

## CHAPTER 2.5

# Patterns of pollen stainability in *Cirsium arizonicum* complex and sympatric thistle populations.

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### INTRODUCTION

Staining for pollen viability provides an estimate of male sterility. Through selective staining of callose walls, which turn green, as opposed to purple-staining protoplasm it is easy to discriminate between empty pollen shells and potentially viable grains (Alexander 1980). Empty pollen is associated with meiotic failure believed to arise from lack of proper pairing between homologous chromosomes. Pollen viability staining has been shown to be useful to detect hybrids (Zonneveld and Van Iren 2001) and is a traditional method to assess hybrids in systematic studies (Thompson *et al.* 1994).

There are numerous reports of putative *Cirsium* hybrids in the literature (Rydberg 1917; Petrak 1917; Cronquist 1955; Moore and Frankton 1967; Gardner 1974; Bloom 1977). In this study I examine the patterns of pollen stain absorption in order to assess its mensural reliability as a taxonomic tool and assess the potential for 50 populations of the *Cirsium arizonicum* complex to represent hybrid zones.

## **MATERIALS AND METHODS**

### **Collections and data**

Thistle collections from 61 locations were used (see Appendix 1 for list of locations), each location being represented by from 1 to 12 capitula, resulting in a total of 278 capitula from 261 plants. Thirty four of these 278 heads had been collected as pairs from 17 individual plants to create a “repeated sampling” dataset.

Pollen was collected from individual florets onto microscope slides. In order to score the viability, the pollen was stained with Alexander’s stain (Alexander 1980), sealed with a ringed cover slide and allowed to absorb the stain for at least two weeks before scoring. In prior examination of pollen viability in *Cirsium* I have found that the individual slide scores increase over the first week and stabilize after the second week.

On each slide, 200 grains were scored for stain uptake under a light microscope. The number stained and the number counted (200 were not always available) were recorded. The viability percentage was calculated from these scores as the number stained divided by the number counted.

### **Analyses**

The univariate distribution of pollen viability scores was examined using JMP In (1989). The scores for individual locations were calculated by the sum of all viable pollen grains for the location divided by the total number of pollen grains examined for the location.

The absolute values of the differences ( $\Delta$ ) between viability percentages of the replicate samples (pairs of heads from individual plants) were calculated. In order to assess whether the observed differences within-plants were different than that expected by random, the  $\Delta$  between viability percentages of all heads with all other heads (for the 278 heads: 35,503 differences) were calculated on an electronic spreadsheet and the univariate distribution of the differences examined.

The distribution of viability percentages was examined in 3 locations that were represented by more than 10 heads.

## RESULTS

As shown in Table 1, most samples gave high viability percentages with 99% being the most common score. The low scoring heads were mixed in locations with high scoring heads and in all locations that were represented by multiple heads, at least one of the heads scored above 90%. The median within-location range was 20%, while the maximum range was 95%

Of the 61 locations, half scored above 90% viable when the pollen counts were pooled. Pooled scores are given in Table 3. Ten locations (19, 64, 31, 57, 37, 4, 18.1, 29, 36, and 48) scored below 80% in the pooled scores. Since even the low scores represent a mixture of high and low scoring plants, it is difficult to interpret these scores effects on population fitness.

Replicate samples did not match well within plants. Most pairs had small differences (0-8.5%) between high viability percentages. Two pairs had small differences (5.5% and 14%) between lower viability percentages and two pairs had very big differences (30% and 77%) between extremely different viability scores (63%:93% and 21%: 98%). What ever causes inviability does not impact a plant uniformly.

Looking at the distribution of all possible differences, finding small differences between heads is to be expected because most heads have high scores . Half of all possible combinations differ from each other by less than 6.5%.

