Wheat domestication: When, Where and How– insight from modern genetics

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THE WEIZMANN INSTITUTE OF SCIENCE, Rehovot, Israel

400 administrative staff

250 professors who head research groups

1,000 M.Sc. and Ph.D. students

850 scientists with Ph.D. degree, engineers and technicians
Triticum turgidum var dicoccoides
Where Cereals come from?
a gift from the gods?
Or a curse?

In the sweat of thy face you shall eat bread..... (Genesis, The Bible)
CHAPTER I.

VARIATION UNDER DOMESTICATION.

Causes of Variability ... Character of Domestic Varieties--Origin of Domestic Varieties from one or more Specie ... Principles of Selection, anciently followed, their Effects ... Methodical and Unconscious Selection ... Unknown Origin of our Domestic Productions ...
The study of the origin of domesticated plants is based on evidence from the following disciplines:

- Folkloristic
- Archaeology
- Botany
- Genetics and Genomics
- Chemistry
- Agronomy
- Climatology
- Anthropology
- History
- Linguistics
Tests used to identify the wild progenitor

- Classical taxonomic approach - morphological similarity.
- Reproductive barrier
- Cytogenetic analysis - chromosomal affinity
- Molecular biology - genetic distance based on markers, or comparative sequence analysis
The three cultivated species of wheat that were recognized by Linnaeus (Species Plantarum, 1753):

**Triticum monococcum** L.

**T. turgidum** L.

**T. aestivum** L.
Theories concerning the site of wheat domestication at the end of the 19th century

Solms-Laubach --> Central Asia (leading theory)

Much --> South of the Baltic sea

De Candolle --> The Euphrates Basin
Found, among spikelets of wild barley collected by Kotschy in Rasheya (Syria) on the Northern slopes of mount Hermon, spikelets which were from a wild origin and looked like wild wheat – supporting De Candole’s proposal of the Euphrates basin origin.
The discovery of Wild emmer wheat in nature

Aaron Aaronsohn (1876-1919)
Wild wheat: 
*Triticum turgidum var dicoccoides*

A fragile spike with a brittle rachis, 2 large grains per spikelet, strongly protected by stiff glumes.
Reproductive evidence: fertile hybrid

Domestic    F1-Hybrid    wild wheat

*Triticum turgidum var. durum*

*Triticum turgidum var. dicoccoides*
The karyotypic evidence: the karyotype of domestic durum wheat is the same as that of wild *dicoccoides* wheat: $n = 14$ chromosomes.

The karyotype of domestic Barley is the same as that of wild barley: $n = 7$ chromosomes.
The cytogenetic evidence:
Chromosome number is the same in durum and dicocoides and there is full pairing between the chromosomes ($2n=28$), 14 pairs

Full

Partial

Low ---&gt; Different species
Evolution of wheat: an history of hybridization, alloploidy and domestication

- **Triticum dicoccoides**
  - Divergence from a common progenitor 2n=2x=14 (~4 MYA)

- **T. durum**
  - Formation of wild wheat 2n=4x=28 (~0.5 MYA)
  - Domesticated primitive wheat 2n=4x=28 (~10,500 Cal BP)
  - Durum wheat (Landraces or modern varieties) 2n=4x=28 (~few hundred YA-present)

- **T. aestivum**
  - Formation of bread wheat 2n=6x=42 (~9,500 Cal BP)

- Hybridization:
  - BBAA
  - BBAADD
Classification of the species of *Triticum* (after van Slageren, 1994)

