



# **“Omics” in risk assessment**

## **Current Status & Highlights from WP1**





## Breeding and Production methods: Claims and Counterclaims

- Sustainability
- Productivity
- Cost
- Nutritional Value
- Safety
- Ethical





## Breeding and Production methods: Claims and Counterclaims

- Sustainability
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## July 2007 “ Organic food 'better' for heart “



Tomatoes contain compounds which are good for the heart. Organic fruit and vegetables may be better for you than conventionally grown crops, US research suggests.

UK Food Standards Agency

"Our long-standing advice on organic food is there can be some nutrient differences but it doesn't mean it's necessarily better for you."



**GMOs**



**EU Scientific Committee**



**European Food Safety Authority**



**Food Safety from Farm to Fork**



**INCREASED  
RISK  
ASSESSMENT**





## Breeding : Likelihood of unintended effects

National Academy of Sciences, 2004

- Selecting from homogeneous population
- Selection from heterogeneous population
- Crossing of existing approved varieties
- rDNA technology (Agrob.) using genes from closely related species
- Conventional pollen based crossing closely related species
- Conventional crossing distant species **and/or embryo rescue**
- Somatic hybridisation
- **Somaclonal variation**
- rDNA biolistic transfer genes from closely related species
- rDNA (Agrob.) transfer genes from distally related species
- rDNA biolistics gene transfer from distally related species
- Mutation breeding, chemical mutagenesis, ionising radiation

**low**

**high**





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**'European consumers have recently been through the mad cow disease crisis, The French AIDS-tainted blood crisis, the Dutch pig plague crisis, the Belgian chicken dioxin crisis, the Belgian Coca-Cola crisis, etc. Therefore, hearing from unsophisticated Americans that their fears are unfounded may not be the best way of proceeding.'**

***Deutsche Bank***





# A level playing field??

## GMOs

Are the most analysed food/feed products on the market

- Compositional analysis = cornerstone of risk assessment process

## Non GMOs – excluding novel foods

There are no legal requirements for other breeding approaches

- Nor for assessing the impact of cultivation
- Nor for assessing impact of environment
- This includes analysis of known toxins e.g. glycoalkaloids

But accidents have happened!!!

Psoralens in celery; glycoalkaloids in potato, E coli 0157 in spinach





# Introgression Breeding

## Exploiting natural diversity



## Chromosome segment moved from from *Solanum Pennettii* into *Solanum esculentum*

Schauer et al 2006

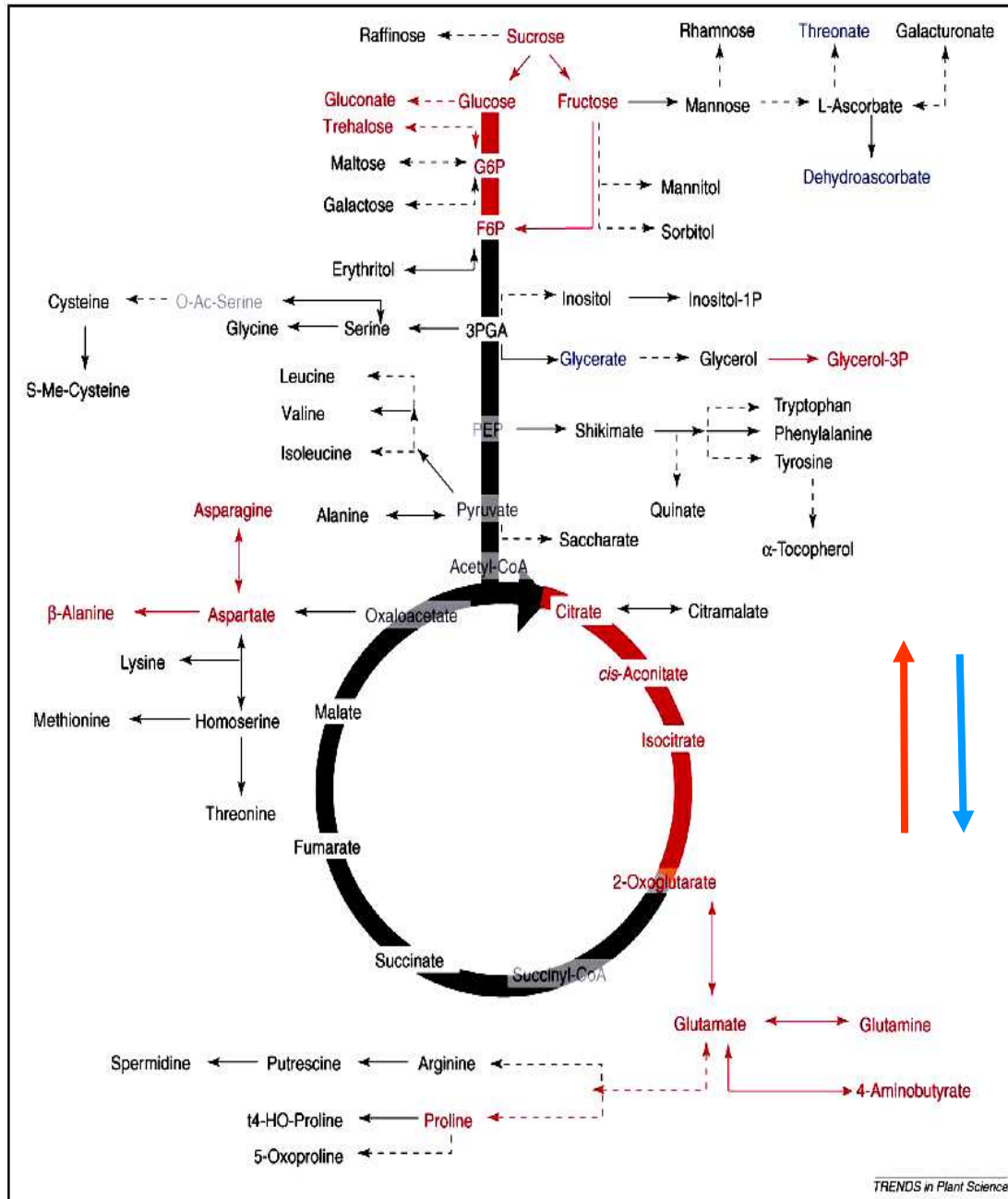


Figure 1. Metabolite profiling for quantitative trait loci (QTLs) determination – an example of a pathway QTL. The introgression of a chromosomal segment of *Solanum pennellii* into *Solanum lycopersicum* (segment 4); for details see Ref. [105]) resulted in reproducible changes in the contents of many metabolites (significant increases are



## International standards

Compositional variation in non-GMOs under a range conditions provides an important benchmark in the risk assessment process

Species- specific guidelines being draw up by OECD etc which deal with relevant nutrients and antinutrients/toxins

This represents a **targeted** approach





## Unintended effects and Uncertainty

- Complexities of processes regulating gene, protein expression and metabolic pathways
- Are we certain that uncertainty about GM safety that generate the metabolites we eat is the only uncertainty or is there as much uncertainty about other crop production systems?





# More is better

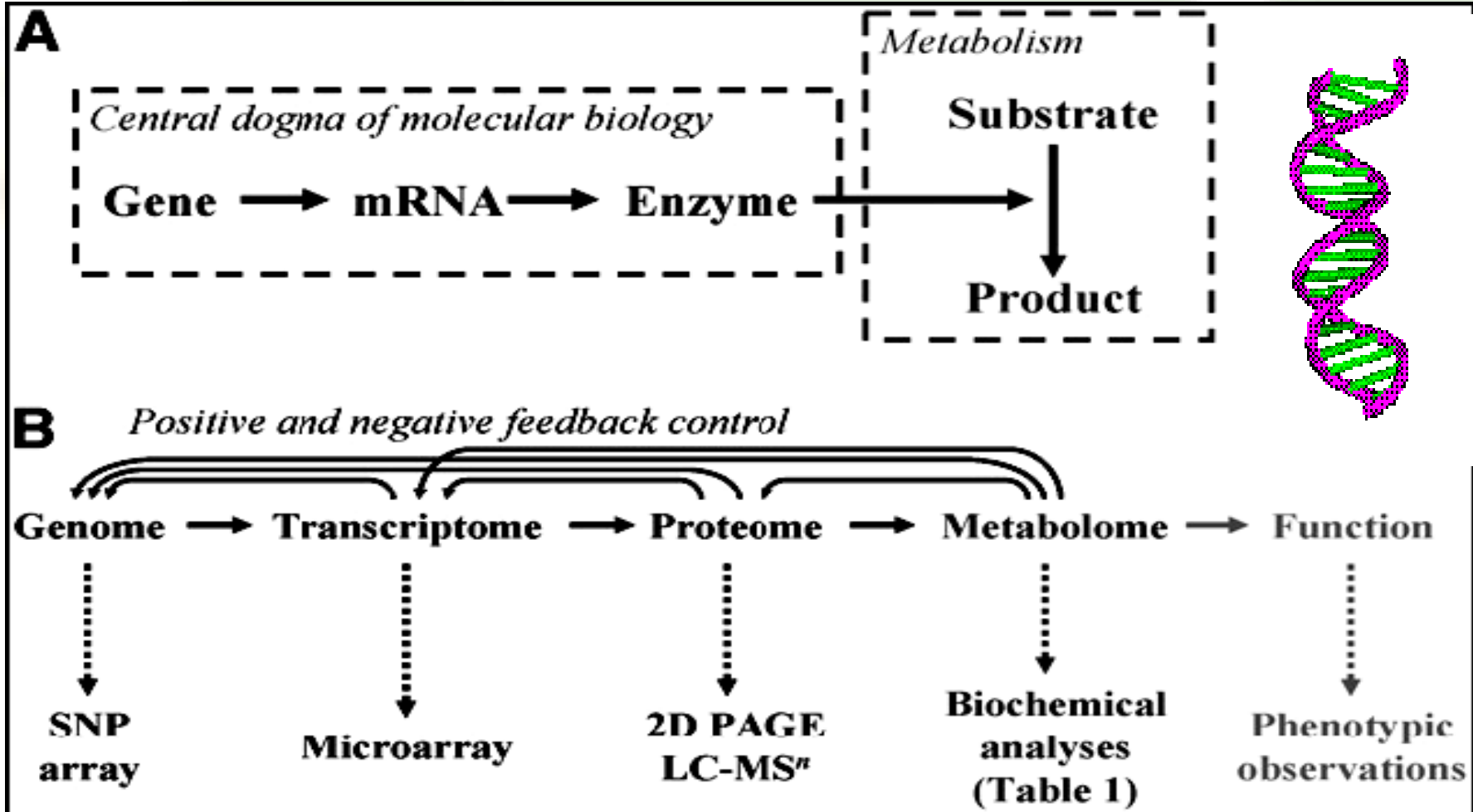
The more things we measure  
the less uncertain we become  
the more confused we get?



