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A test of the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes

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Abstract. It has been theorized that in cross-pollinated polyploid species hybrid vigor is maximized by the frequent occurrence of more than two alleles per chromosomal locus. In polyploid crops this condition of maximum heterozygosity has been reported to be associated with increased yield and optimum field performance. We report herein the first direct test of the maximum heterozygosity hypothesis. Molecular markers were used to examine the association between maximum heterozygosity and several components of yield in three different populations of tetraploid potatoes. The results indicate that the value of maximum heterozygosity is not universal but dependent on the genetic background of the material under evaluation. In a cross between adapted breeding lines, homozygosity was negatively correlated with tuber yield, and maximum heterozygosity was positively correlated with the proportion of tuber yield in the large-size fraction. In contrast, in crosses between adapted and unadapted parents, maximum heterozygosity had no detectable effect on any character. Quantitative trait locus (QTL) analysis of the three populations reveals that, regardless of the genetic background, additive genetic effects are more strongly correlated with the components of yield than are any measures of heterozygosity and that some common QTLs may be influencing yield in all three populations.

Key words: Potato – Heterozygosity – Heterosis – RFLP – Polyploids

Introduction

Unlike diploid individuals, heterozygous polyploids can carry more that two alleles at a given locus. A tetraploid ($2n = 4x$) for example, may have as many as four different alleles at a locus; consequently, each locus has the potential for several first-, second-, and third-order allelic interactions. In the early 1960s a hypothesis emerged that maximum heterozygosity leads to maximum heterosis in polysomic polyploids (reviewed by Bingham 1980; Hemsen 1984). The theory suggests that vigor in autopolyploids is highly dependent on intralocus diversity. Accordingly, autotetraploid genotypes with a high frequency of tetra- or triallelic chromosomal segments ($a_ia_ja_ka_l$ or $a_ia_ia_ja_k$) should be superior in vigor to those that are largely di- or monoallelic. Such an advantage may have implications for the evolution of polyploid species, and efforts have been made to exploit it in crop improvement.

Several experiments have been conducted to test the importance of genetic interactions at multiallelic loci in determining the vigor of autopolyploids such as alfalfa and potato, and much discussion has developed around the theory (Chase 1963; Rowe 1967; Mendiburu et al. 1974; Mendoza and Haynes 1974; Dunbier and Bingham 1975; Mendiburu and Peloquin 1977) These past experiments sought to compare vigor among populations with theoretically different genotypic structures but equivalent gene frequencies. In general, results have suggested that heterosis for forage yield, fertility, and tuber yield increase progressively with the theoretical levels of heterozygosity of populations in these crops. One investigation concluded that there is a possible threshold to this diversity-based heterosis in potato based on diminishing returns observed beyond a certain level of theoretical heterozygosity (Sanford and Hanneman 1982). Moll et al. (1965) also observed a decrease in heterotic expres-

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sion in maize when levels of genetic divergence are very high, and speculated that cummulative differences between isolated populations are manifested in poor hybrid viability.

The maximum heterozygosity hypothesis has had strong implications in the development of strategies for the improvement of autopolyploid crops. In alfalfa, for which cultivars are heterogeneous populations, the hypothesis is applied through attempts to maximize the frequency of highly heterozygous individuals in breeding populations. Potato cultivars are homogeneous and clonally propagated, and breeding methods usually aim to select a highly heterozygous individual possessing a multitude of specific desirable traits from within a heterogeneous population. In both of these cross-pollinating crops, heterogeneous populations or families on which selection is practiced result from the hybridization of heterozygous parental clones.

For potatoes, circumstantial evidence for the dependence of optimum performance on a high degree of heterozygosity is based on observations of (1) inbreeding depression following selfing or somatic doubling and (2) the larger contributions made by wild species genomes (via first division restitution in 4x-2x crosses) than by related genotypes (in 4x -4x crosses) to the vigor of cultivated tetraploids (DeJong and Rowe 1971; Mok and Peloquin 1975; Buso 1986). A critical assumption in these studies has been that relative heterozygosity can be inferred from indirect measures of genetic diversity such as the degree of unrelatedness of parental material. While it is true that a population derived from genetically divergent source parents has the potential to contain many allelic variants, the effects of various combinations of allelic variants in individual genotypes has not been measured directly. Uijtewaal et al. (1987) used 2x and 4x derivatives of monohaploids (1x) to demonstrate that polyploidy itself, or gene dosage regardless of heterozygosity, affects potato performance to a degree that varies with the genotype examined. Recently, molecular genetic markers have been used to measure multilocus heterozygosity in maize (Melchinger et al. 1990) and allelic configurations at mapped chromosomal segments in tomato (de Vicente and Tanksley 1992) in attempts to more critically examine factors contributing to heterosis.

Throughout the experiments reported here we have used molecular markers to test the maximum heterozygosity hypothesis in potato. By selecting the appropriate genetic material and utilizing the genetic map of potato we were able to (1) compare the genetic diversity of progenies derived from pairs of parents varying in their degrees of relatedness and 2) measure the relative heterozygosity of siblings from within each of these progenies. Components of tuber yield were evaluated in a replicated field trial of the clonally propagated progenies in the context of these RFLP-based diversity measurements.