**TABLE 1. DISTRIBUTION OF THE POLLEN STAINABILITY DATA.**

DATASET	Heads	Locations	$\Delta$   within plants	$\Delta$   between all heads
N	278	61	17	38503
MAXIMUM	100%	100 %	0	100
MINIMUM	0%	19 %	77	0
MODE	99% in 31 heads	98% in 6 locations 96% in 6 locations	2% in 6 pairs	0.5% in 2649 pairs
MEDIAN	96%	90.8%	2.5	6.5
MEAN	87.5%	86.6%	9.9	17.
VARIANCE	0.0377	0.0212	0.0352	0.05
SKEWNESS	-2.33	-2.40	3.26	1.67
KURTOSIS	5.29	7.36	11.38	2.24

Location 18, an example of a hybridizing site, contained another thistle species belonging to the *Undulata* group, *C. wheeleri*. Several individuals with intermediate characteristics were observed there. Six pollen scores for *C. wheeleri* at the site averaged 67.6 %, while the specimens appearing to belong to the *C. arizonicum* complex averaged 88%. As shown in Table 2, the overall percentages for Location 18 are high with a few very low scoring individual capitula. Location 36 showed a high degree of morphological variability in floral characters. The occurrence of lower viability in several heads from that site may be indicative of hybridization although no congeners were found in the immediate vicinity. Location 39, a distinctive but unknown taxon, was also examined as a potential hybrid site, because of its proximity to the rare *C. clokeyi*. With 80% of the capitula scoring above 90% viability (not clearly different than locations 18 or 36), there is no clear evidence to support or reject a hybridization theory.

**TABLE 2. POLLEN STAINABILITY IN THREE LOCATIONS AS THREE "STEM AND LEAF" PLOTS.** This type of plot, developed by Tukey (1977), has nothing to do with vegetation, but rather is a graphical/tabular display akin to a histogram, where "leaves" are each a second digit appended to the digit of the "stem" value.

STEM	LOC 18		LOC 36		LOC 39	
	LEAF	COUNT	LEAF	COUNT	LEAF	COUNT
10	00	2			1	1
9	378999	7	13479	5	6678889	7
8	5	1	08	2		
7			1	1		
6					0	1
5	9	1	2579	4	1	1
4						
3	67	2				

## DISCUSSION

The factors which cause within plant variance in pollen stainability cannot simply be nondisjunction through problems in the pairing of homologous chromosomes. The differences within plants can be extreme. These differences could be caused by exogenous factors such as environmental fluctuations or endogenous factors such as heteroplasmy (intra-individual variation of genotype). There might be fluctuation in the factors causing nondisjunction or there might be fluctuation in supportive factors that allow cytokinesis to proceed despite problems with pairing. With these samples being derived from individual florets, there may be some level of variation across the receptacle

creating the heterogeneity observed in this dataset. Although nondisjunction is normally assumed to be the cause, we simply do not have enough information to rule out secondary problems with microgametogenesis in which the lack of stainability might simply reflect death of the microgamete or failure to grow after meiosis. This creates a logical problem in accepting measures of pollen viability as a metric of hybridization. Even if we were not aware of this incongruity, the criteria for according a deme hybrid status is not clear. The differences in the two taxa at the known hybrid site suggested that they were not equally impacted by each other's genome. We have no way to assess if this is a quantitative effect of the direction of introgression or if one taxon is simply more able to cope with the influx of the other's genes. If Location 39 is morphologically distinct and shows some stability of phenotype, given that 20% of the capitula examined had low pollen viability scores do we consider it a potential hybrid or a new taxon when at the known hybrid site only 25% of the capitula showed low scores? I do not think that we have enough understanding of the variation in this data to interpret it in any detail. The ten locations having lowered pollen viability scores need to be viewed with an awareness of their potential meiotic problems.

## **SUMMARY**

Thistles showed high degrees of pollen viability scores within plants and within populations. Questions are raised about the interpretation of this measure of male sterility.

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**APPENDIX A. POLLEN VIABILITY SCORES BY LOCATION IN ORDER OF INCREASING VIABILITY.** Several locations contained taxa that were not part of the *C. arizonicum* complex, but their proximity to study sites prompted their collection as potential introgressants and outgroups. These are marked with OG appended to the location name.

