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ORIGINAL PAPER

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Population development by phenotypic selection with subsequent marker-assisted selection for line extraction in cucumber (*Cucumis sativus* L.)

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Abstract Cucumber (*Cucumis sativus* L.; $2n = 2x = 14$) has a narrow genetic base, and commercial yield of US processing cucumber has plateaued in the last 15 years. Yield may be increased by altering plant architecture to produce unique early flowering (days to flower, DTF), female (gynoecious, GYN), highly branched (multiple lateral branching, MLB), long-fruited (length:diameter ratio, L:D) cultivars with diverse plant statures. The genetic map position of QTL conditioning these quantitatively inherited yield component traits is known, and linked molecular markers may have utility in marker-assisted selection (MAS) programs to increase selection efficiency, and effectiveness. Therefore, a base population (C_0), created by intermating four unique but complementary lines, was subjected to three cycles (C_1–C_3) of phenotypic (PHE) mass selection for DTF, GYN, MLB, and L:D. In tandem, two cycles of marker-assisted backcrossing for these traits began with selected C_2 progeny (C_{2S}) to produce families (F_1[i.e., $C_{2S} \times C_{2S}$], and BC_1 [i.e., $F_1 \times C_{2S}$]) for line extraction, and for comparative analysis of gain from selection by PHE selection, and MAS. Frequencies of marker loci were used to monitor selection-dependent changes during PHE selection, and MAS. Similar gain from selection was detected as a result of PHE selection, and MAS for MLB (~0.3 branches/cycle), and L:D (~0.1 unit increase/cycle) with concomitant changes in frequency at linked marker loci. Although genetic gain was not realized for GYN during PHE selection, the percentage of female flowers of plants subjected to MAS was increased (5.6–9.8% per cycle) depending upon the BC_1 population examined. Selection-dependent changes in frequency were also detected at marker loci linked to female sex expression during MAS. MAS operated to fix favorable alleles that were not exploited by PHE selection in this population, indicating that MAS could be applied for altering plant architecture in cucumber to improve its yield potential.

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Introduction

Genetic markers that are associated with economically important traits can be used by plant breeders as selection tools (Darvasi and Soller 1994). Genetic gain from selection (ΔG) during marker-assisted selection (MAS) has been investigated by computer simulation to evaluate its efficacy (Lande and Thompson 1990; Gimelfarb and Lande 1994; Hospital et al. 1992, 1997; Liu et al. 2004). This has led to the development of modeling software for MAS (Bohn and Melchinger 2000), and strategies for its effective use in plant improvement (Luo et al. 1997; Knapp 1998; Xie and Xu 1998).

Such theoretical appraisals of MAS are indicative of its potential utility for increasing plant improvement efficiency (Hospital et al. 2000). Increased selection efficiency through MAS may be attained through earlier selection and/or by reducing plant population size during selection. However, the efficiency of marker loci as predictors of phenotypic (PHE) variation is dependent upon many factors, and precise predictions of response

to selection are often difficult to define (Staub et al. 1996). Thus, rigorous studies that empirically assess the efficacy of MAS for the improvement of multiple quantitative traits are relatively scarce, and almost non-existent in vegetable crops.

Dramatic allelic shifts associated with economically important traits have been detected during marker-assisted population improvement (Steele et al. 2004; Flint-Garcia et al. 2003; Moreau et al. 2004). Introgression of desirable alleles using MAS has proven particularly effective during backcrossing in several crop species (Wilcox et al. 2002; Lecomte et al. 2004; Thabuis et al. 2004). In cucumber (*Cucumis sativus* L.), mapping of quantitatively inherited traits in a narrow-based U.S. processing cucumber population (i.e., Gy-7 × H-19) led to the identification of QTL associated with yield components (Serquen et al. 1997a; Fazio et al. 2003a) that were successfully used in the marker-assisted backcross introgression of one metric trait, multiple lateral branching (MLB; four QTL) over two cycles of selection (Fazio et al. 2003b).