Materials and methods

Plant material

Three sexual progenies (TT, NT, and PT) from the Cornell University potato breeding program were used in this study. The families were chosen to represent different genetic backgrounds. TT is a cross between two adapted *S. tuberosum* (Tuberosum) cultivars, 'Steuben' and 'Monona'. To generate NT, a Neotuberosum-Tuberosum hybrid (H224-12) was crossed with bulk pollen from the Neotuberosum population previously developed from the primitive *S. tuberosum* spp. *andigena* (Andigena)(Plaisted 1972; Glendinning 1975). Finally, PT is a cross between the interspecific clone DT033 (produced from 4x-2x crosses in which the diploid parent was a Phureja – haploid Tuberosum hybrid) and a Tuberosum advanced selection, NY74. *S. phureja* (Phureja) is a cross-pollinating diploid relative of *S. tuberosum* and, like Andigena, has been used to broaden the genetic base of potato breeding populations (Hanneman and Peloquin 1967; Peloquin et al. 1989). The experiment was initiated with a total of 280 genotypes representing the three families (120 clones from TT and 80 clones of each of NT and PT).

RFLP analysis

Foliar tissue was harvested from greenhouse-grown plants and immediately frozen in liquid nitrogen. DNA was extracted, restriction digested (with *Eco*RI), electrophoresed, and blotted to membranes (HybondN$^+$) as described previously (Bernatzky and Tanksley 1986). The membranes containing digested DNA from each of the 280 plants were hybridized with DNA probes from tomato that had been mapped to loci distributed throughout the potato genome (Bonierbale et al. 1988; Tanksley et al. 1992). A total of 70 probes were utilized, of which 62 were polymorphic and single copy. The chromosomal location of the polymorphic markers is shown in Fig. 1. All probes except *TG20* were assumed to represent single loci on the basis of prior mapping at the diploid level; *TG20* represents duplicate homologous loci on chromosomes 2 and 7. For each probe, the total number of restriction fragments revealed in all three families was determined, and each plant was scored for the presence or absence of each of these fragments. An estimate of genome-wide het-

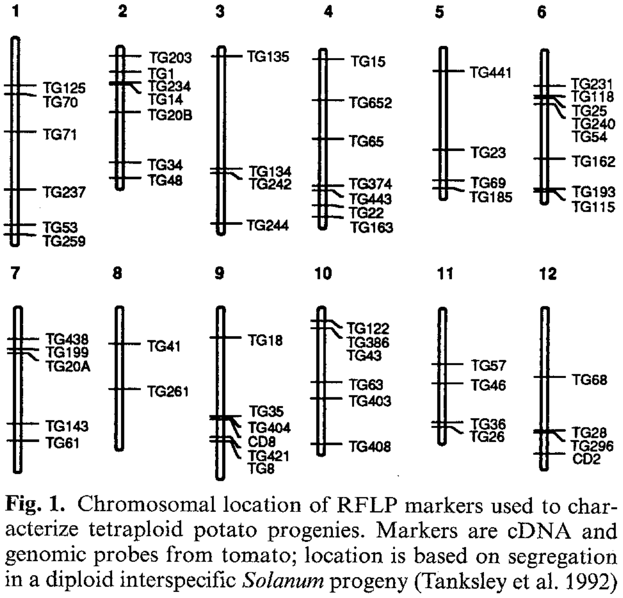

Fig. 1. Chromosomal location of RFLP markers used to characterize tetraploid potato progenies. Markers are cDNA and genomic probes from tomato; location is based on segregation in a diploid interspecific *Solanum* progeny (Tanksley et al. 1992)

erozygosity was calculated for each genotype by dividing the number of restriction fragments present at 62 loci in each individual by the total number of fragments scored for the genotype, generally 317. This calculation yielded the relative heterozygosity index (RHI), previously used by Gebhardt et al. (1989), for each plant. The relative heterozygosity index was used to select less heterozygous and more heterozygous clones from within each family for comparison in yield trials. This interpretation assumes that more fragments will be revealed by a given probe in heterozygous genotypes than in their homozygous counterparts.

Field trials

Tuber multiplication and first selection

Five hills of each clone were planted in the Mt. Pleasant field at Ithaca, N.Y. in May of 1990 in order to produce seed of each genotype for yield trials. Prior to harvest of the multiplication material, selections were made based on the relative heterozygosity index obtained from a preliminary data set of 45 RFLP loci from throughout the genome. To facilitate the selection, each family was characterized by the frequency distribution of its members in various heterozygosity classes as determined by the RHI. Clones with intermediate values of the RHI were left in the field while those at both extremes were harvested. From within each family, 20 'less heterozygous' and 20 'more heterozygous' clones were harvested, graded, and stored for yield trials in the following season.

Yield trial and second selection

A yield trial of 120 clones that had been selected to represent the extremes of heterozygosity in the three populations was planted on May 1, 1991 in Ithaca. A randomized complete block design with three replicates was used for the field experiment. The plots were two rows wide by 15 hills at 9-inch spacings. The cv. 'Atlantic' was entered as a control in 12 replicates. Seed piece size was standardized by cutting tubers. The rows were planted continuously, with the plots delimited by 4 hills of a purple spacer clone. The field was irrigated three times during the growing season and pesticides were applied according to the manufacturers' recommendations. The vines were killed 118 days after planting, on August 26.

Prior to harvest of the yield trial, further selections were made based on the cumulative relative heterozygosity index obtained with the full set of 62 RFLP probes. From this selection 32, 30, and 31 clones from the TT, NT, and PT families respectively, were harvested and washed. Tubers were graded into three size classes: those smaller than 4.8 cm in diameter, those between 4.8 and 6.4 cm, and those larger than 6.4 cm, and the number and yield of tubers (kg) in each class was recorded. A visual rating for tuber appearance (1 = very poor, through 5 = excellent) was assigned to each plot as the tubers came across the grader, and specific gravity was determined from a 4-kg sample of each clone.

