AFLP MARKER ANALYSIS OF MONOPLOID POTATO

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Abstract

Potato haploids have been recent components in protoplast fusion research, strategies to combine wild and cultivated potato germplasm and the generation of economically valuable mutant phenotypes. Additionally, most major genetic mapping and QTL analyses in potato have utilized haploid germplasm to simplify linkage-mapping computations. The accuracy of genetic assumptions concerning the randomness and genetic purity of haploid genomes may directly affect the statistical validity of many results in current potato research. In the present study, AFLP analysis was conducted on two sibling *S. phureja* 'BARD 1-3' monoploid populations derived by androgenesis in anther culture, and gynogenesis through the use of a haploid-inducing pollinator, *S. phureja* 'IVP 101.' Little indication of somaclonal variation and haploid-inducer gene introgression was found in the monoploid band data indicating genomic stability. Segregation of marker alleles that were heterozygous in the parent was distorted from the expected 1: 1 ratio in both populations, ranging from 35% in the gynogenic monoploids (GM) to 46% in the androgenic monoploids (AM). Genetic diversity was more random among the monoploid populations after skewed marker data was removed from phylogenetic analyses. Bilateral and unilateral marker skewness among the monoploids may respectively indicate common and unique segregation distorting loci (SDL) in the haploid genomes. Representatives of both SDL were located on a partial linkage map created using androgenic monoploid data.

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CHAPTER 1

Literature Review

The cultivated potato, *Solanum tuberosum* L. ($2n = 4x = 48$), is an autotetraploid comprising four homologous sets of 12 chromosomes. As such, potato exhibits tetrasomy, a complex inheritance of traits caused by the random pairing of quadrivalents at meiosis. Tetrasomic inheritance may result in tetra-allelism, where four different alleles interact within a single locus (Ross 1986). In a highly heterozygous cross between two tetra-allelic parents, this condition may involve up to eight alleles (36 different genotypes) segregating at a single locus in the hybrid progeny (Meyer *et al*. 1998). Thus, the tetrasomic genetic constitution of *S. tuberosum* creates significant challenges for the potato breeder.

In the area of traditional cultivar production, breeding difficulties arise when epigenetic and epistatic gene effects at complex loci confound strategies to fix desirable, agronomic traits. These gene effects are also responsible for allowing *S. tuberosum* to become a natural reservoir for deleterious and lethal alleles. Although tolerated in tetraploids via overdominance, potato's substantial genetic load causes inbreeding depression when homozygosity is increased through selective breeding or when the tetraploid genome is reduced via haploidization to the diploid level (Ross 1986).

Genetic analyses are arguably the most important areas complicated by autotetraploidy, due to their immense potential for new breeding strategies, including marker-assisted selection (MAS), quantitative trait loci (QTL) mapping and gene pyramiding (Lander and Botstein 1988; Paterson 1996). Genetic analysts generally avoid research at the tetraploid level due to the vast number of calculations required to unravel tetrasomic gene

inheritance and linkage associations (Meyer *et al*. 1998). This has caused efforts in tetraploid mapping to fall behind mapping research in potato populations of lesser ploidy (Luo *et al*. 2000).

Haploid-based breeding techniques have proven successful in circumnavigating the complexity created by autopolyploid genetics. 'Haploids' are plants that have a gametophytic parcel of a sporophytic genome and thus comprise a halved and recombined portion of the parental chromosome set. Although the term 'haploid' is used interchangeably across differing ploidy levels, alternative nomenclature exists that is specifically indicative of chromosome number and parental ploidy. Haploids derived from tetraploid potato are called 'dihaploids' ($2n = 2x = 24$), whereas 'monoploids' ($2n = 1x = 12$), or sometimes 'monohaploids,' are haploids derived from diploid potato and have the basic chromosome number (van Breukelen *et al*. 1977; Ross 1986). Although the induction of haploid plants is generally referred to as 'haploidization,' the various specialized techniques used across the crop spectrum may employ vastly different synthetic and biological mechanisms to achieve their goal. In potato breeding there are two main pathways by which haploid plant formation can be induced: androgenesis and gynogenesis.

Androgenesis is the *in vitro* culturing of whole anthers, or free microspores, on a nutrient rich medium to induce plantlet regeneration from single gametic cells or haploid calli (Fig. 1). For androgenesis to be successful in anther culture, microspores will begin abnormal mitotic divisions that result in direct embryo formation (i.e., embryos that arise from a single cell line and not from callus). Alternatively, microspores may divide into a disorganized mass of cells (callus) from which haploid shoots may eventually regenerate (indirect androgenesis; Veilleux 1996). The ploidy of the resultant plantlets can be

estimated by counting chromosomes in mitotically-dividing root cells (Sopory 1977), counting chloroplasts in guard cell pairs (Singsit and Veilleux 1991) or by using flow cytometric analysis (Owen *et al*. 1988).

Gynogenesis is haploidization via the 'maternal,' or the seed parent's, genome. In this process, specific *S. phureja* ($2n = 2x = 24$) selections, known as 'haploid inducers,' contribute the paternal gametes for pollination of the desired haploid progenitor. The formation of a haploid embryo begins when the egg is either induced into parthenogenesis (Hermsen and Verdenius 1973) or when the zygote experiences spontaneous abortion of the pollen donor's set of chromosomes (Clulow *et al*. 1991). Haploid embryo and plantlet phenotypes are putatively identified by their lack of certain dominant, morphological markers, which have been bred to homozygosity in the pollen donor and are expressed in hybrid embryos. Specifically, these markers are anthocyanin pigmentation on developing embryos (embryo spots) or on plantlet shoots (Fig. 2). Due to a relatively superior haploid-induction frequency and marker pigmentation for early haploid selection, the haploid-inducing clones in frequent application today include *S. phureja* clones 'IVP 35', 'IVP 48' and 'IVP 101' (Ross 1986). The ploidy of gynogenic haploids, like haploids derived via androgenesis, can be confirmed on the basis of root tip chromosome counts or flow cytometry.

Haploid Utility in Potato Breeding

Initially, potato haploids were envisioned as a tool to simplify the breeding of *S. tuberosum* cultivar production by reducing tetraploid germplasm to a diploid breeding level (Chase 1963). As such, haploid breeding programs offer a simpler disomic inheritance as an alternative to the traditional breeding programs encumbered by

tetrasomic segregation. Besides simplifying inheritance ratios and intralocus effects, dihaploid populations can be smaller with a comparable efficiency for breeding (Kotch *et al*. 1992).

A second early motive for the production of haploids was to acquire a 'genetic bridge' between the various genomes of Solanaceae. By this technique, ploidy barriers between the cultivated and wild *Solanum* species can be circumvented by reducing tetraploid *S. tuberosum* to the diploid level and crossing the resultant dihaploids to the wild diploid *Solanum* spp. Novel hybrid germplasm is then incorporated back into tetraploid breeding programs via sexual crosses between *S. tuberosum* and colchicine-doubled *S. tuberosum* dihaploids, or through 4x-2x crosses utilizing unreduced gamete formation. Desirable agronomic characters, such as disease resistance and heat-tolerance, that have been identified in diploid germplasm, can then be easily integrated into tetraploid cultivars (Ross 1986).

Haploid Utility in Genetic Mapping

Initially, genetic marker technology was not capable of handling the scope of computations required to analyze tetrasomic marker inheritance in the cultivated potato. In fact, only with the recent advent of more powerful marker methodologies and faster computerized analyses has mapping research at the 4*x* level begun to move forward. Despite this noticeable advancement, there are still significant obstacles slowing the progress of autotetraploid mapping. These challenges include: 1) the inability of researchers to use multiple dosage marker alleles; 2) distorted segregation ratios of molecular markers due to double reduction; and 3) the high probability for ‘multi-locus markers’ (Luo *et al*. 2000, 2001). Until further technological progress is accomplished,

mapping tetraploid potato will continue to be hampered by inconveniences and sources of error, including a need for large population sizes, limitations in usable marker types, unrealistic gene inheritance assumptions and necessary manual reconstructions of data (Bradshaw *et al*. 1998; Hackett *et al.* 1998; Luo *et al*. 2000).

Because the critical need for genetic maps in modern breeding programs could not be satiated via tetraploid mapping programs, genetic mapping efforts have fostered the most recent rise in haploid application. By basing the potato map on the linkage analyses of diploid progeny, researchers were able to overcome many obstacles associated with tetrasomic inheritance. Bonierbale *et al*. (1988) first demonstrated the feasibility of mapping cultivated potato by using derived dihaploid progeny. Using diploids from the interspecific cross, *S. phureja* × (*S. tuberosum* × *S. chacoense*), the researchers generated a linkage map comprising 606 cM spanning 12 linkage groups. Gebhardt *et al*. (1991) and Jacobs *et al*. (1995) subsequently produced complementary maps using similar dihaploid strategies (Table 1). Together, these dihaploid-based potato maps helped to elucidate the complex evolution of the cultivated potato's genome and provided the seminal insight into the conserved synteny existing between the major crop genomes of the Solanaceae. From this base of genome research, many current gene cloning and mapping strategies have been derived (Paterson 1996).

In addition to their success in broad-scale potato mapping, dihaploid-based molecular analyses have been especially productive in the identification of important disease and pathogen resistance genes. *Phytophthora infestans*, representing one of the most significant and widespread threats to potato yield (Oberhagemann *et al*. 1999), has become a focal point for dihaploid mapping studies. Out of the 11 loci (R1-R11) hypothesized to confer resistance to late blight, four (R1, R3, R6 and R7) have already

been mapped to their respective chromosomes with the aid of dihaploid germplasm (Li *et al*. 1998).

Elkharbotly *et al*. (1994) investigated *P. infestans* resistance using F_1 hybrids with *S. tuberosum* dihaploids as seed parents. Susceptible genotypes were designated as pollinators while the dihaploids segregated for *S. tuberosum* resistance alleles, R1, R3 and R10. The resultant RFLP mapping analyses were successful in verifying a prior study situating the R1 locus on chromosome V and identified the location of R3 to chromosome XI. With similar success, Naess *et al*. (2000) used a *S. tuberosum* dihaploid x *S. berthaultii* hybrid line for backcrossing with a second, *S. tuberosum* dihaploid genotype. The genetic effects of several late blight resistance QTL were mapped using this backcross population, whereby, in one of two field studies conducted, the QTL identified were found to account for up to 66% of the variation in resistance to late blight.

Resistance QTL for potato pathogens other than late blight have also been discovered using the dihaploid-based mapping strategies. Using a population of *S. tuberosum* ssp. *andigena* dihaploids segregating for resistance to the potato cyst nematode, *Globodera rostochiensis*, Pineda *et al*. (1993) found eight RFLP markers linked to the resistance-conferring H1 gene. All markers were localized to chromosome V, with one closely linked marker (2.7 cM) promising to be a good candidate for MAS. Recently, van der Voort *et al*. (2000) used dihaploid-based populations to identify on potato chromosome V two important loci, *Gpa5* and *Gpa6*, which conferred broad-spectrum resistance to *Globodera pallida*.

Valkonen *et al*. (1999) investigated potato resistance to the cauliflower mosaic virus (CMV) by using *S. tuberosum* dihaploid lines. The researchers were able to ascribe CMV

resistance to one locus in one of the segregating populations, while two more loci conferred the major effects for resistance in a second population. Related dihaploid lines were subsequently used by Hermalainen *et al*. (2000), investigating the genetics behind the inhibition of vascular transport of potyvirus-A. This research gathered substantial evidence for the presence of syntenic loci (across solanaceous species) encoding resistances to multiple related potyviruses.

Though perhaps the most important area of potato mapping, resistance QTL have not been the only focus of dihaploid-based QTL research. Simko *et al*. (1999) used a 155-genotype backcross population derived from a dihaploid *S. tuberosum* x *S. berthaultii* interspecific cross to investigate relationships between QTL detected *in vitro* and under greenhouse conditions. Significant QTL found for plant height and early tuberization were similarly detected in both environmental conditions, suggesting convenience and practicality might be found in a breeding program that utilized *in vitro* marker-assisted selection. Freyre *et al*. (1994) investigated QTL controlling tuber dormancy in a mapping population derived by crossing a haploid *S. tuberosum* x *S. chacoense* hybrid with a *S. phureja* pollen donor. Using isozymes, RAPD and RFLP markers, the researchers generated a linkage map comprising 87 loci, 22 of which exhibited a significant association with tuber dormancy. From these results, six QTL map regions were identified that explained 57.5% of the phenotypic variation for tuber dormancy.

Genetic Artifacts in the Haploid Genome

Since the earliest documentation of haploid plant formation, potato breeders have investigated the mechanisms behind haploid embryo induction. Although the biological processes associated with haploidization have yet to be completely deciphered, adequate

models of understanding have been in place for some time. One incomplete area of these models, which is of particular relevance to the great number of ongoing genetic mapping projects, is the origin and nature of 'genetic artifacts' existing in the haploid genome as a result of the haploidization process.

For our purposes, haploidization-derived 'genetic artifacts' can be defined as random genetic sequences that segregate among sibling haploids in a non-Mendelian fashion or are completely foreign to the haploid progenitor. In either case, when analyzed in random samples, a population of haploids exhibiting this 'genetic baggage' would not evenly, or accurately, represent the genetic diversity of the parent. Instead, the depiction would exhibit a preponderance of genomic sequences 'selected' by the biological mechanisms functioning during haploidization.

The implication of these genetic artifacts is seen as potentially great (Clulow *et al*. 1991; Hosaka 1999). Downstream haploid-based research such as cultivar breeding programs or genetic mapping analyses will theoretically exhibit results tainted by the preponderance of nonrandom, or aberrant, haploid genotypes. Consequently, the nature and extent to which the alternate haploidization schemes cultivate these genetic artifacts in their haploid products has been an area of significant investigation.

The genetic artifacts arising in the androgenic haploid genome have not been fully characterized for their general quantity or dispersion. Several studies have revealed that parental genotype is a major prerequisite to success in androgenesis. Androgenic haploid production has been documented to vary among different potato lines (Wenzel and Uhrig 1981; Singsit and Veilleux 1989; Chani *et al*. 2000), and parental clone receptivity to each and all androgenic phases (microspore cell division, regenerability of plants from

androgenic embryos or callus, ploidy level and vigor of regenerated plants) can range from no response at all to highly responsive (Boluarte-Medina and Veilleux, 2002). Parental receptivity has been associated with haploid selection by Meyer *et al*. (1993), where improved genotypic response to anther culture was achieved through recurrent selection of monohaploids (Meyer *et al*. 1993).

