

# On the conservation of the endangered European crab apple (*Malus sylvestris*): threats from hybridization with domesticated apple (*Malus × domestica*).



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## 1 Background

*Malus sylvestris* is a small tree of the Rosaceae family. Its natural distribution range covers the British Islands and continental Europe except for northern Scandinavia and the southern part of the Iberian Peninsula (Fig. 1). The species is influenced severely by habitat modifications and potentially exposed to pronounced hybridization with cultivated relatives (Kleinschmit *et al.*, 2000). Because of its wide distribution and early introduction to Europe, *Malus × domestica* is thought to be the species that has had greatest effect in pollinating the native gene pool of *M. sylvestris*. Hybridization between wild species and their domesticated relatives are likely to reduce the fitness of the wild populations in question and might even lead to their extinction (Rhymer & Simberloff, 1996). The numerous occurrences of phenotypes intermediate of the two hybridizing species, *M. sylvestris* and *M. × domestica*, has led to the hypothesis, that the range of closely related Eurasian species are actually one panmictic species (Kleinschmit *et al.*, 2000).

In the present study, a total of 178 wild individuals from four Danish populations were studied along with a reference sample of 29 old cultivars. Hybridization and genetic variation was studied using ten microsatellite marker loci. A morphological analysis was performed in order to 1) try to identify hybrids occurring in the wild and 2) test for correspondence between phenotype and genotype of the wild individuals studied.



Figure 1 Natural distribution of *Malus sylvestris*.

## 2 Crossability

In general, the species within subfamily Maloideae in Rosaceae is found to have weak genetic barriers to interspecific and intergeneric hybridization (Korban, 1986). In the present study a small scale experiment was set up in order to investigate whether this is also true for the two species *M. sylvestris* and *M. × domestica* (Fig. 2). Controlled pollination was carried out on a total of nine *M. sylvestris* clones by use of two pollen mixtures consisting of (1) five *M. × domestica* cultivars and (2) five individuals of *M. sylvestris*. Fruit set following interspecific (22 of 35 crosses (63%)) and intraspecific (18 of 36 crosses (50%)) was not significantly different.

During the fall 2005 fruits will be harvested and a germination trial carried out in order to further investigate the crossability of the two species.



Figure 2 Isolation of flowers during the pollination trial.

## 3 Microsatellite markers

Large variation was found at the ten microsatellite loci studied as defined by either allele count (A) or gene diversity ( $H_e$ ) (Table 1). Great overlap in allele identities were found between the two species, leaving no chance of identification of hybrids by means of private alleles. Therefore ordination (non-metric multidimensional scaling based on Bowcock *et al.*'s (1994) allele sharing distance) and a model based clustering approach (STRUCTURE, Pritchard *et al.*, 2000) were applied to the data set.

The result was that two genotypically distinct clusters could be identified consisting of wild and cultivated individuals respectively (Fig. 3 and 4). Only very few individuals could be identified as hybrids. In general there was rather poor correspondence between genotypic and morphological indices of hybridization when analysing individual genotypes whereas the correspondence was great at the population level. This is taken to be indicative of genotypically unidentifiable introgression from *M. × domestica*.

Table 1 Description of the loci studied and summary of allelic variation. For each locus are shown the number of individuals scored, n, gene diversity according to Nei (1978),  $H_e$ , and the number of alleles observed, A. Mean for gene diversity and the number of alleles are shown in bold.

