The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2011, **104**, 432–442. With 5 figures

The effects of inbreeding and heat stress on male sterility in *Drosophila melanogaster*

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Received 15 December 2010; revised 7 April 2011; accepted for publication 8 April 2011

Understanding the consequences of inbreeding in combination with stress is important for the persistence of small endangered populations in a changing environment. Inbreeding and stress can influence the population at all stages of the life cycle, and in the last two decades a number of studies have demonstrated inbreeding depression for most life-cycle components, both in laboratory populations and in the wild. Although male fertility is known to be sensitive to temperature extremes, few studies have focused on this life-cycle component. We studied the effects of inbreeding on male sterility in benign and stressful environments using *Drosophila melanogaster* as a model organism. Male sterility was compared in 21 inbred lines and five non-inbred control lines at 25.0 and 29.0 °C. The effect of inbreeding on sterility was significant only at 29.0 °C. This stress-induced increase in sterility indicates an interaction between the effects of inbreeding and high-temperature stress on male sterility. In addition, the stress-induced temporary and permanent sterility showed significant positive correlation, as did stress-induced sterility and the decrease in egg-to-adult viability. This suggests that the observed stress-induced decline in fitness could result from conditionally expressed, recessive deleterious alleles affecting both sterility and viability simultaneously. *Biological Journal of the Linnean Society*, 2011, **104**, 432–442.

ADDITIONAL KEYWORDS: egg-to-adult viability – temperature stress.

INTRODUCTION

The detrimental effects of inbreeding in normally outbreeding organisms have been recognized since Darwin's studies in the late 19th century (Darwin, 1876, 1885). Since then, inbreeding depression has been documented numerous times for both laboratory and domestic species (Lynch & Walsh, 1998; Frankham, Ballou & Briscoe, 2002; Kristensen & Sørensen, 2005; Fox & Reed, 2011). Within the last two decades, data on the occurrence of inbreeding depression in the wild has also been accumulating for many species, including insects, fish, birds, mammals, and plants (e.g. Vrijenhoek, 1994; Keller, 1998; Hedrick & Kalinowski, 2000; Keller & Waller, 2002; Armbruster & Reed, 2005; Frankham, 2005; Kristensen *et al*., 2008a; Fox & Reed, 2011). In fact, there is evidence to support that inbreeding depression is more severe in natural environments (Crnokrak & Roff, 1999; Armbruster & Reed, 2005; Kristensen, Loeschcke & Hoffman, 2008b; Kristensen *et al*., 2008a).

In natural environments, organisms are often exposed to stressful conditions (Parsons, 1993;

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White, 1993; Hoffmann & Parsons, 1997; Hedrick & Kalinowski, 2000), and the increased genetic load expressed by inbreeding might cause populations to be more vulnerable to the effects of stress. This could partly result from the fact that a change in the environment may lead to the exposure of conditionally expressed detrimental alleles, which do not have adverse effects on fitness under benign environmental conditions (Bijlsma, Bundgaard & Van Putten, 1999; Vermeulen & Bijlsma, 2004a, b; Kristensen *et al*., 2008b; Bakker *et al*., 2010).

Increased sensitivity to environmental stress caused by inbreeding has been found in a number of studies, especially in *Drosophila*, where several fitness parameters have been shown to be affected, including viability at different life stages, growth rate, productivity, and female fertility (e.g. Coman & Wallace, 1973; Miller, 1994; Bijlsma *et al*., 1999; Bijlsma, Bundgaard & Boerema, 2000; Armbruster & Reed, 2005). Previous work has shown that short-term heat stress reduces the fecundity of inbred lines more than that of outbred lines of *Drosophila* (Dahlgaard, Krebs & Loeschcke, 1995; Dahlgaard & Hoffmann, 2000; Pedersen, Kristensen & Loeschcke, 2005). However, rather limited work has been performed on male reproductive traits, although impaired male reproductive success, temporary or permanent, could have pronounced consequences, especially in populations with polygynous mating systems (Miller & Hedrick, 1993; Enders & Nunney, 2010). Miller & Hedrick (1993) studied lines homozygous for the second chromosome in *Drosophila melanogaster*, and demonstrated a significant reduction in male mating ability as a result of inbreeding, and a similar result was found by Miller, Glasner & Hedrick (1993) and Enders & Nunney (2010). It has now been clearly established that inbreeding has adverse effects on male fertility in many animals, including insects and mammals (Saccheri *et al*., 2005; Asa *et al*., 2007; Fitzpatrick & Evans, 2009; Zajitschek *et al*., 2009; Okada *et al*., 2011).

Until now, the combined effect of inbreeding and stress on male sterility has, to the best of our knowledge, not been investigated, despite the fact that male fertility (and sterility) in *Drosophila*, and many other species, is known to be strongly affected by stressful temperatures, and more so than other components of fitness (David *et al*., 1983; Jørgensen, Sørensen & Bundgaard, 2006). Because male sterility is sensitive to extreme temperatures, it can therefore be expected to contribute significantly to inbreeding depression in extreme environments (David *et al*., 2005; Jørgensen *et al*., 2006).