FOODS

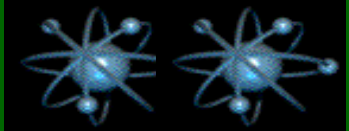


# Controlling composition and phenotype





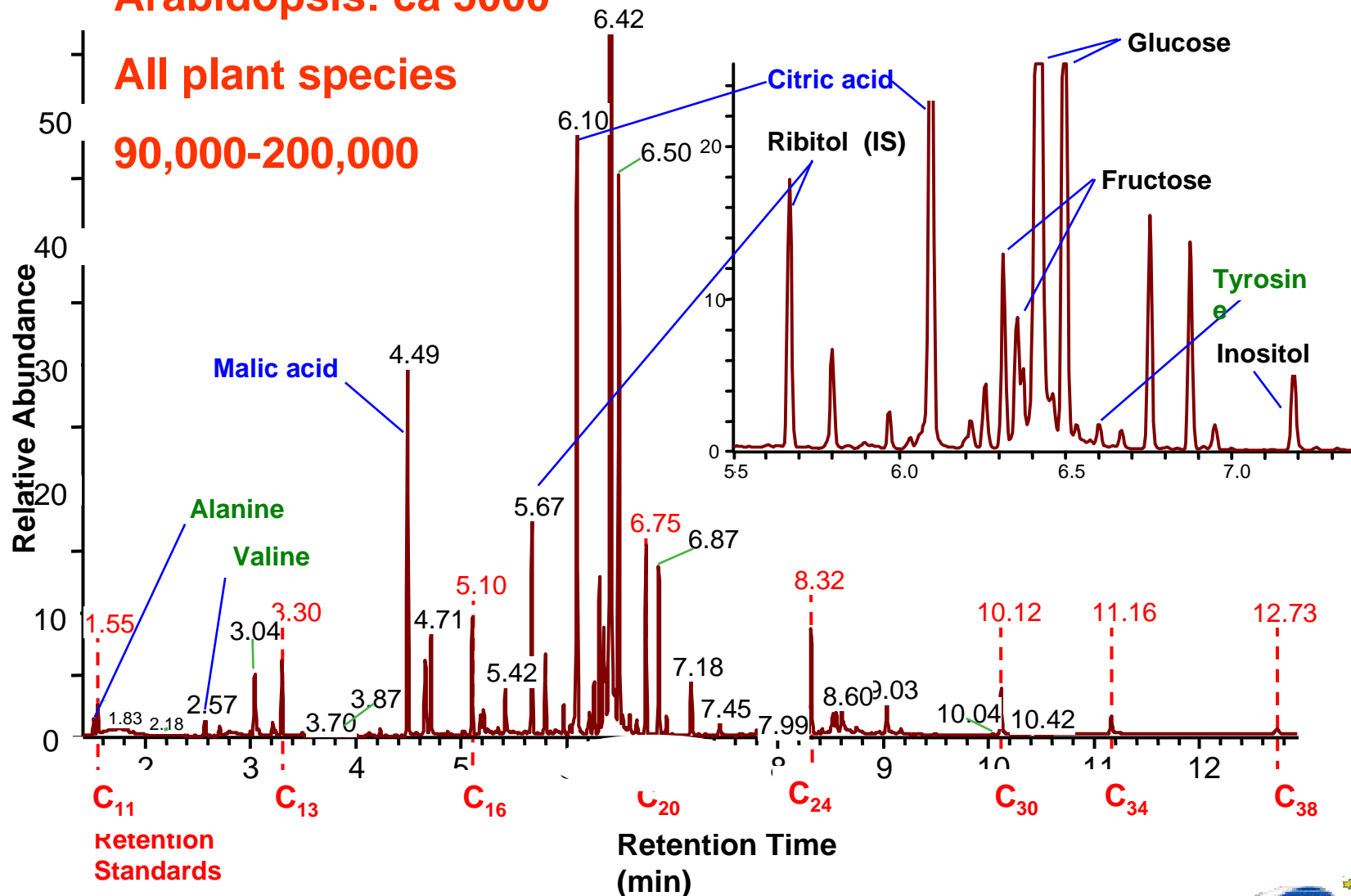
# What do the “Omics” look like?



Arabidopsis: ca 5000

All plant species

90,000-200,000

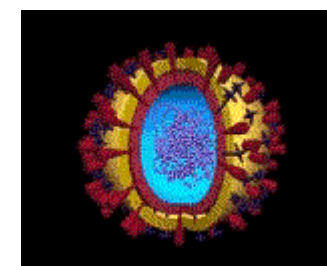
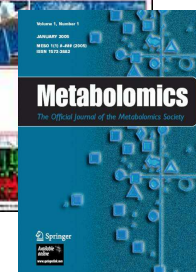
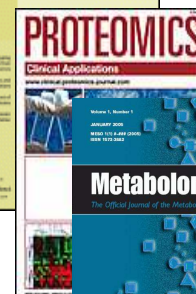
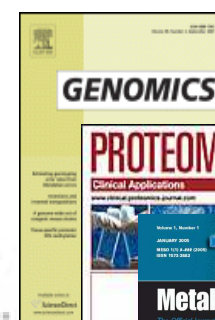
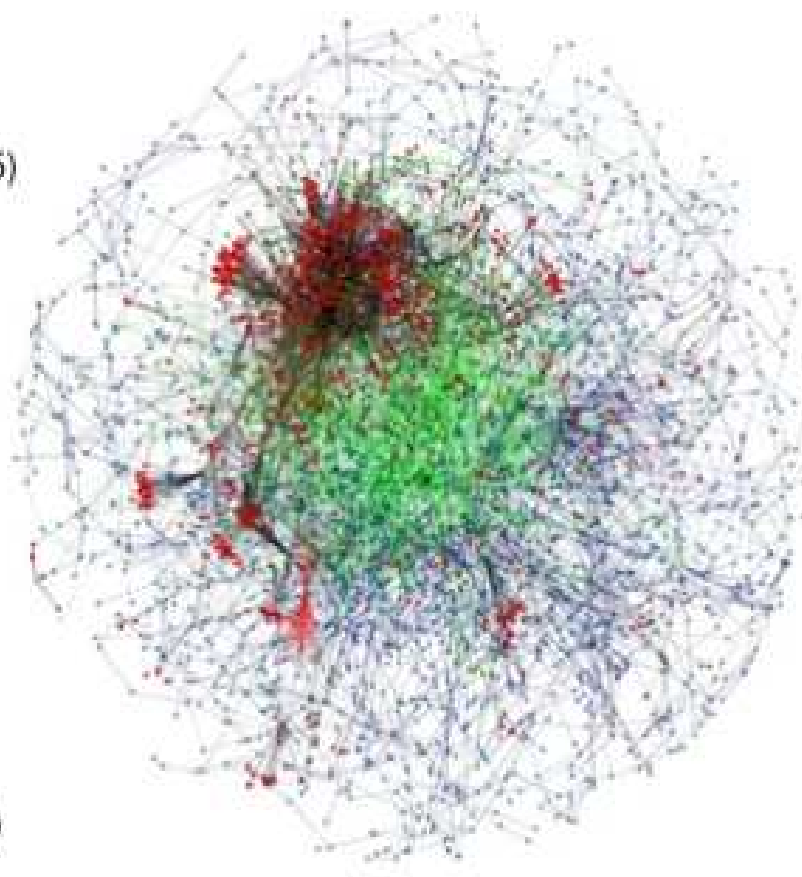




# More “Omes” and more to come

## An interactome of *E. coli*

- Gene (1279)
  - ▲ Protein (702)
  - Protein complex (335)
  - ◆ Metabolite (934)
  - sRNA (16)
- 
- Metabolic reactions (1120)
  - Transcriptional regulation (2724)
  - Protein-metabolite interaction (51)
  - Protein-protein interaction (670)
  - sRNA interaction (25)





## Building “Omics” into risk assessment

“Omics”: Large scale gene, protein, metabolite analysis



Assess potential to differentiate crop (PCA, clusters)



ANOVA:  
Significance values for individual genes, proteins, metabolites driving the changes.  
Magnitude of change



Combining datasets  
“True” extent of variation  
Size of clusters



Metadata: what's really underpinning the variation e.g. genotype, location.



