Theor Appl Genet (1991) 82:337–345

THEORETICAL AND APPLIED GENETICS

Isozyme analysis of progeny derived from (*Allium fistulosum* × *Allium cepa*) × *Allium cepa* *

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Received March 2, 1991; Accepted March 7, 1991
Communicated by H. F. Linskens

Summary. Relatively large quantities of seed were obtained from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa* allowing, for the first time, an extensive study of the heritable traits exhibited by backcross progeny. Two backcross populations, BC1034 and BC1040, distinguished by different *A. fistulosum* parents, were characterized for the isozyme markers *Idh-1*, *Adh-1*, and *Pgi-1*. Statistical methods are described to calculate cell probabilities for a mixed population of F_2 and BC_1 progeny, using an estimate of the fraction of F_2 progeny in the population derived from the isozyme data. Cell probability distributions were calculated for a mixed population with independent pairs of loci and a mixed population with nonindependent pairs of loci. The isozyme loci *Idh-1* and *Pgi-1* appear to be linked, with a map distance estimated at 33 centimorgans (cM) in BC1034 and 42 cM in BC1040. The probability distribution model for linked loci did not account for all of the distorted segregation ratios in *Idh-1* × *Adh-1* or *Pgi-1* × *Adh-1*. The cytological literature does not support linkage between *Idh-1* × *Adh-1* or *Pgi-1* × *Adh-1*. The distorted segregation ratios for these pairs of loci are likely the result of genetic incompatibilities between the two species.

Key words: Interspecific backcross – Linkage probabilities – Onion genetics

Introduction

The Japanese bunching onion, *Allium fistulosum L.*, has many desirable characteristics, such as high solids, disease and insect resistance, and cold hardiness (Jones and Mann 1963; van der Meer and van Benekom 1978). Plant breeders have sought to exploit the genetic variation found in *A. fistulosum* through introgression of these desirable traits into *A. cepa L.*, the cultivated bulb onion (van der Meer and van Benekom 1978; Peffley et al. 1985; Peffley 1986). The reciprocal interspecific cross has been made between these two species (Emsweller and Jones 1935a, b; Levan 1936; Maeda 1937; van der Meer and van Benekom 1978). The F_1 hybrids exhibit a high degree of sterility, but a few backcross (BC) and F_2 individuals have been recovered (Maeda 1937; Levan 1941; Emsweller and Jones 1945; van der Meer and van Benekom 1978). Previous studies focused on the cytological aspects of meiosis and chromosome pairing (Emsweller and Jones 1935b; Levan 1936, 1941; Maeda 1937; van der Meer and van Benekom 1978; Peffley 1986). Emsweller and Jones (1935a) gave a phenotypic description of the parental types used in their study and their F_1 hybrid. Van der Meer and van Benekom (1978) described a subsequent generation derived from the F_1 hybrid, but it is unclear whether progeny were F_2 or BC_1 with *A. fistulosum* as recurrent parent. Relatively little attention has been paid to the description of traits exhibited by the BC_1 generations or to their implication for the successful introgression of the *A. fistulosum* genome into *A. cepa*.

Studies on F_2 or BC_1 progeny have been limited to observations on a few individuals, because controlled crosses of the interspecific hybrids have only produced an occasional seed. In 1985, when a population of F_1 hybrids were open-pollinated in the field with pollen sources restricted to the recurrent *A. cepa* parent, relatively large quantities of seed were obtained. This permitted, for the first time, an extensive study characterizing the heritable traits of interspecific backcross progeny.

* Journal Article No. 1578, Agricultural Experiment Station, New Mexico State University, Las Cruces, NM, USA

The Isozyme alleles expressed at specific gene loci can be used to document the recovery of either parental or the heterozygous genotypes. Progeny from backcross populations were analyzed for isozyme loci previously reported to have polymorphic alleles between *A. fistulosum* and *A. cepa* (Peffley et al. 1985). These loci are: alcohol dehydrogenase (*Adh-1*), glycerate dehydrogenase (*Gdh-1*), isocitrate dehydrogenase (*Idh-1*), phosphoglucoisomerase (*Pgi-1*), and phosphoglucomutase (*Pgm-1*).

A population large enough for heritability studies could only be obtained by open-pollination (massing); thus, it was possible that the population would be a mixture of BC_1 and F_2 or intercross progeny. Isozyme segregation data permitted an estimate of the number of F_2 progeny in the population. The probability distributions for expected cell frequencies could be calculated, allowing the use of Chi-square analysis to test the hypothesis of independent segregation between the loci examined. Previous work (Peffley et al. 1985) suggested possible linkage between the isozyme loci *Idh-1* and *Pgi-1*. An objective of this study was to determine if these populations could be used to estimate linkage and map distance between *Idh-1* and *Pgi-1*.