Analysis of yield trial

Family means for the various measures of yield performance and quality were compared by analysis of variance using SAS (1982). The individual clones were considered to be random effects nested within families. Contrasts among family means were calculated to compare the performance of the three families for total tuber yield, percentage of yield and tuber weight of those in the largest class, tuber number, yield of small tubers, specific gravity, and tuber appearance.

Interpretation of RFLP patterns

Heterogeneity of families

Estimates of the relative genetic diversity of the three populations under study were based on data from 55 discrete RFLP loci. For the purposes of family comparisons, it was necessary to eliminate data from 5 loci for which not all families were scored and from the probe *TG20*, which represents duplicate homologous loci. Relative gene diversities of the three experimental families were estimated according to Nei's formula for 'nucleon diversity' (Nei and Tajima 1981). This measurement describes the level of heterogeneity and summarizes the degree of polymorphism within populations by considering the sum of squared frequencies of restriction fragment patterns at each locus as an indicator of similarity. To measure diversity among the individuals of a group, $h = n(1-\Sigma x_i^2)/(n-1)$, where n is the number of individuals in the group, and x_i is the sample frequency of the i th observed restriction fragment pattern for a given locus. H is the average diversity within the group over all loci examined (in this case, $H = \Sigma h/55$).

Homozygosity and heterozygosity of individuals within families

For each plant included in the yield trial, indexes were calculated to estimate homozygosity and heterozygosity. The first of these indexes was designed to estimate the level of inbreeding (homozygosity) of each clone, while the second indicates the level of heterozygosity at the non-homozygous loci. For each plant, homozygosity was estimated by calculating the percentage of the examined RFLP loci that had only 1 restriction fragment. Heterozygosity was calculated for each plant as the mean number of restriction fragments observed at the loci that exhibited more than 1 fragment. Unlike the relative heterozygosity index (RHI) described earlier, this second measure of heterozygosity is independent of the homozygosity index, and therefore can be used with the homozygosity index to explore correlations between the yield parameters and the heterozygosity of individual genotypes.

Correlations between yield performance and RFLP-based parameters

Assessment of the relationship between homozygosity/heterozygosity and yield performance

The indexes described above were used as independent variables in multiple regression analyses to explain within-family variation in yield performance. Regressions of clone means for each of five yield measurements – total yield, percentage of yield in the >6.4 cm size class, mean tuber weight of tubers larger than 6.4 cm, total tuber number, and specific gravity – on the percentage of loci with only 1 fragment (homozygosity) and the average number of bands at loci with more than 1 fragment (heterozygosity) were conducted within each family using SAS (1982).

Associations between the segregation of specific RFLP fragments and yield parameters

For each family, *t*-tests were conducted to determine the possible associations between the presence or absence of individual restriction fragments and each of the same five yield measurements. Fragments whose segregation patterns showed significant associations ($P<0.02$) with yield measurements within a particular family were selected as candidates for multiple regression in the model selection program *R-square* of SAS.

Results

RFLP data sets

A total of 317 distinct restriction fragments were detected among 280 genotypes by 62 probes. All individuals with 20% or more missing data were eliminated from the data set. This elimination left 108, 76, and 76 plants in the TT, NT and PT families respectively, for comparisons of RFLP patterns among the families. A subset of this RFLP data corresponding to the 93 clones selected for the yield trial (32, 30, and 31, respectively) was used for estimating relationships between the RFLP-based indexes of heterozygosity and the components of tuber yield.

Comparison of RFLP patterns among families: estimates of diversity and heterozygosity

Allelic diversity of clones in the three families

Homozygosity. One measure of a clones's allelic diversity is the percentage of its RFLP loci with only a single restriction fragment, described earlier as the homozygosity index. The greater this percentage, the lower the allelic diversity of the clone. Individuals in the TT family had a significantly higher percentage of loci ($P<0.01$) with only one fragment than did individuals of the NT or PT families indicating the highest degree of homozygosity or inbreeding (29%) (Table 1). The NT hybrid family (24%) and the PT hybrid family (22%) were not significantly different from each other.

Heterozygosity. The heterozygosity statistic estimates the overall level of heterozygosity of an individual, and averages for each family are given in Table 2. This heterozygosity index, being a ratio, does not give a wide spread in mean values, but the variance of the estimates is very low so that by this measure family differences are significant ($P<0.01$). Contrasts for differences among family means showed that PT individuals were on average more heterozygous, followed by NT and TT, with the latter two families not differing significantly from each other. The variances of the estimates of both homozygosity and heterozygosity were smaller for the PT hybrid than for the other two families.

Genetic diversity/heterogeneity of the families

The overall levels of genetic diversity of the three experimental populations estimated by two RFLP-based criteria are presented in Table 3. Nei's H index describes the heterogeneity of each group or the likelihood of finding polymorphism between any two pairs of individuals in a group. Populations with uniform frequencies of restriction fragment patterns among their members are more heterogeneous (higher H values), while groups with a predominant pattern and a few rare patterns are less heterogeneous. Family differences for H were not significant. This indicates that there is practically as much polymorphism among clones from the TT population as there is in the wider crosses.