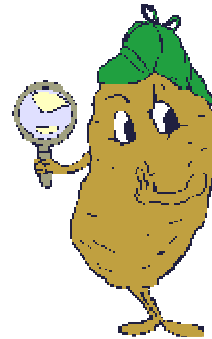
Identify genes proteins, metabolites



Biological and safety relevance



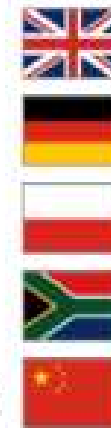
# WP1 – Two model crop species



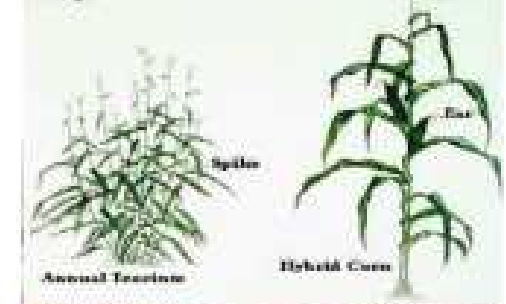
Potato



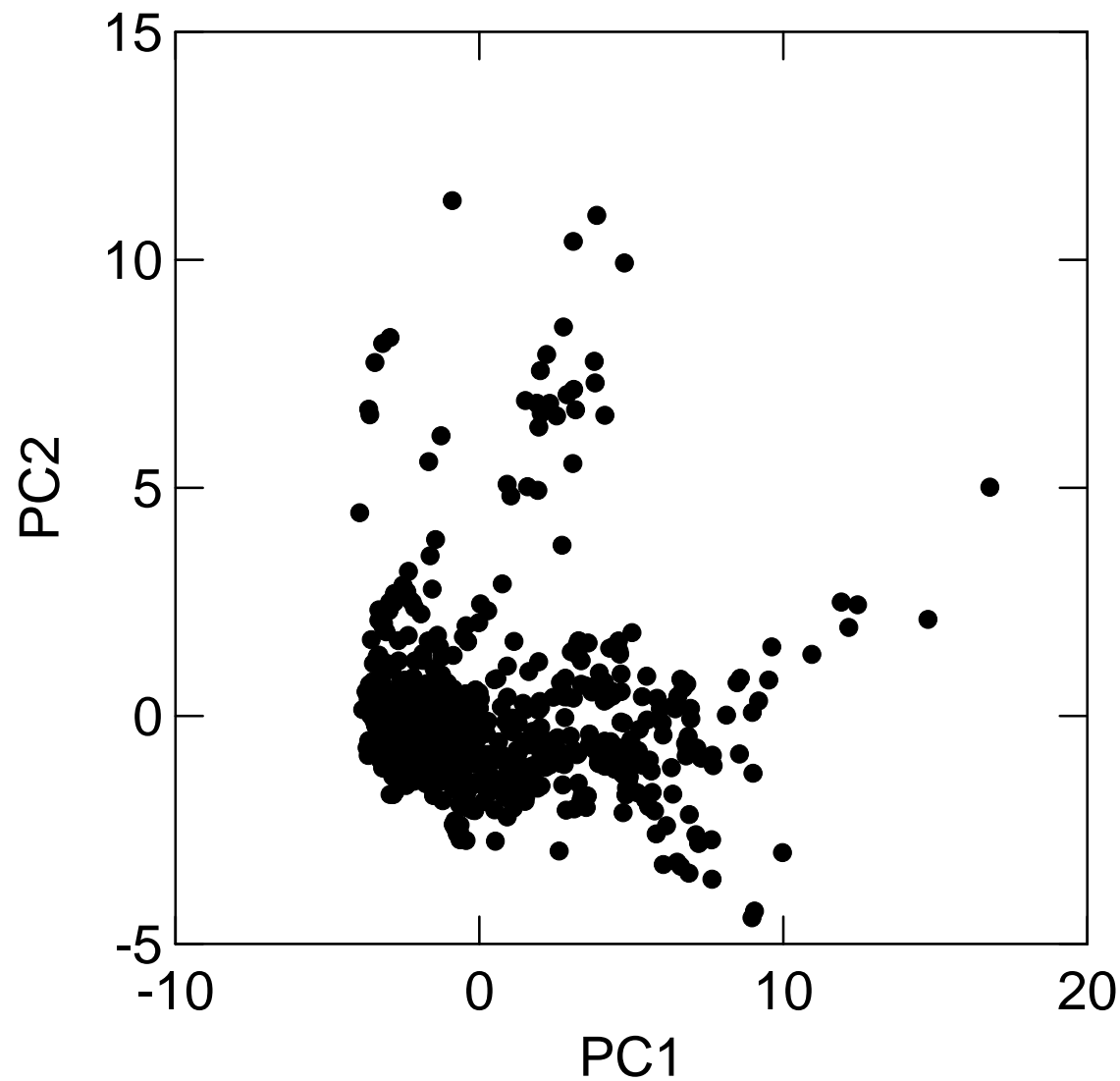
Maize



Modified from Scientific American library.