Materials and methods

Pedigree of backcross populations

The initial F_1 interspecific hybrids were made in the field under screen-covered isolation cages. Ten-plant populations of each parental species were crossed using *A. fistulosum* as the seed parent and *A. cepa* as the pollen parent.

The F_1 hybrid population, 8121, was made using *A. fistulosum* 'Heshiko' (source: Nickerson-Zwaan Seed Co.) and *A. cepa* selection NMSU 792. NMSU 792 is a bolting-resistant selection out of Texas Grano 502 Prr (TG502Prr), a short-day yellow grano onion with tolerance to pink root disease (Prr). The F_1 hybrid population, 8273, resulted from crossing *A. fistulosum* 'Ishikura' (source: Nickerson-Zwaan Seed Co.) and *A. cepa* selection NMSU 8020. NMSU 8020 is a bolting-resistant selection from TG502Prr, comparable to NMSU 792. The interspecific F_1 hybrid populations, 8121 and 8273, each contained 10–20 individuals that were propagated asexually as populations of clones.

The backcrosses were made using 8121 and 8273 as the seed parents and *A. cepa* selection NMSU 8361 as the recurrent parent. NMSU 8361 is a selection for bulb uniformity out of NMSU 792 and is genetically similar to NMSU 792. These backcrosses were made in a field situation in which all other pollen sources, except the F_1 hybrid and the recurrent *A. cepa* parent NMSU 8361, were eliminated. Beehives were placed in the field as pollen vectors. Despite the high degree of sterility in the F_1 hybrid populations, the possibility existed for some F_1 ovules to be fertilized with F_1 pollen nuclei. Such progeny, identified by isozyme analysis, are designated as F_2 generation, but they may be either sibcrosses or true selfs.

Backcross seed harvested from the *A. fistulosum* parent was sown in the greenhouse. The backcross seed harvested from 8121 produced 294 seedlings and the backcross seed harvested from 8273 produced 282 seedlings. These populations were designated 1034 and 1040, respectively.

Isozyme analysis

The backcross populations (1034 and 1040) and samples of the reference populations (parents and corresponding F_1 hybrids) were analyzed electrophoretically for the enzyme loci *Adh-1*, *Idh-1*, and *Pgi-1* following the procedures described in Vallejos and Tanksley (1983). The allele designations assigned by Peffley et al. (1985) are *Adh-1*^{1} and *Adh-1*^{2}, *Idh-1*^{1} and *Pgi-1*^{1} for the *A. cepa* parental type and *Adh-1*^{3}, *Idh-1*^{2}, and *Pgi-1*^{2} for the *A. fistulosum* parental type. In this paper, the subscripts 'cepa,' 'het,' and 'fist' are used to designate a locus homozygous for *A. cepa* alleles, a heterozygous locus carrying one allele of *A. cepa* and one of *A. fistulosum*, and a locus homozygous for *A. fistulosum* alleles, respectively. The progeny were not tested for glycerate dehydrogenase (GDH) or phosphoglucomutase (PGM) because the band resolution of GDH was faint, making it difficult to read (Peffley et al. 1985), and some *A. fistulosum* populations are polymorphic at *Pgm-1*, carrying both the *Pgm-1*^{1} and *Pgm-1*^{2} alleles (Peffley and Orozco-Castillo 1987).

The isozyme analysis was done on root tissue. Onion bulbs were allowed to root in flats of moist sand for 7–10 days. Roots were collected into vials of water and held at 4 °C for at least 12, but not more than 24 h before running the electrophoretic analysis. This enhanced the electrophoretic pattern of alcohol dehydrogenase (ADH), which is induced by anaerobic conditions.

The number of individuals surviving the first growing season was 227 from 1034 and 254 from 1040. Isozyme data for *Idh-1* were obtained on 186 progeny of 1034 and 214 progeny of 1040; 190 progeny of 1034 and 225 progeny of 1040 for *Pgi-1*; 180 progeny of 1034 and 202 progeny of 1040 for *Adh-1*. Isozyme data for some progeny were lacking because weak individuals were lost during screening or there were difficulties in getting a clear band resolution.

Methods of statistical analysis

The hypothesis of independent segregation of alleles can be tested by a Chi-square statistic if some mechanism for obtaining expected cell frequencies is available. Chi-square, $\chi^2 = \sum_{i,j} \frac{(o_{ij} - e_{ij})^2}{e_{ij}}$, is defined as the sum across all the cells (ij) of the observed data (o_{ij}), minus the expected data (e_{ij}), squared and divided by the expected data (e_{ij}) in a two-way contingency table of the data.

