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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
GENEVA

WORKING GROUP ON BIOCHEMICAL AND MOLECULAR TECHNIQUES AND DNA PROFILING IN PARTICULAR

Twelfth Session
Ottawa, Canada, May 11 to 13, 2010

ADDENDUM

EVALUATION OF SIMPLE SEQUENCE REPEAT (SSR) MARKERS FOR IDENTIFICATION OF PEA VARIETIES REGISTERED IN CANADA

Document prepared by experts from Canada

Evaluation of simple sequence repeat (SSR) markers for identification of pea varieties registered in Canada

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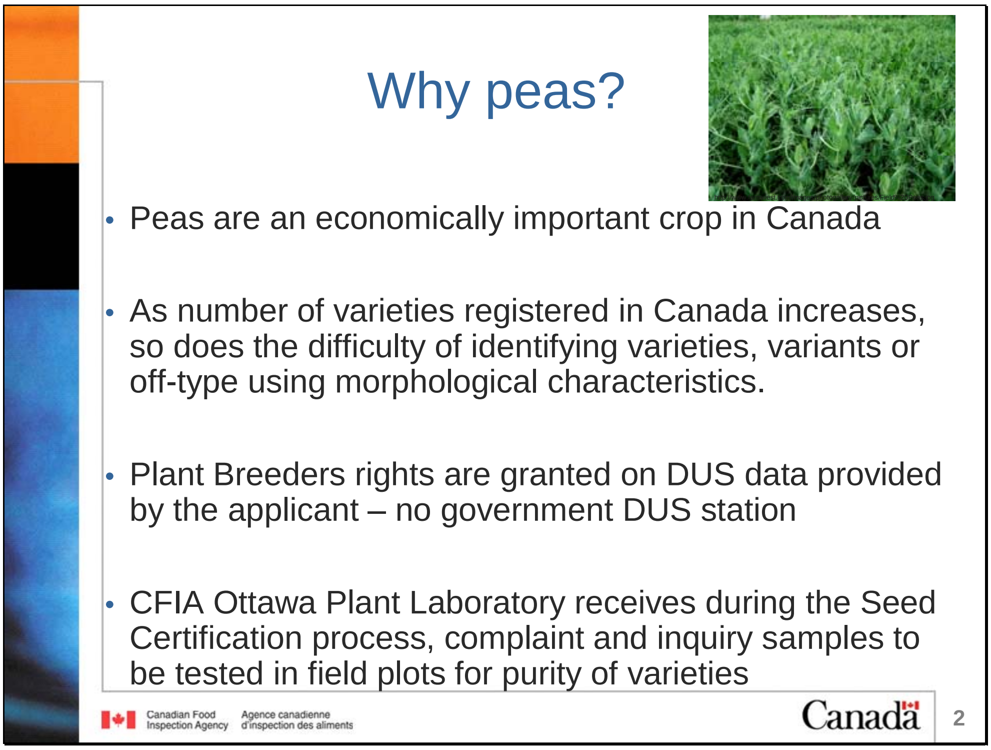
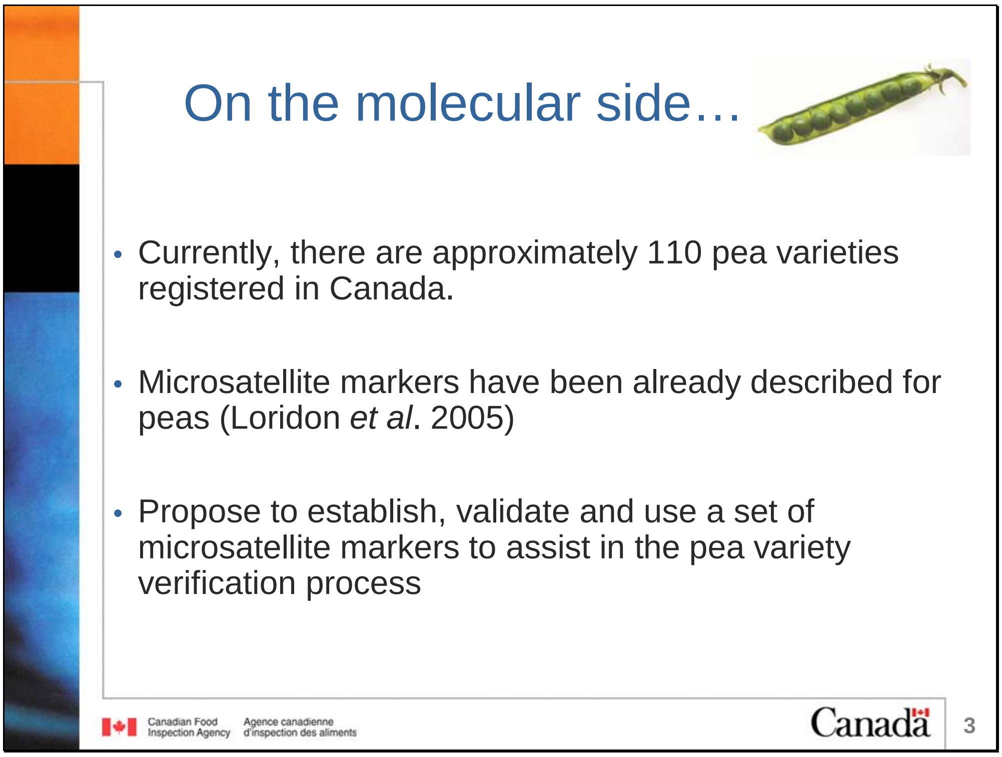


Why peas?

