## Evaluation of maize natural variation

In order to determine whether there was useful natural variation for this trait, we evaluated genetic variation among elite US germplasm (exPVP lines). Crosses between exPVP lines within two prominent heterotic groups of North american maize were made as part of the development of a synthetic population in the greenhouse in the winter of 2018-2019 The parental lines and the crosses that were made in the greenhouse are shown in tables 1 and 2 respectively. The progeny of these crosses were planted in the summer of 2019 and controlled pollinations made between lines, using a bulk of pollen from within the heterotic group. Also included in the cob analysis were several mini maize crosses with the heterotic groups. These crosses did not show any markedly different trends from the exPVP germplasm, apart from low mass ears and cobs and were included in the analysis. Pollinated ears were hand-harvested, shelled, and the cobs ground on a Wiley cutting mill. The mass of the cob and kernels was recorded. Samples of the ground cob were analyzed on a C/N analyzer and the data returned as nitrogen percentage. Kernels were assumed to contain on average 8% protein.

Stiff Stalk						Iodent						
ExPVP	PI	Reg.	Expired	Applicant	Ex	(PVP	PI	Reg.	Expired	Applicant		
CR14	601683	1989	2008	The JC	IB	B14	601658	1989	2010	United		
				Robinson						Agriseeds		
				Seed								
				Company								
4676A	601300	1986	2005	Novartis	09	\$602	559921	1992	2011	United		
				Seeds						Agriseeds		
CS608	560316	1992	2011	United	PH	IG83	601229	1985	2004	Pioneer		
				Agriseeds								
PHG86	601442	1987	2006	Poineer	PH	HG71	601150	1984	2004	Pioneer		
FAPW	600958	1982	2002	DeKalb	PF	IG29	601270	1986	2004	Pioneer		
PHW52	601575	1988	2007	Poineer	IB	B15	601458	1987	2008	DeKalb		
LH209	554612	1991	2011	Holden's	PH	HR55	548804	1991	2010	Pioneer		
				Foundation								
LH206	538010	1990	2009	Holden's	PH	HJ75	601776	1989	2008	Pioneer		
				Foundation								
FBLA	546482	1991	2010	DeKalb	15	538	601658	1989	2010	United		
										Agriseeds		
764	601374	1987	2005	Novartis	L1	.27	601726	1989	2008	Lifaco Seed		
				Seeds						Corporation		
LH220Ht	538011	1990	2009	Holden's	M	Q305	559917	1992	2011	United		
				Foundation						Agriseeds		
PHK35	601777	1989	2008	Poineer	PH	HP55	601784	1989	2008	Pioneer		
PHW17	601777	1987	2005	Poineer	PH	HN82	601783	1989	2008	Pioneer		
LH132	601004	1983	2003	Holden's	PH	HP76	543846	1990	2009	Pioneer		
				Foundation								

Table 1: Parents of the crosses for each heterotic group.

Stiff Stalks			Male Parent							
Female Parent	code	Α	В	С	D	E	F	G		
LH206/CR14	A	AxA	AxB		AxD	AxE		AxG		
FBLA/4676A	В		BxB	BxC	BxD			BxG		
764/CS608	c	CxA		CxC	CxD	CxE		CxG		
LH220Ht/PHG86	D	DxA		DxC	DxD			DxG		
PHK35/FAPW	E		ExB		ExD	ExE		ExG		
PHW17/PHW52	F						FxF			
LH132/LH209	G				GxD	GxE		GxG		
lodent		Male Parent								
Female Parent	Code	1	2	3	4	5	6	7		
IBB14/PHJ75	1				1x4	1x5		1x7		
OS602/1538	2				2x4		2x6	2x7		
PHG83/L 127	3					3x5				
PHG71/MQ305	4				4x4	4x5		4x7		
PHG29/PHP55	5		5x2			5x5	5x6	5x7		
IBB15/PHN82	6						6x6			
PHR55/PHP76	7			7x3			7x6	7x7		

Table 2: Crosses made in the greenhouse during the winter of 2018-2019, along with the parental crosses they were derived from. The seed from the greenhouse was evaluated in the summer of 2019.

Since the cob is entirely maternal tissue a mixed model was fit to the data on 1) nitrogen percentage of cob (NPer) and 2) the cob nitrogen/kernel nitrogen(CN/KN) ratio with random terms accounting for the maternal and paternal contributions, as well as fixed terms for the sample run on the C/N analyzer and two linear fixed terms for the cob mass and the kernel mass. The effects of the parents were extracted from the model and the correlation between the midparent mean effect and the offspring was determined as the narrow sense heritability.

Figure one shows the midparent mean effects for Nper and CN/KN plotted against offsprings values. The narrow-sense heritabilities for Nper and CN/KN were respectively 0.145 and 0.025. The values of the midparent effects in both cases are small, leading to the conclusion that breeding on these traits would have little to no effect in the short term. Figure 2 shows the correlation between traits measured or derived. Furthermore, as figure 3 shows, a plot of CN/KN ratio vs the kernel mass reveals the nitrogen sink relationships. As the kernel mass increases the CN/KN ratio decreases. The mass of open-pollinated ears is commonly between 125-225 grams at production density, a similar mass to those achieved by the controlled pollinations used in this experiment, however under nursery conditions the plant density and hence available nitrogen per plant was much higher than under production scenarios. If open-pollinated ears follow the same trend, then the expected CN/KN ratio in open-pollinated ears would be close to zero. Even if there is genetic variation for this trait, the low variance of the population is expected to make breeding for the trait impracticable.



Figure 1. Total N percentage and CN/KN ratio plots of midparent effect vs offspring values. Points are colored by kernel mass from the cob.

Figure 2. Correlation between the traits measured or derived (CN/KN ratio).





Figure 3. Sink relationships. The kernel mass is plotted on the x axis while the CN/KN is plotted on the Y. The points are sized according to the mass of the cob, and colored according to the nitrogen percentage.