

SNP-based inferences on expression, reproductive ramifications and inheritance of the *Semigamy* gene of cotton (*Gossypium barbadense* L.), a rare angiosperm mutant affecting karyogamy



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Abstract

The *Semigamy* (*Se*) mutant of Pima cotton (*Gossypium barbadense* L. $2n=4x=52$) was first reported in 1963 for its remarkable production of monoembryonic haploids at high frequencies. Our lab has established that akaryogamy is the cytological mechanism responsible for this bizarre form of quasi-apomictic (asexual) reproduction that leads to the production of haploids and chimeras composed of sectors of maternal and paternal origin. In terms of the seed produced, levels of *Se* expression are less than 100%, i.e., some progeny produced by homozygous mutant parents are normal (tetraploid). More knowledge about the mode of gene action and expression of *Semigamy* could be very valuable for breeders and geneticists, such as for mass production of doubled haploids, rapid synthesis of true-breeding genetic stocks and populations of haploids or doubled haploids for genetic mapping. To extend previous studies of *Semigamy*, I analyzed progeny from crosses and reciprocal crosses between a semigamous line (*SeSe*) and heterozygous plants (*Sese*) to investigate differences in *Se* expression in maternal versus paternal heterozygous (*Sese*) parents. Phenotypic seedling markers were used to differentiate parental origins of chimeric haploid sectors, and *Se*-linked SNP markers were used to determine the allelic composition (*Se* vs *se*) of each haploid sector arising from a male or female *Sese* parent. The genotypic results of maternal and paternal haploid sectors differed significantly. This finding strengthens evidence that effects and inheritance of the mutant allele (*Se*) differ according to parent of origin and that a detailed molecular characterization is desirable.

Introduction

Semigamy is a naturally occurring mutation in the cotton species *Gossypium barbadense* that leads to abnormal (semigamous) reproduction, where the gametes undergo syngamy (gametic cell fusion) but the nuclei forego karyogamy (nuclear fusion) (TURCOTTE and FEASTER 1967a).

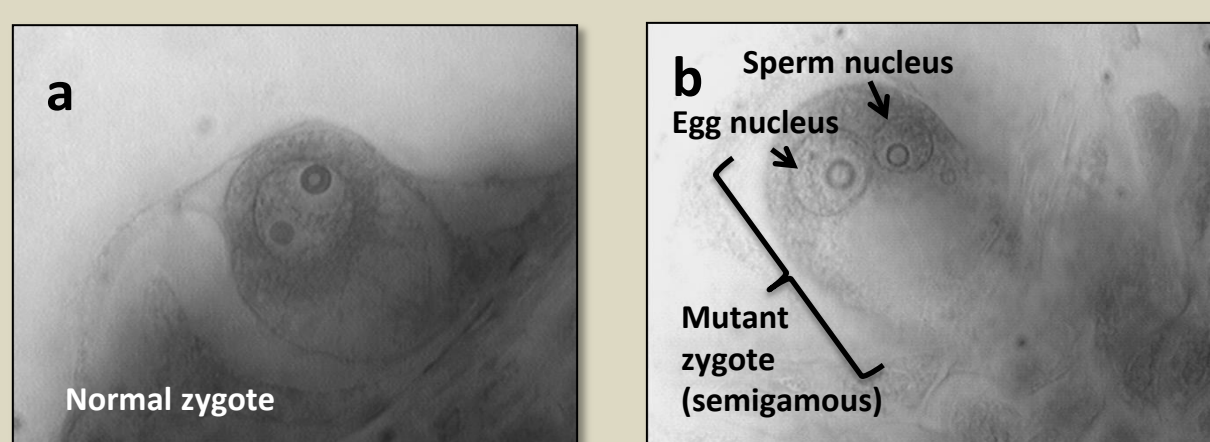


Figure 1. (a) Sexual zygote. The presence of two nucleoli indicates that karyogamy has recently occurred. (b) Semigamous nucellus. Though the nuclei are adjacent to each other, no fusion has occurred. (after Biddle, 2006)

In this mutant of cotton, the non-fused maternal and paternal nuclei of the zygotic heterokaryon undergo mitotic divisions more or less independently, and these subsequently lead to an array of different kinds of embryos, including chimeras, maternal/paternal haploids and normal zygotes (BIDDLE 2006).

