

Hybridization between invasive and native blackberries (*Rubus*) in California

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Summary:

- We are interested in the hybridization of native and introduced species as a mechanism for the evolution of invasiveness.
- The genus *Rubus* includes many invasive plants, and also has a history of evolution through hybridization.
- Using genetic markers, we have identified hybrids:
 - R. ursinus* x *armeniacus* (native Pacific blackberry with invasive Himalayan blackberry)
 - R. ursinus* x *pensilvanicus* (native Pacific blackberry with introduced Pennsylvania blackberry)
- Future studies will:
 - Determine whether hybrids are backcrossing with native *R. ursinus* populations
 - Measure the effect of hybridization on fitness (and potential invasiveness)
 - Compare gene expression of invasive and non-invasive *Rubus* and hybrids
- Management applications:
 - If *R. armeniacus* and other introduced species are genetically contaminating *R. ursinus*, additional control of introduced/invasive and hybrid populations is recommended.
 - Understanding the evolution and genetic basis of invasiveness in *Rubus* will help to regulate species introductions and prioritize the management of introduced species.

Introduction: Biological invasion is often facilitated by rapid adaptive evolution. This provides opportunity to use genetic and genomic tools to understand the basis of invasiveness, since the invasive species may be compared to very closely related non-invasives. Hybridization provides several mechanisms by which invasiveness can evolve in plants: 1) hybrid vigor that is maintained through asexual reproduction, 2) novel combinations of genetic material from the parental species, 3) increased genetic variation that allows local adaptation, and 4) introduction of normally functioning genes so that selection can eliminate harmful mutations.

We have chosen *Rubus* (blackberries and raspberries; Rosaceae) as a genus in which to study the phenomenon of hybridization stimulating invasiveness. *Rubus* includes 11 noxious weeds in the United States alone, and these are taxonomically well distributed within the genus. In particular, Himalayan blackberry (*R. armeniacus* Focke = *R. discolor* Weihe & Nees and *R. procerus* Mueller; Figure 1), a naturalized cultivar originating in Germany, is highly invasive in California and the Pacific Northwest. *Rubus* also has a history of evolution through hybridization. Since there are seven native and at least four naturalized introduced species of *Rubus* in California, it is possible that hybridization could stimulate the evolution of new invasive forms.

The research presented here is a preliminary study in which we identify first-generation hybrids of native *Rubus* with introduced *Rubus* in California. Future studies will assess the invasive potential of hybrids and the degree to which they have sexually recombined with the parent species. We ultimately hope to contribute to a broader understanding of the evolution and genetic basis of invasiveness in *Rubus*, so that invasive species may be more easily predicted and managed.



Figure 1. *R. armeniacus* (Himalayan blackberry) growing in the American River Parkway east of Sacramento, CA.

Materials and Methods: In 2007 and 2008 we collected leaf tissue from 324 *Rubus* individuals at 14 sites across Central and Northern California (Table 1). We collected voucher specimens of each species or type at each site in 2008, and are storing them at the UC Davis Center for Plant Diversity. Our sample set is primarily *R. armeniacus* and *R. ursinus* (Pacific blackberry, native), as these are the species most commonly found in California. Seven other species and cultivars were also collected.

For genetic analysis, we ran six published microsatellites on all samples (Figure 2). Across the sample set, 230 alleles were discovered within these six loci, most of the diversity being in *R. ursinus*. Hybrids were identified by genetic distance using Principal Coordinate Analysis (Figure 3), and were confirmed by visual inspection of genotypes. To determine the maternal (seed) parents of hybrids, we developed and ran two chloroplast markers on our sample. (Chloroplasts contain their own DNA and are inherited from the female parent in most angiosperms.)

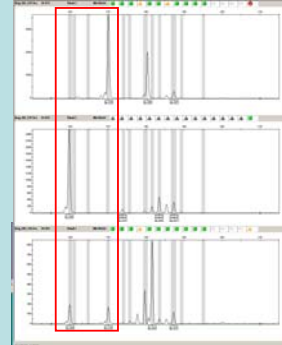


Figure 2. Example of microsatellite data. Microsatellites are repetitive regions of DNA that are highly variable in length. The horizontal axis indicates length, in basepairs, of one microsatellite locus. Multiple peaks (alleles) are present because these individuals are polyploid, having four to eight copies of each chromosome, and hence as many copies of the locus. Small "stutter" peaks are often seen to the left of true alleles. **Top:** an *R. armeniacus* individual. **Center:** an *R. ursinus* individual. **Bottom:** *R. ursinus* x *armeniacus*. Note that the hybrid has peaks characteristic of both parental species.

Results: We saw very little genetic diversity in *R. armeniacus*, and what variation there was originated from mutation rather than sexual recombination. This is consistent with its asexual seed production (apomixis) and its introduction as a cultivar. In contrast, *R. ursinus* showed considerable genetic diversity in both the nuclear (in the cell nucleus) and chloroplast (seed lineage) genomes. This is expected, because *R. ursinus* was sampled in its native range, where it has had millions of years to accumulate genetic diversity. *R. ursinus* is also dioecious (every plant is either male or female, ensuring outcrossing), which is consistent with the low amount of geographic structuring of the nuclear and chloroplast genotypes. Two genetically distinct *R. ursinus* x *armeniacus* hybrids were identified at Caswell Memorial State Park. *R. ursinus* was the maternal parent of both. Both parental species are present at the site, although *R. armeniacus* is a target of eradication. Characters such as leaf shape and color, prickle number and morphology, and stem cross section were intermediate between the two species (Figure 4A, B, D, E). First-year vegetative canes were growing vigorously when we visited.

