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Recent hybrid speciation in *Cardamine* (Brassicaceae) – conversion of nuclear ribosomal ITS sequences in statu nascendi

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Abstract The very recent allopolyploid speciation of *Cardamine insueta* and *Cardamine schulzii* is well documented. We used this system for a further understanding of the evolution of the internal transcribed spacers (ITS) of nuclear ribosomal DNA in recently formed hybrids. The ITS sequencing of the two parent species and the alloploid offspring suggests a synopsis of the types of ITS evolution, reported so far in the literature. We detected homogenization to one parental ITS type with a very strong bias to the maternal sequence. Nevertheless, maintainance of both parental ITS sequences in the alloploids was also recorded. Our data suggest: (1) rapid genomic change in newly formed alloploids, and (2) a multiple origin of *C. insueta* and *C. schulzii*.

Key words ITS · Nuclear ribosomal DNA · Alloploidization · Homogenization · Concerted evolution · *Cardamine*

Introduction

ITS (internal transcribed spacer) sequences of nuclear ribosomal DNA have become a widely used molecular marker for reconstructing angiosperm phylogenies at lower taxonomic levels (Baldwin et al. 1995; Mummenhoff et al. 1997 a). Despite the high copy numbers of the spacer regions, the ITS paralogues are generally uniform due to concerted evolution via gene conversion or unequal crossing over (Baldwin et al. 1995). Particularly at the generic level and below, speciation via alloploidization is an important evolutionary mechanism (Grant 1981; Soltis and Kuzoff 1995). However, by using ITS sequences to study alloploid taxa, strikingly contrasting results have been reported: (1) in *Krigia* (Kim and Jansen 1994) and in *Arabidopsis suecica* (O'Kane et al. 1996) it was shown that both parental ITS sequences have been maintained in the alloploid species; (2) in *Gossypium* the sequences of alloploids have been homogenized to that of either diploid parental species due to concerted evolution (Wendel et al. 1995 a); and (3) chimeric (mosaic like) ITS repeat types that combine distinct parental motifs have been documented in *Gossypium gossypioides* (Wendel et al. 1995 b), *Microseris* (van Houten et al. 1993) and *Microthlaspi* (Mummenhoff et al. 1997 b). Therefore, further studies are needed to understand ITS evolution after hybridization and polyploidization, especially in alloploid species that are of recent origin. The most recent alloploid species studied so far for ITS variation, i.e. *Arabidopsis suecica*, originated approximately 10 000 years ago (Mummenhoff and Hurka 1995; O'Kane et al. 1996). *Cardamine insueta* Urbanska and *Cardamine schulzii* Urbanska represent alloploids that appear to have arisen in this century at Urnerboden in Central Switzerland, a plateau of 5.5 km in length at 1310–1430 m above sea level (reviewed in Urbanska et al. 1997). *C. insueta* is a natural triploid hybrid ($2n = 3x = 24$) between *Cardamine rivularis* auct. non Schur ($2n = 2x = 16$) (for taxonomy see Marhold 1994, 1995) and *Cardamine amara* L. ($2n = 2x = 16$), that arose by fertilization of an unreduced diploid *C. rivularis* auct. gamete with a reduced haploid *C. amara* gamete. The establishment of *C. insueta* coincided with the installation of man-made hay meadows and, therefore, the origin of *C. insueta* may thus date back to approximately 1901, but definitely not earlier (Urbanska et al. 1997). From this alloploid triploid species *C. schulzii* Urbanska ($2n = 6x = 48$) arose by autopolyploidization. This species has also occupied

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man-made habitats that have been available for 50–70 years (Neuffer and Jahncke 1997; Urbanska et al. 1997).

