9

Application of Molecular Cytogenetics in Fundamental and Applied Research of Potato

Tatjana Gavrilenko

ABSTRACT

Potato is the fourth most important crop worldwide. An integrated genetic and physical map is needed to advance genomics research of this important crop species. In this chapter we describe recent results of the application of fluorescence in situ hybridization (FISH) analyses for development of a molecular cytogenetic map of potato, physical mapping in conjunction with the potato genome sequencing project, improvement of structural genomics studies, and comparative mapping of potato and related species. Like FISH, genomic in situ hybridization (GISH) is an important tool for evolutionary genome studies. GISH has been successfully applied to distinguish between subgenomes of wild allopolyploid potato species. Application of molecular cytogenetics to potato genome analysis has greatly advanced both fundamental and applied research of this important crop species.

Keywords: BAC, cytogenetics, FISH, potato

9.1 Introduction

Development of plant molecular cytogenetics was reviewed by Puertas and Naranjo (2008), Jiang and Gill (1994, 2006), and Schwarzacher (2003). The history of potato cytogenetics was reviewed in our previous paper (Gavrilenko 2007). Here we consider achievements in this field of potato research made during recent years with special attention to the application of fluorescence in situ hybridization (FISH) in potato genetics and breeding.

N.I. Vavilov Institute of Plant Industry, B. Morskaya Str. 42/44, 190000, St. Petersburg, Russia; e-mail: *tatjana9972@yandex.ru*

9.2 FISH Karyotyping and Physical Mapping of Potato Chromosomes

Potatoes encompass the tuber-bearing species of section *Petota* of the genus *Solanum* and include diploid, triploid, tetraploid, pentaploid and hexaploid species, with the basic chromosome number of twelve ($x = 12$). Within the karyotype of common potato (*Solanum tuberosum*; $2n = 4x = 48$), identification of all individual chromosomes by conventional methods (such as pachytene karyotyping or Giemsa C-banding) can be difficult (Gavrilenko 2007). Another limitation is the absence of cytogenetic stocks in potato such as monosomic and nullisomic lines or structural chromosome mutants, which allow scientists to locate genetically-mapped DNA markers or genes to specific chromosomes or chromosomal segments. Such an approach was widely used to construct cytogenetic maps in cereals (Künzel et al. 2000; Werner et al. 1992) and *Solanum lycopersicum* (tomato), a close relative of potato (Khush and Rick 1968). These limitations in potato cytogenetics have been overcome by application of fluorescent in situ hybridization (FISH).

FISH is the main technique of molecular cytogenetics. FISH methods use fluorochrome-labeled probes to detect specific DNA sequences in cytological preparations and to localize physically on chromosomes unique, low-copy-number or repetitive DNA sequences (Jiang and Gill 2006). FISH techniques allow one to identify chromosomes and chromosomal segments in plant species with small, numerous chromosomes and in species with morphologically indistinguishable chromosomes. Recently, modifications of basic FISH methods have extended their use in plant genomic research (Jiang and Gill 2006). During recent years, rapid progress has been made in potato FISH-based karyotyping and in construction of integrated chromosomal maps of potato. Application of molecular cytogenetics to potato genome analysis has greatly advanced both fundamental and applied research of this important crop species.

9.2.1 FISH-based Chromosome Identification and Integration of Chromosomal and Genetic Maps of Potato

FISH provides an alternative approach for identification of small plant chromosomes (Jiang and Gill 1994; Jiang et al. 1995). This approach is based on direct hybridization of genetic marker-anchored bacterial artificial chromosome (BAC) clones to mitotic metaphase chromosomes. FISH mapping of BAC clones anchored with previously genetically mapped restriction fragment length polymorphism (RFLP) markers helped to select a set of chromosome-specific cytogenetic DNA markers (CSCDM) (Dong et al. 2000; Song et al. 2000). Using CSCDMs, all 12 linkage groups have been associated with specific potato chromosomes (Dong et al. 2000). This study first integrated genetic linkage groups with chromosomes of potato.

BAC-FISH mapping has also been used for high-resolution mapping of potato pachytene chromosomes. Iovene et al. (2008) reported construction of a cytogenetic map of potato chromosome VI by FISH mapping of BAC clones that contained different amplified fragment length polymorphism (AFLP) markers from linkage group 6 of potato. Potato genotypes included in this FISH mapping effort were the same as those used to develop an ultradense genetic (AFLP) recombinant map of potato (van Os et al. 2006). Two approaches were used for BAC-FISH mapping—reprobing of the same preparations with different probes and mapping of multiple (up to eight different) BAC probes onto the same preparation (Fig. 9-1a, b). In total, 13 BACs were mapped on the short arm and 17 BACs on the long arm of the pachytene chromosome VI with an average interval spacing of ~ 3 cM. Nine BACs were also placed in the pericentromeric heterochromatin region (Iovene et al. 2008).

This chromosomal map helped to reveal the euchromatic or heterochromatic locations of 30 AFLP markers and to evaluate recombination frequencies along chromosome VI. While marker order along both the long and short arms was consistent between the genetic linkage and cytological maps, comparison of the maps revealed that the pericentromeric heterochromatin of potato chromosome VI experiences a suppression of genetic recombination (Iovene et al. 2008). Significant suppression of genetic recombination in the pericentromeric regions of potato has been reported previously, being described as "cold spots for recombination" and revealed as regions with a strong clustering of genetically mapped markers (van Os et al. 2006). However, linkage maps alone are less effective at revealing suppression of recombination than comparison of linkage and physical maps.

In an independent study, another six potato AFLP marker-anchored BAC clones were cytogenetically mapped on the short arm of the chromosome VI of six different diploid potato genotypes (Tang et al. 2008). BAC-FISH mapping revealed minor putative rearrangements in the short arm of chromosome VI in one diploid genotype derived from hybridization of *S. sparsipilum* and *S. tuberosum* (Fig. 9-1c; Tang et al. 2008).

Recently a cytogenetic map of all 12 chromosomes was developed for potato (*S. tuberosum*) using BAC-FISH technology (Tang et al. 2009). In this study a set of 60 AFLP anchored BAC clones (five BACs per chromosome) was located on meiotic chromosomes of a diploid genotype, yielding a standard potato pachytene karyotype.

