Evaluating the Effects of Trembling Aspen Genetics on Insect Communities

Stat 877 project presentation

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May 2, 2019

Background

Plant-insect interactions:

- Plants are known to influence insect community structure
- Plant Diversity is positively correlated with insect diversity.
  - effects of interspecific variation are well studied
  - effects of **intraspecific variation** are less well studied

Populus tremuloides (Trembling Aspen):

- the most wide-spread tree species in North America
- one of the fastest-growing tree species
- incredible amounts of intraspecific variation
- food source for an incredible diversity of herbivores

Genome-wide association analysis (GWA)

Question: How do aspen genetics shape insect communities?

- Herbivorous insects are known to differ by aspen clone (genet) and by aspen traits (size, defense chemistry, nutrition, etc.).
- Is there evidence for genetic drivers other than these physical traits?
- **Are there genomic regions that affect multiple insects?**

GWA of insect incidence on aspen common garden:

- 18 common insect species; present/absent (1/0)
- 1,414 trees from 437 genets
- 8 tree trait covariates
- 4 time periods (longitudinal)
- 114,420 SNP regions

Statistical Model

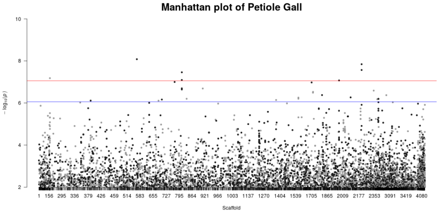
Univariate SNP association model:

$$\mathsf{logit}(p_{ijk_g}) = \beta_0 + \alpha \mathsf{G}_g + x_{jk_g}^{\mathsf{T}} \beta + \varepsilon_{g(j)}$$

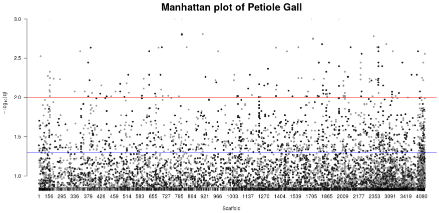
- SNPs considered independently
- p_{ijk_g}: probability of observing > 0 individual insects of species i during survey event j on tree k of genet g
- G_g: SNP-specific genotype of genet g
- x_{jk_g}: vector of observed tree trait covariates for tree k during survey event j
- $\varepsilon_{g(j)}$: nested random effect of genet within survey event
- Storey's q values of coefficient α used to select significant SNPs (.05 cutoff)

A total of 2,059,560 (18 insects $\times$ $114,420$ SNPs) GLMMs were fit.

Diagnostics: Bonferroni vs. Storey's q



Manhattan plot of Petiole Gall



Manhattan plot of Petiole Gall

- Using q values (bottom) allows for discovery of many more potential associations than bonferroni p values (top).
- significant q values contain all significant bonferroni p values

Results: Significant SNPs per Insect

	insect description	SNPs	scaffolds
Green Aphids	free-feeding, specialist (salicaceae)	1788	1020
Petiole Gall	leaf-galling, specialist (populus)	1432	788
Phyllocolpa	leaf-rolling, specialist (salicaceae)	1363	814
Harmandia	leaf-galling, specialist (populus)	174	130
Smokey Aphids	free-feeding, specialist (populus)	90	64
Casebearer Moth	case-bearing, generalist	7	3
Lombardy Mine	leaf-mining, specialist (populus)	1	1
Cottonwood Leaf Mine	leaf-mining, specialist (salicaceae)	1	1
Leaf Edge Mine	leaf-mining, specialist (salicaceae)	0	0
Blotch Mine	leaf-mining, specialist (populus)	0	0
Weevil Mine	leaf-mining, specialist (salicacea)	0	0
Blackmine Beetle	leaf-mining, specialist (populus)	0	0
Leafhoppers	free-feeding, generalist	0	0
Ants	aphid-tending, non-herbivore	0	0
Pale Green Notodontid	free-feeding, specialist (populus)	0	0
Aspen Leaf Beetle	free-feeding, specialist (populus)	0	0
Green Sawfly	free-feeding, specialist (populus)	0	0
Cotton Scale	scale insect, generalist	0	0

There are 4,768 unique significant SNPs.