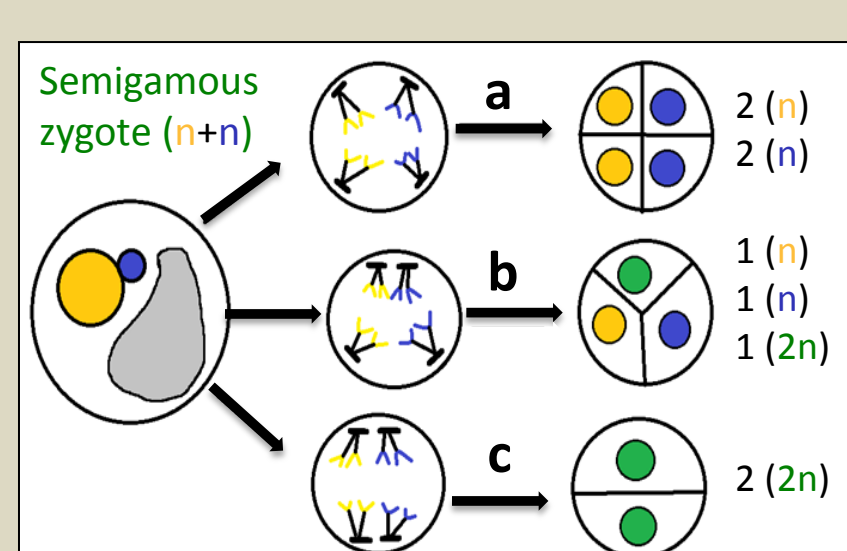
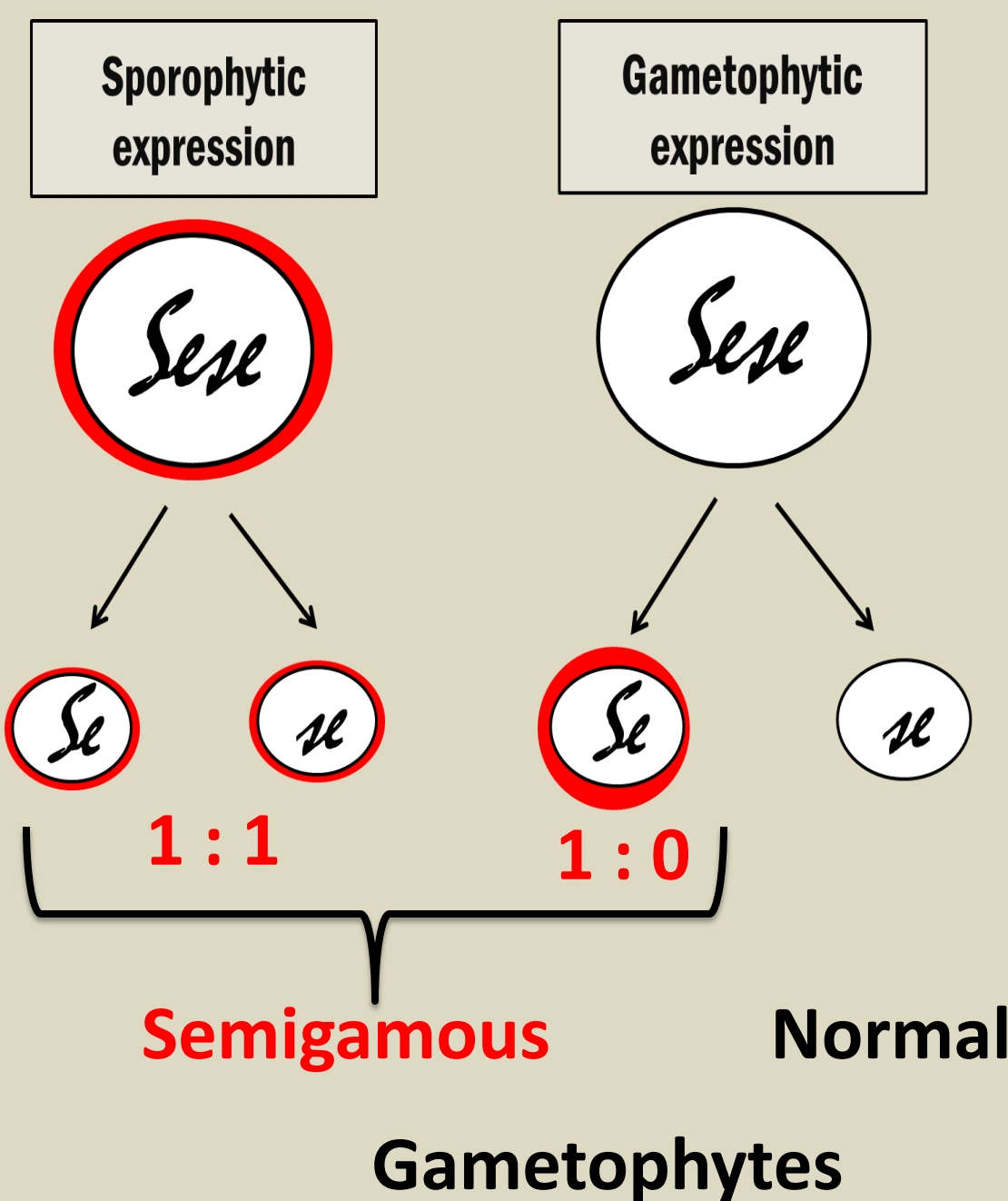


Figure 2. First mitotic divisions of semigamous zygote. Spindle number and orientation determine possible outcomes: (a) **tetrapolar** → 4 haploid cells; (b) **tripolar** → 1 normal + 2 haploid cells, and (c) **bipolar** (single spindle or fused/parallel spindles), → two normal cells.

