15 NS | 1 | 0.00 NS |
| St × Sex | 2 | 0.03 NS | 1 | 0.01 NS |
| F × Sex | 1 | 0.01 NS | 1 | 0.02 NS |
| St × $F$ × Sex | 2 | 0.08 NS | 1 | 0.00 NS |
| Error | 544 | 0.059 | 287 | 0.065 |

Data were percentage survival, arcsin-square-root transformed before analysis.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

## Results

### *Survival to heat stress*

The effect of block within stress was significant for both the younger flies and the older flies (Table 1). In preliminary analyses, no significant interactions with block (stress) were found. Therefore, interactions with block were pooled in the error variance.

The effect of inbreeding on survival (Fig. 2) was significant both for the younger and older flies ($P < 0.01$, Table 1), as was the effect of the stress treatment ($P < 0.001$, Table 1). The sexes were not different in survival after stress (Table 1) nor were any interactions that included sex significant. Therefore, means for males and females were presented together (Fig. 2). The stress × inbreeding interaction was not significant in either experiment although for younger flies, the interaction approached significance ($P = 0.07$), as

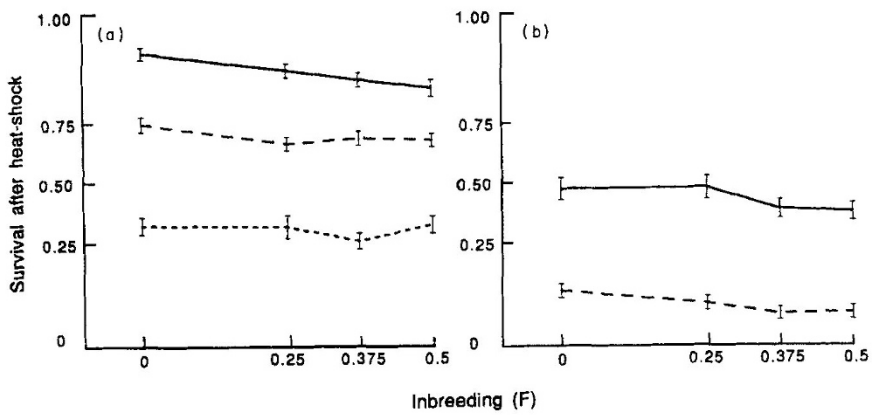


**Fig. 2** Mean survival (± SE) after heat shock of *Drosophila buzzatii* adults at four inbreeding levels, (a) 7–8-day-old flies exposed to heat shock for 88 min (solid line), 103 min (dashed line), or 118 min (fine dashed line) and (b) 16-day-old flies exposed to heat shock for 88 min (solid line) or 103 min (dashed line). The scale is arcsin-square-root transformed. Sample sizes for the 7–8-day-old and 16-day-old flies were respectively 94–96 and 76–78 vials, with about 20 flies per vial.

differences among inbreeding groups declined with increasing stress level.

Analysing inbreeding effects for each stress level (Table 2), the regression coefficient for the effect of inbreeding on survival was significant for the 7–8-day-old flies at 88 min, approached significance ($P<0.1$) for the 103 min treatment and was not significant for the 118 min treatment. For the 16-day-old flies, the regression coefficient was significant at 103 min, and approached significance for the 88 min treatment (Table 2). As all regression coefficients were negative, a combined probability analysis ($\chi^2_{10}=37.5$, $P<0.001$) supports the significance of general inbreeding effects on survival to heat stress. Although inbreeding effects are not expected to be linear (Falconer, 1989), the addition of a polynomial term to the regression models did not significantly improve the explanatory power of the variation among inbreeding levels.

### *Adult mortality without stress*

The inbreeding level had a significant effect ($P<0.001$) on cumulative adult mortality, based on individual ANOVAs for days 3, 6, 9, 12 and 15 (analyses not shown). Significance was due mainly to the higher mortality in the most inbred group (Fig. 3). Cumulative mortality was not significantly different among the three other groups (by Tukey's multiple comparisons test). Across all inbreeding groups, mortality of males was significantly higher than that of females (males $12.3\pm0.8$ per cent, females $5.9\pm0.6$ per cent, $P<0.05$), but no inbreeding level × sex interaction effects were significant. Therefore differences among inbreeding groups in Fig. 2 were presented with results for males and females pooled.