One *R. ursinus* x *pensilvanicus* hybrid was identified at Bidwell Park. *R. ursinus* was the maternal parent. *R. pensilvanicus* is an introduced plant from the Eastern United States, but is not common in Northern California. *R. pensilvanicus* was growing in Bidwell Park near the hybrid, but was not found at any of our other sites. *R. ursinus* was present at the site but not growing as vigorously as *R. pensilvanicus* or the hybrid. Both *R. pensilvanicus* and the hybrid were producing fruit when we visited in late May 2008. There was great variation in leaf morphology even within one cane of the hybrid; leaves had three to seven leaflets, which could be arranged in a pinnate or palmate manner. Stem and prickle morphology was intermediate between the two parental species, while the seven leaflets was a transgressive trait (Figure 4B, C, F).



Figure 4. *Rubus* parental species and hybrids. A. *R. armeniacus*. B. *R. ursinus*. C. *R. pensilvanicus*. D-E. *R. ursinus* x *armeniacus*. F. *R. ursinus* x *pensilvanicus*.

Discussion: First generation hybrids were fairly uncommon in our data set (about 1% of individuals sampled), possibly due to brief overlap of flowering times of parental species, pollinator preference, low interspecies fertility, or other factors. However, the number of molecular markers we used was not sufficient to detect later generation hybrids and backcrosses. Given the high morphological diversity within *Rubus* species, backcrossed individuals might not be obvious from visual inspection either. As we add markers and use more sophisticated software for hybrid detection, it is possible that we will find introduced genetic material introgressing into native *Rubus* species or vice versa. This could cause reduced fitness in *R. ursinus* populations if some of its genes are incompatible with those of other species, or novel gene combinations could cause increased fitness and potential for invasiveness. We will test for these possibilities using common garden experiments. Ultimately we plan to measure gene expression differences between closely related invasive and non-invasive species of *Rubus*, and determine whether hybrids found in California more closely match the invasive or non-invasive profile. This will not only help to prioritize the management of hybrid *Rubus*, but will also give insight into the genetic basis of invasiveness so that it may be predicted in other species.

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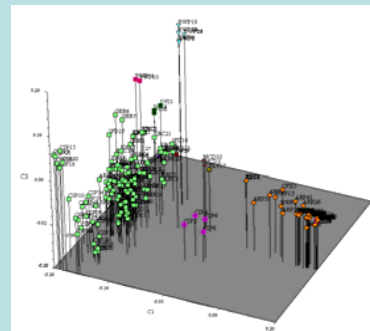


Figure 3. Principal Coordinate Analysis of microsatellite data. Orange circles: *R. armeniacus* (invasive). Light green squares: *R. ursinus* (native). Purple crosses: Putative *R. armeniacus* x *ursinus* hybrids. Light blue down triangles: *R. pensilvanicus* (introduced). Pink X's: Putative *R. pensilvanicus* x *ursinus* hybrids. Green asterisks: Boysenberry, a hybrid cultivar derived from *R. ursinus* and *R. idaeus*. Red circle: *R. idaeus* (cultivar). Purple up-triangle: *R. leucodermis* (native). Brown diamonds: *R. ulmifolius* (introduced). Yellow square: *R. laciniatus* (introduced).

Site	Species collected
King Range Conservation Area	<i>R. ursinus</i> , <i>R. armeniacus</i>
Bidwell Park	<i>R. armeniacus</i> , <i>R. ursinus</i> , <i>R. pensilvanicus</i> , <i>R. ursinus</i> x <i>pensilvanicus</i>
Sierra County	<i>R. armeniacus</i>
Colfax	<i>R. armeniacus</i> , <i>R. leucodermis</i>
Auburn	<i>R. armeniacus</i>
Placerville	<i>R. armeniacus</i> , <i>R. ursinus</i> , <i>R. laciniatus</i> , <i>R. ulmifolius</i>
Knight's Landing	<i>R. ursinus</i> , <i>R. armeniacus</i>
American River Parkway	<i>R. armeniacus</i> , <i>R. ursinus</i> , unidentified thornless cultivar
UC Davis Campus	Boysenberry, <i>R. armeniacus</i> , <i>R. idaeus</i>
Monticello Dam	<i>R. ursinus</i> , <i>R. armeniacus</i> , <i>R. ulmifolius</i>
Marin County	<i>R. armeniacus</i>
Cosumnes River Preserve	<i>R. ursinus</i> , <i>R. armeniacus</i>
Caswell Memorial State Park	<i>R. ursinus</i> , <i>R. ursinus</i> x <i>armeniacus</i> , <i>R. armeniacus</i>
George J. Hatfield Recreation Area	<i>R. ursinus</i>

Table 1. Sites at which *Rubus* leaf tissue was collected for DNA analysis.

