The Genetic Basis of Reduced Inbreeding Depression in Polyploids

Introduction

Inbreeding depression is the reduced fitness of inbred offspring relative to outbred offspring. The level of inbreeding depression has been suggested to determine a plant's mating system, as population genetic theory proposes that individuals will adopt selfing to the extent that inbreeding depression exceeds the associated transmission advantage (Carr and Dudash, 2003). Inbreeding depression is a consistently observable phenomenon; however, its genetic basis continues to be debated. Several hypotheses have been invoked, particularly the dominance, overdominance and epistatic hypotheses (Charlesworth and Charlesworth, 1999). The dominance hypothesis proposes that inbreeding depression occurs as a result of the expression of deleterious recessive alleles as homozygosity increases. The overdominance hypothesis, on the other hand, attributes inbreeding depression to the loss of heterozygotic interactions with higher fitness. (Charlesworth and Charlesworth, 1999). Finally, epistasis can contribute to inbreeding depression by either reducing or enhancing the effects of homozygosity at different loci, but is difficult to detect (Carr and Dudash, 2003).

The genetic basis of inbreeding depression is of interest due to its role in determining plant mating systems, which could potentially be agronomically valuable, along with its potential importance in conservation biology (Husband and Schemske, 1997). Further, the outcrossing of inbred lines can lead to heterosis, a finding that can be harnessed to develop vigorous crop species (e.g. Robins et al., 2007) In an effort to support a genetic hypothesis, several studies have been conducted to isolate quantitative trait loci (QTL) contributing to inbreeding depression and heterosis in a variety of species, including maize (Stuber et al., 1992), rice (Luo et al., 2001),), and rapeseed (Radoev et al., 2008). These studies propose that by characterizing these QTL interactions, we can model the extent of dominance, overdominance and epistasis giving rise to variation in inbred and outbred phenotypes.

Each genetic hypothesis of inbreeding depression is associated with different predictions regarding the consequences of polyploidy on the extent of inbreeding depression. Polyploid success should be associated with a selfing mating system (Soltis and Soltis, 2000), as selfing could facilitate in overcoming minority cytotype disadvantage (Rausch and Morgan, 2005), and polyploids may exhibit reduced inbreeding depression (Husband and Schemske, 1997). Theoretical models based on the dominance hypothesis propose that inbreeding depression should be reduced in polyploids relative to diploids, as the expression of deleterious recessives should be slowed by the nature of tetrasomic inheritance (Husband and Schemske, 1997). Predictions based on the overdominance model are more complex. Bennett (1976) suggests that inbreeding depression in an autotetraploid should be greater, owing to greater accumulation of deleterious recessives with more genomic material. To my knowledge, models determining the contribution of epistatic effects to inbreeding depression in polyploids have not been developed.

Husband and Schemske (1997) empirically demonstrated reduced inbreeding depression in autotetraploids of *Epilobium augustifolium* relative to diploids in the same population. They interpreted significant differences in seed set and cumulative fitness between inbred and outbred autotetraploids and diploids as evidence for the dominance model of inbreeding depression. However, Husband and Schemske (1997) did not these characterize fitness differences at the genetic level.

To my knowledge, no study has ever attributed differences in inbreeding depression between diploid and autotetraploid individuals to the effects of particular loci. Harnessing the knowledge of QTL contributing to inbreeding depression and heterosis, and applying models of the dominance, overdominance and epistatic hypotheses, it should theoretically be possible to quantify the contribution of each of these hypotheses to loci generating phenotypic variation between inbred diploids and polyploids.