Although the families did not differ significantly for H, the number of restriction fragment patterns exhibited in each family were different. NT had a greater number of novel restriction fragment patterns at the loci examined than did PT or TT (Table 3). This difference is probably due to the use of bulk pollen in the Neotuberosum population (NT), which resulted in individuals with rare patterns. As explained earlier, groups with rare patterns are seen as being relatively less heterogeneous by Nei's index than groups with uniform frequencies of patterns, so the differences among families for these two measurements were less significant in terms of Nei's index than in terms of the raw numbers of patterns. For both measures of population diversity, there was a large degree of variation among the loci examined (h), and the family ratings presented represent the means of all loci (H).

Table 1. Family means for the index of homozygosity for 62 loci analyzed

Family	*n*	Mean **	Range	Variance
TT	108	29.0a	12–42	0.33
NT	76	23.6b	5–40	0.33
PT	76	22.1b	13–31	0.15

Values followed by different letters are significantly different at ** $P<0.01$

Table 2. Family means for the relative heterozygosity index (RHI)

Family	*n*	Mean **	Range	Variance
TT	108	0.4772a	0.429–0.540	0.00059
NT	76	0.4807a	0.403–0.523	0.00060
PT	76	0.5028b	0.452–0.538	0.00030

Values followed by different letters are significantly different at ** $P<0.01$

Table 3. Levels of gene diversity of the three populations under study

Family	Relative gene diversity	Number of RFLP patterns **
TT	0.534 ± 0.24	5.29 ± 4.3a
NT	0.570 ± 0.22	7.15 ± 4.2b
PT	0.556 ± 0.24	5.35 ± 4.2a

Values followed by different letters are significantly different at ** $P<0.01$

Table 4. Comparison of family means for yield measurements

Family	*n*	Tuber yield (kg)		Tuber yield for tubers >6.4 cm in diameter				Total tuber number		Yield of tubers <4.8 cm (kg total)	
				Percent of total yield		Tuber weight (kg/tuber)					
		Mean*	Range	Mean***	Range	Mean**	Range	Mean***	Range	Mean***	Range
TT	96	26.11a	17.57–34.37	76.6a	56.0–91.0	0.26a	0.18614–0.38	153a	97–244	1.11a	0.45–2.35
NT	89	23.69b	15.12–38.45	47.7c	20.0–81.0	0.20c	0.16344–0.28	223b	156–358	2.84b	1.06–5.30
PT	92	25.79a	15.84–38.50	59.8b	12.0–82.0	0.22b	0.17252–0.29	209b	127–306	2.42b	0.98–6.43
		Atlantic = 28.19 ± 1.93 CV = 12.70		Atlantic = 79.0 ± 2.9 CV = 9.56		Atlantic = 0.254 ± 0.005 CV = 11.68		Atlantic = 160 ± 19 CV = 12.93		Atlantic = 1.25 ± 0.30 CV = 26.11	

Values followed by different letters are significantly different at * $P < 0.10$, ** $P < 0.01$, and *** $P < 0.001$

Table 5. Comparison of family means for tuber qualities

Family	*n*	Specific gravity		Tuber appearance (scale 1–5)	
		Mean***	Range	Mean**	Range
TT	96	1.0625a	1.055–1.071	2.8a	2.1–3.5
NT	89	1.0750c	1.065–1.088	2.8a	2.0–3.8
PT	91	1.0700b	1.061–1.080	2.3b	1.7–3.0
		Atlantic = 1.0803 ± 0.0005 CV = 0.6023		Atlantic = 3.0 ± 0.33 CV = 10.57	

Values followed by different letters are significantly different at ** $P < 0.01$ and *** $P < 0.001$

Comparison of yield parameters among families

Approximately 30 clones from each family that had been selected to represent the extremes of heterozygosity, were included in the yield trial. Considering the families as main effects, we compared total tuber yield, the distribution of the tuber yield into three size classes, tuber number, specific gravity, and appearance. Tables 4 and 5 present the family means for yield characteristics, the coefficients of variation for the field trials, and the performance of the check variety (cv 'Atlantic') for each yield category. While family differences for the total tuber yield were not highly significant ($P = 0.09$), large differences were observed in the distribution of the yield into size classes. Table 4 shows highly significant differences among families in the tuber yield for tubers greater than 6.4 cm in diameter, the per tuber weight of the large tubers, total tuber number, and the yield of small tubers. All three families differed significantly from each other for the size fractions. TT had the smallest tuber set and the highest yield of large tubers. Each family included clones that were better than and worse than the control cultivar for all categories except specific gravity (Table 5). For this quality, PT and TT were relatively poor (low specific gravity), while NT showed the highest family mean with some clones exceeding the control cultivar. Specific gravity of the tubers varied among families in the order NT > PT > TT.

The families involving less adapted parents, NT and PT, averaged greater numbers of smaller tubers. Perhaps in a longer growing season, the undersized portion of yield would contribute to greater harvest weight in the desirable categories. Both the Tuberosum and the Neotuberosum hybrid were developed from parental material selected for regular shape and high specific gravity, so it was not surprising that the valve for tuber appearance of clones in the Phureja hybrid was generally lower than those in the other two families due to the high proportion of irregular tubers with deep eyes in the former (Table 5).

Tests of inbreeding and heterozygosity and their relationship to yield characteristics

Indexes of homozygosity and heterozygosity were used as predictors in multiple regression models to explain within-family yield variation. Homozygosity was again estimated as the percentage of RFLP loci showing only one fragment, and heterozygosity was estimated by the mean number of restriction fragments at loci showing more than one band. A two-predictor regression model was designed to assess variation in yield that could be explained by these two parameters. Family characteristics for the subset of clones included in this analysis are shown in Table 6. For this subset of clones, the index of homozygosity was still highest for TT and lowest for PT ($P<0.01$), with the latter family exhibiting the smallest variance for the estimate. For the heterozygosity index that does not include homozygous loci, PT has the greatest mean number of fragments per locus, with NT significantly lower and TT intermediate.

