EFFECTS OF INBREEDING ON PRODUCTIVITY AND EXPRESSION OF HEAT SHOCK PROTEINS IN FRUITFLIES

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INTRODUCTION

In farm animals inbreeding has increasingly become a problem as selection programs have become more efficient. Within the world-wide Holstein-Friesian dairy cattle population the effective number (Ne) of animals is estimated to be approximately 30 individuals (Young and Seykora, 1996). Population genetic theory suggests that the level of F increase by $1/2Ne$ per generation (Falconer and Mackay, 1996). Therefore the level of inbreeding within the population will increase dramatically in the future unless precautionary measures are taken. One consequence of inbreeding is inbreeding depression. In addition the level of additive genetic variance is reduced in populations with a small Ne due to random genetic drift. The potential for genetic progress therefore decreases (Falconer and Mackay, 1996). These consequences of a low Ne have been estimated to influence negatively on the economy in farming (Smith \textit{et al.}, 1998).

In this paper we report on an empirical study where \textit{Drosophila} has been used as a model organism to investigate interactions between genetic and environmental stress in a controlled laboratory experiment (experiment 1). Furthermore, Hsp70 expression, which is a physiological response helping organisms to cope with stress (Feder and Hofmann, 1999), is studied in inbred and outbred flies after exposure to different temperatures (experiment 2).

MATERIAL AND METHODS

\textbf{Experiment 1}. A laboratory mass-bred population of \textit{Drosophila buzzatii} was founded from flies collected in spring 1998 on Tenerife, Spain. Lines of four inbreeding levels, F = 0, 0.25, 0.5 and 0.672, were produced by random mating or by full sib mating under standard laboratory conditions. For each level of inbreeding three independent lines were created. All lines were tested for productivity (a multiplicative measure of fecundity and viability) in four different environments: 1) Standard laboratory conditions (control treatment). 2) An environment where stress imposed by chemicals was introduced by the addition of the organophosphorus insecticide dimethoate to the media. 3) An environment where flies were exposed to fluctuating temperature stress. 4) An environment where fluctuating temperature stress and dimethoate was combined.

\textbf{Experiment 2}. The lines with F = 0 and 0.672 (created as described above) were used. Third instar larvae from the two inbreeding groups (lines were pooled) were exposed to stressful (33,
37, 38, 39 or 40°C) or non-stressful temperatures (25 or 29°C) for one hour before being frozen. The inducible Hsp70 was quantified by the ELISA technique using the monoclonal antibody 7.FB (Welte et al., 1993).

**Statistical analysis.** Data were analysed by standard statistical procedures (Zar, 1999). In experiment 1 data were square root transformed before further analysis. To test the effects of inbreeding, treatment and line ANOVAs were used. Variation in productivity was estimated by the coefficient of variation (CV). Lines were pooled within each level of inbreeding, as they were unrelated and independent. Comparisons of CV’s in productivity between environments and levels of inbreeding were performed by variance ratio tests. In experiment 2 Hsp70 level was calculated from six replica ELISA plates. One replicate sample of each treatment and inbreeding level was represented on each plate.

**RESULTS**

**Experiment 1.** The effects of inbreeding (P < 0.0001), treatment (P <0.0001), and line (P< 0.0001) significantly affected productivity. The interaction between inbreeding and treatment was at the boundary of significance (P < 0.0549). When testing productivity in the four environments separately there was a significant effect of inbreeding in two of the three stressful environments : temperature : (P < 0.008) and temperature and dimethoate : (P < 0.0001) (Figure 1). Within the other two conditions (control and dimethoate), the effect of inbreeding was not significant.
CV in productivity was significantly affected by the test treatment (P<0.0001) and inbreeding (P<0.0001). Ratios of CV in productivity between inbred and outbred flies tested in each of the four environments revealed a tendency towards higher CV for highly inbred flies under all environmental conditions (Table 1). The ratio of CV in productivity of control-adapted outbred flies tested in this environment with CV for outbred control-adapted flies tested in the stressful environments showed a weak tendency toward higher CV in productivity for outbred flies tested under stressful conditions (data not shown).

