Evidence for the Limitation of Outcrossing as a Breeding Strategy in Conservation Programs

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Introduction

Captive and endangered animal populations have greater rates of inbreeding due to small population sizes and an insufficient number of unrelated potential mates. If mating between related individuals occurs often, it can have significant negative impacts on fitness and reproductive performance. There are multiple strategies currently available for zoos and other wildlife centers to utilize when making breeding decisions. The two most common strategies to date are maximum avoidance of inbreeding (MAI; Kimura and Crow, 1963) and minimizing kinship (MK; Ballou and Lacy, 1995). MAI works by minimizing any further inbreeding accumulation in the population, but it does not rely on historical pedigree information. MK works by minimizing the overall level of relationship among individuals in a population. MK does use historical pedigree information and is advantageous over MAI when there are unequal contributions of the initial founders to the current population (Montgomery *et al.*, 1997). Minimizing mean kinship, the average of the kinship coefficients between an individual and all other living individuals, while considering demographic constraints, as a breeding strategy appears to have been the most effective to date for retaining the greatest amount of founder genetic diversity (Ballou and Lacy, 1995, Montgomery *et al.*, 1997, Rodriguez-Clark, 1999). Whether this provides a fitness advantage or improves the probability of persistence for populations under natural selective pressures has yet to be determined (Montgomery *et al.*, 1997, Rodriguez-Clark, 1999). In this study, two inbred (moderately and severe) lines and a non-inbred line of the red flour beetle, *Tribolium castaneum*, were used in a series of inter-line matings in an attempt to alleviate inbreeding depression previously observed for fitness and reproductive related traits in the two inbred lines.

Material and methods

Resource lines and crosses. Three laboratory lines of *T. castaneum* (EBLOO, A, and C) were used in this study. These lines have been reproductively isolated for 22 generations in the laboratory. The EBLOO line is a large ($N \approx 2000$), random mating population, with a low estimated average kinship. The A line is a small ($N = 32$), random mating population with moderate estimated average kinship ($F \approx 0.271$). The C line is a small ($N = 32$), random mating population which experienced a 5 generation, severe demographic bottleneck event in generations 2 through 6 after the separation of the lines; it has a high average kinship ($F \approx 0.751$). A detailed description of how the lines were formed is found in Choinière (2008).

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Twenty-seven initial crosses were set up between these lines, including among- and between-line matings. Traits measured for individual crosses included: mean three-day egg lay (egg production), total number of progeny after 18 days (total progeny), proportion of progeny having reached or passed the pupa stage by day 18 (developmental time), and mean pupa progeny weight (pupa weight). Two additional generations of targeted crosses were performed in order to estimate direct and maternal genetic effects for each line, as well as individual and maternal heterosis effects between the three lines. For direct genetic and individual heterosis effects, crosses were performed such that recorded individuals had between 0 and 100% of the effect in increments of 12.5%. For maternal genetic effects and maternal heterosis effects, increments of 25% and 50% were used respectively.

Mating pairs were maintained in a small glass jar on 730mg of 95% enriched flour and 5% brewer's yeast medium in a walk-in incubator with a temperature of 33ºC ± 1ºC and an average humidity of 70% ± 2%.

Statistical analyses. To calculate the individual and maternal heterosis effects, a general linear model adapted from Eisen (1989) was used:

$$Y_{\ell q} = \mu + \sum k_E g_E + \sum k_A g_A + \sum k_C g_C + \sum k_E^M g_E^M + \sum k_A^M g_A^M + \sum k_C^M g_C^M + \sum k_{EA} h_{EA} + \sum k_{EC} h_{EC} + \sum k_{AC} h_{AC} + \sum k_{EA}^M h_{EA}^M + \sum k_{EC}^M h_{EC}^M + \sum k_{AC}^M h_{AC}^M + e_{\ell q}$$

where: $Y_{\ell q}$ is the q^{th} individual of the ℓ^{th} mating type, μ is the overall mean, g_E, g_A and g_C are the average direct genetic effects of the EBLOO, A and C lines, respectively, k_E, k_A, and k_C are the proportions of genes in the progeny contributed by the respective line through either sire or dam, g^M_E, g^M_A, and g^M_C are the average maternal genetic effects of the EBLOO, A and C lines, respectively, k^M_E, k^M_A, and k^M_C are the proportions of genes in the dam from the EBLOO, A and C lines, respectively, h_{EA}, h^M_{EA}, h_{EC}, h^M_{EC}, h_{AC}, and h^M_{AC} are the direct and maternal dominance effects resulting from F_1 combinations in individuals and dams in the EBLOO, A, and C, lines respectively, and k_{EA}, k^M_{EA}, k_{EC}, k^M_{EC}, k_{AC}, and k^M_{AC} are the proportions of loci with one gene from one line and the other from a different line in individuals and dams in the EBLOO, A, and C, lines respectively. $e_{\ell q}$ is the random error term. The model was run nine times, each run excluding one direct genetic effect term and one maternal genetic effect term, due to the dependence in the model (all possible combinations). Significance of heterosis effects was determined based on the significance of the regression coefficient for the effect in the model excluding the EBLOO direct and maternal genetic effects.