## Discussion

One difficulty when analysing interactions between inbreeding and stress is the limited consensus in the literature for how a stress is defined (Hoffmann & Parsons, 1991). Any condition that reduces growth or yield may be considered stressful, as well as treatments that potentially kill the organism. The heat-shock exposures of the type used here approach the tolerance limit of individuals and generally cause severe physiological damage to outbred flies (Krebs & Loeschcke, 1994a). Furthermore, the additive genetic component of variance may change under stress (Ward, 1994). Therefore, predictions for stress interactions with

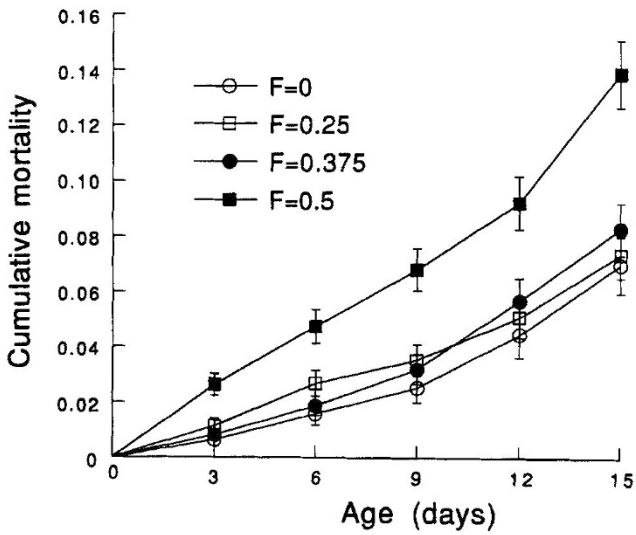


**Fig. 3** Mean cumulative mortality (±SE) of *Drosophila buzzatii* adults in nonstress conditions at four inbreeding levels, scored on days 3, 6, 9, 12 and 15 after eclosure. Sample size was 76–78 vials with about 20 flies per vial.

**Table 2** Regression equations for $F$ on survival of *Drosophila buzzatii* after heat shock, at two ages, across either two (old flies) or three stress levels (young flies), with four inbreeding groups ($F=0$, $F=0.25$, $F=0.375$, $F=0.5$)

| | Regression equation, $F$ on survival: $Y_i=\beta_0+\beta_1(F_i)$ | | |
|---|---|---|---|
| Stress | $\beta_0$(±SE) | $\beta_1$(±SE) | $P$ value for $H_0$: $\beta_1=0$ |
| 7–8 days old | | | |
| 88 min | 1.41(0.03) | −0.35(0.09) | 0.0002 |
| 103 min | 1.05(0.03) | −0.15(0.09) | 0.092 |
| 118 min | 0.57(0.04) | −0.04(0.12) | 0.720 |
| 16 days old | | | |
| 88 min | 0.77(0.04) | −0.25(0.13) | 0.060 |
| 103 min | 0.26(0.03) | −0.23(0.09) | 0.009 |

Data were percentage survival, arcsin-square-root transformed before analysis.

inbreeding depression may depend on the type and severity of the stress administered.

Tolerance against a severe short-term thermal stress declined with increasing inbreeding level both in young and in old adults of *D. buzzatii*. Overall, these results provided further support that inbred individuals are less buffered against environmental variation than are outbred individuals (Lerner, 1954; Zouros *et al.*, 1980; Parsons, 1992). Selection against inbreeding has been proposed to be more intense in stressful environments than in more favourable environments (Komaki, 1982; Levin, 1984), a result supported by several studies on inbred lines and their hybrids (Parsons, 1959; Langridge, 1962; Barlow, 1981). For the severe stress condition, exposure to heat shock, we found that inbreeding effects did not increase with increasing the stress intensity, and that the effect of inbreeding actually may decline at stress conditions approaching physiological limits.