Two gene loci display independence if the probability distribution of allele classes at locus 1 is stocastically independent of the probability distribution of the allele classes at locus 2. Specifically, if P_{ij} denotes the probability of observing the i^{th} allele at the first locus, and the j^{th} allele at the second locus, stochastic independence implies that $P_{ij} = P_{i.} P_{.j}$, where the latter symbols denote the sums of the P_{ij} across the second and first subscripts, respectively. If the loci in question are independent *and* the probabilities for every allele class at both loci are known, the expected frequencies are easily calculated as $e_{ij} = n P_{i.} P_{.j}$. The usual statistical tests of independence calculate e_{ij} based upon this assumption and estimating the $P_{i.}$ and $P_{.j}$ from the data. This method fails when the population is a mixture of two subpopulations defined by different marginal probability distributions for the alleles, even if the loci are segregating independently. The mixed nature of the population can cause the appearance of statistically dependent segregation, although the alleles may be on different chromosomes. Therefore, the usual contingency table analysis is not valid for the populations in this study. The usual Chi-square analysis assumes that the marginal probabilities are unknown. In the situation presented herein, a known genetic model, albeit mixed, provides the marginal probabilities. Thus, if the percentage of F_2 progeny in the mixed population can be estimated, the cell P_{ij}'s can be accurately calculated. The

Table 1. Probability distributions for F_2, BC_1, and mixed population model combining two independent gene loci, denoted *Idh-1* and *Pgi-1*. The probabilities of the F_2 and BC_1 portions of the mixed population denoted γ and $1-\gamma$, respectively

P	*Idh-1* Alleles[a]	*Pgi-1* cepa	*Pgi-1* het	*Pgi-1* fist
F_2 γ	cepa	$P=\frac{1}{16}$	$P=\frac{2}{16}$	$P=\frac{1}{16}$
	het	$P=\frac{2}{16}$	$P=\frac{4}{16}$	$P=\frac{2}{16}$
	fist	$P=\frac{1}{16}$	$P=\frac{2}{16}$	$P=\frac{1}{16}$
BC_1 $1-\gamma$	cepa	$P=\frac{4}{16}$	$P=\frac{4}{16}$	$P=0$
	het	$P=\frac{4}{16}$	$P=\frac{4}{16}$	$P=0$
	fist	$P=0$	$P=0$	$P=0$
Mixed	cepa	$P=\frac{(1\gamma+4(1-\gamma))}{16}$	$P=\frac{(2\gamma+4(1-\gamma))}{16}$	$P=\frac{1}{16}\gamma+0$
	het	$P=\frac{(2\gamma+4(1-\gamma))}{16}$	$P=\frac{(4\gamma+4(1-\gamma))}{16}$	$P=\frac{2}{16}\gamma+0$
	fist	$P=\frac{1}{16}\gamma+0$	$P=\frac{2}{16}\gamma+0$	$P=\frac{1}{16}\gamma+0$

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles

probabilities of the F_2 portion and the BC_1 portion are equal to γ and $1-\gamma$, respectively. The cell probabilities for the mixed population model are γ times the cell probability, given an F_2 probability distribution plus $1-\gamma$ times the cell probability, given a BC_1 probability distribution (Table 1).

An estimate of γ ($\hat{\gamma}$) can be calculated from the number of the progeny identifiable as belonging to the F_2 generation times the probability of being an F_2 progeny. Any individual that is homozygous for *A. fistulosum* alleles at one or more loci is identifiable as an F_2 progeny. Because the principles discussed above apply equally to the situation of three independent loci, the estimate of the percentage of F_2 progeny ($\hat{\gamma}$) in the population was made across data for all three isozymes to give the most accurate estimate of γ. A separate estimate of γ was made for each of the backcross populations under the hypothesis of independent segregation.

Contingency tables were constructed for each of the three possible pairs of gene loci, *Idh-1* by *Pgi-1*, *Idh-1* by *Adh-1*, and *Pgi-1* by *Adh-1*. Each table contains subtables for the populations 1034 and 1040. The contingency table analysis considered only those progeny that had complete isozyme data for both of the loci under consideration. The total progeny considered for each of the loci pairs in the 1034 and 1040 populations were *Idh-1* by *Pgi-1* = 185 and 211, *Idh-1* by *Adh-1* = 177 and 199, and *Pgi-1* by *Adh-1* = 180 and 199, respectively.

Overall Chi-square values and cell contributions to Chi-square values were calculated using expected cell counts, calculated using the cell probabilities developed for a mixed population and independent segregation model (MPI) with known marginal probabilities (Table 1).

The cell probabilities for the populations were calculated using the estimate of γ calculated from the data for the respective populations. The under- or over-representation of the observed cell count is indicated by the sign in parenthesis following the cell contributions to the Chi-square.

