Influence of plant size and population on reproduction in American ginseng

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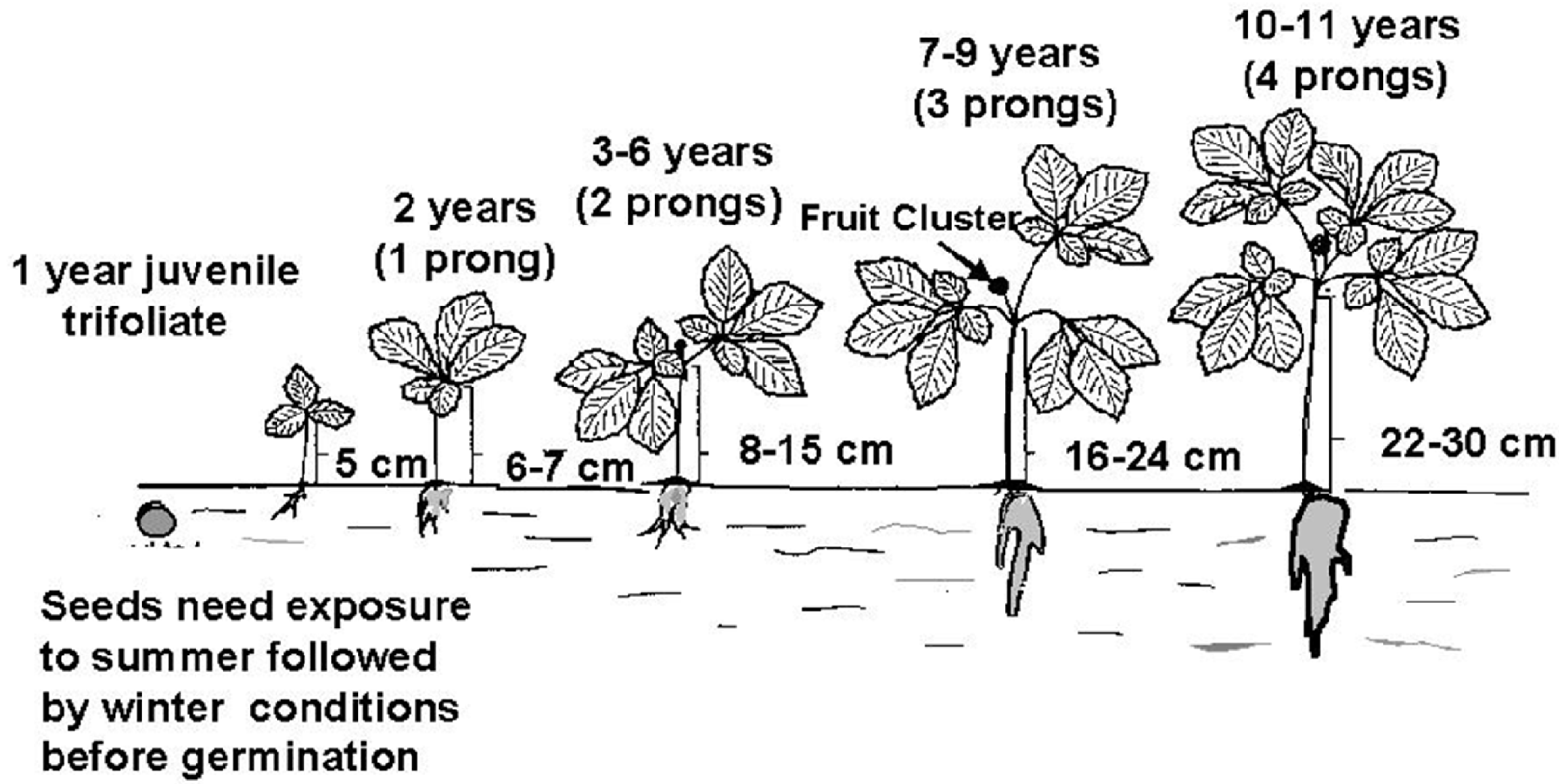


Anderson, Fralish, Armstrong and Benjamin, 1984.

Motivation: Do larger populations of ginseng reproduce better because they are more genetically diverse?

- Do larger populations reproduce more per plant? (flowers, fruits, seeds)
- If so, is it only because they happen to have more big plants (4-leaf)?
- Can we distinguish between the effect of big plants and the effect of the population they reside in (site, genetics)?
- Are the highly reproductive populations more genetically diverse?
- Do the highly reproductive populations have a better environment?

Genetic risks to small populations

- Genetic drift
- Absence of selection except on alleles of large effect (early)
- Fewer potential mates.
- Random loss of alleles.

Current knowledge --

- Plants in small populations have fewer potential mates and are more likely to mate with individuals that are close relatives.
- A number of studies document lower genetic diversity in small populations.
- Rare species are frequently shown to be less genetically diverse than the more common relatives.
- But --relationship between molecular genetic diversity and reproduction is not straightforward.