BAC-FISH mapping has also been an important complement to the potato genome sequencing project. One hundred-fifty-eight BAC clones were assigned to the 12 potato pachytene chromosomes by BAC-FISH (Visser et al. 2009). This helped define the boundary between the euchromatin and pericentromeric heterochromatin and to verify the distal terminal regions of

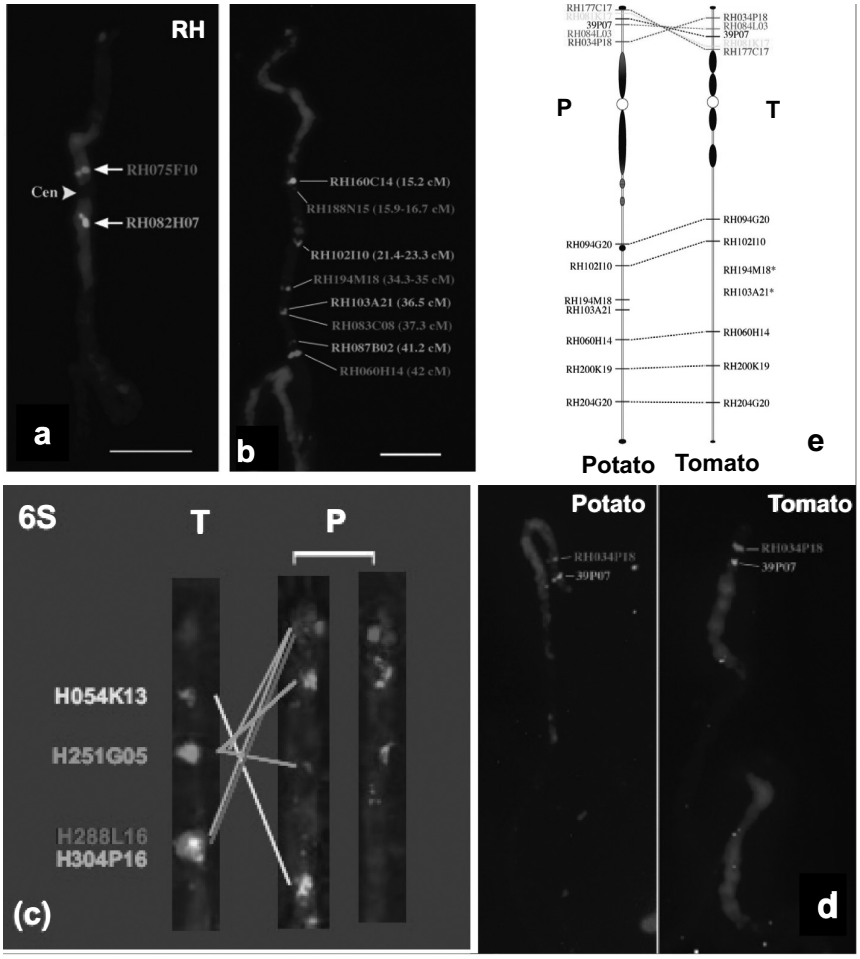

Figure 9-1 FISH mapping of AFLP marker-anchored BAC clones on potato pachytene chromosome VI.

(Panels a-b and d-e – reprinted with permission from Iovene et al. 2008; panel c—reprinted with permission from Tang et al. 2008). (a) Determination of the genetic position of the centromere of potato chromosome VI of genotype RH by FISH mapping of BACs RH075F10 and RH082H07. Scale bar = 5 µm. (b) FISH mapping of eight BACs located in the euchromatic region on the long arm of the chromosome. Scale bar = 5 µm. (c) Cross-species FISH of tomato BACs on the short arm of pachytene chromosome VI of tomato (T) and potato (P) showed inverted order between the homeologues. BAC H251G05 (green) produced a large and small focus on the potato chromosome, suggesting a breakpoint in this BAC and a putative chromosomal rearrangement (Tang et al. 2008). (d) Cross-species FISH of potato BACs on the short arm of pachytene chromosome VI of potato (P) and tomato (T). (e) The comparative chromosomal positions of potato BACs on potato (P) and tomato (T) pachytene chromosome VI. Reproduced with permission of Genetics Society of America, Genetics Editorial Office.

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potato chromosomes (Visser et al. 2009). BAC-FISH also helped to estimate the physical sizes of genetic gaps on chromosome V and VIII and to verify the centromere position of chromosome XII (Tang et al. 2009).

9.2.2 Comparative Mapping of Potato and Related Species using BAC-FISH

BAC-FISH mapping has also been used to study karyotype evolution and the genetic colinearity between genomes of related plant species. In comparative BAC-FISH mapping (or cross-species BAC-FISH painting) DNA probes of one species are hybridized to chromosomes of another species. Recently two independent groups have reported the comparative cytogenetic mapping of potato and tomato chromosome VI. FISH mapping was performed with 15 potato BACs on tomato pachytene chromosome 6 (Iovene et al. 2008) and with 25 tomato BACs on potato chromosomes VI (Tang et al. 2008). Both studies revealed colinearity of the BACs between tomato and potato in the long arm of chromosome VI and an inversion in the euchromatic region of the short arm (6S) (Fig. 9-1c-e). The 6S inversion was not detected by previous comparative genetic linkage mapping in potato and tomato that revealed only paracentric inversions (inversions that did not involve the centromere) in chromosomes V, IX, X, XI and XII (Bonierbale et al. 1988; Gebhardt et al. 1991; Tanksley et al. 1992). Construction of the cytogenetic maps for the remaining chromosomes of potato and their comparative cytogenetic mapping with tomato may provide new evidence of karyotype evolution of two of the most important crops in the Solanaceae.

Besides chromosomal rearrangements, comparative analysis of BAC end sequences revealed differences between potato and tomato at the genomic level in ribosomal DNA (rDNA) content, in telomere-related sequences, and in a number of unclassified sequences, suggesting the importance of repetitive sequences divergence in evolution of these species (Zhu et al. 2008).