Table 7 presents the significant associations found when the indexes of homozygosity and heterozygosity for clones in the yield trial were used as independent variables in multiple regressions to explain within family variation in yield.

TT

In the Tuberosum hybrid, three correlations were significant. A significant association was found for total tuber yield in the two-predictor model. While the partial coefficient for the percentage of homozygous loci was significantly negative ($P=0.0066$), the second predictor, the number of fragments at the non-inbred loci, did not explain significant additional variation. A reduced model with only homozygosity as a predictor describes a negative association ($B=-76.28\pm27.73$; R-squared$=0.20$; $P=0.0100$) between homozygosity and total tuber yield.

For the weight per tuber of the large tubers in TT, the indexes of inbreeding and heterozygosity together explained a significant amount of variation ($P=0.0247$; R-squared$=0.23$). In this full model, *t*-tests for the partial coefficients for inbreeding and heterozygosity indicate that each predictor explains an independent portion of the variability. The weight of the large tubers decreased with increasing levels of homozygosity of the clones ($B=-0.46\pm0.27$) and, in addition, increased ($B=0.26\pm0.14$) with increasing numbers of fragments at the non-inbred loci. The fact that reduced models with each of these predictors alone are also significant ($P=0.0430$, R-squared$=0.13$; and $P=0.0300$, R-squared $=0.15$) adds confidence to this interpretation.

Tuber number was also found to be associated with heterozygosity for the TT family. Multiple regression indicated that the percentage of homozygous loci does not predict the total tuber number but that the level of heterozygosity of the non-inbred loci does. A reduced model with only the second predictor describes a significant negative relationship ($B=-113.5\pm54.62$; R-squared $=0.13$; $P=0.0470$) between tuber number and this index of heterozygosity.

PT

In the Phureja hybrid there was only one significant correlation, total tuber yield with homozygosity, and that was in the opposite direction of the same correlation found for TT. The full regression model (two independent variables) for total tuber yield revealed a positive correlation with homozygosity in PT but no significant relationship with heterozygosity. A reduced model defined a positive relationship ($B=125.13\pm50.09$; R-squared$=0.18$; $P=0.018$) between homozygosity and the percentage of homozygous loci.

NT

No significant correlations were found between homozygosity or heterozygosity and performance in the Neotuberosum hybrid NT or when all entries were taken together without regard for the families.

Correlation of variation in yield performance with the segregation of individual restriction fragments

Using the RFLP data set corresponding to the entries evaluated in the yield trial, we performed *t*-tests to determine the possible association of the presence or absence

Table 6. Family means of clones in the yield trial for the index of homozygosity (percentage of loci with only one fragment) and heterozygosity (the number of fragments at loci with more than one fragment)

Family	*n*	Homozygosity			Heterozygosity		
		Mean**	Range	Variance	Mean**	Range	Variance
TT	32	28.5a	17–37	0.3	2.77ab	2.60–2.98	0.010
NT	30	24.3b	14–41	0.3	2.72b	2.40–2.98	0.023
PT	31	21.2c	15–28	0.1	2.79a	2.57–3.04	0.012

Values followed by different letters are significantly different at ** $P<0.01$

Table 7. Association between yield traits and predictors representing homozygosity and heterozygosity

Family	Trait (response)	Model (predictor)	R-square	Direction of correlation	p-value
TT	Total tuber yield	Reduced model homozygosity	0.20	 −	0.010
TT	Tuber weight for tubers >6.4 cm	Full model homozygosity heterozygosity	0.23	 − +	0.025
TT	Tuber number	Reduced model heterozygosity	0.13	 −	0.047
NT	None	–	ns	ns	ns
PT	Total tuber yield	Reduced model homozygosity	0.18	 +	0.018

ns, Non-significant

Table 8. Variation in yield explained by multiple regression of candidate RFLP bands (predictors) on five traits in three families

Family	Trait	Number of predictors: Considered (tested)	Number of predictors: 'Best' model	R square 'Best' model
TT	Total tuber yield	5	3	0.69
	Weight/tuber >6.4 cm	3	3	0.30
	% of yield >6.4 cm	1	1	0.23
	Tuber number	2	2	0.62
	Specific gravity	2	1	0.20
NT	Total tuber yield	2	2	0.49
	Weight/tuber >6.4 cm	2	2	0.30
	% of yield >6.4 cm	1	1	0.29
	Tuber number	5	3	0.53
	Specific gravity	4	3	0.53
PT	Total tuber yield	7	3	0.56
	Weight/tuber >6.4 cm	8	3	0.64
	% of yield >6.4 cm	11 (7)	2	0.73
	Tuber number	12 (6)	3	0.49
	Specific gravity	5	3	0.53

of specific fragments segregating in each family with the five measurements of yield performance. This analysis was conducted to estimate the additive effects of individual fragments on variation for yield within families (as opposed to overall heterozygosity/homozygosity). As the markers used in this study have previously been mapped in the potato genome, this approach allows the identification of major loci, or clusters of loci, that influence yield traits.