Providing significant evidence for selection pressures unique to androgenesis, Rivard *et al*. (1994, 1996) used RFLP markers to explore the differences between three different potato populations. Two parental lines of the weedy potato relative, *Solanum chacoense* Bitt. ($2n = 2x = 24$), were used alternately to produce two populations each of AC haploids and selfed progeny. Additionally, two lines were created via reciprocal crossing of the same parents. RFLP analyses conducted on these six plant populations revealed certain genomic differences caused by the mode of progeny derivation. Of particular note was the extent of non-Mendelian inheritance of RFLP loci among the AC haploids, where 70% and 46% of total RFLP deviated from the expected 1:1 (presence : absence) band segregation ratio. Moreover, three loci exhibited an extreme bias toward a particular allelic form, wherein two parental alleles were absent in all haploid plants except one and one parental allele exhibited zero inheritance among the haploid plants. The latter allele was also absent in the homozygous form among selfed progeny, indicating the allele had a deleterious, postzygotic effect on viability.

Like with androgenesis, success in gynogenesis is controlled to some extent by parental genotypes (Hermsen and Verdenius 1973); though, the genetic artifacts associated with this mode of selection have been investigated very little. Another source of genetic artifact, which is unique to gynogenesis, has been a point of considerable molecular and morphological scrutiny, however.

Initially, haploids derived from the *S. phureja* haploid-inducing process were thought to originate via parthenogenesis (Hermsen and Verdenius 1973, Peloquin *et al*. 1996). However, Clulow *et al*. (1991) discovered genetic material from the *S. phureja* haploid-inducer present in *S. tuberosum* dihaploid potato DNA. Of 17 dihaploids, 15 exhibited aneuploidy after chromosome counts and only two haploid clones exhibited the expected euploidy. These results suggested that fertilization had occurred between the parental gametes but a partial elimination of *S. phureja* chromosomes was occurring. In subsequent analyses, Clulow *et al*. (1993) found evidence that aneuploids from the haploid-inducing cross expressed leaf isozymes inherited from the *S. phureja* pollinator. The aneuploid plants also differed for their extrachromosomal content, whereby the progeny showing higher percentages of aneuploid cells were more likely to exhibit the pollinators' proteins. Surprisingly, leaf isozymes were also detected in a euploid dihaploid, which theoretically should not have genetic material present from the haploid-inducer. Wilkinson *et al*. (1995) attempted to explain this rare occurrence by using genomic *in situ* hybridization on the putative euploid haploids. From their results, they hypothesized that somatic translocation between *S. phureja* and *S. tuberosum* DNA may have occurred during the haploidization process. The accuracy of their conclusions was later questioned, however, with additional evidence suggesting the putative euploids were actually aneusomatic (Allainguillaume *et al*. 1997). In an additional follow-up study, Clulow *et al*. (1997) used two microsatellite markers and five SSR primers to evaluate the degree and nature of introgression by the haploid-inducer genes. Variable introgression was observed among the populations of dihaploids derived from the different *Solanum tuberosum* cultivars, 'Pentland Crown,' 'Lizen' and 'Brio,' which lead the authors to suggest that the *S. tuberosum* maternal parent can affect this phenomenon.

Monoploid Potato as Mapping Tool

Monoploid potato embodies many positive attributes that make it a desirable germplasm for genetic mapping research. Monoploids individually comprise a gametic set of chromosomes, which represent random permutations of a single parental genome after one cycle of meiosis. As such, they are as informative for creating linkage associations between genetic loci as a population of doubled haploids (DH) or the progeny derived from a conventional, diploid backcross (Snape and Simpson 1981; Paterson 1996). In these three populations, each individual plant displays one newly recombined chromosome per gametic set. Of these three populations, however, monoploids are ultimately the quickest approach toward mapping analyses, as they can be created in a single generation, in contrast to the two generations required for the equally informative DH and backcross progeny (Paterson, 1996). As an additional advantage, monoploids will not exhibit the marker segregation distortions arising from linkage disequilibrium commonly found in sexual crosses or from genetic selection that may arise during chromosome doubling of DH populations (Ross 1986). A genetic map based on monoploids, therefore, should only exhibit the locus associations based on one generation of genetic recombination and genetic artifacts accrued during the haploidization processes.

The mono-allelic state of monoploid loci offers considerable efficiency by minimizing the number of possible genotypes in a population. Where heterozygotes produce 3^n genotypic combinations (n = number of segregating loci), homozygotes create only 2^n combinations (Snape 1988). This reduction in genotypic diversity allows a reduction in the population size required for analysis of phenotypic classes and recombination events (Foisset and Delourme 1996). A monoploid population can also surpass many

population-types in the number of informative markers that are available for linkage analyses, due to all heterozygous loci in the diploid parent acting as potential sites for marker segregation in haploid progeny. Such an immediate wealth of marker loci presents a considerable advantage over the time-consuming identification of simplex markers necessary for polyploid mapping efforts (Wu *et al*. 1992, Meyer *et al*. 1998).

Despite their amenable nature toward genetic mapping and analysis, monoploids exhibit specific disadvantages that prevent them from being ideal potato mapping germplasm. The first disadvantage is found in the lowered viability of monoploid potato resulting from inbreeding depression. This aspect of monoploidy makes it difficult to create and maintain sizable potato populations (van Breukelen 1981; Uijtewaal *et al*. 1987). A second shortcoming to monoploidy, as with diploidy, is the questionable ability by which researchers can extrapolate from data derived from a lesser ploidy level to potato performance at the tetraploid level. Though important QTL can theoretically be broken into smaller components for simpler study at lesser ploidy levels, monoploid/diploid phenotypes cannot characterize QTL alleles as they perform in tetraploid potato, since higher order interaction of alleles are reduced or absent (Bradshaw *et al*. 1998; Xie and Xu 2000). Lastly, the great deal of segregation distortion present in molecular marker data represents an inefficient component to monoploid mapping utility. Molecular investigations of three different monoploid populations has shown 45-70% of the total marker data to be skewed from Mendelian segregation ratios (Rivard *et al*. 1994; Tai *et al*. 2000). Tai *et al*. (2000) addressed this concern with a mapping strategy designed to map monoploid data showing marker skewness. Though their research exhibited success in creating linkage groups centered on theoretical ‘segregation distortion loci’ (SDL) responsible for observed marker skewness, no markers were actually placed on a linkage map of the potato genome.

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Table 1. Comparison of published potato maps (not a comprehensive list). Abbr: *phu*, *S. phureja*; *2xtbr*, a haploid *S. tuberosum*; *chc*, *S. chacoense*; *ber*, *S. berthaultii*; *stn-phu*, a selected clone from a *S. stenotomum-S. phureja* bulk population; *pal*, *S. palustre*; *etub*, *S. etuberosum*; *ver*, *S. vernei*; Isoz, isozyme markers; Mph, morphological markers.

Population	Ploidy	Genotype No.	Marker Type	Marker No.	Marker Skewness	Linkage Group No.	Map Length (cM)	Author
phu x (2x*tbr* x *chc*)	2x	65	RFLP/Isoz	134	Yes; 28%	12	606	Bonierbale *et al* 1988
(2x*tbr* x 2x*tbr*) x 2x*tbr*	2x	67	RFLP	492	Yes; 27%	12	1034	Gebhardt *et al*. 1991
(2x*tbr* x *ber*) x *ber*	2x	155	RFLP	261	No; NA	12	684	Tanksley *et al*. 1992
(2x*tbr* x 2x*tbr*) x 2x*tbr*	2x	67	RFLP/Isoz/Mph	193	Yes; 17%	12	1120	Jacobs *et al*. 1995
chac x *chac*	1x	56	RFLP	58	Yes; 70%	13	308	Rivard *et al*. 1996
chac x *chac*	1x	31	RFLP	57	Yes; 46%	14	463	Rivard *et al*. 1996
4x*tbr x* 4x*tbr*	4x	78	AFLP	231	No; 17%	30	991	Meyer *et al*. 1998
stn-phu x (*chc x phu*)	2x	116	RAPD/RFLP	142	Yes; 30%	12	606	Hosaka 1999
pal x *etub*	2x	76	RFLP	80	Yes; 2.5%	19	704	Perez *et al*. 1999
phu x 2x*tbr*	2x	92	RAPD/AFLP	270	No; NA	12	987	Ghislain *et al*. 2001

Figure 1: Androgenesis. Flower buds are collected from the parental genotype when microspores are in the late uninucleate stage. A cold pre-treatment is performed before placing sterile anthers in flasks of liquid culture medium, maintained at room temperature with no light for 6 weeks. Embryos are then harvested from flasks and cultured on embryo-regeneration medium until plantlet regeneration. The ploidy of the resultant plantlets is determined using flow cytometry.

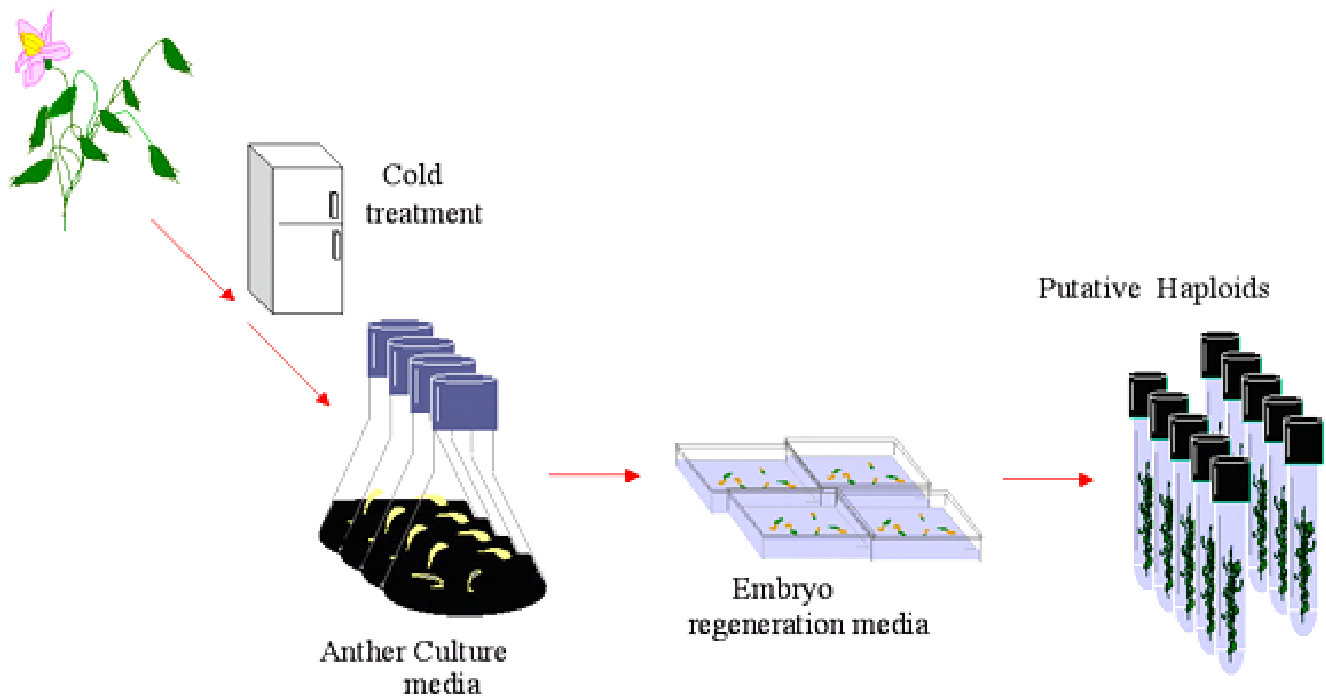
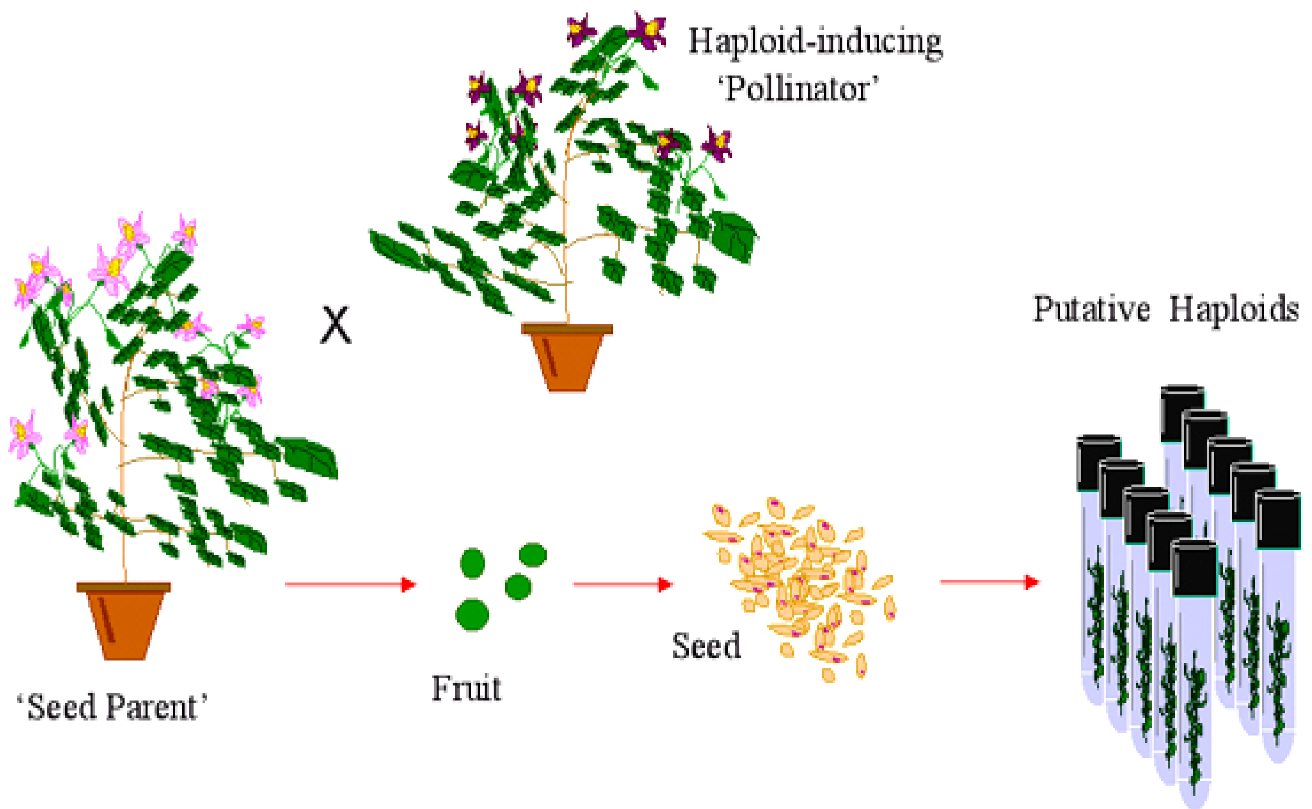


Figure 2: Gynogenesis. The greenhouse-grown seed parent is emasculated and crossed with pollen from the haploid-inducing 'Pollinator.' After 6 weeks, berries are harvested and their seed are extracted. Seed lacking the dominant embryo-spot marker are plated on culture media and germinated in vitro. The ploidy of the resultant plantlets is characterized using flow cytometry.