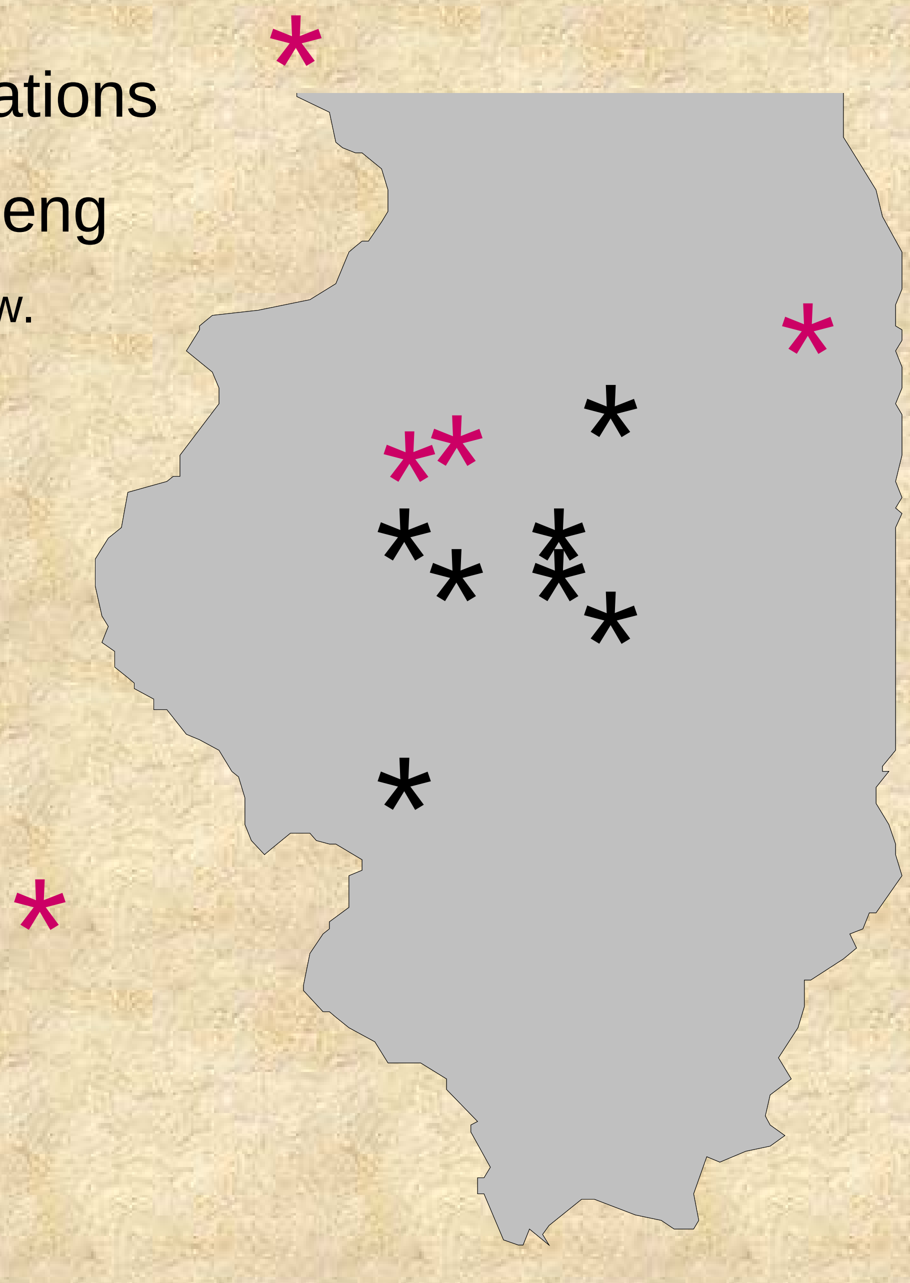
Multi-year study of numerous ginseng populations

- Intensive study of 8 populations (7 IL and 1 WI)
  - Plant characteristics that have greatest effect on maternal reproduction- # leaves, # leaflets, stem height, size of largest leaflet, size of smallest and median leaflet
  - Count flowers, fruits, seeds produced
    - Population reproduction
  - Leaf tissue collected for molecular genetic study
  - Soil samples and site characteristics
- Additional sites added for genetic analysis

Sample populations

American ginseng

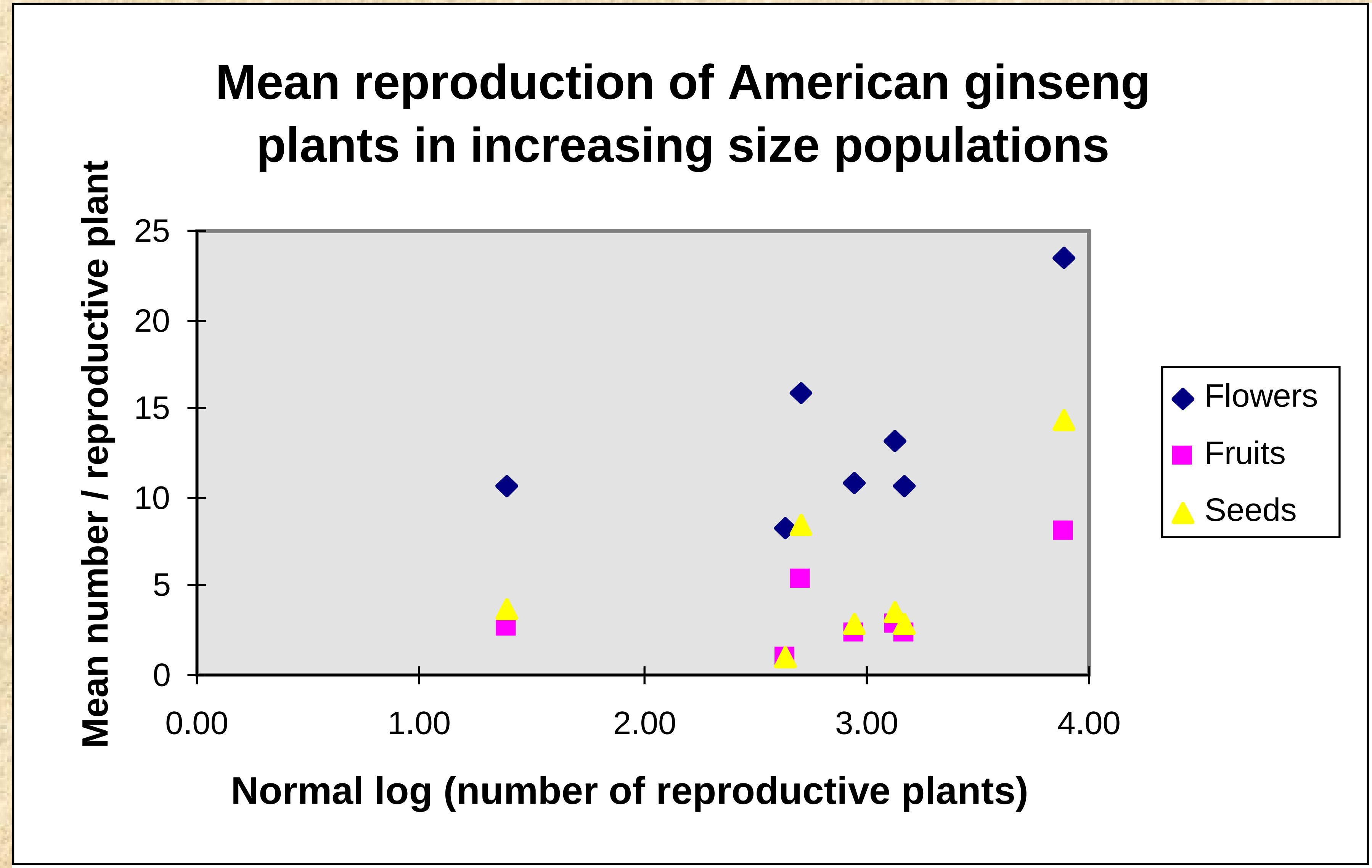
Anderson and Loew.



