Result of Pea testing 2010

At a course at the Department of Agriculture and Ecology, University Life, this Pea testing was implemented

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Frøsamlerne (Danish Seed savers) 2010

Starting point

Our questions and Pea varieties

For comparison of different portions of the Great Holger Kæmpeært - and the comparison of the Great and Small Holger Kæmpeært:

No. 7 - Great Holger Kæmpeært, Mogens Thomsen (PØ)

No. 8 - Great Holger Kæmpeært, Dorthe Hougaard (PØ)

No. 9 - Great Holger Kæmpeært, John Agner Christiansen (PØ)

No. 10 - Great Holger Kæmpeært, Ida Soer Johannessen

No. 11 Great Holger Kæmpeært, Jørgen Kristensen

No. 12 Small Holger Kæmpeært, Ida Soer Johannessen

No. 13 Small Holger Kæmpeært, Jørgen Kristensen

For comparison of Great Holger Kæmpeært and Holger-like peas:

No. 14 Aunt Kirsten's tall pea, Nanna Fyhn

No. 17 HRH the King Christian X Hofært, Molly Hougaard

To "analyze" the breeding grounds affect on variety:

No. 22 Amandas tall pea (grown on clay soil), Ulla Vester

No. 23 Amandas tall pea (grown on sandy soil), Elsa Krogh

To investigate whether Frederick 7th 's pea is old:

No. 16 HRH Frederik 7's pea, Molly Hougaard

For comparison of other tall peas:

No. 3 Local variety from Stevns NGB13919.1, NordGen

No. 4 Stevns tall pea, Nanna Fyhn

No. 5 Videmoseært NGB11759.3, NordGen

No. 6 Videmoseært, Søren Holt

No. 15 Gyrithe tall pea, Ida Soer Johannessen

No. 18 Stangært from Fyn FS 554, NO Crossland

No. 19 Grams tall pea, Søren Holt

No. 20 Maries tall pea FS 240, Søren Holt

No. 21 Jutta tall pea, Søren Holt

No. 24 Jutta tall pea, Elsa Krogh

No. 28 High pea from Susie, Elsa Krogh

No. 29 Karls tall pea, Karen Bredahl

For the investigation of some gray peas / comparison of peas with colored flowers:
No. 25 Brown Pea from Nakskov, Molly Hougaard
No. 26 Errindlev pea, Søren Holt
No. 27 Lollandske rosiner, Søren Holt

Control Peas (ensuring genetic diversity in our material):
No. 1 Jærert NGB11149, NordGen
No. 2 Julita NGB9927.3, NordGen

Other peas (filled vacancies in our study):
30 Std_1-6 (Gråärt NGB11737) Department of Agriculture and Ecology
31 Std_9-5, (Maglaby gråärt NGB14154) Department of Agriculture and Ecology
32 Std_51-2, (Puggor från Ballingslöv-Glimåkra NGB17873) Department of Agriculture and Ecology

Great Holger Kæmpeært and Small Holger Kæmpeært we have included from multiple sources, since we wanted to investigate whether they were different, and examine whether the different sources had actually deviant peas.
Amandas tall pea we included from two sources, then we would see if they had drifted apart over the years where they were grown in very different soils.
Jutta tall pea was included twice, bit of a coincidence - it should prove interesting.
Other varieties are included only once.

Method:

Examination of micro-satellites:
A9 - D21 - AC58 - C20 - AA5 - AC75
These microsatellites are very stereotypical areas of DNA strand consisting of only two bases (A, T, C or G), who during copying of DNA sometimes is duplicated a number of extra times.How many times are often different from variety to variety - ie. that this area can be of different length. These micro-satellites or SSRS belongs to a group of markers that are not so far known to have any meaning for the organism and do not select a gene so they tell us nothing about our varieties. But they are good at distinguishing between related varieties.

