GameteMaker: A Genetic Mapping Simulation Program That Helps Students to Connect Mutant Phenotypes with Underlying, DNA Sequence-Based Genotypes

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Acknowledgements

Road Map

1. B. rapa var. FPsc, a self-compatible and extensively inbred analog of the Wisconsin Fast Plants model system for hands-on education
2. FPsc mutant derivatives
3. Mapping mutants with molecular markers: A goal-oriented approach to genetics education
4. GameteMaker, a genetic mapping app
   1. How the sausage is made
   2. Operations and output of the app
   3. Next steps

FPsc Mutants obtained via EMS mutagenesis

Albino (alb), a simple Mendelian recessive allele
Abnormal leaf (ale), a dominant allele
Elongated internodes (ein), constitutive expression of SAR
GA-deficient1 (gad1), an allelic series unable to synthesize GA

Characteristics of the FPsc variety:

- Self-compatible
- Genetically fixed at loci across the B. rapa genome (est. <1% residual heterozygosity)
- Flowers ~18 DAP, seed-to-seed generation time 7-8 weeks
- Moderately self-fertile: seed yield via spontaneous self-pollination ~75 seeds/plant, assisted self-pollination yields ~150 seeds/plant
- Height at flowering 10” – 12”

The Punnett Square, linkage, and the 3-point cross: Where future geneticists/genomic scientists lose their interest

http://www.biology.ewu.edu/aHerr/Genetics/Bio310/Pages/ch7pages/genchap7.html
http://www.ndsu.edu/pubweb/~mcclean/plsc431/linkage/linkage2.htm
http://www.biologie.msu.edu/caller/Genetics/Recombination/images/punnett.html
Getting from phenotype to genotype: The Holy Grail of genetics education

R500 x FPsc molecular markers

R500 x FPsc molecular genetic map

Mapping ale

Mapping alb

At AS2 data from Wu et al. PNAS 2008
Mapping mutants with molecular markers: ga-deficient dwarf 1 (gad1-1, gad1-2)

Mapping FPsc gad1 mutants: A candidate gene approach

Marker assay RP313 (66 cM A06) applied to R500 x FPsc gad1-2 F2 mapping panel

Chi-Square test of null hypothesis (that gad1 and RP313 assort independently)

Expected: 29 FPsc alleles

(Obs-Exp)^2 / Exp = (44-58)^2 / 58 = 3.37

Chi sq. distr. Critical number (p<0.05) with 1 d.f. = 3.84

Conclusion: Cannot reject the null hypothesis

Molecular mapping experiments have the potential to motivate students to learn fundamental genetic principles through a goal-oriented process:

Get to the gene!

-- Mapping asks students (and teachers!) to view genetic linkage as a useful tool rather than an obscure phenomenon

-- Gene mapping experiments provide a compelling biological context for students to “do” PCR and gels

-- Mapping experiments motivate BLAST and literature searches at both the beginning and end of experiments

-- Mapping genes requires application of higher-order mathematical skills, including statistical analysis

-- Mapping experiments lead ultimately to a genome browser, where students will examine gene models, structural variants, functional annotations, and use links to original literature to inform their conclusions
The "Yeah-but...":

What instructor has the time or budget that might permit their students to conduct a gene-mapping experiment?

The GameteMaker genetic mapping simulation app

Core elements:

1. A macro-enabled MS Excel spreadsheet "recombination engine"

2. A web-based interface that applies student-directed choices to exploit empirically-determined phenotypic images, mapping data (linkage relationships) and PCR/gel images to produce virtual mapping gels

The GameteMaker macro-enabled recombination engine

Base data page

Recombination engine start page

Random gamete pairs

A summing page to determine genotypes at each marker locus
These are the data that will be uploaded to the GameteMaker web site.
**GameteMaker**

### Generate an F2 population

The GameteMaker mapping simulation makes use of virtual F2 populations in which mutant alleles, derived from the Hpc genetic background, are segregating in parallel with alternative alleles provided by the KO8 (yellow-sam6) variety of B. napus.

By using the dialog box below you can specify the size of the F2 population from which you will select individuals, by clicking, that will compose the mapping population. The maximum size of your F2 population is 8,000 segregants, but it is recommended that your initial mapping population should be considerably smaller than that number for use during the "rough mapping" phase of the experiment; the F2 population size can be increased at later stages to provide additional specimens for inclusion in the mapping population.

**F2 population size:**

### Create a mapping population

Select the individuals of the appropriate phenotype class from the simulated population shown here, by clicking left click button of your mouse, and then by clicking right click button of your mouse, to select the mapping population for clicking a second time on selected specimen.

### Select the molecular marker assay that will be applied to your mapping population

Left click on a marker name to select.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Description</th>
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<tbody>
<tr>
<td>M1</td>
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</tr>
<tr>
<td>M2</td>
<td>Marker 2</td>
</tr>
<tr>
<td>M3</td>
<td>Marker 3</td>
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</tbody>
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### PCR recipe (optional)

In hopes of enhancing the realism of the GameteMaker genetic mapping simulation, we provide on this page the opportunity for users to formulate recipes for each round of PCR marker assays. Shown at left, below, is a typical recipe for a single PCR. If directed by your instructor, please "fill in the blank" to create a Master Mix reaction cocktail that will be added to F2 genomic DNA (gDNA) included in the present mapping population.

Each 20 μl reaction gets:

- 1 μl F2 gDNA
- 2 μl 10X PCR buffer
- 2 μl marker-specific fwd primer (2 μM)
- 2 μl marker-specific rev primer (2 μM)
- 10.75 μl H2O

### Summary of experimental parameters

Please review the information below that summarizes the choices made previously in this run of the GameteMaker mapping simulation. If you wish to change parameters you can use the "Reset" button to reset these choices.

Once satisfied with the parameters, click the "Next" button to view a virtual gel image that depicts your simulated experimental results.

- **User name:**
- **Model organism:**
- **Mapping population size:**

### Results of marker assay RPhene (x-cM chr, Ay)

- **Markers:**
- **Genotype:**
- **Map:**

<table>
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<tr>
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<td>Allele</td>
<td>Marker 1 allele</td>
</tr>
<tr>
<td>M2</td>
<td>Allele</td>
<td>Marker 2 allele</td>
</tr>
<tr>
<td>M3</td>
<td>Allele</td>
<td>Marker 3 allele</td>
</tr>
</tbody>
</table>

### Notes:

- **User name:**
- **Model organism:**
- **Mapping population size:**
Status and next steps

The GameteMaker sim is presently being revised on developmental server. Migration to a public server (FPsc.wisc.edu) expected ~March, 2015

Collaborators sought for

- Beta testing
- Implementation/adaptation of the sim for use with other model organisms

FPsc seed stocks available upon email request to swoody@wisc.edu