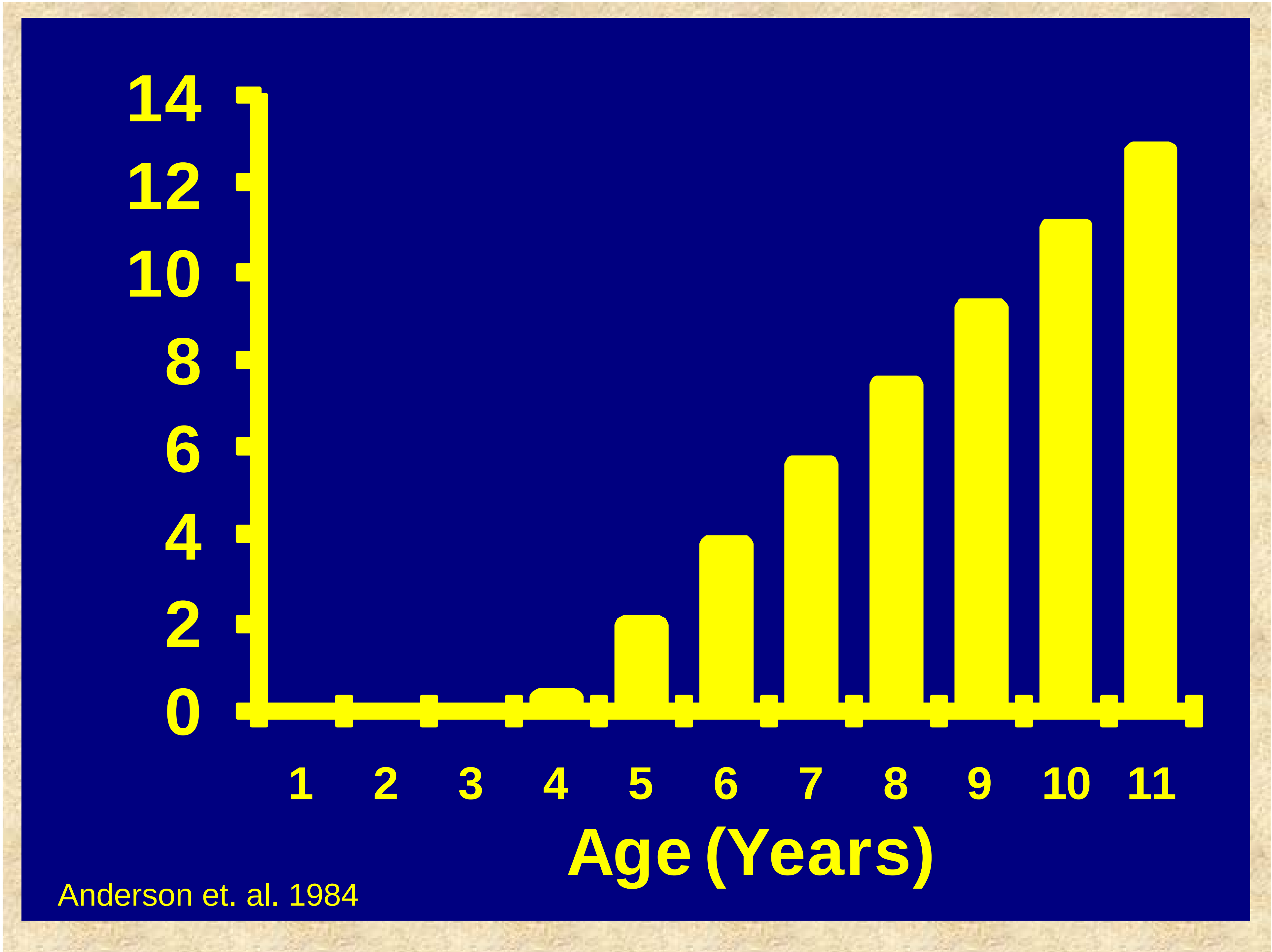
* long-term sites

* additional genetics sites

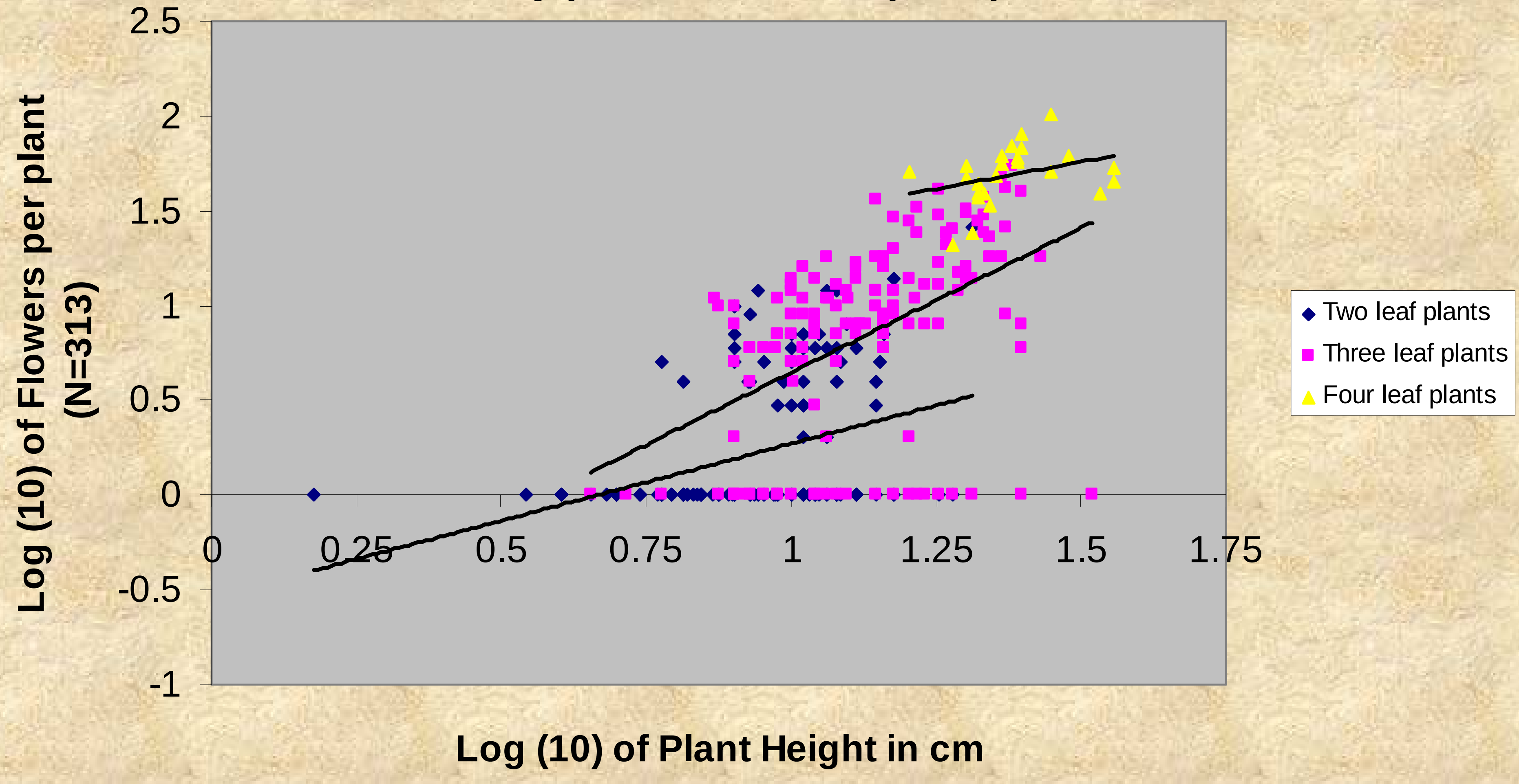
➢A first look (year 1 measurements)



But- the largest population had the most 4-leaf plants.


14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
9
10
11
Age (Years)
Anderson et. al. 1984

Annual reproductive output of American ginseng in eight populations as a function of plant height, by plant size class (2000)



Ginseng plants (yr 2000) N=415

Plant class	n=	# flowers	stem height cm)	area (mm^2)	area2 (mm^2)	area3 (mm^2)	length1 (mm)	length2 (mm)	length3 (mm)
1	109	0	8.43	735.9	390	491.9	46.1	34.9	40.2
2	143	1.46	9.14	1756	270.2	963.4	70.69	29.7	59