This model example of man-influenced hybrid speciation in a small and well-defined area has been documented by both biosystematic and molecular approaches (reviewed in Urbanska et al. 1997). From these analysis it is clear that the maternal parent was *C. rivularis* auct. In the present study we analyze the ITS sequence variation of *C. amara*, *C. rivularis* auct., *C. insueta*, and *C. schulzii* to obtain insights into the dynamics of ITS evolution in a very recent natural hybrid species which has arisen during the present century.

Materials and methods

Plant material

In 1993 living plants of *C. amara* L., *C. rivularis* auct. non Schur, *C. insueta* Urbanska and *C. schulzii* Urbanska were collected at Urnerboden, Central Switzerland. The two hybrid species are restricted to this locality and the total area occupied by *C. insueta* and *C. schulzii* is estimated at 25 ha and 0.5 ha, respectively (Urbanska et al. 1997). We analyzed the ITS regions from three individuals of each taxon. Voucher specimens are deposited at OSBU.

Molecular methods

Fresh leaves were taken from individual plants. Total DNA was isolated following the procedure of Doyle and Doyle (1987). Double-stranded DNA of the ITS1 and ITS2 regions was amplified using the polymerase chain reaction (PCR) protocol given in Mummenhoff et al. (1997 b). Primer 18 F was modified as described in Mummenhoff et al. (1997 b). Amplification products were purified using the Quiaquick PCR Purification Kit (Quiagen, Hilden, Germany). Purified DNAs were sequenced by the dideoxy chain-termination method using the *fmol* kit (Serva, Heidelberg, Germany), following the protocol of Mummenhoff et al. (1997 b). The four primers employed for sequencing both strands of the ITS1 and ITS2 regions were 18 F, 5.8 R, 5.8 F, 25 R (for details see Mummenhoff et al. 1997 b). Boundaries of the coding and spacer regions were determined by a comparison of our sequences with that of *Sinapis alba* L. (Rathgeber and Capesius 1990). DNA sequences were aligned by hand.

Results and discussion

The alignment of ITS1 and ITS2 sequences resulted in a matrix of 457 positions (data not shown) and required the introduction of two gaps (1 bp in length for ITS1 and 2 bp in length for ITS2). The ITS sequence matrix in Table 1 contains those nucleotides (23 positions) that distinguish the maternal parent *C. rivularis* auct. from the paternal parent *C. amara*. There was no sequence variation among the three individuals of the

Table 1 Composition of the ITS sequences of *C. amara*, *C. rivularis* auct., *C. insueta* and *C. schulzii*

Taxon	Ploidy level	Position in the ITS alignment (ITS1)												
		55	56	57	63	122	125	138	214	217	221	225	244	247
Paternal parent														
C. amara 1, 2, 3	2*x*	G	A	G	T	C	T	A	C	C	T	C	G	G
C. insueta 1, 2	3*x*	A	G	A	C	A	–	G	A	C	A	T	A	A
C. insueta 3		A	G	A	C	A	–	G	A	C/T	A	T	A	A
C. schulzii 1	6*x*	A	G	A	C	A	–	G	A	C	A	T	A	A
C. schulzii 2, 3		A	G	A	C	A	–	G	A	T	A	T	A	A
C. rivularis auct. 1, 2, 3	2*x*	A	G	A	C	A	–	G	A	T	A	T	A	A
Maternal parent														

Taxon	Ploidy level	Position in the ITS alignment (ITS2)									
		291	309	340	341	374	391	420	421	436	437
Paternal parent											
C. amara 1, 2, 3	2*x*	T	C	G	C	C	C	T	T	–	–
C. insueta 1, 2	3*x*	C/T	T	G	T	T	T	C	A	T	T
C. insueta 3		C/T	T	A	T	T	T	C	A	T	T
C. schulzii 1	6*x*	C	T	A	T	T	T	C	A	T	T
C. schulzii 2, 3		C/T	T	G	T	T	T	C	A	T	T
C. rivularis auct. 1, 2, 3	2*x*	C	T	A	T	T	T	C	A	T	T
Maternal parent											

Note: This data matrix contains those positions of the complete ITS alignment (not shown) that distinguish the paternal parent *C. amara* from the maternal parent *C. rivularis* auct. Numbers refer to the nucleotide position in the complete alignment. Identical sequences of taxa were merged

parental sequences, respectively. In 20 out of the 23 positions (87%) the sequences of the polyploids have been converted to the ITS sequence information of the maternal parent (*C. rivularis* auct.).