Unique plant architectural traits (i.e., sex expression, MLB, fruiting habit, and development) that have potential for increasing yield in commercial cucumber have been identified in exotic germplasm (Staub and Kupper 1985; Wehner et al. 1989; Wehner 1998). Typically, new, high yielding lines and hybrids are produced through population development followed by line extraction, a process which often requires 5–7 years to accomplish (Staub and Bacher 1997). It would be desirable to shorten the time required for the incorporation of exotic traits into commercial cucumber germplasm. Thus, a project was designed to incorporate exotic traits conditioning plant architecture into commercial cucumber to increase yield. This was accomplished by intermating unique lines differing in plant habit to produce a base population which was subjected to three cycles of recurrent mass selection by phenotype for four yield components. Subsequently, high-yielding lines were extracted through strategic selection by molecular markers, and backcrossing. The objectives of this study were to determine if gain from selection was realized: (1) over three cycles of PHE mass selection (population improvement), and, if so, whether there was concomitant changes in frequencies of markers linked to QTL associated with those traits under selection, and (2) by marker-assisted backcrossing (line extraction) after population improvement with selection-dependent allelic changes. Such information would allow for the development of strategies for more efficient incorporation of exotic traits into commercial cucumber germplasm.

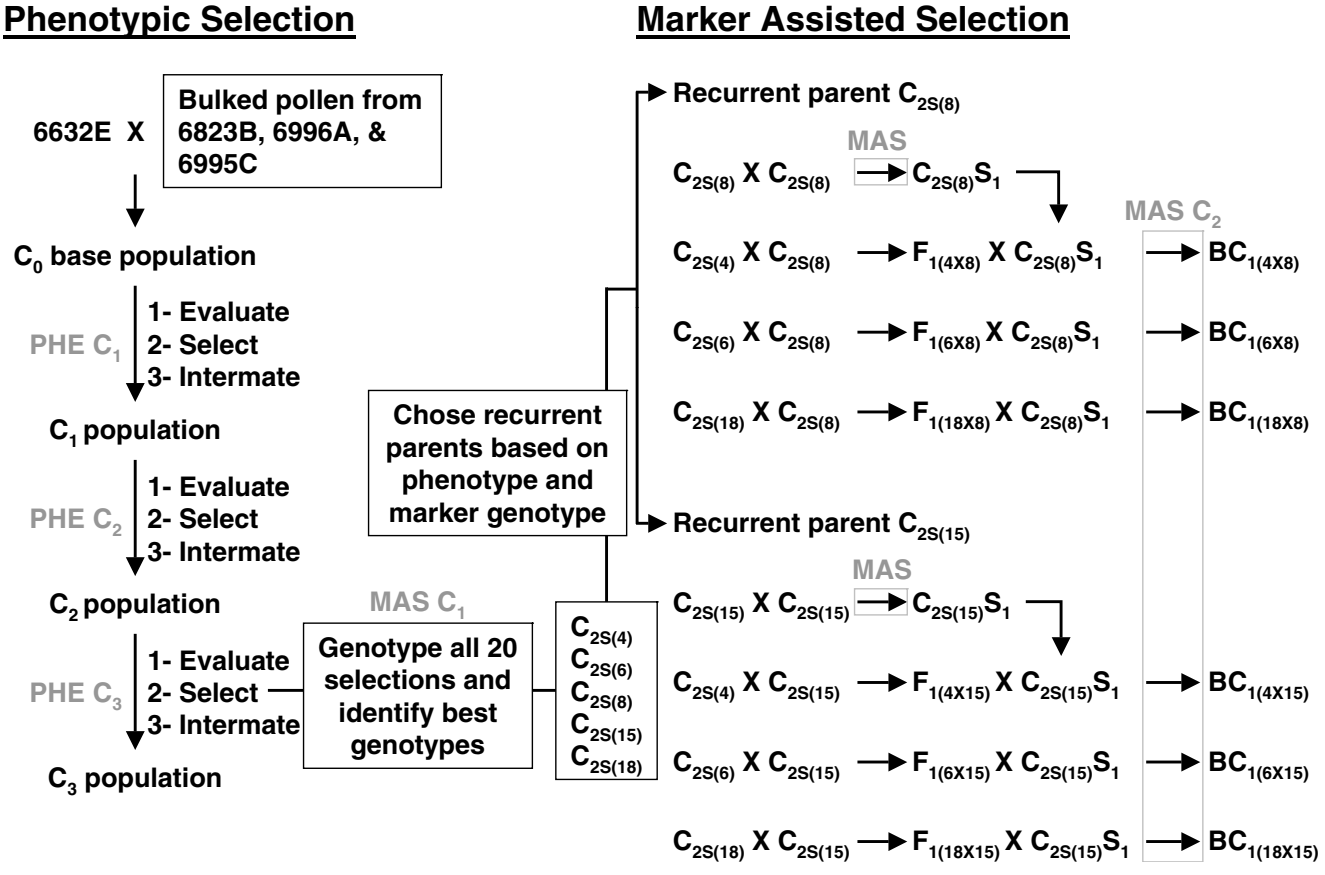


Fig. 1 Schematic of population development by PHE selection (PHE C1-3) followed by marker-assisted line extraction (MAS; see text)

Materials and methods

Germplasm

A genetically diverse but complementary array of four inbred lines was used as parents for population development. These contrasting phenotypes were drawn from the U.S. Department of Agriculture (USDA) cucumber breeding program, Madison, WI, because of their potential contribution to the base population for the earliness, sex expression, branching, and fruit length:diameter (L:D) ratio. Lines 6996A and 6995C were drawn from a recombinant inbred line (RIL) population (F_9), and possess differing vegetative characteristics (Fazio et al. 2003a). The determinate, unilateral branching (~1.3 branches) line 