Extreme temperatures increase the deleterious consequences of inbreeding under laboratory and semi-natural conditions

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The majority of experimental studies of the effects of population bottlenecks on fitness are performed under laboratory conditions, which do not account for the environmental complexity that populations face in nature. In this study, we test inbreeding depression in multiple replicates of inbred when compared with non-inbred lines of Drosophila melanogaster under different temperature conditions. Egg-to-adult viability, developmental time and sex ratio of emerging adults are studied under low, intermediate and high temperatures under laboratory as well as semi-natural conditions. The results show inbreeding depression for egg-to-adult viability. The level of inbreeding depression is highly dependent on test temperature and is observed only at low and high temperatures. Inbreeding did not affect the developmental time or the sex ratio of emerging adults. However, temperature affected the sex ratio with more females relative to males emerging at low temperatures, suggesting that selection against males in pre-adult life stages is stronger at low temperatures. The coefficient of variation (CV) of egg-to-adult viability within and among lines is higher for inbred flies and generally increases at stressful temperatures. Our results contribute to knowledge on the environmental dependency of inbreeding under different environmental conditions and emphasize that climate change may impact negatively on fitness through synergistic interactions with the genotype.

Keywords: inbreeding×environment interactions; inbreeding depression; temperature stress; phenotypic variance; semi-natural environments

1. INTRODUCTION

A decline in population size is often accompanied by loss of genetic variability and inbreeding depression, which in turn may impact on the extinction risk of threatened populations and the evolution of inbreeding avoidance behaviour and physiological responses (Cmnokrak & Roff 1999; Hedrick & Kalinowski 2000; Amos & Balmford 2001; Keller & Waller 2002; Spielman et al. 2004; Kristensen et al. 2006; O’Grady et al. 2006). Apart from genetic concerns, environmental stress induced by, for example, climate change and pollution imposes direct threats to the persistence of populations (World Conservation Monitoring Centre 1992; Hoffmann & Parsons 1997; Baillie et al. 2004).

The relative contributions of genetic versus ecological factors to extinction probabilities in wild populations are still being debated (Caro & Laurenson 1994; Caughley 1994; Frankham 2005; Jamieson et al. 2006).

The deleterious consequences of inbreeding depend on the genetic load carried by a population and may be trait and environment dependent (Armbruster & Reed 2005; Szulkin & Sheldon 2007; Kristensen et al. 2008a).

A number of recent studies have suggested that the deleterious consequences of inbreeding are reinforced under harsh environmental conditions (Keller et al. 2002; Reed et al. 2003, 2007; Da Silva et al. 2006), and that studies investigating inbreeding effects under laboratory conditions that are often benign may not be representative for consequences of inbreeding in variable natural environments (Chen 1993; Pray et al. 1994; Kristensen et al. 2008a). On the other hand, studies of the effects of inbreeding and interactions between inbreeding and the environment in natural populations often suffer from low replication, incomplete pedigree information, lack of proper controls and the fact that the presumed neutral molecular markers often used may not reflect levels of detrimental and adaptive genetic variation. Therefore, most individual field studies lack sufficient statistical power to uncover detailed patterns of inbreeding effects in nature (Szulkin & Sheldon 2007). Experiments performed under semi-natural conditions with replicated populations with known levels of inbreeding may therefore be a useful supplement for increasing our knowledge of the importance of inbreeding×environment interactions on fitness.

Temperature is one of the most important variables that determine distribution and abundance of species (Cossins & Bowler 1987; Hoffmann & Parsons 1997).
The mean global temperature is increasing at an unprecedented rate and weather conditions are becoming more unpredictable, increasing the frequency of more extreme temperatures (IPCC 2007). This poses great challenges to all organisms and is one factor why distribution maps are currently changing and population sizes decreasing for many species (Perry et al. 2005; Walther et al. 2005; IPCC 2007). Therefore, potential effects of interactions between genotype and temperature on fitness should be investigated with respect to their impact on extinction risk and hence biodiversity.