The aim of this study was to investigate the effect of inbreeding and high-temperature stress on male sterility using *D. melanogaster* as a model organism. More specifically, we examined: (1) how inbreeding and high-temperature stress affect male sterility; and (2) whether stress-induced male sterility and egg-to-adult viability are correlated. Sterility was divided into temporary male sterility (male sterility during heat stress that disappears after returning to benign temperatures) and permanent male sterility (male sterility that remains after males have returned to benign temperatures for an extended period of time). We also investigated whether these two measures of sterility were correlated. We studied both temporary and permanent male sterility, because it is known from studies with *Drosophila* that males exposed to temperature stress during development remain sterile for some period of time after exposure, and some may become permanently sterile, depending on the severity of stress and their genetic composition (Vollmer *et al*., 2004; Jørgensen *et al*., 2006).

MATERIAL AND METHODS

Experimental flies

In this experiment, 23 inbred lines and five non-inbred (control) lines of *D. melanogaster* were used, all originating from the mass population Groningen 83 (G83). G83 was originally founded by 403 female flies caught at a fruit market in Groningen, the Netherlands, in 1983, and has been kept as a large mass population since then (see Vermeulen, Bijlsma & Loeschcke, 2008). Previous work has shown the G83 population to exhibit substantial levels of genetic variation (Bijlsma *et al*., 1999), and therefore the inbreeding coefficient of the base population could be assumed to be fairly low. All lines were kept in large vials (68-mL plastic tubes, 83 mm high and 36 mm diameter) with 20 mL of a standard laboratory medium sprinkled with dry yeast. The unstressed lines were held under standard laboratory conditions (25.0 ± 1.0 °C, 55% relative humidity, 12-h light/12-h dark cycle), and crowding was avoided throughout the experiment. All flies used in the experiments were collected as virgins within 8 h of eclosion. Males and females were kept in separate small 23-mL plastic tubes (75 mm high and 23.5 mm diameter) containing 6 mL of medium, and females were between 3 and 5-days old when used in the experiment. All flies were gently anaesthetized with CO_2 when handled.

Inbreeding procedure

The inbred lines were established from the G83 population by seven consecutive generations of full-sib matings, generating a theoretically expected inbreeding level, F, of approximately 0.785. The inbreeding level was calculated as the inbreeding coefficient in generation t with recurrent sib mating,

$F_t = ¼ + ½F_{t-1} + ¼F_{t-2}$ (Falconer & Mackay, 1996). For each line in each generation, five full-sib matings were set up in individual vials. In order to minimize the loss of lines during inbreeding, the five full-sib pairs chosen to establish the next generation were selected from one of the five vials in the previous generation that produced the most offspring. Thus, the inbreeding procedure selected for higher productivity, and allowed for within-line purging of highly deleterious alleles, such as recessive lethal and highly detrimental alleles (Wang, 2000).

Except for three lines that were established in 1998 (lines ic4, ic11, and ic14), all lines were established in 1999 and thereafter maintained as large bottle populations (350–450 individuals per generation) until they were used for the present experiment in 2001–2002. Most lines were therefore used 2–3 years after they had been inbred. Although some new spontaneous mutations may have occurred during this period, previous studies on inbred lines from the same base population and similar levels of inbreeding have shown that fitness rebound during this period is negligible (Bijlsma *et al.*, 2000).

Experimental procedure

Viability

In order to identify the optimal temperature for the sterility experiment (see description below), two preliminary experiments were carried out investigating egg-to-adult viability and male sterility, respectively, for the inbred lines at a range of temperatures around 28.5 °C. Male sterility was assessed as described below.

For 22 of the inbred lines (one was left out because of poor performance), egg-to-adult viability was determined by allowing 50 pairs of between 3- and 5-day old flies to lay eggs for 12 h in a vial containing a small plastic tray ($8 \times 44 \times 44$ mm) with medium that was lightly sprinkled with dry yeast. After oviposition, eggs were collected and transferred to small vials. For each line, 400 eggs were collected and placed in 16 small vials with medium (25 eggs per vial), and four vials were incubated at each of the temperatures: 25.0, 27.5, 28.0 and 28.5 °C. When all flies had emerged, adult flies were collected and counted. Viability was calculated as the proportion of flies emerging from each vial relative to the number of eggs.

The pilot experiments showed egg-to-adult viability to be fairly high at 28.5 °C for most inbred lines (see Results, Fig. 1), and sterility to be induced in a narrow temperature interval between 28.5 and 29.0 °C (data not shown). Accordingly, we set the stress temperature in the sterility experiment to 29.0 °C in order to ensure that substantial levels of sterility were induced. Two inbred lines (33 and 34) showed very low egg-to-adult viability and overall poor performance, and were therefore not included in the sterility experiment.

Sterility

The sterility experiment was performed on 21 inbred lines and divided into three blocks with the same experimental outline for each block: for each line, six large vials with medium, each containing approximately 40 pairs of flies between 3- and 5-days old, were incubated at 25.0 °C. The flies were allowed to lay eggs for 24 h and were then removed. The eggs were incubated at either 25.0 °C (control) or 29.0 °C (stress). All incubators used were set on a 12-h light/12-h dark cycle and were equipped with fans, ensuring a homogeneous temperature (±0.5 °C). Vials were regularly rotated to ensure a homogeneous treatment. To preserve humidity, trays with water were placed in the bottom of the incubator and the stoppers were wetted every second day. This ensured that sterility differences were not caused by desiccation.