| Locus               | A  | Size range | Wild populations |              | Cultivars |            |              |       |
|---------------------|----|------------|------------------|--------------|-----------|------------|--------------|-------|
|                     |    |            | n                | $H_e$        | n         | $H_e$      |              |       |
| NZ5804 <sup>a</sup> | 8  | 90-110     | 178              | 7            | 0.690     | 29         | 7            | 0.774 |
| NZ2364 <sup>a</sup> | 14 | 83-116     | 178              | 12           | 0.666     | 29         | 12           | 0.851 |
| CB0160 <sup>b</sup> | 17 | 162-219    | 178              | 17           | 0.862     | 29         | 12           | 0.898 |
| CB0160 <sup>c</sup> | 20 | 103-148    | 178              | 20           | 0.854     | 29         | 7            | 0.842 |
| CB0160 <sup>d</sup> | 18 | 87-140     | 178              | 17           | 0.851     | 29         | 9            | 0.804 |
| CB0207 <sup>b</sup> | 14 | 94-131     | 178              | 12           | 0.774     | 29         | 8            | 0.780 |
| CB0207 <sup>c</sup> | 18 | 104-149    | 177              | 18           | 0.748     | 29         | 11           | 0.854 |
| CB0206 <sup>b</sup> | 26 | 202-288    | 178              | 26           | 0.886     | 29         | 13           | 0.903 |
| CB0211 <sup>c</sup> | 15 | 207-239    | 178              | 13           | 0.803     | 29         | 11           | 0.878 |
| CB0446 <sup>c</sup> | 17 | 148-203    | 178              | 15           | 0.857     | 29         | 13           | 0.874 |
|                     |    |            | <b>18.8</b>      | <b>0.795</b> |           | <b>9.8</b> | <b>0.846</b> |       |

Marker loci from <sup>a</sup> Guilford *et al.* (1997), <sup>b</sup> Gianfranceschi *et al.* (1998), <sup>c</sup> Liebhard *et al.* (2002)

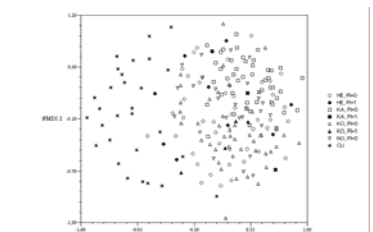


Figure 3 Ordination of individuals based on pairwise allele sharing distances,  $D_{as}$ . Labeling according to sampling location and morphology as given by the pubescence index (PI) described under 'Morphology'.



Figure 4 Proportional assignment,  $q_i$ , of individual genotypes to each of the  $K=2$  inferred clusters in the model based admixture analysis. Individuals are grouped by sampling location.



## 4 Morphology

Analyses of hybridization between *M. sylvestris* and *M. × domestica* has to date been dominated by morphological studies (eg. Remmy & Gruber, 1993) and a wide range of characters has been suggested for the differentiation between the two species and their hybrids (Wagner, 1996). In the present study pubescence of the basal part of long shoots and the inferior surface of leaves from long and spur shoots was described in August on a scale from (0) to (3). The scale ranges from (0): hairless to (3): felted where values above (1) (sparsely hairy; hairs restricted to the main nerves) is indicative of hybrid origin and (3) is typical for *M. × domestica*. Pubescence data was combined into a pubescence index, where (1) indicates hybrid character at one or more of the studied organs and (0) means 'wild type'. Fruits were registered as being of either 'wild' or hybrid type.

From the pubescence index a total of 16 individuals out of the 178 studied were identified as possible hybrids. Fruit characters could only be obtained from ca 40% of the individuals. Both investigations found the proportion of morphologically identified hybrids to vary strongly between the four studied populations (Fig. 5 and 6).

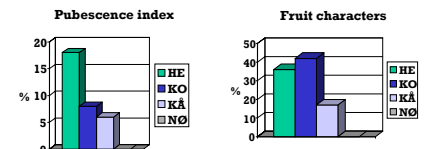


Figure 5 Proportion of individuals in the four populations studied showing signs of hybridization according to pubescence of investigated organs.

Figure 6 Proportion of individuals studied showing signs of hybridization according to fruit character traits.

## 5 Phenology

In addition to genetic incompatibility, difference in flowering phenology is another potential hindrance to interspecific hybridization under natural conditions. In the present study, flowering was registered in two wild populations of *M. sylvestris* along with two nearby orchards of *M. × domestica*. The individuals of the latter represented a range of early to late flowering cultivars.

Beside relatively large variation in individual flowering time within populations of *M. sylvestris*, a marked difference between the two species was observed (Fig. 7).