The intercept generated from each model was used to create a new data set with a factorial arrangement by direct genetic line and maternal genetic line, based on the premise that the intercept would be the average effect for that specific combination when all other effects are removed from the data. Pooled residuals and original degrees of freedom from the previous model were used to calculate statistical significance between pairwise contrasts of the means for each trait.

Results and Discussion

Most direct genetic effects were significantly different from one another for all four traits measured (egg production, total progeny, developmental time, and pupa weight), P<0.05, except lines EBLOO and A, which did not differ significantly for egg production, and lines A and C, which did not differ significantly for developmental time (Figure 1a). The direct

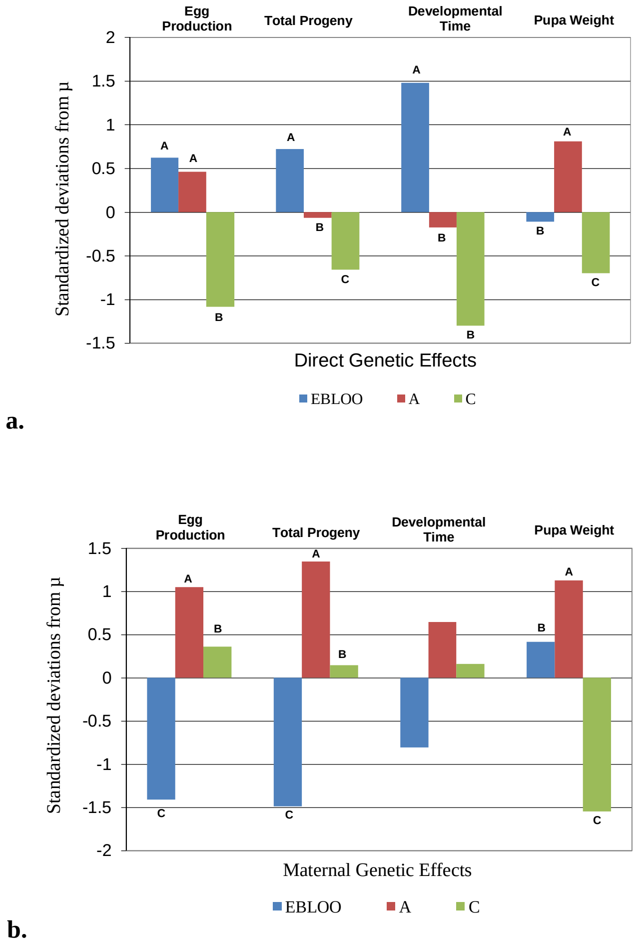

Figure 1: Direct genetic (a) and maternal genetic (b) effect means expressed as standardized deviations from the overall population mean. Groupings (A, B, and C) within traits are significantly different at α=0.05. Populations are ordered in ascending order of inbreeding accumulation from left to right, within each trait.

genetic effect of the A and C lines compared to the EBLOO line demonstrates the negative impact that inbreeding accumulation can have on fitness. The highly inbred line, C, had the lowest mean fitness for all four traits. Maternal genetic effects were all significantly different from one another for egg production, total progeny, and pupa weight, however there were no maternal genetic effects observed for developmental time (Figure 1b). Moderate inbreeding appeared to have a favorable impact on the maternal genetic effect.

There was no evidence of positive heterosis for egg production, total progeny or developmental time. Significant positive heterosis for pupa weight was only observed between the EBLOO and C lines, 0.0016 ± 0.0007 (β_i ± SE, P=0.0322). The lack of positive heterosis between genetic lines for fitness related traits suggests that introducing unrelated individuals into inbred captive populations may only have marginal success in improving growth and reproduction.

There was a significant negative effect of heterosis between the EBLOO and A lines for egg production, -0.075 ± 0.036 (β_i ± SE, P=0.0379) and total progeny, -0.281 ± 0.130 (β_i ± SE, P=0.0317), illustrating the potential for negative effects on fitness (outbreeding depression) when unrelated individuals are introduced into a small, inbred population. No significant maternal heterosis was observed in this study.

Conclusion

The decrease observed in egg production, total progeny, developmental time and pupa weight as inbreeding increases emphasizes the importance of MK as a breeding strategy to maximize the genetic effective population size of small, captive populations. In support of Lynch (1991), the lack of significant positive heterosis and the presence of significant negative heterosis observed after three generations of crosses, suggest that outcrossing may not be an effective breeding strategy to alleviate the effects of inbreeding depression.

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