<table>
<thead>
<tr>
<th>Species</th>
<th>Genomes</th>
<th>Wild</th>
<th>Domesticated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diploid (2n=14)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. urartu</em></td>
<td>A</td>
<td>all</td>
<td>-</td>
</tr>
<tr>
<td><em>T. monococcum</em></td>
<td>A&lt;sup&gt;m&lt;/sup&gt;</td>
<td>ssp. <em>aegilopoides</em></td>
<td>ssp. <em>monococcum</em> (domest. einkorn)</td>
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<tr>
<td></td>
<td></td>
<td>(wild einkorn)</td>
<td></td>
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<tr>
<td><strong>Tetraploid (2n=28)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><em>T. timopheevii</em></td>
<td>GA</td>
<td>ssp. <em>armeniacum</em></td>
<td>ssp. <em>timopheevii</em></td>
</tr>
<tr>
<td><em>T. turgidum</em></td>
<td>BA</td>
<td>ssp. <em>dicocoides</em></td>
<td>ssp. <em>dicococum</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(wild emmer)</td>
<td>ssp. <em>parvicolossum</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ssp. <em>durum</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ssp. <em>turgidum</em></td>
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<td></td>
<td></td>
<td></td>
<td>ssp. <em>polonicum</em></td>
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<td></td>
<td></td>
<td></td>
<td>ssp. <em>carthlicum</em></td>
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<tr>
<td><strong>Hexaploid (2n=42)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. zhukovskiyi</em></td>
<td>GAA&lt;sup&gt;m&lt;/sup&gt;</td>
<td>-</td>
<td>ssp. <em>zhukovskiyi</em></td>
</tr>
<tr>
<td><em>T. aestivum</em></td>
<td>BAD</td>
<td>-</td>
<td>ssp. <em>spelta</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ssp. <em>macha</em></td>
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<td></td>
<td></td>
<td></td>
<td>ssp. <em>vavilovii</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ssp. <em>aestivum</em></td>
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<td></td>
<td></td>
<td></td>
<td>ssp. <em>compactum</em></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ssp. <em>sphaeroococcum</em></td>
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</tbody>
</table>

* Extinct, described by Kislev (1980).
Figure 2 | Phylogenetic trees showing a single origin for domesticated varieties of einkorn and barley. The trees are based on amplified-fragment length polymorphism (AFLP) data from a | 288 loci and 388 accessions for einkorn (Triticum monococcum) and b | 400 loci and 374 accessions for barley (Hordeum vulgare). KD, Karacadag region.
How did a species with a limited habitat become the largest grown crop worldwide – today 225 million hectares
Changes involved in the transition from wild into domesticated wheat

Wild form -------> Domesticated (hulled) -------> Domesticated (free-threshing)

<table>
<thead>
<tr>
<th>Loss of seed dispersal mechanism</th>
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<tbody>
<tr>
<td>Loss of grain protection (stiff glumes)</td>
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</tbody>
</table>
Changes involved in the transition from wild into domesticated wheat

Selection for a more compact spike (Q)

Selection for multiple seeds per spikelets

Fig. 1.—Diagrams of wheat heads of varying density. A, lax; B, dense; C, compact. (After Watkins (1939)).

Fig. 2.—Diagram of a wheat spikelet. A, glumes; B, rachilla; C, floret; D, lemma; E, palea; F, grain; G, awn.
Evolution of tetraploid *turgidum* wheat, genome 2n=4x

*T. dicocoides* (wild lines)
- Fragile, hulled
- 2 grains/spikelet

*T. dicoccum* (primitive varieties)
- Non-Fragile, hulled
- 2 grains/spikelet

*T. durum*
- landraces
  - Non-Fragile, Free threshing
  - > 2 grains/spikelet
- Modern varieties
  - Non-Fragile, Semi-dwarf
  - Free threshing
  - > 2 grains/spikelet
How did a species with a limited habitat become the largest grown crop worldwide – today 225 million hectares

The archaeological evidence:

Tracking the where and when of domestication through the analysis of botanical findings and diagnostic features such as:

- Non fragile rachis,
- non-hulled types,
- ancient DNA
Wild wheat together with early domestic types are found in Neolithic sites in the fertile crescent.
Spikelets from fragile versus non-brittle spikes