The probability distribution under the alternate hypothesis of linked loci for the mixed population model (MPL) can be developed considering the likelihood of crossing over. The probability of crossing over is denoted by $\mathbf{m}$ where $0<\mathbf{m}<0.5$. The probability distribution of two independent loci is 0.25 for each of the possible genotypes of the gametes produced. The probability distributions for crossover gametes are $\frac{(1-\mathbf{m})}{2}$ = homozygous for either *A. cepa* or *A. fistulosum* alleles at both loci, $\frac{\mathbf{m}}{2}$ = heterozygous carrying one *A. cepa* and one *A. fistulosum* allele at both loci.

The probabilities of the F_2 portion and the BC_1 portion of the mixed population are γ and $1-\gamma$, respectively. Cell probability distributions for the mixed and linked loci model are γ times the cell probability of crossover gametes, given an F_2 probability distribution plus $1-\gamma$ times the cell probability distribution of crossover gametes, given a BC_1 probability distribution (Table 2). An estimate of $\mathbf{m}$ can be obtained by numerically maximizing the likelihood function. This provides the value of $\mathbf{m}$ most consistent with the observed data. The cell probabilities for the

Table 2. Probability distributions for F_2, BC_1, and mixed population model combining two linked gene loci, denoted *Pgi-1* and *Idh-1*. The probabilities of the F_2 and BC_1 portions of the mixed population equal γ and $1-\gamma$, respectively. The probability of crossing over between loci is **m**. All entries below are divided by 4; divisor omitted for readibility

P	*Idh-1* Alleles[a]	*Pgi-1* cepa	*Pgi-1* het	*Pgi-1* fist
F_2 γ	cepa	$P=(1-\mathbf{m})^2$	$P=2\mathbf{m}(1-\mathbf{m})$	$P=\mathbf{m}^2$
	het	$P=2\mathbf{m}(1-\mathbf{m})$	$P=2(1-\mathbf{m})^2+2\mathbf{m}^2$	$P=2\mathbf{m}(1-\mathbf{m})$
	fist	$P=\mathbf{m}^2$	$P=2\mathbf{m}(1-\mathbf{m})$	$P=(1-\mathbf{m})^2$
BC_1 $1-\gamma$	cepa	$P=2(1-\mathbf{m})$	$P=2\mathbf{m}$	$P=0$
	het	$P=2(1-\mathbf{m})$	$P=2\mathbf{m}$	$P=0$
	fist	$P=0$	$P=0$	$P=0$
Mixed	cepa	$P=\gamma((1-\mathbf{m})^2)+1-\gamma(2(1-\mathbf{m}))$	$P=\gamma(2\mathbf{m}(1-\mathbf{m}))+1-\gamma(2\mathbf{m})$	$P=\gamma(\mathbf{m}^2)$
	het	$P=\gamma(2\mathbf{m}(1-\mathbf{m}))+1-\gamma(2\mathbf{m})$	$P=\gamma(2(1-\mathbf{m})^2+2\mathbf{m}^2)+1-\gamma(2(1-\mathbf{m}))$	$P=\gamma(2\mathbf{m}(1-\mathbf{m}))+0$
	fist	$P=\gamma(\mathbf{m}^2)+0$	$P=\gamma(2\mathbf{m}(1-\mathbf{m}))+0$	$P=\gamma(1-\mathbf{m})^2+0$

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles

Table 3. Contingency table for gene loci *Idh-1* by *Pgi-1* for populations 1034 and 1040 derived from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa* ($\hat{\gamma}=0.094$ and 0.074 for 1034 and 1040, respectively)

Families	1034			1040		
Alleles[a]	*Pgi-1*			*Pgi-1*		
Idh-1	cepa	het	fist	cepa	het	fist
cepa	59[b]	36	0	73	27	1
	5.2853[c]	1.6611 (–)[d]	0.8209 (–)	10.6468	11.2059 (–)	0.0043
het	39	42	4	41	66	2
	0.7051 (–)	0.3905 (–)	3.3868	1.9176 (–)	3.3282	0.0087
fist	2	1	2	0	0	1
	1.6934	0.2509 (–)	1.6934	0.9363 (–)	1.8726 (–)	0.0043
Chi-square[e]		15.8876			29.9249	

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles
[b] Observed cell count
[c] Cell contribution to overall Chi-square
[d] Indicates sign of observed cell count minus expected cell count
[e] $df=7$, $P\leq 0.01=18.5$, $P\leq 0.05=14.1$

populations using the MPL probability distributions resulted in different estimates for γ. These estimates of γ were calculated based on the number of F_2 progeny identified in each population for each pair of loci. Therefore, a different value for gamma was used to calculate the expected cell frequencies for each population for each pair of loci. The observed data for each pair of loci were tested for fit to the MPL model when the Chi-square value was significant using the MPI model.

Under the MPI model the test statistic has 7 *df*. The degrees of freedom for a χ^2 statistic are calculated by subtracting the number of constraints imposed by the null hypothesis from the number of cells. In the case of the MPI model, there are nine cells and two constraints ($o_{33}=n-o_{11}-\ldots o_{23}$, and $\hat{\gamma}$ estimated from the data), hence 7 *df*. Under the linked model (MPL), we have 6 *df* because **m** is added to the list of constraints.