For TT, NT, and PT, respectively, 150, 200, and 160 *t*-tests were conducted for each of the five yield parameters. The number of tests corresponds to the number of fragments, of the 317 scored in total, which segregated in each family. From among the resulting 2,550 *t*-tests, 70 (or 2.7%) were identified as being significant ($P > 0.02$), and the corresponding bands were considered as candidates in regression models against the respective measure of yield performance. When the number of candidates considered was greater than eight, a 'most significant subset' was chosen for testing on the basis of probability values. The number of restriction fragments 'considered' and 'tested' as predictor variables for each trait-family combinatin, and the number of predictors in the 'best' model are presented in Table 8 with the corresponding R-squared values. The number of fragments 'considered' corresponds to those indicated as being significant by the *t*-tests, and the number 'tested' indicates those used as the most significant subset for entry into multiple regression models.

In general, when several candidate bands were tested, models with two or three predictors were the most efficient at explaining the respective variation. For example, when seven fragments (with $P < 0.02$ in *t*-tests) segregating in PT were considered as predictors of the 'percentage of tuber yield > 6.4 cm in diameter', two of them together explained 73% of the variation. Using this approach, for almost all traits in all families, specific fragments at 3 loci would predict an average of 50% of the variability. This is roughly double the best correlations found when indexes of homozygosity or heterozygosity were used as predictors for the same yield characteristics (Table 7 vs Table 8). Regression diagnostics were not performed for the 'best models', so significance levels are not presented.

In these tests using individual fragments as predictors for within-family variation in the distribution of yield into size fractions, the highest correlations were found in the Phureja hybrid family, PT (see R-squared values in Table 7). As this cross is between adapted and unadapted parental material, it is here that we might expect to see the greatest effect of major genes that contribute to adaptation, such as those influencing earliness and concentration of tuber set. Among the three families, individual fragments, as well as the indexes of heterozygosity and homozygosity, were the least successful at the prediction of variability in yield for NT.

Map position of markers correlated with yield performance

Fragments whose segregation patterns showed significant associations with the measured yield characteristics correspond to 26 mapped loci from seven of the potato chromosomes (Fig. 2). While some of the implicated loci, such as those shown on chromosomes 1, 4, 5, and 7 are correlated with yield traits only in single families, others are correlated with the characteristics in two or even all three families. For example, regions of chromosome 2 (around *TG1-TG14*) and chromosome 9 (around *TG35-TG421*), each represented by 4 markers, both seem to contribute to all of the measured traits in all of the families, and 1 locus on chromosome 12 influences both total tuber yield and specific gravity in two families. From this data we are not able to determine the parental source of the implicated restriction fragments, but due to the close proximity of fragments with significant effects on the measured characters in more than one family, it is likely that they are linked to genes influencing yield and/or adapatation. Loci on chromosomes 2 and 5 of potato have also been implicated in segregation for earliness of tuberization on *S. tuberosum-S. berthaultii* hybrids (van den Berg et al. 1992).

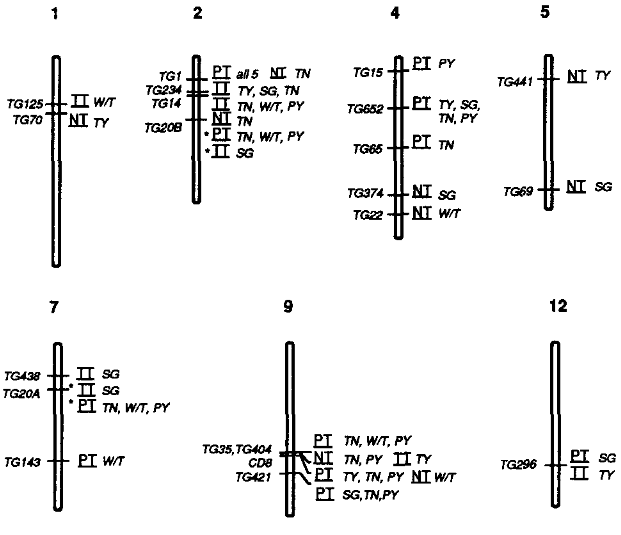

Fig. 2. Map location of restriction fragments predicting variation for five yield components in tetraploid potato progenies TT, NT, and PT (see text). *TY* Total yield of all tubers, *WT* weight per tuber of tubers in large-size fraction (> 6.4 cm), *PY* percentage of yield in large-size fraction (> 6.4 cm), *TN* tuber number; *SG* specific gravity; *all 5* variation for all five measurements listed previously. Segregation for traits listed by asterisks are correlated with fragment from the marker *TG20*, which hybridizes to duplicate loci on *chromosomes 2* and *7*; the data do not enable the assignment of particular fragments to discrete loci in this case

Discussion

The experiment reported here was designed to quantify the levels and effects of inbreeding and heterozygosity of individuals, and the heterogeneity of populations resulting from various hybrid combinations of potato breeding material. Three hybrid families were chosen to represent populations likely to vary in their levels of allelic diversity. TT was expected to have the lowest level of diversity of the three groups since it was derived from a cross between two parents with the Tuberosum background from a variety selection program. With one Tuberosum hybrid parent and one Neotuberosum parent, NT was expected to have greater diversity than TT due to the influx of alleles from selected *S. tuberosum* ssp. *andigena* material. PT combined a selected Tuberosum clone with an unselected source of germ plasm, Phureja, and was therefore expected to show the greatest allelic diversity of the three groups.

Results from these studies confirm the expectations for allelic diversity. Comparisons of family means for these indexes showed that the TT has the highest degree of inbreeding and that the Phureja hybrid PT has the highest degree of heterozygosity (Tables 1 and 2). On the basis of the number of different restriction fragment patterns observed within each population, the NT family was found to have a larger variety of allelic configurations at the loci measured. However, the heterogeneity (*H*) of each population, estimated by considering the frequencies of restriction fragment patterns within each one was not significantly different from each other (Table 3). The bulk pollen used in the development of NT apparently contributed a larger number of 'rare' patterns to its progeny, thereby decreasing the overall heterogeneity relative to the clone-by-clone hybrids. Due to the arrangement of restriction fragments into patterns at tetraploid loci, even the family with the largest degree of inbreeding is able to maintain considerable genetic heterogeneity.