Most published studies on responses to extreme temperatures and the importance of interactions between genotypes and thermal conditions have been performed in the laboratory at constant low or high temperatures (Bijlsma et al. 1999; Dahlgaard & Hoffmann 2000; Kristensen et al. 2003). They therefore do not take into account the potential importance of daily and seasonal fluctuations in temperature, humidity and light intensity. Thus, the ecological relevance of such studies can be questioned (Gibbs 1999; Harshman & Hoffmann 2000; Kristensen et al. 2007, 2008a,b). Therefore, there is a need to study how inbreeding affects the mean and variance of fitness-related traits in highly replicated experiments and under different ecologically relevant climatic conditions. Such studies are of crucial importance for evaluating the impact of climate change on biodiversity and for designing sustainable conservation strategies for small and isolated populations.

In this highly replicated study with the model organism Drosophila melanogaster, we test the hypothesis that the level of inbreeding depression is dependent on climatic conditions. Egg-to-adult viability, developmental time and sex ratio of emerging adults are tested in eight inbred and eight non-inbred control lines under low, intermediate and high temperatures, both under laboratory and semi-natural conditions. By testing flies under natural temperature, humidity and daylight conditions, we attempted to move beyond the limitations of laboratory investigations of inbreeding × temperature interactions. The results reveal strong interactions between breeding regime (inbred versus non-inbred) and temperature for egg-to-adult viability and developmental time, with inbreeding depression observed only for egg-to-adult viability at low and high temperatures, and not at presumably more benign intermediate temperatures.

2. MATERIAL AND METHODS

(a) Inbreeding procedure and maintenance of the lines in the laboratory

Inbred and non-inbred control lines were generated from a genetically diverse mass population of D. melanogaster (see Bublij & Loeschcke 2005 for details). Eight independent lines with expected equivalent levels of inbreeding (F = 0.67) were obtained through five generations of full-sib mating (see Kristensen et al. 2005 and Pedersen et al. 2005 for details on the inbreeding procedure and maintenance of stocks). After reaching the desired level of inbreeding, all lines were flushed to a minimum of 500 individuals (within two generations). Eight non-inbred lines, each founded by approximately 500 breeding individuals, were established at the time when inbreeding was initiated. The non-inbred and flushed inbred lines were thereafter held at high N_e (> 1000). Throughout and following the inbreeding procedure, all flies were maintained in a climate room (25 ± 0.2°C, 40–60% relative humidity, 12 L: 12 D cycle). The lines used in this study were established in 2004 and kept at the University of Aarhus, Denmark, for approximately 75 generations before the present experiments were carried out.

(b) Experiments on inbreeding effects under laboratory and semi-natural conditions

Eight inbred and eight non-inbred control lines were tested for egg-to-adult viability, developmental time and sex ratio under laboratory and semi-natural conditions at low, intermediate and high temperatures. All experiments were performed in the same generation. Twenty eggs were collected into each of 10 vials per line, location (laboratory and semi-natural) and temperature treatment (low, intermediate and high). The total number of eggs and vials used were, respectively, 19 200 and 960. All tests in the laboratory were performed in incubators at the University of Aarhus, Denmark, whereas tests under semi-natural conditions were performed in Denmark (low temperatures) or Queensland, Australia (intermediate and high temperatures). Two generations before the experiments were performed, we sent developing larvae from all 16 lines to Australia. These were maintained at the University of Queensland for two generations at standard laboratory conditions prior to collection of the eggs that were used in the experiments. All eggs were collected in the laboratory at 25°C at the University of Aarhus, Denmark, or the University of Queensland, Australia. Thereafter, the vials were randomized and transferred to incubators in the laboratory or to a field site (woodland) within 2–3 hours after being collected. In the field, the vials with eggs from all lines were randomized in racks and placed approximately 1.5 m above ground hanging from a tree and shaded from direct sunlight. Temperatures in the incubators were 5.5°C at night (01.00–06.00) and 14.5°C during the day (06.00–01.00) for the low temperature treatment, constant 25°C for the intermediate temperature treatment, and 20°C at night (20.00–08.00) and 33.5°C during the day (08.00–20.00) for the high temperature treatment. At the intermediate temperature treatments (25°C), the light was on from 08.00 to 20.00 (12 L : 12 D cycles), whereas in the other treatments the light was on from ‘day’ temperatures (19 L : 5 D for the low and high temperature treatments, respectively). Mean temperatures throughout development at low, intermediate and high temperatures were, respectively, 12.6, 25 and 26.8°C under laboratory conditions, and 12.4, 21.6 and 24.6°C under semi-natural conditions in the field (figure 1).

