Microsatellites distinguish sexual vs. apomictic reproduction in spontaneous *Rubus* hybrids

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Introduction

Hybridization between native and introduced species has contributed to the evolution of many invasive plant taxa. One means by which hybridization can lead to invasiveness is through fixed heterosis, whereby F1 hybrids experience fitness benefits due to dominance and overdominance effects and pass these benefits on to their offspring via asexual reproduction. Alternatively, sexual recombination in such hybrids may allow for novel trait combinations as well as introgression into either parent species, which could be detrimental to the native species or beneficial to the non-native species.

Native and non-native blackberry (*Rubus* subgen. *Rubus*) species often occur sympatrically in the Western United States. In a previous study using microsatellites and chloroplast markers, we identified two wild clones of *R. ursinus* x *armeniacus* and one of *R. ursinus* x *pensilvanicus*. These are hybrids between native and non-native species, where the native parent produces seed sexually and the non-native parent produces most of its seed clonally via pseudogamous apomixis (Table 1).

The objective of this study is to determine whether these spontaneous hybrids produce seed sexually, by apomixis, or a mixture of the two. Our results have implications for how a novel hybrid can evolve into an invasive species.

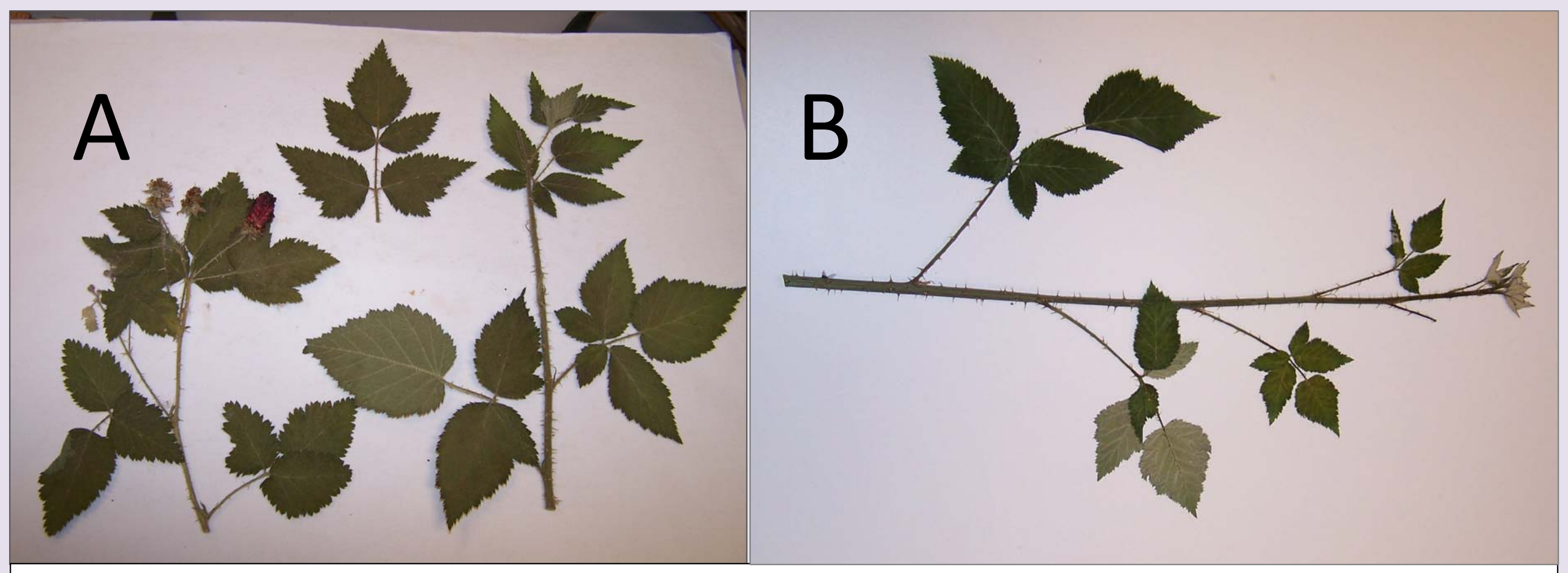


Figure 1. a) *Rubus ursinus* x *pensilvanicus* cuttings collected in Chico, California. b) *R. ursinus* x *armeniacus* cutting collected in Caswell Memorial State Park, CA.

Species	Ploidy	Native range	Seed production	Status in CA
R. ursinus	8	Western US, BC	Sexual	native
R. pensilvanicus	4	Eastern US	Facultative pseudogamous apomict	introduced, naturalized
R. armeniacus	4	Germany	Facultative pseudogamous apomict	introduced, highly invasive

Table 1. Parent species of spontaneous hybrids in this study.