We worked in 4 groups. Every team isolated DNA from up to 8 pea varieties (Quick & Dirty isolation of genomics DNA with NaOH (caustic soda)), a total of 29 varieties, 30 Std_1-6, (Gråärt NGB11737), 31 Std_9-5, (Maglaby gråårt NGB14154) and 32 Std_51-2 (Puggor från Ballingslöv-Glimåkra NGB17873) were already prepared by the Department of Agriculture and Ecology.Small pieces of leaves were cooled to inhibit the natural enzymes that would degrade the DNA before we could do it. A sodium hydroxide dilution were added and the mixture boiled 1 minute. Then finely ground mixture with the tip of a thin plastic rod, a buffer was added to neutralize the caustic soda and the mixture was centrifuged. Bottom total leaf stones in a green area on top of creamy DNA material in a clear liquid. Samples were collected from the clear fluid with isolated DNA to the next step, so we had isolated DNA from all 32 ærtesorter. Samples was kept on ice.

In the next step all the groups was working with isolated DNA from each of the 32 varieties. Groups 1 and 3 propagated micro satellites A9, D21 and AC58. Groups 2 and 4 propagated micro satellites C20 AA5 and AC75.
To propagate micro satellites, the isolated DNA was mixed with a primer, which we already knew would bind to the DNA strand, just before and after the current micro-satellite, and an enzyme, Taq DNA polymerase, which copied the micro satellite, starting from the primer. Various aids are added to provide the necessary energy, dye micro satellites respectively blue, green and yellow, and maintain the correct acidity. After going through about 40 repli cyclic temperature changes in a PCR machine.
At 94 °, where DNA strand separated
64-55 °, where the primer binds to the DNA strand, and
72 °, where Taq DNA polymerase copies the DNA strand from the primer and the forward (micro-satellite is copied).
By the repeated changes in temperature, we multiplied the selected microsatellites exponentially. The first iterations gave very little material, but for each iteration, there was more material to copy. Centrifuge. Now we had for each variety, two tubes with each micro-satellite, a total of 2x6x32 = 384 test tubes.

In the last step, each micro-satellite sorted by length. In capillaries with polymer exposed to an electric field that causes the negatively charged micro-satellites to migrate up

through the tube. Short molecules move faster through the gel than long. There is a sort, as detected by a photocell that detects the color of micro-satellite.
Each group mixes its 3 colored micro-satellites in a test tube for each pea variety. This saves time in the sorting process, and because of colors the photocell knows difference of micro satellites. There's also added a red colored sample with molecules of known length. They indicate a scale of molecular size, so we end up with a result where we for the various ærtesorter each microsatellites molecular size.

Result:

C20 and AA5 deleted. One didn't stain, so we did not profit from it. The other showed no variation, all peas had the same variant, it could not distinguish between pea varieties.

Table 1

Acc.	A9	A9	AC58	AC58	D21	D21	AC75	AC75
01_Jærert	385	385	230	230	282	282	287	287
02_Julita	383	383	236	236	298	298	281	281
03_Lokals	383	383	236	236	294	294	281	281
04_Stevns	383	383	236	236	294	294	281	281
05_Videmo	383	383	236	236	298	298	281	281
06_Videmo	383	383	236	236	298	298	281	281
07_store	383	383	236	236	294	294	281	281
08_store	383	383	236	236	294	294	281	281
09_store	383	383	236	236	294	294	281	281
10_store	383	383	236	236	294	294	281	281
11_store	383	383	236	236	294	294	281	281
12_lille	383	383	236	236	294	294	281	281
13_lille	383	383	236	236	294	294	281	281
14_Faste	383	383	226	226	294	294	281	281
15_Gyrit	383	383	260	260	294	294		
16_HKH_F	403	403	236	236	298	298	279	279
17_HKH_C	383	383	236	236	294	294	281	281
18_Stang	403	403	260	260	302	302	279	279
19_Grams	383	383	236	236	298	298	281	281
20_Marie	383	383	236	236	294	294	281	281
21_Jutta	403	403	260	260	294	294	279	279
22_Amand	387	387	260	260	248	248	281	281
23_Amand	387	387	260	260	248	248	281	281
24_Jutta	383	383	236	236	294	294	285	285
25_Brun_Æ	385	385	220	220	248	248	281	281
26_Errin	405	405	230	230	227	227	275	275
27_Lollan	385	385	220	220	248	248	281	281
28_Høj_ær	383	383	236	236	294	294	281	281
29_Karls	387	387	236	236	302	302	279	279
30_Std_1-6	383	383	236	236	298	298	281	281
31_Std_9-5	383	383	220	236	294	294	281	281
32_Std_51-2	379	379	226	226			285	285