3	138	10.5	14.24	2827	328.8	1474	90.11	31.6	74.69
4	25	49.7	24.24	5771	651.7	2691	129.04	43.32	101.56

Total plants	# leaves	Total flowers
109	1	0
144	2	209
138	3	1461
25	4	1243

Separating the effect of plant size from population identity

What we learned

- All size variables are important
- Number of seeds is most affected by number of flowers
- Number of flowers is most affected by almost everything else in plant size

NMS variables, 1999 data

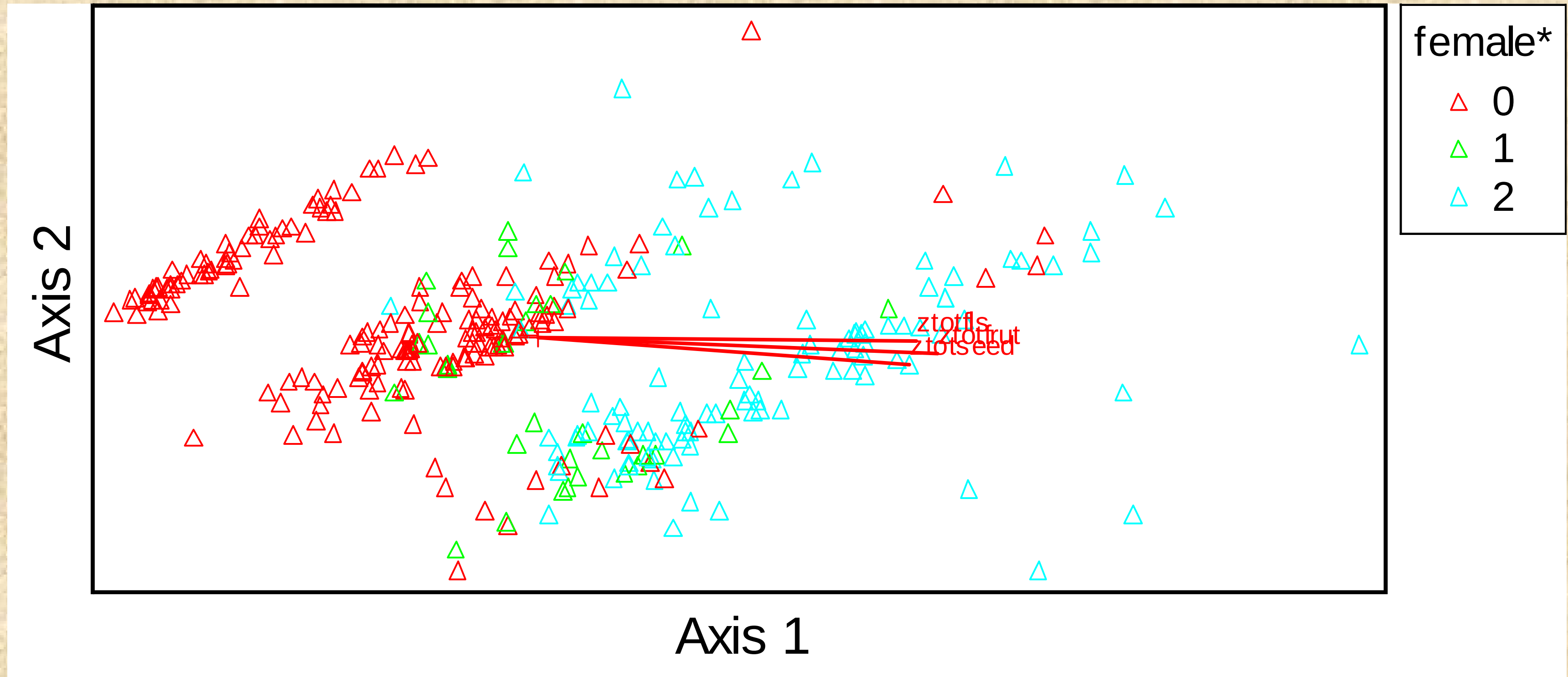
- Number of leaves
- Number of leaflets
- Stem height
- Largest leaflet: length, width, area
- Z-transformed variables to remove the influence of the absolute magnitude of values.

