

A Historical Evaluation of High Molecular Weight Glutenin Subunit Frequencies



Colleen Roseborough¹, Jeff Leonard¹, Andrew Ross¹, Hilary Gunn¹, Teepakorn Kongraksawech¹, Adam Heesacker¹ and Robert Zemetra¹

United States Department of Agriculture
National Institute of Food and Agriculture

¹Department of Crop and Soil Science, Oregon State University, Corvallis, OR

Introduction

The incidence of celiac disease and sensitivity to gluten appears to have increased in recent years in the United States, leading to questions regarding changes over time in the composition of gluten (glutenin and gliadin) in wheat and whether these changes could be the cause for this proposed increase in gluten sensitivity and celiac disease. These changes would have occurred when new germplasm was introduced into the U.S. wheat breeding programs and/or when there was an increased emphasis on selection for end-use quality in hard and soft winter wheat. These changes could be due to direct genotypic selection for the major glutenin subunits (5+10 – hard wheat, 2+12 – soft wheat) in the D genome or indirect for the minor glutenin subunits on the A and B genome due to phenotypic selection for end-use quality.

Objectives:

1. Determine if there has been any shift in the major and minor glutenin subunits in wheat cultivars grown in the Pacific Northwest (PNW) and United States over the last century.
2. Identify any changes in the glutenin subunits that occurred during the last twenty years that match the proposed timeline for the increase in the incidence of gluten sensitivity and celiac disease in the U.S. population.

Materials & Methods:

122 top U.S production varieties dating from the 1900's to 2011 (see Table.1.) were collected and grown in a greenhouse. Germplasm was chosen by top production acreage (Bonjean and Angus 2001) in the U.S. Plants were grown to maturity and grain was harvested and milled using a CEMOTEC 1090 sample mill. High molecular weight glutenins were extracted using a modified 4 step extraction protocol (Uthayakumaran, Listiohadi et al. 2006) using 100 mg of sample flour. Extraction supernatant was analyzed using an Agilent Bioanalyzer 2100 and Protein 230 kit. Protein bands were analyzed and identified according to Jonnala, Ramakanth S, et al. (Jonnala, Ramakanth S, et al. 2010; Marchetti-Deschmann, Lehner et al. 2011) (see Figure.1.).

Class	1800	1910's	1920's	1930's	1940's	1950's	1960's	1970's	1980's	1990's	2000's
Hard	0	2	6	5	12	9	10	14	20	14	30
Soft	2	22	6	8	15	20	8	10	14	4	14

Table 1. Number (N=) of plants within prospective decade. These numbers were used to calculate allelic frequencies of the Glu A-1, Glu B-1 and Glu D-1 subunits.

