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Estimating heritability of disease resistance and factors that contribute to long-term survival in butternut (*Juglans cinerea* L.)



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Critical points for management

Cold-tolerant hard mast species, potentially useful for reclamation, once much more common. Still favored by herbalists and native Americans as a medicine.

Butternut numbers are declining rapidly

- Butternut canker disease: Kills all ages in all parts of the range

- Tree age and isolation

- Regeneration is poor

 - Hybridization with Japanese walnut confounds seed supply

- Butternut is listed as endangered in Canada

Existing genetic diversity is probably sufficient but threatened

Seed cannot be stored so trees must be maintained as living populations

Current germplasm collection efforts and genetic screening efforts

- UT Knoxville

- Hardwood Tree Improvement and Regeneration Center/Purdue/TNC



Symptoms of butternut canker

Juglans cinerea

Former range of butternut

500 0 500 1000 1500 Kilometers



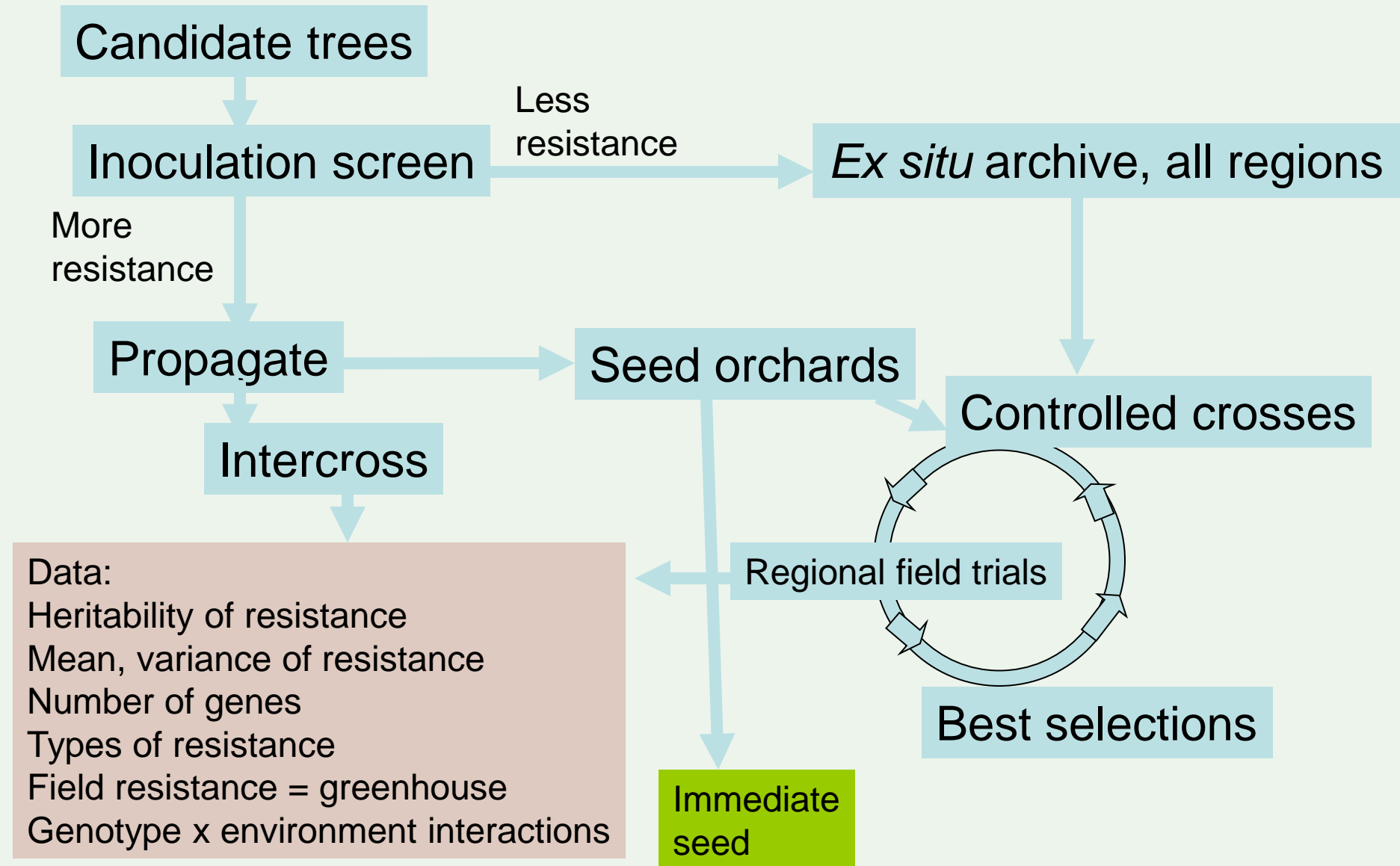
Red dots show
current range



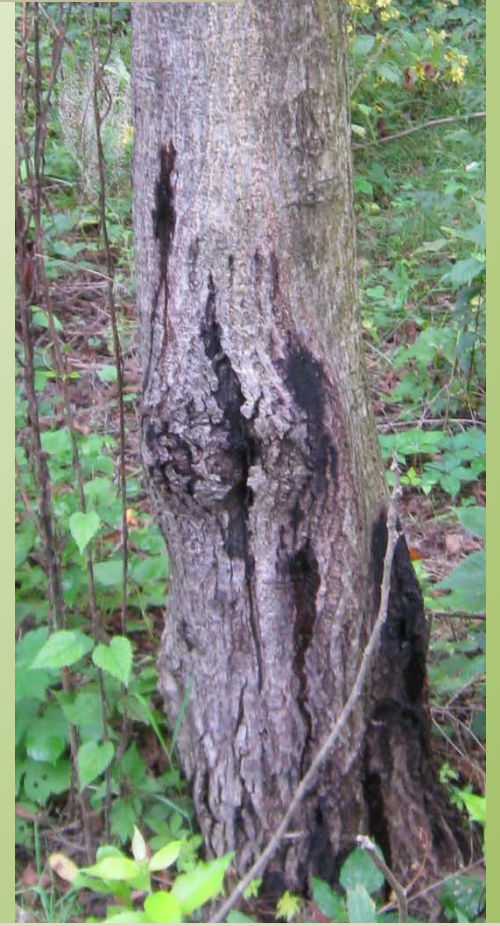
Phenotypically resistant trees have remained healthy and can reach merchantable size



BREEDING RESISTANT BUTTERNUT



2 to 4 years later, inoculated cankers heal or new cankers form at margins – our bottomland tests have high natural disease pressure



Artificial stem inoculations do not correlate well with natural infection phenotypes

Approaches to understanding observed variation in susceptibility in wild butternut populations

1. Mantel tests to determine relationship between pairwise relatedness and disease phenotypes. (Relatedness determined with 15 SSRs.)
2. Regression (Ritland, 2000) of phenotypic similarity on pairwise relatedness.
3. Identification of 'pseudo-families' and calculation of variance within and among families for traits of interest.
4. Logistic regression to determine environmental effects on survival