Correlation of original size variables with NMS axes (1999 data)

Pearson and Kendall Correlations with Ordination Axes N= 317

Axis:	1		2	
	r	r-sq	r	r-sq
zleaf	.824	.679	-.655	.428
zleaflet	.847	.717	-.645	.417
zheight	.904	.817	-.144	.021
zlong	.959	.920	-.018	.000
zwide	.945	.892	.026	.001
zarea	.952	.907	.062	.004

NMS ordination of 317 ginseng plants (year 1) based on plant size traits. Vectors represent the strength and direction of correlation of reproductive variables, with the plant size axes. Axis 1 separates smaller plants at left and larger at right.



Symbol colors: red=non-reproductive, yellow=flowers, green=fruits & seeds

MANCOVA RESULTS (1999 data)

- PLANT SIZE was significant for production of flowers, fruits and seeds in individual plants (P<0.0001)

- Populations (with plant size as covariate) significantly predicted reproductive output (P<0.0001), also.

MEANING --

- There was a difference among populations even if plant size was accounted for.

- The difference was not related to population size.

Repeated with yr 2000 data

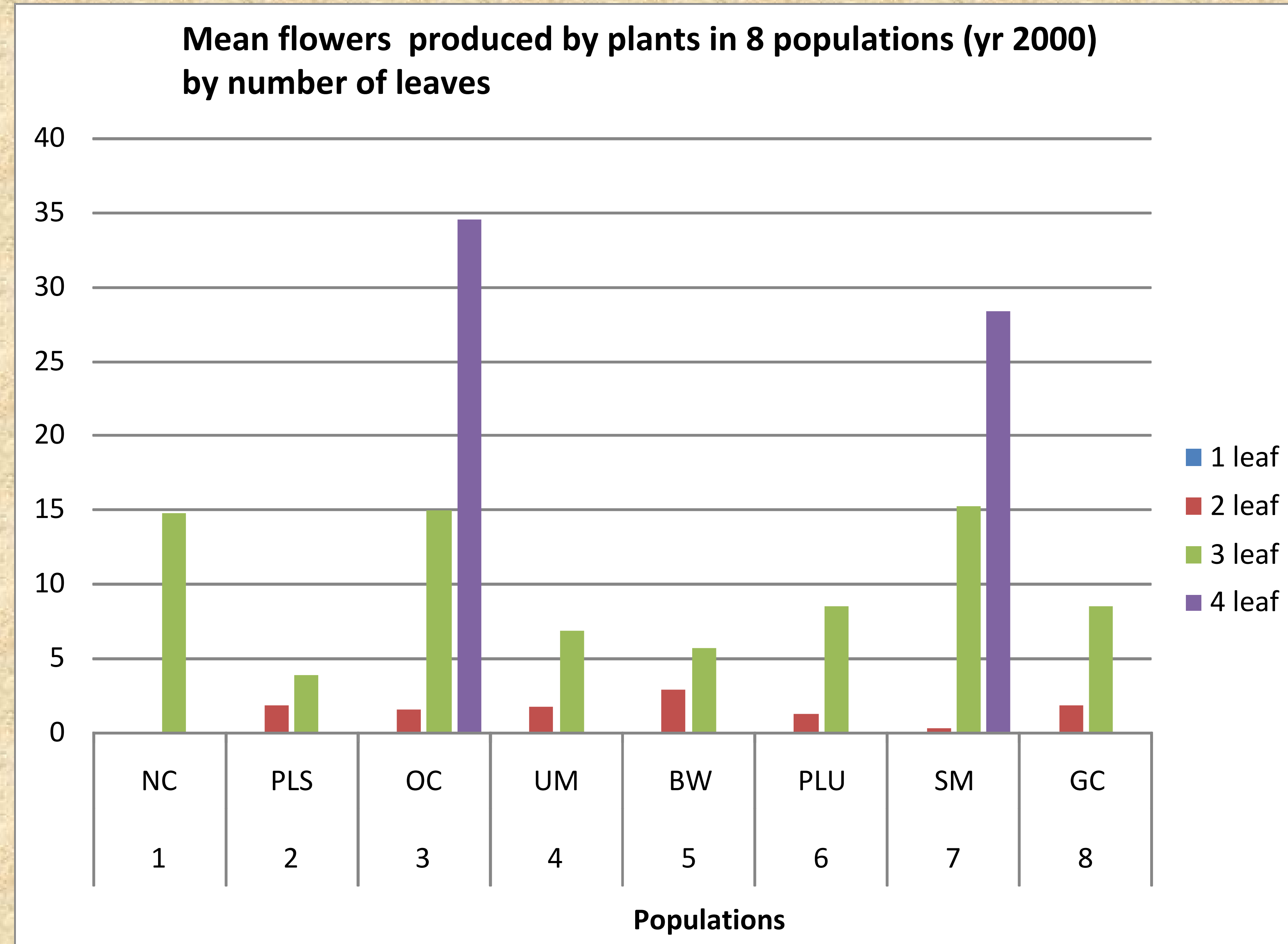
- More plants, additional larger population, additional leaflets.
- Only # total reproductive units (flowers), not fruits and seeds.