Egg-to-adult viability was estimated as the proportion of eggs, which survive to adulthood in each vial, out of the initial 20 eggs per vial; developmental time as the time taken from egg laying to emergence of adults (for adults that actually emerge from pupae—counted daily) and the sex ratio as the number of males emerging relative to the total number of flies emerging. All traits were investigated at both localities and for all three temperature regimes.

(c) Analyses

Nested ANOVAs were used to test the effects of location (laboratory or semi-natural), temperature (low, intermediate or high) and breeding regime (inbred or non-inbred) on egg-to-adult viability, developmental time and sex ratio. Males and females were pooled for ease of interpretation of...
the results for the traits egg-to-adult viability and developmental time (analyses of the sexes treated separately did not lead to different conclusions, results not shown). Line was nested within breeding regime as a random factor. In the full factorial nested ANOVAs, the error degrees of freedom were 913 for developmental time and sex ratio, whereas the error degrees of freedom were 934 for egg-to-adult viability. The difference is due to the fact that some vials did not produce any emerging adults. Differences among inbred and non-inbred lines for all three traits were tested separately for each location/temperature regime by nested ANOVAs with line nested within breeding regime as a random factor and with breeding regime as a fixed factor. Significances were adjusted according to the sequential Bonferroni correction.

Within line CVs were estimated for egg-to-adult viability (CVs are only presented for this trait as inbreeding depression was not observed for the other two traits investigated) across the 10 replicate vials per line at each location and temperature. In total, 96 within line CV estimates were obtained (2 locations × 3 temperatures × 16 lines (8 inbred and 8 outbred)). CVs were also calculated across lines within each location and temperature. Average egg-to-adult viability estimates were calculated for all locations, temperatures and lines, and the CVs were obtained based on these line means. In total, 12 across line CV estimates were obtained (2 locations × 3 temperatures × 2 breeding regimes). ANOVA models were used to test differences in within and across line CVs with location, temperature and breeding regime as fixed factors.

3. RESULTS

(a) Egg-to-adult viability

Location, temperature and breeding regime all had a significant effect on egg-to-adult viability (location: F\textsubscript{1,934} = 7.24, \(p < 0.01\); temperature: F\textsubscript{2,934} = 293.59, \(p < 0.0001\); breeding regime: F\textsubscript{1,934} = 23.93, \(p < 0.001\)). Egg-to-adult viability was higher at intermediate temperatures and generally lowest at low temperatures (figure 2). Egg-to-adult viability was higher under semi-natural relative to laboratory conditions at high temperatures (figure 2). Across locations and temperatures, viability was 22.5% lower in inbred relative to non-inbred flies.

(b) Developmental time

The fixed factors location and temperature affected developmental time significantly, whereas breeding regime did not impact on developmental time (location: F\textsubscript{1,913} = 43.35, \(p < 0.0001\); temperature: F\textsubscript{2,913} = 28607.50, \(p < 0.0001\); breeding regime: F\textsubscript{1,913} = 1.92, n.s.). Developmental time was almost four times longer at low temperatures, both under laboratory and semi-natural conditions, when compared with intermediate or high temperatures, slightly longer at intermediate temperatures in semi-natural when compared with laboratory conditions, and slightly longer at high temperatures under laboratory conditions when compared with semi-natural conditions (figure 3).

Figure 1. Temperatures in the semi-natural environments during the experimental periods for low (grey curve), intermediate (light grey curve) and high (black curve) temperatures. Development at low temperatures lasted for up to 50 days and therefore temperature registrations also lasted this long.

Figure 2. Proportion egg-to-adult viability ± s.e. for development at low, intermediate and high temperatures under laboratory and semi-natural conditions. Significant differences in egg-to-adult viability between inbred (black bars) and non-inbred (grey bars) lines at different temperatures are indicated (***, \(p < 0.001\); table 1).
Table 1. Results from nested ANOVAs testing effects of line (d.f. = 14 in all cases) and breeding regime (d.f. = 1) for each location/temperature for the traits egg-to-adult viability, developmental time and sex ratio (error d.f. = 144 in all cases). (Significance adjusted according to the sequential Bonferroni correction. n.s., non-significant; (n.s.), significant before but not after Bonferroni correction. **p<0.001, *p<0.01, ’p<0.05.)