Butternut at Slocum's Woods, 2001-2012



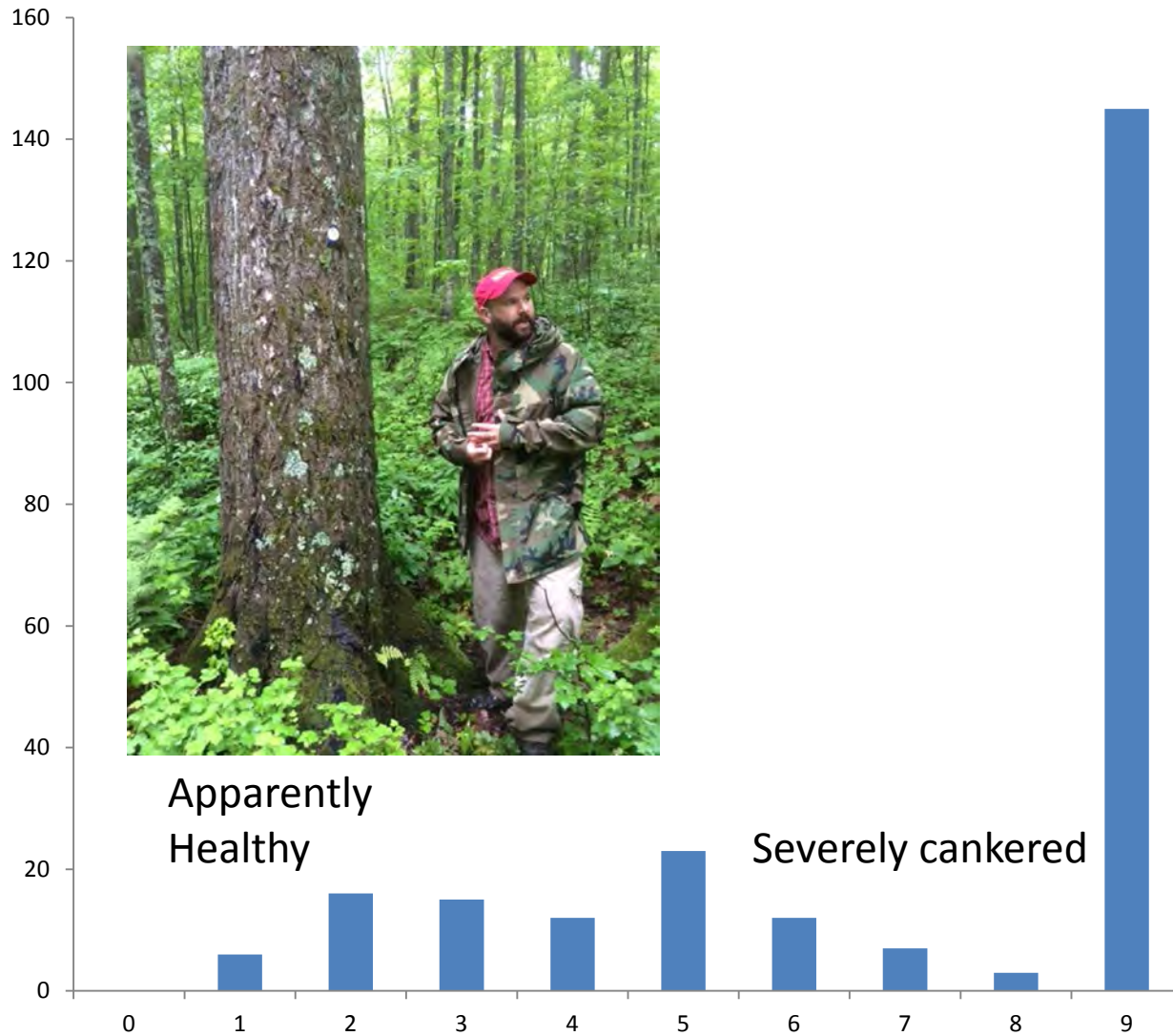
0 40 80 160 Meters

- ◆ Live trees with cankers 2001
- ◆ Trees without cankers 2001
- Live butternuts 2012

	Living trees	Dead trees	Found 2012	GPS	Disease index	Bark color	Health (qualitative)	DNA
2001	341	200	141	X		X	X	
2003	302	--	93			X	X	X
2012	113	39		X	X	X		X

Disease index derived from PCA of traits including number of cankers below DBH, limbs cankered, percent circumference cankered, number of basal cankers, live crown ratio, % crown dieback

Distribution of butternut disease scores



Lower disease index was correlated with dark bark $r=0.39$, $p=0.001$, as calculated by Mantel test



■ No. of trees in range

Disease score was based on a combination of traits chosen using principal components

Mantel tests of relatedness matrices and pairwise phenotypic similarity matrices in two butternut populations.

†Right-tailed p-value (999 permutations).

Significance with * $\alpha=0.10$; ** $\alpha=0.05$, *** $\alpha<0.01$.