6996A is gynoecious (GYN), relatively early flowering (~41 DTF), and produces fruit that are of relatively short length (L:D = ~2.8). In contrast, line 6995C is indeterminate, monoecious, and late flowering (~47 DTF), but produces fruit of intermediate length (L:D = ~3.0) in a MLB (~2.9 branches) background. Line 6823B is indeterminate and possesses a comparatively late (~43 DTF) and monoecious flowering habit in a MLB (~4.0 branches) background and produces relatively long fruit (L:D = ~3.3). This line originated from a cross between a parent (H-19) of the RIL population and a USDA elite processing line whose progeny were then selected for H-19 attributes to create 6823B. The relatively early flowering (~40 days to flower after planting [DTF] under WI conditions), multiple disease resistant, GYN line 6632E is indeterminate and possesses a unilateral branching habit (~1.5 laterals) that bears fruit with a L:D of about 2.7 (industry minimum is at least 2.8). This line does not have either H-19 or Gy-7 in its pedigree.

Population development

Population development involved three cycles (C) of PHE recurrent mass selection (Fig. 1). The base population (C_0) was produced in a greenhouse in Madison, WI in 2000 using bulk pollen (from at least five flowers) from lines 6823B, 6996A, and 6995C crossed onto line 6632E. Subsequently, three populations (C_1, C_2 and C_3) were obtained as selection was practiced on 400 (2001; C_0), 600 (2002; C_1), and 600 (2003; C_2) plants for earliness, gynoecy, MLB, and high L:D values to capture at least 20 plants per cycle under open field conditions at the University of Wisconsin Experiment Station, Hancock, WI (UWESH; Planefield loamy sand [Typic Udipsament; sandy, mixed, mesic]). PHE selection was accomplished by establishing thresholds for each trait (i.e., earliness 50 DTF, gynoecy >50% female flowers on the first 10 nodes, MLB >4 branches, and L:D >2.8) and selecting plants with the highest values of all four traits above these thresholds. In each selection block (year), meristems of unique plants [20 C_0 plants (selection intensity, $i = 2.063$); 20 C_1 plants, $i = 2.219$, and 20 C_2 plants, $i = 2.219$] were taken for cloning as rooted cuttings, and then random mated using bulk

Table 1 Characteristics of molecular markers defined in a genetic map of cucumber (*Cucumis sativus* L.) constructed by Fazio et al. (2003) that were used for MAS in this study