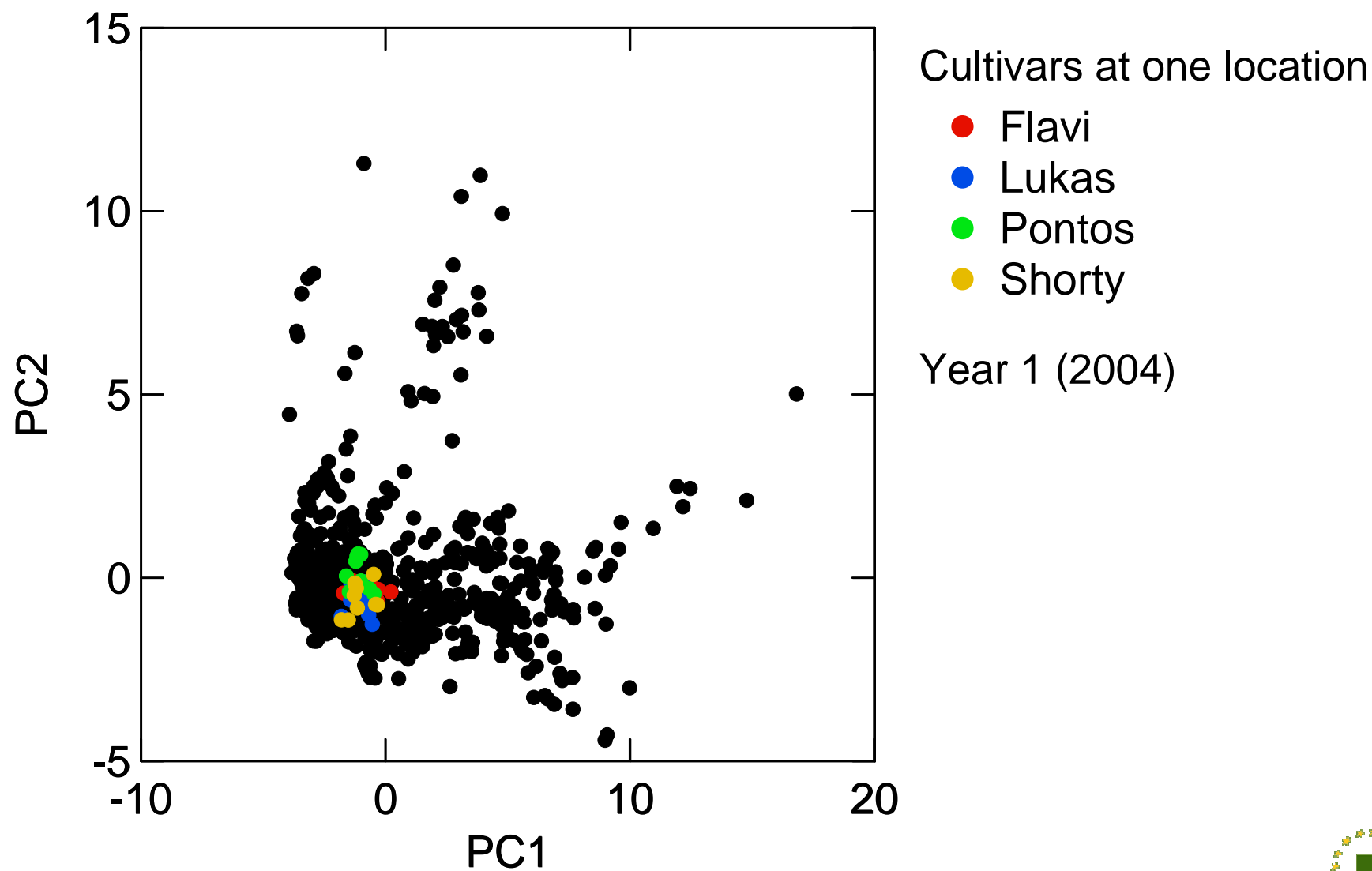


# PCA with pooled samples

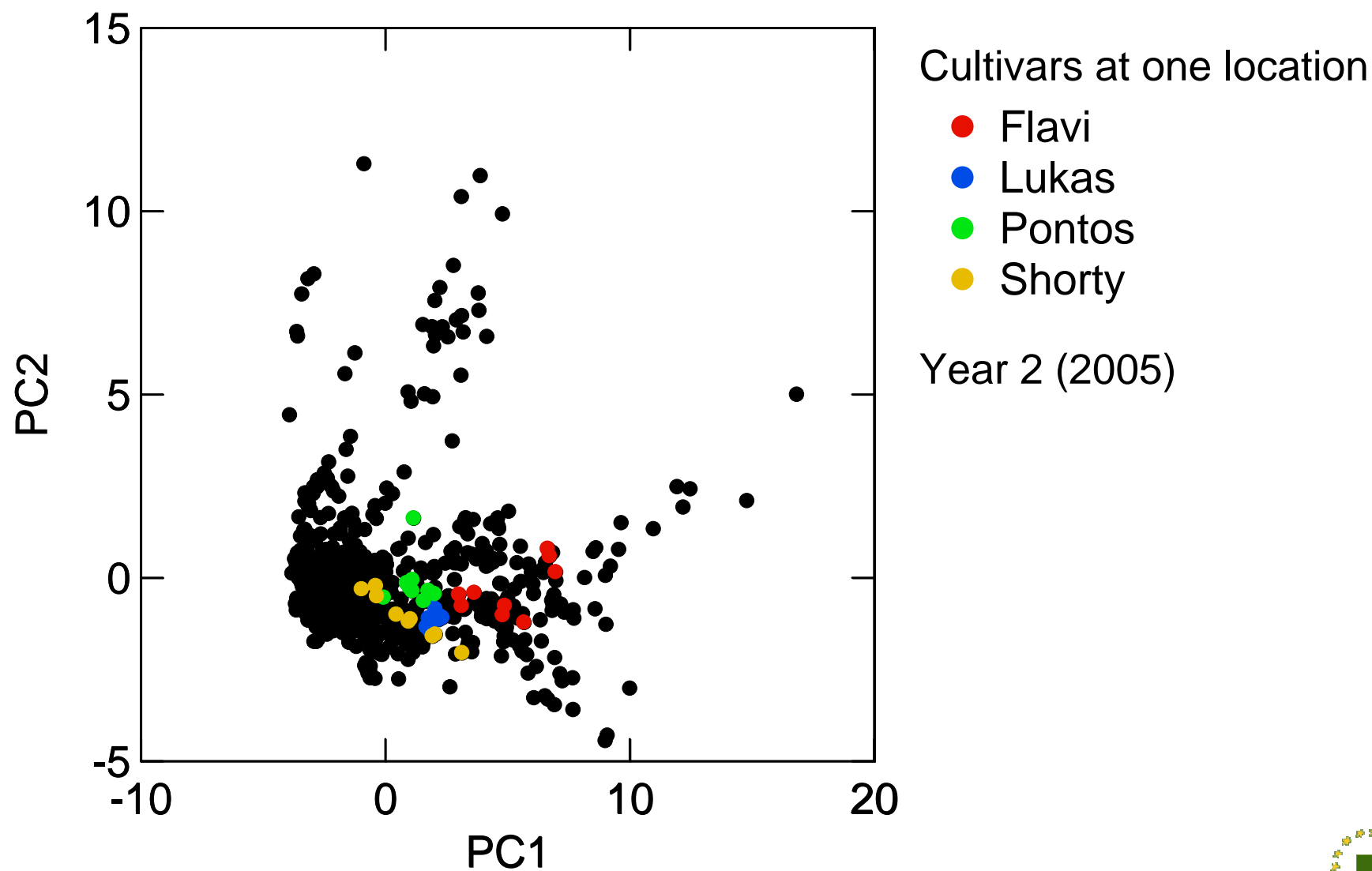


pooled samples from all  
experiments

# PCA with pooled samples

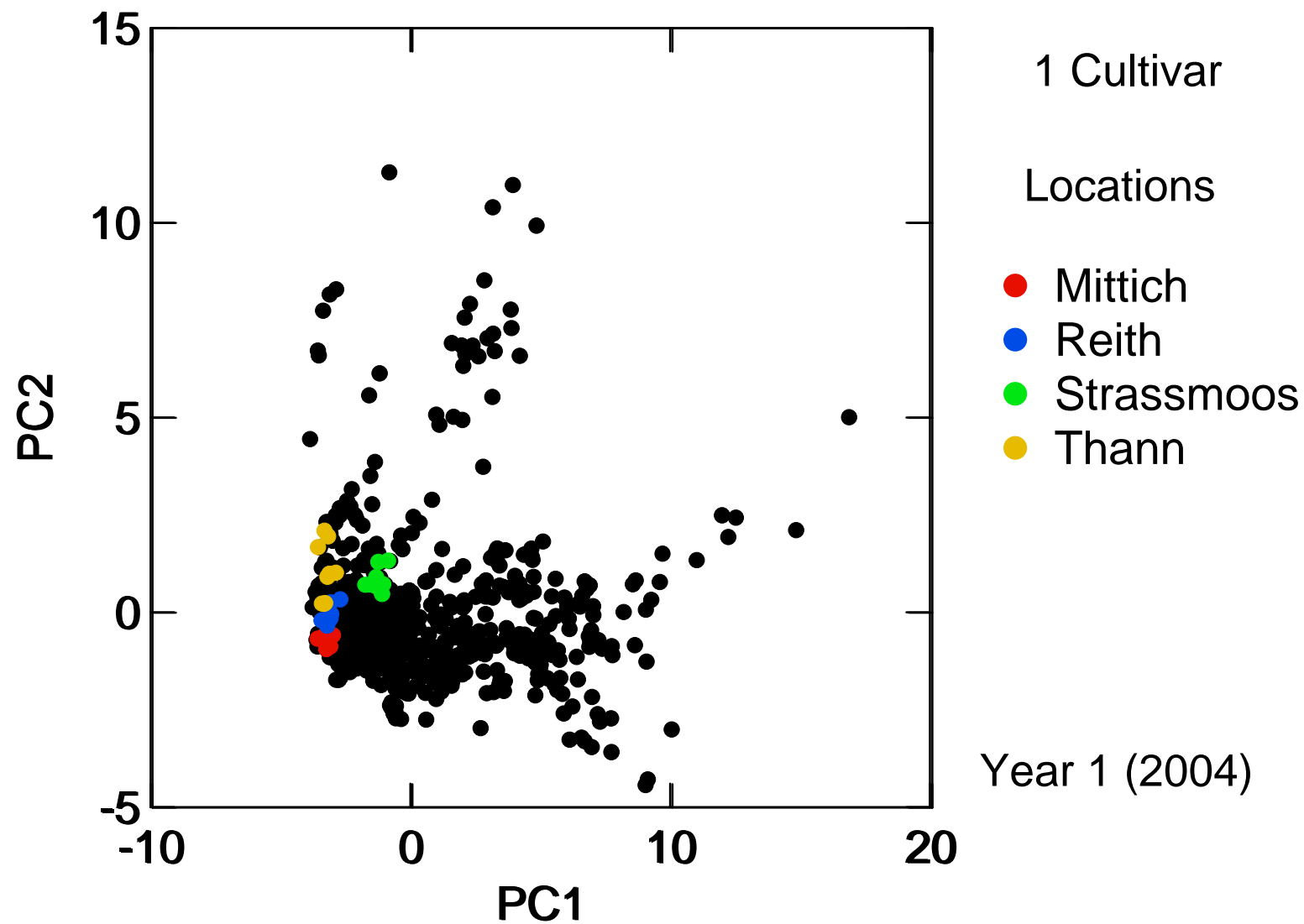


# PCA with pooled samples

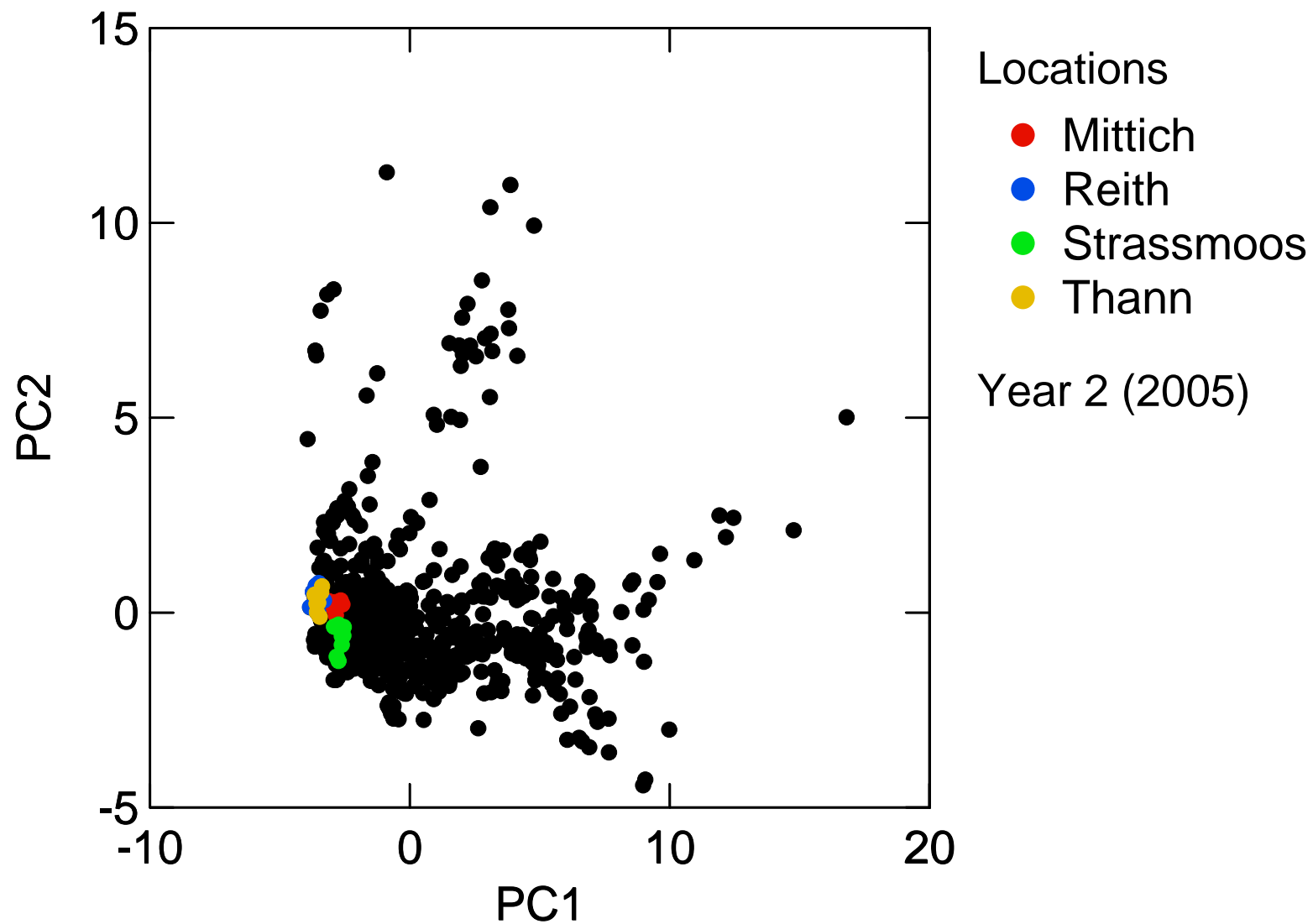




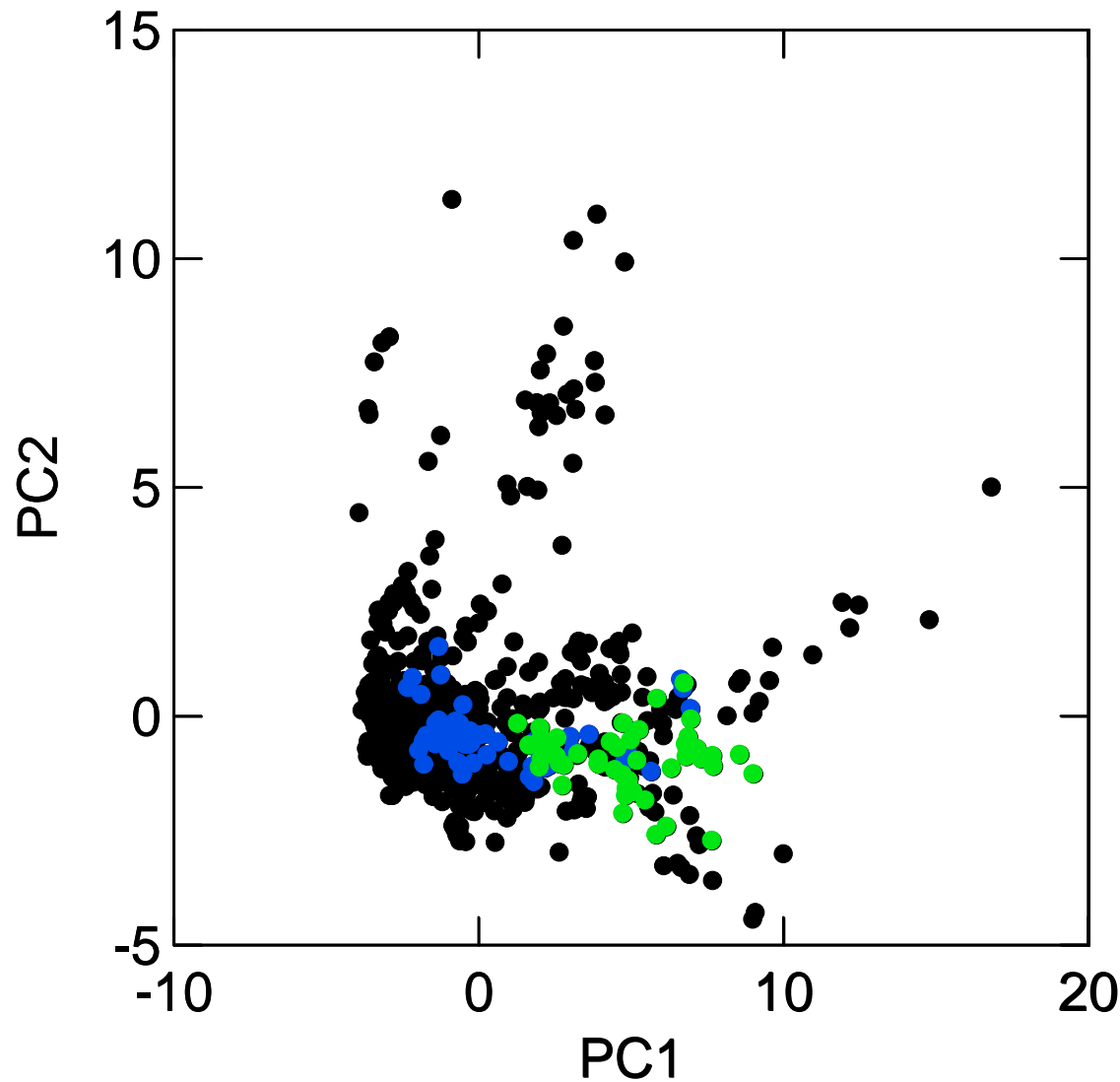
# PCA with pooled samples



# PCA with pooled samples

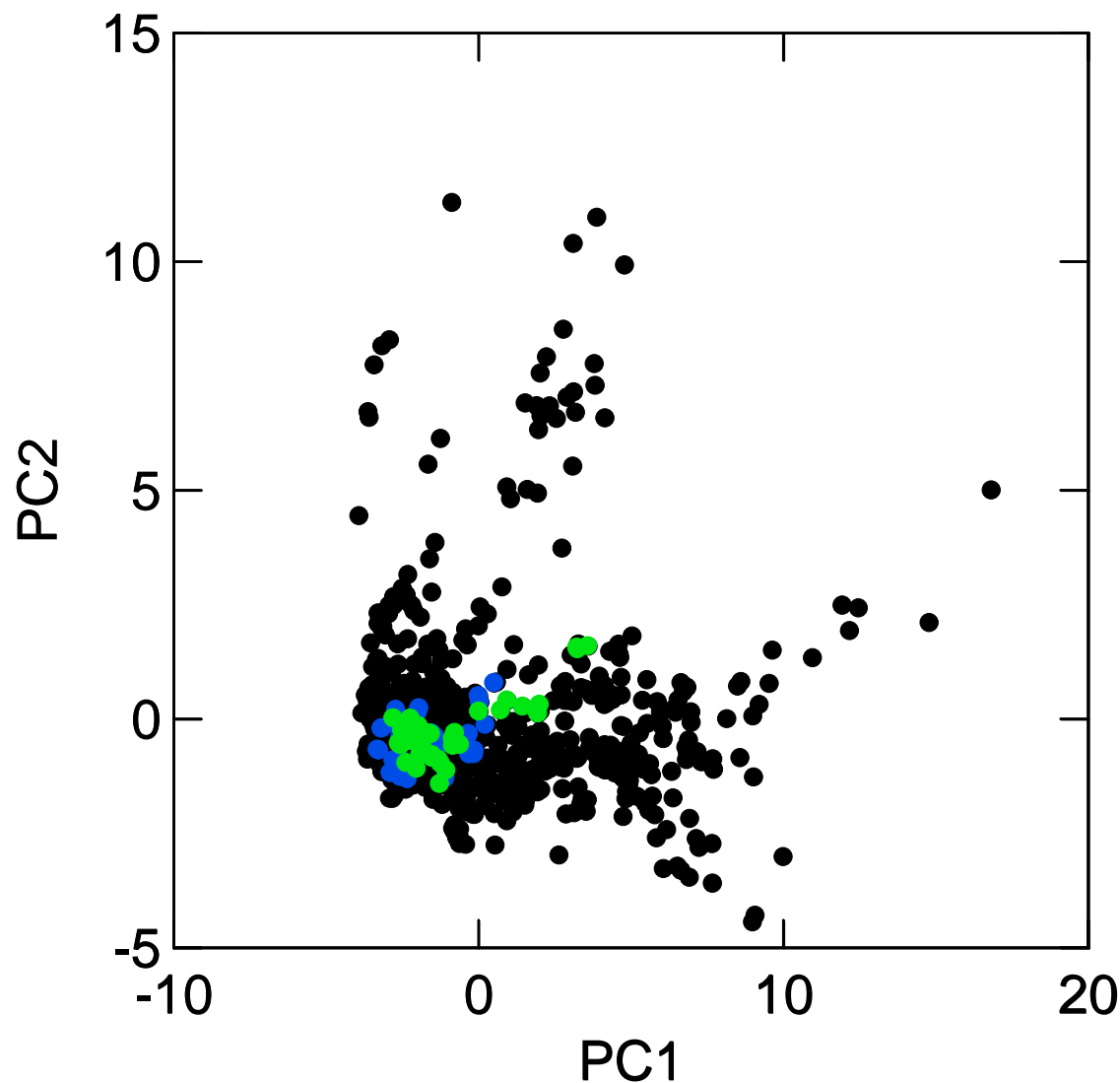