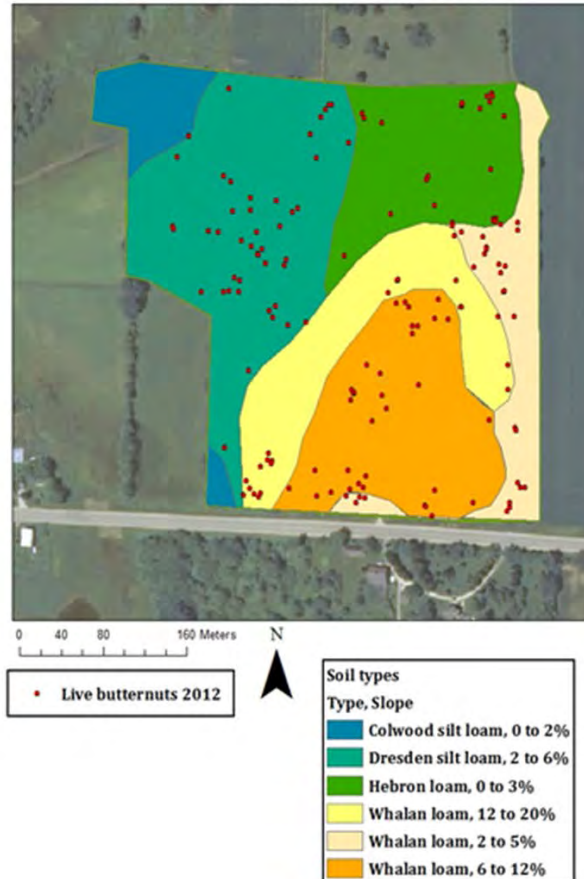
^aMantel test not performed because results were far from significant in first set of tests.

Lynch and Ritland r_{ij}		SLOW n=113		KMSF n=28	
Phenotype measure		Correlation	p-value†	Correlation	p-value†
Disease index		0.01232	0.15	-0.01803	0.662
Bark color		0.00832	0.228	-0.03268	0.766
Number of basal cankers		0.02481	.041** *	-0.02842	0.738
Number of cankers below DBH		0.01669	0.13	0.07876	.047** *
Percent of trunk cankered		0.00725	0.282	0.01009	0.351
Percent scaffold limbs dead		0.03442	0.014** *	-0.1471	0.999
Canker containment		0.00297	0.397	0.05406	0.107
Crown vigor		0.00587	0.303	-0.06831	0.953
Epicormic branching		0.01133	0.186	0.02729	0.224
MLRELATE r_{ij}					
Disease index		0.00684	0.268	- ^a	-
Bark color		0.00247	0.388	-	-
Number of basal cankers		0.03371	.006*** *	-	-
Number of cankers below DBH		0.02199	.046** *	0.06555	0.068* *
Percent scaffold limbs dead		0.02822	0.028** *	-	-
Epicormic branching		0.00832	0.24	-	-

Factors influencing butternut survival under heavy canker disease pressure

- We examined a large population (current n=113) of butternut in southern Wisconsin with a long history of canker disease occurrence and monitoring
- Mortality between 2001 and 2012= 67%, canker incidence in 2012=99%
- Trees on thin soil over limestone bedrock (orange/yellow on map) were significantly more likely to survive 2001-2012 than those on deep silt alluvium (green/blue)
- Trees with dark bark were more likely to survive than those with light bark

Butternut at Slocum's Woods, 2001-2012



Estimates of true variance of relatedness (Ritland 1996) computed using SpaGeDI (Hardy and Vekemans 2007) and regression coefficients of bark color and disease resistance in SLOW butternuts sampled in 2012 (n=113).

