Pollinator-Driven Nonrandom Mating Impacts in Red Clover Polycross Progeny

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Background

Proximal nonrandom mating and its impact on breeding efforts have been studied in wind-pollinated grass synthetics[3,4] but has been largely overlooked in insect-pollinated forage legumes. Two models of red clover (*Trifolium pratense* L.) mating systems have been described in detail: one assumes random mating[2] and the other nonrandom mating resulting from hierarchical fecundity rates[5]. However, random mating is rarely observed in natural or agricultural settings[1], and neither model accounts for potential pollinator-driven, proximity-based nonrandom mating. Because inbreeding can cause notable declines in plant vigor[6], it is of interest to quantify the potential contribution of pollinators to inbreeding during the creation of synthetic red clover cultivars.

Figure 1. Typical red clover polycross cage setup. Note the bumble bee (*Bombus impatiens* Cr.) hive circled in green.

Objectives

1. Determine whether insect-mediated pollination in diploid red clover polycrosses contributes to proximal nonrandom mating, which would be evidenced by unexpectedly high frequencies of full siblings compared to model expectations
2. Compare effective polycross sizes observed to those estimated from existing red clover mating models to determine if pollinator behavior significantly increases inbreeding in subsequent synthetic generations

Methodology

1. Polycross selection

1. >100 genotyped progeny with known paternity
2. Clonal parents removed

350 Polycrosses
Conducted at the U.S. Dairy Forage Research Center 2006 - 2021

→

33 Polycrosses
Size (*N*): [8,93]
Progeny: [119,2181]

2. Calculating effective polycross size (N_e)

1. Classify relationships between all pairwise combinations of observed progeny

Full sibs = p_g 2 common parents	Half sibs = p_h 1 common parent	Unrelated = p_m no common parents

2. Calculate the observed coefficient of inbreeding (F)[2]

Equation 1: $F = \frac{1}{4}p_g + \frac{1}{8}p_h$

3. Derive equation for N_e by setting N = [2,100] as a function of F (assuming random mating[2])

Equation 2: $N_e = 0.5F^{-1}$

3. Modeling effective polycross size

1. Calculate expected frequencies of all possible progeny given modeled pollen-parent and observed seed-parent fecundities

Weibull model (N_e^w)

i. Rank fecundity of pollen-parents (Pp_i) based upon observed seed yields

ii. Calculate frequency of progeny with a given pollen-parent (Pf_i) following a Weibull distribution[5]

$$Pf_i = \frac{1}{N}\left[-1 + k\left(-\ln\left(1 - \frac{Pp_i}{N+1}\right)\right)^{\frac{1}{\lambda}}\right] + \frac{1}{N}$$

Uniform model (N_e^u)

Calculate frequency of progeny with a given pollen-parent (Pf_i) following a uniform distribution $\quad Pf_i = N^{-1}$

2. Calculate expected p_g, p_h, and p_m values from the joint probability and relationship of all pairwise combinations of possible progeny
3. Use Equation 1 to calculate coefficients of inbreeding expected from both models
4. Use Equation 2 to calculate effective polycross sizes expected from both models

4. Quantifying pollinator contributions to nonrandom mating & inbreeding

1. Assess differences between p_g and p_h values observed and estimated from Uniform and Weibull models
   a) Create linear regressions of p_g and p_h as a function of effective polycross size and compare regression lines via ANCOVA and Tukey adjusted post-hoc pairwise comparisons
   b) Compare p_g and p_h for individual polycrosses and across the dataset with binomial tests

➢ An increased frequency of proximal nonrandom mating events would result in higher full sibling, but not half sibling, frequencies observed compared to estimates from both models across all polycross sizes

2. Set N_e^u and N_e^w as a function of N_e to create linear regressions and compare regression slopes to $\beta = 1$ (complete parity between N_e and N_e^u or N_e^w) with a one-sample t-test

➢ Significant increases in inbreeding due to pollinator-driven nonrandom mating would result in smaller effective polycross sizes observed compared to estimates derived from both models

