



Effects of Selection Intensity on Genetic Drift and Disease Resistance in Alfalfa

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Legacy Seeds

Plant Breeding = Information + Decisions

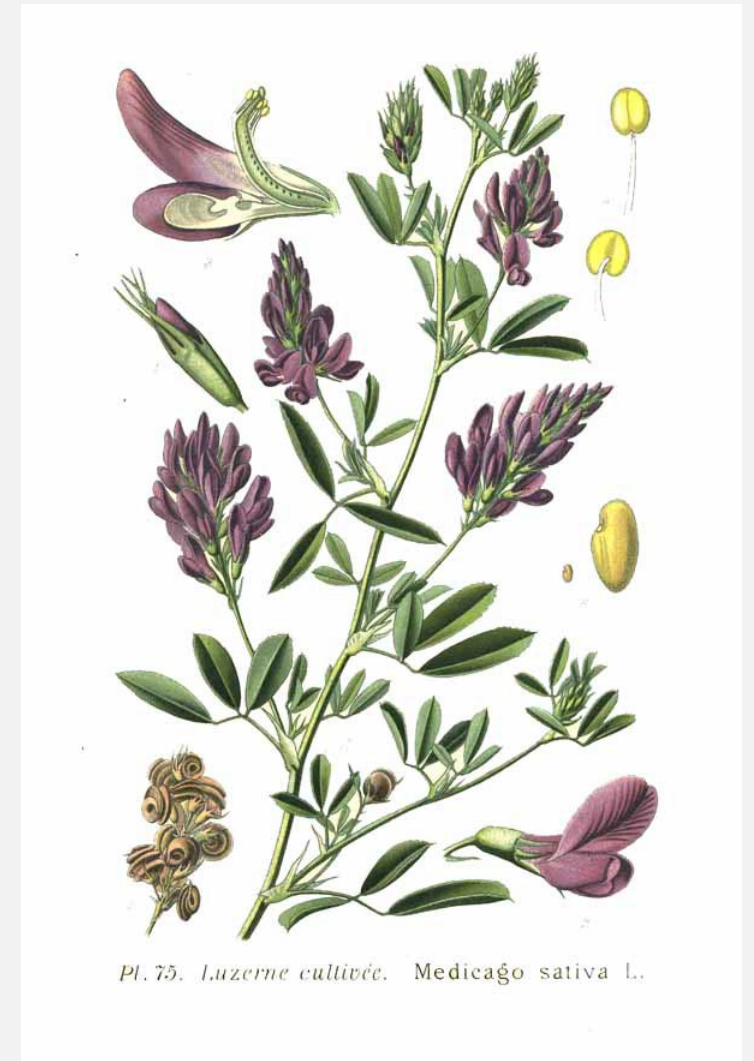


Background



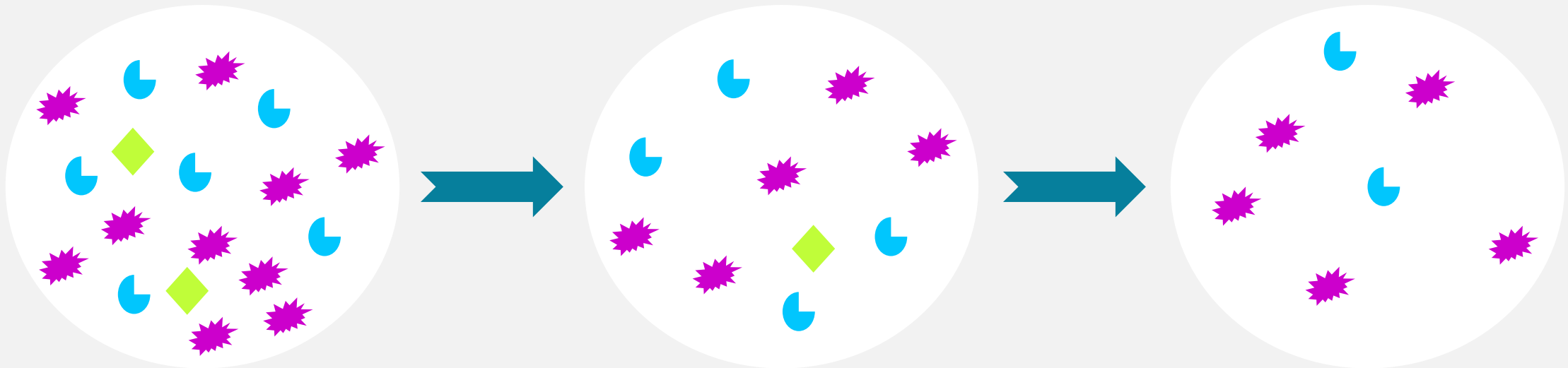
Alfalfa (*Medicago sativa*)