Glume forklets in archaeological remains

Fig. 7. Glume forklets, the diagnostic elements for the recognition of hulled wheats in archaeological remains. A—Einkorn. B—Emmer. Late Neolithic Goljamo Delcevo, Bulgaria. (Hopf 1975b.)
Rounded grains and rachis segments are diagnostic of domestic wheat types.
Fig. 8. Rachis segments, the diagnostic elements for the recognition of free-threshing wheats in archaeological remains. Upper row: rachis fragments separated from among threshed grains of modern bread wheat (left) and durum wheat (right). Lower row: Carbonized rachis fragments of free-threshing wheats. Neolithic Tell Ramad, Syria. (van Zeist 1976.)
Barley spikelets from Netiv Hagdud (early neolithic site), characteristic of wild (left = smooth abscission scar) or domesticated (right = roughly broken spikelet segment)

Barley spikelet fragments from Netiv Hagdud. The specimen on the left, characteristic of wild barley, shows a smooth, elliptical abscission scar at the connecting node, where the next spikelet up the ear has cleanly broken away. The “domesticated node” specimen on the right, in contrast, shows a small fragment of the lower part of the next spikelet up the ear still attached at the connecting node.
Modern examples of dehiscent wild (A, B, C) and domestic non-dehiscent einkorn wheat (D, E)

Archaeological specimens of wheat and Barley sorted as wild/domestic/intermediate in sites from early and late Neolithic

K Tanno, and G Willcox Science 2006;311:1886-1886
Chronology for the late Epipalaeolithic and the Neolithic periods in the Levant, the western flank of the Fertile Crescent

<table>
<thead>
<tr>
<th>Date (BP)</th>
<th>Period</th>
<th>Major events in wheat cultivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>15,000 - 11,500</td>
<td>Late Epipalaeolithic (Natufian)</td>
<td>Harvesting from wild emmer and einkorn stands - agrotechnical development</td>
</tr>
<tr>
<td>11,500 - 10,500</td>
<td>Prepottery Neolithic A (PPNA)</td>
<td>Cultivation of brittle forms of emmer and einkorn - the first phase of cultivation</td>
</tr>
<tr>
<td>10,500 - 8,500</td>
<td>Prepottery Neolithic B (PPNB)</td>
<td>Appearance of non-brittle emmer and einkorn, naked tetraploid wheat, and naked hexaploid wheat - the second phase of cultivation</td>
</tr>
<tr>
<td>8,500 - 6,700</td>
<td>Pottery Neolithic</td>
<td>Spread of wheat culture to central Asia, southern Europe and Egypt - expansion of agriculture</td>
</tr>
</tbody>
</table>

Moshe Feldman (Origin of cultivated wheats, 2001)
Archaeological dating of wheat domestication

- Pottery Neolithic
  - 8,000 Cal BP
  - 7,800 Cal BP
  - Pre-pottery Neolithic B
  - Pre-pottery Neolithic A
  - Epi-Palaeolithic
    - Younger Dryas (cold climate)
  - Early farming sites in South-eastern Turkey
  - Abundant wild cereals
  - Naked seeds of wheat at Abu Hureyra
  - Remains of domesticated founder crops all over the Fertile Crescent
  - Naked seeds of wheat in Damascus basin sites (T. parviococum)
  - Abu Hureyra II (domesticated rye, emmer, einkorn, barley)
  - Intermediate
  - Abu Hureyra I (abundant wild cereals)
  - Wild rye cultivated
  - Abu Hureyra founded
  - Wild barley seeds at O halab II
Speed of expansion of wheat culture: ~ 1 km/year
Genetics provides unbiased but indirect evidence of location of domestication (could be affected by gene flow and genetic drift), can also provide some dating.

Archeology provides direct evidence, but sometime ambiguous interpretation (fragility is not a perfect criterion).

Ancient DNA evidence: could link between genetics and archeology and solve ambiguous cases, e.g. looking at sequence of fragility gene.