Results and discussion

Estimate of F_2 progeny

The estimate of F_2 progeny ($\hat{\gamma}$) in the populations using the MPI model was 0.094 (9.4%) for 1034 and 0.074 (7.4%) for 1040. These estimates of γ are higher than anticipated, given the high degree of sterility observed in the F_1 interspecific crosses (Emsweller and Jones 1935a, b, 1945; Levan 1936; Maeda 1937; and van der Meer and van Benekom 1978). The estimates of γ under the MPL probability distributions for the pairs of loci *Idh-1* by *Pgi-1*, *Idh-1* by *Adh-1*, and *Pgi-1* by *Adh-1* in population 1034 are 0.108, 0.08, and 0.098, respectively. The esti-

Table 4. Contingency table for gene loci *Idh-1* by *Adh-1* in populations 1034 and 1040 derived from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa* ($\hat{\gamma}=0.094$ and 0.074 for 1034 and 1040, respectively)

Families	1034			1040		
Alleles[a]	*Adh-1*			*Adh-1*		
Idh-1	cepa	het	fist	cepa	het	fist
cepa	60[b]	31	0	62	27	2
	8.6569[c]	2.9588 (–)[d]	1.0400 (–)	4.7130	9.1765 (–)	1.4128
het	31	49	2	50	55	2
	2.9359 (–)	0.5099	0.0031 (–)	0.0847	0.5540	0.0310
fist	3	1	0	0	1	0
	3.6948	0.5606 (–)	1.0399 (–)	0.8831 (–)	0.3323 (–)	0.8831 (–)
Chi-square[e]		21.4226			18.0704	

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles
[b] Observed cell count
[c] Cell contribution to overall Chi-square
[d] Indicates sign of observed cell count minus expected cell count
[e] $df=7$, $P\leq 0.01=18.5$, $P\leq 0.05=14.1$

Table 5. Contingency table for gene loci *Pgi-1* by *Adh-1* for populations 1034 and 1040 derived from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa* ($\hat{\gamma}=0.094$ and 0.074 for 1034 and 1040, respectively)

Families	1034			1040		
Alleles[a]	*Adh-1*			*Adh-1*		
Pgi-1	cepa	het	fist	cepa	het	fist
cepa	58[b]	37	1	72	35	1
	6.2531[c]	0.8076 (–)[d]	0.0031 (–)	13.1626	3.5133 (–)	0.0155
het	35	42	1	39	45	3
	1.4498 (–)	0.2000 (–)	0.5878 (–)	1.6820 (–)	0.4535 (–)	0.8620
fist	2	4	0	2	2	0
	0.8400	1.6800	1.0575 (–)	1.4127	0.0310 (–)	0.8830 (–)
Chi-square[e]		12.8789			22.0157	

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles
[b] Observed cell count
[c] Cell contribution to overall Chi-square
[d] Indicates sign of observed cell count minus expected cell count
[e] $df=7$, $P\leq 0.01=18.5$, $P\leq 0.05=14.1$

mates of γ using the MPL probability distributions for these pairs of loci in population 1040 are 0.048, 0.059, and 0.088.

The MPI model contingency Chi-square analysis for population 1034 resulted in a significant Chi-square value at $P=0.01$ for the pair of loci *Idh-1* by *Adh-1* (Table 4). The Chi-square value for the pair of loci *Idh-1* by *Pgi-1* (Table 3) was significant at $P=0.05$. The Chi-square value for the pair of loci *Pgi-1* by *Adh-1* (Table 5) was not significant at $P=0.05$. Using the MPI model, population 1040 had significant Chi-square values at $P=0.01$ for all three pairs of loci (Tables 3, 4, 5).

The statistically significant Chi-square values under the MPI model indicate that nonindependent segregation occurred between all pairs of loci, except *Pgi-1* by *Adh-1* in the 1034 population. Chi-square analyses using the MPL model were run on all pairs of loci for both populations (Tables 6, 7, 8).