<table>
<thead>
<tr>
<th>Breeding regime</th>
<th>Line</th>
<th>Egg-to-adult viability</th>
<th>Developmental time</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low laboratory</td>
<td>***</td>
<td>(n.s.)</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>Low semi-natural</td>
<td>***</td>
<td>***</td>
<td>n.s.</td>
<td>(n.s.)</td>
</tr>
<tr>
<td>Intermediate laboratory</td>
<td>(n.s.)</td>
<td>***</td>
<td>n.s.</td>
<td>(n.s.)</td>
</tr>
<tr>
<td>Intermediate semi-natural</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>*</td>
</tr>
<tr>
<td>High laboratory</td>
<td>***</td>
<td>(n.s.)</td>
<td>***</td>
<td>n.s.</td>
</tr>
<tr>
<td>High semi-natural</td>
<td>n.s.</td>
<td>***</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Lines differed significantly in developmental time ($F_{14,913} = 2.98, p<0.001$). The interaction between location and temperature was significant ($F_{2,913} = 161.07, p<0.0001$), showing that the impact of temperature on developmental time differed between laboratory and field conditions. This result, however, may be affected by the fact that the low, intermediate and high temperature regimes were not identical under laboratory and semi-natural conditions. The interaction between breeding regime and temperature was highly significant ($F_{2,913} = 8.58, p<0.001$), showing that inbreeding effects on developmental time depend on the temperature conditions. This interaction was primarily caused by slower development for inbred lines at low temperatures in the laboratory, a tendency in the opposite direction at high temperatures in the laboratory, and no difference between developmental time for inbred and outbred lines at the remaining locations/temperatures (figure 3). The interaction between location and breeding regime was not significant ($F_{1,913} = 2.94, n.s.$), but the three-way interaction among location, temperature and breeding regime was significant ($F_{2,913} = 11.89, p<0.0001$). Even though the overall test showed no effect of breeding regime, we tested for inbreeding depression and line effects in the two locations and for the three temperature treatments. After Bonferroni correction, developmental time was not significantly different among breeding regimes in any of the comparisons, but significant line effects were observed in some comparisons (table 1).

(c) Sex ratio
The sex ratio was significantly affected by temperature ($F_{2,913} = 9.26, p<0.0001$). This effect was mainly due to the lower sex ratio (a higher number of females emerging) at low temperatures (figure 4). At intermediate and high temperatures, 50.3 and 50.0% of the emerging flies were males, whereas at low temperatures on average across breeding regimes and localities only 44.5% of the emerging flies were males. None of the other fixed effects, interactions among them, nor line significantly affected the sex ratio (location: $F_{1,913} = 2.71, n.s.$; breeding regime: $F_{1,913} = 1.76, n.s.$; location x temperature: $F_{2,913} = 1.72, n.s.$; temperature x breeding regime: $F_{2,913} = 0.46, n.s.$; location x breeding regime: $F_{1,913} = 1.47, n.s.$; location x temperature x breeding regime: $F_{2,913} = 0.20, n.s.$; line: $F_{4,913} = 0.76, n.s.$). Neither breeding regime nor line significantly affected sex ratio when tested for the separate locations/temperatures (table 1).

(d) The effects of location, temperature and inbreeding on the coefficient of variation in egg-to-adult viability
CVs based on egg-to-adult viability in the 10 replicate vials per line were calculated for all lines, locations and temperatures. Average values are presented in table 2. ANOVA results show that location, temperature and breeding regime all had significant effects on the within line CVs (location: $F_{1,84} = 4.01, p<0.05$; temperature: $F_{2,84} = 9.51, p<0.001$; breeding regime: $F_{1,84} = 11.46, p<0.01$). The CVs were on average higher for the inbred lines relative to the outbred control lines and higher at low and high temperatures relative to intermediate temperatures. The interaction between location and temperature was significant, whereas none of the other interaction terms affected the CVs (location x temperature: $F_{2,84} = 11.46, p<0.01$; temperature x breeding regime: $F_{2,84} = 1.79, n.s.$; location x breeding regime: $F_{1,84} = 0.67, n.s.$; location x temperature x breeding regime: $F_{2,84} = 1.01, n.s.$).