My Experiment

a) **Question:** For my experiment I plan to ask,
   1) Are there differences in the extent of inbreeding depression in *Medicago sativa* L. diploids and autotetraploids?
   2) If so, can we use analyses identifying viability loci to quantify the extent of dominant, overdominant and epistatic interactions in giving rise to these differences?

b) **Study system:** For my study system, I plan on using F1 hybrids of two subspecies of *Medicago sativa – M. sativa* subsp. *sativa* and *M. sativa* subsp. *falcata*. *M. sativa* is the preferred organism for my study because severe inbreeding depression has been demonstrated (Li and Brummer, 2009). I plan on using subspecies hybrids in my study, as inbreeding is too severe in the domestic line *M. sativa* subsp. *sativa* to allow for a large and adequately sampled F2 generation. Diploid and autotetraploid lines are readily available in this species. Further, due to its economic value as a forage crop, a genetic linkage map is also available, thus genetic markers do not need to be constructed.

c) **Methods:** The methodology for my study is as follows: 1) Using a sample that is genotypically diverse, make *Medicago sativa* F1 hybrids by randomly crossing diploid and autotetraploid *M. sativa* subsp. *sativa* and *M. sativa* subsp. *falcata* within their respective cytotypes. 2) Generate selfed and outcrossed F2 generations by experimentally selfing and outcrossing F1 hybrids. 3) Compare fitness attributes of selfed and outcrossed F2 offspring, particularly seed biomass yield, number of seeds per pod, and seedling survival to ensure that inbreeding depression has occurred. Conduct a chi-squared test to demonstrate that there are significant differences in fitness attributes between selfed and outcrossed F2 offspring, and between cytotypes in selfed F2 offspring. 4) Genotype F2 offspring using SSR and AFLP markers, where possible using previously developed primers (Julier et al., 2003). Use a high density of markers in regions known to be closely linked to loci of interest that contribute to offspring seed set (Robins et al., 2007). A large number of markers coupled with knowledge of QTL determining fertility should provide enhanced statistical power to detect distorted segregation ratios governed by fitness differences (Fu and Ritland, 1994b). 5) Apply the graphical method of Fu and Ritland (1994a, 1994b, 1996) to observed frequencies of genotypic markers in the selfed F2 population. These methods propose that markers linked to loci that reduce offspring fitness by inbreeding depression should be disproportionally absent from the inbred population. Through knowledge of heterozygosity of marker loci, recombination rate, and segregation ratios, the method is used to rate the consistency of marker data with predictions for overdominant, partially dominant, and epistatic interactions (Fu and Ritland, 1996). 6) Conduct a chi-squared

test to determine whether the effects of overdominance, dominance, and epistasis on inbreeding depression by viability loci are statistically significant between ploidy levels.

d) **Predicted results and significance:** Based on theory, I predict that inbreeding depression will be less severe in autotetraploid *M. sativa*, and that this difference will be due to the expression of fewer deleterious recessive alleles. That is, if statistically significant differences between cytotypes in expression of particular viability loci are observed, the segregation of these loci should be consistent with the dominance hypothesis. I predict that any differences between ploidy levels in the expression of overdominant loci and epistatic interactions will not be statistically significant, due to the inconsistency of theory regarding these interactions. If any differences are observed that are consistent with a particular hypothesis, this will be the first study to empirically attribute differences in inbreeding depression between ploidy levels to a genetic basis. In this case, more studies will need to be conducted in other taxa and examining differences at other loci to demonstrate the generalizability of our findings.

e) **Drawbacks:** Due to the fact that no study of this kind has ever been conducted, and a lack of preliminary data, the possible results of this experimental procedure are difficult to predict. Whether the methods described above possess enough statistical power to detect differences in mechanisms of inbreeding depression between cytotypes is debatable. I propose remedying this problem by using large samples of individuals, controlling for environmental interactions by growing individuals at multiple plots, and saturating genetic coverage with a large number of genotypic markers. Because I plan to use F1 hybrids of *M. sativa*, any results observed may be an artifact of artificial hybrid breakdown rather than inbreeding depression *per se.* In addition, detected differences between ploidy levels may be due to genotypic differences, though the use of large samples with genotypic diversity should reduce the significance of this problem. Nonetheless, this proposal provides a provisional preliminary framework for generating an answer to a novel question.

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