With inbreeding, a decrease in mean performance for most characters related to fitness is expected (Falconer, 1989). Additionally, the variance of traits may increase in response to inbreeding even when the mean for the trait declines (Hauser *et al.*, 1994). Consequently, the mode of selection imposed by the environment becomes important for predicting the outcome of inbreeding. At the lower stress intensities, groups with high inbreeding levels may contain individuals that die under low stress or nonstress conditions. For older flies, mortality after the conditioning treatment was 5.4 per cent in the most inbred group whereas it was about 3 per cent in the three other groups, and mortality at 25°C also was higher for the most inbred flies (Fig. 3). At the opposite extreme, where a stress kills most individuals, an increase in the variance with inbreeding would create the possibility of some individuals in all inbreeding groups withstanding the stress treatment, and the differences in survivorship among groups would decrease with a decrease in the number of survivors. This effect was most clear in young flies stressed for 88 min compared with those stressed for longer durations. A similar comparison cannot be made for the older flies where the lower stress still caused 50 per cent mortality.

The effect of inbreeding on heat-shock tolerance may best be explained by the expression of rare recessive alleles that reduce fitness under hot conditions. The dominance hypothesis of inbreeding depression, i.e. that rare deleterious rather than overdominant genes cause the observed fitness decline, is favoured in most studies (Simmons & Crow, 1977; Wright, 1977; Charlesworth & Charlesworth, 1987; Savolainen, 1994). Kacser & Burns (1981) also emphasized that most deleterious mutations are recessive or partially recessive. For heat-shock tolerance, dominance effects may result from a class of alleles that are deleterious recessive at high temperatures, but selectively neutral at lower temperatures (Langridge, 1962, 1968). The presence of temperature sensitive alleles would cause a continuous reduction in survival to heat stress following increased inbreeding both in young and old flies. Alternatively, survival after a heat shock would be reduced by inbreeding if tolerance to stress has a cost that enlarges pre-existing differences in fitness among individuals that differ in inbreeding level (Hauser, 1994).

The 16-day-old flies were much more susceptible to heat stress than were the younger flies, demonstrating ageing effects on survival to heat stress for an age range below that at which mortality is common without stress (Partridge, 1988; Partridge & Barton, 1993). However, effects of inbreeding on stress tolerance were similar in both age groups despite apparent selection against weak individuals, at least in the most inbred group. Mortality without stress between days 9 and 15 was about 5 per cent of the outbred flies and about 7 per cent of the most inbred group. This suggests that the cause of mortality without stress may be independent of that under the stress treatment. Temperature sensitive alleles, if present, would contribute similarly to the reduction in survival of flies at all ages because natural selection against these alleles would not occur until exposure to the high temperature.

In the experiments presented here, two factors could have reduced inbreeding effects on heat tolerance below that which may occur naturally. First, maintenance of isofemale lines for several generations will have caused some purging of deleterious alleles through slow inbreeding within lines. Secondly, during preparation of inbred lines by full-sib mating (Fig. 1), pairs that were not productive were eliminated providing the possibility for further purging of deleterious alleles, as pairs carrying a highly deleterious allele, albeit one expressed at 25°C, would not be very productive. As a consequence, actual inbreeding levels would be lower than those predicted from coancestry.

One deviation was observed between these and previous experiments on heat-shock tolerance in *D. buzzatii*. Survival after heat stress generally is greater for males than for females (Loeschcke *et al.*, 1994; Krebs & Loeschcke, 1994a). Here we found no significant sex differences or sex by stress interaction terms on survival, although survival of males was numerically greater than that of females at the two lower stress levels. At the highest stress level, survival of females numerically exceeded that of males. Changes in which sex survives better have also been observed as stress levels were increased in other experiments

(unpublished data). The cause of this apparent sex–stress interaction, however, is not understood.