CHAPTER 2

Introduction

Nonrandom and aberrant haploid genotypes propagated by the alternate haploidization mechanisms, androgenesis and gynogenesis, are issues of current relevance to modern potato breeding programs. Haploids have been recent components in protoplast fusion research (Oberwalder *et al*. 1998; Bastia *et al*. 2001), strategies to combine wild and cultivated potato germplasm (Carputo *et al*. 1997; Serquen and Peloquin 1996; Rokka *et al*. 1998a, 1998b) and the generation of economically valuable mutant phenotypes (Hoogkamp *et al*. 2000; Vanderleij 1991, 1992). Moreover, most major genetic mapping and QTL analyses in potato have utilized haploid germplasm to simplify linkage-mapping computations (Ewing *et al*. 2000; Naess *et al*. 2000; Milbourne *et al*. 1998). Consequently, the accuracy of genetic assumptions concerning the randomness and genetic purity of haploid genomes may directly affect the statistical validity of many results in current potato research (Wilkinson *et al*. 1995; Clulow *et al*. 1997).

Rivard *et al*. (1996) used molecular markers to demonstrate that a nonrandom preponderance of certain marker alleles existed among anther-derived monoploids. They only used at most 27 RFLP clones, however, to demonstrate the extent of marker skewness, while many of the markers were clustered around only a few specific loci. Thus the extent to which the anther-derived haploid genomes were affected by segregation distortion remained to be adequately investigated. The molecular and phenotypic analyses conducted on the *Solanum phureja*-induced dihaploids presented substantial evidence concerning the nature and presence of the haploid-inducer genetic material in dihaploid potatoes (Clulow *et al*. 1991, 1993; Wilkinson *et al*. 1995);

however, the degree of genome dispersion and the consistency in the rate of genetic translocation of the inducer-genes are concerns that have yet to be fully addressed.

Along with haploidization, molecular markers are considered to be among the two most essential tools in potato mapping research. Using molecular-based approaches, genome analysts can achieve linkage map saturation formerly unattainable with traditional morphological and isozyme markers, thereby greatly increasing the precision at which traits can be located and studied (Gebhardt and Valkonen 2001). Amplified fragment length polymorphism (AFLP) markers are one of the most frequently used molecular markers in crop research today. Based in the polymerase chain reaction (PCR) and implemented with commonly practiced molecular techniques, the AFLP method is considered both DNA- and cost-efficient, and thus has gained popularity in genome mapping research (Blears et al. 1998). AFLP markers are generally considered to be dominantly inherited, wherein homozygosity and heterozygosity cannot be distinguished on the basis of band presence. Some studies, however, have demonstrated AFLPs can be used as codominant markers with limited success, on the basis of quantifying PCR products (Castiglioni et al. 1999; Piepho and Koch 2001). One of the most prominent aspects of AFLP markers is their excellent primer to band production ratio (multiplex ratio; Meyer et al. 1998). In potato, several studies have highlighted the ability of AFLP markers to generate many polymorphic bands between diploid and tetraploid genotypes (van Eck et al. 1995; Meyer et al. 1998; Kim et al. 1998). One study in particular enhanced the usefulness of AFLP markers for potato by creating a reference pool of 733 AFLP markers designed to provide interspecific anchors for the cultivated potato map (van der Voort *et al*. 1997a, 1998). The reference pool was created on the basis of marker allele comigration, wherein AFLP bands shared between fingerprints of multiple *Solanum* spp. exhibited identical mobility and corresponded to identical chromosomal

locations. The AFLP anchors are expected to provide an invaluable resource for the anchoring of various *Solanum* spp. maps to that of common potato.

Comparing agronomic performance between androgenic and gynogenic *S. phureja* Juz. & Buk. monoploid populations, Lough *et al*. (2001) recently addressed the practical implications of inadvertent genotypic selection at the haploid level. In 15 of 17 comparisons of morphological data, where a significant difference was due to derivation, the androgenic monoploids were superior in performance relative to their gynogenic counterparts. The superior traits among the androgenic monoploids included leaf length and width, leaflet number and tuber yield. In contrast, the gynogenic monoploids excelled over the androgenic monoploids for only one trait (plant height). Comparison of nuclear DNA content between the monoploid populations indicated that the androgenic monoploids showed evidence of increased endopolyploidization. Though they could not make definitive conclusions from their data, the researchers hypothesized that the population differences may have arisen from incorporation of haploid-inducer genetic material, differential selection pressures or genomic changes inherent in the haploid-derivation processes.

The monoploid populations previously characterized by Lough *et al*. (2001) represent ideal components for molecular-marker based research investigating the selective nature of potato haploidization mechanisms, as well as a platform for further characterizing monoploids for their utility in genome mapping. Toward this end, our present research objectives are: 1) to generate amplified fragment length polymorphism (AFLP) marker data for androgenic and gynogenic monoploids derived from a single *S. phureja* parental genotype; 2) to carry out comparative χ^2 analyses and phylogenetic analyses in an attempt to characterize haploidization-specific genetic artifacts; and 3) to create and qualitatively

evaluate a linkage map using the AFLP data derived from the androgenic monoploid population (Fig. 1).

Materials and Methods

Plant material

The highly heterozygous, diploid ($2n = 2x = 24$) *Solanum phureja* Juz. & Buk. clone BARD 1-3, selected from the adapted population (Haynes 1972) derived from PI 225669, was used as the parental genotype for deriving androgenic and gynogenic potato monoploids. BARD 1-3 was chosen as the haploid progenitor for its high androgenic and gynogenic monoploid production capability. *Solanum phureja* clone IVP-101, originally developed by Hersen and Verdenium (1973) from a cross between *S. phureja* PIs 225702 and 225682, served as the haploid-inducing pollinator during the creation of the gynogenic monoploids. IVP 101 is commonly used for its superior haploid induction capabilities (Hutten *et al*. 1993). The androgenic and gynogenic monoploid populations were previously created (1997–1999) as the basis for research comparing the agronomic potential and nuclear content of the monoploid populations (Cutright, 1998; Lough *et al*. 2001).

The androgenic monoploid population consisted of 58 genotypes (maintained *in vitro*) and served as the mapping population. Androgenesis was performed by collecting flower buds from *S. phureja* BARD 1-3 when the microspores were in the late uninucleate stage. A three-day cold pre-treatment was given before placing sterile anthers in flasks of liquid culture medium, which were maintained on a shaker (125 rpm) at room-temp with no light for 6 weeks. Subsequently, embryos were harvested from the flasks and maintained

on embryo-regeneration medium until plantlet regeneration. Ploidy was gauged using flow-cytometry and the monoploid plantlets were maintained *in vitro*. Details for this method have been previously published (Cutright, 1998; Lough *et al*.2001).

The population of 30 gynogenic monoploids yielded AFLP data used in comparing the degree and type of marker skewness found between the monoploid populations, as well as for investigating haploid-inducer gene introgression. Gynogenesis was performed using greenhouse-grown plants by pollinating the seed parent, *S. phureja* clone BARD 1-3, with pollen from the haploid-inducing pollinator, *S. phureja* clone IVP 101. Berries were harvested after six weeks. Seeds lacking the IVP 101 dominant embryo-spot marker were plated on culture media and germinated *in vitro*. Seedlings were evaluated for their ploidy using flow cytometry and putative monoploids were maintained *in vitro* via frequent subculture. Details for this process have been previously published (Cutright, 1998; Lough *et al*. 2001).

DNA extraction

DNA extraction was performed as a modified version of the procedure by Doyle and Doyle (1987). Fresh leaf material (1.5 g) from greenhouse-grown and *in vitro* specimens was frozen in liquid nitrogen and finely ground using mortar and pestle. Nucleic acids were released from cells by incubation (1 h 60°C) with CTAB extraction buffer [2% CTAB, 100 mM Tris, 1.4 M NaCl, 20 mM EDTA] and were separated from extraneous plant debris using centrifugation and a chloroform gradient. DNA stock solutions were suspended in TE buffer and maintained at –80°C for long-term storage. Quantitation of nucleic acid and protein concentration per sample was performed via ultraviolet

spectrometry. Additional DNA solutions were prepared from each stock solution to make the 'working' concentration (250 ng/µl) to be used in AFLP marker analysis.

Amplified fragment length polymorphism (AFLP) analysis

Eight primer combinations were used to generate AFLP data using DNA from the two monoploid populations, BARD 1-3, and the haploid-inducing pollinator, IVP-101. The generation of AFLP data was performed according to Vos *et al*. (1995) with slight modifications to DNA and reagent concentrations. Sample DNA (2 µl at 250 ng/µl) was digested in an incubatory reaction (3 h, 37ºC) with *EcoR*1 and *Mse1* restriction enzymes. Oligonucleotide adaptors specific to enzyme restriction sites were ligated to fragmented DNA through incubation (3 h, 37°C) with DNA ligase. A selective PCR '+1/+1 pre-amplification' step was performed using primers designed to amplify the DNA fragment between the adaptor sequence as well as one additional nucleotide within the fragment sequence. The polymerase chain reaction comprised 30 cycles of denaturation (94º C, 60 sec), annealing (60ºC, 30 secs) and primer extension (72º C, 30 sec). A second amplification was performed using the +1/+1 DNA and new primers comprising three-nucleotide extensions. Amplified DNA fragments were separated on a 4.5% polyacrylamide gel using 60 watts for 2 h. The gel was transferred to Whatman 3MM paper and vacuum dried. Marker data was visualized, initially, by exposing gels to autoradiographic film. This method was eventually substituted with more sensitive phosphorimaging techniques, enacted through exposing the dried gel to a phosphorimager screen. The screen was "developed" using the STORM phosphorimager (Molecular Dynamics, Amersham Biosciences, Piscataway, NJ). Molecular Dynamics' *Image Quant* (v 5.2) software was used to digitally visualize fragment data and *Convert*

16 to 8 software (v 1.5a; Molecular Dynamics) was used to convert *ImageQuant* GEL files to TIFF image format.

Film-based marker data were scored and recorded by hand. Phosphorimager-derived marker data were analyzed using *Photoshop* software (v 6.0; Adobe) to digitally enhance TIFF images and to convert the TIFF files to BMP files for use in *AFLP CrossChecker* (v 2.91; Wageningen Univ), which was used to score and convert the band data to text format. Data were archived in Microsoft *Excel*, which also provided a platform for performing χ^2 analyses and recording band data associated with IVP-specific markers and putative somaclonal variants. Data were combined in Microsoft *Excel* before a final transformation into the ASCII text file format used for importing raw locus data into *MapMaker/EXP* computer software.

Chi-square "Goodness-of-fit" Analysis

Barring the event of gametic selection, heterozygous loci in BARD 1-3 are expected to yield polymorphic loci in the haploid progeny. Due to the dominant nature of AFLP markers, a 1:1 presence to absence ratio of marker alleles is expected at each polymorphic locus. The χ^2 goodness-of-fit analysis was used to test marker segregation data among the 69 polymorphic loci common to both populations of monoploids. χ^2 statistics at each locus were tested for significance at the $P < 0.05$ and $P < 0.01$ level using one degree-of-freedom.

Phylogenetic Analysis

A phylogenetic analysis was performed using the RESTDIST program within the *PHYLIP* software package (v 3.6; Felsenstein 1989). Genetic distances between individual genotypes were based on the proportion of shared band fragments between any two specimens. Calculations were performed using a modified equation (RESTDIST default) of the 'restriction sites distance method' described by Nei and Li (1979). AFLP data included in this analysis consisted of the 69 loci common to both monoploid populations. Two analyses were conducted using total marker data and a data subset that excluded skewed markers ($P < 0.05$). Phylogenetic trees were drawn using the distance matrix method in the FITCH program, which implements the "Fitch-Margoliash and Least-Squares Distance Method" and is a component of the *PHYLIP* software package.

Linkage analysis of marker loci

Linkage analyses of marker data were performed under the F_2 backcross model of *MapMaker/EXP* (v 3.0; Lander *et al*. 1987). Marker data input consisted of a '1' for band presence and a '0' for a band absence. To enable *MapMaker/EXP* to consider markers linked in repulsion phase, we duplicated all marker data in reversed format, so that a band presence was symbolized with a '0' and a band absence was indicated by a '1.' Both forward and reverse formats for marker data were combined in the same data set for linkage group analysis. Linkage groups were created using two-point linkage analysis (LOD >4, cM < 30) between all markers. Local permutations of marker data within linkage groups were ordered at LOD > 3 using *MapMaker/EXP* "order" and "ripple" commands. Map distance in centiMorgans (cM) was estimated using the Kosambi calculation. Marker data exhibiting 2 < LOD < 3 were added to linkage groups sparingly and only after LOD >3 framework orders were in place. Two linkage maps were created according to the method described above by alternately using: 1) marker

data comprising skewed and non-skewed markers; 2) marker data consisting of non-skewed markers only. *MapMaker/EXP* linkage map data were compiled in ASCII text format to be used as input for drawing visual representations of the linkage groups using *GGT* software (Wageningen Univ., The Netherlands).

Additional linkage groups were created according to the method described by Tai *et al*. (2000), with a slight modification. Initially, one locus was selected from the total pool of skewed markers on the basis of unexcelled skewness (i.e., exhibiting the highest χ^2). This marker was then used as a "seed" for the creation of a group of markers linked with a putative segregation-distorting locus (SDL). All markers directly linked with the "seed" locus were included within this group if the additional markers also met the proposed genetic expectations for the theoretical SDL. The modification of the original strategy was performed at this point and consisted of first determining which marker class (see Results, Table 4) described the majority of grouped markers, followed by the exclusion of all markers that did not fit the marker class. This triple-screened group of markers was then ordered in *MapMaker/EXP* at LOD >3. Markers not included in the initial grouping were then used for the creation of additional SDL linkage groups by repeating the entire method on the reduced pool of skewed markers. *MapMaker/EXP* linkage map data were compiled in ASCII text format to be used as input for drawing visual representations of the linkage groups using *GGT* software (Wageningen Univ., The Netherlands).

Results

Marker Harvest

The quantity of useable AFLP markers generated in this study was less than anticipated, resulting from limitations in the electrophoresis apparatus. As a necessary P^{32}–related safety precaution, laboratory protocol restricted electrophoresis to a shortened duration (< 2 h) and therefore limited overall fragment migration in the gels. As a consequence, DNA sequence fragments of close molecular weight were combined as single loci on the gel, known as 'homoplasic bands' (Ghislain *et al*. 2001) or 'multi-locus markers' (Luo *et al.* 2000). These marker-aggregates were apparent as dense, oversized bands and occasionally prevented accurate grading of four to five different marker loci. Due to their inherently confusing nature, all putative homoplasic markers were excluded from subsequent analyses, thus accounting for a substantial reduction in the overall marker count (Figure 2).