Materials and Methods

DNA was extracted from parent plants as well as from seeds and, when possible, germinated seedlings. All individuals were genotyped with five microsatellite markers, which were scored using an Applied Biosystems 3100 Genetic Analyzer.

Microsatellite genotypes were imported into the statistical software R using the package POLYSAT (Clark and Jasieniuk, *Mol. Ecol. Res.* In press). Each seed and seedling was then assigned a score to indicate dissimilarity from the genotype of the mother plant. Scores were calculated in the following way:

- For each locus, alleles were identified that were present in the mother genotype but not in the offspring genotype and vice versa.
- Points were added for each missing or extra allele. If the allele was larger than all others in the genotype (potentially the result of allelic dropout), 0.5 points were added. If the allele was within one repeat length of another allele in either genotype (potential stutter or mutation), 0.5 points were added. For all other extra or missing alleles, 1 point was added.
- Scores were averaged across loci and divided by ploidy (4 for *R. armeniacus* and *R. pensilvanicus*, 8 for *R. ursinus*, and 6 for hybrids).

Scores were plotted in the histograms shown in Figures 2 and 3. Normal distributions of scores from the parent species, whose reproductive mode is known, were calculated. These distributions were used to calculate the posterior probability that an individual is a clone of its mother plant, given its score.

$$P(\text{apomictic}|\text{score}) = \frac{P(\text{score}|\text{apomictic})}{P(\text{score}|\text{apomictic}) + P(\text{score}|\text{sexual})}$$

Genotype scores from the hybrids were compared to the posterior probability curve in order to infer reproductive mode.

Results and Discussion

Scores of sexual and clonal offspring of the parent species were sufficiently different to assign most offspring of the hybrids to one category or the other. The two *R. ursinus* x *armeniacus* hybrids produced approximately equal amounts of seed sexually and by apomixis, while the *R. ursinus* x *pensilvanicus* hybrid produced nearly all of its seed by apomixis. However, in both cases, a disproportionately high number of seeds that germinated were sexually produced.

Due to the low germination rate of apomictic seed produced by the *Rubus* hybrids in this study, it is unlikely that these hybrids could become invasive via fixed heterosis. However, they possess the ability to continue evolving via sexual recombination. Therefore, hybrid *Rubus* populations should be periodically monitored in case eradication becomes necessary to prevent invasive spread.