- Outcrossing autotetraploid ($2n = 4x = 32$)
- Highly heterozygous
- Severe inbreeding depression
- Nitrogen-fixing perennial legume
- US hay production 52.6 million tons over 16.6 million acres



Genetic Drift

- Random change in allele frequency
- Bottleneck, loss of variation
- Rare alleles lost in small populations



Alfalfa Breeding

- Developed as a synthetic variety
 - Number of parents selected “Syn0”
 - Narrow or Broad
- Improving multiple traits simultaneously
 - Genetic resistance to multiple diseases, yield, quality, persistence
- Broad-based remain stable



Space-Planted Nurseries



Plant Parents are Dug Up



Leafcutter Bees in the Cages



New Experimentals are Tested



Goals and Hypothesis

Goal

Create broad-based and narrow-based synthetics selected on field vigor

Goal

Compare disease resistance and DNA markers of original populations with broad and narrow selections

Hypothesis

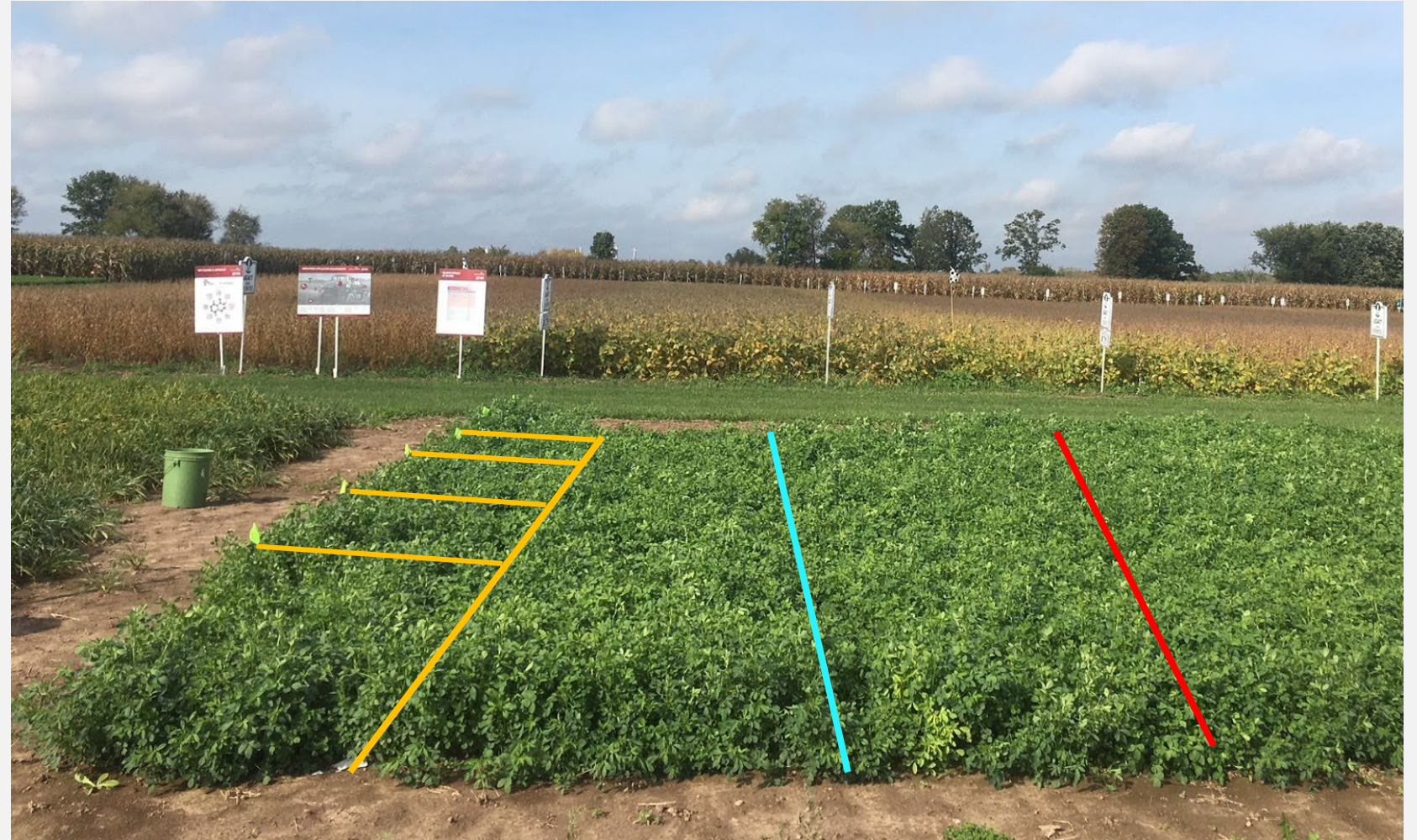
More change in the narrow selections due to genetic drift