The problem: Most conserved seeds are charred and not suitable for DNA extraction.
Phytoliths are silica deposits in plant tissues. They are abundant in archeological sites (in ashes, sediments etc..) Their shape is typical of certain species/tissues.
Phytoliths were isolated from modern fresh, modern dry, and ancient sediments

New methods were devised to dissolve silica in conditions that do not damage DNA

New methods to isolate organic materials from silicified phytoliths reveal fragmented glycoproteins but no DNA

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\textsuperscript{c}Department of Anthropology, Harvard University, 11 Divinity Avenue, Cambridge, MA 02138, USA
Conclusions part 1:

- Einkorn wheat: Genetic and Archaeological evidence support domestication in the Karacadag region at the early Neolithic.

- Barley: Genetic and Archaeological evidence support domestication in the Jordan Valley region at the early Neolithic.

- Emmer wheat: Genetic and Archaeological evidence are not yet conclusive – must have happened somewhere in the levantine corridor, sometime between PPNA and PPNB (11-10,000 yrs BP).

- Domestication was a gradual process over extended periods of mixed culture of wild and domestic types or ? The first domestic types had a phenotype that resembles the wild wheats (partially fragile) ?

- Ancient DNA could solve some ambiguities but is limited by the quality and quantity of the samples.
Possible reasons for the Agricultural Revolution

• Population pressure and growth of large communities

• Reduction in food sources: because of climatic changes (the Younger Dryas)

• Spread of the technological breakthrough
I. During the transition from wild to cultivated

1. Non-brittle spike
2. Free-threshing spike (naked grains)
3. Non-dormant seeds
4. Uniform and rapid germination
5. Erect plants
6. Increased grain size
7. More spikelets per spike (?)
Modifications that occurred during the three phases of wheat cultivation

II. During 10,000 years of cultivation in polymorphic fields

1. Adaptation to new, sometimes extreme, regional environments
2. Increased plant height
3. Development of canopy with wide horizontal leaves
4. Increased competitiveness with other wheat genotypes and weeds
5. Modifications in processes that control phenology
6. Increased grain number per spikelet
7. Improved seed retention (non-shattering)
8. Improved technological properties of grains
III. During cultivation in monomorphic fields due to modern breeding procedures in the last century

1. Increased yield in densely planted fields; reduced intragenotypic competition
2. Canopy with erect leaves
3. Reduced height
4. Enhanced response to fertilizers and agrochemicals
5. Increased resistance to grain shattering
6. Increased resistance to diseases and pests
7. Lodging resistance
8. Improved harvest index
9. Improved baking and bread-making quality
Wild emmer wheat: 
*Triticum dicoccoides*

Domesticated wheat: 
*T. turgidum var durum*

What **genes** and what **metabolites** were affected in the process of domestication?

~11,000 years of domestication

Fragile rachis

Non-fragile rachis
Differential expression of genes and Copy number variation during tetraploid wheat evolution

We used a microarray with 160,000 probes consisting of ~60mer oligos designed for ~40,000 unigenes and 400 transposons
Transcripts that were up-regulated in green tissues of young plants of domesticated wheat

Putative Annotation

- Caffeic acid O-methyltransferase
- Phenylalanine ammonia lyase
- Peroxidase
- Putative bifunctional nuclease
- Ribulose 1,5-bisphosphate carboxylase
- RuBisCO chain precursor
- RuBisCO large subunit
- RuBisCO small subunit
- Sucrose synthase

Lignin biosynthesis (strengthened straw)

Carbon metabolism

RT-PCR validation
Leaves dicoccoides = LD
Leaves durum = LR

(Sharon Ayal, PhD)
The plant cell wall is built of Cellulose, hemicellulose and lignin—The most abundant polymers on the planet

Cellulose and hemicellulose are sources of sugar for fermentation.
Wheat straw as feedstock for biofuel

- Abundant ~ 700,000,000 t/year
- Cheap
- Does not compete with food
- Poor digestibility due to high lignin
Straw digestibility without pretreatment among 48 wheat lines

Reducing sugars concentration [mM] after enzymatic hydrolysis of Wheat Straw Samples for 24 h

Enzyme Loading: 30 FPU/g + BGlu-188
Biomass Loading: 50 g/L

Designer Energy

5 fold differences in digestibility—Lignin is lower in digestible lines
Evolution of tetraploid *turgidum* wheat, genome 2n=4x