9.2.3 FISH Mapping of Chromosomal Domains and Various Repetitive Sequences

The FISH technique has been successfully applied for physical mapping on potato chromosomes of specific chromosomal domains, such as centromere, heterochromatin, and nucleolar organizer regions and different types of repeats.

FISH mapping of (AFLP) marker-anchored BAC clones revealed the genetic position of the centromeres on potato chromosomes VI (Fig. 9-1a; Iovene et al. 2008) and XII (Tang et al. 2009). This approach also helped to establish the distribution of heterochromatic regions along chromosome

VI: the genetic position of the euchromatin–heterochromatin boundaries, the pericentromeric heterochromatin and the interstitial heterochromatic knob on the long arm of this chromosome (Iovene et al. 2008; Visser et al. 2009).

The FISH technique has also been used to assess the chromosomal position and number of ribosomal DNA tandem repeats in the potato karyotype. 5S rDNA and 45S rDNA loci were positioned as distinct loci on potato chromosomes I and II by simultaneous hybridization of rDNA probes and CSCDMs, each labeled with a different fluorochrome (Dong et al. 2000). Thus, both repeats (45S and 5S rDNA) can also be used for identification of individual chromosomes in potato. Similar numbers and locations of 5S rDNA and 45S rDNA loci have been detected in other tested diploid potato species; in the analyzed polyploid species the number of these loci corresponds to species ploidy level (Fig. 9-2c-e).

FISH has also revealed the number and chromosomal distribution of other tandem repeats on potato chromosomes. For example, intergenic spacer (IGS)-related repeats have been located by FISH to a pericentromeric heterochromatic region on a single chromosome of *S. tuberosum* (Stupar et al. 2002). Another type of tandem repeat, the interstitial telomeric repeat (ITR), has been found mainly in centromeric and pericentromeric regions of potato chromosomes (Tek and Jiang 2004). Tang et al. (2008) suggested that these telomere sequences possibly resulted from inversion events. Both repeats (IGS and ITR) were mapped by FISH to a different number of chromosomes in several potato species and were highly diverged in structure and copy number among the tested species (Stupar et al. 2002; Tek and Jiang 2004). Also using FISH mapping, BACs containing telomere-related repeats have been localized in centromeric and pericentromeric regions of several chromosomes of *S. tuberosum* (Zhu et al. 2008).

Besides ITRs and IGSs, repeats that are widely distributed among potato species, species-specific repeats have also been found in *Solanum* using Southern blot analysis. FISH has been subsequently used to visualize their chromosomal locations in specific species. The tandemly repeated element Sobo from the diploid potato species *S. bulbocastanum* was not detected in closely related Mexican potatoes or in other, more distantly related *Solanum* species (Tek et al. 2005). At the same time, intraspecific variation was detected in the presence/absence of this repeat among the tested accessions of *S. bulbocastanum*. Sequence analysis revealed that the Sobo repeat includes regions which display similarity to the long terminal repeat (LTR) of a retrotransposon (Tek et al. 2005). Sobo was mapped as a single locus in the pericentromeric region of chromosome VII using simultaneous BAC-FISH mapping of CSCDMs and a BAC clone containing the Sobo repeat.

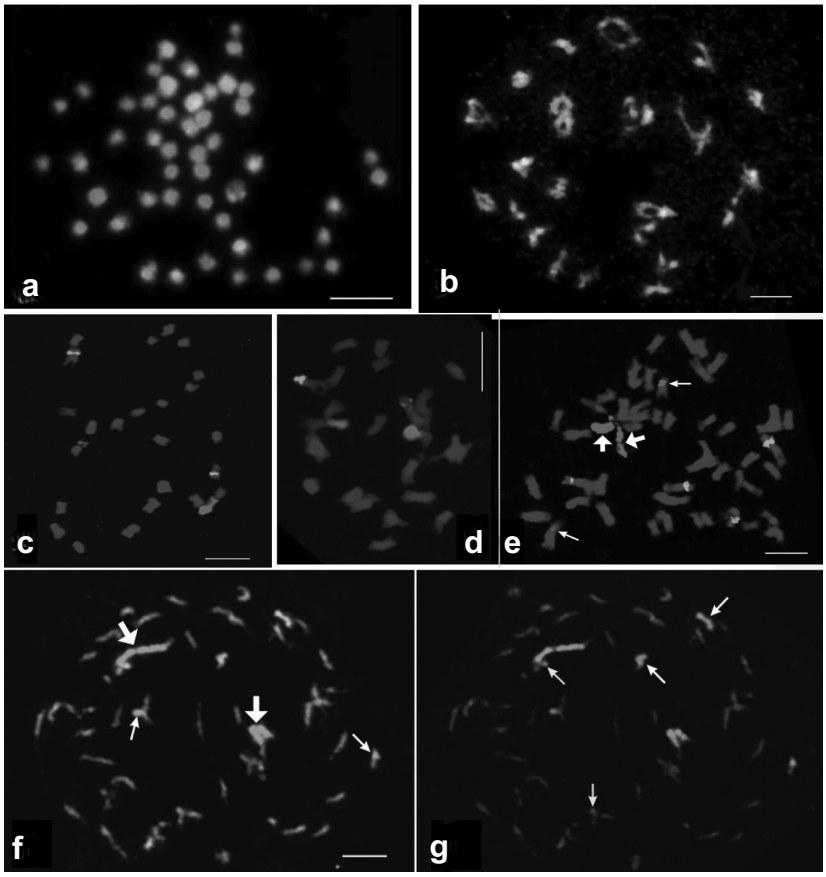

Figure 9-2 GISH analysis of series *Longipedicellata* tetraploid species *Solanum stoloniferum* ($2n = 4x = 48$) reprinted with permission from Pendinen et al. 2008. (a) Somatic chromosomes of *S. stoloniferum* probed with labeled DNA from its putative diploid ($2n = 2x = 24$) progenitor species - *S. verrucosum* (red) and *S. jamesii* (green). (b) GISH analysis of chromosomal pairing in diakinesis of *S. stoloniferum*. Pairing between *A* genome chromosomes (red, detected by labeled DNA of *S. verrucosum*) and *B* genome chromosomes (green, detected by labeled DNA of *S. jamesii*) were not observed.