When adult flies emerged, males were collected and individually mated to non-inbred, unstressed 2- to 5-day-old virgin females from the G83 base population (control females) in small vials containing medium. For each line 20 vials each containing one male and two control females were set up. Mating was allowed for 7 days in the stressful environment (29.0 °C). Then females were replaced and vials were transferred to 25.0 °C, where mating was allowed for an additional 14 days. Flies were transferred to a fresh vial every second day, and dead females were replaced. Vials were regularly examined for larvae, and when larvae were observed, the male was scored as fertile and the vial discarded. In vials where no larvae were observed, males were scored as sterile at that time point, and scored again at the following examination. If males died after having been classified as sterile, they were regarded as being sterile throughout the experiment. This resulted in a slightly inflated measure of permanent sterility and an equivalent underestimation of temporary sterility, especially for inbred lines with poor overall survival.

Inbreeding depression was measured as the percentage increase in sterility. Sterility was determined as the proportion of sterile males relative to all males, and was recorded at three time points: immediately after heat stress, after 7 days of recovery, and after 14 days of recovery. These three time points were chosen in order to detect possible differences in the duration of sterility, as temporarily sterile males should normally recover fertility within a maximum of 10 days (Rohmer *et al.*, 2004). Sterility immediately after heat stress is hereafter referred to as initial sterility, and sterility after heat stress followed by 14 days of recovery is hereafter referred to as permanent sterility.

As we initially used only one control line, but wanted to have some information about the variation among control lines, and to improve statistical power, an experimental block with four additional control lines was assayed for sterility at 25.0 and 29.0 °C. To align this block with the initial three blocks, three inbred lines (12, 14, and ic4) were tested at the same time. The results were analysed using PROC MIXED, with sterility being a function of temperature, line, their interaction, and a block and replicate effect. Blocks were treated as random effects. As the values observed for the inbred lines did not differ significantly between replicates ($F_{1,58} = 2.69$, $P = 0.107$), we assumed that the test conditions in this last experiment were similar to the initial experiment. Also, the five control lines were extremely similar both with respect to survival and sterility at both temperatures, as they were only marginally affected by the two treatments (see the Results section). As such, the five control lines were treated as true replicates. To keep the number of replicates the same for all inbred lines, the additional data obtained for the inbred lines 12, 14, and ic4 in this latter block were not used in the analysis.

Data analysis

Viability

Egg-to-adult viability was analysed using the PROC MIXED procedure of SAS with egg-to-adult viability being a function of temperature, line, and their interaction, allowing for a random block effect.

Sterility

The structure of the data meant that block effects could not be included in the statistical model. Assuming no block effects, the sterility data were pooled across blocks and the number of sterile flies within line and temperature groups was for each time point assumed to be the outcome of a binomial experiment. Data were analysed separately for each time point by a generalized linear mixed model with log-link and a random line effect. The population distribution of the random line effect was assumed to be the same for inbred and control lines, and the relative risk of sterility at high, relative to low, temperature was assumed to be the same for all lines within the inbreeding level. Data were analysed by the NLMIXED procedure of SAS (SAS Institute Inc., 1999–2001). The temperature effect was quantified for inbred and control lines, respectively, by the relative risk of sterility at high, relative to low, temperature, and compared by statistical significance testing. Within temperature groups, the effect of inbreeding on sterility could be quantified by the median over lines of the relative risk of sterility for inbred relative to control lines.

Temporary sterility was calculated at both temperatures as the difference between initial and permanent