A further reduction in marker count arose from data variation between the two monoploid populations, wherein the gynogenic monoploids exhibited band data lower in quality than that in their androgenic counterparts. The inferior gynogenic fingerprints likely arose from error during DNA extraction, since additional AFLP sample preparations of problematic genotypes (comprising original DNA solutions) did not improve band data. Poor AFLP data, which sporadically arose in a small number of genotypes (4-5), took the form of altered patterns of blurry bands. Despite this complication, we were able to collect a considerable amount of gynogenic monoploid data for six of the eight primer combinations. Primer combination sets #4 and #6 (Table 2), however, yielded data too poor for consideration in almost all of the gynogenic monoploids. This primer specific result indicates that, in the majority of gynogenic DNA samples, there is a slight contaminating variable that affects primer specificity or PCR stringency, such as foreign exonucleases or high phenolics, and that the variable is most prominent in a few individuals. The observable decrease in the total number of AFLP markers graded for the

gynogenic population reflects our pursuit of the most reliable polymorphic locus data (Table 1). A small, redundant subset of gynogenic AFLP fingerprints (data not shown), completely redone with newly extracted DNA, supported our belief that we selected the most reliable band data for analysis.

Chi-square comparative analysis

In the androgenic population the eight primer combinations yielded roughly 340 loci with 99 loci (30%) being polymorphic. Nearly half (46%) of the polymorphic loci exhibited deviations from Mendelian segregation ratios (Table 1), with one primer combination contributing as low as 9% of the total number of skewed markers and another contributing up to 20% of the total marker skewness (Table 2). Of the 99 polymorphic marker loci derived from the androgenic population, 69 loci were also present in good, scoring condition in the gynogenic monoploids. Based on data from these 69 loci, the χ^2 'goodness-of-fit' analysis was used to compare marker segregation data between the AM and GM populations. Relative to the χ^2 results derived from the gynogenic monoploid data, the AM population data showed a slight increase in the total number of skewed markers and a substantial increase in the intensity of marker skewness. In the androgenic monoploids, 17% of the polymorphic markers were skewed at the $P < 0.05$ level, whereas nearly 70% were skewed at the $P < 0.01$ level. In the gynogenic population, 43% of the skewed markers were significant at the $P < 0.05$ level and only 26% were skewed at the $P < 0.01$ level (Table 3).

Nine configurations summarize the alternating directions of marker skewness ($P < 0.01$) between the monoploid populations (Table 4). Rows 1 – 4 in the table pertain to markers that indicate a common selective pressure present operating in both the AM and GM

monoploids. The band alleles for these individual marker loci are either significantly higher or significantly lower than Mendelian expectations in both the AM and GM populations. In contrast, rows 5 – 9 in the table exhibit configurations that allude to population-specific marker skewness, as one population shows a Mendelian inheritance of marker alleles while the other does not. The number of marker loci at which the AM population shows marker skewness while the gynogenic population does not (10 counts), is conspicuously higher than the opposite instance (4 counts). The only marker configuration type not present in the table is one in which a marker allele is preferentially selected for in one monoploid population and selected against in the other population.

Origins of marker loci

AFLP marker alleles present in monoploid potato populations are expected to be derived from their parental genome; however, some monoploid alleles may originate in the haploid-inducing pollinator or from somaclonal variation during tissue culture. The AFLP loci observed in both monoploid populations provide marker evidence for all three possible origins (Table 5). As expected, monoploid alleles originating from BARD 1-3 are the majority case, representing 99% of the total marker count. These alleles were either homozygous in BARD 1-3 and monomorphic in the monoploids (65%), or they were heterozygous in BARD 1-3 and polymorphic in the monoploids (35%).

BARD 1-3 and IVP 101 exhibited AFLP fingerprints that were 87% homologous (Table 5). Approximately 32% of these BARD/IVP shared loci segregated in the monoploids, while the other 68% was in a monomorphic state in BARD 1-3 and the monoploid genotypes. Markers present in the monoploids and BARD 1-3, but not also present in

IVP 101, were only 7% of the total. These data suggest that a close genetic similarity exists between IVP 101 and the monoploid genotypes.

IVP-specific AFLP markers were in small number, representing 6% of the total band count. Bands specific to IVP assumed a variety of forms (Figure 3). Common forms were either bands that were confusable with co-migrating bands exclusive to BARD 1-3 and the monoploids, or bands that were homoplasic to loci shared among all *S. phureja* genotypes. Less frequent IVP markers comprised conspicuously identifiable bands, or bands that were also shared with the gynogenic monoploids. Three different IVP-specific loci were observed to appear in select members of the gynogenic monoploids. Two of these loci appeared in two gynogenic genotypes while the other locus appeared in only one. None of the monoploids showed more than one instance of IVP-specific genetic material. An instance of bands uniquely present in the monoploids consisted of one marker locus showing a band-configuration in three gynogenic genotypes.

Phylogenetic analysis

Figure 4 shows a phylogenetic tree based on skewed and Mendelian marker data. Based on the length of separation between the most remote genotypes, the androgenic monoploids appear to comprise a greater range of genetic diversity. The gynogenic monoploids show greater genotypic differences between close relatives. Throughout, the tree illustrates population-specific branch clusters, where members of the two groups of monoploids seldom integrate. The phylogenetic tree based on markers showing only Mendelian inheritance is shown in Figure 5. This tree is different from the former tree in a number of ways. The gynogenic genotypes appear to have an increased range of genetic diversity so that they have become comparable to the androgenic monoploids in

that respect. The differences between terminal branch pairs of the gynogenic population have also become more like their androgenic monoploid counterparts, with a few conspicuous exceptions. Despite this increased similarity between the two populations, some population-specific clustering remains. This clustering is most evident in the small cloistered groups occurring among the gynogenic monoploids, but can also be seen in some androgenic-specific branches on the upper peripheral of the tree.

Linkage map analysis

Two different linkage maps were created using *MapMaker/EXP* and *GGT* software with AFLP data derived from the androgenic monoploid population. Because the AFLP 30-330bp DNA ladder (Gibco-BRL) did not suffice in anchoring the linkage groups to a common potato map, all linkage groups are presently anonymous with respect to actual potato chromosomes.

The first map, shown in Figure 6, was created with a mapping analysis using all 99 polymorphic marker loci. This map is referred to as the Comprehensive Marker (CM) map, and reflects the all-inclusive marker placement strategy of early potato mapping efforts. The CM map comprises 67 markers spread among 11 linkage groups, with a total distance of 634 cM. A total of 16 markers was assigned to an "unlinked" category and are not shown on the map. Linkage groups I and IX are composed entirely of highly skewed markers; the other skewed markers present in the map are sporadically situated among six other linkage groups. Two areas of clustered markers, exhibiting reduced recombination frequencies, are prominent in this map, with the most densecluster occurring on linkage group III. This cluster is composed of eight markers without a single recombination event between them. The second cluster, located on CM linkage

group I, is less conspicuous than the first, being composed of just four loci in a 10 cM range.

Figure 7 displays the results from the second linkage analysis, which included only the 59 androgenic markers exhibiting Mendelian segregation ratios. These linkage groups are referred to as the Mendelian Markers (MM) map and are reflective of current mapping strategies that exclude skewed markers from analysis. In this map, 43 markers disperse among seven linkage groups, spanning a genetic distance of 319 cM. The remaining 16 markers were categorized as unlinked loci. Most of the locus orders in this map are syntenic to those in the CM map, with a few exceptions. The loci on CM linkage groups I and IX are completely absent in the MM map, since CM-I and CM-IX were composed entirely of skewed markers. All marker loci from CM linkage groups VII and VIII are likewise absent from the MM map, since 50% of their loci were excluded from the MM map due to marker skewness and the remaining markers were sequestered in the unlinked category. Thus four CM linkage groups were eliminated as a result of excluding skewed markers from the MM map. Most of the remaining differences between the CM and MM maps are nominal and represent only a minor reduction in total cM; however, MM linkage group V underwent a notable transformation that the other MM groups did not. When MM-V lost its one skewed marker (C1_28), the marker C8_48 filled the gap. It was this new pairing that influenced an equalizing response in the internal marker ordering of MM-V, thereby resulting in a slightly different marker ordering than what was originally present in CM-V.

The third linkage analysis was performed using a modification of the strategy described by Tai *et al*. (2000). The Skewness Map (SM) comprises linkage groups of skewed markers grouped around their theoretical SDL. Sixteen markers were assembled in four

linkage groups, spanning a distance of 157 cM (Figure 8). The largest linkage group satisfied both the theoretical expectations and the marker class screen: however its final distance intervals correlated poorly with expected results. The second linkage group provided an image highly consistent with expected results. The third and fourth groups were consistent with expectations, but are too small to judge their accuracy.

Discussion

The substantial amount of AFLP data generated in this study, via the eight AFLP primer combinations, allowed us to complete the majority of our research objectives. A considerable obstacle to our progress, however, originated from an unforeseen and problematic aspect of the AFLP marker system. Specifically, this was the difficulty found in accurately grading our AFLP marker data.

The high multiplex ratio is considered one of the great advantages to AFLP analysis; however, our experience adds to a list of studies suggesting high multiplex ratios coupled with high potato heterozygosity can represent an unwieldy combination. The general problem stems from the fact that AFLP markers are dominantly inherited, and thus act as alleles and not loci (van der Voort *et al*. 1997b). Consequently, a potentially vast number of allelic forms may be amplified in an AFLP potato fingerprint, due to the great genetic diversity of the *Solanum* spp. Naturally, this is where the high productivity of the AFLP system arises; however, as a result of this general marker abundance in a gel, a substantial number of AFLP bands may have identical or nearly identical mobility, which will respectively allude to chromosomal locus alleles or to comigrating DNA fragments from different chromosomal loci. Therefore, to accurately grade an AFLP fingerprint, one needs to discern 'coincident comigration' of different DNA fragments from the

comigration of chromosomal locus alleles, especially for the correct implementation of the published AFLP-anchors (van der Voort *et al*. 1997a). If a marker analyst employs careful scrutiny of band data, van der Voort *et al*. (1997a) estimate the probability of coincident comigration of bands arising by chance (i.e., not due to same locus origin) to be 0.03, and therefore of nominal impact to map-anchoring attempts. However, the crucial assumption underlying this low probability of error is that "small" differences in band mobility will be detected during the grading process. Van der Voort *et al*. (1997a) clarify "small" differences as alterations in nucleotide composition, not just fragment size. Thus, to avoid collecting erroneous data, the average marker analyst is expected to scrutinize their autoradiograms at every segregating locus for comigrating 'allele-imposters.' Yet this may not always be feasible, as imperceptible differences between alleles were observed by the authors only after sequencing efforts; e.g., two nearly identical fragments differed only by a 46 nucleotide stretch in two 240 bp fragments (van der Voort *et al*. 1997a).

Three recent studies evidence the inherent difficulty in discerning comigrating DNA fragments from comigrating chromosomal locus alleles. Using a tetraploid mapping population and 39 AFLP primer combinations, Meyer *et al*. (1998) generated a total of 1066 polymorphic markers, but found that only 573 (54%) were "considered scorable within the required accuracy." They noted that some of the discarded markers exhibited differential band intensities. Even from the select pool of bands, the researchers observed a percentage (24%) of the markers to fit poorly into the expected segregation classes. Due to the inefficient marker harvest (only 10% of the marker total were mapped), the researchers concluded that AFLP markers would be "abandoned" for SSR markers in the future.

Ghislain *et al*. (2001) used several marker types (AFLP, RAPD, RFLP and SSR) to create and anchor genetic maps of a *S. phureja* x dihaploid *S. tuberosum* F_1 hybrid population. Because no reference was made to the number of primer combinations used, or to the actual percentage of skewed markers found, it is difficult to say how efficient the AFLP markers were in their population. However the researchers observed that the AFLP markers showed an increased frequency of homoplasic band formation relative to the other marker types, declaring that 15 and 18% homoplasic bands were observed in the first and second parental maps, respectively. Moreover, despite performing electrophoresis with both their own samples and the five reference DNA samples used in the comigrating AFLP marker study (van der Voort et al. 1997a), the researchers observed that anchoring their maps was "more accurate" using RFLP and SSR markers, thus implying that inconsistency, or some other error, was experienced using the AFLP anchors. Van der Voort *et al*. (1997b) also noted mixed success in applying their own comigrating AFLP-anchor strategy to a different mapping population. In their study, an insufficient number of comigrating AFLP markers was found (from a pool of 196 segregating markers) to anchor one of their linkage groups to chromosome V, and only the minimum (3 markers) was found to anchor the correct linkage group to chromosome XII. Since their study only focused on those two chromosomes, no additional data were reported concerning the anchoring success for the remaining linkage groups. The researchers did conclude, however, that RFLP markers remain the "more accurate" means for identifying chromosomal intervals. The 733 AFLP markers serving as a reference map, on the other hand, are not as useful as RFLP markers to many mapping projects since the allelic heterozygosity of the *Solanum* spp. reduces AFLP-anchor application (van der Voort *et al*. 1997b). The researchers' failed attempt to apply the AFLP-anchors to a second more distantly related mapping population (*S. bulbocastanum* x *S. tuberosum*; van der Voort *et al*. 1999) provides additional evidence for this assertion.

It comes as little surprise, therefore, that our marker total was reduced from what was originally expected. The increased rate of polymorphism among our monoploids, coupled with reduced electrophoresis, increased the number of ambiguousalleles that had to be graded,at times resulting in complex banding patterns that had to be excluded from analysis (Figure 2). Moreover, no anchors could be found for our linkage maps with a suitable level of confidence, due to the difference in resolution quality between the published AFLP-anchor fingerprints (van der Voort *et al*. 1997a) and our own gels. Yet, given the questionable success presented in the mapping studies that ran parental DNA from the AFLP-anchor study (van der Voort *et al*. 1997b; Ghislain et al. 2001), it is evident that the strategy may be too sensitive for the grading complexity inherent in thepotato-AFLP marker system.

Despite the trouble experienced in discerning reliable from unreliable band data, a substantial amount of trustworthy marker information was generated for the later analyses. One immediately evident aspect of the AFLP data, upon first analysis, was the visible lack of haploid-inducer genetic material in the gynogenic AFLP fingerprints. Haploid-inducing ‘pollinators’ have been observed to contribute DNA to potato haploids in the form of extrachromosomal fragments (Clulow *et al*. 1991, 1993) or as DNA sequences directly integrated into the haploid genome (Wilkinson *et al*. 1995). In total, there were three markers suspected of being putative haploid-inducer genetic material, wherein five different gynogenic monoploids displayed band patterns that were otherwise unique to IVP 101. A fourth candidate marker comprised bands completely unique to three specific monoploids, which also could represent fragmented inducer-DNA (Clulow et al. 1993). Compared with related research, our findings represent a marked reduction in haploid-inducer gene introgression of haploid potatoes. Clulow *et al*. (1991) analyzed

ten aneusomatic potato dihaploids (*S. tuberosum*) and found four clones (40%) that exhibited RFLP evidence of inducer-specific genetic material. Using related germplasm, Waugh *et al*. (1992) found 13 of 15 dihaploid clones to exhibit RAPD markers specific to the haploid-inducer. The validity of both studies was later corroborated with additional research using morphological markers, leaf isozymes, Southern blot and GISH (genomic in situ hybridization) analyses for gene-introgression detection (Clulow *et al*. 1993; Wilkinson *et al*. 1995; Allainguillaume *et al*. 1997). Considering the contrast of these results with our own, it seems possible that the dihaploid germplasm used in the above research was more susceptible to gene introgression than that used in our study. This is not an unreasonable hypothesis considering the different developmental backgrounds of the haploid germplasm in question. Crosses between *S. tuberosum* (4*x*) and *S. phureja* (2*x*) have been shown to yield both diploid (dihaploid) and triploid offspring. The triploid offspring, by means of chromosome elimination, are hypothesized to be the origins of the dihaploids in the above research (Clulow *et al*. 1991). Our gynogenic monoploids, derived from an intraploidy (2*x*) *S. phureja* x *S. phureja* cross, originated from a pool of predominantly diploid and monoploid seeds, and thus may not have the same developmental complexity as *S. tuberosum* dihaploids. In fact, it is possible that the majority of our monoploids actually arose from parthenogenesis, the initially hypothesized gynogenic mechanism (Hermsen and Verdenius 1973; Rowe 1974). Chromosomal elimination, on the other hand, would have more importance for the survival rate of triploid gametes, rather than monoploid or diploid gametes.