(c-e) FISH mapping of 45S rDNA (red) and 5S rDNA (green) in (e) tetraploid *S. stoloniferum* and its putative diploid progenitor species (c) *S. verrucosum* and (d) *S. jamesii*. In *S. stoloniferum*, 45S rDNA hybridization sites were observed (large and small arrows). (f) Somatic chromosomes of *S. stoloniferum* probed with labeled genomic DNA from *S. verrucosum* (red) and *S. andreanum* (green). A large fragment (big arrows) and a small fragment (small arrows) from two pairs of *S. stoloniferum* chromosomes showed bright green color. Color differentiation was not observed on the rest of the chromosomes. (g) The same metaphase cell as in (f) was hybridized with 45S ribosomal RNA gene probe (red). The two large white arrows and two small yellow arrows FISH sites are not located on the chromosomes with color differentiation in GISH analysis. Scale bars = 5 µm.

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9.2.4 Physical Mapping of Genes

BAC-FISH has also been used for mapping of genes on specific chromosomes of potato. Thus, the major late blight resistance gene of wild diploid species *S. bulbocastanum* was physically mapped to a single region on chromosome VIII, a region subsequently dubbed *RB* ("Resistance from *S. bulbocastanum*") (Dong et al. 2000; Song et al. 2003). Then, physical mapping and contig construction for the *RB* region were performed using a BAC walking method with high-resolution genetic mapping (Bradeen et al. 2003). The *RB* gene was subsequently cloned and transformed into susceptible potato varieties (Song et al. 2003).

Given the significance of potato late blight disease, from a practical point of view, this is the most important example of the integration of BAC-FISH mapping together and other potato genomic resources and approaches.

9.3 Studying Natural and Artificial Polyploids by Genomic In Situ Hybridization

A special type of FISH—genomic in situ hybridization (GISH) is an excellent technique to differentiate genomes of parental species in natural and artificial polyploids. Now this method substitutes for conventional meiotic pairing analysis of polyploids.

GISH is based on the use of total genomic DNA of one of the parental species as a labeled probe and non-labeled DNA of the second parent as a competitor. The ability to discriminate chromatin of different genomes depends on the amount and divergence of repeated sequences. The standard GISH protocol allows one to distinguish genomes sharing 80–85% or less sequence homology (Schwarzacher et al. 1989). Cross hybridization in GISH experiments is suppressed by an excess of unlabeled blocking DNA. This standard method helps to discriminate genomes of species that are not very closely related by homology such as potato and *S. etuberosum* or *S. brevidens* (from *Solanum* section *Etuberosum*) (Gavrilenko 2007; Gavrilenko et al. 2002, 2003) or potato and *S. nigrum* (from *Solanum* section *Morella*) (Horsman et al. 2001). As a guideline, GISH can easily discriminate between parental chromatin of potato and distantly related species belonging to another section, however, distinguishing chromatin of species that are more closely related, such as species within section *Petota*, is more difficult.

Multicolor GISH might be helpful in discriminating genomes of species that have closer affinities to each other. This modification uses mixtures of differently labeled genomic DNA of both parental species and simultaneous hybridization of both probes with chromosomes of allopolyploids. The extent of cross hybridization between genomic DNA of parental species can be decreased by adjusting the stringency conditions in the GISH

experiments. Recently, Iovene et al. (2007) reported successful GISH painting of somatic hybrids between species within section *Petota*—*S. tuberosum* and *S. bulbocastanum,* using multicolor GISH with some modification (i.e. adjusting the time of post-hybridization rinses).

Besides GISH, FISH was successfully used to differentiate closely related parental genomes in polyploids using as probes dispersed repetitive DNA sequences which are specific to different component genomes of allopolyploid (Zhang et al. 2004).

9.3.1 Studying Polyploidy in Potato Species—Traditional and Molecular Cytogenetics

GISH is a powerful technique to study allopolyploidy and to analyze genome affinity between polyploid species and their diploid progenitors. In this section we review polyploidy cytogenetics in potato and highlight achievements that were made in the study of polyploidy using GISH and FISH.

9.3.1.1 Polyploid Complexes in Relation to Species Distribution and Genetic Diversity

Petota is a complex section comprised of about 200 tuber-bearing potato species. Potato species are distributed widely in 16 countries and grow under extremely diverse climatic conditions from sea level to 4,500 m and from the southwestern USA, throughout the tropical highlands of Mexico, Central America and the Andes, to Argentina, Chile and Uruguay (Hijmans and Spooner 2001).

Potato species exist as a polyploid series ranging from diploid to hexaploid (Rybin 1929, 1933) with the same basic chromosome number of $x = 12$ for all species. Of 191 (184 wild and 7 cultivated) potato species with known chromosome numbers, 126 (66%) are exclusively diploids ($2n = 2x = 24$) (Table 9-1). Diploids occupy the greatest geographic area within the section *Petota* as well as the northern and southern extremes of the distribution of potato species (Hijmans et al. 2007). Forty-four potato species (23%) are exclusively polyploids [4.2% are triploid ($2n = 3x = 36$), 12.6% are tetraploid ($2n = 4x = 48$), 1% are pentaploid ($2n = 5x = 60$) and 5.2% are hexaploid ($2n = 6x = 72$)] (Table 9-1). Multiple cytotypes exist in 21 species (11%), mostly at the diploid and triploid levels; five species have three different cytotypes (Hijmans et al. 2007).

Polyploidization has played an important role in the distribution of plant species and in their adaptive evolution (Grant 1971; Soltis et al. 2004). Analysis of the ecological and geographic distribution of wild potato species with different ploidy levels showed that polyploids occupy a smaller geographic area than diploid species, but polyploids more frequently occur

at ecological extremes (Hijmans et al. 2007). Thus, higher-level polyploids of wild potato species consistently occur in colder areas than diploids. Polyploids also occur in areas that are wetter than those occupied by diploids, although triploids tend to occur in warmer and drier areas than diploids of wild potato species (Hijmans et al. 2007).