- Peas are an economically important crop in Canada
- As number of varieties registered in Canada increases, so does the difficulty of identifying varieties, variants or off-type using morphological characteristics.
- Plant Breeders rights are granted on DUS data provided by the applicant – no government DUS station
- CFIA Ottawa Plant Laboratory receives during the Seed Certification process, complaint and inquiry samples to be tested in field plots for purity of varieties



On the molecular side…

- Currently, there are approximately 110 pea varieties registered in Canada.
- Microsatellite markers have been already described for peas (Loridon *et al*. 2005)
- Propose to establish, validate and use a set of microsatellite markers to assist in the pea variety verification process

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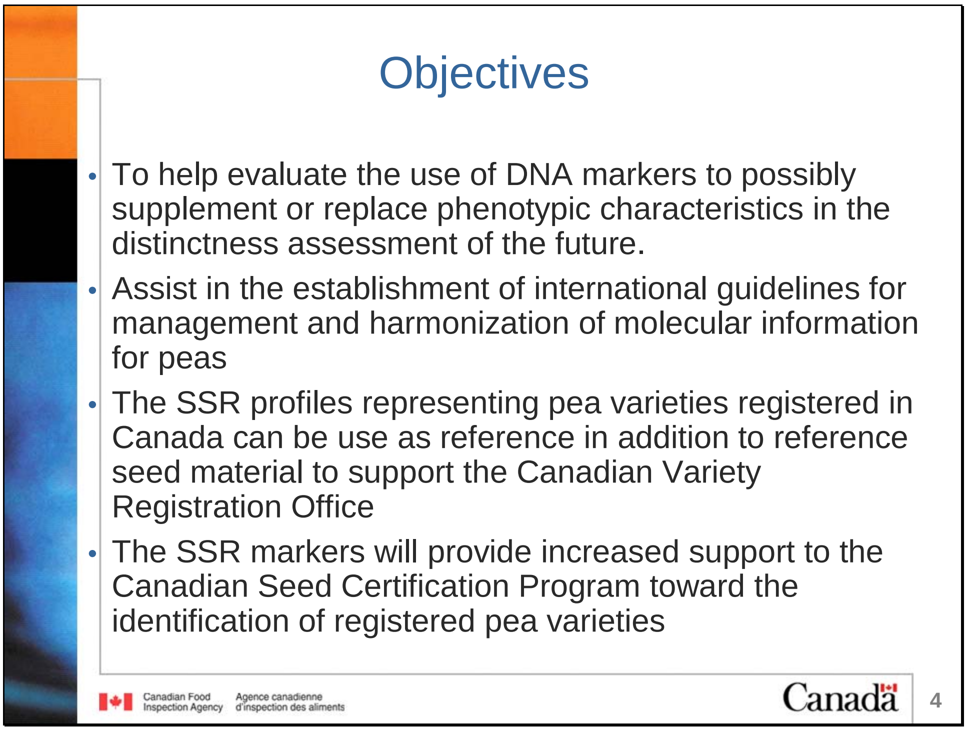

Objectives

- To help evaluate the use of DNA markers to possibly supplement or replace phenotypic characteristics in the distinctness assessment of the future.
- Assist in the establishment of international guidelines for management and harmonization of molecular information for peas
- The SSR profiles representing pea varieties registered in Canada can be use as reference in addition to reference seed material to support the Canadian Variety Registration Office
- The SSR markers will provide increased support to the Canadian Seed Certification Program toward the identification of registered pea varieties

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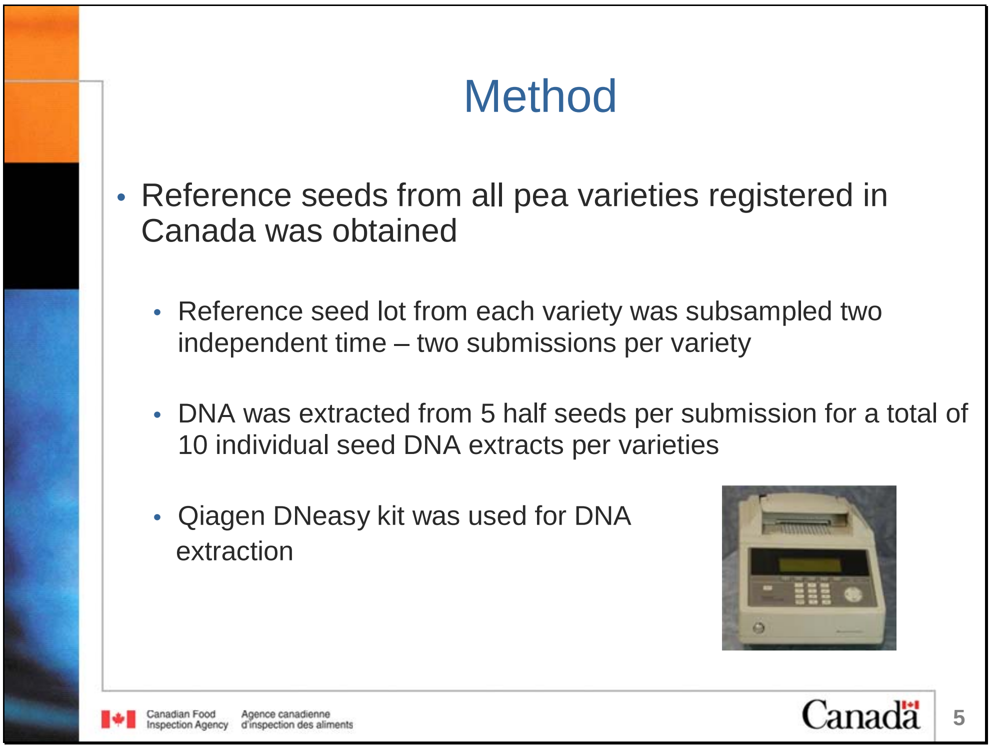
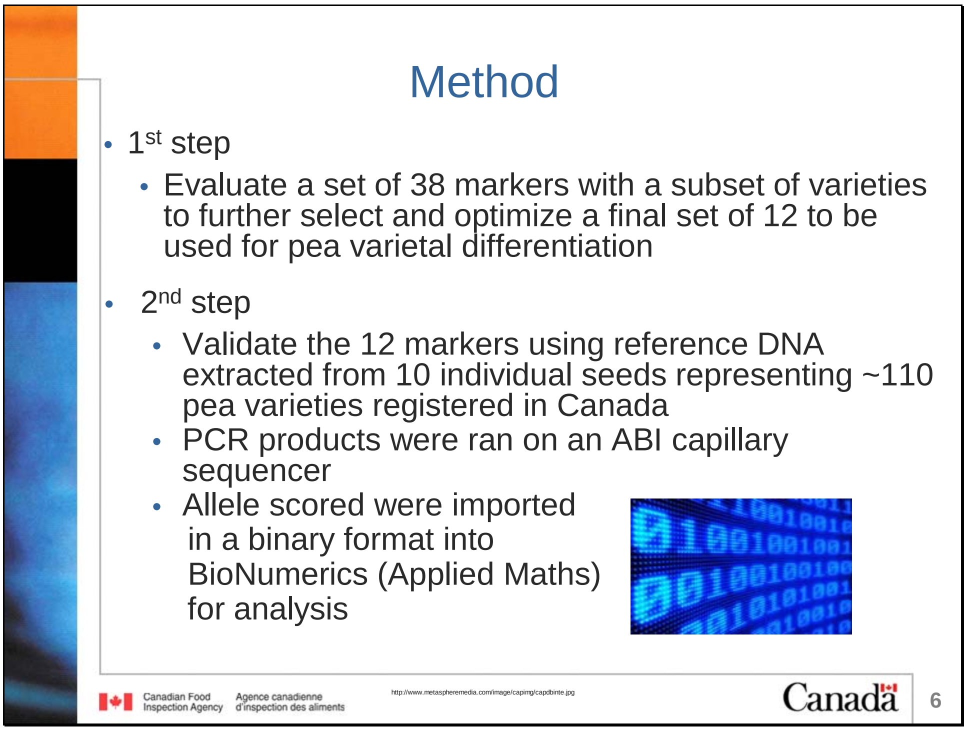