As an alternative hypothesis to that described above, haploid-inducer gene introgression may indeed be present in our monoploid populations, but our detection strategy (i.e., AFLP markers) may not be sensitive enough to detect it. The extremely close genetic relationship between the diploids used in this study can be observed in the low amount of

unique marker data between them. The difference between the two diploids includes 17 bands specific to BARD 1-3 and 14 bands specific to IVP 101. The haploid-inducing pollinator and monoploids, therefore, are similar enough that unique IVP 101 genetic material may actually incorporate into the gynogenic genotypes (i.e. as either extrachromosomal or intrachromosomal fragments) without being conspicuous. Until analysis is conducted at a more sensitive level (e.g., AFLP resolution on a sequencing gel), our conclusions will be limited to speculation.

The alternate hypotheses discussed above have an important, shared implication between them, which is specifically valuable to the ongoing investigations in our lab. Whether gene introgression is occurring in the GM population but cannot be observed due to its close relationship with IVP 101, or whether little to no gene introgression is occurring at all, it becomes evident that the substantial differences found between the AM and GM agronomic performances are not likely due to the introgression of inferior traits from the haploid-inducer, as formerly hypothesized (Lough *et al*. 2001). In fact, the AFLP data suggests that few, if any, radical changes occurred to the majority of gynogenic genotypes as a result of incidental IVP genetic material introgression. Presumably, a more influential genetic difference must exist between the monoploid populations to impart such a significant effect on their relative field and greenhouse performances.

The fact that somaclonal variation (in the form of nonparental bands) was largely undetected in our monoploid populations is a little more difficult to explain than the lack of inducer-gene introgression. Typically, at least some evidence for somaclonal variation has been observed whenever tissue culture regenerants have been thoroughly investigated (Veilleux and Johnson 1998). Our results are not completely anomalous, however. Rivard *et al* (1994) found high genomic stability (i.e., no marker evidence was found for

gametoclonal or somaclonal variation) present in their androgenic monoploids, irrespective of their callus or non-callus origins. The researchers did note that several spontaneous doubled haploids occurred in their monoploid populations, but these genotypes were never a source of non-parental marker alleles. Yet, Rivard et al. (1994) used only 73 RFLP probes, which can be expected to provide sparse illumination of the potato genome. Our study, on the other hand, with a higher number of bands analyzed, offers good corroborating evidence that monoploid genotypes may be as genetically stable as the 2x and 4x ploidy levels. A possible reason for their seemingly stable nature is that the hemizygous nature of monoploids may make them particularly sensitive to various forms of somaclonal variation, such as chromosomal aberrations, point mutations or transposon activation, which may result in the early extinction of affected genotypes. On the other hand, one of the major sources of somaclonal variation, spontaneous DNA methylation, may escape undetected in our AFLP data, since both *Eco*R1 and *Mse*1 are not methylation-sensitive restriction enzymes. To investigate this area, new AFLP reactions would be required using a pair of isoschizomers; but even then it would be possible that some DNA methylation, given the vast expanse of the potato genome, would go undetected.

Due to their putatively low impact on the monoploid genomes, both haploid-inducer gene introgression and somaclonal variation seem to be unlikely origins for the great difference in agronomic performance observed between the androgenic and gynogenic monoploids (Lough et al. 2001). The marker skewness data, on the other hand, provides a plethora of genetic variability between the two monoploid populations. In the androgenic monoploid population, the percentage (40%) of polymorphic markers skewed from the expected Mendelian ratio was near the range already cited in previous monoploid studies. Rivard *et al*. (1996) found 70% of their RFLP markers to be skewed

in one *S. chacoense* anther-derived monoploid population and 46% in another population . It is the latter population, however, that has a developmental background more similar to our population of monoploids, which were generated from direct embryogenesis during anther culture. The population of monoploids exhibiting 70% skewed segregation was derived from an intermediate callus stage (indirect embryogenesis). Tai *et al*. (2000) also found 45% of their RAPD markers skewed in their androgenic monoploids, however, the origin of their population was mixed (embryo/callus). Considering these general results, it seems evident that three of the four monoploid populations discussed above show only moderately elevated levels of marker skewness, relative to published mapping populations, with our population exhibiting the least of the three.

For potato, whether or not gynogenic haploids show marker segregation distortion trends comparable to androgenic haploids is presently unknown. In our study, the χ^2 based on markers common to both monoploid populations (Table 3) appear to suggest that the androgenic monoploids exhibited the highest degree of segregation distortion, with 54% of their skewed loci significant at the $P < 0.01$ level. However, due to the considerable difference between the AM and GM population sizes and concomitant inequality in statistical power, the higher percentage of marker skewness in the AM population may be misleading. Subsamples of the AM population were created to investigate this problem of unequal comparison. The subsamples (replicated three times) were created with 25 randomly selected AM genotypes pooled from the original 58 AM, and χ^2 were performed with the new reduced tallies (data not shown). The identity and proportion of loci skewed in these AM subsamples were substantially different from that found in the original AM population, with the most variation being among loci skewed at the $P < 0.05$ level. Markers skewed at $P < 0.01$ in the subsets were more reliable, however, and varied nominally. Assuming the AM and GM populations comprise similar genotypic variance,

these results acknowledge a possible statistical instability to the χ^2 of the GM population, which is equivalent in size to the AM subsamples. To minimize the consideration of unreliable and misleading data, observations at the $P < 0.05$ level were thus excluded from Table 4, which illustrates the variety of marker skewness found in our monoploid populations. The statistical power conferred to the sizeable AM population limited the number of discarded $P < 0.05$ observations to five, whereas a total of 11 GM observations were excluded at the same probability "cut-off." Due to this data manipulation, Table 4 may not be a reliable source for quantitative comparisons between the populations; however, in contrast, Table 4 should be a reliable indication of the types of marker skewness found among the AM and GM monoploid populations.

It is evident that marker skewness in our monoploid populations falls within two general classes, presumably reflecting marker associations with different segregation distorting loci (SDL; Tai *et al*. 2000). The first marker class (rows 1–4; Table 4) is composed of markers similarly skewed in the AM and GM populations; i.e., marker band alleles are overabundant, or under-represented, at the same loci in both populations. Theoretically, SDL that elicit this bilateral effect (hereafter 'bilateral SDL') may be one of two types. On one hand, bilateral SDL may comprise deleterious alleles that are recessive in the heterozygous, diploid parent, but hinder, or preclude, survival in the hemizygous monoploid gametes or plantlets. Alternatively, bilateral SDL may comprise genes that play a role in an evolutionary fork common to androgenic and gynogenic monoploids during haploidization. This might be, for example, the ability to survive in tissue culture, or the ability to spontaneously double in ploidy during subculture; either of these cases would selectively exclude specific genotypes from both monoploid populations, and thus cause marker skewness around the same SDL in both populations. With the present data, it is impossible to determine the exact nature of the bilateral SDL in our monoploids;

however, due to the strong selective force these SDL represent to the monoploid genotypes and their related molecular data, a further investigation into the identity of the SDL may uncover areas of deleterious loci or areas important to haploidization in the diploid genome.

The second type of marker skewness (rows 5–9; Table 6) originates from a different class of SDL, since selective pressures are evident in one monoploid population but not in the other. In this ‘unilateral SDL’ class, the culture stages unique to either haploidization scheme can be held primarily accountable. Loci eliciting marker segregation distortion solely in the androgenic population, for example, may comprise alleles necessary for survival in anther culture, such as embryo formation in liquid media or shoot regeneration *in vitro*. Alternatively, unilateral SDL may comprise deleterious loci that are present in both monoploid populations, though deleterious in only one. This scenario is expected to occur when one haploid-inducing process “pampers” haploid plantlets more than the other (Lough et al. 2001). As an example, the protective, nutritive tissue of the endosperm has been hypothesized to confer better survival rates to gynogenic embryos than what might be found for developing embryos during anther culture. For either of the above unilateral SDL cases, however, the unique survival conditions inherent in the alternate haploidization processes are mainly responsible for the skewed marker alleles. Therefore, it is these marker alleles that offer the most attractive candidates for MAS breeding. It has already been shown that parental genotypes can be selected for better haploid production levels (Meyer et al. 1993). Depending on their specific identity, the markers associated with putative unilateral SDL in our monoploid populations might be used in the future to create PCR-based assays for regions of the genome important to haploidization. In this way a more direct method can be created for developing efficient parental genotypes for haploid production.

As a visual representation of the marker data, the phylogenetic trees illustrate the genetic relationships (as a function of shared marker alleles) among the monoploid populations. The χ^2 analyses discussed above are therefore described, in part, in the Figure 4 phylogenetic tree, which includes both skewed and non-skewed markers. Though it is not possible to view the influence of specific, skewed marker loci over the genetic distances present in the tree, cumulative effects of marker skewness can be readily observed. Barring the possibility of any type of genetic selection, or other sources of data differentiation between the two populations, it is expected that each terminal "branch" of the tree should be nearly equidistant from the next. Moreover, the red and blue colored-branch ends (representing the AM and GM clones) should be dispersed randomly and thus fairly integrated. The tree that is observed in Figure 4, however, illustrates the opposite case. At a glance, the AM and GM populations show a visible degree of nonrandom branch separations among them in the form of clustered branches on the tree. This clustering is largely due to the markers uniquely skewed in either population, a fact evident by comparing the tree in Figure 4 with the tree solely composed of non-skewed markers (Fig. 5); the latter exhibits an increased integration of AM and GM genotypes, whereas the former does not. Clustering is still evident in Figure 5, despite the exclusion of markers skewed at the $P < 0.05$ level. However, the majority of the clustering (top cluster) belongs to the gynogenic genotypes with the most problematic AFLP fingerprints; or, in other words, the clustering is due to missing data rather than persistent similarities in marker "phenotype." In those gynogenic samples, the elevated proportion of missing data effectively amplifies the weight of the data that is present, but does not necessarily reflect actual genotype. Thus, the top cluster should be discounted from the Figure 5 tree. In contrast, the small bottom cluster of gynogenic genotypes does not show an excess of missing data, rather the tight clustering is reflective of a unique

configuration of AFLP alleles. Overall, however, the act of eliminating the skewed markers from the data set appears to have integrated the marker-based phenotypes of the two monoploid populations to a great extent. Due to the variable levels of missing data per each genotype, however, this can only be seen as an interesting preliminary assessment. Additional data is required for a more detailed phylogenetic analyses before this observation gains substantial support. A detailed summary of missing data points and branching-characteristics, per genotype, would be particularly useful for determining the effect of missing data on genetic distance calculations. Until then, however, we will make speculations concerning our present results. Acknowledging that the phylogenetic analyses were based on statistics similar to those used by MAPMAKER (Felsenstein 1989; Lander *et al*. 1987), we might speculate that selectively excluding monoploid marker skewness data has a practical application for mapping. By combining the data from our sibling AM and GM populations (already stripped of their skewed marker data) we might be able to increase our mapping population size while maintaining an integrated phenotypic array. In this way a valid framework map, though containing an unknown number of genetic gaps, can be created for the parental genotype in question. Some studies have already shown success in combining male and female gametes by using computer software that accounts for different recombination rates (Jacobs *et al*. 1995).

Besides addressing key issues in plant breeding, haploids represent a unique tool for tackling issues in potato mapping. Specifically, monoploids comprise extremely basic haplotypes, compared to their diploid and tetraploid counterparts, and therefore monoploids present a unique ability for QTL mapping by simplifying epistatic and epigenetic allelic interactions. Our AFLP data from the AM population allowed us to

investigate the direct utility of monoploids for genetic map construction, as well as investigate some of the unique marker-types revealed in the χ^2 analyses.

The CM map embodies the strategy of early research in potato genome mapping, where the inclusion of skewed markers was commonly practiced (Table 6). The early mapping analyses are largely outdated, however, given the present direction in genome mapping, identifiable by the active exclusion of marker skewness data. Thus, the CM map was created mainly for comparing the suitability of our monoploid mapping population with the more commonplace mapping populations. In the CM map, 67 markers are spread among 11 linkage groups, with a total distance of 634 centiMorgans (cM). Overall, the CM map length compares well with several diploid and monoploid maps, though many of them employed an increased number of markers (Table 6). This favorable result may be due to the increased number of observable recombination events in our mapping population; however, the fact that our map only includes 11 linkage groups out of the expected 12 indicates the genetic distance is not necessarily indicative of good genome coverage. Furthermore, it is likely that the greatest proportion of genetic distance (i.e. group CM-I) is actually composed of erroneous linkage assignments, since the CM-I linkage group is composed entirely of skewed markers. In MA*PMAKER/EXP*, skewed markers cause recombination frequencies to inaccurately represent genetic distance and may instigate the creation of spurious linkage groups (Lander *et al*. 1987; Foisset and Delourme 1996).

The MM map was based on markers showing only Mendelian segregation ratios to satisfy the basic mapping assumptions employed by MA*PMAKER/EXP*. The result was a reduced number of linkage groups (seven total) and a reduction in total genetic distance covered. The remaining linkage groups generally show good homology with the CM

map. The cluster on MM-III is interesting for its great density of loci, quite anomalous with respect to the other linkage groups. This centralized cluster pattern has been seen in other crop species as well, though the central clusters are found among all the linkage groups. In maize (Castiglione *et al*. 1999), soybean (Young *et al*. 1999) and tomato (Haansta *et al*. 1999), these centralized chromosomal clusters have been associated with the *EcoR1*-based AFLP primer combinations, which show preferential targeting of the heterochromatic regions surrounding the centromeres. The *Pst1*-based AFLP primer combinations, on the other hand, are sensitive to cytosine-methylation in the centromere regions, and have been observed to provide better genome coverage of the more distal areas of the chromosomes. Therefore, in our MM map, it is likely that the central loci of the large linkage groups and most of the loci in our smaller linkage groups are actually situated near the centromeres of a select number of the potato chromosomes.