Idh-1 by Pgi-1

Nonindependent segregation between the two loci may be caused by the location of both loci within the same linkage group or any number of other barriers to inde-

Table 6. Contingency table for gene loci *Idh-1* by *Pgi-1*, testing the mixed population and linked loci model ($\hat{\gamma}=0.108$ and 0.048; $\mathbf{m}=0.420$ and 0.330 for 1034 and 1040, respectively) for populations 1034 and 1040 derived from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa*

Families	1034			1040		
Alleles[a]	*Pgi-1*			*Pgi-1*		
Idh-1	cepa	het	fist	cepa	het	fist
cepa	59[b]	36	0	73	27	1
	1.8065[c]	0.0320 (–)[d]	0.8800 (–)	0.3052	1.5385 (–)	1.8514
het	39	42	4	41	66	2
	0.0984	2.2756 (–)	1.0144	1.3260	0.2421 (–)	0.6914
fist	2	1	2	0	0	1
	1.4255	0.8415 (–)	0.0610	0.2800 (–)	1.1200 (–)	0.0172 (–)
Chi-square[e]		8.4347			7.3718	

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles
[b] Observed cell count
[c] Cell contribution to overall Chi-square
[d] Indicates sign of observed cell count minus expected cell count
[e] $df=6$, $P\leq 0.01=15.1$, $P\leq 0.05=11.1$

Table 7. Contingency table for gene loci *Idh-1* by *Adh-1*, testing the mixed population and linked loci model ($\hat{\gamma}=0.080$ and 0.059; $\mathbf{m}=0.380$ and 0.410 for 1034 and 1040, respectively) for populations 1034 and 1040 derived from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa*

Families	1034			1040		
Alleles[a]	*Adh-1*			*Adh-1*		
Idh-1	cepa	het	fist	cepa	het	fist
cepa	60[b]	31	0	62	27	2
	1.2844[c]	0.0795 (–)[d]	0.5100 (–)	0.5856	4.1219 (–)	4.6533
het	31	49	2	50	55	2
	0.0795 (–)	0.5026 (–)	0.0652	2.6083	0.1835 (–)	0.2369
fist	3	1	0	0	1	0
	12.1571	0.2688 (–)	1.3600 (–)	0.4900 (–)	0.1242 (–)	1.0200 (–)
Chi-square[e]		16.3070			14.0238	

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles
[b] Observed cell count
[c] Cell contribution to overall Chi-square
[d] Indicates sign of observed cell count minus expected cell count
[e] $df=6$, $P\leq 0.01=15.1$, $P\leq 0.05=11.1$

pendent assortment (Hermsen 1977; Graves 1988). The MPL Chi-square values for *Idh-1* by *Pgi-1* were not significant at either $P=0.01$ or $P=0.05$ (Table 6). The linked loci model for the mixed population adequately accounts for the deviation of the observed data from the expected cell frequencies calculated using the MPI model. Peffley et al. (1985) reported evidence suggesting the linkage of the *Idh-1* and *Pgi-1* loci in *A. fistulosum*. If these loci are linked, it is not a close association. A large percentage of recombinant progeny representing frequent crossover events were recovered. The map distance is estimated to be 33 cM in 1034 and 42 cM in 1040 using the MPL probability distributions.

Idh-1 by Adh-1

The observed data for *Idh-1* by *Adh-1* does not fit the MPI model, as evidenced by the significant Chi-square values for both populations (Table 4). The Chi-square values calculated using the MPL model were still signifi-

Table 8. Contingency table for gene loci *Pgi-1* by *Adh-1*, testing the mixed population and linked loci model ($\hat{y}=0.098$ and 0.088; $\mathbf{m}=0.430$ and 0.400 for 1034 and 1040, respectively) for populations 1034 and 1040 derived from the interspecific backcross (*A. fistulosum* × *A. cepa*) × *A. cepa*

Families	1034			1040		
Alleles[a]	*Adh-1*			*Adh-1*		
Pgi-1	cepa	het	fist	cepa	het	fist
cepa	58[b]	37	1	72	35	1
	2.2193[c]	0.0001 (–)[d]	0.0395	4.5584	0.3010 (–)	0.1286
het	35	42	1	39	45	3
	0.1156 (–)	1.5149 (–)	0.6230 (–)	0.0094 (–)	3.3220 (–)	0.3857
fist	2	4	0	2	2	0
	1.6980	1.5674	1.4300 (–)	2.4143	0.0048 (–)	1.5800 (–)
Chi-square[e]		9.2079			12.7041	

[a] cepa = homozygous for *A. cepa* alleles
het = heterozygous
fist = homozygous for *A. fistulosum* alleles
[b] Observed cell count
[c] Cell contribution to overall Chi-square
[d] Indicates sign of observed cell count minus expected cell count
[e] $df=6$, $P\leq 0.01=15.1$, $P\leq 0.05=11.1$

cant at $P=0.05$ (Table 7). The hypothesis of linkage between the two loci accounted for a large portion of the deviation of the observed data from the expected cell frequencies calculated using the MPI model, but not for all of it. Electrophoretic and cytogenetic evidence suggests, in *A. fistulosum*, that it is unlikely these two genes are linked (Peffley et al. 1985; Peffley et al. 1988; Peffley and Mangum 1990). Homoeology between the *A. fistulosum* and *A. cepa* genomes is generally assumed because of the bivalent pairing of heteromorphic chromosomes observed in the interspecific hybrids (Emsweller and Jones 1935a, b, 1945; Maeda 1937; Levan 1941; Peffley 1986). Normal bivalent pairing in the interspecific F_1 hybrid between *A. fistulosum* and *A. cepa* has been reported to vary from 50% (Maeda 1937) to 70% (Emsweller and Jones 1945). Maeda (1937) and Emsweller and Jones (1945) reported approximately 50% bivalent pairing in the few backcross progeny that each examined. Meiotic studies frequently show the satellite chromosomes of *A. fistulosum* and *A. cepa* paired forming normal bivalents (Maeda 1937; Levan 1941; Emsweller and Jones 1945). This suggests that some degree of homology exists between the satellite chromosomes of these species. Peffley (1986) proposed at least one translocation, involving the satellite chromosome, and one inversion differentiates these species.