To get an estimate of the variation across lines, egg-to-adult viability estimates were calculated for all lines in the respective locations and temperatures. The CVs were calculated on the basis of these line means (table 2). Results show that inbreeding increased CVs (breeding regime: $F_{1,12} = 20.70, p<0.01$), whereas neither location
and high temperatures (figure 2), whereas the interaction inbreeding depression was observed only at stressful low viability and developmental time. For egg-to-adult viability, this question is affirmative for the traits egg-to-adult components were temperature dependent. The answer to was whether the consequences of inbreeding on fitness The major hypothesis that we set out to test in this study 4. DISCUSSION studies showing that inbreeding depression may be more trait expected not to be closely linked to fitness. The result is very much in accordance with recent studies showing that inbreeding depression may be more severe under harsh environmental conditions for traits closely linked to fitness (Keller et al. 2002; Armbruster & Reed 2005; Da Silva et al. 2006; Reed et al. 2007; Szulkin & Sheldon 2007). However, our results also show that consequences of inbreeding are highly line and trait specific and emphasize that studies on inbreeding depression should be performed under different environmental conditions, be highly replicated and several life-history traits should be investigated in order to get detailed knowledge on the genetic load of any specific population. Given the high absolute level of inbreeding of the inbred lines investigated in this study, we were surprised to see that only relatively low levels of inbreeding depression were observed. This may be explained by the history of the investigated lines. They have been held in the laboratory for 75 generations after the bottleneck events. Deleterious alleles may have been purged and mutations may have introduced new variation during that time. However, despite these circumstances, we observed strong interactions between inbreeding and temperature on the fitness traits egg-to-adult viability and developmental time. This indicates that purging of deleterious alleles is environment specific and therefore may not be of great use in conservation practice. Inbreeding levels approaching 0.7 are probably rare in nature (Crnokrak & Roff 1999; Szulkin & Sheldon 2007), but qualitatively we expect the results presented in this paper to be representative for inbreeding×environment interactions involving less inbred individuals. Interestingly, deleterious consequences of inbreeding were mainly observed in the laboratory at low and high temperatures. At low temperatures in the laboratory, inbreeding depression was observed for the trait egg-to-adult viability and a non-significant trend towards longer developmental time for inbred relative to non-inbred flies and a skewed sex ratio among the two breeding regimes were observed. At high temperatures under laboratory conditions, inbreeding depression for egg-to-adult viability was observed, whereas no significant inbreeding depression was observed at high temperatures under semi-natural conditions for this trait. This trend towards increased inbreeding depression at low and high temperatures under laboratory conditions relative to at low and high temperatures under semi-natural conditions could be caused by locality-specific variation in the temperature regimes. Under laboratory conditions, the mean low and high temperatures were 12.6 and 26.8°C, respectively, whereas these were 12.4 and 24.6°C, respectively, under semi-natural conditions. However, the main difference between the laboratory and semi-natural temperature regimes was not the absolute levels, but that under semi-natural conditions temperatures decreased gradually in late afternoons and nights and increased gradually in the mornings, whereas under laboratory conditions the temperature shifts were sudden and fast (within 30 min). Under semi-natural conditions, flies therefore have more chance of acclimating to daily changes in temperatures leading to plastic physiological changes that are known to be important for survival (Hoffmann et al. 2003; Chown & Terblanche 2007; Kristensen et al. 2008b). The distinct temperature distributions under laboratory and semi-natural conditions could explain the different results obtained at the two locations. The laboratory high temperature conditions

**Table 2. CVs for egg-to-adult viability. The estimates are the average CVs for the eight lines (mean within line CVs) and CVs of the across line means (across line CVs) for all temperatures, locations and breeding regimes.**

