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Intimate stories told by the grain proteins of Australian wheat and Thai rice .... to the police, to breeders, to grain growers, to grain handlers, to processors and to us as consumers

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Abstract - All cereal grains have genetic ("intimate") information "locked up" in the grain proteins. Accessing this information requires suitable methods of protein extraction and fractionation. Here we describe how this research approach can reveal information about stolen wheat - reliable evidence to be accepted in court. Plant breeders also rely on the grain-protein "semantides" to predict the type of grain quality (even from just a single grain), thus to produce new varieties of suitable quality. That same semantic information can subsequently be used after harvest to ensure that grain deliveries of the appropriate quality type are binned together, but separate from grain of different quality. Information about the grain proteins is also critical to the processing and consumption of grain-based foods.

Keywords: Variety identification, quality attribute, genome, capillary electrophoresis

1. Introduction

Wheat and rice (with maize) are the most important food sources for the world (Table 1). Both rice and wheat crops are important to our two countries: Thailand produces and exports much more rice than wheat, whereas Australia produces and exports much more wheat than rice (Table 2). In both cases, grain-protein composition can tell "intimate stories" that may not be readily elucidated by other means.

Table 1. The three major cereal grains in tonnes (t) - maize, wheat and rice production during 2019, according to FAO.org.

	World production
Maize (corn)	1,148,487,300 t
Wheat	765,769,600 t
Rice (as paddy)	755,473,800 t

Table 2. Comparison of the production and exported quantities of rice and wheat for Thailand and Australia during 2019, in tonnes (t), according to FAO.org.

	Rice production	**Wheat production**	**Rice export**	**Wheat export**
Thailand	28,357,000 t	1,351 t	7,581,000 t*	12 t
Australia	67,000 t	17,598,000 t	15 t	9,592,000 t

* Thai Rice Exporters Association

1.1 Grain proteins as "semantides"- carriers of intimate information

The term "semantide" was coined by Linus Pauling (Nobel Prize winner) and Emile Zuckerkandl in the 1960s, meaning molecules that carry genetic information. Proteins (and polypeptides) are considered to be "tertiary semantides". They are "tertiary" because they arise by translation from messenger RNAs ("secondary semantides"). DNA (the gene) is considered a "primary semantide". This sequence of "meaningfulness" is illustrated in Figure 1.

Thus, the proteins of the cereal-grain endosperm carry important information about the genetics (genes, genotype) of the grain (Figure 1). Thus, the grain proteins are valuable markers of identity, being readily extracted from the crushed grain (even a single grain) and being readily analysed for their composition (e.g., by gel electrophoresis). However, as Figure 1 also shows, growth environment can also modify the "meaningfulness" of the protein composition (the proteome).

1.2 Genome

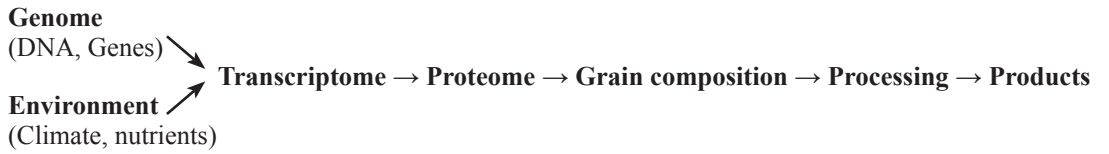

Figure 1. The biochemical chain of events through to food/feed production.

1.3 Products

Environment

Here, we explain how grain-protein composition can tell "intimate stories" about the origins, identity and quality characteristics of individual grains, thus providing information at all stages of the Grain Chain-from breeder to consumer (Figure 2) (Wrigley & Gooding, 2009; Wrigley, 2016).

Plant Breeder → Grain Grower → Grain handling → Processing → Retail → Consumer

Figure 2. The Grain Chain-from breeder to consumer (Wrigley, 2016).

2. The Police

2.1 Grain proteins tell true stories to the police

Here is a true detective story about wheat proteins solving a crime. Imagine an enormous truck racing through the back roads of north-western New South Wales (Australia). Suddenly it screeches to a stop. Officers of the Roads & Traffic Authority have their car parked across the narrow road. The roads officers approach the truck, intent on charging the driver with being over-weight for these roads. But the driver has locked himself inside the cabin of the big truck. The officers can make no contact. The situation is at a stalemate. They contact the police.

The police arrive; but they are less concerned about the over-weight charge and more interested in the likelihood of finding the fifty tonnes of grain stolen overnight from a silo further north-west. Eventually the driver is out and being interrogated. Reconstructed conversation:

Police: "What's your load?"
Driver: "Just grain."
Police: "Wheat? Where did you get it? Where are you taking it?"
Driver: "I just got it from a farmer back there. He had just stripped it off his main paddock. I have to take it to this address near Sydney. Now can I go?"
Police: "Not so fast. This load looks like the 50 tonnes of Prime Hard wheat out-loaded from a silo last night in Narrabri. And the address where you are taking it is a poultry farm. Prime Hard wheat isn't just chickenfeed! More likely it's the Prime Hard wheat stolen from the silo last night."
Driver: "What? You can't pin that on me! I know nothing about stolen grain. I don't know if it's wheat or what. Anyway, I'm just the driver. I was told to get this load to this place, so that's what I'm doing. Now can I go?"
Police: "No! If your story is true, why would you be taking back roads? We are taking grain samples for analysis. Your truck is impounded!"

"Wheat is wheat!"