LOCATION	HEADS	COUNTED	VIABLE	PERCENT	LOC NAME
19	1	200	38	0.19	Riggs Flt. AZ OG
64	3	600	318	0.53	Whipple Tr. UT
31	5	898	512	0.57	Escalante Cyn CO
57	3	200	118	0.59	Henrieville UT
37	2	377	233	0.618	Ladrone Pk. NM
4	3	477	321	0.673	Lukachukai AZ
18.1	6	571	386	0.676	Mt Graham OG
29	5	1000	688	0.688	Douglas Ps. CO
36	12	2304	1813	0.787	Gubernador Cyn NM
48	5	1000	795	0.795	Bald Rdg UT OG
7	4	800	643	0.804	Grand Cyn AZ
25	6	1200	977	0.814	Bear Cyn AZ
34	7	1096	925	0.844	Clanton Drw NM
53	5	1000	846	0.846	Antimony Cr. UT
13	6	1200	1022	0.852	Pioneer Ps AZ
42	4	783	669	0.854	Mt. Rose NV
12	5	960	822	0.856	Red Lake AZ
10	4	800	696	0.87	Moenave AZ
8	6	1018	890	0.874	Shonto AZ
54	6	1200	1049	0.874	Red Cyn UT
55	4	400	351	0.878	Burr Rd. UT
11	6	1065	935	0.878	Bitter Sp AZ
61	5	1000	882	0.882	Moki Dugway UT
18	15	2370	2090	0.882	Swift Tr. AZ
41	5	1000	884	0.884	Spooner Lk NV
38	3	300	270	0.9	Kings Cyn NV
39	10	1749	1580	0.903	Kyle Cyn NV
59	4	800	725	0.906	Willow Cr. UT OG
35	6	1000	908	0.908	Sunspot NM
22	3	351	319	0.909	2 <sup>nd</sup> Mesa AZ
51	4	692	630	0.91	Lilia Cyn Rd UT OG
2	4	800	730	0.913	Chinle AZ
50	5	1000	918	0.918	Horse Cyn UT
15	2	400	368	0.92	Sierra Ancha AZ
52	5	955	879	0.92	Sego Cyn UT
60	5	881	823	0.934	Bears Ears UT
17	1	200	187	0.935	Chelsey Flt. AZ OG
3	9	1800	1687	0.937	Nazllini AZ
33	6	1200	1129	0.941	Windy Pt CO OG
32	5	748	712	0.952	Nucla CO OG
45	5	1000	955	0.955	Whitmore UT
20	8	1456	1390	0.955	Hualpai Mts. AZ
56	2	400	383	0.958	Spencer Flt UT
9	5	1000	959	0.959	The Gap AZ
62	9	1800	1726	0.959	Moki Dugway UT
27	5	1000	962	0.962	Goodwin AZ

**APPENDIX A (CONT.)**

LOCATION	HEADS	COUNTED	VIABLE	PERCENT	LOC NAME
28	6	1200	1156	0.963	Yellowjacket CO
47	2	400	386	0.965	Joes Vly UT
49	1	200	194	0.97	Bald Ridge UT OG
58	2	400	389	0.973	Cedar City UT
5	2	400	390	0.975	Carr Cyn AZ
1	5	1000	978	0.978	Cyn de Chelly AZ
14	1	200	196	0.98	Rosecreek AZ
23	5	1000	980	0.98	Showlow Lk. AZ
24	6	1200	1177	0.981	Kitt Pk AZ
30	5	1000	983	0.983	Coal Glch CO OG
16	4	800	789	0.986	Tonto Vlg AZ
40	1	200	198	0.99	Mt Charleston NV
63	1	200	198	0.99	Pine VI Mts UT OG
6	5	1000	991	0.991	Jacob Lk AZ
26	4	800	796	0.995	Crownking AZ