The principle differences in yield characteristics among the three populations studied were in the distribution of yield into various size categories. Primitive potato species often produce large numbers of small tubers, while adapted varieties are selected to produce smaller numbers of larger tubers. This tendency was seen in the smaller set of larger tubers in the most adapted family, TT (Table 4), and has been reported in previous studies of populations derived from Tuberosum and Neotuberosum hybrids (Glendining 1969; Cubillos 1974; Tam and Tai 1977).

Inbreeding and heterogeneity effects

Independent indexes of the homozygosity and heterozygosity of clones were able to predict a small but significant amount of the variation for the distribution of tuber yield in two of the experimental populations. In the most adapted family, TT, three correlations contributed evidence in support of the concept that inbreeding had a negative effect on the development of the large-size fraction and, in addition, that maximum heterozygosity seems to have a positive effect. Total tuber yield was also negatively correlated with the index of homozygosity, or inbreeding, in this cross. In the Phureja hybrid family L40, an opposite effect of homozygosity was seen. In this adapted by unadapted cross, the degree of homozygosity was positively correlated with total tuber yield, and intralocus heterozygosity did not make a significant contribution. From this outcome, it appears that the additional restriction fragment variability associated with the Phureja germ plasm does not contribute positively to yield. For this set of populations, the association between heterozygosity and yield seems to be limited by the degree of adaptation of the parental clones. This is in agreement with the concept that while genetic diversity of parental clones is beneficial to maximizing heterosis for yield, the alien sources of germ plasm should undergo previous selection for adaptation (Eberhardt 1971; Plaisted 1972; Mendoza and Haynes 1974). Although significant, the correlations found in this study between heterozygosity and yield were small, especially in comparison to additive effects (see next section).

Our results indicate that a measure of homozygosity has as much predictive values for yield traits as do measures of heterozygosity. One explanation for the greater significance and different effects of the negative relationship between homozygosity and yield performance in the TT family as compared to the more diverse crosses could relate to a difference in the breeding histories of the populations. In TT a RFLP is likely to reflect homozygosity over a larger chromosome segment, due to linkage disequilibrium from crossing related material, than the same marker would represent in a population that has experienced more genetic exchange. In this case the actual homozygosity of TT would be underestimated by our measurement, relative to the other crosses, and the effect would seem greater.

Additive effects

By considering the segregation of individual restriction fragments as well as the indexes of heterozygosity, we attempted to assess the relative importance of maximum heterozygosity and additive genic effects in determining yield variation among clones. The present yield data was regressed on both types of predictors, and stronger correlations were found with specific combinations of individual fragments than with the indexes of heterozygosity. From this we can conclude that the presence of specific alleles or fragment patterns is more important than overall restriction fragment heterozygosity for at least 50% of the variability in the genetic material tested. This suggests that the heterosis for tuber yield seen when wild species are crossed with elite material may be largely due to the additive combination of genes for reduced tuber set and large tuber size in the resulting progeny. Similar observations have been made by Masson and Peloquin (1987) and E. Zimnoch-Guzowska (personal communication). Melchinger et al. (1990) found poor associations between their RFLP-based estimate of genetic distance and heterosis in crosses of unrelated maize lines, and these authors suggest that in light of evidence that important quantitative trait loci affecting yield are located only on certain chromosomal segments, estimates of heterozygosity based on arbitrarily chosen RFLP loci may be unsuitable to predict actual heterotic response. In the present study, the largest effects of segregation at discrete loci, relative to the correlations with heterozygosity, were found in PT, the widest cross. De Vicente and Tanksley (1993) also reported that overall heterozygosity in a wide cross with tomato was not as well correlated with measurements of vigor as were certain configurations of specific chromosomal segments.

Consensus of quantitative trait loci affecting yield: implications for the use of unadapted germ plasm

The importance of specific fragments from mapped markers in governing yield components in potato should be further investigated with larger segregating populations, with attention paid to their parental source, before major loci influencing these traits can be located with confidence. Furthermore, as the yield trial reported here was conducted in only one season, we have not been able to estimate interactions between genotypes, years/locations, and yield, and must confine our conclusions to expressions of performance in a single year and location. However, these data suggest a consensus across different genetic backgrounds of important loci for yield. A homeologous counterpart of the potato chromosomal region around *TG125-TG70* on chromosome 1 identified in this study as important for yield components in two families has also been implicated in a component of yield in tomato (de Vicente and Tanksley 1993). Given the conserved nature of chromosomal contents between tomato and potato (Bonierbale et al. 1988), it may be that orthologous genes have been identified in these two studies.

In the development of modern cultivars, unadapted germ plasm has often been used as a source for adding desirable qualitative traits, but it has been used less commonly for the improvement of quantitative characters. Genes for lack of adaptation often interfere with good performance in crosses between adapted and unadapted clones, thus creating problems in identifying superior recombinants; and while gentic variability is enhanced, mean performance is reduced. In order to take advantage of non-adapted germ plasm to improve crops for complex traits, long-term selection programs aimed at eliminating undesirable characters and developing adapted populations with a broad genetic base are often necessary (Eberhardt 1971; Plaisted 1972). If lack of adaptation is controlled by major genes, it may be valuable to identify and apply selectable markers to unadapted germ plasm source populations in order to shorten the period of time required for the development of broadly based populations that are adapted to a target area. In this way advantage could be taken of genetic variability to create potential heterotic combinations without the undesirable consequences of the adapted by unadapted cross.