Huh? "Wheat is wheat! That's all!", isn't it? How could the police determine whether this is the allegedly stolen grain or grain just harvested from the grower's paddock, as asserted by the driver? The police took grain samples from three parts of the grain load-the front, centre, and rear of the load. The samples were sealed, and the labels were signed by the driver and attending police.

In due course, the police delivered the grain samples to me (CW) at the CSIRO laboratories in Sydney, with instructions to determine whether the truck's grain is all one wheat variety (as would be expected from the driver's story of being harvested from one paddock) or if it is a mixture of the specific Prime Hard varieties that had been delivered to the grain silo in Narrabri. It was impressive that the police took pains to establish "continuity of identity" for the samples. The officer who took the samples from the truck was the one who delivered them to me (CW) in Sydney, ensuring that I signed for the samples and that they were kept locked up until their analysis. This proof of identity would be important in the ensuing court case.

2.2 The story told by wheat-grain proteins to the police, one grain at a time

For some decades at the CSIRO Wheat Research Unit (North Ryde, Sydney, Australia), we have been using electrophoretic procedures for fractionating the gliadin proteins of wheat, as extracted from grain (even from just a single grain) (Wrigley *et al.*, 1982). In this way, we can obtain a 'gliadin fingerprint', characteristic of each Australian wheat variety. The gliadin proteins represent about 50% of wheat storage (gluten-forming) protein; they are a good target for this purpose, since they are readily extractable from flour or crushed grain (using 6% urea solution) and giving electrophoretic patterns that distinguish between many wheat varieties. The grain proteins are thus more easily extracted and analysed than the DNA of the grain. This forensic assignment about stolen wheat was a good test for our ability to identify varietal identity for a mixed sample, one grain at a time.

We knew that grain-by-grain analysis would be needed, but our first approach was to grind up some of each of the three forensic samples, thus combining many grains to represent each sample. If the suspect grain load had really been taken from a single paddock (as claimed by the driver), then the electrophoretic pattern for a ground sample should be simple, representing a single wheat variety. If, however, the load were the grain stolen from the silo, the electrophoretic pattern would be complex, being the combined electrophoretic patterns representing the specific varieties that had been delivered prior to the alleged theft.

The outcome? The electrophoretic pattern of the suspect grain showed a complex pattern, indicative of many varieties, and certainly not the simple pattern for one variety that would match the driver's story. But this result alone would be insufficient evidence in a court of law. We needed to identify one grain at a time. In this way, we could show what mix of varieties was present, so that the varietal mix of the truck's load could be compared to the known combination of Prime Hard varieties that had previously been delivered into the Narrabri silo.

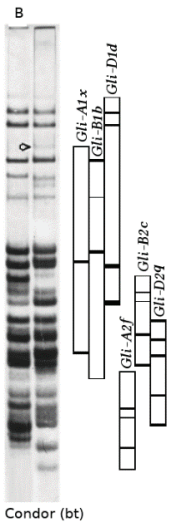

Figure 3. Gliadin gel electrophoresis pattern for one of the varieties in the grain load, namely Condor (at right: "bt" = specific biotype), shown beside the pattern ("B") for the variety Bezostaya (a universal standard cultivar, left pattern).

The Condor blocks of gliadin bands are shown diagrammatically indicating the gliadin bands relating to the six sets of gliadin alleles for its six chromosomes, which together make up the whole Condor gliadin pattern in the photo. The full set of Gli alleles for this variety can thus be shown as:

***Gli-A1x*, *Gli-B1b*, *Gli-D1d* and *Gli-A2f*, *Gli-B2c* and *Gli-D2q*.**

We determined the varietal identity for each of 140 single grains, in total, from the samples taken by police, based on their gliadin electrophoretic patterns (Table 3). There was a mix of five varieties; all were varieties that would be expected to be present in the Australian Prime Hard grade at that time. This mix also matched the mix of varieties received into the silo involved, according to the grain handler's records.

But how could the identification of 140 grains indicate the composition of a 50-tonne load of grain? Established statistical methods are available to answer that question. Accordingly, "confidence limits" were calculated (Table 3) to indicate the probability (at 90% confidence level) of how broad these percentages might be when comparing the compositions of the grain samples with the contents of the truck. The variety Timgalen was clearly the major component of the combined three sub-samples, but its proportion was unlikely to be more than 80% of the grain in the full truckload. These results thus indicated that the driver's story was most unlikely when he claimed that the truck's load was harvested from a grower's paddock.

Table 3. Wheat varieties (as %ages) identified in the combined three samples provided by police from the 50-tonne truckload.

Prime Hard Variety	Variety proportions (140 grains)	
	Actual %ages	Confidence limits
Timgalen	73%	65-80%
Gatcher	9%	4-13%
Kite	8%	3-13%
Winglen	4%	1-7%
Condor	5%	1-9%
Unidentified	2% (3 grains)	0-5%

Adapted from Wrigley *et al.* (1982).

In due course, I (CW) was called to give evidence in the Narrabri Court House to describe the results of our analyses and the procedures that had led us to this evidence. (Imagine explaining to the court how to perform gel electrophoresis!) I described:

- how we had extracted gliadin proteins into 6% urea solution from a milled sample (of many grains) and also from 140 crushed grains individually;
- how we made a slab of gel material, connected via electrolyte to electrodes to provide electrical charge;
- how we applied the extracted gliadin proteins into separate depressions in the gel slab (Wrigley *et al.*, 1982; Lookhart & Wrigley, 1995);
- how we applied a DC voltage to move the proteins inside the gel medium until the individual proteins were spread out along the gel according to their charge properties; and finally...
- how we stained the protein bands to produce results, such as in Figure 3.