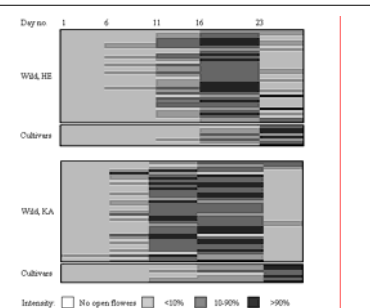


Figure 7 Flowering period for wild and cultivated individuals at sampling location HE (top) and KA (bottom). Day number one was 05.05.05 and last day of registration (23) was 28.05.05. Intensity of flowering is given in percentage of the observed number of flower buds on each tree.

## References

- Gianfranceschi, L., Seglias, N., Tarchini, R., Komjanc, M., Gessler, C., 1998. Simple sequence repeats for the genetic analysis of apple. *Theoretical and Applied Genetics* 96: 1069-1076.
- Guilford, P., Pankab, S., Zhu, J.M., Rikkarik, E., Gardiner, S., Bassett, H., Foster, R., 1997. Microsatellites in *Malus × domestica* (apple): Abundance, polymorphism and cultivar identification. *Theoretical and Applied Genetics* 94: 249-254.
- Kleinschmit, J., Stephan, R., Wagner, I., 2000. Wild fruit trees (*Prunus avium*, *Malus sylvestris* and *Pyrus pyramidalis*) genetic resources conservation strategy. EUFORGEN, International Plant Genetic Resources Institute. [http://www.ipgr.cgiar.org/networks/euforgen/Networks/Noble\\_Hardwoods/Strategies/WildFruitConsStrategy.htm](http://www.ipgr.cgiar.org/networks/euforgen/Networks/Noble_Hardwoods/Strategies/WildFruitConsStrategy.htm).
- Korban, S.S., 1986. Interspecific hybridization in Malus. *Hortscience* 21: 41-48.
- Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., van de Weg, E., Gessler, C., 2002. Development and characterisation of 140 new microsatellites in apple (*Malus × domestica* Borkh.). *Molecular Breeding* 10: 217-241.
- Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.
- Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.
- Remmy, K., Gruber, F., 1993. Untersuchungen zur Verbreitung und Morphologie des Wildapfels (*Malus sylvestris* (L.) Mill.). *Mitteilungen der Deutschen Dendrologischen Gesellschaft* 81: 71-94 (summary in English).
- Rhymer, J.M., Simberloff, D., 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* 27: 83-109.
- Wagner, I., 1996. Zusammenstellung morphologischer Merkmale und der Birnsäurigen (*Pyrus*). *Mitteilungen der Deutschen Dendrologischen Gesellschaft* 82: 87-108 (summary in English).

## 6 Conclusion

Based on molecular data, the present study found individuals of *M. sylvestris* from wild Danish populations and cultivars of *M. × domestica* to form two discrete clusters. This indicates that pronounced admixture between the two species is not present. At the population level, a high correspondence was found between geographic isolation from *M. × domestica* and genotypic and morphological indices of hybridization. As expected, isolated populations appeared less affected by hybridization than poorly isolated populations. Isolated 'pure' *M. sylvestris* populations could thus be identified indicating that genetic isolation of *in situ* protected populations is both possible and advisable. However, morphological and molecular evidence of hybridization was found to be divergent at the individual level. This is suggestive of some historical introgression into the *M. sylvestris* gene pool, and indicates that relying exclusively on either morphological or molecular characters as diagnostic markers in studies of hybridization between *M. × domestica* and *M. sylvestris* might lead to fallible results. Difference in flowering phenology was observed and could in part explain the low level of contemporary hybridization under Danish conditions.

For further information about hybridization between *M. × domestica* and *M. sylvestris* in an ecological perspective:

- Coart, E., Vekemans, X., Smulders, M.J.M., Wagner, I., van Huylenbroeck, J., van Bockstaele, E., Roldán-Ruiz, I., 2003. Genetic variation in the endangered wild apple (*Malus sylvestris* (L.) Mill.) in Belgium as revealed by amplified fragment length polymorphism and microsatellite markers. *Molecular Ecology* 12: 845-857.
- Larsen, A.S., Asmussen, C.B., Coart, E., Orlík, D.C., Kjær, E.D., 2005. Hybridization and genetic variation in Danish populations of European crab apple (*Malus sylvestris*). (Submitted)