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(wild lines)

Fragile, hulled
2 grains/spikelet

**T. dicoccum**
(primitive varieties)

Non-Fragile, hulled
2 grains/spikelet

**T. durum**

landraces

Non-Fragile, hulled
2 grains/spikelet

Modern varieties

Non-Fragile, Semi-dwarf
Free threshing
> 2 grains/spikelet

> 2 grains/spikelet
Clustering genes by expression patterns throughout domestication

- Genes up-regulated after domestication but not related to high yield in modern wheat: X
- Genes down-regulated (or lost) after domestication: +
- Genes correlated with the degree of domestication (■)
- Genes not correlated with domestication but with breeding of modern lines: ▲
- Genes with no correlation to domestication: ●

Expression level

Domestication degree
Embryo Transcripts clusters

Cluster 1 (1144 probes)

Cluster 2 (1045 probes)

Cluster 3 (988 probes)

Endosperm Transcripts clusters

Cluster 1 (1806 probes)

Cluster 2 (1162 probes)

Cluster 5 (583 probes)

Cluster 6 (272 probes)

Cluster 9 (274 probes)

Cluster 10 (249 probes)

W=Wild
P=Primitive
L=Landrace
M=Modern
Transcripts Embryo Cluster 2-

Genes involved in germination? Dormancy?

Gene Ontology
- 20769/20772, 539/539
- GO:0005575 cellular_component
  - 16840/20772, 388/539

- molecular_function
  - 17573/20772, 465/539

- nutrient reservoir activity
  - 243/20772, 75/539

- enzyme regulator activity
  - 398/20772, 32/539

- extracellular region
  - 640/20772, 32/539

- virion
  - 30/20772, 8/539

- GO:000152 metabolic process
  - 10929/20772, 218/539

- GO:0009877 cellular process
  - 9350/20772, 205/539

- GO:00433470 macromolecular metabolic process
  - 5426/20772, 114/539

- GO:0044237 cellular metabolic process
  - 7411/20772, 149/539

- GO:0042445 hormone metabolic process
  - 1458/20772, 46/539

- GO:0009550 auxin metabolic process
  - 1446/20772, 46/539

alpha-amylase inhibitor activity
- 39/20772, 11/539

- enzyme inhibitor activity
  - 270/20772, 25/539

- process
  - 39

- scale
Transcripts Endosperm Cluster 5

Genes for trafficking in the endosperm. Vesicles for protein bodies - Starch or lipids?
What are the changes in metabolite composition that occurred during wheat domestication (Do we eat the same wheat as our Neolithic ancestors?)

We analyzed secondary and primary metabolites in the endosperm and embryo of wheat grains.

Primary= carbohydrates, lipids, proteins

Secondary= alkaloids, phenols, terpenoids etc…
Materials & methods for analytical chemistry:

GC-MS

LC-MS-Q-Tof
PCA of wheat dry embryo analyzed with UPLC-QTOF-MS in negative mode.
LC-MS on dry endosperm

Metabolites changes during the domestication of wheat

- Conserved
- Decreased in domestication
- Increased in domestication
- Variable
Conclusions:

Domestication was associated with extensive changes in gene expression, copy number variation, and metabolite composition.

Transcriptome data indicates genes/pathways that were affected by domestication.

Genes involved in lignin biosynthesis were up-regulated during wheat evolution, suggesting that wild wheats may enable to improve straw composition for bioethanol.

Metabolic profiling shows distinct patterns for the different evolutionary stages of wheat.
Conclusion cont.

The knowledge gained from a study of wheat evolution can be used to continue and strengthen certain trends for yield increase (e.g. carbon fixation).

Conversely, we may want to reverse trends which caused the loss of desirable traits, e.g. specific metabolites, or digestible straw composition.

Human selected wheat for a few things they knew and for many things they did not know.
Thanks to:

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Rivka Elbaum (HUJI)
Ely Morag (Designer Energy)