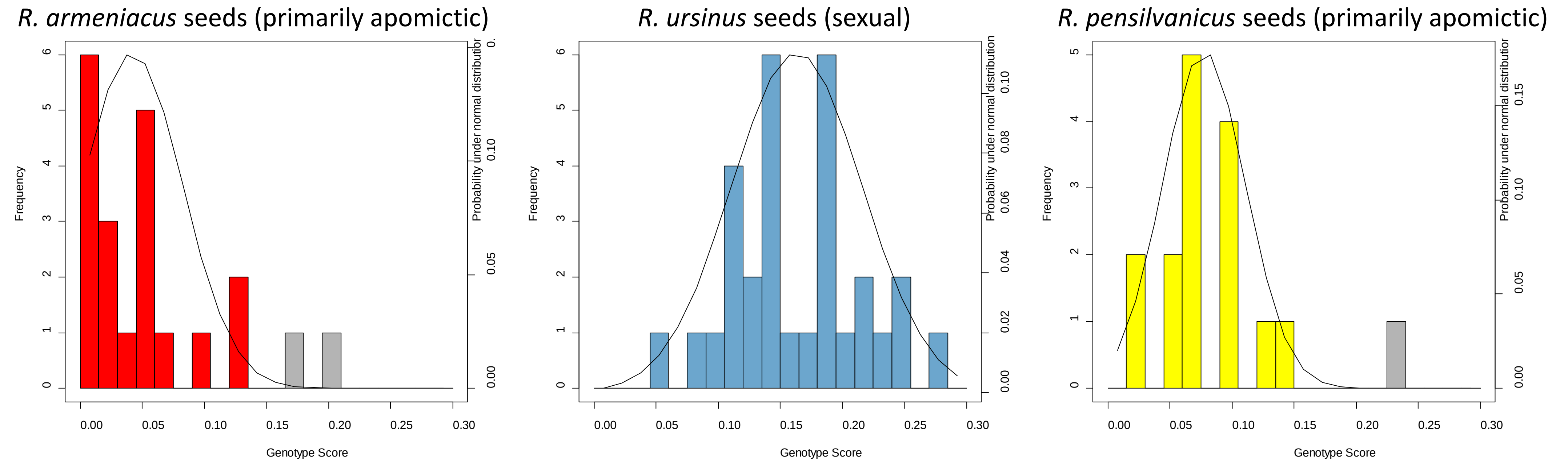
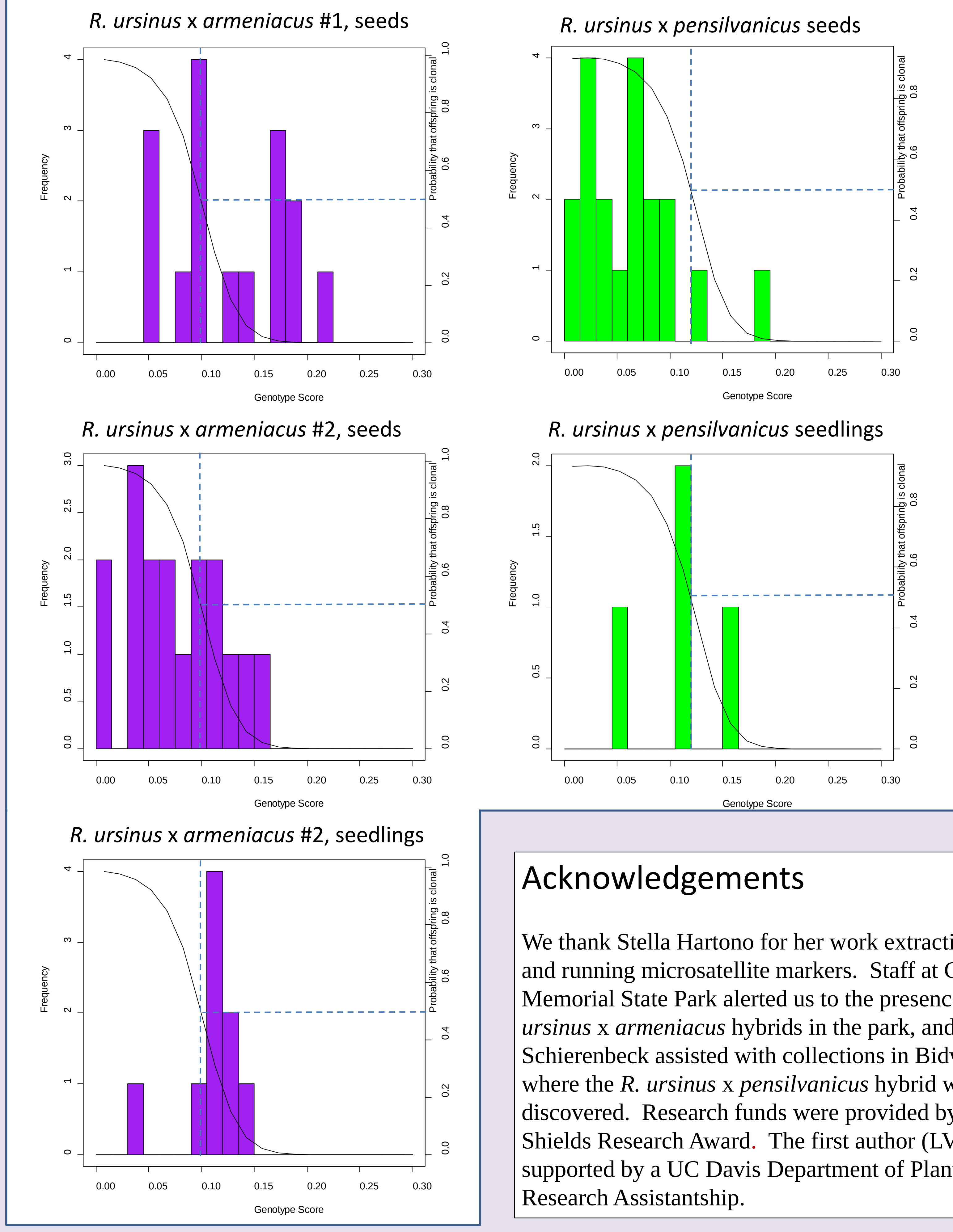


Figure 2. Histograms of genotype scores of offspring of the parent species. Because *R. armeniacus* and *R. pensilvanicus* produce approximately 10% of their seed sexually, grey bars indicate presumed sexually-produced offspring that were omitted when fitting normal curves to the data.

Figure 3. Histograms of genotype scores of offspring of hybrids. Curves indicate the posterior probability of an individual being a clone of the mother plant, based on apomictic and sexual score distributions of the parent species. Dashed lines indicate the score at which there is an equal likelihood that an individual was sexually produced as clonally produced.



Acknowledgements

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