Method

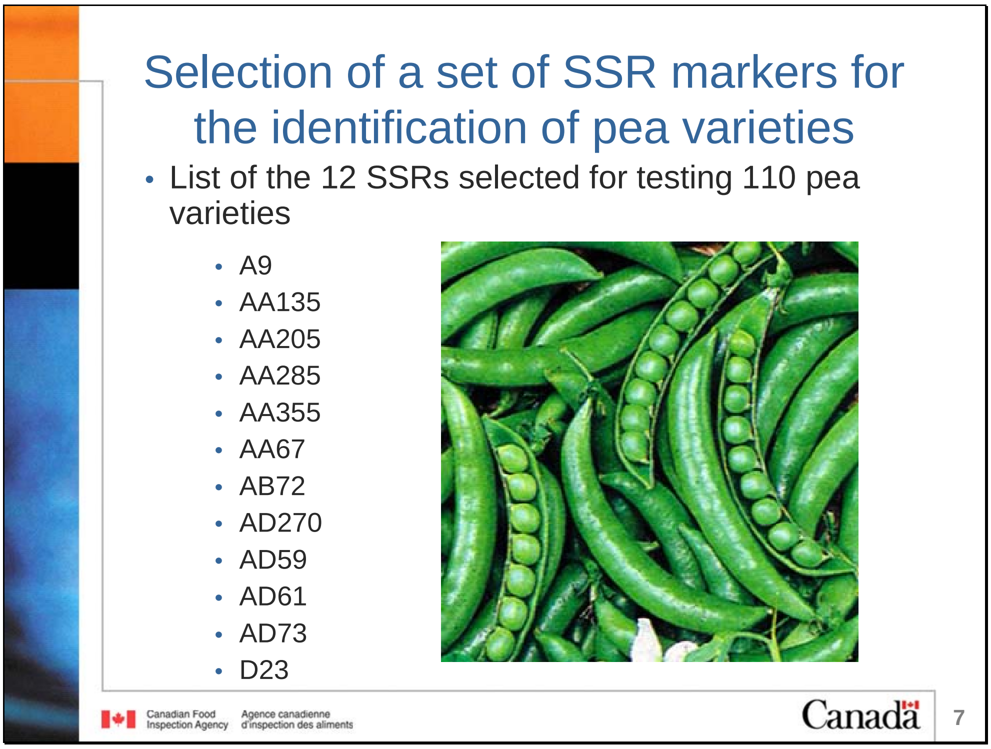
- Reference seeds from all pea varieties registered in Canada was obtained
  - Reference seed lot from each variety was subsampled two independent time – two submissions per variety
  - DNA was extracted from 5 half seeds per submission for a total of 10 individual seed DNA extracts per varieties
  - Qiagen DNeasy kit was used for DNA extraction



Method

- 1st step
  - Evaluate a set of 38 markers with a subset of varieties to further select and optimize a final set of 12 to be used for pea varietal differentiation
- 2nd step
  - Validate the 12 markers using reference DNA extracted from 10 individual seeds representing ~110 pea varieties registered in Canada
  - PCR products were ran on an ABI capillary sequencer
  - Allele scored were imported in a binary format into BioNumerics (Applied Maths) for analysis

http://www.metaspheremedia.com/image/capimg/capdbinte.jpg



Selection of a set of SSR markers for the identification of pea varieties

- List of the 12 SSRs selected for testing 110 pea varieties
  - A9
  - AA135
  - AA205
  - AA285
  - AA355
  - AA67
  - AB72
  - AD270
  - AD59
  - AD61
  - AD73
  - D23

