Three-factor inheritance of aleurone color speckling in Navajo Robin's Egg and Hopi Speckled open pollinated varieties of maize—summary of research performed in Urbana, Illinois.

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Various systems of aleurone color mutability in South American maize land races have been isolated and characterized. Stippling produced by *R1-st* alleles has been identified in Andean land races (Brink RA, unpublished; Williams WM. 1972. Variability of the *R-stippled* gene in maize. Ph.D. Thesis, Univ. Wisconsin, Madison); sectoring induced by mutable alleles of the *Enr1 r1* haplotype-specific aleurone color enhancer has been identified in northern South American land races (Stinard PS, Kermicle J, and Sachs M. 2009. J Hered 100:217-228; Gonella JA and Peterson PA. 1977. Genetics 85:629-645); and aleurone color marbling conditioned by an *R1-mb* allele has been identified in Pisccorunto maize from Peru (http://www.maizegdb.org/cgi-bin/displayvarrecord.cgi?id=9017398). Although exhaustive surveys have not been conducted, these previously described systems of mutability have not been reported in native North American maize land races. Nevertheless, there are North American land races with systems of variegated aleurone color that have not previously been characterized. We describe here the characterization of one such system: Three factor inheritance for aleurone color speckling found in two North American open pollinated varieties of tribal maize (see Goncalves Butruille *et al.*, this MNL, for companion article).

Seeds of the open pollinated variety Navajo Robin's Egg Corn (NREC) with purple aleurone color speckling on colorless background (Figure 1) were obtained from Abundant Life Seed Foundation, Port Townsend, Washington. The sectors of speckling on kernels of NREC do not have well-defined borders, but are more diffuse and reminiscent of *r1* mottling or the endosperm blotching of *Pl1-Bh*. Crosses of NREC to the open pollinated variety Hopi Speckled Maize obtained from Native Seed Search, Tucson, Arizona produced speckled kernels in both the F1 and F2, indicating that the speckling is due to the same system. Initial crosses of NREC to aleurone color testers for *a1*, *a2*, *c1*, *c2*, and *r1* produced full colored kernels, indicating complementation. F2's of NREC with the Stock Center's full colored aleurone (ACR) standard produced a very low frequency of speckled kernels, approximating a 63:1 ratio of full color to speckled. To further characterize the nature of NREC speckling and to determine the number of genetic factors involved, test crosses were performed as follows: NREC was crossed to a stock carrying the nonparamutagenic self-colored *R1-sc:124* allele (and all other genes needed for aleurone color) in a W22 background, and the F1 was backcrossed by NREC to generate test cross ears. Kernel counts from the test cross ears indicated 7:1 segregation of full color to speckled aleurone (Table 1). All deviations from 7:1 were nonsignificant at the 0.10 level.

We hypothesized that the 7:1 test cross ratios observed were due to the independent assortment of three recessive triplicate factors, *i.e.* kernels need to be homozygous for all three factors in order to produce the speckled aleurone observed in the NREC line. In order to test this hypothesis, full colored kernels from the test cross ears were planted, and the resulting plants were crossed again by NREC. Kernels from these ears were scored for full color *vs.* speckled. If the system involves independently assorting triplicate factors, we would expect to obtain 7:1, 3:1, and 1:1 ratios for full color to speckled on these second generation back cross ears. The results are presented in Table 2. Of 66 ears, 63 gave chi-square values that didn't differ significantly from 7:1, 3:1, or 1:1 ratios. One ear gave a 3:1 chi-square significant at the .10 level, one ear gave a 3:1 chi-square significant at the .05 level, and one ear gave a 7:1 chi-square significant at the .01 level. Given the population size, such deviations would not be unexpected. Furthermore, based on independent assortment, the number of ears with 7:1, 3:1, and 1:1 ratios respectively would be expected to occur in a ratio of 1:3:3. Our observed number of ears matching these ratios (13, 33, and 20), did not differ significantly from the 1:3:3 ratio (chi-square = 4.57). Thus, the data from the second generation back cross ears match what would be expected for three independently assorting triplicate factors.

In order to further characterize the NREC factors, crosses of NREC were made to various aleurone color tester lines, yielding interesting results. As mentioned above, crosses of NREC to the Stock Center's *r1* tester (in M14/W22 background) produced full colored kernels. Crosses of NREC to *r1* introgressed into W23 also produced full colored kernels. However, crosses of NREC to *r1* introgressed into Oh43 produced speckled kernels. This cross was repeated using an independent *r1 wx1* Oh43 conversion, also producing speckled kernels in the F1. From these results we deduced that the *r1* locus is likely one of the factors involved in the speckling phenomenon, and that Oh43 is homozygous for the other two speckling factors, but M14, W22, and W23 are not.