# PCA with pooled samples



- 2 locations with different input systems  
Schönbrunn (organic)  
Frankendorf (conventional)
- 2 years  
2004  
2005
- 3 cultivars  
Lukas  
Amadeo  
Flavi

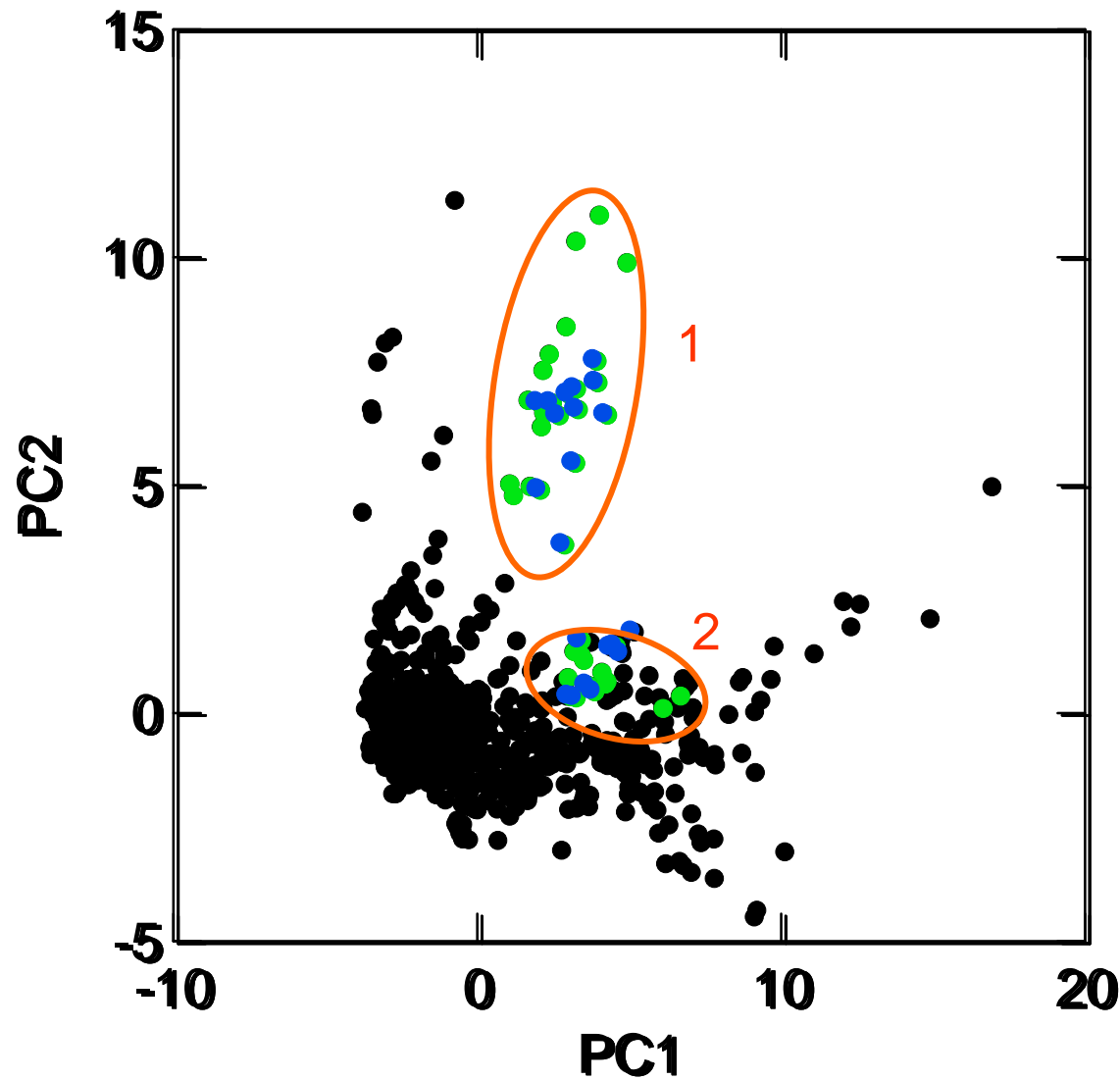
# PCA with pooled samples



- 1 location with different input systems  
Scheyern (organic)  
Scheyern (conventional)
- 1 year  
2006
- 4 cultivars  
Lukas  
Amadeo  
Flavi  
Gavott