ANOVA: flowers per plant = population identity and both size variables

- Without the size variables,
  - populations differ significantly in total flowers produced.
  - Population 7 differs from all but 1 population.

- With the size variables,
  - the overall model significantly predicts total flowers produced.
- But only the size variables are significant.

Population identity does NOT predict flowers produced, independently of plant size.

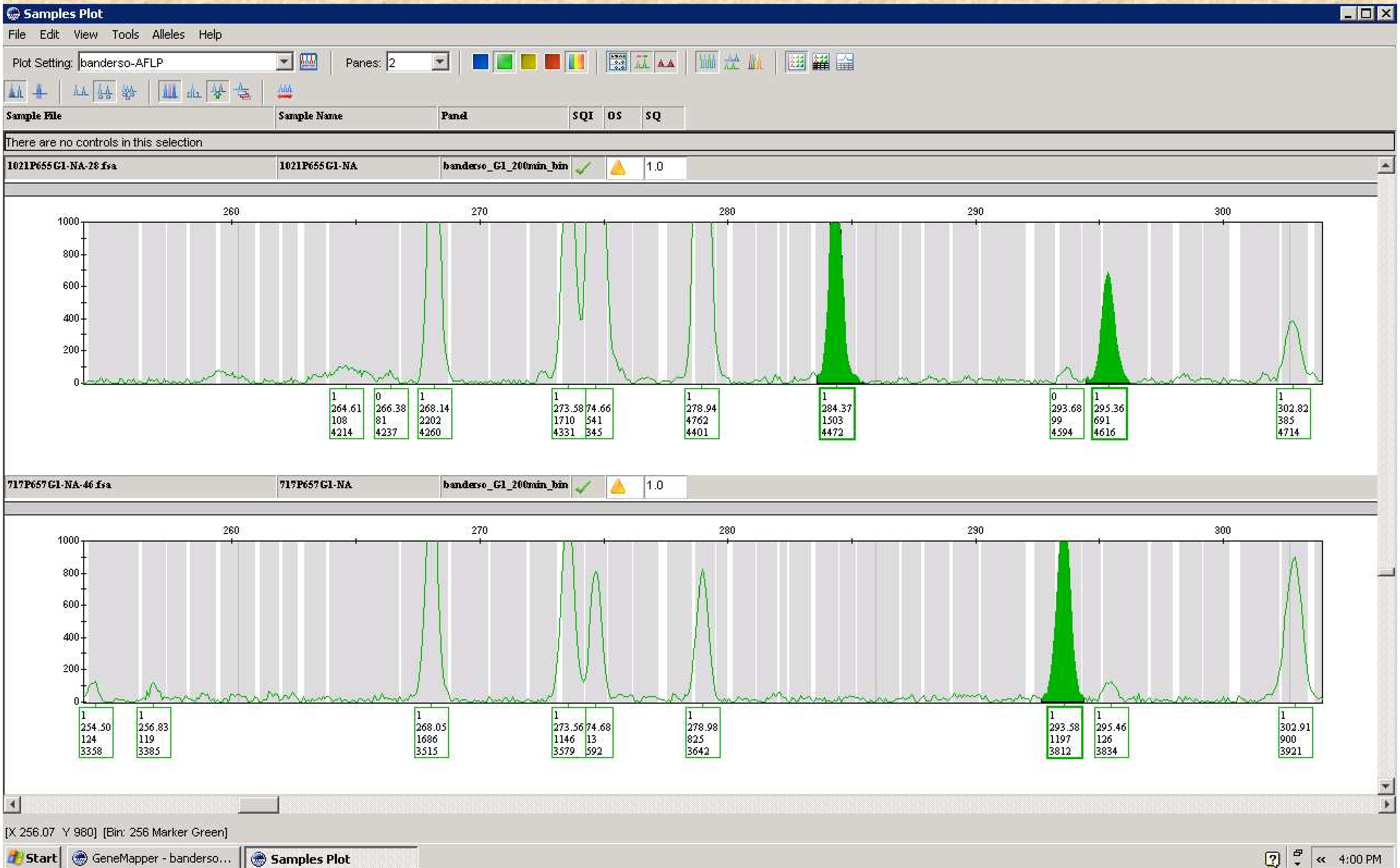

Mean flowers produced by plants in 8 populations (yr 2000) by number of leaves
40
35
30
25
20
15
10
5
0
NC
1
PLS
2
OC
3
UM
4
BW
5
PLU
6
SM
7
GC
8
Populations
1 leaf
2 leaf
3 leaf
4 leaf

Are differences in populations related to genetic diversity?