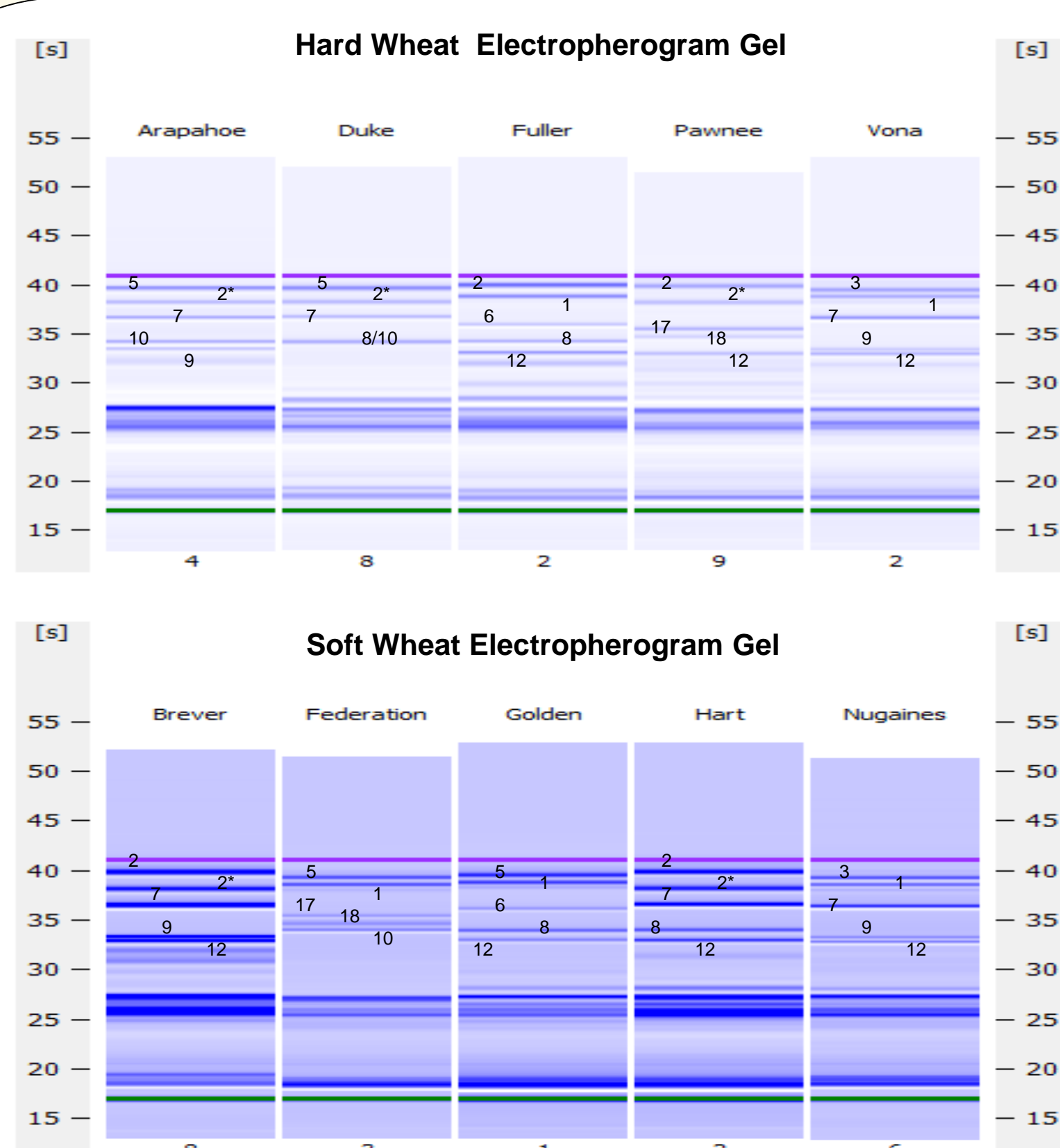