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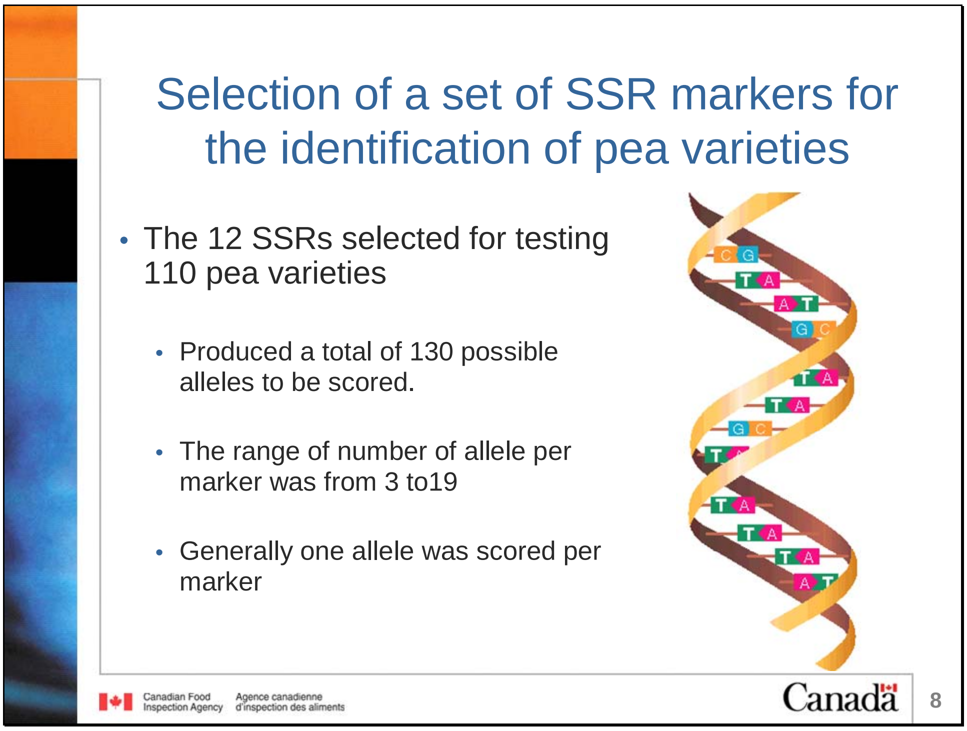

Selection of a set of SSR markers for the identification of pea varieties

- The 12 SSRs selected for testing 110 pea varieties
  - Produced a total of 130 possible alleles to be scored.
  - The range of number of allele per marker was from 3 to19
  - Generally one allele was scored per marker