Results: Common Associations

- Of these 4,768 unique SNPs, There are **84** SNPs with multiple insect associations. These will be our SNPs of interest.
- 4 of these had associations with 3 insects and were contained in 1 gene:

SNP.name	qval.Harmandia	qval.Phyllocolpa	qval.Petiole.Gall
Potra002191:26587	0.0193784	0.0123563	0.0286529
Potra002191:26642	0.0226734	0.0091460	0.0258001
Potra002191:26644	0.0226734	0.0091460	0.0258001
Potra002191:26650	0.0236877	0.0116280	0.0234547

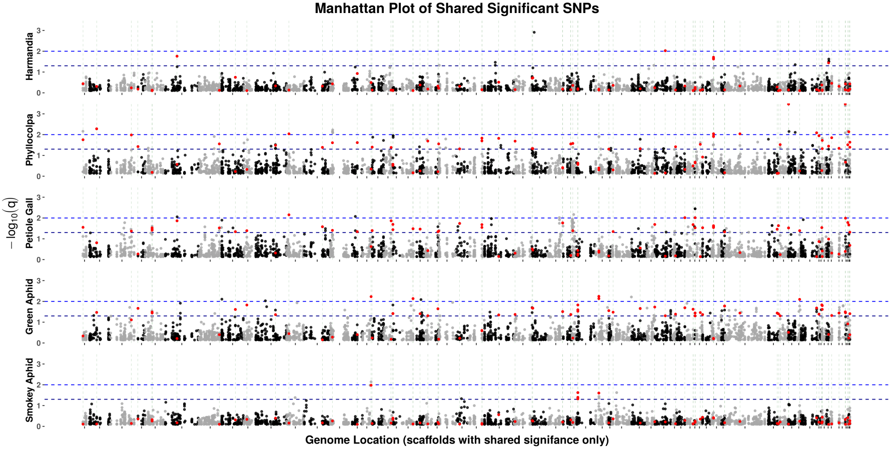
- They are within 63 base pairs (LD/same gene)

Note that these are the 2 **leaf-galling** and 1 **leaf-rolling** species. They are tightly associated with leaves. However, the gene annotation is not very insightful:

Potri Gene	%match	Descr.	avg allele. freq
Potri|011G157300.1	98.97	Uncharacterized oxidoreductase C663	0.136

(transmembrane oxireductases create electron-transport chains in chloroplasts and mitochondria)

Results: Multiple-Manhattan Plot



Results: Imputed Gene Function

Top matched genes with annotations imputed from congeneric *Populus trichocarpa*:

Potri Gene	%match	Descr.	avg allele. freq
Potri|006G267600.1	99.66	protein phosphatase 2C	0.110
Potri|013G036000.1	99.44	tRNA-specific adenosine deaminase	0.204
Potri|007G058500.1	99.02	1-deoxy-D-xylulose-5-phosphate synthase	0.226
Potri|014G079900.1	99.01	dependent malic enzyme	0.112
Potri|003G045300.1	99.01	multivesicular body protein	0.143
Potri|011G157300.1	98.97	Uncharacterized oxidoreductase C663	0.136
Potri|006G249800.1	98.63	Probable boron transporter	0.139
Potri|016G142100.1	98.60	Unknown protein 1	0.150
Potri|001G369000.1	98.59	Transmembrane emp24 domain-containing protein	0.205
Potri|016G037900.1	98.40	STRICTOSIDINE SYNTHASE-LIKE	0.170
Potri|006G082900.1	98.38	Kinesin-like protein	0.218
Potri|010G014100.1	98.33	repeat-containing protein	0.141
Potri|015G112500.1	98.16	hydrolase domain-containing	0.126
Potri|014G156100.1	98.00	CSC1-like protein	0.137
Potri|001G113500.1	98.00	domain-containing protein	0.403
Potri|005G061600.1	97.99	glucuronate:xylan alpha	0.183
Potri|002G009100.1	97.98	Bromodomain-containing protein	0.230
Potri|010G164400.1	97.93	uncharacterized protein LOC105116966 isoform X1	0.156
Potri|006G149900.1	97.81	RNA-binding protein	0.150
Potri|001G253900.1	97.81	Protein TIC 62, chloroplastic	0.222
Potri|001G253800.1	97.67	Sugar transport protein	0.211

These annotations are vague and will require more research to determine which make biological sense.

"*A. t.* **glucuronate:xylan alpha-glucuronosyltransferase 1**: Glycosyltransferase required for the addition of both glucuronic acid and 4-O-methylglucuronic acid branches to xylan in stem cell walls. . . . is responsible for almost all of the substitutions of the xylan backbone in stem

Discussion

Conclusions

- Evidence of genomic regions that influence individual insects and communities
- These genomic regions are independent of the observed tree traits

Future Directions

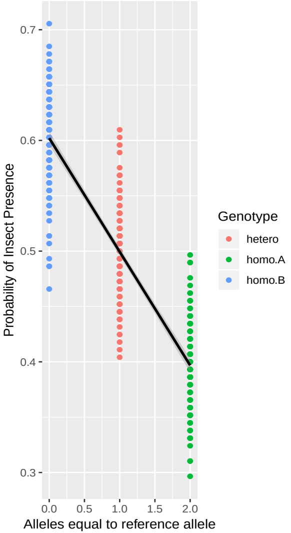
- More thoroughly research gene annotations from congeneric *Populus trichocarpa* and model *Arabidopsis thaliana* to check biological function where possible
- Compare with other methods (multi-SNP, BLUP association, GBlups, etc)
    - A similar study on this garden found only 2 insect associations with < 12 total significant SNPs using BLUP association and a more lenient inclusion cutoff ($q < 0.1$) in 2015
- Include more tree traits and environmental variables and interactions