Amplified fragment length polymorphism

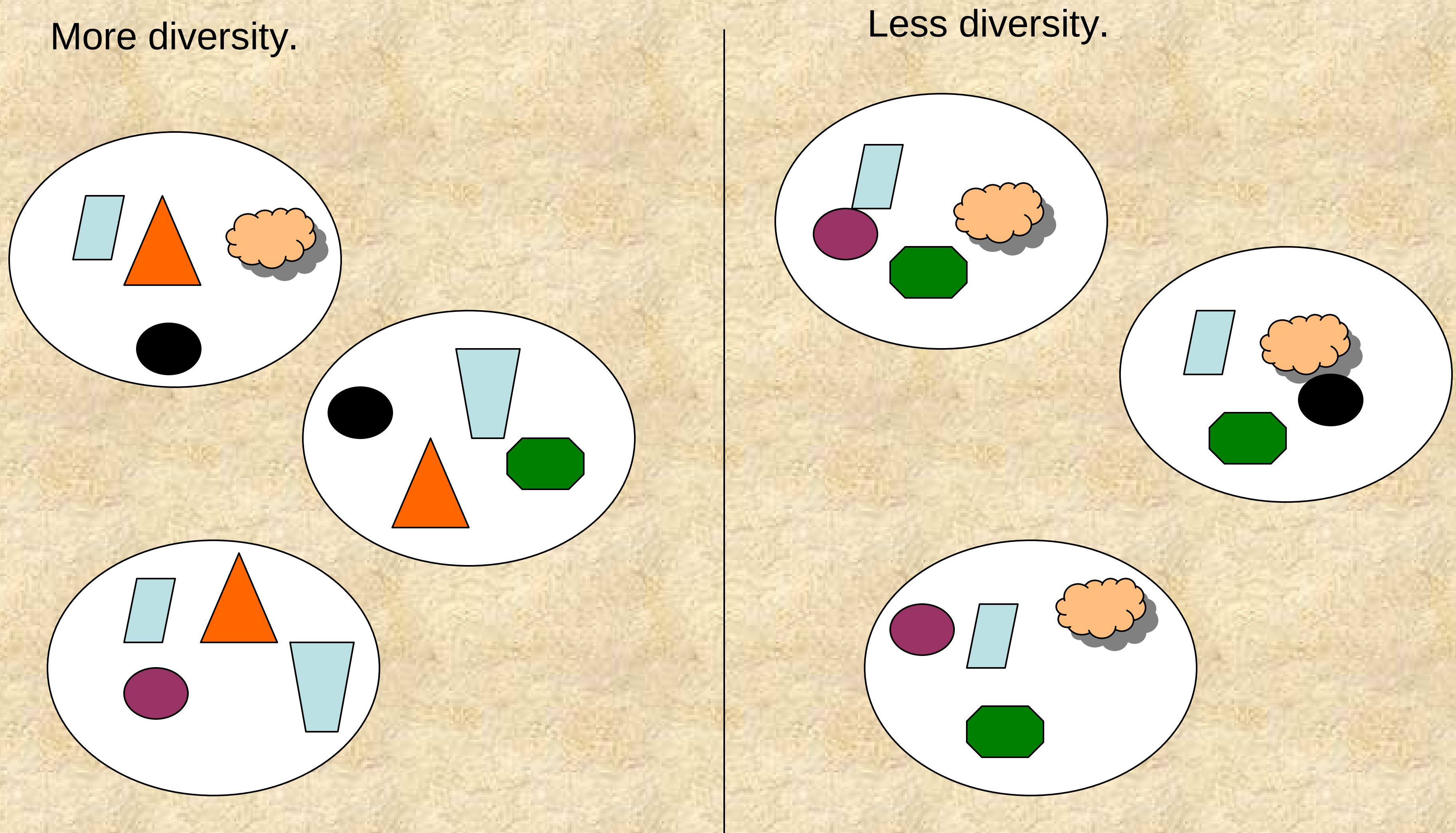
- Neutral DNA – does not code for genes that are expressed.
- Inherited in Mendelian fashion, can be mapped.
- No prior knowledge of genome
- Highly repeatable, many alleles detected.
- Expensive, technically detailed
- Dominant marker– can’t detect heterozygosity.

Polymorphic loci are present in some plants, not in others.



Partitioning of genetic diversity

- AMOVA (Analysis of Molecular Variance)
- How much of the total genetic diversity resides within populations separately
- Compared to how much resides in the whole population.



More diversity between groups than within groups.

AMOVA, CONT'D

- High % within populations means
  - Each population is different from others.
  - Populations are not very well connected by pollinators and seed dispersal.
- High % among populations means
  - Each population is mostly like all the other populations.
  - Populations are more likely to be connected by pollinators and seed dispersal.

A test of 12 populations based on pair-wise differences

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	11	524.722	1.72867 Va	5.52
Within populations	114	3370.381	29.56475 Vb	94.48
Total	125	3895.103	31.29341	
Fixation Index	FST :	0.05524		

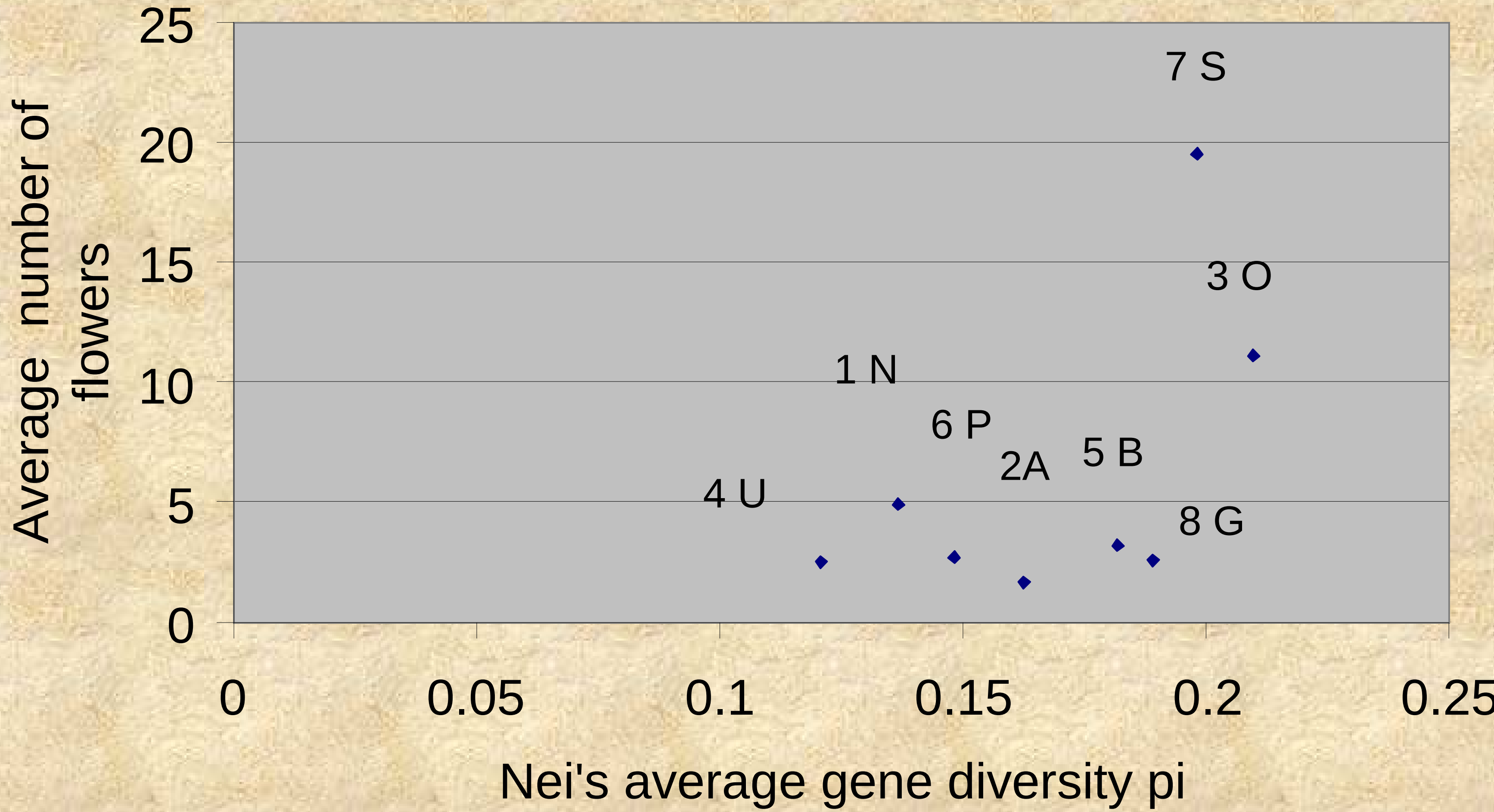
Conclusion: Each population is very different from others.