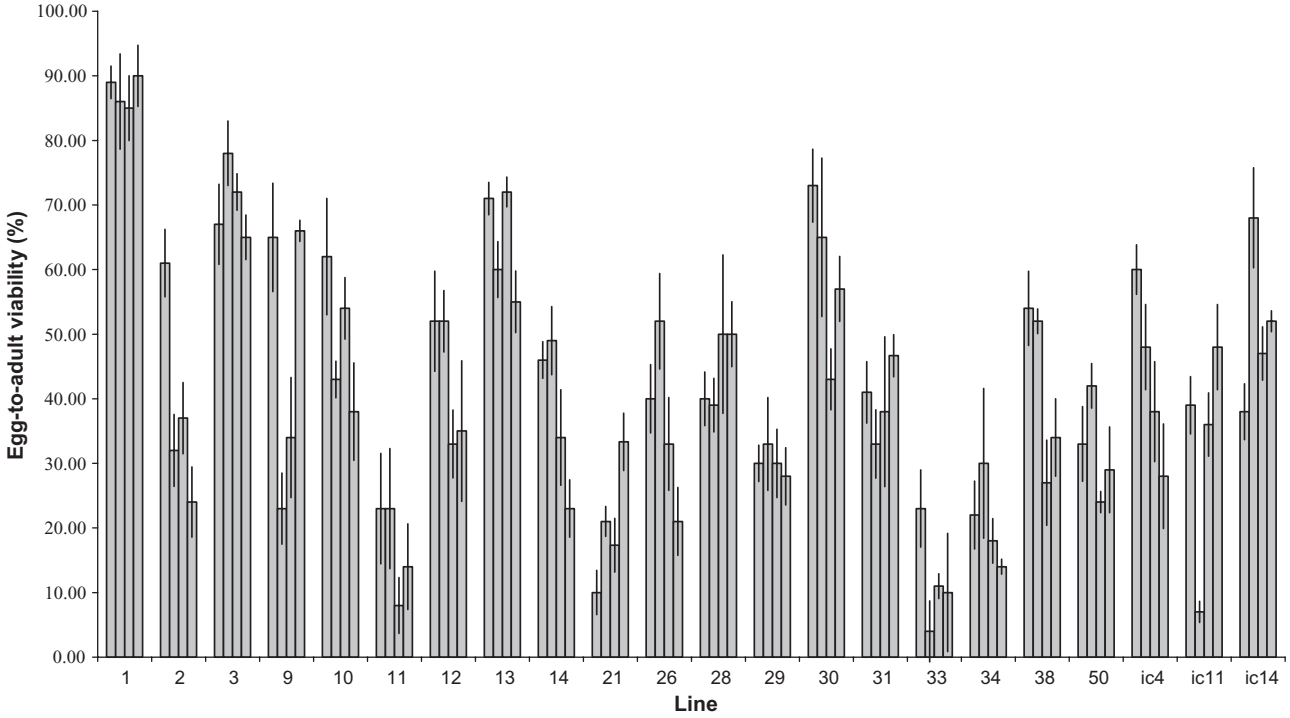

Figure 1. Mean egg-to-adult viability ± SE for 22 inbred lines (1–ic14) of *Drosophila melanogaster*. The bars represent four different temperature treatment levels: 25.0, 27.5, 28.0 and 28.5 °C (from left to right for each line).

sterility. Stress-induced sterility was calculated for initial, temporary, and permanent sterility as the difference between sterility at 29.0 and 25.0 °C. Correlations between stress-induced temporary and stress-induced permanent sterility were analysed by means of the nonparametric Spearman's rank correlation procedure. This was performed for permanent sterility, defined both as sterility after 14 days and as sterility after 7 days in order to see if scoring dead sterile flies as sterile flies affected the result. The same statistical analysis was applied for the correlation between stress-induced initial sterility and stress-induced decreased egg-to-adult viability. The stress-induced decrease in egg-to-adult viability was calculated based on the results of the pilot experiment, as the difference in egg-to-adult viability between 25.0 and 28.5 °C. The correlation between sterility and egg-to-adult viability was based on the assumption that egg-to-adult viability was the same in males and females.

To obtain a measure of how sensitive male fertility (i.e. fertility = 100% – sterility%) is in relation to the applied temperature stress, we calculated the following stress index:

Relative stress sensitivity = (fertility at 25.0 °C – fertility at 29.0 °C)/(fertility at 25.0 °C).

The statistical analyses were all performed using SAS 9.2 (SAS Institute Inc., 1999–2001).

RESULTS

Viability

As can be expected from the inbreeding process, the pilot experiment showed large variation in egg-to-adult viability among inbred lines (Fig. 1): some lines (e.g. 1, 3, 13, and 30) show a viability between 70 and 90%, which is close to the viability generally observed for this base population (Bijlsma *et al.*, 1999; Joubert & Bijlsma, 2010), whereas other lines (e.g. 11, 33, and 34) show a conspicuous low viability. This is supported by a highly significant line effect in the ANOVA ($F_{21,261} = 34.34$, $P < 0.0001$). Viability is also significantly affected by temperature ($F_{3,261} = 9.61$, $P < 0.0001$), with on average the highest viability at 25.0 °C (mean ± SE: 47.2 ± 1.29) and lower viability at the higher temperatures (42.7 ± 1.29, 38.2 ± 1.30, and 39.1 ± 1.31 at 27.5, 28, and 28.5 °C, respectively). The difference in mean viability between 25.0 °C and the highest temperatures of 28.0 and 28.5 °C was found to be significant at the 5% level (data not shown). However, again the response is highly variable among lines (e.g. compare lines 33 and ic4 with lines 1 and 29), and is supported by the significant interaction between line and temperature ($F_{63,261} = 3.07$, $P < 0.0001$). This indicates that the highest temperatures impose thermal stress on the inbred lines.

Sterility

Temporary and permanent sterility at 25.0 and 29.0 °C are shown in Figure 2. The mean sterility for the inbred lines ranged from 0.42% for temporary sterility at 25.0 °C to 20.21% for permanent sterility at 29.0 °C, as compared with 0 and 2.69%, respectively, for the controls.

Within temperature groups, the effect of inbreeding on sterility was significant only at 29.0 °C. The median over lines of the relative risk of sterility at 29.0 °C for inbred relative to control lines was 5.85 (95% CI 2.32–14.70, $P = 0.001$) for initial sterility, 5.92 (95% CI 2.29–15.30, $P = 0.001$) for sterility at the second recording, and 5.53 (95% CI 2.14–14.30, $P = 0.001$) for sterility at the third recording, i.e. the ratios were both significantly different from 1. At 25.0 °C, the median over lines of the relative risk of sterility for inbred relative to control lines was 3.78 (95% CI 0.74–19.2, $P = 0.104$) for initial sterility, 3.45 (95% CI 0.69–17.20, $P = 0.126$) for sterility at the second recording, and 3.40 (95% CI 0.68–17.00, $P = 0.130$) for sterility at the third recording, i.e. the ratios were not significantly different from 1.