Results

Pollinator preferences increase proximal nonrandom mating events

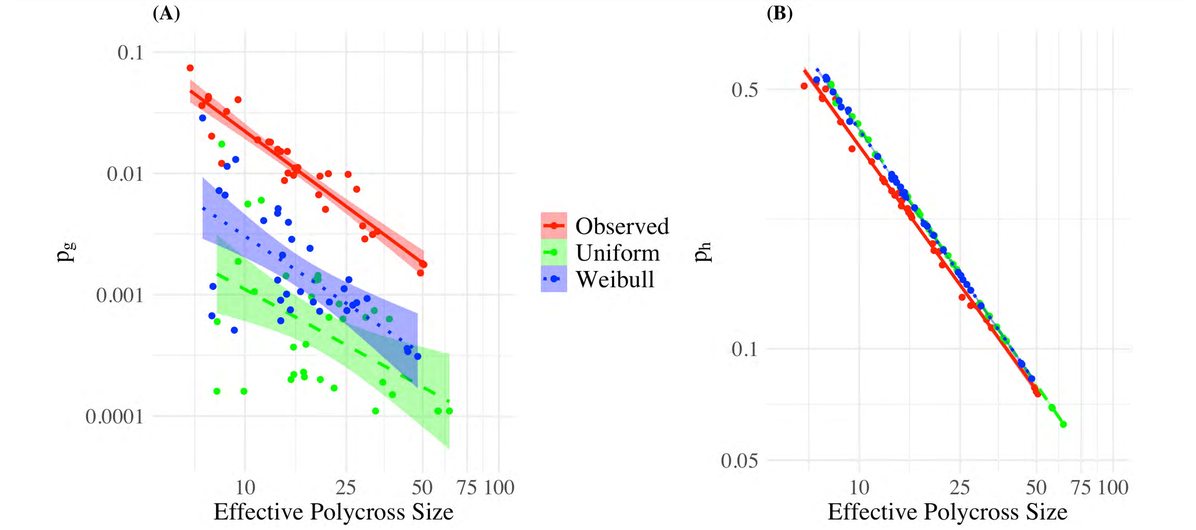

Figure 2. Log transformed linear models of full sibling (A) and half sibling (B) frequencies as a function of effective polycross size. Shaded regions represent standard errors. Observed p_g values are significantly higher than those estimated from the Uniform ($p < 0.001$) or Weibull ($p < 0.001$) models across all polycross sizes. Observed p_h values are lower than those estimated from either model in smaller polycrosses but converge as polycross size increases.

Pollinator-driven nonrandom mating does not significantly increase inbreeding

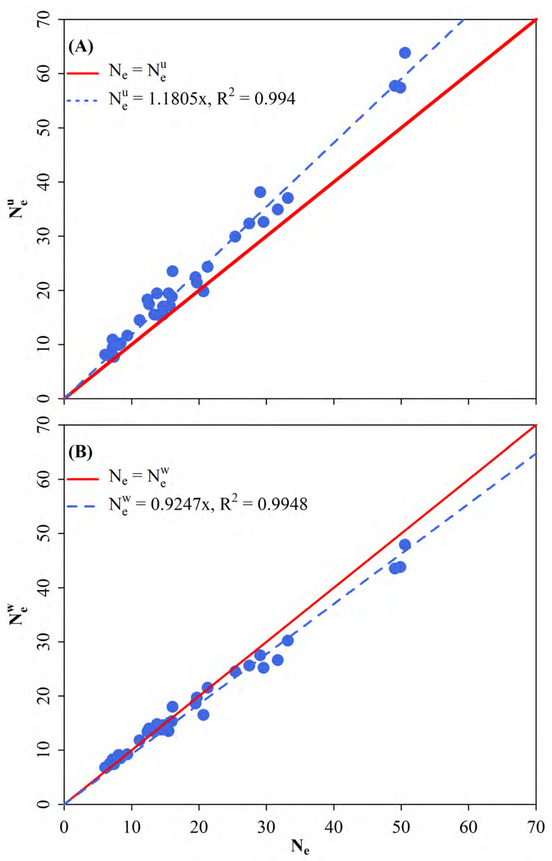

Figure 3. Linear regressions of effective polycross sizes observed as a function of those estimated from the Uniform (A) and Weibull (B) models. N_e^u is significantly overestimated ($p < 0.001$), especially as N_e increases. N_e^w values are closer to those observed and are slightly underestimated as N_e increases ($p < 0.001$).