Materials and Methods



Field Rating and Selection

- Six populations
 - LS1505, LS1601, LS1602, LS1603, LS1605, WAPH-5
- Vigor rating (1-5) twice
- Three selection treatments
 - Original, Broad, Narrow



Selection Intensity

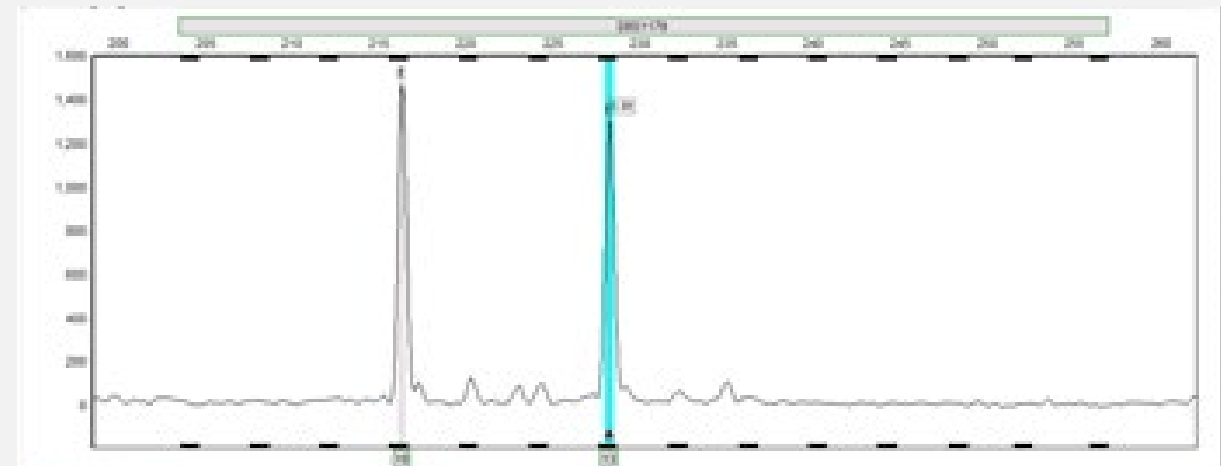
Population	Nursery Location	Nursery Size	Broad Selection			Narrow Selection		
			Plants Selected	Selection Intensity	Polycross Size	Plants Selected	Selection Intensity	Polycross Size
LS 1505	Evansville	500	115	23.0%	86	6	1.2%	6
LS 1601	Waupaca	500	130	26.0%	73	6	1.2%	6
LS 1602	Evansville	275	69	22.0%	58	3	1.1%	6
	Waupaca	275	52			3		
LS 1603	Waupaca	500	96	19.2%	64	6	1.2%	6
LS 1605	Evansville	310	54	16.9%	65	3	0.9%	6
	Waupaca	310	51			3		
WAPH-5	Evansville	500	93	18.6%	56	6	1.2%	6