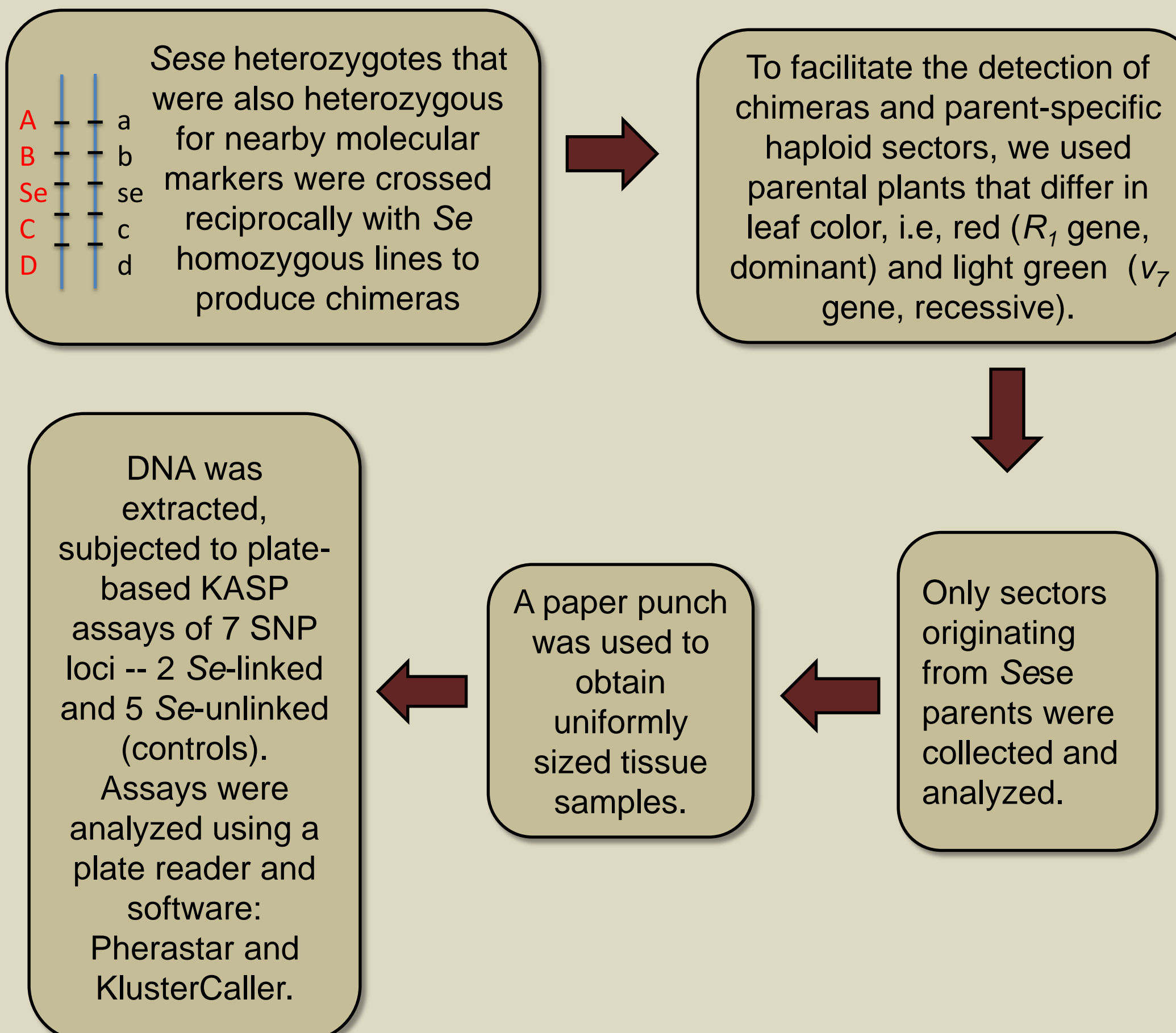
Despite its history and intriguing features, the location of *Semigamy* gene in the cotton (*G. barbadense*) genome is still unclear, as well as its precise mechanism. Our lab has established its cytological mechanism, akaryogamy (Biddle 2006), and subsequently used cytological, molecular, Next Generation Sequencing and bioinformatics to identify SSR and SNP molecular markers potentially linked to the *Semigamy* gene (Stelly lab, unpublished data). Mapping the gene and determining its mode of gene action and expression could be very valuable for breeders and geneticists, who would like to use it to mass-produce doubled haploids, rapidly synthesize true-breeding genetic stocks and breeding lines, and convert cytoplasm (cytoplasmic substitution).