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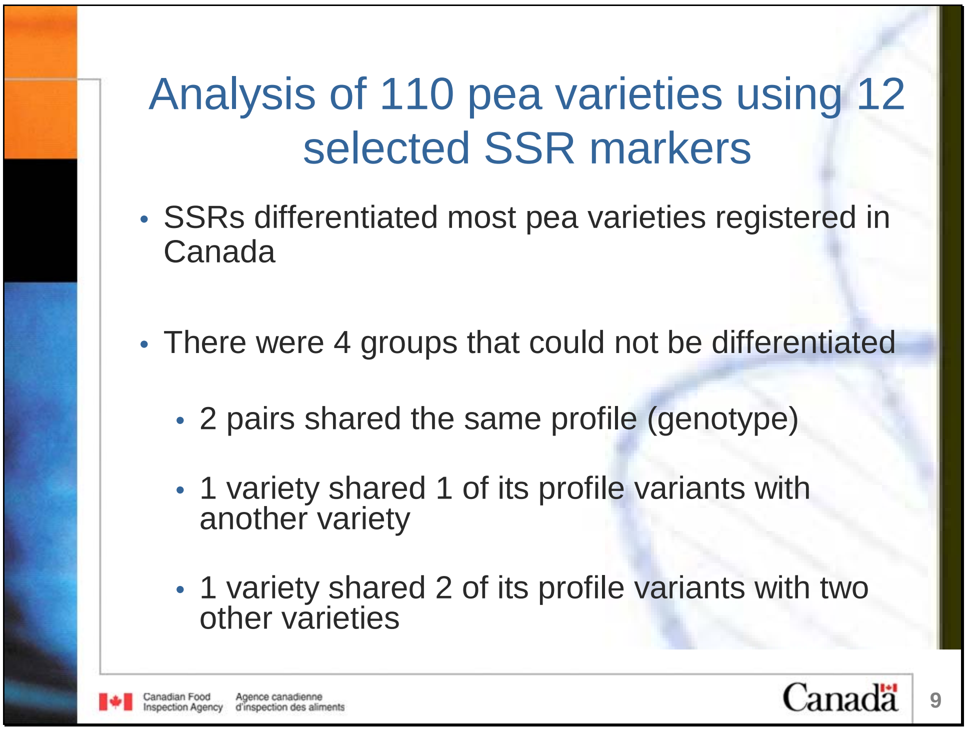
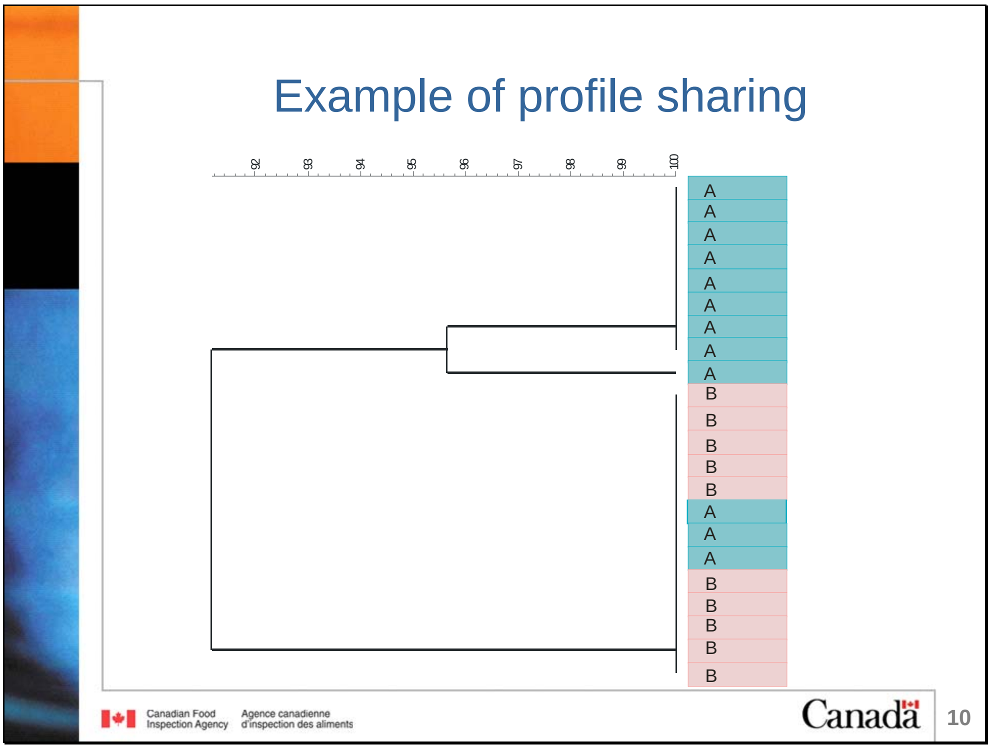
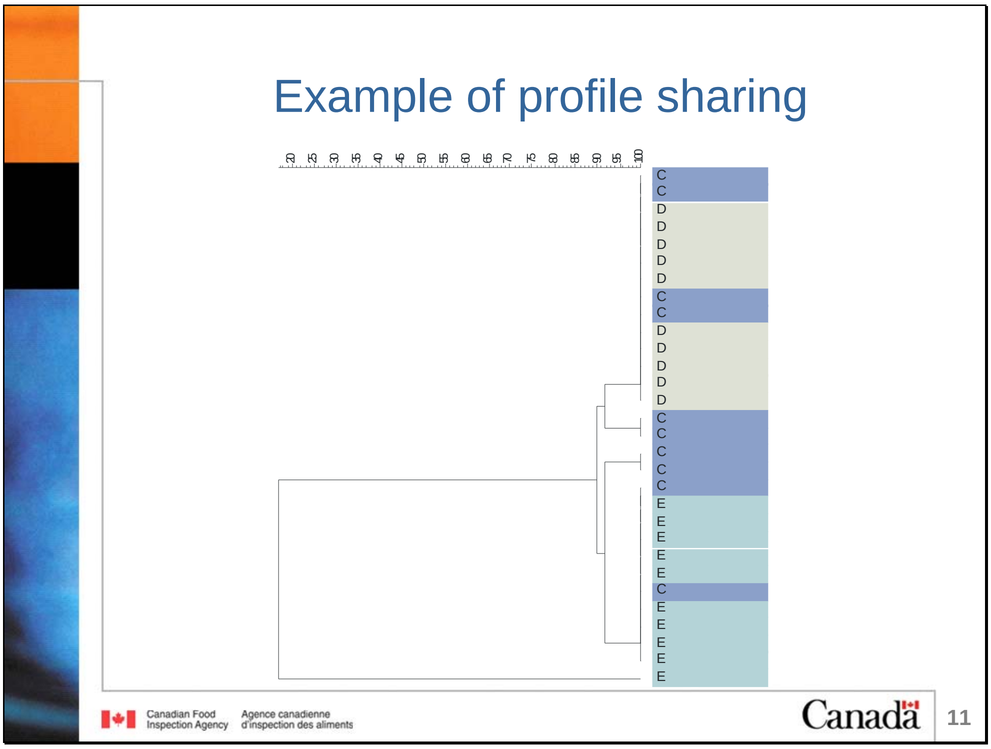


Analysis of 110 pea varieties using 12 selected SSR markers

- SSRs differentiated most pea varieties registered in Canada
- There were 4 groups that could not be differentiated
  - 2 pairs shared the same profile (genotype)
  - 1 variety shared 1 of its profile variants with another variety
  - 1 variety shared 2 of its profile variants with two other varieties