It is well known that environmental factors stimulate the production of unreduced gametes, resulting in polyploidization (Grant 1971; McHale 1983; Ramsey and Schemske 1998; Soltis et al. 2004). For potato species, a high frequency of unreduced gametes has been reported: 1.9-36.3% for unreduced pollen, and 4.9–22.6% for unreduced eggs (Werner and Peloquin 1991). These facts suggest an important role of sexual polyploidization in the formation of polyploid complexes in potatoes (den Nijs and Peloquin 1977).

Millions of years of adaptation to various ecological and geographic areas have created significant genetic diversity among the wild potato species, including genomic divergence, creation of polyploid complexes, and auto- and allopolyploid formation. The genetic mechanisms involved in diversification of diploid potato species are largely unresolved. Matsubayashi (1991) hypothesized that genomic variants of diploid potato species differ from each other by cryptic structural differences. Dvořák (1983) proposed that rapid evolution of non-coding sequences plays an important role in genome differentiation of diploid potato species. Recent FISH studies indicate that genome differentiation of potato species both at inter- and intraspecific levels might be due to divergence in nucleotide sequences and amplification of different classes of repetitive DNA (Stupar et al. 2002; Tek and Jiang 2004; Tek et al. 2005).

At larger taxonomic scales, gross chromosomal rearrangements including inversions, translocations and transpositions have occurred during evolutionary divergence of potato species from other, non-potato *Solanum* species. Genome rearrangements have been confirmed between potato and *Solanum* subgenus *Pachystemonum* (sections *Lycopersicum*, *Etuberosum*, *Juglandifolium*) and *Solanum* subgenus *Leptostemonum* through comparative mapping studies (Tanksley et al. 1992; Perez et al. 1999; Donganlar et al. 2002; Pertuze et al. 2002) and by BAC-FISH mapping (Iovene et al. 2008; Tang et al. 2008). Despite gross structural rearrangements, a high level of genetic colinearity between these various *Solanum* species is noted. It appears that differences in repetitive sequence (rather than gene) content and composition may have a higher importance in divergence of potato and related *Solanum* species (Zhu et al. 2008). Future research combining molecular cytogenetics with other genomic resources will benefit our understanding of genetic mechanisms involved in diversification of diploid potato species and in genome evolution within potato species as well as between potato and related *Solanum* species.

Table 9-1 Chromosome numbers in wild and **cultivated** potato species (Hijmans et al. 2007, with modifications).

Chromosome number	No. (%)	Species
Species with exclusive chromosome number		
$2n = 2x = 24$	126 (66%)	*S. achacachense, S. acroglossum, S. acroscopicum, S. alandiae, S. albornozii, S. amayanum, S. ambosinum, S. anamatophilum, S. ancophilum, S. ancoripae, S. x arachuayum, S. ariduphilum, S. arnezii, S. augustii, S. avilesii, S. ayacuchense, S. aymaraesense, S. berthaultii, S. bill-hookeri, S. x blanco-galdosii, S. boliviense, S. x bruecheri, S. buesii, S. bukasovii, S. burkartii, S. cajamarquense, S. calacalinum, S. cantense, S. chacoense, S. chancayense, S. chilliasense, S. chillonanum, S. chiquidenum, S. chomatophilum, S. clarum, S. coelestipetalum, S. contumazaense, S. x doddsii, S. dolichocremastrum, S. ehrenbergii, S. gandarillasii, S. gracilifrons, S. guzmanguense, S. hastiforme, S. hintonii, S. huancabambense, S. huancavelicae, S. huarochiriense, S. humectophilum, S. hypacrarthrum, S. incahuasinum, S. incamayoense, S. incasicum, S. infundibuliforme, S. ingifolium, S. irosinum, S. jalcae, S. jamesii, S. kurtzianum, S. laxissimum, S. lesteri, S. lignicaule, S. limbaniense, S. x litusinum, S. longiusculus, S. lopez-camarenae, S. marinasense, S. megistacrolobum, S. x michoacanum, S. minutifoliolum, S. mochiquense, S. morelliforme, S. neocardenasii, S. neorossii, S. neovavilovii, S. okadae, S. olmosense, S. orophilum, S. ortegae, S. pampasense, S. paucissectum, S. peloquinianum, S. pillahuatense, S. pinnatisectum, S. piurae, S. puchupuchense, S. raphanifolium, S. raquialatum, S. regularifolium, S. rhomboideilanceolatum, S. x ruiz-lealii, S. salasianum, S. xsambucinum, S. sanctae-rosae, S. sandemanii, S. santolallae, S. sarasarae, S. sawyeri, S. saxatilis, S. scabrifolium, S. schenckii, S. x setulosistylum, S. simplicissimum, S. soestii, S. spegazzinii, S. stenophyllidium, S. tacnaense, S. tapojense, S. tarapatanum, S. tarijense, S. tarnii, S. taulisense, S. trifidum, S. trinitense, S. urubambae, S. velardei, S. venturii, S. vernei, S. vidaurrei, S. violaceimarmoratum, S. virgultorum, S. wittmackii, S. yamobambense,* ***S. ajanhurii, S. phureja, S. stenotomum***

Exclusively polyploids with single cytotype		
$2n = 3x = 36$	8 (4.2%)	*S. burtonii, S. calvescens, S. flavoviridens, S. x indunii, S. x neoweberbaueri, S. x viirsoii,* ***S. chaucha, S. juzepczukii***
$2n = 4x = 48$	24 (12.6%)	*S. acaule, S. agrimonifolium, S. bombycinum, S. colombianum, S. flahaultii, S. garcia-barrigae, S. hjertingii, S. hoopesii, S. lobbianum, S. longiconicum, S. neovalenzuelae, S. nubicola, S. orocense, S. otites, S. oxycarpum, S. pamplonense, S. paucijugum, S. solisii, S. stoloniferum, S. subpanduratum, S. x sucrense, S. tuquerrense, S. ugentii,* ***S. tuberosum***
$2n = 5x = 60$	2 (1%)	*S. x edinense,* ***S. curtilobum***
$2n = 6x = 72$	10 (5.2%)	*S. albicans, S. guerreroense, S. hougasii, S. iopetalum, S. jaenense, S. moscopanum, S. nemorosum, S. neovargasii, S. sucubunense, S. tundalomense*
Subtotal	44 (23%)	
Species with multiple cytotypes		
$2x + 3x$	12	*S. bulbocastanum, S. candolleanum, S. cardiophyllum, S. commersonii, S. immite, S. maglia, S. medians, S. microdontum, S. multiinterruptum, S. x rechei, S. sogarandinum, S. yungasense*
$2x + 4x$	5	*S. andreanum, S. brevicaule, S. leptophyes, S. polyadenium, S. sparsipilum*
$3x + 6x$	1	*S. vallis-mexici*
$5x + 6x$	1	*S. demissum*
$2x + 3x + 4x$	1	*S. verrucosum*
$2x + 4x + 6x$	1	*S. oplocense*
Subtotal	21 (11%)	
Total	191	