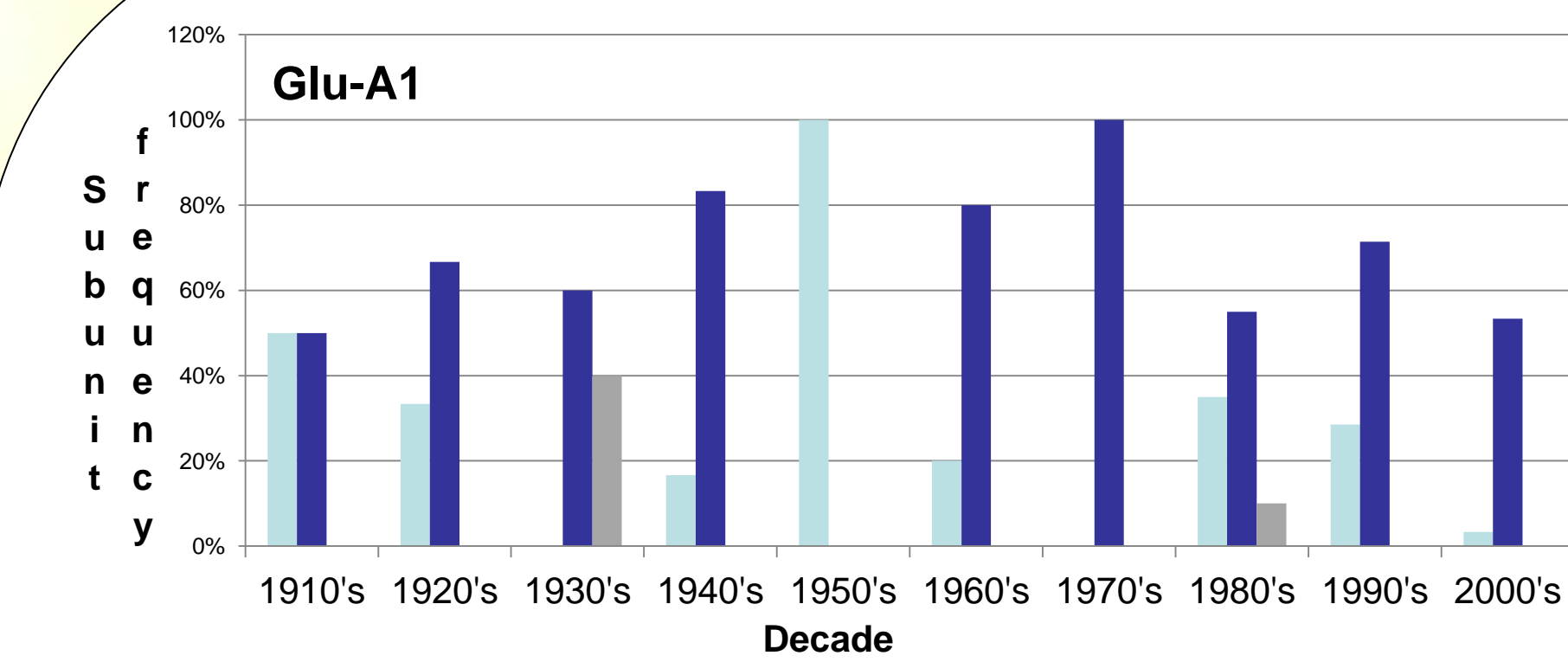