Our results correspond in some degree to the findings in *Gossypium*; however, in that study the ITS sequences of the alloploids have been nearly completely homogenized to that of either parental species, due to concerted evolution (Wendel et al. 1995 a). Our example shows that such a homogenization may be a rapid process (less than 100 generations) given the fact that the hybrid species *C. insueta* was not established before approximately 1900. The studies of Song et al. (1995) on synthetic polyploids of *Brassica* also demonstrated that extensive genome changes can occur during each of just five generations. Song et al. (1988, 1995) further suggested that the nuclear DNA composition of each natural and one out of three synthetic *Brassica* alloploids are more closely related to the diploid maternal parent that contributed the cytoplasm to that polyploid. This is in agreement with our results. This would mean that the cytoplasmatic donor plays an important role in the formation of a new polyploid. In *Cardamine*, concerted evolution is apparently operating, given the high homogeneity of ITS sequences within the polyploids. However, this conclusion may not generally be valid for the Brassicaceae. In tetra- and hexa-ploid individuals of *Microthlaspi perfoliatum* (Brassicaceae) Mummenhoff et al. (1997 b) found a chimeric ITS type that combines motifs from both parental lineages, a scenario also reported for *Gossypium* (Wendel et al. 1995 b) and *Microseris* (van Houten et al. 1993).

Our evidence for rapid rDNA conversion in *Cardamine* hybrids may be explained by a PCR artefact, due to an asymmetric genome constitution. *C. insueta* (genome constitution RRA) and *C. schulzii* (RRRRAA) have two/four copies of *C. rivularis* (R) chromosomes and only one/two copies of *C. amara* (A) chromosomes, respectively. Thus, the copy number of one rDNA variant (*C. rivularis* auct.) is double the number of the other variant (*C. amara*) in both hybrids. It may be, that the PCR products obtained from the hybrid species are strongly biased to that of the predominant parental (*C. rivularis* auct.) ITS type. In order to exclude such a PCR artefact we mixed the DNAs isolated from the parent species in a ratio of 2 : 1. This corresponds to the expected ratio of the parental rDNA types in the hybrid species due to the genome constitution of RRA and RRRRAA in *C. insueta* and *C. schulzii*, respectively. In this control experiment we detected both parental ITS sequences, suggesting that the ITS sequences we obtained from the hybrid species do not suffer from PCR artefacts.

In positions 217 and 340 the paternal nucleotide is found in some of the polyploid accessions. This would mean that homogenization to the paternal ITS type is also possible. Homogenization to both parental sequences, however with a strong bias to the maternal parent, would indicate a somewhat mosaic-like structure. The detection of either parental nucleotide at a certain position in different individuals of the polyploid hybrids (Table 1) points to a multiple origin of *C. insueta* and *C. schulzii*. A multiple origin of *C. insueta* was previously suggested by Neuffer and Jahncke (1997) whereas *C. schulzii* appears to have originated only once, in contrast to our results.

At two positions (217: *C. insueta* 3; and 291: *C. insueta* 1, 2, 3 and *C. schulzii* 2, 3) we detected additivity/polymorphisms of both parental ITS types within individual plants. The maintainance of both parental sequences indicates that the hybridization event was recent (O'Kane et al. 1996). Our analysis in *Cardamine* seems to represent a study of ITS evolution in statu nascendi. In less than 100 years the homogenization of 87% of the variable nucleotide positions to the maternal parent (cytoplasmic donor) was completed, suggesting that "the native cell cytoplasm may provide a selection pressure on portions of the foreign nuclear genome stabilizing the newly produced polyploid by establishing a harmonious relationship between cytoplasmic and nuclear genomes" (Soltis and Soltis 1995). It may be speculated that the remaining two nucleotide positions, that show additivity of the parental ITS types, may be homogenized in the near future.