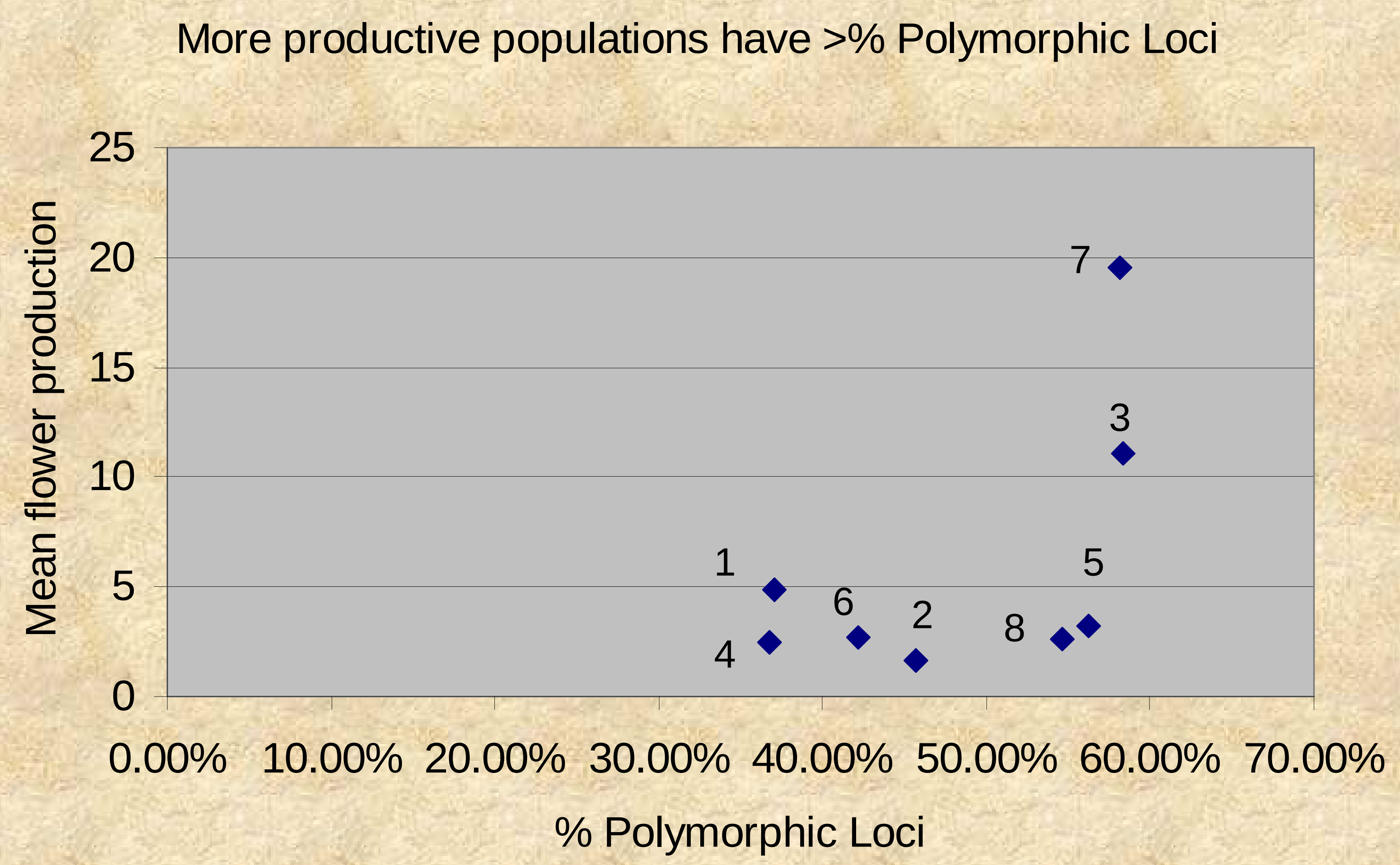
Not well connected.

AMOVA partitioning of AFLP diversity from the literature

- Sweden -- willow leaf rust --mostly not sexual reproduction
  - 97.5% within locations
- Illinois bundle flower --accessions related to crop development
  - 83% of diversity explained by 2 major clusters
- Little bluestem grass, including cultivars (wind pollinated)
  - >91 % of total variation is between cultivars
- Nicotiana attenuata - a post-fire annual in montane western US
  - 88% within populations
  - Among populations within sites 8.72%

Genetically diverse populations produce more flowers





Populations are numbered from smallest (1) to largest (8). Here the most diverse are not necessarily the largest.

What have we learned

- Crucial importance of big plants.
- Differences between populations in reproduction may be mostly due to differences in size of plants.
- Important to maintain genetic diversity in order to maintain higher reproduction.
- Maintain connectivity between populations.

But --

- Are populations productive because they are genetically diverse, or are they genetically diverse because they haven’t been harvested heavily.

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