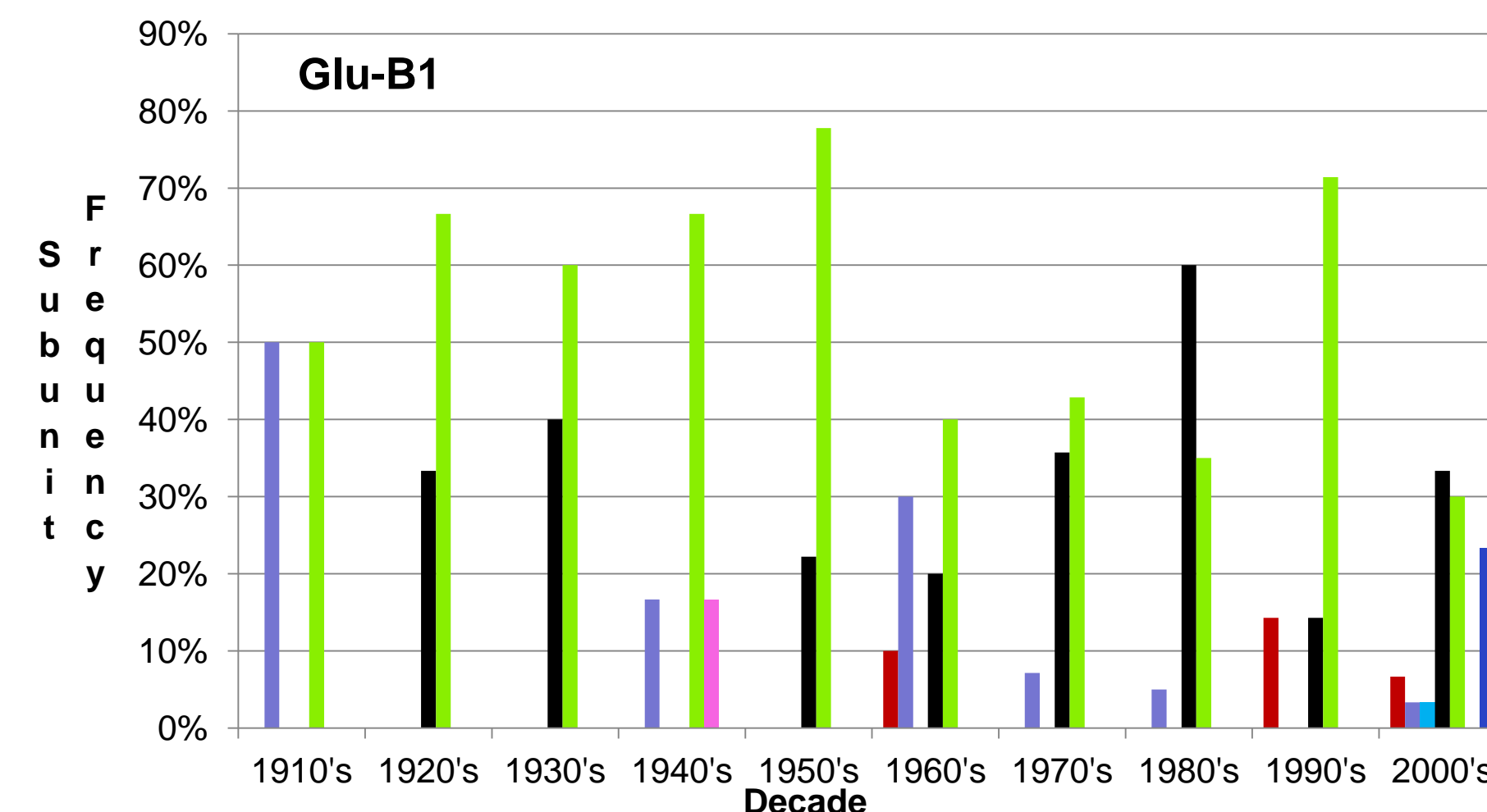