Typically, a genetic map is considered “complete” when the number of linkage groups equals the gametic number of chromosomes and when the markers are spaced at an average of 5% recombination intervals (Paterson 1996). Therefore, in our case, the MM map cannot be expected to represent any sizable portion of the diploid potato genome, which is estimated to be around 1120 cM (Jacobs *et al*. 1995). Though some partial genome maps have been used in successful QTL mapping attempts (Meyer *et al*. 1998; Bradshaw et al. 1998), we can assume the MM map will be most productive as a trait-mapping tool only after additional servicing. Primarily, a general marker fortification effort would be required to increase the linkage group number and genetic distance covered to the acceptable standards already determined by the major potato maps. Amendments should also include markers that anchor our linkage groups to existing maps. A number of research groups have published possible anchors for the *S. phureja* genome (Hosaka *et al*.1988; Ghislain *et al*. 2001) using RAPD and SSR markers that may

prove more easily applied to our population than the published AFLP-anchors. The eventual anchoring of our map will validate and identify the presently mapped linkage groups, as well as associate our markers with reputable maps. Moreover, the gaps in our map, due to the aforementioned varieties of marker skewness, can be located in the *S. phureja* genome with the use of chromosome specific markers. This additional information will be key to understanding the genome coverage-limitations of our map. After these specific amendments have been accomplished, we will gain a more accurate illustration of the *S. phureja* genome, as well as an increased number of markers to choose from when investigating specific areas of interest on our map.

The areas of special interest on our maps are those that pertain to the genetic mechanisms behind androgenesis and gynogenesis. Despite the inadequacy with which the MM map covers the potato genome, we can still observe the presence of some of these important areas on our map. One such area consists of markers skewed in the gynogenic population and Mendelian in the androgenic population, which are thought to involve one or more unilateral SDL important to gynogenesis. The four markers clustered on MM-III (Fig. 9) exhibit the same type of unique segregation pattern (i.e. unilateral marker skewness in the gynogenic monoploids) and have been ordered relatively close together on the linkage map. This consistency gives credence to a hypothetical locus that may affect the differential viability of gynogenic monoploids. After anchoring MM-III to a published map we will be able to foster further investigative research into the validity and importance of this SDL.

We used the strategy described by Tai *et al*. (2000) to order markers skewed in the androgenic population into meaningful linkage groups. The original strategy by Tai *et al*. (2000) was proposed by using an example based on 25 skewed RAPD markers taken

from a population of 25 anther-derived monoploids (haploid *S. tuberosum* x *S. chacoense*). Their example was successful in identifying and mapping four putative SDL in perfect accord with the corresponding genetic expectations described by the authors (see Tai *et al.* 2000). When we applied the strategy to our AFLP data, however, we did not gain the same consistency between theory and product. Instead, SDL linkage groups comprised map intervals that were extended beyond expected distances and markers that did not show expected relationships with their "seed" loci. Our modified strategy, using the additional marker-screening step, partially improved the results, wherein one linkage group was created that highly resembled the theoretical expectations (SM-II; Fig. 8). Two additional linkage groups seemed compliant with the expectations, though they were too small to allow an accurate judgement (SM-III and SM-IV). The most consistent SDL group (SM-II), composed of putative unilaterally skewed markers important to the androgenic population, represents a successful interaction between the theory created by Tai *et al*. and our experimental observations based on the comparative χ^2 analyses. This collaboration thus supports the SDL linkage group as well as the individual identities of the markers contained within. Additional research may discover the possible importance these markers have in the androgenic population and ultimately aid in MAS for parental genotypes more efficient in haploid production.

As noted above, the majority of remaining skewed markers could not be placed into informative SDL groups. Therefore, either the value of our data or the methodology used in mapping the data is of questionable value. If both areas were equally adequate, then realistic SDL linkage relationships among most, if not all, of the skewed markers should have been found. The quality of the data has already been discussed. Some error can be expected to arise from mistaken segregation ratios present in undetected homoplasic loci, where different comigrating DNA fragments may confuse the grading process. But our

screening methods were fairly conservative and any data that indicated homoplasic band formations was excluded from later analyses. Additionally, the overall marker skewness attained for our androgenic population was less, not more, than that seen in other similar mapping populations (Rivard *et al*. 1994; Tai *et al*. 2000), which suggests our marker skewness data set is not enlarged with the presence of erroneously scored data. Therefore, the reason for the poor SDL mapping results should not solely be described by problematic data.

Tai *et al*.'s (2000) methodology employs certain assumptions that also might cause inconsistent success in mapping our skewed marker data. The foremost case for this point is that the negatively selective SDL alleles (versus the viable allele) in Tai's strategy are expected to exercise complete lethality over the monoploid genotypes, and thus affect linked markers accordingly. Yet, many stages of the haploidization process have been observed to be quantitative in nature, such as parent receptivity to haploidization (Singsit and Veilleux 1989), plantlet success *in vitro* (Meyer et al. 1993), and overall haploid vigor (Lough et al. 2001). In these cases, whether or not an SDL leads to the extinction of a haploid plantlet depends mainly on the complementary haplotype (i.e., synergistic gene effects), and not a rigid assumption of lethality. It follows therefore that linkage groups based on distorted marker segregation ratios actually observed in our monoploids may not always resemble the linkage groups based on Tai's genetic expectations.

The second area of concern is more speculative in nature, as it considers the presumed limit in the types of SDL predicted to exist in the monoploid genome. A maximum of three different SDL was considered by Tai *et al*. (2000). Two SDL were composed of single selective loci, while the third addressed the possibility of two selective alleles

being situated at a single locus. The latter comprised two alleles that were independent of each other and completely lethal to the monoploid genotype, which ignores once again that the alleles might actually interact with each other either in a mutually exclusive or synergistic fashion. Certainly additional and more complex SDL scenarios may result from the gradual evolution of genetic sequence deletions, substitutions and recombinational hotspots present throughout the potato genome.

At this point, the exact origin of our general failure to create the expected SDL linkage groups cannot be known. The questions concerning the purity of the data are perhaps the easiest to address, since repeated AFLP reactions may indicate an answer in one way or the other. Testing the soundness of Tai's strategy is a more difficult issue, since success and failure in applying the strategy will likely be intermixed, depending on the similarity between the theoretical SDL of the strategy and the actual SDL within the genotypes in question.

Conclusion

In this study, we investigated the extent to which androgenesis and gynogenesis affect the genomes of their monoploid products. Little to no evidence of genetic material from the haploid-inducer was present in our gynogenic monoploids, which suggests that BARD 1-3 and the monoploids were too genetically similar to IVP to incur noticeable changes, or that monoploids derived from diploid parents are less prone to gene introgression than their dihaploid counterparts. Chi-square analyses of AFLP data revealed multiple classes of marker segregation distortion present in both monoploid populations. Bilateral SDL were observed to produce marker skewness at the same loci in both populations, whereas unilateral SDL produced marker skewness in only one population. The unilateral SDL

are expected to have unique relevance to the haploidization mechanisms themselves and may eventually prove useful in marker-assisted selection for parental genotypes amenable to haploidization. The phylogenetic analyses in this study suggested that the AFLP marker data included unique AM and GM-specific marker configurations that appeared to become nominal in presence after skewed marker data were removed from the analysis. The androgenic monoploid population served as an initial attempt at map construction and provided a means for investigating a unilateral SDL in the gynogenic population, as well as for investigating a unilateral SDL in the androgenic population. The latter SDL was mapped with a high degree of confidence using a strategy detailed by Tai *et al*. (2000).

Beneficial mapping projects could be initiated on the basis of this research. Because little foreign DNA or marker patterns were observed in our monoploid populations, there is good evidence that they are stable germplasm for genetic mapping or other genetic analyses. Moreover, the phylogenetic analyses demonstrated that the removal of population-specific skewed marker data may allow the populations to be combined to exhibit a larger population with an integrated genetic diversity. The putative unilaterally skewed markers could be alternately assigned to the monoploid map by selectively excluding and including the appropriate marker/genotype data (Figure 9) and subsequently aid in determining their importance to androgenesis and gynogenesis. The putative bilaterally skewed markers, on the other hand, could be used to study the genetic basis for genetic load, or be used for the MAS screening of parental genotypes that promise higher monoploid production capacity.

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Table 1: Marker statistics for androgenic (AM) and gynogenic (GM) monoploids based on individual AFLP marker totals.

	Genotypes[a]	Primer Comb.	Polymorphic Loci	Skewed Loci [b]
AM	58	8	99	46 (46%)
GM	28	6	69	24 (35%)

[a] Number varies among primer combinations

[b] Values at $P < 0.05$

Table 2: Relative productivity of AFLP primer combinations as based on data derived from the androgenic monoploid population.

Primer Combination	Total No. of Bands[a]	No. of Polymorphic Loci	No. of Skewed Loci	% Skewed (P<.05)
E+AAC/M+CAC	40	13	9	69%
E+AAC/M+CAG	40	13	6	46%
E+ACA/M+CAC	40	13	7	54%
E+ACA/M+CTG	45	10	4	40%
E+ACA/M+CAG	45	14	5	36%
E+AGA/M+CAT	55	12	4	33%
E+AAC/M+CTG	40	14	7	50%
E+ACA/M+CCT	35	13	4	23%

[a] Variation in resolution and fragment separation among gels precludes an exact locus count

Table 3: Chi-square results based on 69 loci common to both monoploid populations.

	Genotypes[a]	Polymorphic Loci	Total No. Skewed	P < 0.05	P < 0.01	P < 0.001
AM	58	69	29	5 (17[b])	4 (14)	20 (69)
GM	26	69	24	10 (44)	7 (30)	6 (26)

[a] Number varies with primer combination

[b] Numbers in parentheses represent percentages of total no. skewed

Table 4: Nine configurations of marker skewness in the androgenic and gynogenic monoploid populations. Configuration type is composed of the similarity or dissimilarity between the 1*x* populations for allelic segregation patterns of 69 skewed loci. Marker bands in overabundance (+) or marker bands underrepresented (-) could be linked with segregation distortion loci (SDL) in the form of deleterious loci or loci enhancing gametic survival (e.g., haploidization mechanism-specific genes). SDL types in the table were one of two kinds: 1) an SDL enacting a bilateral marker skewness, consisting of similar band preferences between the two populations; or 2) an SDL enacting a unilateral marker skewness. Mendelian (M), all present (++) and all absent (0) band configurations are also noted.

Configuration	SDL Type	Andro	Gyno	No. of Loci ($P< 0.01$)
1	1	+	+	2
2	1	-	-	2
3	1	++	+	3
4	1	0	-	2
5	2	M	+	3
6	2	M	-	1
7	2	+	M	6
8	2	-	M	4
9	2	++	M	1

Table 5: Origins of loci present (P), absent (A) or segregating (S) in the *S. phureja* genotypes. BARD 1-3, as the haploid-progenitor, is believed to donate the majority of bands to the monoploids, while IVP 101 may or may not include the same bands. Bands not present in BARD 1-3 but present in IVP and in some (or none) maternally-derived monoploids are considered to be specific to the IVP-101 genome.

BARD 1-3	IVP 101	Monoploids	Number of Loci[a]
P	P	P	145
P	P	S	67
P	A	P	4
P	A	S	13
A	P	A	14
A	P	P	3
A	A	P	1

[a] Derived from six primer combinations

Table 6: Comparison of published potato maps (not a comprehensive list). Abbr: *phu*, *S. phureja*; *2xtbr*, a haploid *S. tuberosum*; *chc*, *S. chacoense*; *ber*, *S. berthaultii*; *stn-phu*, a selected clone from a *S. stenotomum-S. phureja* bulk population; *pal*, *S. palustre*; *etub*, *S. etuberosum*; *ver*, *S. vernei*; Isoz, isozyme markers; Mph, morphological markers.

Population	Ploidy	Genotype No.	Marker Type	Marker No.	Marker Skewness	Linkage Group No.	Map Length (cM)	Author
phu x (2x*tbr* x *chc*)	2x	65	RFLP/Isoz	134	Yes; 28%	12	606	Bonierbale *et al* 1988
(2x*tbr* x 2x*tbr*) x 2x*tbr*	2x	67	RFLP	492	Yes; 27%	12	1034	Gebhardt *et al*. 1991
(2x*tbr* x *ber*) x *ber*	2x	155	RFLP	261	No; NA	12	684	Tanksley *et al*. 1992
(2x*tbr* x 2x*tbr*) x 2x*tbr*	2x	67	RFLP/Isoz/Mph	193	Yes; 17%	12	1120	Jacobs *et al*. 1995
chac x *chac*	1x	56	RFLP	58	Yes; 70%	13	308	Rivard *et al*. 1996
chac x *chac*	1x	31	RFLP	57	Yes; 46%	14	463	Rivard *et al*. 1996
4x*tbr x* 4x*tbr*	4x	78	AFLP	231	No; 17%	30	991	Meyer *et al*. 1998
stn-phu x (*chc x phu*)	2x	116	RAPD/RFLP	142	Yes; 30%	12	606	Hosaka 1999
pal x *etub*	2x	76	RFLP	80	Yes; 2.5%	19	704	Perez *et al*. 1999
phu x 2x*tbr*	2x	92	RAPD/AFLP	270	No; NA	12	987	Ghislain *et al*. 2001

Figure 1: Experimental framework of the present study. AFLP molecular marker data, based on DNA extracted from two *in vitro* monoploid populations, was used to determine the origin of monoploid genetic material, characterize marker skewness and provide the raw data for phylogenetic and linkage analyses.