My evidence was accepted in court, namely, that the varietal composition of the truckload was similar to that in the silo, and that the varietal mix did not agree with the driver's account of obtaining the load (likely to be a single variety) from a farmer's paddock. Additional evidence also indicted the grain thief. But most importantly, the grain proteins had 'told their story'!

Why test grain proteins? According to Figure 1, examination of the grain's DNA should provide information closer to the actual genotype (variety) of the grain. Yes, that is true, but the examination of DNA composition is more difficult and time-consuming, compared to study of the grain proteins. Nevertheless, we have developed a "rapid" DNA-based method of identification (Tran-Dinh *et al.*, 2009), but it is still easier to extract grain proteins for electrophoretic examination.

3. In the lab: Faster analysis

3.1 Fractionation of grain proteins by capillary electrophoresis

Even faster and more convenient than gel electrophoresis (Figure 3) is the newer method of capillary electrophoresis (Siriamornpun *et al.*, 2001). Figure 4 shows the differences between several Australian wheat varieties with respect to their grain proteins, extracted from the crushed grain, and fractionated by the capillary electrophoresis equipment illustrated on the right of Figure 5. Fractionation is based on the relative sizes of the proteins, as indicated by the calibration "ladder" (in kiloDaltons) at the left of Figure 4.

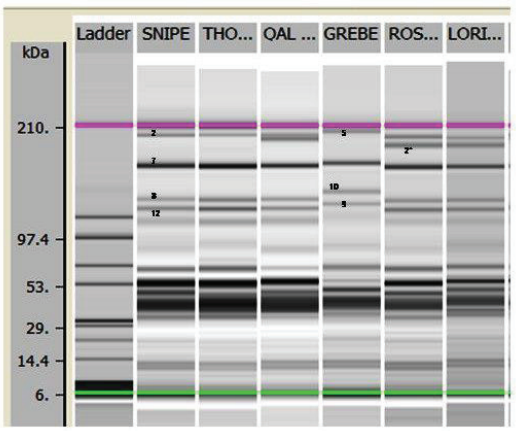

Figure 4. Patterns for the grain proteins of six Australian wheat varieties, fractionated by capillary electrophoresis in the equipment illustrated in Figure 5. Adapted from Uthayakumaran *et al.* (2005).

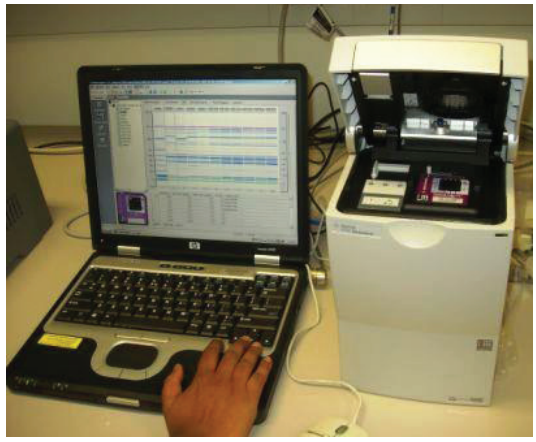

Figure 5. Equipment for capillary electrophoresis, suitable for proteins extracted from crushed grains of either wheat or rice. The resulting protein patterns can be seen on the computer screen.

3.2 Intimate stories are also told by the grain proteins of rice

All cereal grains have genetic information "locked up" in the grain proteins. Accessing the information requires suitable methods of protein extraction and fractionation. Siri has developed relevant methodology suitable for rice, using gel electrophoresis or capillary electrophoresis (Figures 5 and 6). In these cases, the methods of fractionation both involve protein extraction from the crushed grain (either brown or white rice) with detergent (SDS) and with mercaptoethanol to break the disulfide bonds of the grain proteins.

3.3 "Rice is rice!"

Could we also just say that "Rice is rice-no variations in quality?" Certainly not! Table 4 gives a few examples of Thai rice varieties, showing how they differ in aspects of grain quality-most significantly in starch properties, ranging from 6.1% to 26.5% amylose content (Siriamornpun *et al.*, 2005).

Table 4. Quality attributes of important Thai rice varieties (Adapted from Siriamornpun *et al.*, 2005)

Variety	Amylose content	Glutinous?	Fragrant?	Hardness
KDML 105	16.7%	No	Yes	Soft
Ko-Kho 15 (RD 15)	17.2%	No	Yes	Soft
Prathum Thani 1 (PTT 1)	26.5%	No	No	Hard
Kularb Dang (KD)	7.1%	No	No	Soft
Sanpatong 1 (SPT1)	6.1%	Yes	No	Soft

Can the grain proteins of rice also "tell intimate stories" about rice varietal identity? If it cannot be said that "Rice is rice! That's all!", how can we distinguish between varieties and between quality types of rice? Given the large differences in amylose content shown in the examples of Table 4, starch properties would be a good guide to distinguishing that aspect of quality type-for example, by using the Rapid ViscoAnalyzer (Ross *et al.*, 1987). However, a more exacting method is needed to distinguish between varieties that differ in other aspects of quality. That approach involves analysis of grain-protein composition by, for example, capillary electrophoresis (Figures 4 and 5).

3.4 Grain-protein composition for identifying rice varieties

The story of rice varieties is reflected by the quotation below.

"Siri has had experience about the "intimate stories" told by rice-grain proteins. This experience happened just after finishing her PhD in Australia and returning to Thailand. She took some undergraduate students to visit a big rice-milling company near a rice-growing region. She was curious to ask about the identification of rice varieties (or quality types). This is what the owner said, at the front desk where truckloads of paddy rice first came in."

