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Cytoplasmic genome diversity in the cultivated apple – Short Communication

T. Mikami, K. Kitazaki, Y. Kishima

Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan

Abstract

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The cultivated apple is one of the most common and important fruit crops in temperate regions. Phylogenetic analysis using a wide array of apple genotypes could give insights into the origin and domestication history of this crop. Maternally inherited mitochondrial and chloroplast DNAs have been utilised to characterise the cytoplasmic diversity within the apple germplasm collection and to elucidate the relationships between the cytoplasm types defined. This review focuses on the molecular basis of changes in the mitochondrial genome giving rise to diverse cytoplasm types. The possible maternal lineage of the cultivated apple is also discussed.

Keywords: genomic rearrangements; *Malus* × *domestica* Borkh.; maternal lineage; mitochondrial genome; phylogenetic relationship

The cultivated apple (*Malus* × *domestica* Borkh.) is one of the most ubiquitous and economically important fruit crops in temperate zones. Over 7,000 commercial cultivars have been described in the literature (Way et al. 1990; Janick et al. 1996; Hokanson et al. 2001; Hancock 2004; Patzak et al. 2009), but modern apple orchards are dominated by a limited number of cultivars (Janick et al. 1996; Noiton, Alspach 1996; Patzak et al. 2012). Many breeding programmes utilise only a few well-known cultivars in controlled crosses for commercial apple production (Janick et al. 1996; Noiton, Alspach 1996). Consequently, most apple breeders work with a narrow germplasm base. Such a limited germplasm base has made identifying possible sources of diversity an important aim of apple crop improvement efforts.

Analysis of plant organelle genomes could provide information about the cytoplasmic diversity of crop plants, which is a significant issue if genetic vulnerability. Due, in part, to uniform cytoplasm types, it is to be avoided. Moreover, both chloroplastic (cp) and mitochondrial (mt) DNA polymorphisms might allow maternal lineages to be followed, because in most plant species, including apple, chloroplasts and/or mitochondria are inherited exclusively from the maternal parent (Ishikawa et al. 1992; Robinson et al. 2001; Harris et al. 2002). In apple, cpDNAs and mtDNAs were used to characterise the cytoplasmic diversity within a wide range of germplasm accessions (Ishikawa et al. 1992; Kato et al. 1993a,b, 2012; Savolainen et al. 1995; Robinson et al. 2001; Coart et al. 2006; Wakatsuki et al. 2011; Kitazaki et al. 2013). The objective of this paper is to present a brief overview of progress in our understanding of the distribution of cytoplasmic diversity in apple germplasm collections and of genetic relationships between accessions.

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Chloroplast DNA polymorphism

The chloroplast genome contains several variable regions, the sequences of which were exploited for phylogenetic analysis (Coart et al. 2006). In a survey of 40 apple cultivars, for example, two cpDNA haplotypes were distinguished by nucleotide differences found in the *atp*B-*rbc*L spacer region (Savolainen et al. 1995). Similarly, Robinson et al. (2001) showed that nine apple cultivars could be divided into two groups depending on the occurrence of a duplication in the cp *mat*K-3'*trn*K spacer.

In a large study by Coart et al. (2006), a number of cpDNA markers were used to unravel phylogenetic relationships among 634 wild and 422 domesticated apple accessions. The *mat*K gene locus and two other intergenic regions, *trnH*/*trnK1* and *trnD*/*trnT*, were found to exhibit sufficient variation to define 16 different chloroplast haplotypes. It was of immense interest that *M.* × *domestica* and the wild European crabapple *M. sylvestris* (L.) Mill. share eight chloroplast haplotypes, suggesting a much closer relationship between these two species than presently appreciated. Additionally, the three most frequent chloroplast haplotypes of both species turn out to be nearly absent from the wild Central Asian species *M. sieversii* (Lebed.) examined. This is unexpected because *M. sieversii* was described as the main maternal ancestor of the domesticated apple (Robinson et al. 2001; Harris et al. 2002).