Extra Slides

Extra Slides: Potential Issues (Simulated Data)

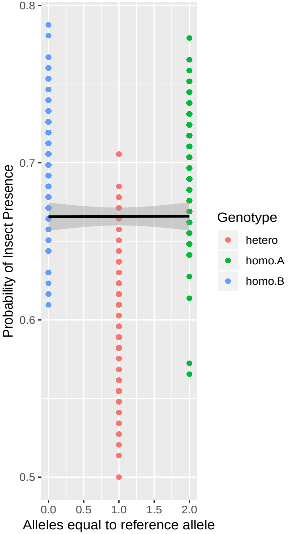
Association with SNP 1 Hypothesized Situation

Equal distribution of alleles



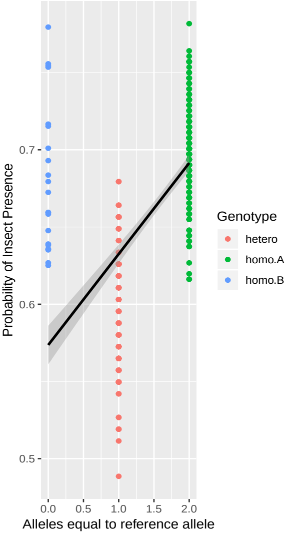
Association with SNP 3: Heterozygote Advantage

Equal distribution of alleles



Association with SNP 2: Heterozygote Advantage

Uneven distribution of alleles



BLUPs: Linear Mixed Model (LMM) Definition

Consider the following model for some response Y on observation j:

$$Y_j = \mu + X_j\beta + Z_j\tau + \varepsilon$$

- $E(Y_j = X_j\beta)$
- X_j and Z_j are vectors of independent variables for the jth observation
- β is an unknown vector of regression parameters (fixed effects)
- τ is an unknown vector of **random effects**
  - $E(\tau) = 0$ and $Var(\tau) = G$; $G = Cov(\tau_m, \tau_k)$ for all m and k
- ε is an unknown vector of random errors
  - $E(\varepsilon) = 0$ and $Var(\varepsilon) = R$

LMM: Parameter Estimation

$$Y_j = \mu + X_j\beta + Z_j\tau + \varepsilon$$

- $\tau \sim N(0, G)$, $\varepsilon \sim N(0, R)$, and $Cov(\tau, \varepsilon) = 0$
- Find coefficients $\hat{\beta}$ and $\hat{\tau}$ such that they:
  - minimize variance prediction error $Var(Y_j - \hat{Y}_j)$
  - are constrained by $E(Y_j - \hat{Y}_j) = 0$ $(Bias(\hat{\theta}) = E(\hat{\theta}) - \theta)$
- $\hat{\beta}$ is a vector of best linear unbiased estimators (BLUEs) of fixed effects
- $\hat{\tau}$ is a vector of best linear unbiased predictors (BLUPs) of fixed effects
- conditional variances (covariance matrices for β and ε) are often unknown and are estimated as a nuisance parameter with Bayesian EM algorithm (`nlme`,`lme4`, etc.)

Take-Home Message

BLUPs are constrained coefficienct estimates of random effects.

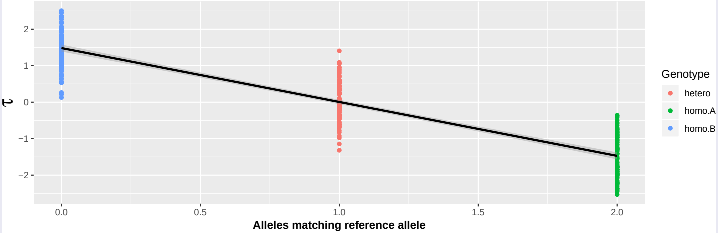
Modern GWA Models

- First fit LMM for a trait of interest (Size) with β_i fixed effects and τ_g random effects:

$$\text{Size} = \mu + \beta_1(\text{PGs}) + \beta_2(\text{CTs}) + \cdots + \beta_n(\text{Trait}_n) + \tau_g(\text{Genet}_g) + \varepsilon$$

- Then conduct a linear association analysis between the size-specific BLUPs τ and the Genotype of at SNP s of each genet g:

$$\tau_g = \mu + \alpha(\text{Genotype}_{s,g}) + \varepsilon$$


τ
Alleles matching reference allele
Genotype
hetero
homo.A
homo.B