# PCA with pooled samples

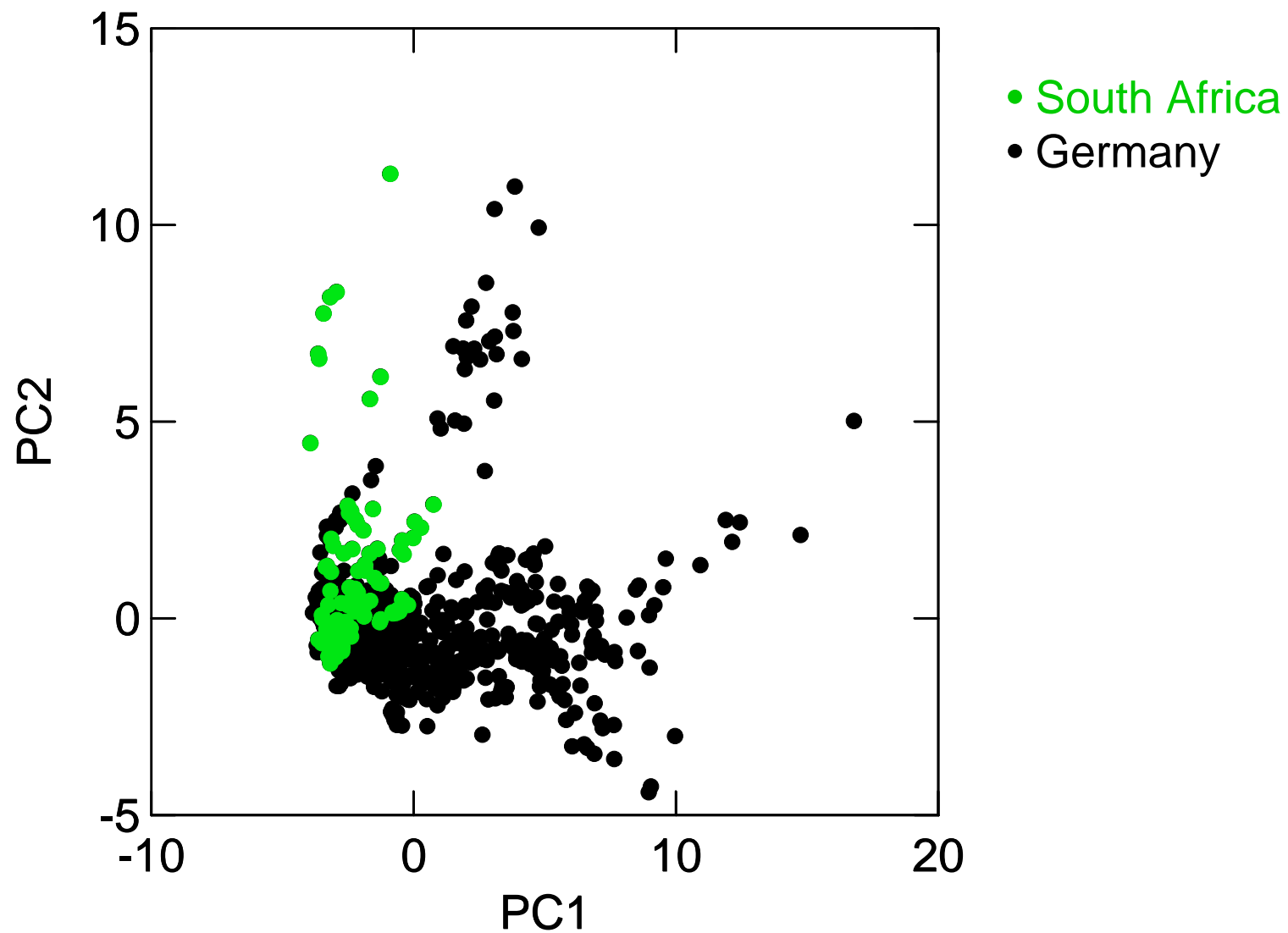


Locations

- 1 Neuhof
- 2 Pfaffenhofen

- isogenic maize (Cv. DKC3420)
- Bt-maize (Cv. TXP 138-F)

## PCA with pooled samples





# German and S. African Maize

## Proteomics

- Cultivars, harvest groups, locations and seasons can be separated
- The effect of cultivar seem to be stronger than that of the location
- Slight separation has observed between some GM and non-GM lines

# Observations

## Transcriptomics

- differences found for samples similar to other ~omics technologies
- largest variation due to cultivars, locations and years of harvest