The results from Coart et al. (2006) are in line with those recently reported based on the analysis of a dataset including 46 completely or nearly completely sequenced chloroplast genomes sampled across the genus *Malus* (Nikiforova et al. 2013): the latter study indicated that *M. sylvestris* contributed the chloroplast genome to the majority of *M.* × *domestica* cultivars they examined. Note, however, that the domesticated apple probably did not arise from a single event over a short period of time, but from evolution extending over thousands of years. It may be assumed that *M.* × *domestica* was initially domesticated from *M. sieversii* and subsequently received a significant cytoplasmic contribution from *M. sylvestris*.

Mitochondrial DNA polymorphism

Four different cytoplasm types. Four apple cytoplasmic groups were differentiated by polymorphisms detected with mitochondrial *cox1* and *atp9* gene probes (Ishikawa et al. 1992; Kato et al. 1993a): Golden Delicious type, Delicious type, McIntosh type, and Dolgo Crab type. A large number of European, the US and Canadian ancient cultivars are placed in Golden Delicious, Delicious, and McIntosh types, whereas Chinese crab apples are included in either Delicious or Dolgo Crab type. In order to understand the molecular basis of mitochondrial genome variation giving rise to these diverse cytoplasm types, mtDNA rearrangements involving the *cox1* and *atp9* loci were analysed recently.

***cox*1 locus**. Wakatsuki et al. (2011) showed that cvs Golden Delicious and Delicious each contain an intact *cox1* gene copy (*G-cox1* and *D-cox1*, respectively) and a truncated copy (*G-*Ψ*cox1* and *D-*Ψ*cox1*), and that the two intact and two truncated copies have an 1,115-bp segment in common (Fig. 1). Ancestor/descendant relationships cannot yet be inferred between the *G-cox1* and *D-cox1* arrangements yet. However, on the premise that the ancestral form was the *G-cox1* arrangement, Wakatsuki et al. (2011) proposed the hypothesis that the 1,115-bp segment containing part of *G-cox1* was duplicated in an unknown progenitor genome, thereby generating *G-*Ψ*cox1* (Fig. 1). This was followed by homologous recombination across the 1,115-bp repeat, which gave rise to the *D-cox1* and *D-*Ψ*cox1* arrangements (Fig. 1).

Intriguingly, the *G-cox1* and *G-*Ψ*cox1* sequences are maintained at high copy number in the Golden Delicious and Dolgo Crab cytoplasm type cultivars examined so far, whereas the two sequences were found as substoichiometric forms in the Delicious and McIntosh type cultivars (Wakatsuki et al. 2011; Kitazaki et al. 2013) (Table 1). By contrast, the Delicious and McIntosh type cultivars contained predominantly forms of *D-cox1* and *D-*Ψ*cox1*. Moreover, these two sequences occurred at either substoichiometric or undetectable levels within the mitochondrial genomes of the Golden Delicious and Dolgo Crab type cultivars (Table 1).

***atp9* locus**. The *atp9* gene sequence of Golden Delicious cultivars exists in one intact version and two truncated versions (Ψ*atp9-1* and Ψ*atp9-2*) (Kato et al. 2012). The Ψ*atp9-1* sequence was predominant in the Golden Delicious, McIntosh and Dolgo Crab type cultivars examined but present substoichiometrically in Delicious type cultivars (Kato et al. 2012; Kitazaki et al. 2013) (Table 1). Ψ*atp9-1* was suggested to originate in a homologous recombina-