With this set of experimental materials, we have found that maximum heterozygosity influences the components of tuber yield in a favorable direction when adapted germ plasm sources are combined. A reduction in tuber number and an increase in tuber size were associated with increased heterozygosity of adapted clones as measured by RFLPs. In such populations, the degree of inbreeding or homozygosity of the clones is as important as heterozygosity, detracting from both total tuber weight and the size of large tubers. Additive genic effects, measured by the segregation of individual restriction fragments, were more strongly related to the components of tuber yield than was heterozygosity.

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References

Bernatzky R, Tanksley SD (1986) Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887–898

Bingham ET (1980) Maximizing heterozygosity in autopolyploids. In: Lewis WH (ed) Polyploidy: Biological relevance. Plenum Press, New York, pp 471–489

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Buso IA (1986) Evaluation of families and clones from 4x-2x screening schemes in potato. PhD thesis, University of Wisconsin, Madison

Chase SS (1963) Analytical breeding in *Solanum tuberosum* L. – a scheme using parthenotes and other diploid stocks. Can J Genet Cytol 5:359–363

Cubillos AG (1974) The yielding ability of autotetraploid *Solanum tuberosum* L. intra- and inter-group F_1 hybrids. PhD thesis, Cornell University, Ithaca, N.Y.

DeJong H, Rowe PR (1971) Inbreeding in cultivated diploid potatoes. Potato Res 14:74–83

de Vicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics (in press)

Dunbier MW, Bingham ET (1975) Maximum heterozygosity in alfalfa: results using haploid-derived autotetraploids. Crop Sci 15:527–531

Eberhardt SA (1971) Regional maize diallels with US and semi-exotic varieties. Crop Sci 11:911–914

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Urig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum.* Theor Appl Genet 78:65–75

Glendinning DR (1969) The performance of progenies obtained by crossing Groups Andigena and Tuberosum of *Solanum tuberosum.* Eur Potato J 12:13–19

Glendinning DR (1975) Neo-Tuberosum: new potato breeding material. 3. Characteristics and variability of Neo-Tuberosum, its potential value in breeding. Potato Res 18:351–362

Hanneman RE, Peloquin SJ (1967) Crossability of 24-chromosome potato hybrids with 48-chromosome cultivars. Eur Potato J 10:62–72

Hermsen JGTh (1984) Nature, evolution and breeding of polyploids. Iowa State J Res 58:411–412

Masson MF, Peloquin SJ (1987) Heterosis for tuber yields and total soloid content with 4x-2x FDR crosses in potato. In: Jellis GJ, Richardson DE (eds) The production of new potato varieties; technological advances. Cambridge Univ Press, Cambridge pp 213–217

Melchinger AE, Lee M, Lamkey KR, Woodman WL (1990) Genetic diversity for restriction fragment length polymorphisms: Relation to estimated genetic effects in maize inbreds. Crop Sci 30:1033–1040

Mendiburu AO, Peloquin SJ (1977) The significance of 2n gametes in potato breeding. Theor Appl Genet 49:53–61

Mendiburu AO, Peloquin SJ, Mok DWS (1974) Breeding with haploids and 2n gametes. In: Kasha KI (ed) Haploids in higher plants. Advances and potential. Proc 1st Int Symp University of Guelph, Guelph, Ontario

Mendoza HA, Haynes FL (1974) Genetic basis of heterosis for yield in the autotetraploid potato. Theor Appl Genet 45:21–25

Mok DWS, Peloquin SJ (1975) Breeding value of 2n pollen (diplandroids) in tetraploid x diploid crosses in potatoes. Theor Appl Genet 46:307–314

Moll RH, Lonquist JH, Fortuno JV, Johnson EC (1965) The relationship of heterosis and genetic divergence in maize. Genetics 52:139–144

Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163

Peloquin SJ, Yerk GL, Werner JE, Darmo E (1989) Potato breeding with haploids and 2n gametes. Genome 31:1000–1004

Plaisted RL (1972) Utilization of germ plasm in breeding programs – use of cultivated tetraploids. In: French ER (ed) Prospects for the potato in the developing world. Cento International de la Papa, Lima, Peru, pp 90–99

Rowe PR (1967) Performance of diploid and vegetatively doubled Phureja-Haploid Tuberosum hybrids. Am Potato J 44:195–203

Sanford JC, Hanneman RE (1982) A possible heterotic threshold in potato and its implications for breeding. Theor Appl Genet 61:151–159

SAS User's Guide (1982) Sas Institute Inc, Cary, N. C.

Tarn TR, Tai GCC (1977) Heterosis and variation of yield components in F1 hybrids between Group Tuberosum and Group Andigena potatoes. Crop Sci 17:517–521

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovanoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder GS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes Genetics

Uijtewaal BA, Jacobsen E, Hermsen JGTh (1987) Morphology and vigor of monoploid potato clones, their corresponding diploids and tetraploids and their heterozygous diploid parent. Euphytica 36:745–753

Van den Berg J, Bonierbale MW, Ewing EE, Plaisted RL, Tanksley SD (1992) Use of RFLP-linkage to detect loci affecting tuberization. In: 76th Annu Meet Potato Assoc Am. New Brunswick, Canada, p (abstr)