Figure.1. Representation of different subunit combinations.

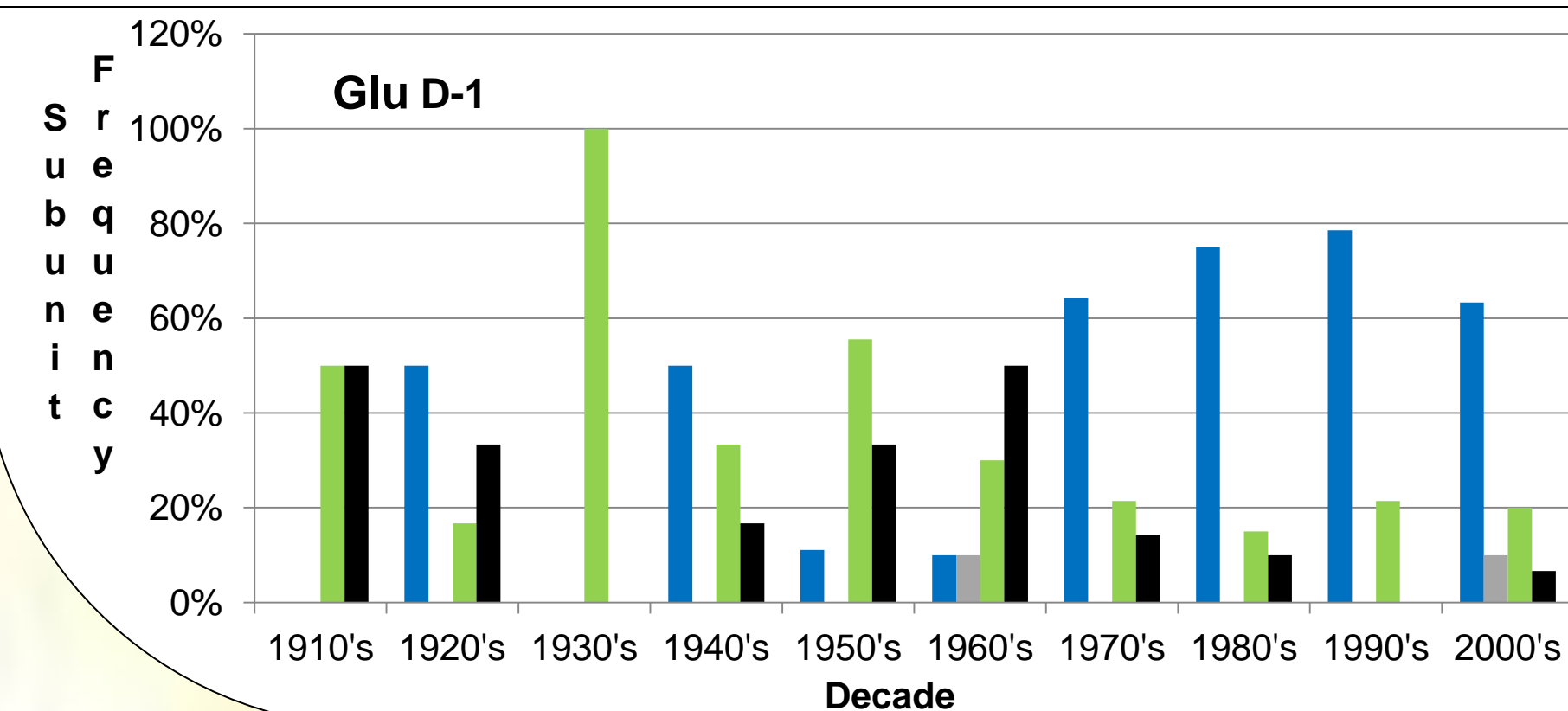
Results: Hard Wheat



- 1: Equal to 2* in the 1910's, dominated in the 1950's. Not in the 1970's germplasm. 35% frequency in the 1980's and decreased each decade since.
- 2*: Has been in wheat germplasm at a frequency of 50% or higher since the 1910's, but absent in the 1950's.
- null: Has a frequency of 40% (1930's) or less throughout the decades.