Peffley and Currah (1988) mapped the *Adh-1* locus to the satellite chromosome in *A. fistulosum*, placing it on the long arm. Peffley and Mangum (1990) presented evidence of intergenic recombination between the two satellite chromosomes of *A. fistulosum* and *A. cepa* in a progeny of a trisomic known to be carrying the *A. fistulosum* satellite chromosome as the trisome, using the *Adh-1* locus and the satellite as markers. The recombinant chromosome consisted of the short arm of the *A. cepa* chromosome, marked by its tiny satellite, and the long arm of the *A. fistulosum* chromosome, marked by its smaller length and the expression of the *A. fistulosum* allele for the *Adh-1* locus. This evidence supports the assumption that the *Adh-1* locus is located on the satellite chromosome in *A. cepa* as well as in *A. fistulosum*. Peffley et al. (1985) reported the presence of *A. fistulosum* alleles for *Pgi-1* in the *Idh-1* trisomic addition line that carried a nonsatellite *A. fistulosum* chromosome as the trisome. This suggests the *Pgi-1* and *Idh-1* loci are on the same chromosome in *A. fistulosum*, and that chromosome is not a satellite chromosome. *Adh-1* and *Idh-1* loci appeared to segregate independently in Peffley's material. *Idh-1* and *Pgi-1* loci are probably on a different chromosome than the *Adh-1* locus in *A. cepa* as well, in which case *Idh-1* could not be linked with *Adh-1*.

A closer look at the MPI contingency table for *Idh-1* by *Adh-1* (Table 4) shows that the greatest cell contributions to the Chi-square for *Idh-1* by *Adh-1*, in both 1034 and 1040, are from the cells representing the $Idh\text{-}1_{cepa}$ $Adh\text{-}1_{cepa}$ and $Idh\text{-}1_{cepa}$ $Adh\text{-}1_{het}$ genotypes. The cell contributions to the overall Chi-square values, for the cells representing the genotypes homozygous for *A. cepa* alleles at both loci, indicate a preference for this genotype that is overrepresented in both populations. Selection against those genotypes heterozygous at one or both loci is apparent; the observed data in these cells are underrepresented. In population 1034, there is an overrepresentation of F_2 progeny of the gentoype $Idh\text{-}1_{fist}$ $Adh\text{-}1_{cepa}$.

The contradiction between the data from populations 1034 and 1040 for the loci pair *Idh-1* by *Adh-1* may reflect the different *A. fistulosum* seed parents in the interspecific F_1 hybrids. Researchers have reported various abnormalities in chromosome pairing in both the F_1 and the backcross generations. These abnormalities seem to occur at different rates, dependent upon the parental cultivars used in the interspecific crosses (Emsweller and Jones 1945; Peffley 1986).

Pgi-1 by Adh-1

The highly significant ($P=0.01$) Chi-square value for population 1040 using the MPI model indicates nonindependent segregation, but the nonsignificant ($P=0.05$) Chi-square value for population 1034 indicates independent segregation of the two loci (Table 5). The Chi-square value calculated using the MPL model for linked loci was significant ($P=0.05$) for the 1040 population, but was not significant ($P=0.05$) for the 1034 population (Table 8). If the *Idh-1* and *Pgi-1* loci are linked and on a different chromosome than *Adh-1* locus, it is not possible for the loci *Pgi-1* and *Adh-1* to be on the same chromosome.

The contingency table for *Pgi-1* by *Adh-1* with cell contributions and Chi-square calculated using the MPI model show similar preference for, and selection against, certain genotypes as previously observed for the *Idh-1* by *Adh-1* pair of loci. The cells representing the genotype $Pgi\text{-}1_{cepa}$ $Adh\text{-}1_{cepa}$ make the largest contribution to the overall Chi-square being greatly overrepresented. The cells representing genotypes heterozygous at one or both loci are consistently underrepresented. Distortion of the expected segregation under the assumption of independent loci can be explained by the existence of barrier(s) to nonindependent segregation other than linkage.