The relative risk of sterility at high relative to low temperature was highly significant for inbred flies ranging from 6.49 (95% CI 4.53–9.28, $P < 0.0001$) for permanent sterility to 7.74 (95% CI 5.57–10.75, $P < 0.0001$) for initial sterility. In the control lines the relative risk of sterility at high relative to low temperature was only significant at the first sterility recording, i.e. for initial sterility (5.00, 95% CI 1.03–24.37, $P = 0.047$). For both of the two following time points, the relative risk was 4.00 (95% CI 0.79–20.14, $P = 0.090$). It was thus not possible to demonstrate an interaction between temperature and level of inbreeding, most likely because of the low number of control lines.

For inbred lines, permanent and temporary stress-induced sterility showed a positive correlation, although this was only significant for permanent sterility defined as sterility remaining after 7 days (Fig. 3) (7 days, $\rho = 0.445$, $P = 0.043$; 14 days, $\rho = 0.425$, $P = 0.055$). Stress-induced initial sterility and stress-induced decrease in egg-to-adult viability was also significantly positive correlated (Fig. 4) ($\rho = 0.557$, $P = 0.011$).

Figure 5 reveals that the mean relative sensitivity of the inbred lines (0.23 ± 0.051) is considerably higher than the mean of the controls (0.03 ± 0.019), and 20 out of the 21 inbred lines tested show a sensitivity that is higher than the average of the controls (binomial test, $P < 0.0001$). Moreover, the