<table>
<thead>
<tr>
<th>temperature/location</th>
<th>breeding regime</th>
<th>mean within line CVs</th>
<th>across line CVs</th>
</tr>
</thead>
<tbody>
<tr>
<td>low laboratory</td>
<td>control</td>
<td>21.73</td>
<td>17.05</td>
</tr>
<tr>
<td></td>
<td>inbred</td>
<td>67.46</td>
<td>53.61</td>
</tr>
<tr>
<td>low semi-natural</td>
<td>control</td>
<td>27.60</td>
<td>11.16</td>
</tr>
<tr>
<td></td>
<td>inbred</td>
<td>48.93</td>
<td>42.89</td>
</tr>
<tr>
<td>intermediate</td>
<td>control</td>
<td>11.42</td>
<td>2.91</td>
</tr>
<tr>
<td>laboratory</td>
<td>inbred</td>
<td>12.68</td>
<td>8.63</td>
</tr>
<tr>
<td>intermediate</td>
<td>control</td>
<td>16.51</td>
<td>13.72</td>
</tr>
<tr>
<td>semi-natural</td>
<td>inbred</td>
<td>30.83</td>
<td>46.78</td>
</tr>
<tr>
<td>high laboratory</td>
<td>control</td>
<td>53.50</td>
<td>7.77</td>
</tr>
<tr>
<td></td>
<td>inbred</td>
<td>78.00</td>
<td>47.33</td>
</tr>
<tr>
<td>high semi-natural</td>
<td>control</td>
<td>22.45</td>
<td>14.58</td>
</tr>
<tr>
<td></td>
<td>inbred</td>
<td>30.38</td>
<td>49.14</td>
</tr>
</tbody>
</table>

nor temperature impacted on the estimates (location: $F_{1,12}=1.06$, n.s.; temperature: $F_{2,12}=1.58$, n.s.).

**4. DISCUSSION**

The major hypothesis that we set out to test in this study was whether the consequences of inbreeding on fitness components were temperature dependent. The answer to this question is affirmative for the traits egg-to-adult viability and developmental time. For egg-to-adult viability, inbreeding depression was observed only at stressful low and high temperatures (figure 2), whereas the interaction between inbreeding and temperature on developmental time was mainly due to a non-significant trend towards longer development for inbred flies under cold conditions in the laboratory. No effects of inbreeding or inbreeding×temperature interaction were detected for the sex ratio, a trait expected not to be closely linked to fitness. This result is very much in accordance with recent studies showing that inbreeding depression may be more severe under harsh environmental conditions for traits closely linked to fitness (Keller et al. 2002; Armbruster & Reed 2005; Da Silva et al. 2006; Reed et al. 2007; Szulkin & Sheldon 2007). However, our results also show that consequences of inbreeding are highly line and trait specific and emphasize that studies on inbreeding depression should be performed under different environmental conditions, be highly replicated and several life-history traits should be investigated in order to get detailed knowledge on the genetic load of any specific population. Given the high absolute level of inbreeding of the inbred lines investigated in this study, we were surprised to see that only relatively low levels of inbreeding depression were observed. This may be explained by the history of the investigated lines. They have been held in the laboratory for 75 generations after the bottleneck events. Deleterious alleles may have been purged and mutations may have introduced new variation during that time. However, despite these circumstances, we observed strong interactions between inbreeding and temperature on the fitness traits egg-to-adult viability and developmental time. This indicates that purging of deleterious alleles is environment specific and therefore may not be of great use in conservation practice. Inbreeding levels approaching 0.7 are probably rare in nature (Crnokrak & Roff 1999; Szulkin & Sheldon 2007), but qualitatively we expect the results presented in this paper to be representative for inbreeding×environment interactions involving less inbred individuals. Interestingly, deleterious consequences of inbreeding were mainly observed in the laboratory at low and high temperatures. At low temperatures in the laboratory, inbreeding depression was observed for the trait egg-to-adult viability and a non-significant trend towards longer developmental time for inbred relative to non-inbred flies and a skewed sex ratio among the two breeding regimes were observed. At high temperatures under laboratory conditions, inbreeding depression for egg-to-adult viability was observed, whereas no significant inbreeding depression was observed at high temperatures under semi-natural conditions for this trait. This trend towards increased inbreeding depression at low and high temperatures under laboratory conditions relative to at low and high temperatures under semi-natural conditions could be caused by locality-specific variation in the temperature regimes. Under laboratory conditions, the mean low and high temperatures were 12.6 and 26.8°C, respectively, whereas these were 12.4 and 24.6°C, respectively, under semi-natural conditions. However, the main difference between the laboratory and semi-natural temperature regimes was not the absolute levels, but that under semi-natural conditions temperatures decreased gradually in late afternoons and nights and increased gradually in the mornings, whereas under laboratory conditions the temperature shifts were sudden and fast (within 30 min). Under semi-natural conditions, flies therefore have more chance of acclimating to daily changes in temperatures leading to plastic physiological changes that are known to be important for survival (Hoffmann et al. 2003; Chown & Terblanche 2007; Kristensen et al. 2008b). The distinct temperature distributions under laboratory and semi-natural conditions could explain the different results obtained at the two locations. The laboratory high temperature conditions