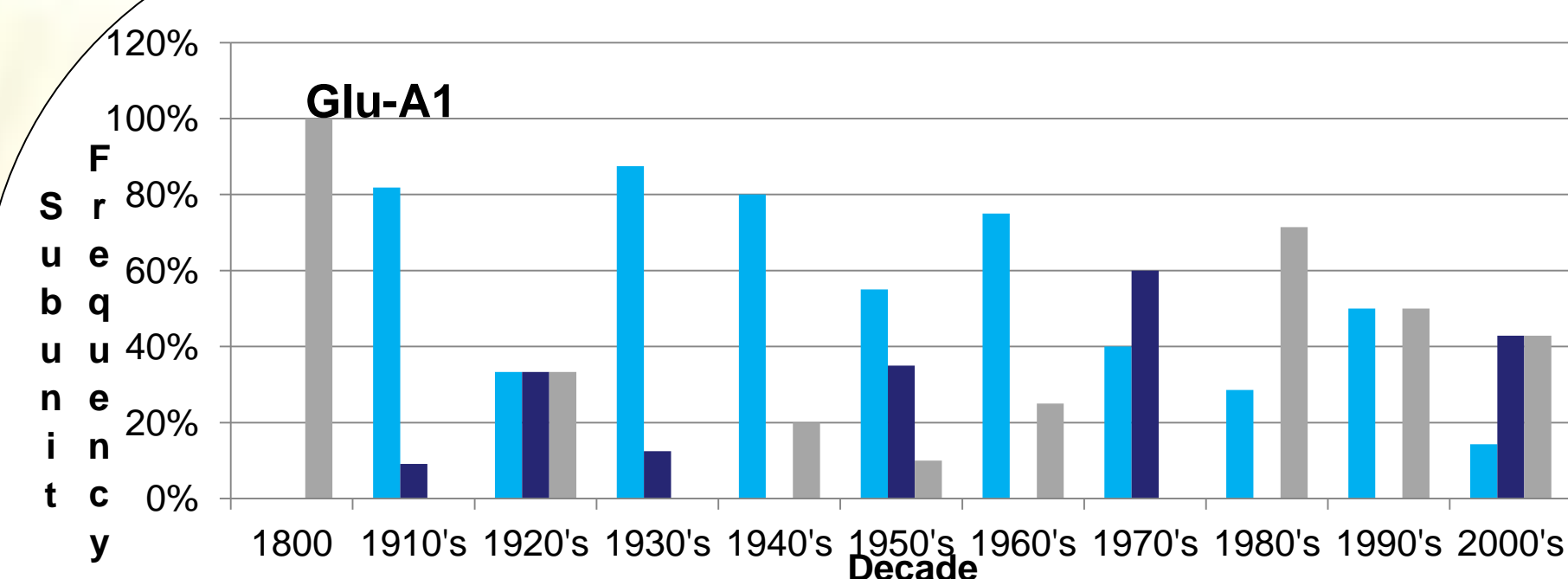


- 7: Present alone during the 1960's and recent varieties (1990-2000's).
- 6+8: 50% frequency in the 1910's, absent in the 1920's and 1930's. Had a 17% frequency in 1940's, peaks in the 1960's at 30%. & steadily decreases in later decades.
- 6+9: Novel combination for hard wheat as of the 2000's.
- 7+8: Appears in the 1920's, reaches a frequency of 40% by the 1930's. Decreases through the 1960's, peaks in the 1980's.
- 7+9: The dominate subunit combination through all decades except for 1980's and 2000's.
- 17+18: Present in the 1940's at a low frequency.
- 20*: A close approximation and needs further evaluation; however, this allele only appears present in the 2000's.