The potential decline in fitness that follows inbreeding has received much attention. Our results emphasize that stress tolerance also may be reduced by inbreeding. Inbreeding in conjunction with drift may deplete genetic variation and therefore the ability of organisms to adapt to stressful environments also may be diminished. For *D. buzzatii*, the occurrence of inbreeding and exposure to high temperatures is common in nature and thus purging of alleles that specifically reduce tolerance against high temperatures may occur. Consequently, the effects of inbreeding on temperature tolerance potentially may be larger for boreal *Drosophila* species that experience heat stress in nature less often.

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## References

BARKER, J. S. F. AND MULLEY, J. C. 1976. Isoenzyme variation in natural populations of *Drosophila buzzatii*. *Evolution*, **30**, 213–233.

BARLOW, R. 1981. Experimental evidence for interaction between heterosis and environment in animals. *Anim. Breed. Abst.*, **49**, 715–737.

BEARDMORE, J. A. 1983. Extinction, survival, and genetic variation. In: Schonewald-Cox, C. M., Chambers, S. M., MacBryde, B. and Thomas, W. L. (eds) *Genetics and Conservation*, pp. 125–151. Benjamin/Cummings, London.

CHARLESWORTH, D. AND CHARLESWORTH, B. 1987. Inbreeding depression and its evolutionary consequences. *Ann. Rev. Ecol. Syst.*, **18**, 237–268.

DIDOMENICO, B. J., BUGAISKY, G. E. AND LINDQUIST, S. 1982a. The heat shock response is self-regulated at both the transcriptional and posttranscriptional levels. *Cell*, **31**, 593–603.

DIDOMENICO, B. J., BUGAISKY, G. E. AND LINDQUIST, S. 1982b. Heat shock and recovery are mediated by different translational mechanisms. *Proc. Natl. Acad. Sci. U.S.A.*, **79**, 6181–6185.

DUDASH, M. R. 1990. Relative fitness of selfed and outcrossed progeny in a self-compatible, protandrous species, *Sabatia angularis* L. (Gentianaceae): a comparison in three environments. *Evolution*, **44**, 1129–1139.

EHIOBU, N. G., GODDARD, M. E. AND TAYLOR, J. F. 1989. Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*. *Theor. Appl. Genet.*, **77**, 123–127.

FALCONER, D. S. 1989. *Introduction to Quantitative Genetics*, 3rd edn. John Wiley and Sons, New York.

GRIFFING, B. AND LANGRIDGE, J. 1963. Phenotypic stability of growth in the self-fertilized species, *Arabidopsis thaliana*. In: Hanson, W. D. and Robinson, H. F. (eds) *Symposium on Statistical Genetics and Plant Breeding*, Publ. 982. National Academy of Sciences-National Research Council, Washington, DC.

HAUSER, T. P. 1994. *Inbreeding Depression in Plants: Experimental Results and Evolutionary Implications*. Ph.D. Thesis, University of Aarhus, Århus, Denmark.

HAUSER, T. P., DAMGAARD, C. AND LOESCHCKE, V. 1994. Effects of inbreeding in small plant populations: expectations, and implications for conservation. In: Loeschcke, V., Tomiuk, J. and Jain, S. K. (eds) *Conservation Genetics*, pp. 115–130. Birkhäuser, Basel.

HOFFMANN, A. A. AND PARSONS, P. A. 1991. *Evolutionary Genetics and Environmental Stress*. Oxford Science Publications, Oxford.

KACSER, H. AND BURNS, J. A. 1981. The molecular basis of dominance. *Genetics*, **97**, 639–666.

KIDWELL, J. F. AND KIDWELL, M. M. 1966. The effects of inbreeding on body weight and abdominal chaeta number in *Drosophila melanogaster*. *Can. J. Genet. Cytol.*, **8**, 207–215.

KOMAKI, M. K. 1982. Inbreeding depression and concealed deleterious mutations in buckwheat populations, *Fagopyrum esculentum*. *Jap. J. Genet.*, **57**, 361–370.

KREBS, R. A. AND LOESCHCKE, V. 1994a. Response to environmental change: genetic variation and fitness in *Drosophila buzzatii* following temperature stress. In: Loeschcke, V., Tomiuk, J. and Jain, S. K. (eds) *Conservation Genetics*, pp. 309–321. Birkhäuser, Basel.