# The Detective Work Begins

Beyond Clusters

Identifying the drivers of difference



# Further examples of complex dataset analysis

## Analysis of Variance (ANOVA)

To identify individual genes which are differentially expressed

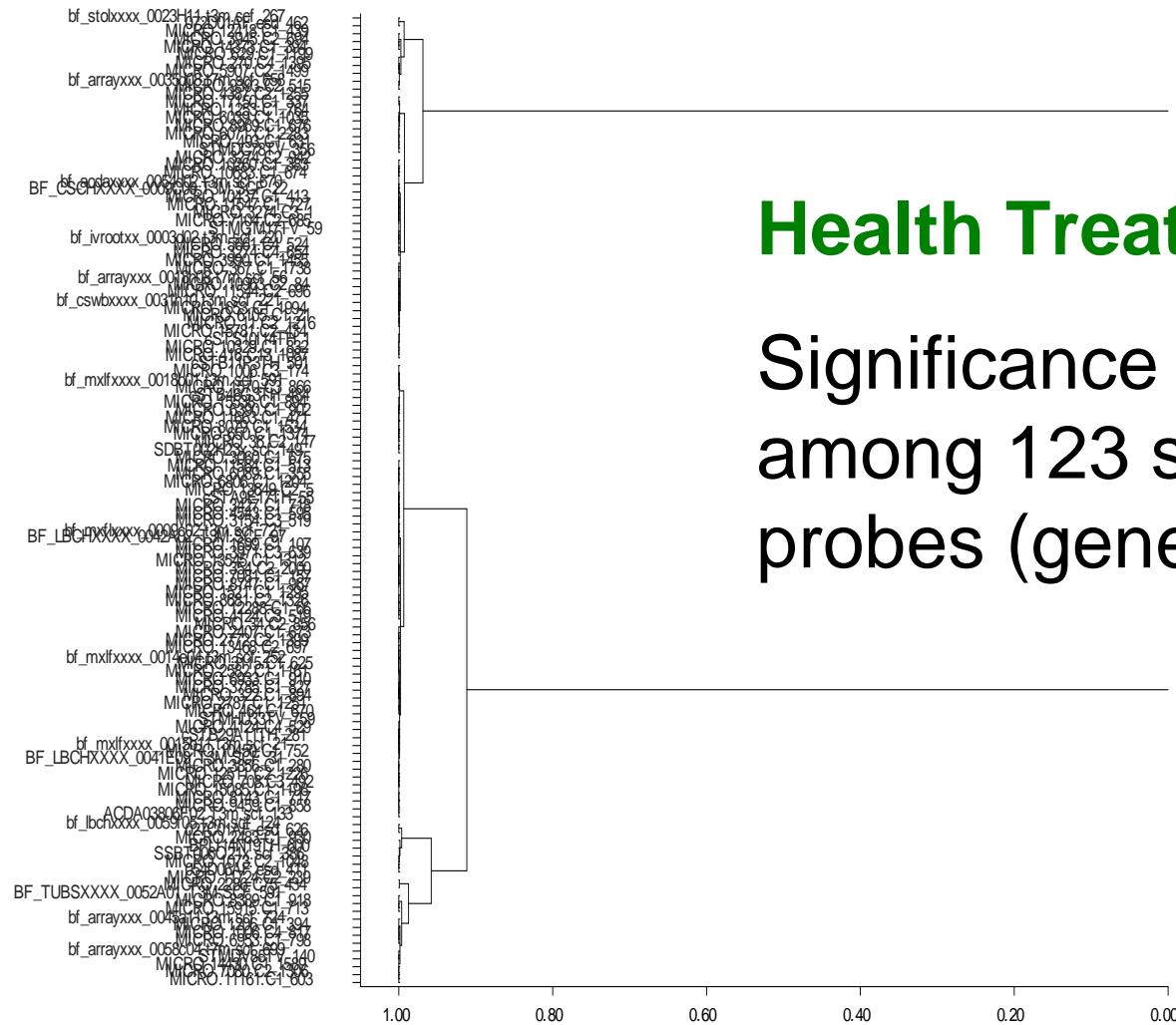
428148 genes measured

Reduced to 17500 after filtering out genes with

- missing values
- every treatment mean below 100



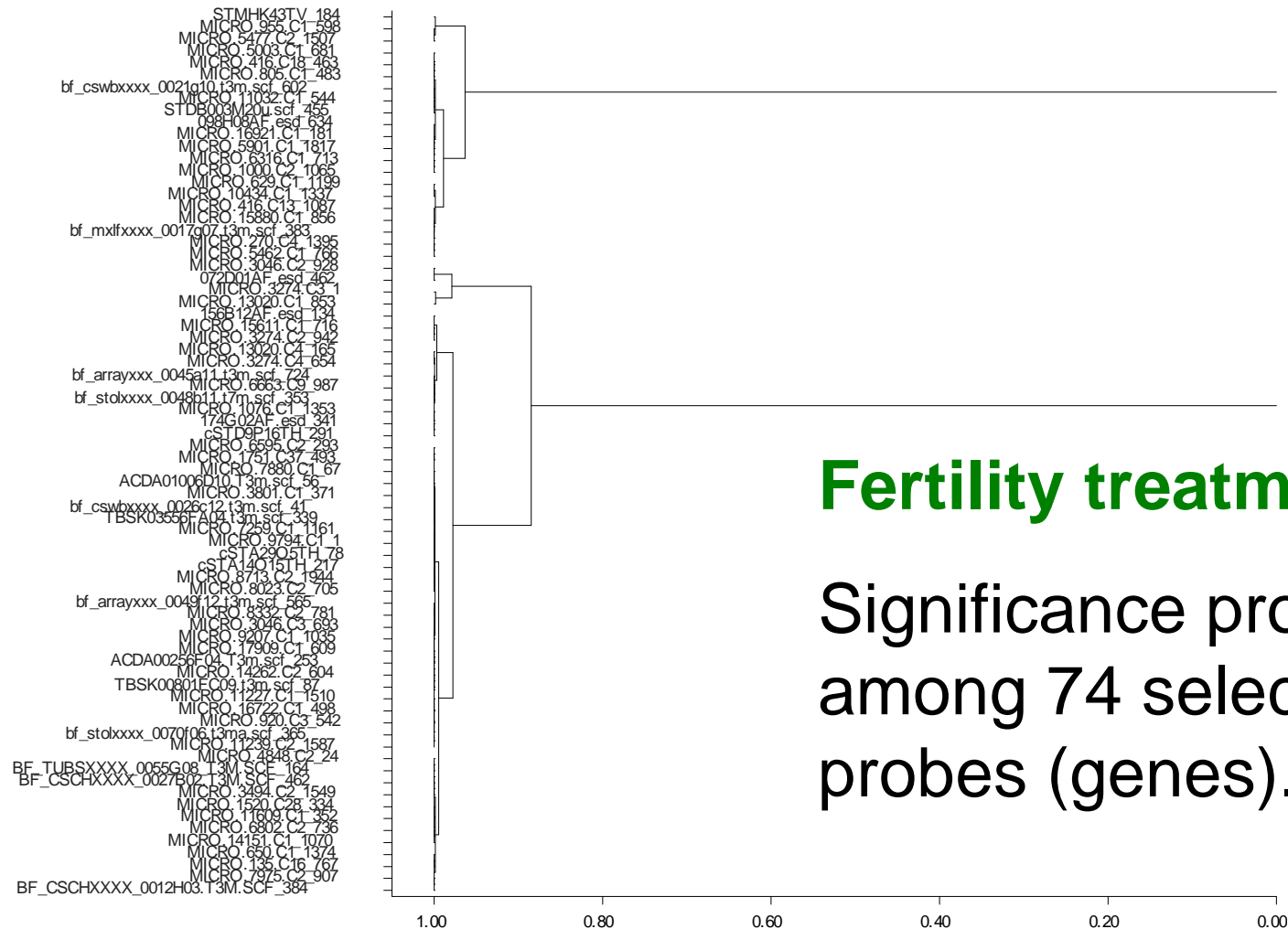
## Organic vs Conventional: 2005 Microarrays



## Health Treatment:

Significance profiles  
among 123 selected  
probes (genes).

# Organic vs Conventional: 2005 Microarrays

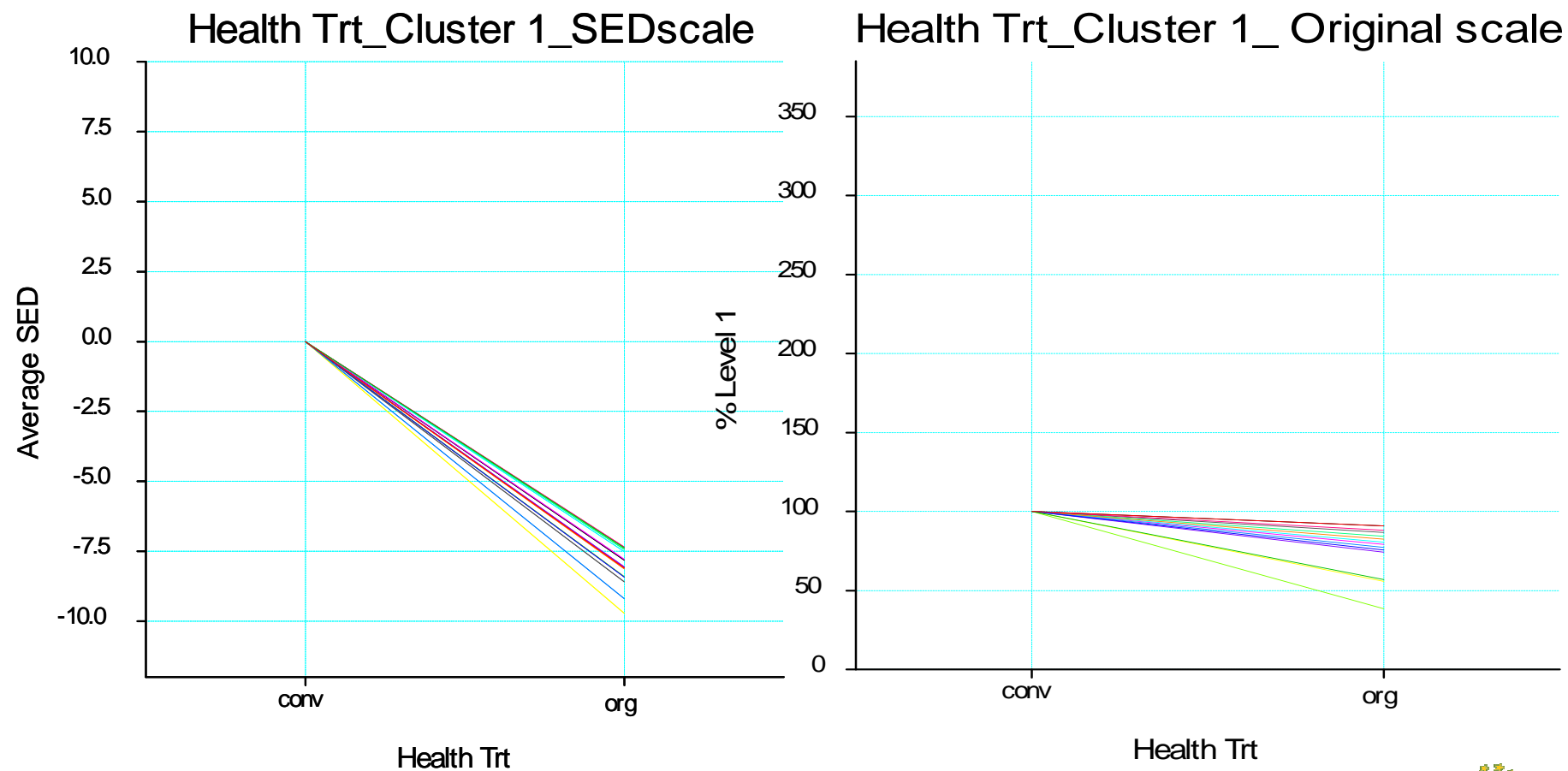


**Fertility treatment:**

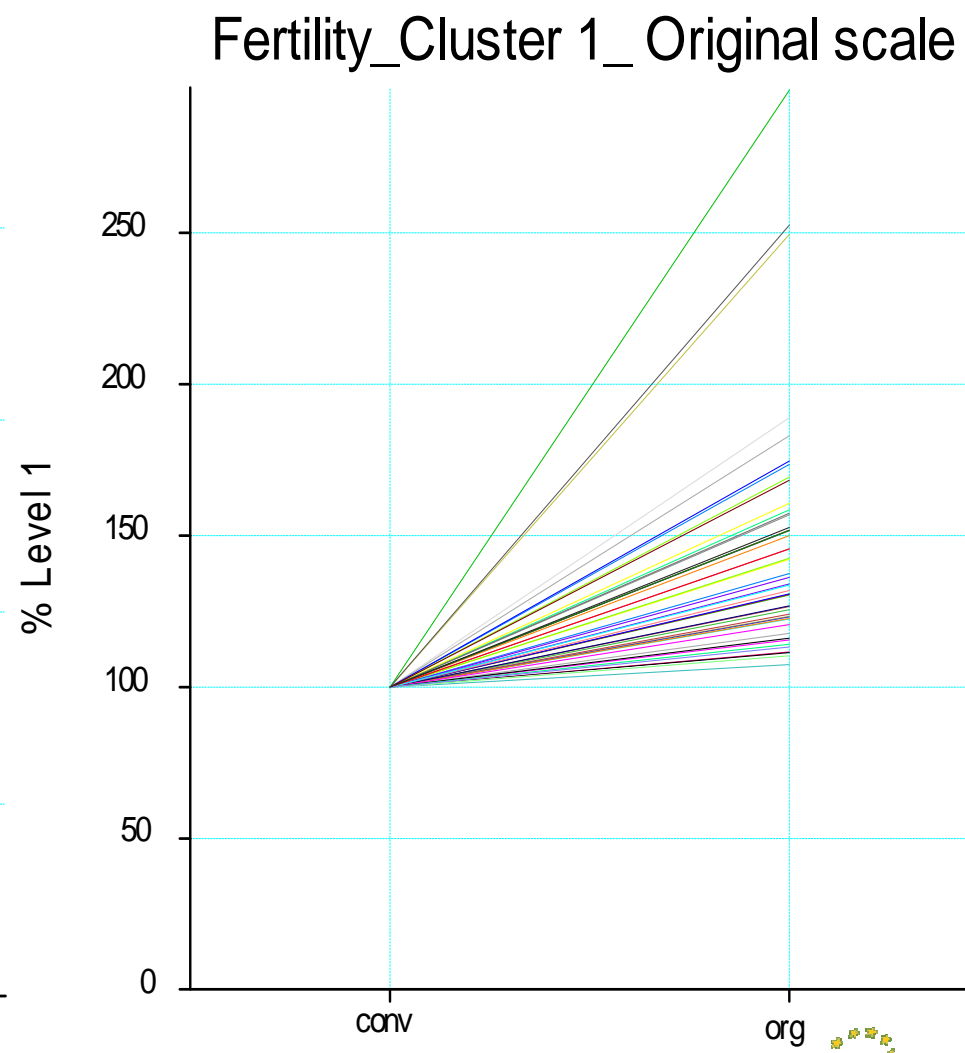
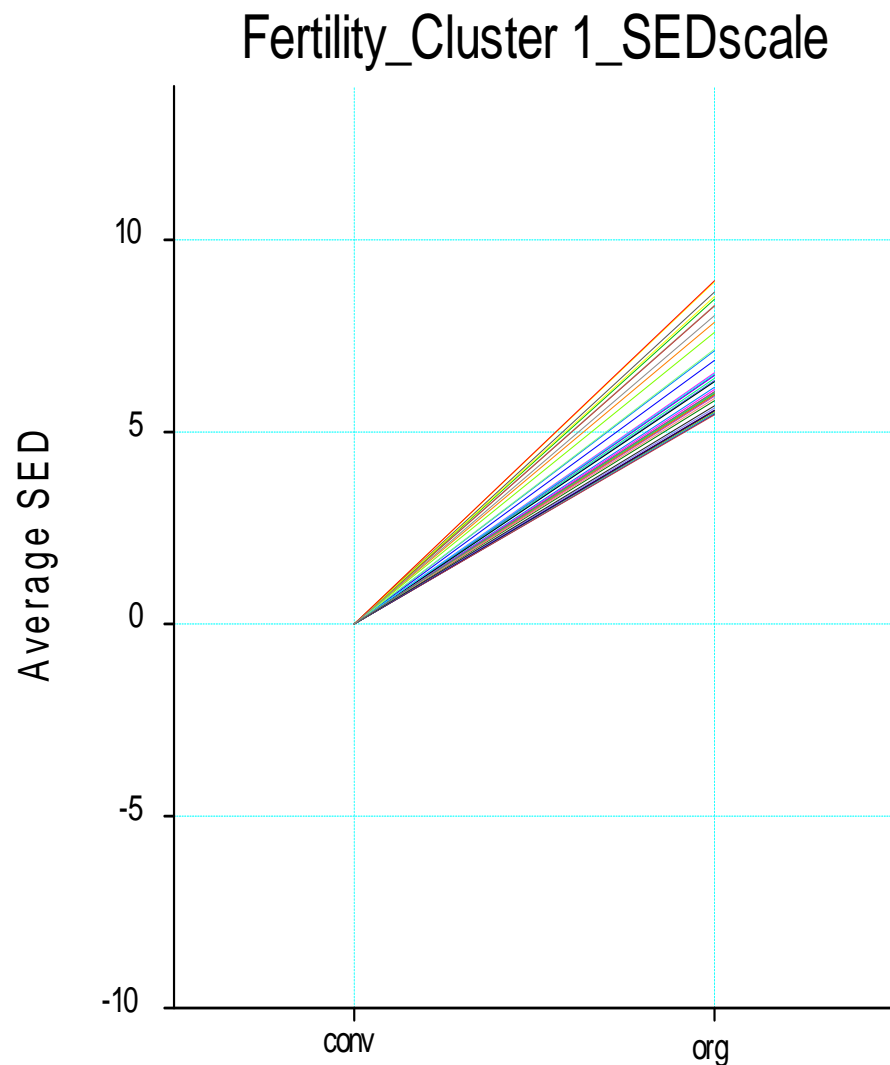
Significance profiles  
among 74 selected  
probes (genes).