9.3.1.2 Traditional Concepts of Types and Origin of Polyploid Potato Species

Polyploidy is an important mechanism in plant speciation (Stebbins 1950; Grant 1971). There are two major types of polyploids: (1) autopolyploids (e.g., *AAAA*, in which *A* represents a copy of the entire genome complement received from species parent *A*) received their homologous chromosomal sets from the same species; they may show irregular meiosis, with a high frequency of multivalents at metaphase I, sterility, or a very low level of fertility. Autopolyploids may also undergo bivalent pairing, but there is no restriction of which two homologs pair. (2) Allopolyploids (e.g., *AABB*, in which *A* and *B* represent a copy of the entire genome complement received from species parent *A* and *B*, respectively) received their homoeologous chromosomal sets from different species and show regular meiosis with bivalent pairing and an extremely low frequency of multivalents. Some of the polyploids are classified as segmental allopolyploids (e.g., $A_1A_1A_2A_2$) representing species with chromosome homology somewhere in between that of allopolyploids and autopolyploids. The component genomes of segmental allopolyploids are somewhat differentiated, but pairing of chromosomes from different component genomes (e.g., A_1A_2) is still possible.

In formation of natural autopolyploids, somatic doubling has been less common than sexual polyploidization through production of unreduced gametes (Soltis et al. 2004). Self-pollination or intraspecific crosses through fertilization of unreduced gametes leads to formation of autopolyploids. Additionally, the ability to form unreduced gametes provides opportunity for hybridization between species with divergent genomes that possess different ploidy levels and/or different endosperm balance numbers (EBNs). Traditionally, identification of the type of polyploidy and development of genome concepts have been based on the analysis of meiosis of species and interspecific hybrids. According to early genome concepts, autopolyploids are less common in potatoes than strict or segmental allopolyploids (Table 9-2). Multiple cytotypes (as *AAA*, *AAAA* or higher levels) of diploid potato species (*AA*) may represent strict autopolyploids (Gavrilenko 2007).

Polyploid species of the series *Tuberosa* and *Acaulia* have been classified as segmental allopolyploids (Matsubayashi 1991). Segmental allopolyploidy can also be proposed for the 4*x* species *S*. *tuquerrense* of the *Piurana* series (Gavrilenko 2007). Diploid progenitors (A^aA^a, A^tA^t, A^sA^s) of segmental polyploids have been proposed but not positively identified by Matsubayashi (1991). Wild polyploid species of the series *Longipedicellata*, *Demissa*, *Conicibaccata*, and *Piurana* have been considered by several researchers to be strict allopolyploids based on their regular bivalent pairing (Hawkes 1958, 1990; Marks 1955, 1965; Irikura 1976; Ramanna and Hermsen 1979; Lopez and Hawkes 1991; Matsubayashi 1991).

Table 9-2 Genomic relationships of potato species based on different genomic concepts.

Series (Hawkes 1990)	Ploidy	Genome formulae according to the concept of: Classical cytogenetics		*Waxy, NIA* sequences	GISH
		Irikura 1976	Matsubayashi 1991	Spooner et al. 2008; Rodríguez and Spooner 2009	Pendinen et al. 2008
Cuneoalata, Megistacroloba, Commersoniana	2*x*		AA	AA	
Yungasensa	2*x*	AA	AA	AA	
Ingaefolia, Olmosiana	2*x*		A^iA^i, A^oA^{od}		
Morelliformia	2*x*		A^mA^m	BB	
Polyadenia	2*x*		$A^{po}A^{po}$	BB	
Bulbocastana	2*x*		A^bA^b	BB	
Pinnatisecta	2*x*	BB	$A^{pi}A^{pi'}$	BB	B^xB^x
Acaulia	4*x*	AAB^aB^a	AAA^aA^a		
	6*x*		$AAA^aA^aXX^b$		
Tuberosa	2*x*	AA	AA, AA^a	AA	AA
	3*x*		AAA^t		
	4*x*	AAAA	AAA^tA^t, AAA^sA^s	AAAA	
	5*x*		$AAAAA^aA^t$		
Conicibaccata	2*x*		$A^{c1}A^{c1}$, $A^{c2}A^{c2}$	AA	
	4*x*		$A^{c1}A^{c1}C'C'$	AAPP	
Longipedicellata	4*x*	AAB^sB^s	AABB	AABB	AAB^xB^x
Piurana	2*x*		A^PA^P	PP	PP
	4*x*		A^PA^PPP	PPPP	
Demissa	6*x*	$AAB^sB^sB^dB^d$	$AADDD'D'$	AABBPP for *Iopetala* Group	

In general, there are frequent contradictions in the determination of the origin of the natural potato allopolyploids (Gavrilenko 2007). Here we consider two of the most distinctive classical hypotheses developed by Irikura (1976) and Matsubayashi (1991) (Table 9-2). According to Irikura (1976), allopolyploid species share one common component genome *A* but differ from one another by the genomic variants of the second component genome *B*. The 2*x* species *S. verrucosum* was proposed as a donor of the *A* component genome in allopolyploids and the 2*x* species *S. cardiophyllum* was proposed as a possible donor of the *B* component genome of Mexican tetraploids. However, no experimental evidence establishing the *B* genome progenitor was provided by Irikura (1976). Matsubayashi (1991) recognized five genomes (*A, B, C, D, P*) in potato species. No existing diploid species with the *B, C, D* and *P* genomes nor putative progenitors of these distinct genomes have been identified. According to Matsubayashi (1991), all diploid potato species comprise one major genomic group *A* that combines very similar genomic variants designated as genome formula *A* with superscripts corresponding to each taxonomical series (Table 9-2). Allopolyploid species share one common component genome *A* and differed from each other by their second merged component genome *B, C, D* or *P* (Matsubayashi 1991) (Table 9-2). Diploid species *S. verrucosum* was suggested as the contributor of the *A* component genome to Central American allopolyploids (Bains 1951; Marks 1955, 1965; Matsubayashi 1991).