KREBS, R. A. AND LOESCHCKE, V. 1994b. Effects of exposure to short-term heat stress on fitness components in *Drosophila melanogaster*. *J. Evol. Biol.*, **7**, 39–49.

LANGRIDGE, J. 1962. A genetic and molecular basis for heterosis in *Arabidopsis* and *Drosophila*. *Am. Nat.*, **96**, 5–27.

LANGRIDGE, J. 1968. Thermal responses of mutant enzymes and temperature limits to growth. *Mol. Gen. Genet.*, **103**, 116–126.

LERNER, M. I. 1954. *Genetic Homeostasis*. Oliver and Boyd, Edinburgh.

LEVIN, D. A. 1984. Inbreeding depression and proximity-dependent crossing success in *Phlox drummondii*. *Evolution*, **38**, 116–127.

LINDQUIST, S. 1986. The heat-shock response. *Ann. Rev. Biochem.*, **55**, 1151–1191

LOESCHCKE, V., KREBS, R. A. AND BARKER, J. S. F. 1994. Genetic variation for resistance and acclimation to high temperature stress in *Drosophila buzzatii*. *Biol. J. Linn. Soc.*, **52**, 83–92.

MAYNARD SMITH, J. 1956. Acclimatization to high temperatures in inbred and outbred *Drosophila subobscura*. *J. Genet.*, **54**, 497–505.

PARTRIDGE, L. 1988. Lifetime reproductive success in *Drosophila*. In: Clutton-Brock, T. H. (ed) *Reproductive Success*,

pp. 11–23. University of Chicago Press, Chicago.

PARTRIDGE, L. AND BARTON, N. H. 1993. Optimality, mutation and the evolution of ageing. *Nature*, **362**, 305–311.

PARSONS, P. A. 1959. Genotypic-environmental interactions for various temperatures in *Drosophila melanogaster*. *Genetics*, **44**, 1325–1333.

PARSONS, P. A. 1992. Fluctuating asymmetry: a biological monitor of environmental and genomic stress. *Heredity*, **68**, 361–364.

PEDERSON, D. G. 1968. Environmental stress, heterozygote advantage and genotype-environment interaction in *Arabidopsis*. *Heredity*, **23**, 127–138.

PROUT, T. AND BARKER, J. S. F. 1989. Ecological aspects of the heritability of body size in *Drosophila buzzatii*. *Genetics*, **123**, 803–813.

PROUT, T. AND BARKER, J. S. F. 1993. *F* statistics in *Drosophila buzzatii*: selection, population size and inbreeding. *Genetics*, **134**, 369–375.

RUBAN, P. S., CUNNINGHAM, E. P. AND SHARP, P. M. 1988. Heterosis × nutrition interaction in *Drosophila melanogaster*. *Theor. Appl. Genet.*, **76**, 136–142.

SAS INSTITUTE 1989. *SAS/Stat Users Guide*. SAS Institute Inc., Cary, North Carolina.

SAVOLAINEN, O. 1994. Genetic variation and fitness: conservation lessons from pines. In: Loeschcke V., Tomiuk, J. and Jain, S. K. (eds) *Conservation Genetics*, pp. 27–36. Birkhäuser, Basel.

SIMMONS, M. J. AND CROW, J. F. 1977. Mutations affecting fitness in *Drosophila* populations. *Ann. Rev. Genet.*, **11**, 49–78.

THOMAS, R. H. AND BARKER, J. S. F. 1990. Breeding structure of natural populations of *Drosophila buzzatii*: effects of the distribution of larval substrates. *Heredity*, **64**, 355–365.

WARD, P. J. 1994. Parent–offspring regression and extreme environments. *Heredity*, **72**, 574–581.

WRIGHT, S. 1977. *Evolution and the Genetics of Populations*, vol. 3, *Experimental Results and Evolutionary Deductions*. University of Chicago Press, Chicago.

ZOUROS, E., SINGH, S. M. AND MILES, H. E. 1980. Growth rate in oysters: an overdominant phenotype and its possible explanation. *Evolution*, **34**, 856–867.