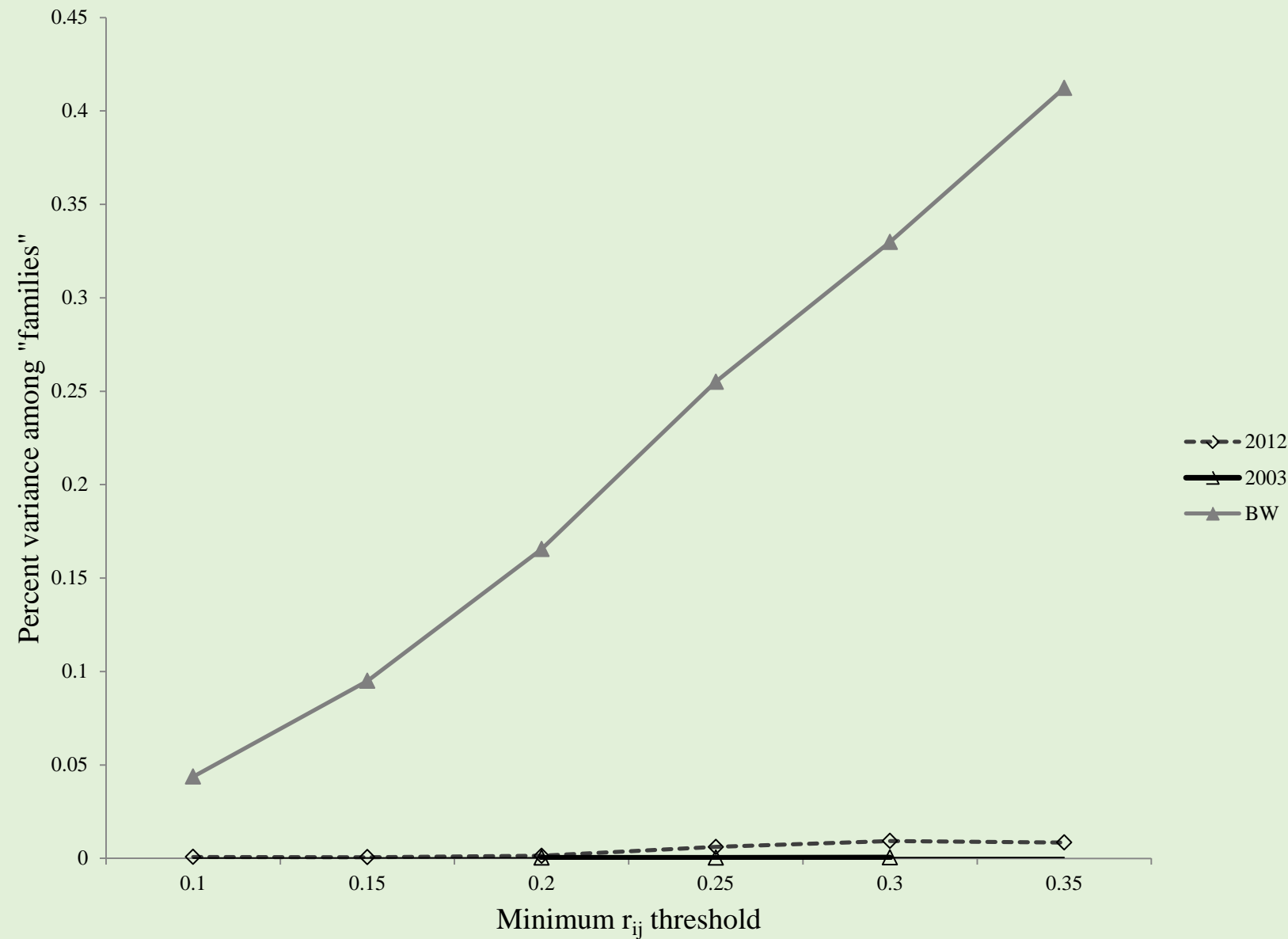
^aAll standard errors computed by jackknifing over loci.

^bSlope estimate for the regression of pairwise phenotypic similarity on pairwise relatedness.

Estimator	Variance of r_{ij} (S.E.) ^a	Disease index h^2 (S.E.) ^b	Bark color h^2 (S.E.)
Queller and Goodnight (1989)	0.00527 (0.00231)	-0.0011 (0.00634)	-0.0052 (0.00743)
Lynch and Ritland (1999)	0.00065 (0.00285)	0.00330 (0.002)	0.00081 (0.00167)
Wang (2002)	0.00424 (0.00170)	0.00007 (0.01098)	0.00027 (0.0085)



Variance among Pseudo-families for height (black walnut) and disease index (butternut).





Conclude:

1. Wild populations can be used to understand heritability of disease responses
2. Some components of butternut canker disease response have low heritability
3. Site has large effect on survival
4. Breeding will probably rely on hybridization with congener

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