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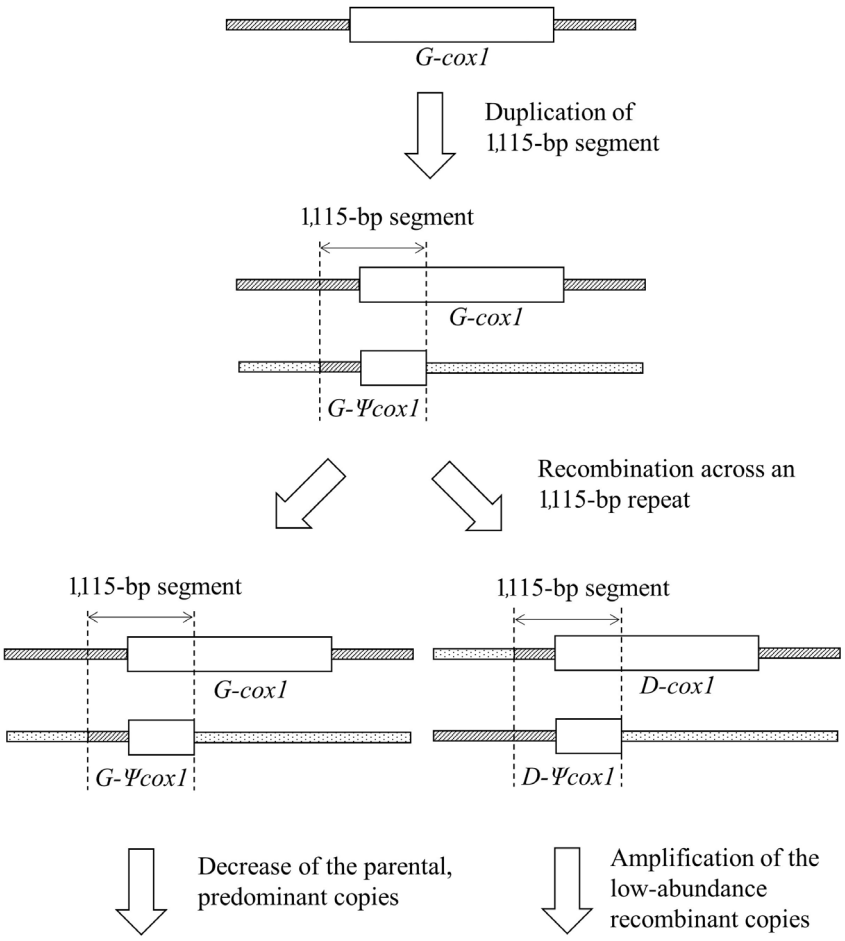

Fig. 1. The presumed structural rearrangements occurred in the cox1 locus, giving rise to diverse cytoplasm types in the cultivated apple

1,115-bp segment containing the 5' coding and its flanking sequences of the progenitor cox1 (*G-cox*1 is hypothesized to be the progenitor form) was duplicated in an ancestral genome to yield a pseudo-*cox*1 copy (*G*-Ψ*cox*1). The *G-cox*1 arrangement subsequently recombined with the G-Ψ*cox*1 arrangement via the 1,115-bp segment, resulting in the generation of the descendant *cox*1 (*D-cox*1) and pseudo-cox1 (*D*-Ψ*cox*1) arrangements. The low-abundance recombined copies consequently coexisted with the parental, predominant copies. This was followed by the change of gene copy numbers, generating novel mitochondrial genome types

tion event mediated by a short repeat in an ancestral mitochondrial genome, and was preferentially amplified in the lineage that led to the Golden Delicious type mitochondrial genome (Kato et al. 2012). On the other hand, Ψ*atp9-2* was revealed to be present in high abundance irrespective of the cytoplasm type (Kato et al. 2012; Kitazaki et al. 2013).

Possible maternal lineages in cultivated apple

Small repeated sequences (shorter than 1,000 bp according to Palmer 1992) are widespread in the mitochondrial genomes of many plant species, and infrequent recombination across the small repeats leads to heteroplasmy. The low-abundance recombined products coexist with the predominant parental forms. An important consequence of mtDNA heteroplasmy is that substoichiometric forms can be amplified and become the predominant form, resulting in the appearance of a novel mitochondrial genome type (Fig. 1). This process, termed substoichiometric shifting (Mackenzie 2007; Kűhn, Gualberto 2012), appears to play a key role in the evolution of apple mtDNA.

Based on mtDNA stoichiometric shifting in combination with cpDNA mutations, Kitazaki et al.