Pollinations



DNA Extraction and Molecular Marker Amplification

- Leaf tissue for Broad and Narrow; seed tissue for Original
- Multiplex polymerase chain reaction (PCR) with 15 simple sequence repeat (SSRs) primer pairs
- Used GeneMarker Version V1.91 to visualize and interpret
- Output was 0s and 1s
- Estimated allele frequencies with:
 - $(p + q)^4 = p^4 + 4p^3q + 6p^2q^2 + 4pq^3 + q^4$
- Common alleles (frequency > 0.05)
- Calculated change in allele frequency and richness



Disease Screens

- Anthracnose (*Colletotrichum trifolii*) Race 1
 - Fungal pathogen, foliar disease
 - One dominant resistance gene “AN1”
- Phytophthora Root Rot (*Phytophthora medicaginis*)
 - Oomycete pathogen
 - Two complementary resistance genes “Pm1” and “Pm2”
- Aphanomyces Root Rot (*Aphanomyces euteiches*) Race 2
 - Oomycete pathogen
 - At least two resistance genes
- Fusarium Wilt (*Fusarium oxysporum* f. sp. *medicaginis*)
 - Fungal pathogen, vascular disease
 - At least two resistance genes, completely dominant “FW1” and incompletely dominant “FW2”



Results and Discussion



Allele Frequency Change

Treatment was significant ($P=0.0009$) for all alleles and for common alleles ($P=0.0051$)

Population	Treatments Compared	Average Absolute Value % Allele Frequency Change	
		All Alleles	Common Alleles
WAPH-5	OG – N	87.38	48.86
WAPH-5	OG – B	44.57	24.18
LS1601	OG – N	71.55	49.80
LS1601	OG – B	33.52	20.97
LS1602	OG – N	98.63	50.18
LS1602	OG – B	45.87	24.19
LS1603	OG – N	107.96	52.46
LS1603	OG – B	35.40	15.68
LS1605	OG – N	89.95	77.96
LS1605	OG – B	35.00	19.20
LS Mean	OG – N	91.09 a†	55.85 a
LS Mean	OG – B	38.87 b	20.84 b

† Letters indicate significant difference based on a Tukey Test at $P < 0.05$.

Allele Richness Change: All

Treatment was significant ($P < 0.0001$)

Population	Treatment	Total Alleles Observed	% of 131 Possible Alleles Observed	% of Alleles Observed in OG
WAPH-5	N	78	59.54	74.29
WAPH-5	B	106	80.92	100.9
WAPH-5	OG	105	80.15	100.0
LS1601	N	64	48.85	62.14
LS1601	B	96	73.28	93.20
LS1601	OG	103	78.63	100.0
LS1602	N	62	47.33	62.00
LS1602	B	87	66.41	87.00
LS1602	OG	100	76.34	100.0
LS1603	N	69	52.67	66.99
LS1603	B	98	74.81	95.15
LS1603	OG	103	78.63	100.0
LS1605	N	64	48.85	65.31
LS1605	B	97	74.05	98.98
LS1605	OG	98	74.81	100.0
LS Mean	N	-	-	66.1 a†
LS Mean	B	-	-	95.0 b
LS Mean	OG	-	-	100.0 b

† Letters indicate significant difference based on a Tukey test at $P < 0.05$.