Siri: "How can you tell the variety of each rice truckload?"

Owner: "It's easy. I just use my experience."

Siri: "How?"

Owner: "Using my eyes, hands, and sometimes I chew it (after I de-husk the paddy by hand). I have been doing so all my life."

"All her life" was a lot of experience because the owner was about 60 years old. Furthermore, she was so confident that no one could dispute her judgement. To her, it wasn't like "Rice is rice! That's all!" She could tell what variety and quality type was being delivered; thus. she could also tell the value of each load and what payment was due to the rice grower!

Rice varieties differ in amylose content (Table 4). A higher amylose content is associated with a more desirable grain texture. Of the varieties in Table 4, the most expensive rice in Thailand is Khao Dawk Mali 105 (KDML 105); it is well known as 'Thai jasmine rice'. It is a long-grain indica type, fragrant (aromatic), with good cooking quality. It is physically similar to some non-fragrant rice types, which are cheaper. RD 15 is the second in desirability, and PTT 1 is considerably cheaper. A further complication in the rice trade is the dilution of a consignment of a more expensive varieties by grain of a lower value. When Thai jasmine rice is mixed up with cheaper rice types, it is difficult to distinguish between these rice types by visual examination.

So, Siri came back from this experience with ideas of how to identify and distinguish between varieties of rice using more subjective, acceptable, and scientific methods. At that time, methods of genetic identification (DNA-based) were being developed in Thailand (see Figure 1), but such methodologies were inaccessible for the faraway sites of practical rice delivery and trade. There was thus the likelihood that rice farmers could have the quality of their rice deliveries judged subjectively, and consequently downgraded and judged to be of lower value by the rice buyer, with no way

to dispute the decision. There was clearly a need for better ways of establishing the variety and quality type of rice delivered at harvest. That is how Siri's research on rice variety identification started.

Good distinctions were obtained using either SDS gel electrophoresis or capillary electrophoresis, applicable to both wheat and rice (Siriamornpun *et al.*, 2001, 2004a, 2004b). Figure 6 shows the capillary electrophoresis patterns for some Thai rice varieties. These fractionation methods have permitted the unequivocal identification and distinction between Thai rice varieties. The grain proteins are again **telling their intimate stories**.

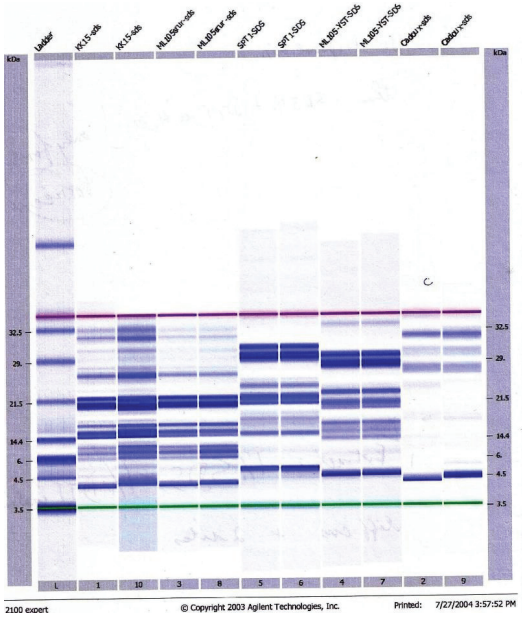

Figure 6. The composition of grain proteins of several Thai rice varieties, fractionated by capillary electrophoresis (Siriamornpun *et al.*, 2001).

3.5 Grain quality in the grain chain

Identification of variety is important at the stages of sowing and grain receival (Figure 2), because the grower needs to know that the seed sown is appropriate for the growth time and conditions. Variety is also important when the grower delivers that mature grain after harvest, because the grain handler must keep grain deliveries of similar quality type together and separate from grain of different quality type. These considerations apply to bother rice and wheat. In both cases, segregation of delivered grain takes account of the quality attributes that have been "built in" by the breeder; thus, to suit the distinct markets and end-product suitability, e.g., for making bread versus noodles, etc. (Table 5). When the grain is delivered at harvest, varietal identity is usually used as an indication of end-product suitability, because the grain handler cannot test for grain-quality attributes in the rush of harvest time. Thus, varieties of similar quality types are accepted together into a relevant "quality class". Variety identification for this purpose can ultimately be performed by analysis of grain-protein composition for both wheat and rice.

4. The breeder

So, it is the responsibility of the breeder, back at the start of the Grain Chain (Figure 2) to "build in" the desired type of grain quality for the new varieties that are registered. Table 5 lists several uses of wheat and the grain-quality attributes that are needed for each use. In the late stages of the breeding process, the breeder can test for actual grain quality (e.g., dough properties or suitability for bread making) but it requires significant amounts of grain to perform these tests. But at a very early stage of breeding (years earlier!), the breeder can find out the "intimate stories" about end-use quality by testing for grain-protein composition; that requires only half a grain, so that the germ end of

the grain can be retained to be grown on into a plant, if specific half-grains show promising quality attributes, based on protein composition. However, this approach requires that genetic connections must be established between protein components and quality attributes.

Table 5. Uses of wheat grain, each requiring a distinct type of grain quality. Adapted from Wrigley *et al.* (2009a).