9.3.1.3 Genome Evolution of Potato Polyploids and Their Relationships with Putative Diploid Progenitors—Genome Research

9.3.1.3.1 Molecular Phylogenetics

The evolutionary history of potato polyploid species has been studied using DNA markers and organellar and nuclear phylogenies. Close relationships between the diploid species *S. verrucosum* (2*n* = 2*x* = 24, *AA*) and polyploid species of the series *Longipedicellata*, *Demissa* and *Acaulia* have been supported by plastid DNA phylogenies (Spooner and Sytsma 1992) and AFLP results (Kardolus 1998).

DNA sequence analyses of the single copy nuclear Granule-Bound Starch Synthase I (*GBSSI*) or *Waxy* gene (Spooner et al. 2008) and the nitrate reductase (*NIA*) gene (Rodríguez and Spooner 2009) revealed the type of polyploidy and the putative diploid progenitors of polyploid potato species. The allopolyploid nature was supported for species of the series *Longipedicellata*, *Conicibaccata,* and the informal *Iopetala* group consisting of *S. hougasii*, *S. iopetalum* and *S. schenckii* (Table 9-2). *GBSSI* and *NIA* sequence analyses (Spooner et al. 2008; Rodríguez and Spooner 2009) support a close phylogenetic relationship of polyploids within series *Conicibaccata* and

Piurana and the *Iopetala* Group, with the diploid species in series *Piurana* possessing the *P* genome (A^P genome of Matsubayashi 1991; Table 9-2).

Based on *GBSSI* and NIA data, the diploid species *S. verrucosum* (or a species closely related to *S. verrucosum*) could be the *A* genome contributor to tetraploid species of the series *Longipedicellata* (*AABB*) and hexaploids of the series *Demissa*. Clade-specific (not species-specific) progenitors have been detected for the *B* genome donor of *Longipedicellata* species which encompass 13 remaining North and Central American diploid species of series *Pinnatisecta*, *Polyadenia* and *Bulbocastana* (Clade 1+2 in Spooner et al. 2008; Rodríguez and Spooner 2009, correspondingly). Based on a high degree of sequence conservation at the *RB* (syn. *Rpi-blb1*) locus, Wang et al. (2008) postulate that *S. bulbocastanum* may be one of the progenitors of the 4x *S. stoloniferum* (series *Longipedicellata*).

9.3.1.3.2 GISH and FISH of Potato Polyploid Species

Polyploid origin of the tetraploid species of the series **Longipedicellata:** In the study of natural polyploids of potato, GISH analysis was first applied to investigate genome origin of Mexican tetraploid species of the series *Longipedicellata* (*S. stoloniferum* and *S. hjertingii*) (Pendinen et al. 2008). Diploid putative progenitors for *S. stoloniferum* and *S. hjertingii* were selected based on prior hypotheses of classical genome analyses (Irikura 1976; Marks 1965; Matsubayashi 1991) and the nuclear DNA sequence-based phylogenies of Spooner et al. (2008) and Rodríguez and Spooner (2009). Specifically, *S. verrucosum* was considered as the donor of the *A* genome and diploid Mexican species from the series *Pinnatisecta* (*S. jamesii*, *S. cardiophyllum, S. ehrenbergii*) were considered as donors of the *B* genome (Table 9-2). Multicolor GISH was used to discriminate genomes of putative diploid ancestors of Mexican tetraploid species using a mixture of differently labeled genomic DNA of both parental diploid species and simultaneous hybridization of both probes to chromosomes of tetraploids. The differential painting of chromosomes of two component genomes of all five tested accessions of the tetraploid species *S. stoloniferum* and *S. hjertingii* indicates the phylogenetic distances between ancestral genomes (*A* and *B*) (Fig. 9-2, a-b). Thus, GISH results showed that species of the series *Longipedicellata* originated through merging two divergent ancestral genomes (*A* and *B*) and supported an *AABB* genome constitution predicted by other authors (Table 9-2). Symbol *B* (rather than $A^{Pi}A^{Pi}$, as used by Matsubayashi 1991) has been subsequently adopted to denote the genomes of Mexican diploid species of the series *Pinnatisecta* ($2n = 2x = 24$, *BB*), reflecting their homology to the second subgenome *B* of the allotetraploid Mexican species *S. stoloniferum* and *S. hjertingii* (Pendinen et al. 2008; Table 9-2). GISH provides evidence of the strict allopolyploid nature of Mexican tetraploid species of the series

Longipedicellata because bivalent pairing of *S. stoloniferum* and *S. hjertingii* is restricted to the pairing within ancestral parental homologues (Pendinen et al. 2008; Fig. 9-2b).

In addition to GISH analyses, the species listed above were also analyzed by FISH using 5S and 45S rDNA probes. All analyzed diploid species have one terminally located 45S rDNA locus on one chromosome pair and one 5S rDNA locus on another chromosome pair. Allotetraploid species have two chromosome pairs with one 45S rDNA locus and two other pairs of chromosomes with one 5S rDNA locus (Fig. 9-2, c-e). FISH analysis revealed different sizes of the 45S rDNA regions in allotetraploid species, indicating that the 45S rDNA regions of subgenomes *A* and *B* of *S. stoloniferum* changed during coevolution of *A* and *B* component genomes (Pendinen et al. 2008). GISH and FISH results provide important information about the nature and origin of wild polyploid potato species, as well as about their relationships with putative diploid progenitors.