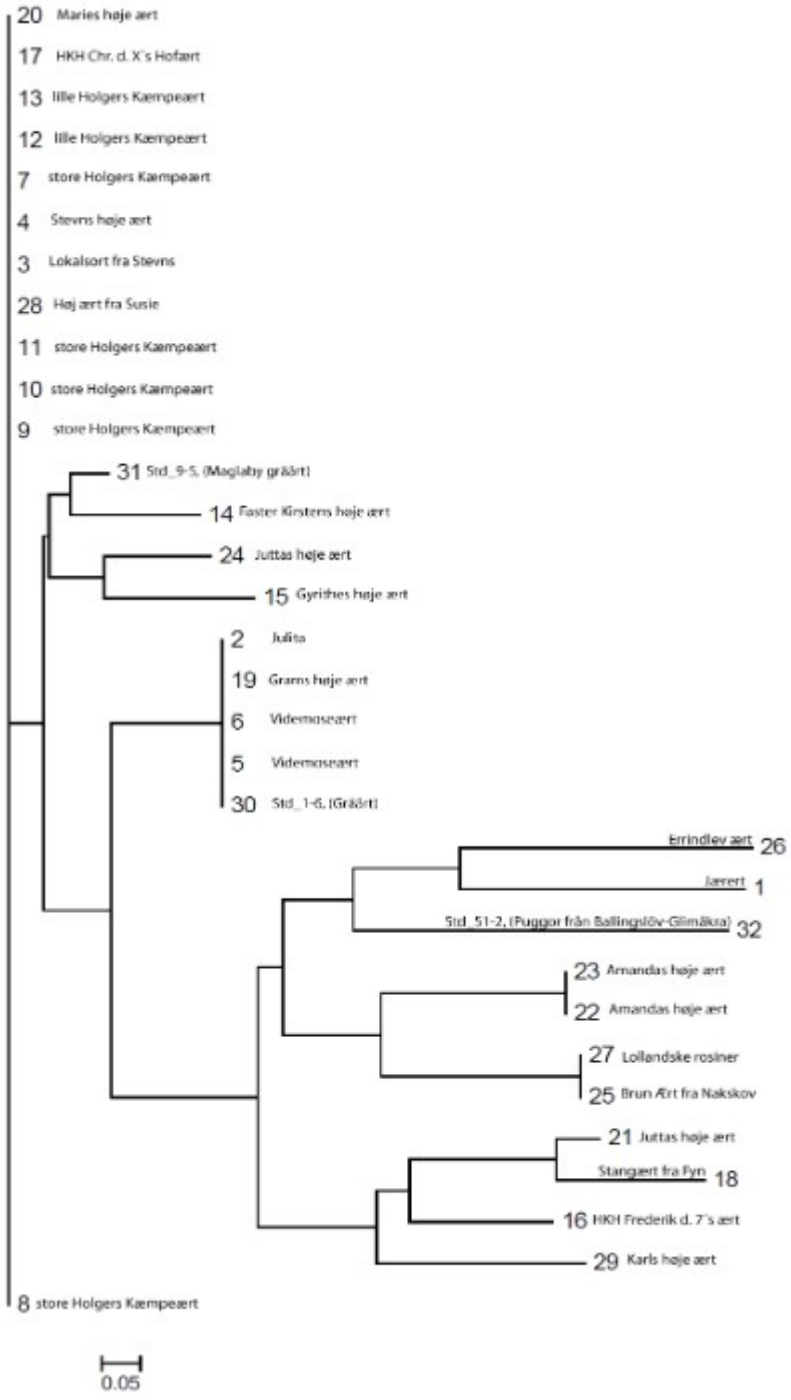
The numbers indicate the length of micro-satellites , ie number of times base pairs repeated.