Example of profile sharing



Example of profile sharing

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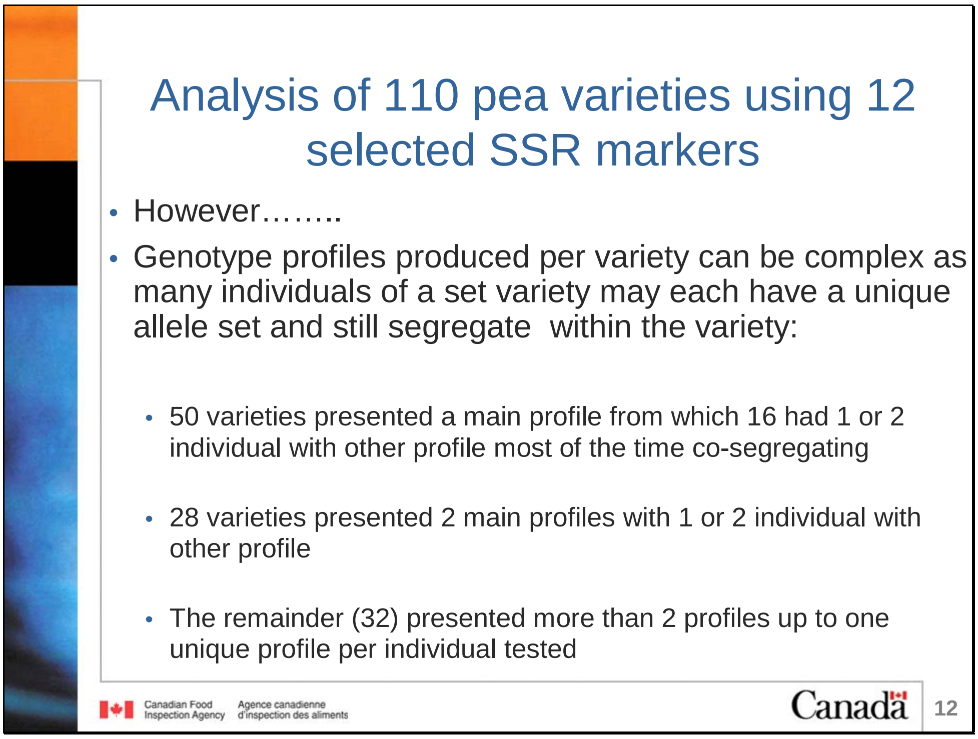

Analysis of 110 pea varieties using 12 selected SSR markers

- However……..
- Genotype profiles produced per variety can be complex as many individuals of a set variety may each have a unique allele set and still segregate within the variety:
  - 50 varieties presented a main profile from which 16 had 1 or 2 individual with other profile most of the time co-segregating
  - 28 varieties presented 2 main profiles with 1 or 2 individual with other profile
  - The remainder (32) presented more than 2 profiles up to one unique profile per individual tested

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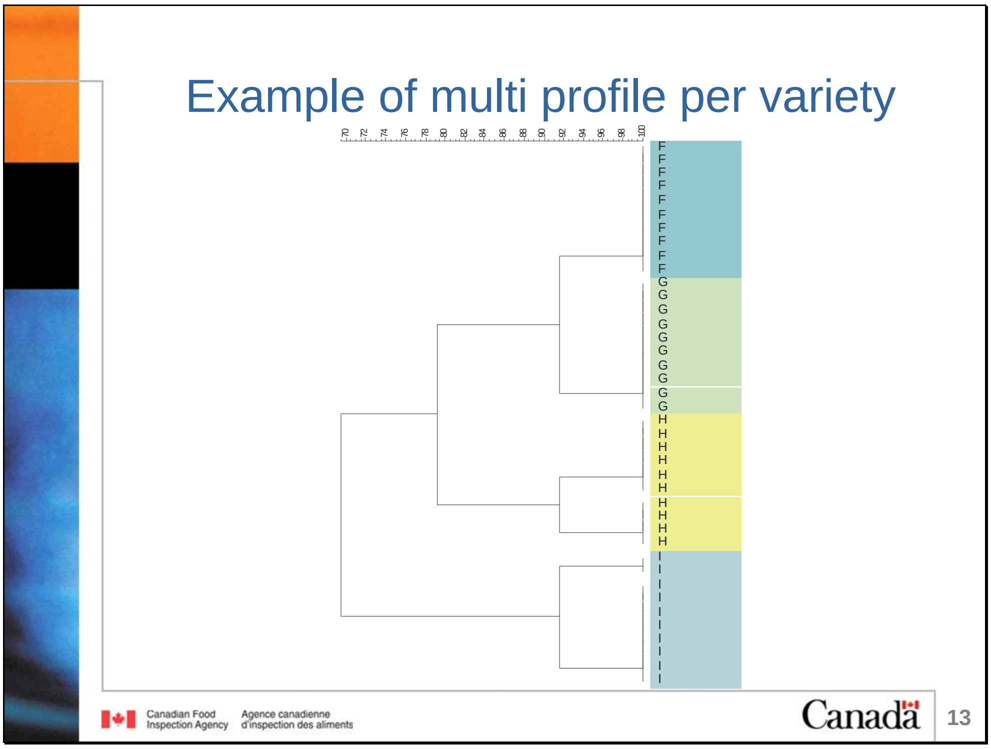

Example of multi profile per variety

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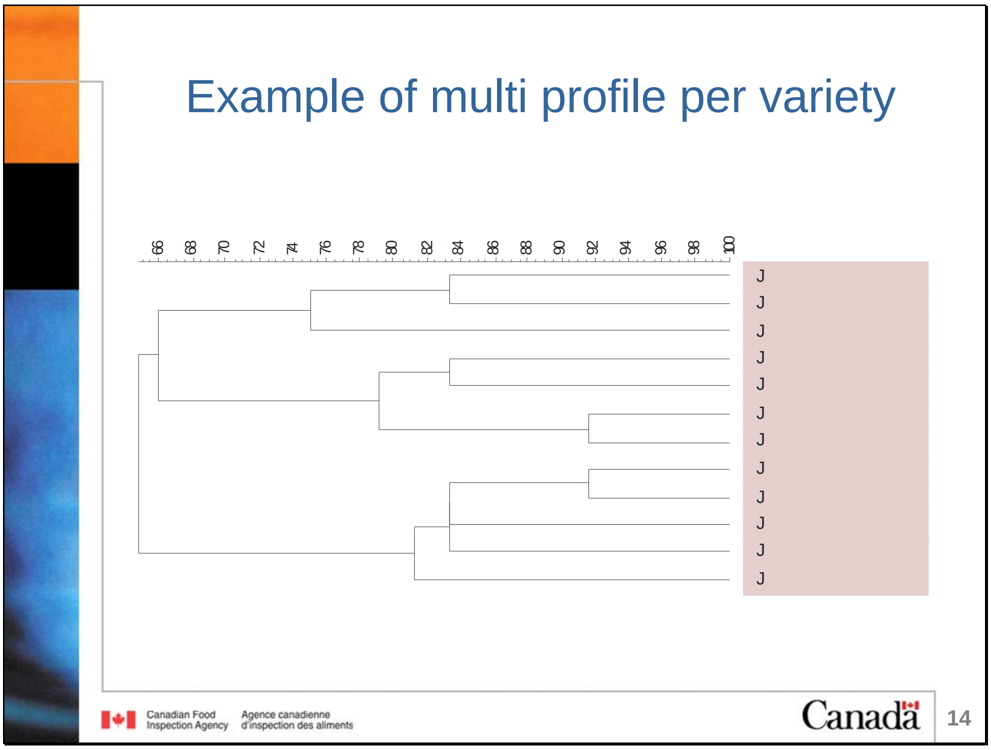