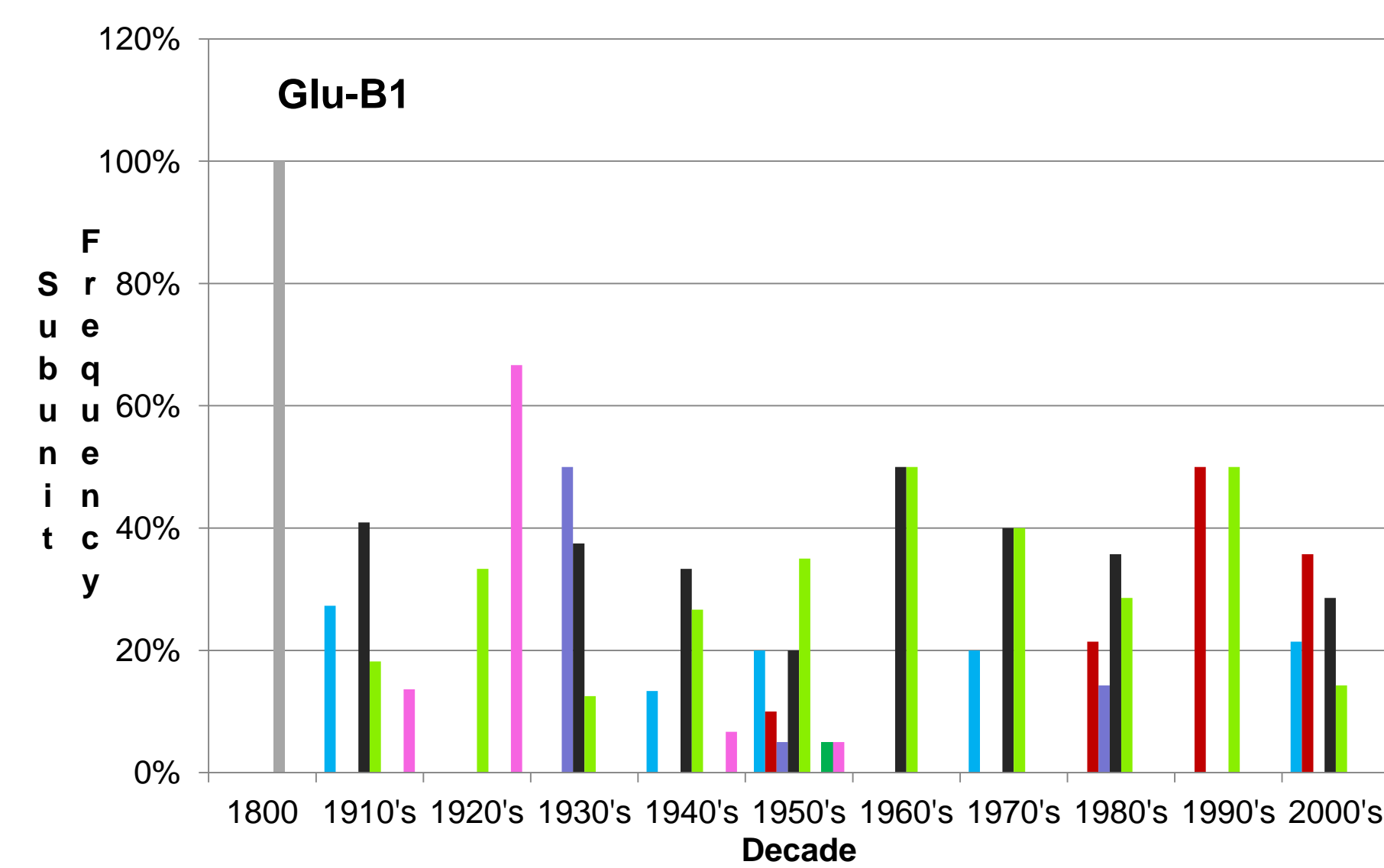


- 5+10: Appears in the 1920's and the 1940's. Decreases through the 1950's-1960's, main combination from the 1970's on.
- 5+12: Present in the 1960's and 2000's at a frequency of 10%.
- 2+12: Dominating combination up to 1950's then decreases.
- 3+12: Has a frequency of 50% in the 1910's and 1960's, continuously declines after the 1960's.