Test crosses of NREC to an Oh43 conversion of *R1-g* produced 1:1 ratios of full colored to speckled kernels (1428 full color : 1384 speckled, 1:1 chi-square = 0.688, NS). This confirms that Oh43 is homozygous recessive for two of the speckling factors, and the 1:1 segregation is due to segregation at the *r1* locus (*R1-g* vs. the *R1* allele present in NREC, *R1-NREC*). Even though *R1-NREC* behaves as a dominant for aleurone color in crosses to the Stock Center's *r1* tester in the absence of speckling factors, it apparently acts as a recessive relative to *R1-g* and *R1-sc:124* with respect to response to NREC speckling factors.

Test crosses of NREC to a W23 conversion of *R1-r* produced 3:1 ratios of full colored to speckled kernels (746 full color : 266 speckled, 3:1 chi-square = 0.891, NS). Test crosses of NREC to a W23 conversion of *R1-Randolph* produced a 1:1 ratio of full color to speckled kernels (1499 full color : 1570 speckled, 1:1 chi-square = 1.642, NS). Since the difference between these two stocks is the *R1* allele and not the genetic background, we conclude that the W23 background is homozygous recessive for one speckling factor, and what differentiates between the 3:1 segregation and the 1:1 segregation is the *R1* allele. In other words, *R1-Randolph* is susceptible to NREC speckling, but *R1-r* is not.

From these data, we conclude that speckling in NREC requires three factors: a permissive (e. g. *R1-NREC* or *R1-Randolph*) allele at the *r1* locus, and homozygous recessive factors at two other independent loci. Thus the genotype of *r1* ^Oh43 is *r1 r1 fac1 fac1 fac2 fac2*, where *fac1* and *fac2* represent recessive alleles at the two independent speckling loci. NREC is *R1-permissive R1-permissive fac1 fac1 fac2 fac2*. *R1-sc:124* ^W22 is *R1-nonpermissive R1-nonpermissive Fac1 Fac1 Fac2 Fac2*; *R1-g* ^Oh43 is *R1-nonpermissive R1-nonpermissive fac1 fac1 fac2 fac2*; *R1-r* ^W23 is *R1-nonpermissive R1-nonpermissive fac1 fac1 Fac2 Fac2*; and *R1-Randolph* ^W23 is *R1-permissive R1-permissive fac1 fac1 Fac2 Fac2*.

Jerry Kermicle initially referred to a similar speckling phenomenon as "Four Corners mottling" because it was identified in varieties of speckled maize from Native American open pollinated varieties from the Four Corners region of the Southwestern United States. Studies in Wisconsin (see Goncalves Butruille *et al.*, this MNL, for companion article) found that this speckling system requires a permissive *r1* allele (the strongest effect being found among certain *R1-d* haplotypes, although certain other haplotypes show a weaker effect) and two recessive factors named *mot1* and *mot2* for *mottling* factors. Tests of allelism were performed in Urbana and revealed the NREC system to be identical to the Four Corners mottling system. Separate *mot1* and *mot2* testers from Wisconsin were used to show that the COOP's W23 lines are homozygous recessive for *mot1*. We all concur that the two independent factors should be called *mot1* and *mot2*.

Figure 1. Kernels on a self pollinated ear of Navajo Robin's Egg Corn.

Table 1. Counts of full color (Cl) and speckled (spk) kernels from ears of the test cross: [*R1-sc:124* X NREC] X NREC.

female parent	**Cl**	**spk**	**7:1 $\chi 2$**
2003P-139-1	231	35	0.105
2003P-139-2	331	44	0.202
2003P-139-3	255	30	1.015
2003P-139-4	241	39	0.522
2003P-139-5	312	44	0.006
2003P-139-7	363	50	0.058
2003P-139-8	269	44	0.694
2003P-139-9	337	48	0.000
2003P-139-10	288	52	2.427
Totals	2627	386	0.267

Table 2. Counts of full color (Cl) and speckled (spk) kernels from ears of the test cross: [[*R1-sc:124* X NREC] X NREC] X NREC.