Allele Richness Change: Common

Treatment was significant (P=0.0023)

Population	Treatment	Total Alleles Observed	% of 59 Possible Alleles Observed	% of Alleles Observed in OG
WAPH-5	N	48	81.36	88.89
WAPH-5	B	48	81.36	88.89
WAPH-5	OG	54	91.53	100.0
LS1601	N	45	76.27	85.19
LS1601	B	53	89.83	98.15
LS1601	OG	54	91.53	100.0
LS1602	N	46	77.97	90.20
LS1602	B	52	88.14	101.96
LS1602	OG	51	86.44	100.0
LS1603	N	49	83.05	89.09
LS1603	B	55	93.22	100.0
LS1603	OG	55	93.22	100.0
LS1605	N	42	71.19	89.36
LS1605	B	50	84.75	106.38
LS1605	OG	48	81.36	100.0
LS Mean	N	-	-	88.5 a
LS Mean	B	-	-	99.1 b
LS Mean	OG	-	-	100.0 b

Disease Resistance Change

Population	Treatment	Anthracnose	Phytophthora	Aphanomyces Resistance	Aphanomyces DSI	Fusarium Resistance	Fusarium DSI
		----- % -----			- Score †	---- % ----	--Score--
WAPH-5	N	13.7 <i>b</i> ‡	36.2	55.6	2.62	49.3	1.85 <i>a</i>
WAPH-5	B	30.3 <i>a</i>	35.1	46.8	2.70	33.3	2.67 <i>b</i>
WAPH-5	OG	17.2 <i>b</i>	46.6	65.8	2.49	32.8	2.56 <i>ab</i>
LS1601	N	77.7 <i>a</i>	53.4	28.0	3.02	41.1 <i>b</i>	2.16
LS1601	B	66.1 <i>ab</i>	53.6	44.0	2.66	46.4 <i>ab</i>	1.91
LS1601	OG	57.5 <i>b</i>	45.5	39.3	2.84	56.2 <i>a</i>	1.65
LS1602	N	91.2 <i>a</i>	48.7	29.2	3.02	46.2	2.11 <i>b</i>
LS1602	B	70.9 <i>b</i>	56.2	12.0	3.26	48.0	1.83 <i>ab</i>
LS1602	OG	53.6 <i>c</i>	53.4	29.1	3.18	40.6	1.83 <i>a</i>
LS1603	N	54.3	63.2	31.2	2.92	77.2	0.99 <i>a</i>
LS1603	B	64.5	49.7	35.4	3.00	56.8	1.67 <i>b</i>
LS1603	OG	51.0	51.2	33.7	3.21	71.3	1.20 <i>a</i>
LS1605	N	55.6 <i>a</i>	78.5	19.2	3.04	71.4 <i>a</i>	1.85 <i>a</i>
LS1605	B	48.1 <i>ab</i>	77.6	20.0	3.13	60.9 <i>ab</i>	2.67 <i>b</i>
LS1605	OG	44.2 <i>b</i>	65.3	22.0	3.27	52.8 <i>b</i>	2.56 <i>ab</i>

† DSI score is on a 1 to 5 scale for Aphanomyces and 0 to 5 scale for Fusarium, with lower values indicating more resistance.

‡ Significant ($P < 0.05$) mean comparisons from Tukey Tests for comparisons within each population, among the three selection treatments: original (OG), narrow- (N), and broad- (B) based.

Conclusions

- Found evidence of genetic drift using SSRs
 - Narrow selections had greater allele frequency change
 - Narrow selections had fewer alleles and reduced genetic diversity
 - # of alfalfa plants selected had a significant effect
- Found evidence of potential genetic drift in Anthracnose and FW
 - One R-gene for Anthracnose in alfalfa, otherwise multigenic
 - Polyploids experience less pressure from genetic drift
- Safe side = broad-based synthetics

THANK YOU

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QUESTIONS