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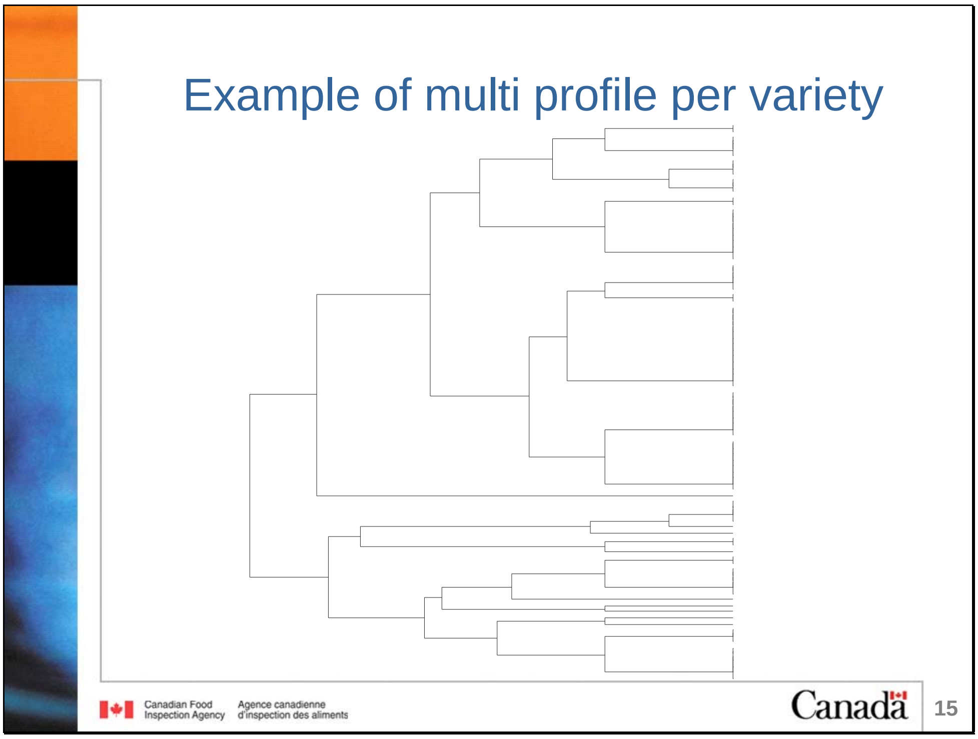
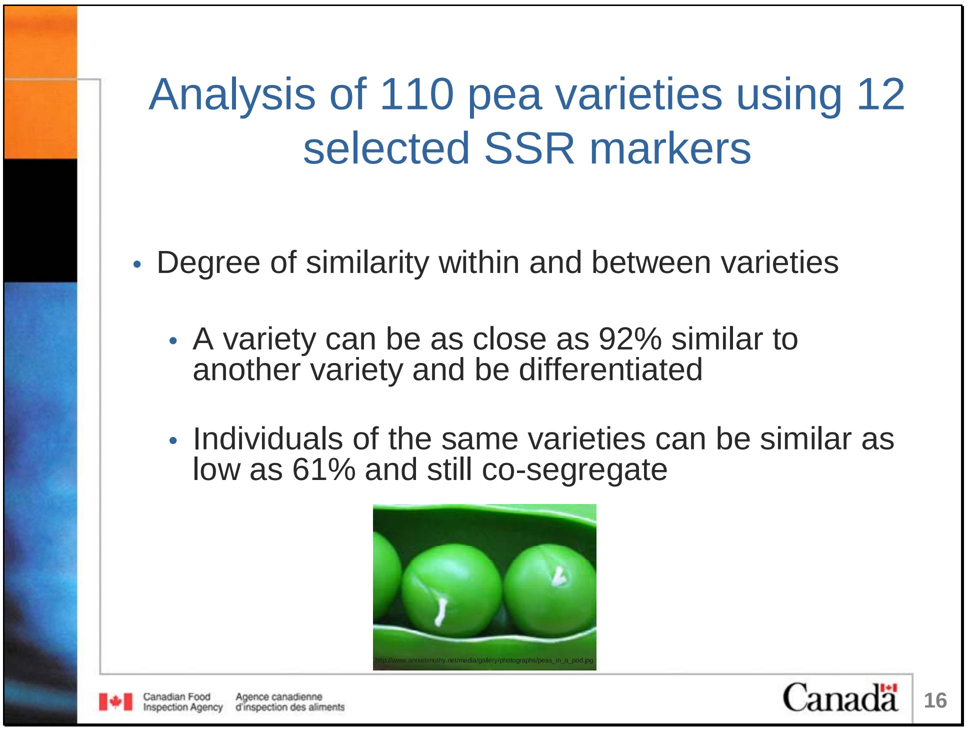