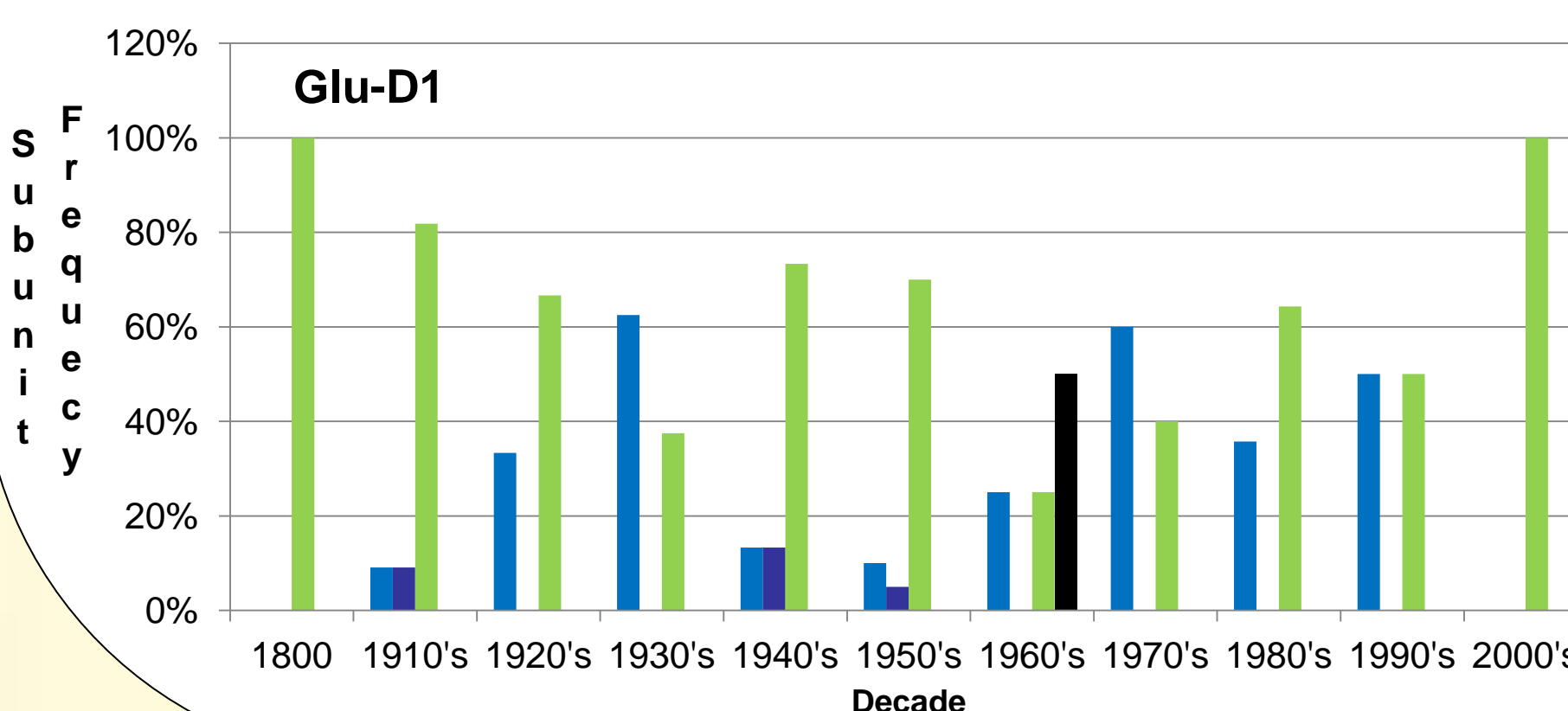
Results: Soft Wheat



- 1: Main subunit through the 1930's, declining in subsequent years.
- 2*: Low frequency in the 1910's, then steadily increases peaking in 1970's. Absent for 2 decades reappearing in the 2000's at ~50% frequency.
- Null: Present in the 1800's, decreasing through the 1950's. Peaks in the 1980's then slowly declines to present day.



- 6: Has frequency of ~20% from the 1910's-2000's.
- 7: Appears alone in the 1950's, increasing through the 1980's, peaking in the 1990's then slightly declines in the 2000's.
- 6+8: Appears with a frequency of 50% in the 1930's then decreases and is absent after the 1980's.
- 7+8: In the 1910's has a frequency of 40%, declining through the 1950's. Peaks in the 1960's then again declines through the 2000's.
- 7+9: Very fluid frequency from the 1910's to the 2000's.
- 8+17: Only in seen in 1800.
- 13+16: Present in the 1950's at a low frequency.
- 17+18: Peaks in the 1920's, declining through the 1950's. This combination is absent from the 1960's on.



- 5+10: Is the main subunits in the 1930's and 1970's, absent by the 2000's.
- 5+12: Has low frequency in the 1910's, 1940's and the 1950's.
- 2+12: Main subunits through the decades except for 1930's, 1960's and 1970's. By 2000's, it is only subunit combination.
- 3+12: Has a frequency of 50% in the 1960's.

Discussion/Conclusion

1. In cultivars grown after 2000 there was a shift in the major glutenin subunits observed in both the hard and soft wheat cultivars with 5-10 subunits being the primary subunits found in hard wheat and 2-12 subunits being the only D genome subunits found in soft wheat. This is most likely due to increased use of genomic selection for these sub-units and the increased emphasis on selection for end-use quality, especially in the soft white winter wheat breeding programs.
2. Variation was observed for the minor glutenin subunits over time in both hard and soft wheat cultivars with some minor subunits increasing or decreasing during various decades. Some of the minor subunits that increased in the 1990s and 2000s cultivars may warrant further study in relationship to glutenin sensitivity.

Future Research

Research is currently underway to determine if there has been a change in the presence and amount of the gliadin epitopes in wheat that induce celiac disease.

References

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Acknowledgements

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