Product	Protein content	Grain hardness	Dough strength	Dough development time
Pan breads	>12%	Hard	Strong	High
Flat breads	10-12%	Hard	Medium	Medium
Steamed breads	10-12%	Medium/Hard	Medium	Medium
Noodles	11-13%	Soft/Hard	Medium	Medium
Cookies, cakes	8-9%	Very soft	Weak	Low
Pasta	>13%	Durum, hard	Strong	High
Starch-gluten	>13%	Soft preferred	Medium	High
Bioethanol	Low	Soft preferred	Not relevant	

4.1 Wheat-quality type can be told to the breeder by grain-protein composition

Analysis of gliadin composition (as Gli alleles) proved suitable for the forensic analyses described above, and also for variety identification at grain receival during grain handling, further along the Grain Chain (Figure 2). Figure 3 illustrates how the gliadin alleles of the variety Condor may be deduced from its whole electrophoretic pattern. The diagrams beside the gel pattern of Figure 3 show that the many gel bands are separated out to identify the specific gliadins relating to each of the three genomes of wheat (genomes A, B and D). Thence, the gliadin composition of this variety can be reduced to the set of allele letters in Figure 3. Similarly, the alleles of the glutenin subunits are indicated by the allele letters in Table 7.

However, the glutenin polypeptides are more significant indicators of dough quality (Tables 6 and 7) than are the gliadins. Thus, glutenin alleles (especially the Glu-1 alleles) are more appropriate to the needs of the wheat breeder when selecting for genotypes with dough properties suited to the respective food product that is targeted for the new variety. Analysis of grain-protein composition is a valuable means for the breeder to predict ("foretell") quality type after making a cross, long before the progeny has been multiplied to provide enough grain to mill and process into bread (or whatever product is the breeding target). For each of the various products made from wheat, there is a range of contrasting (and competing) grain-quality goals, as summarised in Table 5.

Table 6. Specific proteins (and corresponding genes) as indicators/contributors to specific aspects of wheat-grain quality. Adapted from Wrigley *et al.* (2009a).

Locus (gene) designations	Polypeptides involved	Relevant quality attributes
Gli-1	Gliadin proteins	Modest contributions to dough properties
Gli-2	Gliadin proteins	
Glu-1	HMW subunits glutenin of	Major contributions to dough strength
Glu-3	LMW subunits glutenin of	Significant contributions to dough extensibility
Pin-a	Puroindoline a	Grain hardness
Pin-b	Puroindoline b	Grain hardness
Wx-1	Granule-bound starch synthase	Starch properties

Table 6 lists the various aspects of grain-protein composition that can be used by the wheat breeder to select for quality attributes when selecting suitable parent lines and when segregating the single grains of progeny. In both cases, the breeder considers the presence (or absence) of "indicator proteins" in turn predictive of the relevant genes/alleles (see Figure 1). Of these grain-quality attributes (grain hardness, dough and starch properties), dough properties are the most difficult to evaluate during breeding, due to the need to grow up enough grain for milling into flour and mixing into dough. It is thus essential that suitable gene/protein markers have been developed to assist the breeder in selecting for dough strength. This approach of predicting grain-quality potential at the single-seed stage of propagation permits the discard of inappropriate grain types, thus saving a few years of propagating potentially useless progeny.

Table 6 shows that the high-molecular-weight (HMW) subunits of glutenin (***Glu-1*** loci) make major contributions to dough strength, complemented by the low-molecular-weight (LMW) subunits of glutenin (***Glu-3*** loci). The individual Glu-1 alleles differ in the extent of their contributions to dough strength, as shown by their respective rankings (Table 7). These rankings tell the breeder valuable "stories" about grain-protein composition, permitting a tailored approach in selecting the alleles most likely to provide the quality attributes needed for the target grain quality, e.g.,

- pan bread (sandwich loaf) needs ...
- strong dough (Table 5), so the breeder would seek ...
- Glu-1 alleles (HMW glutenin subunits for dough strength, Table 6) with ...
- Combinations of Glu-1 alleles in the upper half of Table 7 for the three genomes of wheat.
    - Conversely, a breeding aim for a cake-flour wheat would involve parent lines with Glu-1 alleles in the lower half of Table 7, resulting in the required dough weakness (Table 5).

Table 7. Correspondence between dough-strength rankings and allele designations (shown as lower-case letters) for combinations of HMW subunits of glutenin (Glu-1 loci contributed by each of the three wheat genomes [A, B and D]). Adapted from Wrigley *et al.* (2009b).

Dough-strength score	Glu-A1	Glu-B1	Glu-D1
5		al	
4			d
3	a b	f	
2		u i	
1	c	c	a c
0		d e	b f

4.2 Puzzles for the breeder - Wheat

Tables 5, 6 and 7 illustrate how a wheat breeder is faced with a complex puzzle in trying to select for a specific combinations of quality attributes, as in Table 5, to produce a new variety that would be suited for a particular end use. For example, the breeder may have made a cross between a hard and a soft wheat, but then need to select for progeny that would be hard-grained. It is important to know that grain hardness is controlled by the genes pin a and pin b (Table 6), coding for the polypeptides called puroindolines. Selection of progeny containing an appropriate puroindoline marker protein provides an efficient means of selecting progeny that would be hard-grained. A similar strategy may be applied to using marker proteins shown in Table 6 to select for other quality attributes, e.g., suitable glutenin polymers to select for dough strength, or specific granule-bound starch synthase proteins to identify which progeny could be expected to have suitable starch-pasting quality.