Table 1. Full [FS] and half sibling [HS] counts and effective polycross sizes observed and expected from Uniform and Weibull models. Effective polycross size is also calculated from progeny frequencies observed and expected across the total 16,120 progeny in the dataset. Significant deviations from observed values at $p < 0.05$ are noted (*).

	OBSERVED			UNIFORM			WEIBULL		
POLYCROSS	FS	HS	N_e	FS	HS	N_e^u	FS	HS	N_e^w
C276	2	91	49.89	0*	82	57.40	0	107	43.78
C328WS	13	260	16.13	1*	195*	23.45	3*	251	18.01
C584Y07	2	84	49.07	0*	74	57.72	0	98	43.46
COM14	2	44	19.49	0*	41	22.41	0*	49	18.55
CR06Top	6	61	7.16	0*	47*	10.87	1*	60	8.33
DGC328	5	61	8.45	1*	56	10.25	2*	66	8.49
EL11	2	32	14.66	0*	31	16.99	0*	36	14.56
EL12	2	99	29.61	0*	94	32.64	1	121*	25.16
EL13	1	44	14.29	0*	44	15.16	0	48	13.81
EL14	1	20	31.75	0*	19	34.85	0	25	26.56
EL16	1	25	20.72	0*	27	19.78	0	32	16.52
GSI18	30	208	6.07	7*	187*	8.08	12*	216	6.80
HE08	9	149	12.37	0*	113*	18.26	2*	150	13.42
HEDURG13	6	64	7.16	0*	57	9.38	1*	66	7.89
HEN09	4	90	15.51	0*	76	19.39	2	107	13.48
LE08	12	100	9.37	2*	96	11.57	4*	118*	9.17
LEN09	5	118	15.99	1*	108	18.83	1*	132	15.25
LP08	12	210	11.20	1*	180*	14.50	3*	217	11.83
LT18	1	29	15.68	0*	29	17.12	0	32	15.08
ST16	1	34	14.74	0*	35	15.55	0	39	13.80
ST17	2	32	13.42	0*	31	15.51	0*	35	13.43
ST19	2	34	12.58	0*	28	17.36	0*	34	14.04
ST20	3	81	7.38	0*	83	7.74	0*	87	7.40
TOP16	7	248	33.20	1*	233	36.98	2*	285*	30.23
TOP17	4	66	21.27	0*	64	24.27	0*	72	21.47
TOP18	2	32	19.71	0*	33	21.41	0*	36	19.65
TOP19	4	50	25.45	0*	48	29.89	0*	58	24.55
TOP20	1	18	27.50	0	17	32.25	0	21	25.62
TOP21	5	75	6.75	0*	74	7.76	0*	77	7.47
Vis09	16	267	13.81	1*	210*	19.36	4*	270	14.80
WI21	3	134	50.62	0*	111*	63.81	1*	147	47.85
WT12	2	77	8.08	0*	66	9.89	0*	72	9.06
Yld09	3	120	29.05	0*	97*	38.06	1*	133	27.51
TOTAL	170 1.1%	3054 18.9%	18.99	18* 0.1%	2684* 16.6%	23.70	42* 0.3%	3298* 20.5%	19.07

Conclusions

- Pollinator behavior increases nonrandom mating in red clover polycrosses
  - Significantly greater full sibling frequencies are observed compared with model expectations
  - This indicates ***more frequent pollination events between adjacent plants***
- Pollinator-driven nonrandom mating ***did not*** result in significant increases in inbreeding in subsequent generations beyond those already accounted for by the Weibull model of nonrandom mating

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