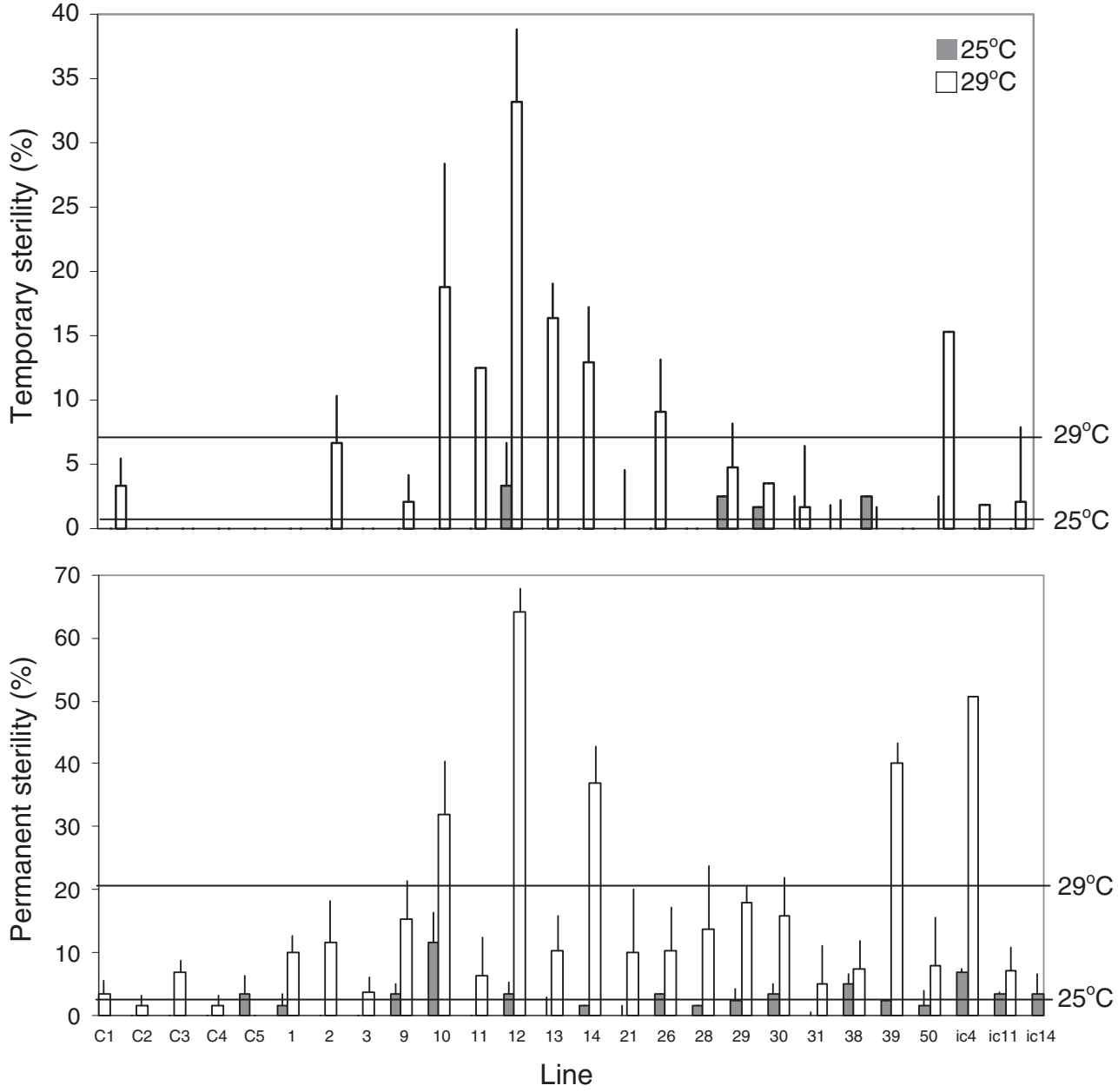

Figure 2. Temporary sterility ± SE (top) and permanent sterility (bottom), measured after 7 days of stress followed by 14 days of recovery, ± S.E. for five non-inbred control replicates (C1–C5) and 21 inbred lines of *Drosophila melanogaster* (1–ic14) at 25.0 °C (black bars) and 29.0 °C (white bars). The overall mean sterility for inbred lines only is indicated by horizontal lines: lower line, 25.0 °C; upper line, 29.0 °C. The mean sterility of the non-inbred controls is not shown in the figure, as the values are too small to be distinguished from the *x*-axis (temporary sterility, 0.0 and 0.67%; permanent sterility, 0.68 and 2.69%; at 25.0 and 29.0 °C, respectively, for the controls). Note the difference in the scale of the *y*-axis.

inbred lines exhibited high variance in heat stress sensitivity (e.g. compare the two extreme lines 3 and 12), almost all inbred lines were more sensitive than the controls, and the mean relative sensitivity was considerably higher for inbred lines as compared with the control lines (Fig. 5). This large variation among lines in stress sensitivity suggests strong genotype-by-environment interaction for sterility. The duration of temporary sterility was 7 days or less for most lines (data not shown). Lines that had not gained fertility after 7 days of recovery were kept for an additional 7 days to determine if the duration of stress-induced sterility varied among lines. Roughly one-third of the lines showed some further improvement in fertility

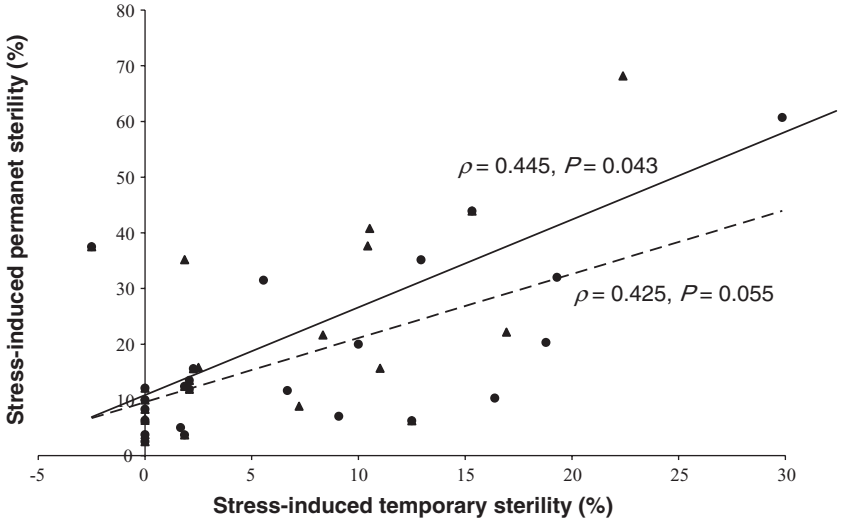

Figure 3. Correlation between stress-induced permanent and stress-induced temporary sterility for 21 inbred lines of *Drosophila melanogaster*. Two correlations are shown: one defining permanent sterility as sterility after 7 days of recovery (▲, solid line), and one defining permanent sterility as sterility after 14 days (●, dashed line), respectively.

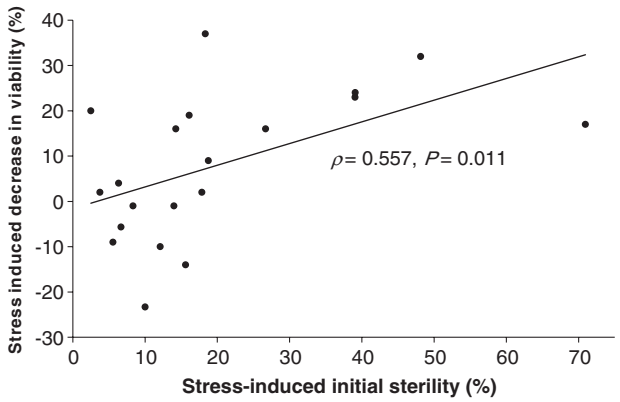

Figure 4. Correlation between stress-induced initial sterility at 29.0 °C and stress-induced decrease in egg-to-adult viability at 28.5 °C for inbred lines of *Drosophila melanogaster*.

in the following 7 days. However, in all cases the improvement in the second period was substantially less than that of the first period. The difference in the duration of temporary sterility between inbred and control lines could, unfortunately, not be analysed using the NLMIXED procedure, because for many lines too few flies were sterile at the first recording.