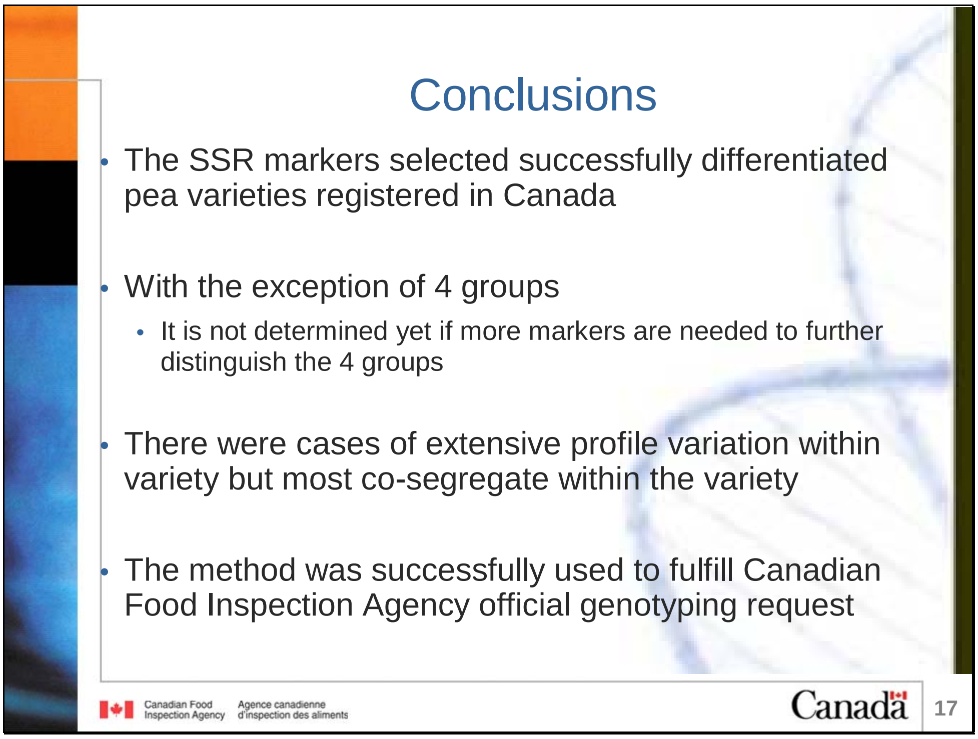
Example of multi profile per variety



Analysis of 110 pea varieties using 12 selected SSR markers

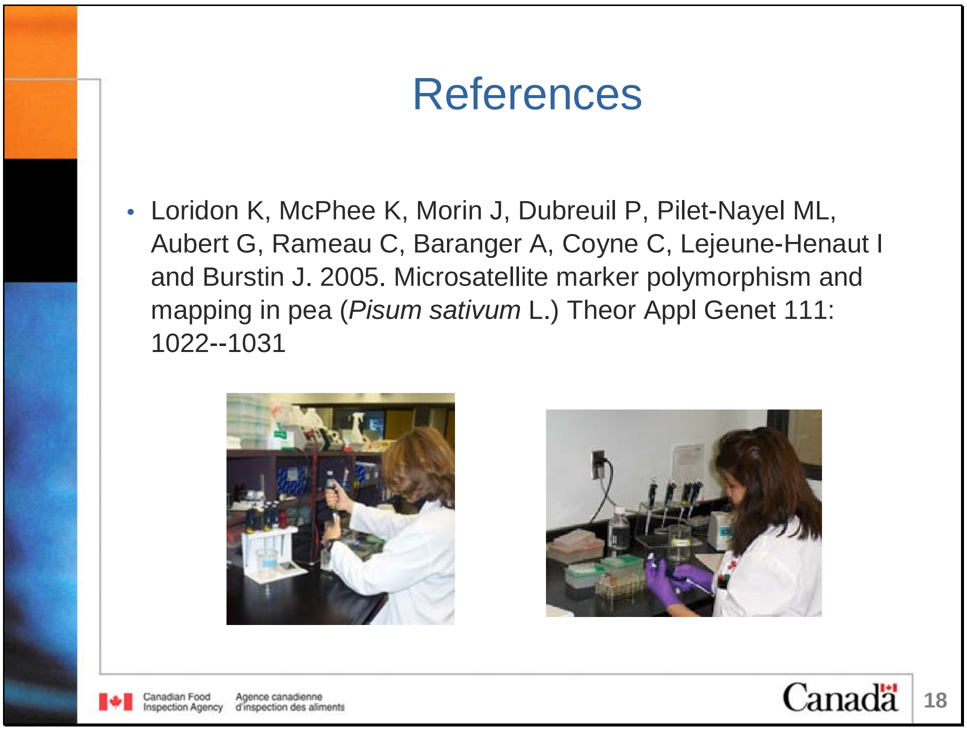
- Degree of similarity within and between varieties
  - A variety can be as close as 92% similar to another variety and be differentiated
  - Individuals of the same varieties can be similar as low as 61% and still co-segregate

http://www.annietimothy.net/media/gallery/photographs/peas_in_a_pod.jpg



Conclusions

- The SSR markers selected successfully differentiated pea varieties registered in Canada
- With the exception of 4 groups
  - It is not determined yet if more markers are needed to further distinguish the 4 groups
- There were cases of extensive profile variation within variety but most co-segregate within the variety
- The method was successfully used to fulfill Canadian Food Inspection Agency official genotyping request



References

- Loridon K, McPhee K, Morin J, Dubreuil P, Pilet-Nayel ML, Aubert G, Rameau C, Baranger A, Coyne C, Lejeune-Henaut I and Burstin J. 2005. Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.) Theor Appl Genet 111: 1022--1031

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