The breeder's puzzle of genes X attributes can be illustrated with the jigsaw puzzle in Figure 7. Across the horizontal axis (bottom of figure) are the several quality attributes that the breeder needs to select. At left (vertical axis) are the potential genetic markers at various levels-marker compounds through to actual DNA (genes). For example, grain hardness for wheat (quality attribute, bottom row) is caused by the friabilin proteins that contribute adherence between the starch granules in the wheat-grain endosperm, thus contributing to the hardness of the grain. The respective genes (pin a and pin b) code for the specific adherence proteins (purindolines). The breeder has the choice of selecting for grain hardness at the levels of the genes (DNA - pin a or pin b) or specific proteins/ polypeptides. Similarly, marker proteins and specific genes are known for other (but not all) of the wheat-quality attributes.

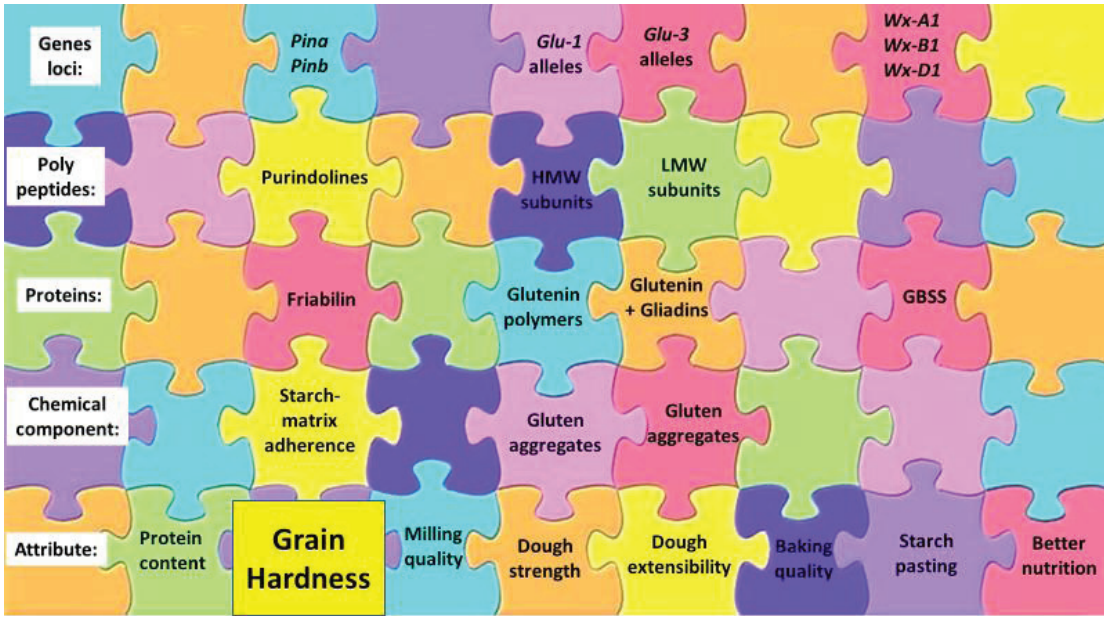

Figure 7. The choices for a wheat breeder, selecting for grain qualities, are represented by the two-dimensional jigsaw grid of quality attributes (horizontal axis) X marker molecules (vertical axis) ranging from specific proteins/polypeptides to specific genes.

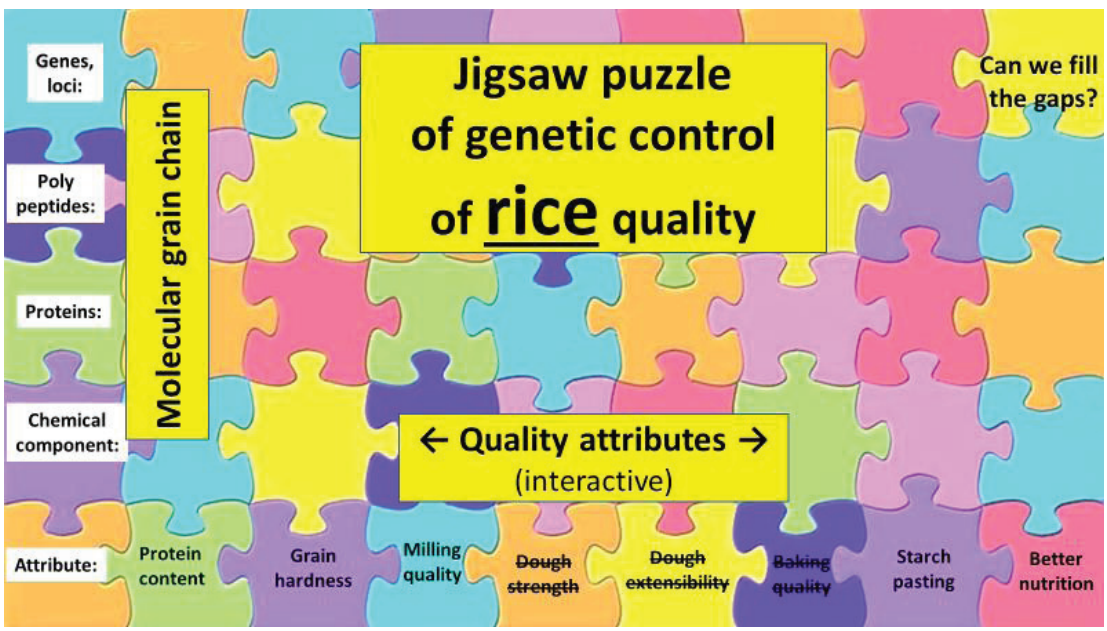

Figure 8. The choices for a rice breeder, expressed similarly to the puzzle for wheat.