Female parent	Cl	spk	1:1 $\chi2$	3:1 $\chi2$	7:1 $\chi2$
2004-2705-1	257	80	92.964	0.286[1]	38.919
2004-2705-2	352	54	218.729	29.639	0.238[1]
2004-2705-3	289	98	94.266	0.022[1]	58.180
2004-2705-4	217	85	57.695	1.594[1]	67.589
2004-2705-5	172	23	113.851	18.135	0.089[1]
2004-2705-6	358	106	136.862	1.149[1]	45.399
2004-2705-7	409	142	129.381	0.175[1]	88.728
2004-2705-8	266	104	70.930	1.906[1]	82.411
2004-2706-1	184	178	0.099[1]	112.799	445.084
2004-2706-2	180	179	0.003[1]	118.337	458.149
2004-2706-3	149	22	94.322	13.429	0.021[1]
2004-2706-4	149	136	0.593[1]	78.457	323.213
2004-2706-6	254	89	79.373	0.164[1]	56.710
2004-2706-7	180	27	113.087	15.783	0.0559[1]
2004-2706-8	319	108	104.265	0.020[1]	63.891
2004-2707-1	236	209	1.638[1]	114.518	483.316
2004-2707-2	311	113	92.462	0.616[1]	77.628
2004-2707-3	298	92	108.810	0.414[1]	43.852
2004-2707-4	197	66	65.251	0.001[1]	38.145
2004-2707-5	245	96	65.106	1.807[1]	76.384
2004-2707-6	168	168	0.000[1]	112.000	432.000
2004-2707-7	213	204	0.194[1]	127.259	505.730
2004-2707-8	227	218	0.182[1]	136.576	541.702
2004-2707-9	239	216	1.163[1]	122.550	508.800
2004-2707-10	355	122	113.813	0.085[1]	74.574
2004-2707-12	366	127	115.864	0.152[1]	79.261
2004-2808-1	87	91	0.090[1]	64.787	242.777
2004-2808-2	443	69	273.195	36.260	0.446[1]
2004-2808-3	243	249	0.073[1]	172.098	653.310
2004-2808-4	254	241	0.341[1]	148.122	592.638
2004-2808-5	284	38	187.938	29.917	0.144[1]
2004-2808-6	233	69	89.060	0.746[1]	29.565
2004-2808-8	182	79	40.648	3.863[3]	75.337
2004-2808-9	131	37	52.595	0.794[1]	13.932
2004-2808-11	231	210	1.000[1]	120.333	497.286
2004-2709-1	146	165	1.161[1]	130.548	467.653
2004-2709-2	277	99	84.266	0.355[1]	65.751
2004-2709-4	307	109	94.240	0.321[1]	71.407
2004-2709-5	303	86	121.051	1.735[1]	32.832
2004-2709-7	178	157	1.316[1]	85.422	361.723
2004-2709-8	197	225	1.858[1]	180.477	642.818
2004-2709-9	291	85	112.862	1.149[1]	35.112
2004-2710-1	186	186	0.000[1]	124.000	478.286
2004-2710-2	130	120	0.400[1]	70.533	288.057
2004-2710-4	223	83	64.052	0.736[1]	59.834
2004-2710-5	231	238	0.104[1]	165.806	627.239
2004-2710-6	217	84	58.767	1.357[1]	65.326
2004-2710-8	218	231	0.376[1]	167.502	622.717

2004-2711-2	279	93	93.000	0.000[1]	53.143
2004-2711-3	437	162	126.252	1.336[1]	115.862
2004-2711-5	314	41	209.941	34.254	0.293[1]
2004-2711-7	456	64	295.508	44.677	0.018[1]
2004-2711-8	288	45	177.324	23.432	0.313[1]
2004-2711-9	214	76	65.669	0.225[1]	49.815
2004-2711-10	284	103	84.654	0.538[1]	70.494
2004-2711-11	180	29	109.096	13.794	0.362[1]
2004-2711-12	421	58	275.092	42.456	0.067[1]
2004-2712-1	287	104	85.650	0.533[1]	71.056
2004-2712-2	162	56	51.541	0.055[1]	34.666
2004-2712-3	270	42	166.615	22.154	0.264[1]
2004-2712-5	324	118	96.009	0.679[1]	81.449
2004-2712-6	285	102	86.535	0.380[1]	67.937
2004-2712-7	125	134	0.313[1]	98.750	364.572
2004-2712-8	105	48	21.235	3.314[2]	49.824
2004-2712-9	264	20	209.634	48.845	7.734[4]
2004-2712-10	354	121	114.293	0.057[1]	73.097

[1] $p > 0.1$ (NS)
[2] $p < 0.1$
[3] $p < 0.05$
[4] $p < 0.01$
$p < 0.001$ (no highlight or superscript)