Table 1. Ratios between estimates of CV’s in productivity. All comparisons were tested by variance ratio tests. Significant differences between CV’s are indicated (*** = P < 0.001)

<table>
<thead>
<tr>
<th>Environment</th>
<th>F = 0/F = 0.25</th>
<th>F = 0/F = 0.5</th>
<th>F = 0/F = 0.672</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.82</td>
<td>0.55***</td>
<td>0.71</td>
</tr>
<tr>
<td>Dim.</td>
<td>1.29</td>
<td>0.64</td>
<td>0.84</td>
</tr>
<tr>
<td>Temp.</td>
<td>1.06</td>
<td>0.73</td>
<td>0.56***</td>
</tr>
<tr>
<td>Temp. and Dim.</td>
<td>1.5</td>
<td>0.85</td>
<td>0.68</td>
</tr>
</tbody>
</table>

**Experiment 2.** Inbreeding (P<0.001) and temperature (P<0.001) affected Hsp70 expression significantly. Data were subsequently divided into two sets for further analysis according to temperatures that normally induce Hsp70 (33, 37, 38, 39 or 40°C) in Drosophila buzzatii larvae, and those that normally do not (25 and 29°C). In both sets, there was a significant effect of inbreeding on Hsp70 expression (inducing temperatures: P<0.001, non-inducing temperatures: P<0.001), but the temperature effect was significant only for the high temperature set (inducing temperatures: P<0.001). Pairwise comparisons of Hsp70 expression in inbred and outbred larvae at each temperature showed that inbred larvae expressed significantly more Hsp70 except at 38 and 40°C (Figure 2).

![Figure 2. Hsp70 expression illustrated on a logarithmic scale (mean ±S.E.) in inbred (F=0.672) or outbred (F=0) larvae after exposure to 25, 29, 33, 37, 38, 39 or 40°C. Differences in Hsp70 expression between inbred and outbred larvae were tested by t-tests. Significant differences are indicated (*p<0.05, **p<0.01, *** p<0.001)
DISCUSSION

Experiment 1. To test effects of environmental changes, lines adapted to control conditions were exposed to stressful environments. An effect of inbreeding and treatment as well as an interaction term on the border of significance were observed. This result indicates that the negative impact of inbreeding is most severe under sub-optimal conditions. In a favourable environment inbreeding depression may be absent or of minor importance (Figure 1). Inbreeding and treatment significantly affected the CV in productivity. Within each test treatment there was a tendency toward higher CV at higher levels of inbreeding (Table 1). A reason for this may be that inbreeding increases the sensitivity to environmental changes. Furthermore, we observed a tendency toward higher CV’s under exposure to stressful environments. This shows that the novelty of environments is of importance for the expression of phenotypic variation.

A highly significant effect of line was observed (Figure 1). This is in concordance with most other studies on inbreeding, revealing significant population effects (Lynch, 1988). Specific populations in given environments can preserve high long-term fitness even though they are highly inbred, whereas other populations may suffer (Figure 1).

Experiment 2. Inbreeding depression as explained by the widely accepted partial dominance hypothesis is caused by the expression of deleterious recessive alleles. Increased homozygosity may increase the proportion of proteins having non-native conformation due to deleterious alleles being expressed. These proteins often have severe problems with folding leaving them in a conformation that may be deleterious (Gregersen et al., 2001). Our data indicate that an upregulation of Hsp70 is necessary even at non-stressful conditions in order to restore protein homeostasis in inbred populations, i.e. by facilitating correct protein folding/refolding or by disposal by degradation of non-functional proteins.

CONCLUSION

We conclude that inbreeding can severely effect fitness of inbred lines of Drosophila buzzatii, especially when exposed to stressful environmental conditions. We also observed a tendency toward increased CV in productivity at higher levels of inbreeding, and at harsh environmental conditions. Furthermore, our results reveal an upregulation of Hsp70 expression in inbred fruitflies under both stressful and benign temperatures.

REFERENCES