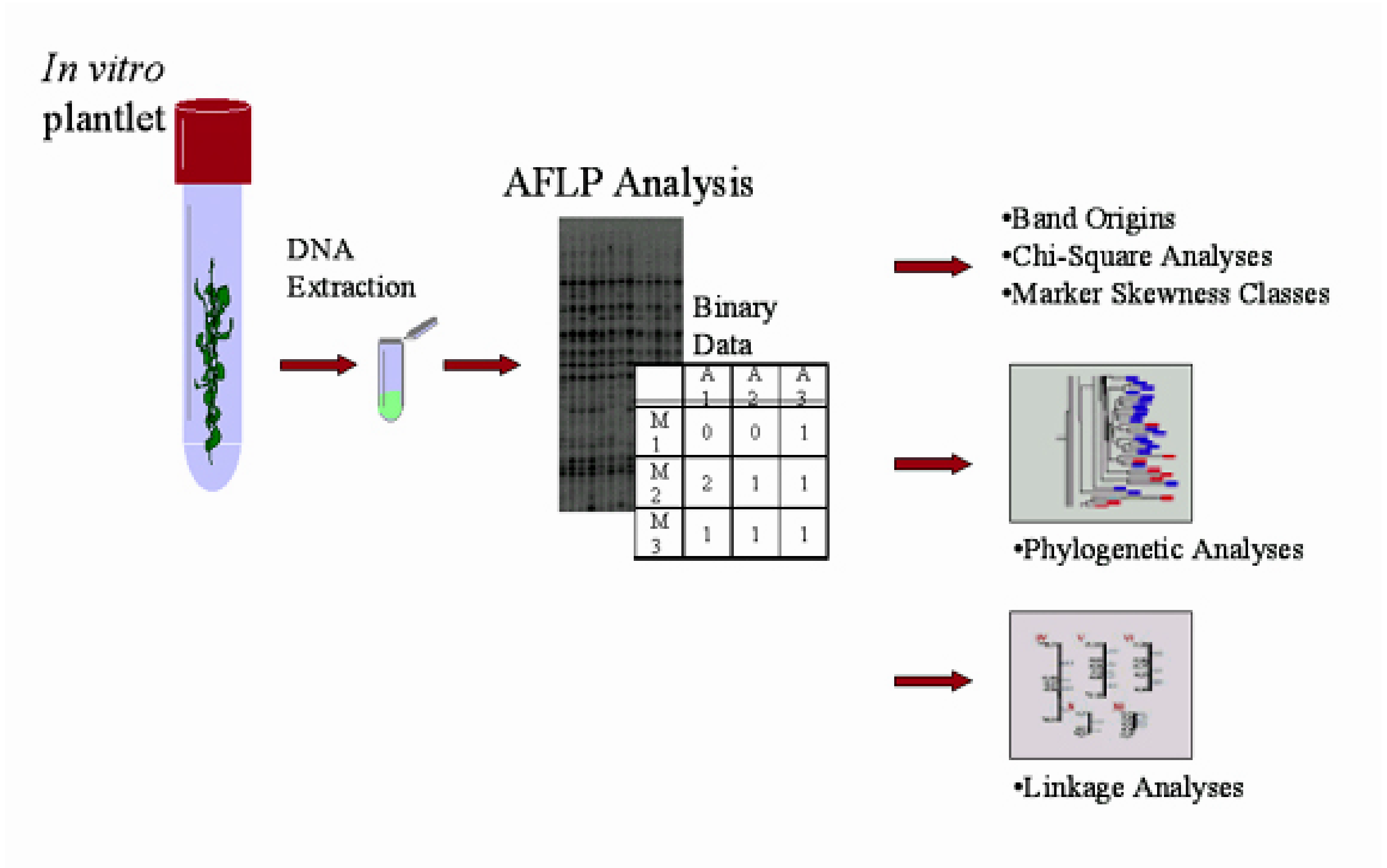


Figure 2: A section of an AFLP gel, illustrating an assortment of typical marker loci: a) monomorphic locus; b) polymorphic locus with marker alleles segregating in a 1:1 ratio; c) complex polymorphic locus with 2 - 3 bands in close proximity; d) complex polymorphic locus exhibiting homoplasic bands (left arrow), in which single marker alleles are too ambiguous to identify (right arrow); e) polymorphic locus exhibiting marker skewness; f) polymorphic locus showing both marker skewness and homoplasic band formation (lane 7).

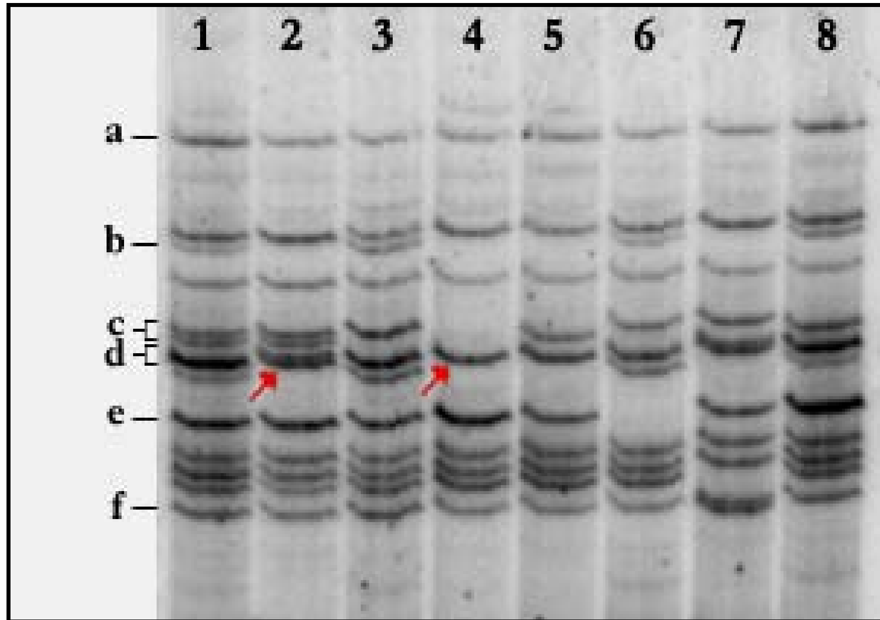


Figure 3: AFLP fingerprints of the haploid-inducer, IVP 101 (lane1), with four gynogenic monoploids (lanes 2-5). This gel illustrates the types of unique bands observed in IVP 101: a) a putative "escapee" gene is evidenced by this marker (faint) only present in IVP and the monoploid in lane 4; b) a more common IVP-specific marker, showing a homoplasic locus unique to IVP; c and d) two examples of rare IVP-specific bands, which were atypical in that they were relatively easy to identify and grade.

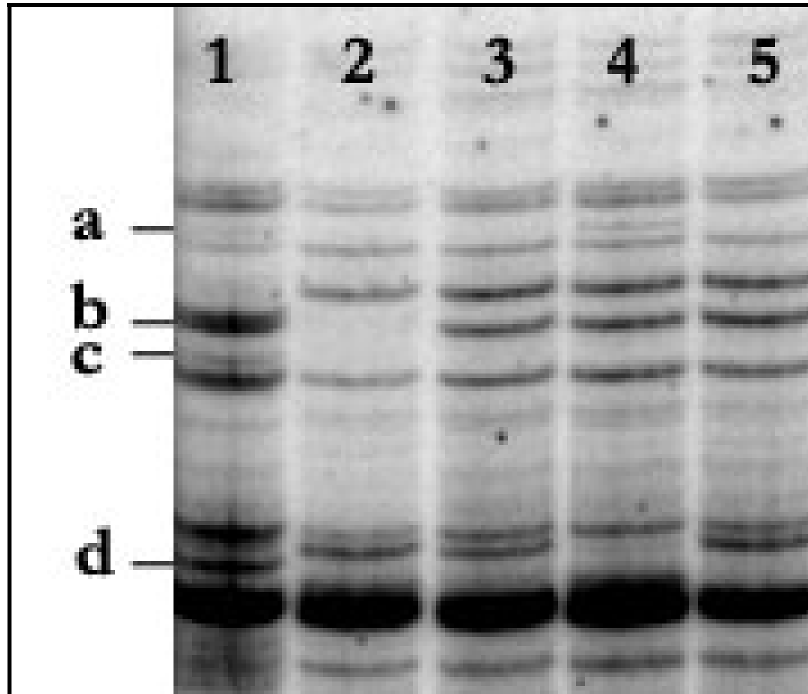


Figure 4: Phylogenetic tree comprising androgenic (blue) and gynogenic (red) monoploids. Analyses based on 69 markers common to both populations. Distances are relative to the proportion of band alleles, out of the total, that are shared between two individuals. Distance calculations were performed using a modified equation of the 'restriction sites distance method' described by Nei and Li (1979).

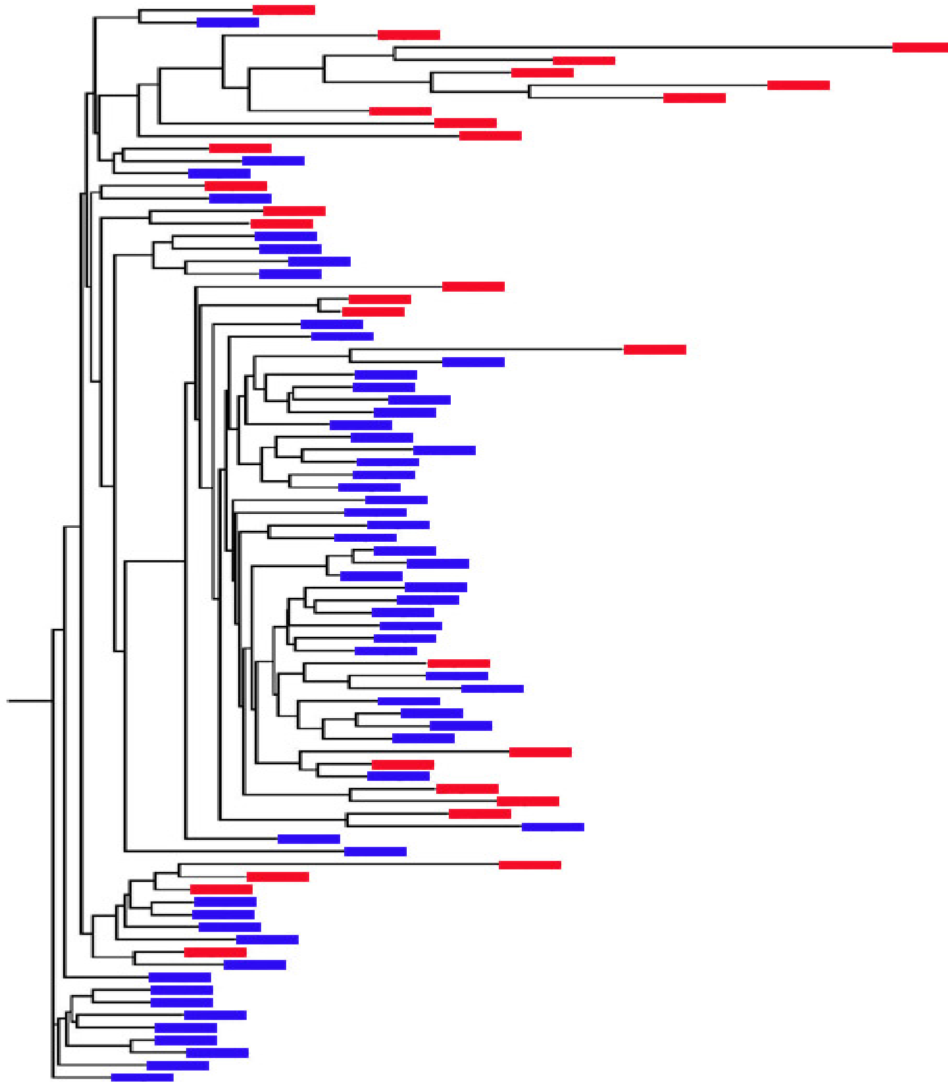


Figure 5: Phylogenetic tree comprising androgenic (blue) and gynogenic monoploids (red). Analyses based on 32 non-skewed markers common to both populations. Distances are relative to the proportion of band alleles, out of the total, that are shared between two individuals. Distance calculations were performed using a modified equation of the 'restriction sites distance method' described by Nei and Li (1979).

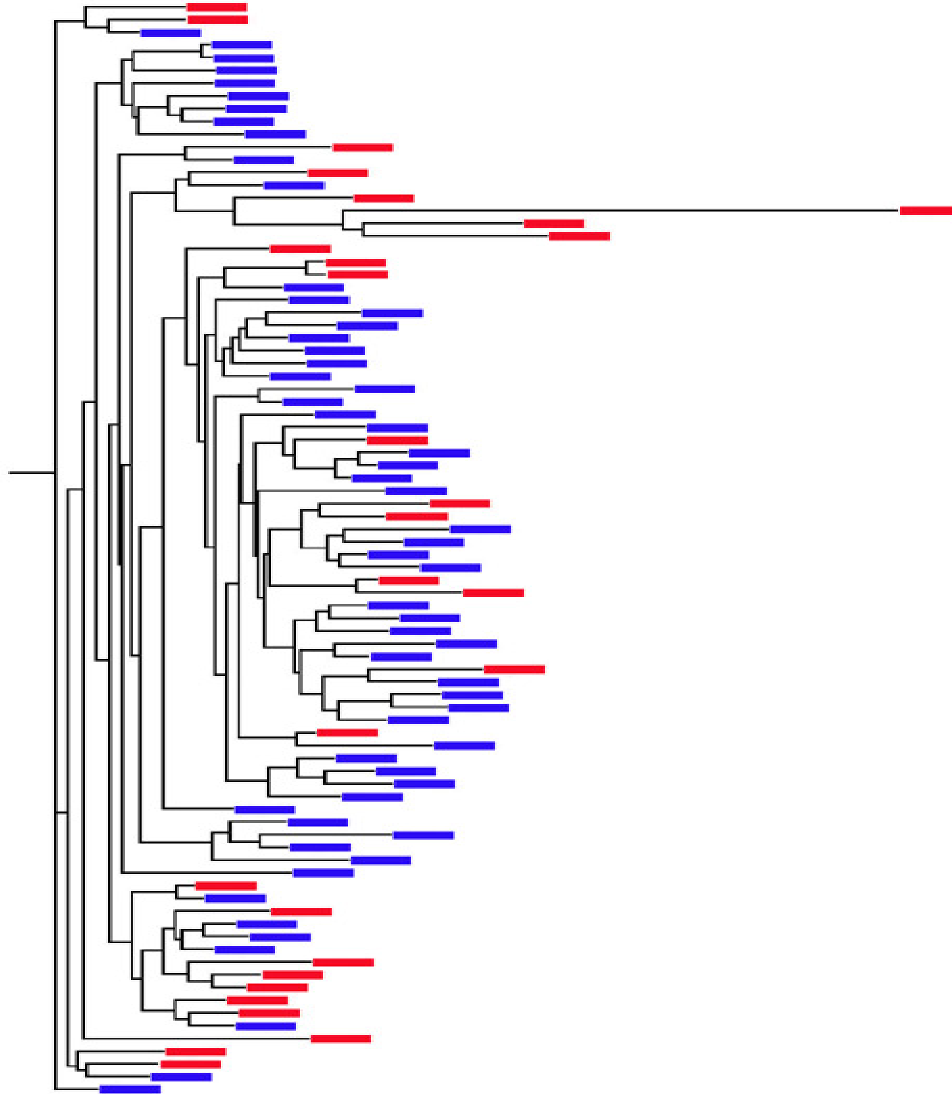


Figure 6: Molecular linkage map base on the androgenic monoploid *S. phureja* genome, including skewed and Mendelian markers. Linkage groups are named and ordered on the basis of size in centiMorgans (cM). Single and double asterisks designate markers skewed at the P < 0.05 and P < 0.01 level, respectively. Genetic distance intervals in cM for each linkage group are recorded on right side of staff. Name designations for markers are on the left side of the staff. Markers in the same linkage group and linked in coupling have a 'c' as a prefix, whereas markers linked in repulsion have an 'r' as a prefix. The first number in the marker name indicates primer combination (see key; Table 2), while the characters immediately following indicate a specific marker.