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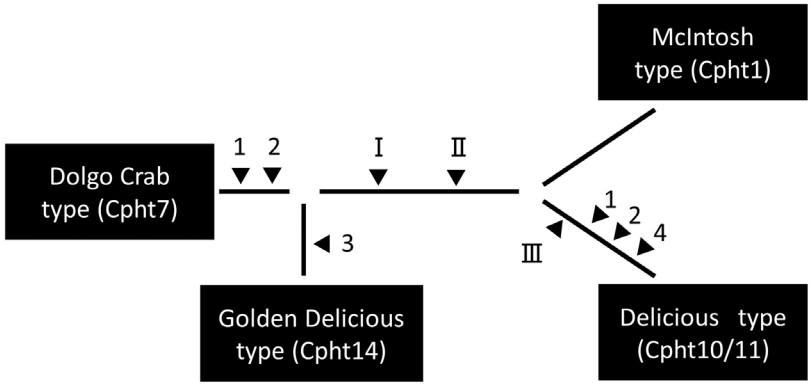

Fig. 2. Relationships among four apple cytoplasm types

roman numerals indicate the changes in the mitochondrial gene loci, which occurred along the path: I – copy number shifts in the *D-cox1* and *D-*Ψ*cox1* sequences; II – copy number shifts in the *G-cox1* and *G-*Ψ*cox1* sequences; III – copy number shifts in the Ψ*atp9-1* sequences. Chloroplast mutations (indicated by Arabic numerals) detected are also shown (see Coart et al. 2006 and Kitazaki et al. 2013 for details): 1 – mutations in the *trn*H/*trn*K1 region; 2 – mutations in the *trn*D/*trn*T region; 3 – a duplication in *mat*K; 4 – transversion in *mat*K

Table 1. Summary of four apple cytoplasm types defined by chloroplast (cp) DNA and mitochondrial (mt) DNA

Cytoplasm type	Cp haplotype	Mt gene copy				
		G-cox1	*G-*Ψ*cox1*	*D-cox1*	*D-*Ψ*cox1*	Ψ*atp9-1*
Golden Delicious	Cpht14	P	P	UD	S	P
Delicious	Cpht10/Cpht11	S	S	P	P	S
McIntosh	Cpht1	S	S	P	P	P
Dolgo Crab	Cpht7	P	P	S	S	P

UD – undetectable; P – predominant form; S – substoichiometric form; Cp – haplotypes are according to Coart et al. (2006)

(2013) built a parsimonious network of apple cytoplasm types showing possible phylogenetic relationships. Four cytoplasm types were connected by five branches (Fig. 2). Because no outgroups were included, the network drawn cannot be polarised. However, it might be appropriate to consider Dolgo Crab cytoplasm type to be ancestral because the chloroplast haplotype (cpht 7) found in this cytoplasm type has never been observed in *M.* × *domestica* (Coart et al. 2006; Kitazaki et al. 2013) (Table 1). If this were the case, one could presume that from Dolgo Crab cytoplasm type, two lineages appeared, distinguishing Golden Delicious cytoplasm type from the other two cytoplasm types (Fig. 2). Dolgo Crab and Golden Delicious cytoplasm types differ by the presence or absence of a duplication in cp *mat*K locus. Amplification of the *D-cox1* and *D-*Ψ*cox1* sequences, as well as the decrease in the *G-cox1* and *G-*Ψ*cox1* sequences may have led to McIntosh cytoplasm type (Fig. 2). In addition, a shift in stoichiometry of the Ψ*atp9-1* sequence and chloroplast mutations may have separated the Delicious cytoplasm type from the McIntosh cytoplasm type (Fig. 2). Nevertheless, there is still much to be discovered about the genetic relationship of apple germplasm accessions. Further research is required with a broader survey of genotypes, which could allow us to better understand the origin and maternal lineages of the cultivated apple.

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Corresponding author:

Dr. Yuji Kishima, Research Faculty of Agriculture, Hokkaido University, Sapporo 060-8589, Japan
phone: + 81 11 706 2439, e-mail: kishima@abs.agr.hokudai.ac.jp