Pea varieties 15 Gyrithe tall pea and 32 Std_51-2 each lack data from one micro-satellite, probably because the primer could not bind because of variation in DNA strand immediately before the micro-satellite.

31 Std_9-5, (Maglaby gråärt) shows unexpected variation in the micro satellite AC58, it may be due to variation in the variety, which of NordGen is designated as land-race, and that the two samples taken from two different individuals. Looking at the photo of the seeds in NordGen database SESTO you also see significant variation in color pattern on the peas, something that normal is hereditary.

It's hard to survey data in tabular form. It is possible to manipulate the data mathematically in different ways, so you get a more visual presentation.

First, a dendrogram. (Figure 1)
It is based on the distance between the varieties. It built up hierarchically, first find the two closest. Then beaten their two lines into one line, and will be treated as a single unit.
Again, find the two nearest, which then merged etc.
One must be aware that it is in no way be read as a family tree!



Ill. 1

Another way to visualize distances is to make a principal coordinate analysis, configured in two dimensions.

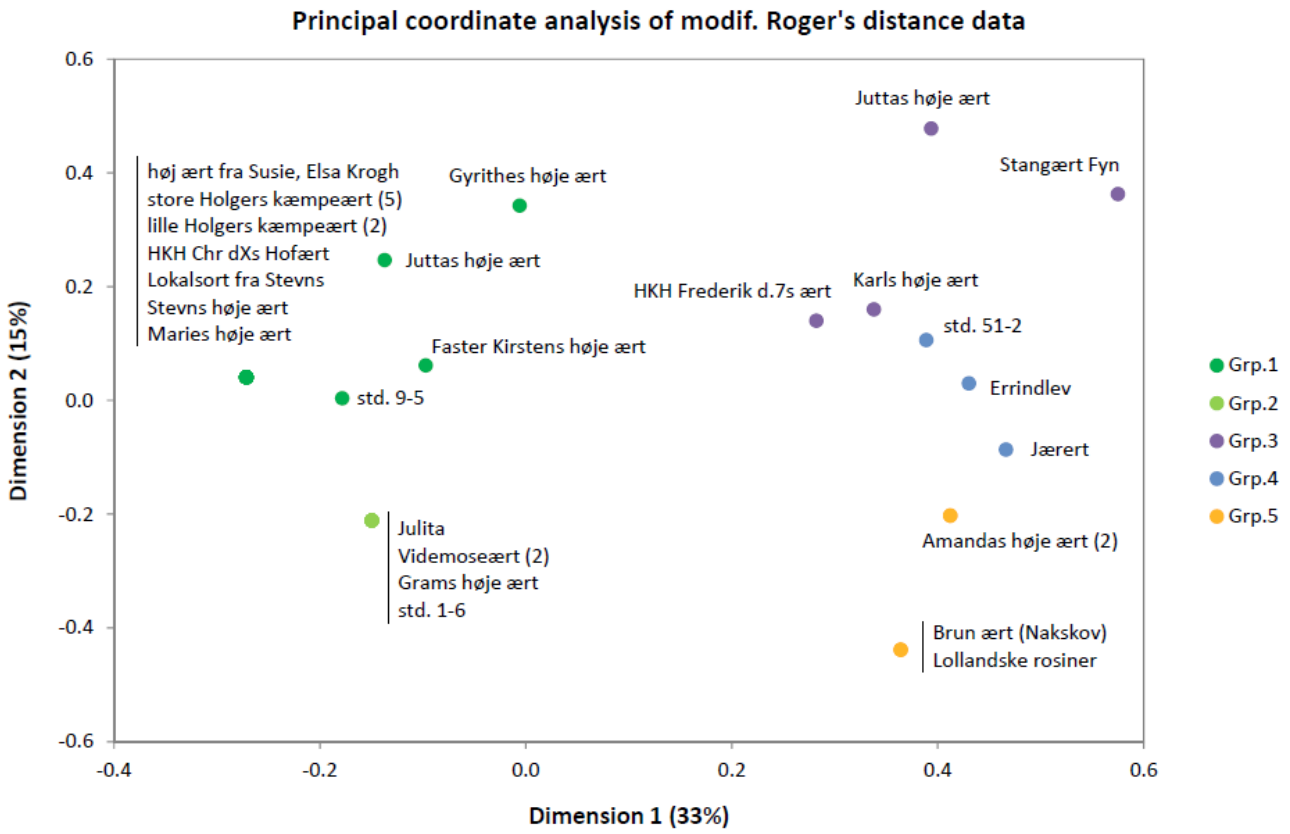


Figure 2Principal coordinate analysis

In the above Figure 2 shows how the peas are grouped in two dimensions. The dimensions are mathematical ways to express several independent variations in a single image. It is not as accurate as the exact numbers of micro-satellite length. In return it gives a visual image we humans quickly grasp. Some would also prefer that presentation rather dendrogram.