4.2 Puzzles for the breeder - rice

Figure 8 illustrates how a similar jigsaw strategy should operate in rice breeding. In the case of rice, however, we have less knowledge of the marker proteins/genes for the many quality attributes. For rice, the significant attributes relate largely to starch properties, including cooking quality (indicated by apparent amylose content), gelatinisation temperature and gel consistency. Other considerations are milling quality (yield of pearled rice), appearance (including chalkiness) and nutritional quality. Grain-protein markers are not yet so well established for rice qualities as they are for wheat.

4.3 Computer-based estimation of desired grain-protein composition for wheat-dough quality

For wheat-quality marker proteins, it has been possible to combine the information in Tables 5 to 7 into a database (Bekes & Wrigley, 2013), combining dough quality and glutenin alleles from over 8,500 wheat varieties from 80 countries, sourced from 220 publications. It includes alleles for both HMW- and LMW-glutenin subunits:

- HMW-glutenin subunits:
  - 23 alleles of Glu-A1;
  - 87 alleles of Glu-B1; and
  - 25 alleles of Glu-D1; and
- LMW-glutenin subunits:
  - 11 alleles of Glu-A3;
  - 13 alleles of Glu-B3; and
  - 12 alleles of Glu-D3.

The database can be searched according to:

- the name of a variety,
- its country of origin, or
- the glutenin (Glu) allele(s) in any combination of alleles, thus to predict:
  - dough properties as determined using the Extensograph (Figure 9), with both:
  - the height (strength, Rmax) and
  - the length (extensibility, Ext) of the Extensogram of dough for the respective variety.

The example is given by Bekes and Wrigley (2013) of the Australian variety Aroona. Based on its glutenin alleles (Glu-A1a, Glu-B1b/c, Glu-D1a, Glu-A3c, Glu-B3b and Glu-D3b), its Rmax and Ext are predicted to be 465 and 20.7 cm, respectively (see example in Figure 9). The database can be used, for example, to determine the potential of a specific variety to suit a process requiring a specific range of dough properties or to assess the potential suitability of specific parent lines when planning crossbreeding to produce a new variety of desired quality.

The set of glutenin alleles (above) for the variety Aroona illustrates a common difficulty of polymorphisms in registered varieties worldwide (Metakovsky *et al.*, 2020). This polymorphism appears for Aroona at the Glu-B1 locus, for which there are two distinct alleles, namely, b and c. Furthermore, there are multiple biotypes for all the varieties listed in Table 8. In

each case, it is evident that the biotypes are genuine descendant of the parent lines. The variety Gamenya, for example, has two biotypes at the Gli-A1 locus; alleles g and a are inherited respectively from parent lines Gabo and Mentana; so, the two biotypes are genuine descendants from the original cross. This phenomenon creates uncertainties for the prediction system if the biotypes differ significantly in their respective contributions to dough properties and if their respective proportions vary under different growth conditions.

Thus, to indicate the suitability of the flour for processing, e.g., into bread, cakes or cookies. The maximum height of the stretching curve is designated the Rmax; the overall length of the curve is the extensibility (Ext.) of the dough sample. The two traces are produced by repeat tests on two dough samples of the same variety, thereby providing an indication of the reproducibility of this test procedure.

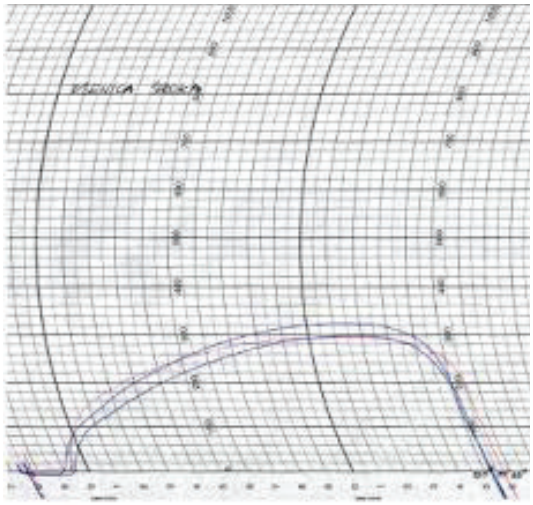

Figure 9. The result of testing (by stretching) a sample of dough made from wheat flour in the Extensograph,

Table 8. Gliadin-allele compositions of Australian non-uniform cultivars (in bold)and of relevant parent lines (not bold). Adapted from Metakovsky *et al.* (2019c).

Cultivar	Alleles at the Gli loci						Year of release
	A1	B1	D1	A2	B2	D2	
Gamenya	***g+a***	***b***	***f***	***c***	***c***	***t***	1958
Gabo	*g*	*b*	*f*	*c*	*c*	*t*	
Mentana	a	k	a	e	h	j	
Insignia	***af***	***i***	***i+g***	***a***	***i***	***i***	1946
Ghurka	*f*	*i*	*g+a*	*a*	*ab*	*g*	
Ranee	af	i	a+i	a+c	i	q+i	
Suneca	***o***	***d***	***f***	***m***	***c***	***m+j***	1982
Ciano-67	*o*	*d*	*f*	*f*	*c*	*j*	
Spica	o	b	a	m	c+o	m	
Timgalen	***g***	***b***	***f***	***c***	***c***	***q+t***	1967
Winglen	*m*	*b*	*g*	*f*	*aq*	*q*	
Gabo	g	b	f	c	c	t	

5. The grain grower

5.1 Seed for sowing: Mixed-up stories for breeders and grain growers

In anticipation of sowing seed in a new season, the grain grower buys "certified seed", expecting to sow clean seed that is genetically uniform, true to the variety label and free of impurities. This uniformity was assumed for our analysis of varieties in the case of the stolen wheat (Table 3) [although the two biotypes of Condor and of Timgalen (Table 8) were known and had to be taken into consideration]. The gliadin-allele compositions of Australian non-uniform cultivars (in bold) and of relevant parent lines (not bold) are listed in Table 8. These 'older' varieties were assumed to be homogeneous at the time of their release, but that assessment would have been mainly based on the appearance of the plants and the grain. More recently, each of these varieties is seen to be genetically heterogeneous, each being represented by more than one gliadin pattern.