In another series of multicolor GISH experiments, the DNA from *S. verrucosum* was labeled in red and the DNA from diploid South American species *S. andreanum* and *S. piurae* in green and both probes were hybridized with chromosomes of Mexican tetraploids (Pendinen et al. 2008). Most of the *S. stoloniferum* and *S. hjertingii* chromosomes were not preferentially labeled in either red or green in GISH (Fig. 9-2f). This result indicates that the genomes of *S. andreanum* and *S. piurae* have diverged from both the *A* genome of *S. verrucosum* and the *B* genome of diploid Mexican species (*S. cardiophyllum*, *S. ehrenbergii*, and *S. jamesii*). So GISH results do not contradict the genome formula *PP* (not equivalent to A^pA^p of Matsubayashi 1991) proposed by Spooner et al. (2008) for diploid species of the series *Piurana*. Only two pairs of chromosomes in *S. stoloniferum* (as well as *S. hjertingii*) had segments labeled in green due to the hybridization to the *S. andreanum* probe (Fig. 9-2f). FISH analysis (Fig. 9-2g) showed that these two pairs of chromosomes were not associated with the 45S rDNA (Pendinen et al. 2008). Thus, GISH results indicate that the genomes of diploid South-American species of the series *Piurana* [*S. piurae* and *S. andreanum* ($2n = 2x = 24$, *PP*)] and the genomes of tetraploid Mexican species [*S. stoloniferum* and *S. hjertingii* ($2n = 4x = 48$, *AABB*)] have homologous segments only on two chromosome pairs of allotetraploids (Pendinen et al. 2008). Partial homology of the two chromosome pairs of the *B* genome of Mexican tetraploid species with the *PP* genomes of *S. andreanum* (as well as *S. piurae*) indicates that the *B* genome itself may be of hybrid origin (B^x—Table 9-2) and that possible hybridization and introgression occurred between *S. andreanum*, *S. piurae* (or their progenitors or other species closely related to them) and the *B*-genome donor species.

9.4 Practical Applications of Molecular Cytogenetics

Despite the wide genetic diversity that exists in wild potatoes and closely related species of the genus *Solanum*, only 10% of potato species have been explored for use in breeding programs (Budin and Gavrilenko 1994). Many of the wild species that are highly resistant to pathogens and pests are also reproductively isolated from cultivated tetraploid potato and hence difficult to include in classical breeding programs. Therefore most genes for resistance to pathogens and pests present in modern potato varieties have been introgressed from closely related potato species. Utilization of closely related germplasm in practical breeding has resulted in the narrow genetic basis of modern potato varieties (Ross 1986). To broaden the genetic base of the common potato gene pool and to combine different resistance genes introgressed from wild *Solanum* species, various methods have been used including ploidy manipulations and bridge crosses, embryo rescue, hormone treatments, reciprocal crosses and protoplast fusion (Jansky 2006). However, this is only the first step towards successful introgression of alien genetic material into potato. The potato breeder must, over several subsequent generations, select for genotypes carrying the desired genes or alleles from the wild donor while simultaneously restoring or achieving all desirable phenotypic attributes of cultivated potato.

Success of introgression of alien genetic material into the cultivated potato genome can be monitored using cytogenetical studies of species and interspecific hybrids and their derivatives. Conventional cytogenetic analysis of potato is hampered by the relatively small size of potato chromosomes, their slight differences in morphology and similar karyotypes across potato species. FISH and GISH have greatly advanced researchers' abilities to monitor alien chromatin.

Significant progress in introgressive hybridization and breeding may be achieved when we have knowledge about genome evolution of natural wild polyploids and about genome composition of artificial polyploids. Several examples illustrate this statement.

Knowledge about the ancestors of wild polyploid species can provide excellent support for searching for desirable genes in numerous diverse germplasm collections and predict the success of introgressive hybridization. Thus, GISH and nuclear phylogeny studies undoubtedly indicate that one of the diploid progenitors of Mexican polyploids is a *B* genome diploid species of series *Pinnatisecta* or *Bulbocastana* (Clade 1 in Spooner et al. 2008). The diploids of these series are important sources of useful genes for resistance to pathogens and pests (Hawkes 1994) and there is a high probability that their resistance genes could be also found in Mexican allotetraploids. Experimental confirmation of this hypothesis was provided by Wang et al. (2008) who showed the presence of functional

homologues of the *RB* (syn. *Rpi-blb1*) gene of *S. bulbocastanum* (2*x*, *BB*) in *S. stoloniferum* (4*x*, *AABB*). Significantly, allopolyploids can be much more easily crossed directly with cultivated potato (Adiwilaga and Brown 1991) than can 1 EBN *B* genome diploids [from which genes can be accessed for potato improvement only through the use of complex, multispecies bridge crosses (Hermsen 1994; Jansky 2006) or somatic hybridization (Ward et al. 1994; Thieme et al. 1997, 2008; Helgeson et al. 1998)]. Therefore, knowledge about ancestors of natural polyploids can help to better plan strategies for potato improvement.

Application of GISH and FISH techniques to genome analysis of artificial potato polyploids was reviewed in our previous paper (Gavrilenko 2007). GISH has been successfully used for identification of alien introgression in interspecific somatic hybrids of potato and their sexual progenies (Gavrilenko 2007). GISH combined with FISH using CSCDMs as probes helped to identify alien chromosomes in addition and substitution lines in a potato background (Dong et al. 2001, 2005; Tek et al. 2004). In situ hybridization techniques helped identify not only alien chromatin but also mapped a late blight resistance gene of *S. bulbocastanum* to potato chromosome VIII (Dong et al. 2000).

FISH is very useful for programs directed at positional cloning agronomically important genes, providing information about the physical distances between markers along a chromosome and about their positional orientation relative to chromosomal landmarks.

The results of comparative BAC-FISH mapping across different taxa may have practical implications in predicting the success of introgressing genes from divergent wild species into cultivated potato.

In summary, use of molecular cytogenetics together with other genomics techniques will be of great help in better understanding genome evolution of species within the section *Petota* and will help in designing more efficient breeding programs for potato.

Acknowledgments

I thank Prof. Jiming Jiang for helpful suggestions and providing Figs. 9-1a, b, d, e; the Genetics Society of America, Genetics Editorial Office for permission to publish Figs 9-1a, b, c, d, e and National Research Council Canada Research Press for permission to publish Fig. 9-2.

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