Dimension 1 explains more of the difference between varieties than the second dimension The colors refer to a division into 5 groups.

Groupings

It makes sense to group the peas.

2 groups is the safest breakdown, but 5 groups is also reasonable.

By splitting into 2 groups, where groups 1 and 2 in the above Figure 2 represents the one and group 3-5 constitute the second group, obtained a very clear division, where all the peas in each group closest to the peas from their own group. It is a right-left split, according to dimension 1

By splitting into 5 groups, we see some peculiarities where for example Amandas tall pea is closer to Jærert than other peas in own group. This classification into 5 groups is not as obvious as the division into 2 groups. However, there is not some quite surprising things.

As seed savers can probably also see a fairness in the division into 7 groups, because then we get separated Amandas tall pea from the two Lolland field peas, brown pea from Nakskov and Lollandske rosiner.
On the other hand, is there something weird and not credible with Std_51-2 (Puggor från Ballingslöv-Glimåkra), which then are grouped with Aunt Kirsten's tall pea and No.24 Jutta tall pea. O ur lecturers are, from our data alone are not likely to accept a division into 7 groups, but note how strong a division into 2 groups, and 5 groups also acceptable
Note that the peas location on the figure above is independent of how many groups we're doing.

By splitting into 5 groups, it can be configured as in Figure 3 below This is another way to visualize the variation in our peas.