DISCUSSION

The main results of this study are that inbreeding and stress increase male sterility, and that viability and male sterility are correlated when inbred lines are exposed to high temperature. The results from the sterility experiment showed that male sterility, both initial and permanent, increased significantly with temperature stress for inbred flies, whereas only initial sterility increased significantly for non-inbred flies. Within temperature groups, the effect of inbreeding was only significant when flies were exposed to the stressful temperature. Comparing sterility at 25.0 and 29.0 °C, inbreeding on average increased temporary and permanent sterility by no less than ten-fold (cf. Fig. 2). In addition, the relative sensitivity to stress was estimated to increase almost ten-fold when flies were inbred (cf. Fig. 5). These results are consistent with previous studies showing synergistic effects of environmental and genetic stress on fitness traits (e.g. Bijlsma *et al*., 2000; Armbruster & Reed, 2005; Joubert & Bijlsma, 2010; Fox & Reed, 2011).

In *D. melanogaster*, male fertility has previously been shown to decrease at both high and low temperatures. This species can complete its development at temperatures between 11.0 and 32.0 °C (David *et al*., 1983), but in temperate populations, males are only fertile within the range from 13.0 to 30.0 °C (Cohet, 1973; David *et al*., 1983, 2005). Stress-induced sterility at 29.0 °C is normally only temporary, as fertility is regained within approximately 3.5 days of returning the flies to 25.0 °C (Rohmer *et al*., 2004), although the duration of the recovery period depends on the severity of the heat stress. Temperature-induced sterility is believed to result from an arrest of spermatogenesis, and is induced within a very narrow temperature interval both at high and low threshold temperatures (David, Arens & Cohet, 1971; Chakir *et al*., 2002). Our results are generally consistent with these data. At 25.0 °C, temporary sterility was very

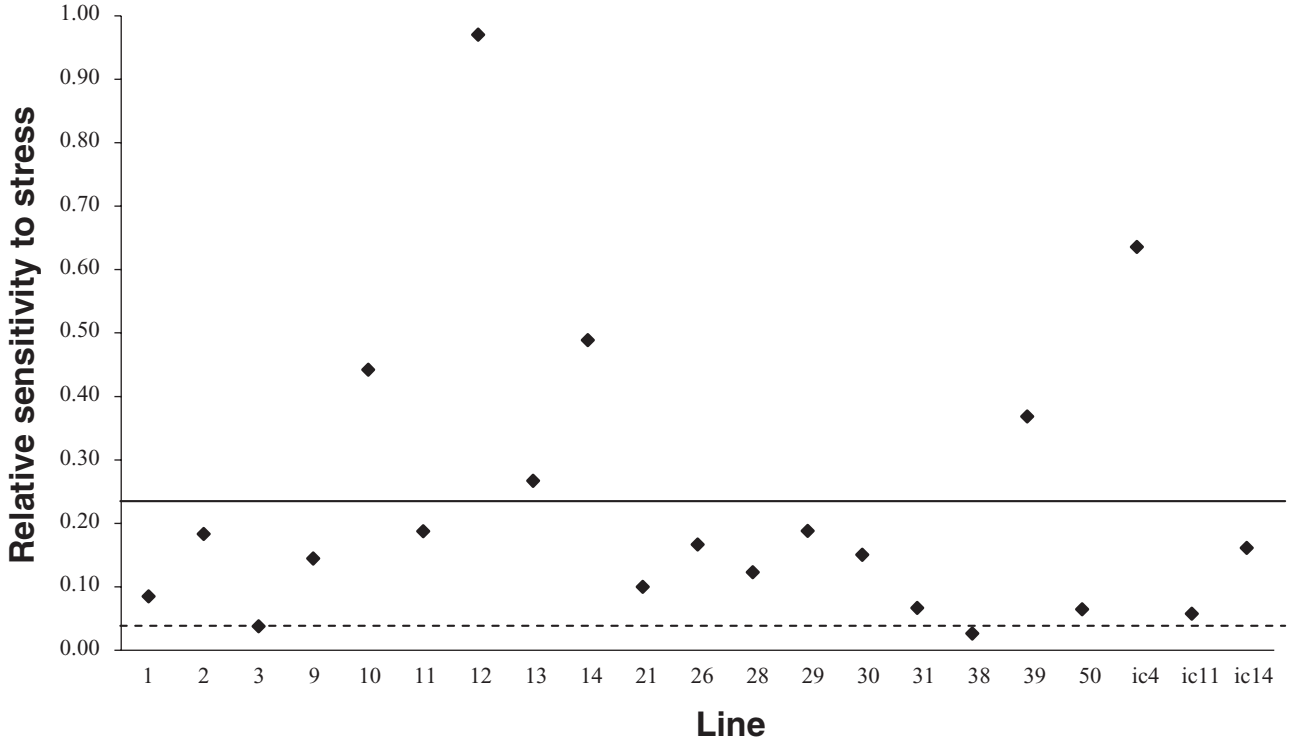

Figure 5. The relative sensitivity of male sterility to the temperature stress applied for 21 inbred lines of *Drosophila melanogaster* (1–ic14). The horizontal lines indicate the mean relative sensitivity to stress averaged over all inbred lines (full line) and the control lines (broken line).

low, with only four of the inbred lines exhibiting a considerable level of sterility. Temporary sterility increased greatly at 29.0 °C, and the results of the preliminary experiment indicated this increase to be induced between 28.5 and 29.0 °C. The duration of the stress-induced temporary sterile period for most lines was less than 7 days. However, ten lines did recover further fertility in the following 7 days, confirming previous results showing that genetic factors influence the duration of the recovery time (Kærsgaard *et al*., 2000; Chakir *et al*., 2002; Rohmer *et al*., 2004; Vollmer *et al*., 2004).