Approach

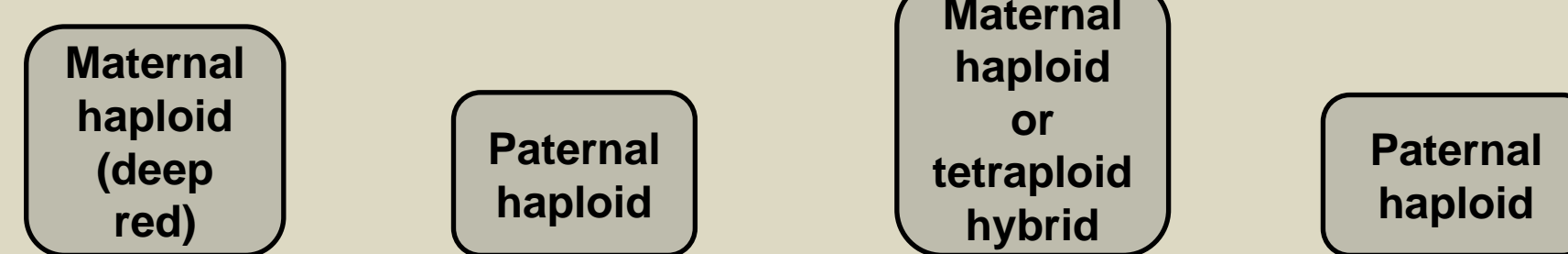
Crosses and reciprocal crosses between a semigamous line (*SeSe*) and heterozygous F1 and BC1F1 plants (*Sese*) were analyzed to investigate differences between male and female *Se* expression according to allelic inheritance from maternal and paternal heterozygous (*Sese*) parents. Mutant and wild-type alleles would be found randomly in the sectorial tissues if *Se* expression relies exclusively on sporophytic transcription, whereas only mutant alleles would be found if expression relies exclusively on gametophytic transcription.



Materials and Methods



Sese Rr W * *SeSe rr w*



Results and Discussion

The genotyping of maternal and paternal haploid sectors with *Se*-linked SNPs revealed that all maternal haploid sectors originating from *Sese* heterozygous females were mutant (*Se*), with the exception of one recombinant. The biased recovery indicates that the semigamous reproduction requires megagametophytic expression of the *Semigamy* allele.

Allele	Female origin		Male origin	
	SNP-A	SNP-E	SNP-A	SNP-E
<i>Se</i>	34	33	33	30
<i>se</i>	0	1*	11	13

* Recombinant

In contrast, similar analysis of haploid sectors originating from *Sese* heterozygous males presented both mutant and wild type alleles. However, paternal haploid sectors were skewed towards the mutant allele, and did not consist of a 50:50 mutant:wild allelic distribution. The frequencies of the *Se* allele among sectors from *Sese* male versus *Sese* female parents were statistically significant ($p < 0.05$).

The ratios of *Se:se* in haploid sectors from *Sese* males does not follow expectations based on determination that is exclusively sporophytic (1:1, $p < 0.01$) or gametophytic (1:0, $p=0$). The observed ratios might involve the following: (1) presence of epistatic interactions between genes in the male genome before/during gamete formation, (2) differential selection against the wild type allele, where selection is stronger in female than male heterozygotes, (3) there are more recombination events between the *Se*-linked SNP loci and *Se* gene in the male than in the female, (4) the paternal effect is limited -- influencing but not determining if fertilization and early development occur semigamously or normally.

Conclusions

The natural occurrence of a gene that causes apomixis-like reproduction in a major crop like cotton is a rare and exciting event, with potential benefits to future agriculture. This present study has shown that expression of the *Semigamy* gene is different in female versus male parents. The results also indicate that expression of the *Semigamy* gene is highly gametophytic on the female side, and at least partially gametophytic on the male side. Further studies are needed to fully define *Semigamy* expression, and characterize mechanisms at the molecular level. These should help scientists realize the full potential of this gene in cotton and perhaps other crops.

References

- Biddle, K. D., 2006. An analysis of alternative forms of plant reproduction using *Gossypium barbadense* and *Arabidopsis thaliana*, pp. Texas A&M University.
Turcotte, E., and C. V. Feaster, 1967a. Semigamy in Pima cotton. *Journal of Heredity* 58: 55-57.