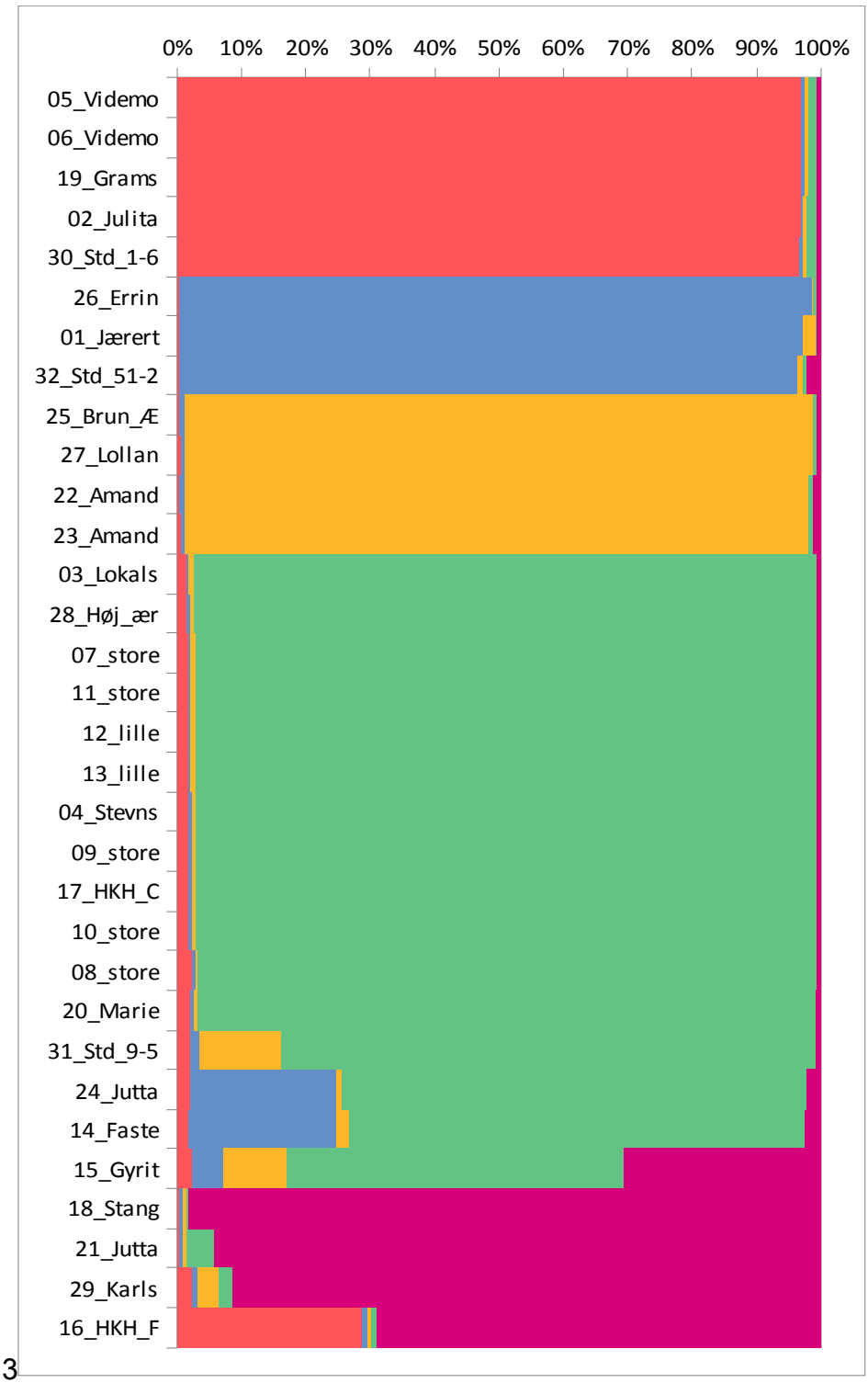


Figure 3

Gr.1: Green in figure 3.Gyrithe tall pea, Aunt Kirsten's tall pea, No.24 Jutta tall pea and 31 Std_9-5 (Maglaby gråärt) is distinctly different from each other and others.The other shows no signs of being different that we must look at their phenotypes (the properties of the plant which originates from its genes and can be observed when we grow it). Large and Small Holger is indistinguishable from one another, so Little Holger is probably a variation of Big Holger, not an interference of another variety.

Gr.2: Coral red on Figure 3These show no sign of being different that we must look at their phenotypes. It is reasonable to assume that the two Videmoseært are identical.

Gr.3: Dark red.They are all different from each other and others.

Gr.4: Blue.They are different from each other and others.

Gr.5: Yellow.The two Lolland gray peas with large seeds show no sign of being different, we must look at their phenotypes.
Both Amanda shows no sign of being different. Thankfully.

21 Jutta tall pea and 24 Jutta tall pea should be identical, but they are not at all, and none of them are identical to others of the peas in the experiment. It should be further explored, for example. by looking at the phenotype.