Decades ago, we proposed procedures and nomenclature to characterise the various types of off-types that may occur in registered varieties (Appleyard *et al.*, 1979). Subsequent studies of seed homogeneity have involved interactions with the Russian scientist, Dr Eugene Metakovsky, initially when he was a visiting scientist at the CSIRO Wheat Research Unit in the 1980s, following on from interactions with his "boss" in Moscow, Dr Alexei Sozinov. In Eugene's studies of single grains of several Australian varieties, there were more than one gliadin pattern for each of some varieties (Metakovsky *et al.*, 1990). Fast forward a few decades. Eugene Metakovsky, now interacting with me (CW) from Spain, has taken these studies world-wide to include a few hundred cultivars, still by determining gliadin composition using gel electrophoresis (Metakovsky *et al.*, 2019a, 2019b, 2019c, 2020). We have offered three major reasons to explain the non-uniformity of wheats globally:

1. A foreign/unrelated cultivar is present as a contaminant, possibly mixed in by error during seed processing.
2. A distinct genotype as the result of accidental out-crossing with wheats growing nearby during propagation.
3. Sister lines, thus authentic progeny from the original cross, but present due to inadequate sub-sampling by the breeder (possibly even intentional). These may be regarded as valid biotypes, as they share the same pedigree as the main genotype that was registered. Table 8 shows examples of these biotypes.

5.2 Global genetic heterogeneity of wheat, based on grain-protein analysis

These recent studies have involved a collection of 1,060 wheat cultivars, from more than 20 countries in Europe and North America, plus Australia and several regions of the former USSR. The studies involved 193 gliadin alleles, including nine different new alleles and two null alleles.

with the extent of genetic heterogeneity differing across countries of origin (Metakovsky *et al.*, 2021).

We observed an extreme case of such non-homogeneity in the Russian variety Saratovskaya-29; the minor differences in non-homogeneity for the majority of cultivars are eclipsed by this popular variety. However, the success of Saratovskaya-29 may be partly due to its non-homogeneity, so that at least a few of its biotypes may be best suited to the growth environment in a certain season and situation. Depending on the growth site, the samples of Saratovskaya-29 showed significant differences of dough quality, due to varying proportions of the biotypes which differed in dough properties.

6. The grain handler

6.1 Grain proteins tell grain handlers if the correct variety is delivered

Premium wheat classes specify varietal composition, so that only wheats of suitable grain quality are included in a premium grade at harvest. On-the-spot identification of wheat variety is used to check if the declaration of variety is correct for delivered grain. In the past, varietal identification has depended on the expertise of inspectors to recognise differences in visual grain appearance "on-the-spot". More recently, biochemical tests have offered objective verification of variety, such as the use of gel electrophoresis of gliadin composition (such as in Figure 3). More rapid and convenient methods are now available for analysis of gliadin composition, such as capillary electrophoresis, as illustrated in Figures 4 to 6 (Uthayakumaran *et al.*, 2005; Siriamornpun *et al.*, 2004a, 2004b). This technology permits the verification of varietal identity within minutes, making the methodology suitable for use at grain receival. It is applicable to wheat, rice and other grains.

6.2 The processor

It can be a long journey for harvested grain to reach the mill, bakery and supermarket, as illustrated in Figure 2, possibly across a continent or even overseas. In any case, the aim of the grain handler and the marketing authority is to provide the miller or baker with grain (wheat or rice) of defined grain quality, thus to suit the processor's specifications, whilst also providing the grower with optimised value addition, based on grain quality. For wheat, grain composition is a significant part of these specifications, so that the dough-forming gluten proteins are appropriate to processing and baking (Table 5). In the case of rice, variety composition is also important to ensure uniformity of grain quality (Table 4). Thus, the processor is dependent on all the previous steps of the Grain Chain (Figure 2).

6.3 Us as consumers

How do the stories told by grain proteins relate to us as consumers?

We, as consumers/buyers, exercise our choice of product and of retail source. For example, do we prefer the bread of the small baker or supermarket bread? Do we prefer a sticky style of rice, or jasmine or basmati?

More important, is there a matter of dietary intolerance for any members of our family with respect to the food or grain type? This question relates especially to those with coeliac disease, due to the gluten proteins of wheat. For example, my

brother and cousin have coeliac disease. That means that they cannot tolerate wheat gluten in their diet. If they eat any wheat-based food, the gluten proteins cause various horrible symptoms, such as bloating, flatulence, diarrhoea, fatigue, weakness and lethargy. There are also other forms of dietary intolerance/allergy associated with wheat proteins (Bekes *et al.*, 2017). Nevertheless, are there possibilities for reduced immunogenicity to gluten (Rustgi *et al.*, 2021)?

Yet fortunately, most of us are not subject to these restrictions. The baking of gluten-free bread is possible, but difficult. Rice, on the other hand, is hypoallergenic; there is a low risk of abnormal dietary reactions when we eat rice-based foods.

So, what will be your choice when you dine later today-rice-based or wheat-based dishes?

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