Conclusions

Statistical methods are given to calculate expected segregation ratios in a mixed population of BC_1 and F_2 progeny, based on an accurate estimate of the fraction of F_2 progeny in the population. Progeny homozygous for *A. fistulosum* alleles at one or more loci were used to estimate the percentage of F_2 progeny. The average estimates were 9.5 and 6.7% for 1034 and 1040, respectively. The estimates of the F_2 progeny varied under the hypothesis of independence or linkage. The best fit of the data was found for *Idh-1* by *Pgi-1* using the MPL model (linkage hypothesis), with the estimates of F_2 progeny calculated to be 10.8 and 4.8% for 1034 and 1040, respectively.

The nonindependent segregation shown between the *Idh-1* and *Pgi-1* loci supports the suggestion that these loci are linked, but preference was shown for genotypes homozygous for *A. cepa* alleles at both loci, and selection against those heterozygous at one or both loci was observed. It is probable that the nonindependent segregation between *Idh-1* by *Adh-1* and *Pgi-1* by *Adh-1* is the result of barriers to independent segregation other than linkage. Linkage between *Idh-1* and *Adh-1* or *Pgi-1* and *Adh-1* seems highly improbable, given the electrophoretic and cytological evidence to the contrary presented in the scientific literature.

Graves (1988) cites numerous examples of genic and chromosome instability in interspecific plant and animal hybrids, resulting in a wide range of abnormalities including preferential chromosome segregation, genomic reorganization, high mutation rates, selective repression of one parental allele, and nucleolar dominance. Graves refers to these abnormalities as the genomic shock phenomena. It is possible that such genomic shock may account for the aberrant segregation ratios observed in the isozyme data for *Idh-1* × *Adh-1* and *Pgi-1* × *Adh-1*. These populations of BC_1 progeny from the interspecific cross between *A. fistulosum* and *A. cepa* are probably not useful for genetic mapping studies. These data suggest that genes and chromosomes may not maintain their normal structure and function in these particular interspecific environments.

Recombination between the genomes of *A. cepa* and *A. fistulosum* for *Idh-1* and *Pgi-1* was demonstrated by 40% of the population for 1034 and 32% for 1040. It is obvious from these data that relatively frequent genetic recombination occurs between these two species, at least in specific regions of the genomes. Cytological evidence of intergenic recombination between *A. fistulosum* and *A. cepa* has been presented by Peffley and Mangum (1990) as well.

References

Emsweller SL, Jones HA (1935a) An interspecific hybrid in *Allium*. Hilgardia 9:265–273

Emsweller SL, Jones HA (1935b) Meiosis in *A. fistulosum*, *A. cepa*, and their F_1 hybrid. Hilgardia 9:277–294

Emsweller SL, Jones HA (1945) Further studies on the chiasmata of the *A. cepa* × *A. fistulosum* hybrid and its derivatives. Am J Bot 32:370–379

Graves JAM (1988) Genome instability in interspecific cell hybrids. I. Unstable expression of genes in divergent cell hybrids. J Genet 67:9–22

Hermsen JGTH (1977) General considerations on interspecific hybridization. In: Proc 8th EUCARPIA Congr, Madrid, Spain

Jones HA, Mann LK (1963) Onions and their allies. Interscience, New York p 32

Levan A (1936) Die Zytologie von *A. cepa* × *A. fistulosum*. Hereditas 21:195–214

Levan A (1941) The cytology of the species hybrid *A. cepa* × *A. fistulosum* and its polyploid derivatives. Heredity 27:253–272

Maeda T (1937) Chasma studies in *A. fistulosum, A. cepa*, and their F_1, F_2, and backcross hybrids. Jpn J Genet 13:146–159

Meer QP van der, Benekom JL van (1978) Improving the onion crop (*A. cepa* L.) by transfer of characters from *A. fistulosum* L. Biul Warzywniczy 22:87–91

Peffley EB (1986) Evidence for chromosomal differentiation of *Allium fistulosum* and *A. cepa*. J Am Soc Hortic Sci 111:126–129

Peffley EB, Currah L (1988) The chromosomal locations of enzyme-coding genes *Adh-1* and *Pgi-1* in *Allium fistulosum* L. Theor Appl Genet 75:945–949

Peffley EB, Mangum P (1990) Introgression of *A. fistulosum* L. into *A. cepa* L.: cytogenetic evidence. Theor Appl Genet 79:113–118

Peffley EB, Orozco-Castillo C (1987) Polymorphism of isozymes within plant introductions of *Allium cepa* L. and *A. fistulosum* L. HortScience 22(5):956–957

Peffley EB, Corgan JN, Horak KE, Tanksley SD (1985) Electrophoretic analysis of *Allium* alien addition lines. Theor Appl Genet 71:176–184

Vallejos CE, Tanksley SD (1983) Segregation of isozyme markers and cold tolerance in an interspecific backcross of tomato. Theor Appl Genet 66:241–247