The possible groupings can also set up in the following way. (Table 2)

2 groups	5 groups	7 groups
Great Holger Kæmpeært (5) small Holger Kæmpeært (2) HRH King Christian X Hofært Local variety from Stevns Stevns tall pea Maries tall pea Std_9-5 *(Maglaby gråårt)* Gyrithe tall pea Tall pea from Susie Aunt Kirsten's tall pea No.24 Jutta tall pea	Great Holger Kæmpeært (5) small Holger Kæmpeært (2) HRH King Christian X Hofært Local variety from Stevns Stevns tall pea Maries tall pea Std_9-5 *(Maglaby gråårt)* Gyrithe tall pea Tall pea from Susie	Great Holger Kæmpeært (5) small Holger Kæmpeært (2) HRH King Christian X Hofært Local variety from Stevns Stevns tall pea Maries tall pea Std_9-5 *(Maglaby gråårt)* Gyrithe tall pea Tall pea from Susie
	Aunt Kirsten's tall pea No.24 Jutta tall pea	Aunt Kirsten's tall pea No.24 Jutta tall pea Std_51-2, *(från Puggor Ballingslöv-Glimåkra)*
Julita Videmoseært (2) Grams tall pea Std_1-6, *(Gråärt)*	Julita Videmoseært (2) Grams tall pea	Julita Videmoseært (2) Grams tall pea

	Std_1-6, *(Gråärt)*	Std_1-6, *(Gråärt)*
No.21 Jutta tall pea Stangært from Fyn HRH Frederik 7's pea Karls tall pea	No.21 Jutta tall pea Stangært from Fyn HRH Frederik 7's pea Karls tall pea	No.21 Jutta tall pea Stangært from Fyn HRH Frederik 7's pea Karls tall pea
Std_51-2, *(från Puggor Ballingslöv-Glimåkra)* Errindlev pea Jærert	Std_51-2, *(från Puggor Ballingslöv-Glimåkra)* Errindlev pea Jærert	Errindlev pea Jærert
Amandas tall pea (2) Brown Pea from Nakskov Lollandske rosiner	Amandas tall pea (2) Brown Pea from Nakskov Lollandske rosiner	Amandas tall pea (2) Brown Pea from Nakskov Lollandske rosiner

Table 2

Notice how Std_51-2, *(från Puggor Ballingslöv-Glimåkra)* make a tremendous leaps from 5 groups of 7 groups.

Answer our questions

For comparison of different portions of the Great Holger Kæmpeært - and the comparison of the Great and Small Holger Kæmpeært:

We found no difference between the various portions of the Great and Small Holger.

For comparison of Great Holger Kæmpeært and Holger-like peas:

14 Aunt Kirsten's tall pea is different from every other pea in the experiment.
17 HRH the King Christian X Hofært shows no sign of being different from Holger peas.

To "analyze" the breeding ground Affect variety:
We found no difference between Amanda from sandy and clay soils from cultivation through a number of years.

To investigate whether Frederick 7th 's pea is old:
16 HRH Frederik 7's pea is different from all other investigated peas, but whether it is old we can not know from this study.

For comparison of other tall peas:
We managed to compare many tall old peas and have them grouped in a meaningful way. It has also become clear which are unique and what is perhaps the same variety with different names and cultural history, and therefore should be investigated with other methods.

For the investigation of some gray peas / comparison of peas with colored flowers:
26 Errindlev pea is different from the other investigated peas.
25 Brown Pea from Nakskov and 27 Lollandske rosiner shows no sign of being different, but there may be phenotypic differences as well.
Note also that gray peas are distributed across most groups!

So there are varieties that we now know is different from all others.
There are also groups of varieties, where we should check whether there are phenotypic differences. It is a great advantage that it is now clearly delineated the varieties to be compared by cultivation experiments. There is probably also varieties that are identical with each other. It is not something that can be resolved by this trial, which is only suitable to detect differences in the 4 areas (micro-satellites), we got useful results from. Whether they are identical in many more microsatellites, we can not know before we make a new attempt!

Thanks to Gunter Backes and Jihad Orabi, both for the good course and the material afterwards.They also provide all tables and figures except Table 2