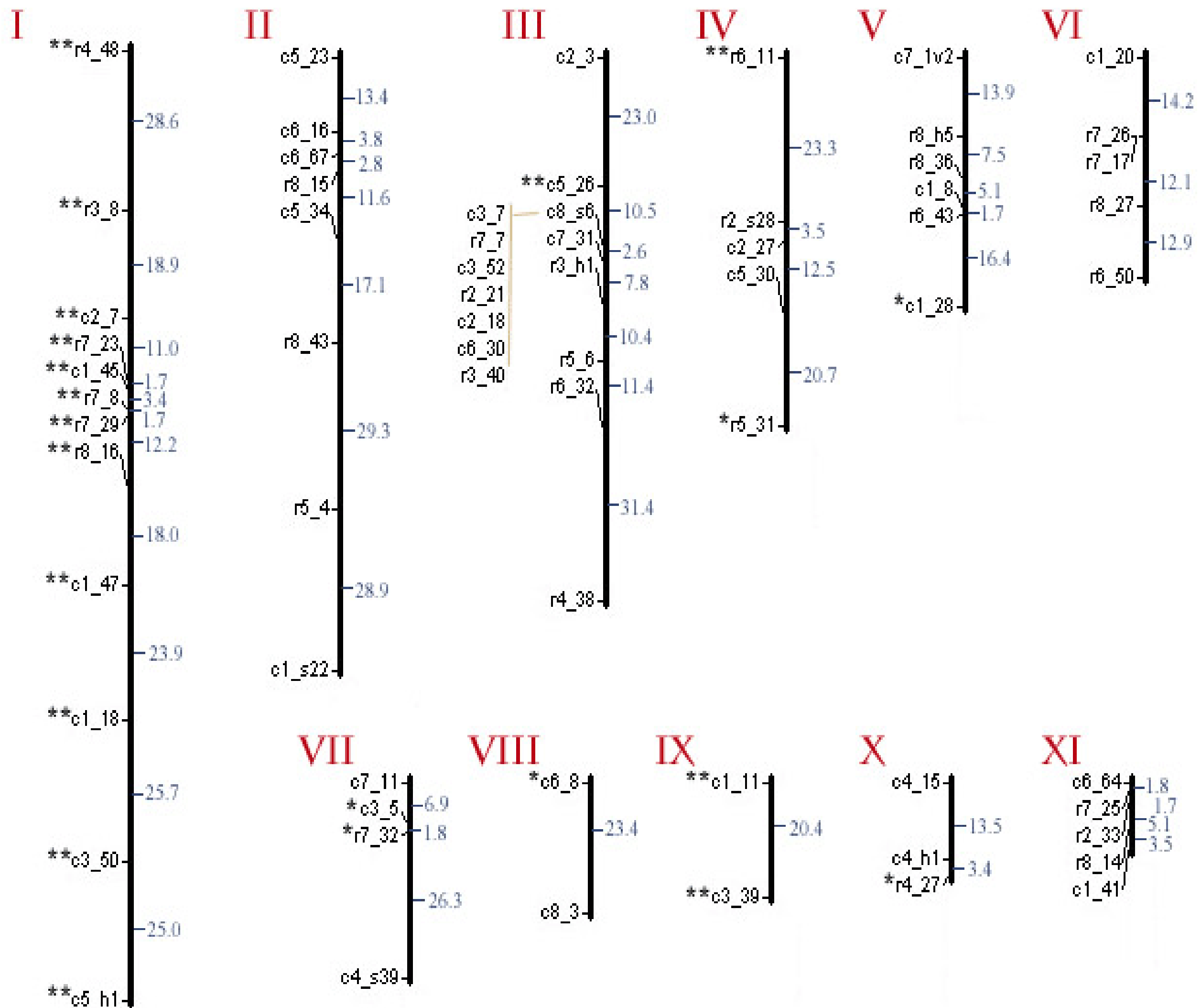


Figure 7: Molecular linkage map of androgenic monoploid *S. phureja* genome, comprising Mendelian markers only. Linkage groups are named on the basis of shared loci with the CM linkage groups. Genetic distance intervals in centiMorgans (cM) for each linkage group are recorded on right side of staff. Name designations for markers are on the left side of the staff. Markers in the same linkage group and linked in coupling have a ‘c’ as a prefix, whereas markers linked in repulsion have an ‘r’ as a prefix. The first number in the marker name indicates primer combination (see key; Table 2), while the characters immediately following indicate a specific marker.

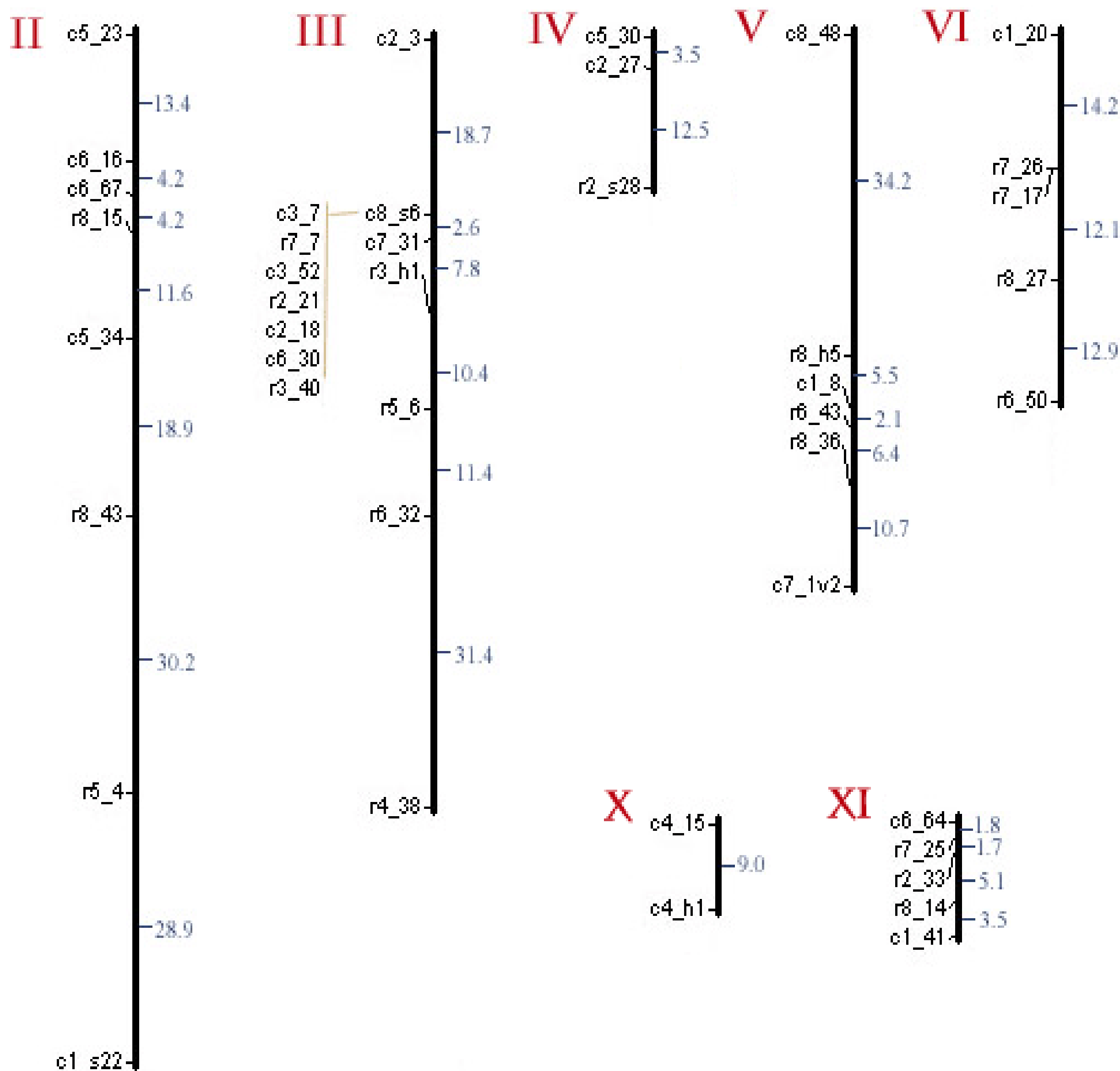


Figure 8: Linkage groups of putative SDL areas in the *S. phureja* genome, mapped according to Tai *et al*. (2000). Name designations for each linkage group was based on CM linkage groups that comprised homologous loci. Single and double asterisks designate markers skewed at the P < 0.05 and P < 0.01 level, respectively. Distance intervals between loci are in cM and are recorded on the right side of the staff. Name designations for markers are on the left side of the staff. Markers in the same linkage group and linked in coupling have a ‘c’ as a prefix, whereas markers linked in repulsion have an ‘r’ as a prefix. The first number in the marker name indicates primer combination (see key; Table 2), while the characters immediately following indicate a specific marker. In linkage groups showing orders and genetic distances consistent with the theoretical expectations, green boxes indicate the theoretical placement of the SDL causing segregation distortion.

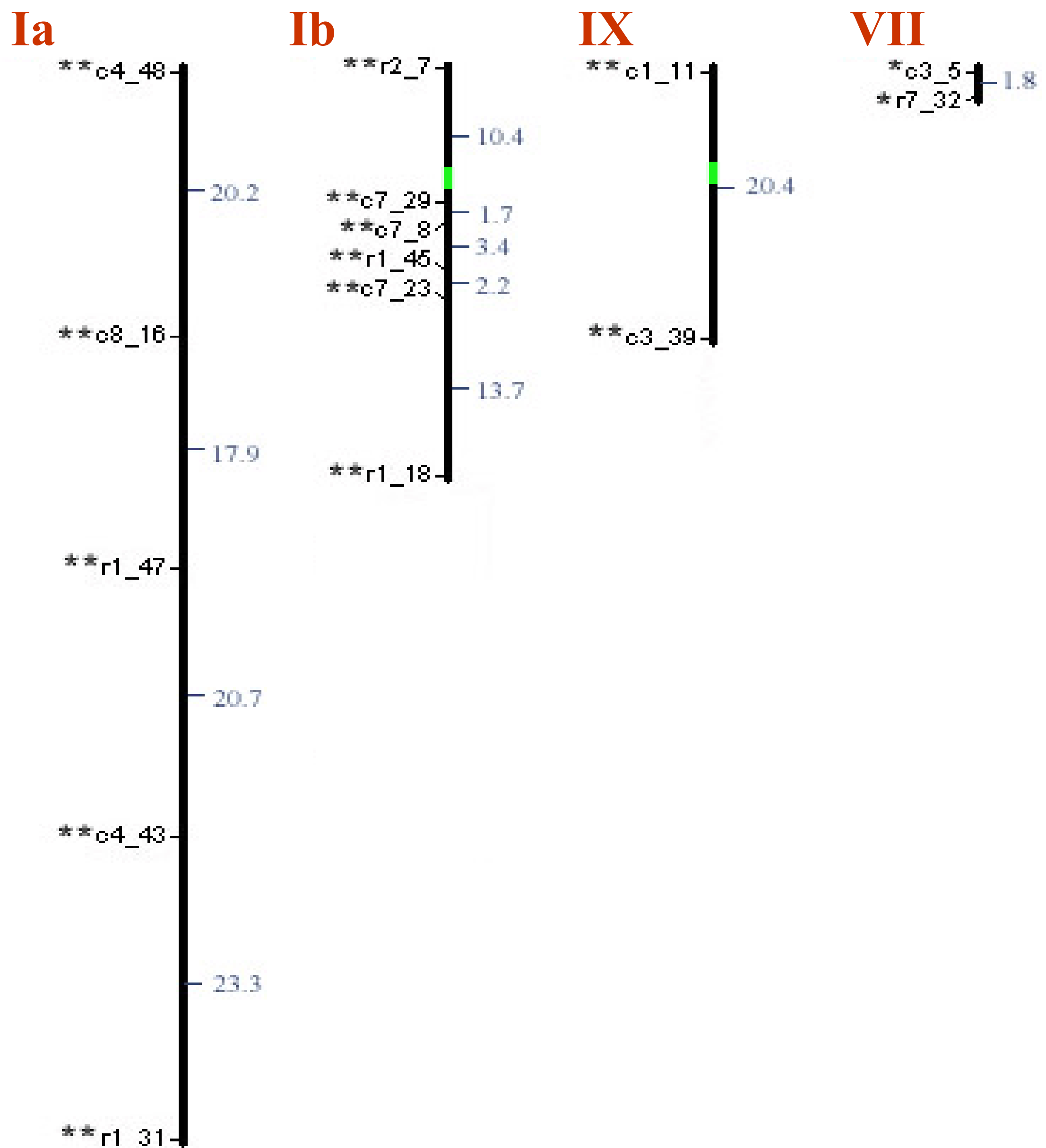
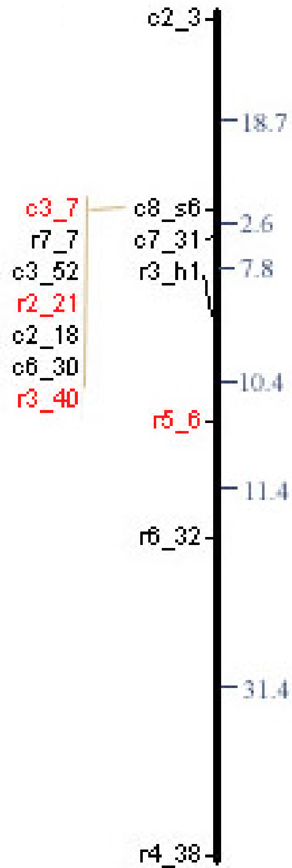


Figure 9: Linkage group III from the Mendelian Markers (MM) map. Marker names are on the left side of the staff, while distance intervals in cM are on the right. Markers exhibiting skewed segregation ratios in the gynogenic population, but not in the androgenic population, are highlighted in red and are associated with a putative unilateral SDL important to gynogenesis.



Vita

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Personal History

Born in Seoul, South Korea on May 27, 1976

Education

1. Woodbridge Senior High School, Woodbridge, Virginia, Sept. 1990-June 1994

2. West Virginia University, Morgantown, West Virginia, Aug. 1994-May 1996

3. B.S., Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, Jan. 1997-June 1999

4. M.S., Horticulture, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, Aug. 1999-May 2002

Professional Experience

Employer: Virginia Polytechnic Institute and State University
Type of Employment: Internship: Campus Gardens Design and Maintenance
Inclusive dates: May 1997-Aug 1997

Employer: Virginia Polytechnic Institute and State University
Type of Employment: Graduate Teaching Assistantship
Inclusive dates: Aug 1999-May 2001

Professional Memberships and Awards

Member, Virginia Tech Horticulture Club 1997-1999

President, Virginia Tech Horticulture Club 1998-1999

Member, Pi Alpha Xi Honor Society 1998-Present

Recipient of Biological Sciences Initiative grant, Virginia Tech, 1999

Member and Program Coordinator, XYZ Student Art Gallery, 2000-2001

Member, Mensa, 2000-Present