Unlike temporary sterility, permanent sterility was observed for almost every inbred line: 16 out of 22 lines at 25.0 °C and all lines at 29.0 °C (Fig. 2). Furthermore, permanent sterility contributed more to the initial stress-induced sterility than temporary sterility. This is not surprising as male fertility in *D. melanogaster* is governed by a complex genetic control system involving many loci. Estimates of 1000 to more than 2000 mutations are believed to be able to cause male sterility. Most of these mutations are recessive, and many are temperature sensitive (Lindsley & Tokuyasu, 1980; Wakimoto, Lindsley & Herrera, 2004). This explains the high frequency of permanent sterility in the inbred lines as well as the increase in permanent sterility at the high temperature, as the novel environment unmasks conditionally expressed recessive deleterious alleles that are only detrimental at the restrictive temperature, as suggested for adult and pupal mortality by Vermeulen & Bijlsma (2004a, b) and Bijlsma *et al*. (2010).

It should be mentioned that permanent sterility is likely to be overestimated in the experiment, especially at 29.0 °C, because flies that were classified as sterile were classified as being permanently sterile if they died during the course of the experiment. The average proportion of inbred flies that died while sterile was 1% (25.0 °C) and 9% (29.0 °C) at the second time of recording, and 2% (25.0 °C) and 16% (29.0 °C) at the third sterility recording. The average proportion of control flies that died while sterile was always less than 1%. However, as the statistical analysis of sterility at both recordings gave the same result, and the correlation between temporary and permanent sterility differed very little depending on whether the second or third sterility recording was set as permanent sterility (cf. Fig. 3), the effect of this classification is expected to by minor. The observation that stress-induced temporary and permanent sterility were correlated (cf. Fig. 3) implies that, at least in part, the same genes affect both temporary and

permanent sterility (pleiotropy), or that the alleles of the responsible genes are in linkage disequilibrium.

The observation that the initial male sterility directly after heat stress and stress-induced egg-to-adult viability are correlated is somewhat surprising. The alleles responsible for sterility are possibly either in linkage disequilibrium with alleles reducing egg-to-adult viability or exert pleiotropic effects on egg-to-adult viability. Inbreeding may increase the strength of such a correlation among fitness components if rare deleterious recessive alleles have negative pleiotropic effects on both fitness components. This is supported by the study of Shellenbarger & Cross (1979), who found that eleven out of 93 stocks of *D. melanogaster*, originally isolated as temperature-sensitive lethals, turned out to be temperature-sensitive male steriles as well. Furthermore, Geer, Lischwe & Murphy (1983) found that many loci of which the product functions in non-reproductive tissue could mutate to a form that causes male sterility as a secondary effect. These results support the view that male sterility can be caused by two types of mutations: mutations with detrimental effects solely acting on stages in spermatogenesis, and/or the development of reproductive tissue and mutations with detrimental effects disrupting more basic metabolic events also present in non-reproductive tissue. However, it should be mentioned that the correlation between sterility and egg-to-adult viability could also be caused by the overall fitness of the inbred lines being so low that homeostasis could not be maintained, and consequently both reproduction and survival was reduced. However, as the inbreeding procedure exerted selection for high productivity, this is not very likely.

A high level of variation in both temporary and permanent sterility was observed among the inbred lines, reflecting their differential sensitivity to environmental conditions. As the lines differ genetically because of random drift and fixation for different alleles, specifically for conditionally expressed deleterious alleles, the high level of genetic variation among lines is to be expected. The same phenomenon can explain the high variation observed for viability in the preliminary experiment (cf. Fig. 1).

Our results show that inbreeding does increase male sterility, both temporary and permanent. The increased level of permanent sterility would of course have a higher impact on the fitness of individual males in natural populations than temporary sterility, but the importance of temporary sterility should not be ignored, as age at sexual maturity is a principal life-history trait (Stearns, 1992). The importance of induced temporary sterility on male fitness would depend on the duration of the sterile period, and as the expected lifespan of *D. melanogaster* in wild populations is estimated to range from a few days to a few weeks (Rosewell & Shorrocks, 1987; Turelli & Hoffmann, 1995), a difference of only a few days may have a great impact on the total reproductive output of males in nature.

The processes underlying spermatogenesis in *Drosophila* are very similar to the processes in other animal species, e.g. mammals, and the basic principles of sperm structure are well conserved throughout the animal kingdom (Hackstein, Hochstenbach & Pearson, 2000). The results of this study are therefore likely to be of relevance to other species as well.

ACKNOWLEDGEMENTS

The authors would like to thank Torsten Nygaard Kristensen and Stuart Barker, as well as three anonymous reviewers, for significant contributions and constructive comments on the manuscript. Also thanks to Desiree Joubert (Population and Conservation Genetics, University of Groningen, the Netherlands) for kindly providing the inbred lines. This research was supported by a grant from the Danish Natural Science Council to J.B. (no. 51-00-0298).

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