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References

Baldwin BG, Sanderson MJ, Porter JM, Wojciechowski MF, Campbell CS, Donoghue MJ (1995) The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Mo Bot Gard 82 : 247–277

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19 : 11–15

Grant V (1981) Plant speciation, 2nd edn. Columbia University Press, New York

Houten WHJ van, Scarlett N, Bachmann K (1993) Nuclear DNA markers of the Australian tetraploid *Microseris scapigera* and its North American diploid relatives. Theor Appl Genet 87 : 498–505

Kim KJ, Jansen RK (1994) Comparisions of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia*): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. Plant Syst Evol 190 : 157–185

Marhold K (1994) Taxonomy of the genus *Cardamine* L. (Cruciferae) in the Carpathians and Pannnonia. 1. *Cardamine pratensis* group. Folia Geobot Phytotax 29 : 335–374

Marhold K (1995) *Cardamine rivularis* auct. non Schur in the Eastern Alps. Carinthia II (Sonderheft) 53 : 101–102

Mummenhoff K, Hurka H (1995) Allopolyploid origin of *Arabidopsis suecica* (Fries) Norrlin: evidence from chloroplast and genome markers. Bot Acta 108 : 403–466

Mummenhoff K, Franzke A, Koch M (1997 a) Molecular data reveal convergence in fruit characters used in the classification of *Thlaspi* s. l. (Brassicaceae). Bot J Linn Soc 125 : 183–199

Mummenhoff K, Franzke A, Koch M (1997 b) Molecular phylogenetics of *Thlaspi* s.l. (Brassicaceae) based on chloroplast DNA restriction-site variation and sequences of the internal transcribed spacers of nuclear ribosomal DNA. Can J Bot 75 : 469–482

Neuffer B, Jahncke P (1997) RAPD analysis of hybridization events in *Cardamin*e (Brassicaceae). Folia Geobot Phytotax 23 : 57–67

O'Kane Jr. SL, Schaal BA, Al-Shehbaz IA (1996) The origins of *Arabidopsis suecica* as indicated by nuclear rDNA sequences. Systematic Bot 21 : 559–566

Rathgeber J, Capesius I (1990) Nucleotide sequence of the intergenetic spacer and the 18S ribosomal RNA gene from mustard (*Sinapis alba*) DNA. Nucleic Acids Res 18 : 1288

Song K, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLPs). 1. Genome evolution of diploid and amphiploid species. Theor Appl Genet 75 : 784–794

Song K, Lu P, Tang K, Osborn TC (1995) Rapid genome change in synthetic polyploids of Brassicaceae and its implications for polyploid evolution. Proc Natl Acad Sci USA 92 : 7719–7723

Soltis DE, Kuzoff RK (1995) Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). Evolution 49 : 727–742

Soltis DE, Soltis PS (1995) The dynamic nature of polyploid genomes. Proc Natl Acad Sci USA 92 : 8089–8091

Urbanska KM, Hurka H, Landolt E, Neuffer B, Mummenhoff K (1997) Hybridization and evolution in *Cardamine* (Brassicaceae) at Urnerboden, Central Switzerland: biosystematic and molecular evidence. Plant Syst Evol 204 : 233–256

Wendel JF, Schnabel A, Seelanan T (1995 a) Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). Proc Natl Acad Sci USA 92 : 280–284

Wendel JF, Schnabel A, Seelanan T (1995 b) An unusual ribosomal DNA sequence from *Gossypium gossypioides* reveals ancient, cryptic, intergenomic introgression. Mol Phylog Evol 4 : 298–313