![Figure 4. Sex ratio ± s.e. (defined as the number of male flies emerging relative to the total number of emerging adults) for development at low, intermediate and high temperatures under laboratory and semi-natural conditions. Grey bars, non-inbred; black bars, inbred.](http://rspb.royalsocietypublishing.org/Downloaded from `http://rspb.royalsocietypublishing.org/Downloaded from http://rspb.royalsocietypublishing.org/`)
indeed seem to be more stressful relative to the high 
temperatures investigated under semi-natural conditions, 
since egg-to-adult viability is much lower in both the 
inbred and non-inbred flies in the laboratory.

The within and across line CVs in egg-to-adult viability 
showed that inbreeding as well as temperature significantly 
impacted on these estimates. The overall conclusions 
were that within as well as across line CVs increase with 
inbreeding and that within line CVs tend to be higher 
when development takes place at sub-optimal (low or high) 
temperatures (table 2). What do these results tell us? According to quantitative genetic theory, one would 
expect the within line (additive) genetic variance to decrease and the between line genetic variance to increase 
with inbreeding (Falconer & MacKay 1996). However, 
changes in non-genetic variance components in inbred lines make it hard to come up with clear expectations of 
the consequences of inbreeding on variances at the phenotype level. Inbreeding may increase the environ-
mental variance, which can be interpreted as increased 
environmental sensitivity (Fowler & Whitlock 1999; 
Whitlock & Fowler 1999; Kristensen et al. 2005). This 
could explain the increased CVs observed within the 
inbred lines in the current study. Also, low and high 
temperatures lead to increased CVs within lines. This may 
be caused by increased expression of genetic variation that 
is hidden under benign temperatures, as suggested by 
Rutherford & Lindquist (1998). Obviously, this hypothesis 
needs to be tested in more detail. The increase in 
in within line CVs (and a non-significant trend in the same 
direction across lines) observed at low and high tempera-
tures is interesting because it has been suggested that 
inbreeding depression should be more pronounced in 
environments that increase the variability of fitness traits 
(Szulkin & Sheldon 2007; Waller et al. 2008). As discussed 
recently by Szulkin & Sheldon (2007), increased variabil-
ity as a consequence of stressful environmental conditions will make the detection of statistically signi-
ficant inbreeding depression and interactions between 
genotypes and environments difficult. Performing 
replicated experiments with high statistical power under 
semi-natural conditions may therefore be the best way to 
increase our understanding of inbreeding depression and 
inbreeding×environment interactions.

Interestingly, the sex ratio of emerging flies was skewed 
towards more females relative to males emerging at low 
temperatures. It is well known that mated adult female 
D. melanogaster are less cold resistant relative to males 
(Rako & Hoffmann 2006; Jensen et al. 2007). However, 
Jensen et al. (2007) presented data indicating that in pupal 
stages selection may actually be stronger on males relative 
to females at low temperatures. The mechanism behind 
this finding is unknown, but our data indicate that 
selection on eggs, larvae and/or pupae is stronger in 
males relative to females at low developmental tempera-
tures. If this is a general phenomenon, the evolutionary 
significance should be investigated in more detail.

5. CONCLUSION

In the current study, we find that inbreeding effects are 
trait and temperature dependent, with inbreeding 
depression observed only at stressful low and high tempe-
ratures under laboratory and semi-natural conditions 
(only at low temperatures) for the trait egg-to-adult 
viability. We also show that the selection pressure during 
development is higher for males relative to females at low 
temperatures. Furthermore, variances (quantified by CVs) 
in egg-to-adult viability are higher within and among lines 
with inbreeding than without inbreeding, and increase at 
stressful temperatures relative to benign intermediate 
temperatures. The synergistic interactions between 
inbreeding and temperature observed in this study are of 
concern in relation to climatic change and emphasize that 
(i) climate change may have more severe negative 
consequences for inbred relative to outbred populations and 
(ii) small populations exposed to inbreeding should 
be investigated under a range of ecologically relevant environ-
mental conditions and for several traits to verify whether 
inbreeding is a threat for their long-term persistence.

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