# Organic vs Conventional: 2005 Microarrays



# Organic vs Conventional: 2005 Microarrays



Fertility

Fertility



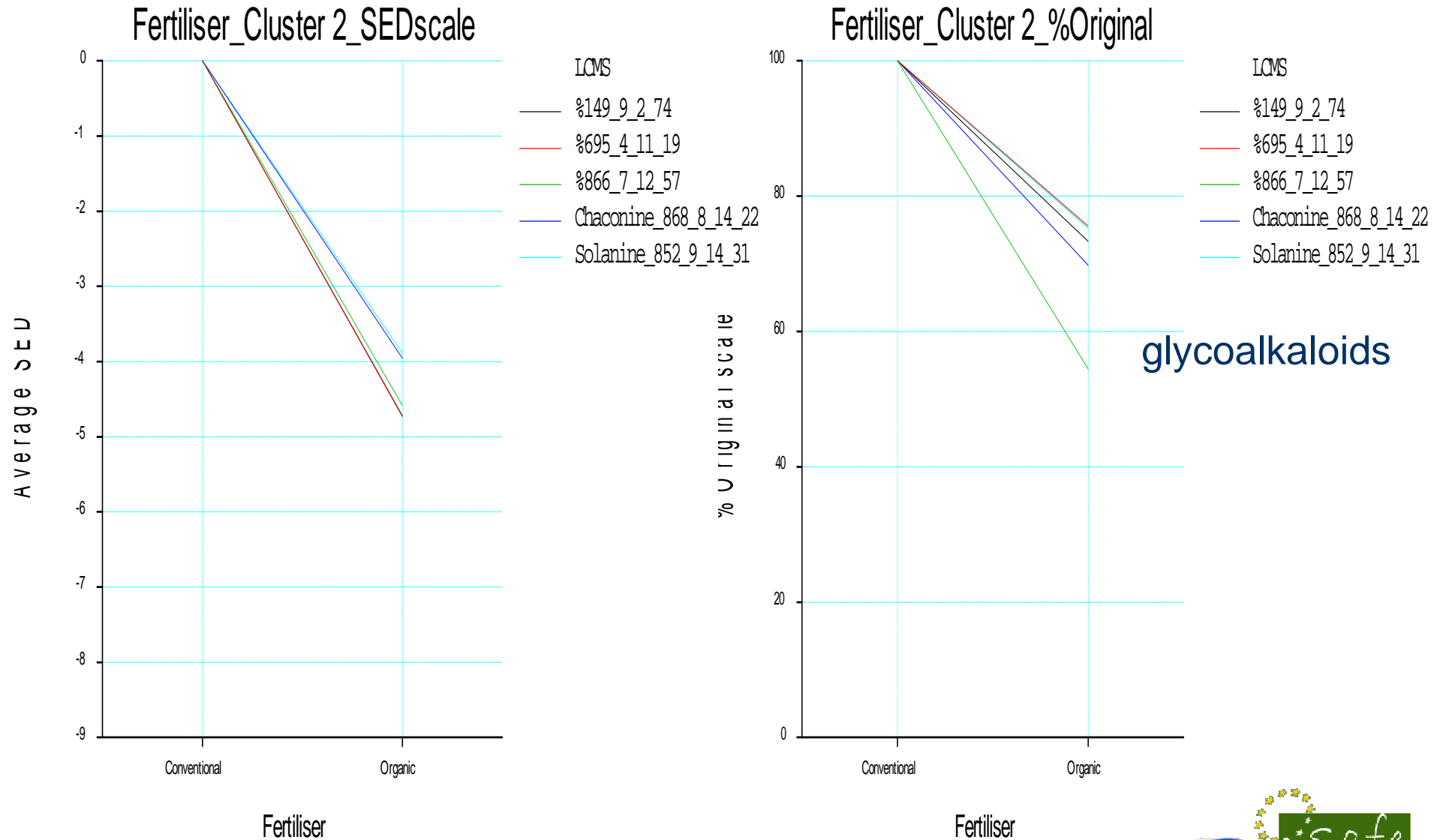
# Organic vs Conventional: 2005 Microarrays

## Fertility Treatment

Cluster	Probe	Annotation	SED	% Difference
1	MICRO.6802.C2_736	putative protein [Arabidopsis thaliana]	6.3	116
1	bf_cswbxxx_0026c12.t3m.scf_41	No Hits Found	5.6	131
1	174G02AF.esd_341	glucose-6-phosphate/phosphate translocator 2 [Solanum tuberosum]	8.5	297
1	MICRO.8713.C2_1944	nitrite transporter [Cucumis sativus]	5.5	131
1	BF_CSCHXXX_0027B02.T3M.SCF_462	putative protein [Arabidopsis thaliana]	6.5	134
1	MICRO.3494.C2_1549	12-oxophytodienoate reductase 3 [Lycopersicon esculentum]	6.5	115
1	cSTD9P16TH_291	No Hits Found	8.5	142
1	MICRO.15611.C1_716	unknown [Arabidopsis thaliana]	7.9	150
1	MICRO.7880.C1_67	No Hits Found	5.5	143
1	MICRO.1520.C28_334	No Hits Found	6.4	159
1	MICRO.3046.C3_693	unknown [Arabidopsis thaliana]	6.2	174
1	MICRO.11609.C1_352	UDP-glycosyltransferase 85A8 [Stevia rebaudiana]	6.3	136
1	MICRO.17909.C1_609	Unknown protein	6.0	112
1	MICRO.4848.C2_24	small blue copper protein Bcp1 [Boea crassifolia]	6.5	152
1	MICRO.6663.C9_987	5-lipoxygenase [Solanum tuberosum]	8.3	183
1	bf_arrayxxx_0045a11.t3m.scf_724	putative allantoinase [Solanum tuberosum]	8.3	127
1	bf_stolxxx_0048b11.t7m.scf_353	No Hits Found	8.3	249
1	cSTA14O15TH_217	putative ADP,ATP carrier [Arabidopsis thaliana]	5.5	125



# Organic vs Conventional: 2005 Metabolomics



# Reality Check

- Few complete crop genomes sequenced (but many partially and ongoing)
- Many gaps, unknown gene function in high % cases
- When/where to measure gene expression for safety
- Correlation between gene and protein expression poor
- Expensive for high throughput
- Proteome size and complexity an issue for complete coverage
- Crop databases poor, difficulties in annotation
- Can be high throughput
- Not high throughput
- Metabolome “pool” in a single species smaller than other “omes”
- Still limited metabolite coverage, need for database building
- Large datasets provide statistical challenge
- Combining inter lab databases to assess the extent of “true” variation (in support of risk assessment) is a major challenge

instrument bias, operator bias, instrument sensitivity  
common methodologies, sampling,  
SOPs



# Nevertheless.....

- Even now, valid and robust comparisons can be made in experiments with appropriate controls and comparators included
- There are many published examples of where the technologies are adding value to our knowledge of metabolism, crop composition and developmental processes
- Omics approaches would complement, on a case by case basis, targeted analysis and not replace them e.g. crops with modified metabolism to improve nutritional profile
- They could be usefully deployed in the breeding (experimental) process to pick up issues early. This would include screening of diverse, wild germplasm collections now used in breeding.

# The End