Marker	Marker type	Linkage group	Map position (cM)	Parent[a]	Multiple × group[b]	QTL associations (mapping parent and LOD score)[c]
CSWCT28	SSR	1	5.0	G&H		DTF(H, 7.1), MLB(H, 10.4), GYN(G, 13.0), L:D(H, 5.7)
OP-AG1-1	RAPD	1	31.8	G		DTF(H, 6.4), MLB(H, 11.6), GYN(G, 7.3)
AJ6SCAR	SCAR	1	61.4	G	3	MLB(H, 3.3)
BC523SCAR	SCAR	1	66.5	G	2	MLB(H, 3.3)
AW14SCAR	SCAR	3	3.9	G&H	1	GYN(G, 5.1)
CSWTAAA01	SSR	4	34.1	G&H	2	MLB(H, 4.6)
OP-AI4	RAPD	5	101.0	G		GYN(G, 3.0)
OP-AO12	RAPD	5	117.3	G		GYN(G, 3.0)
OP-AI10	RAPD	6	22.5	H		L:D(G, 7.3)
AK5SCAR	SCAR	6	33.0	G	2	MLB(H, 3.0)
M8SCAR	SCAR	6	39.1	H		MLB(H, 3.0)
OP-W7-1	RAPD	6	83.4	H		GYN(G, 4.1)
L19-2SCAR	SCAR	6	115.0	H	1	MLB(G, 4.2), GYN(G, 4.1)
BC515	RAPD	7	0.0	H		L:D(H, 4.2)
L19-1SCAR	SCAR	7	9.9	H	3	L:D(H, 4.2)

SSR simple sequence repeat; *RAPD* random amplified polymorphic DNA; *SCAR* sequence characterized amplified region

[a]Allelic constitution based on mapping parents H-19 and Gy-7 (synom. G421) used by Fazio et al. 2003a, where G = present in Gy-7, H = present in H-19, G&H = present in Gy-7 and H-19 (codominant marker)

[b]Markers used in multiplex were placed in multiplexing groups (1, 2, or 3)

[c]Markers associated with QTL for DTF = earliness, MLB = multiple lateral branching, GYN = gynoecious, and L:D = length to diameter ratio. The parentheses contain the parent contributing the QTL (G = Gy-7, H = H-19) followed by the highest LOD score for each QTL obtained from multiple field trials (Serquen et al. 1997b; Fazio et al. 2003a, b) is shown in parentheses

pollen from five male flowers to pollinate each female flower.

Backcrossing for line extraction

Line extraction was implemented using specific C_2 selections (C_{2S}) based on their field performance and their molecular marker profile (Tables 1, 2). Five of the 20 C_2 selections [C_{2S} plants designated 4 ($C_{2S(4)}$), 6 ($C_{2S(6)}$), 8 ($C_{2S(8)}$), 15 ($C_{2S(15)}$), and 18 ($C_{2S(18)}$); Fig. 1] possessing relatively high values (i.e., at or above selection thresholds) for the traits examined and ideal marker phenotypes (i.e., desired allelic composition) were used to make hybrid progeny. While C_{2S} plants 15 and 8 were used as the paternal parents, plants 4, 6, and 18 were employed as maternal parents for the production of hybrid progeny. Matings lead to production of the following F_1 hybrid progeny: 4×8, 6×8, 18×8, 4×15, 6×15, and 18×15 (Fig. 1).

Molecular analysis was initially performed on each of the selected C_{2S} individuals (4, 6, 8, 15, and 18) and then on at least 10 individuals of selfed (plants 15 and 8) and hybrid progeny (Fig. 1). Original plants 15 and 8 were self-pollinated, and selfed progeny were evaluated using markers to identify individuals that were subsequently used as recurrent parents in the production of BC_1 families. The selfed progeny of plants 15 and 8 were used for BC family construction since the floral production of the cloned original plants did not coincide with the timing of hybrid development (i.e., the chemical induced sex conversion of pistillate to staminate flowers; Atsmon and Tabback 1979). Those F_1 individuals having the most ideotypic marker profile (i.e., preferred parental constitution) were then used in backcrossing to the selfed progeny of plants 15 and 8 (recurrent parents; designated as a subscript notation, e.g., $BC_{1,8}$) possessing parental marker constitutions to produce BC_1 progeny (Table 1). Plants used as recurrent parents used in backcrossing were GYN under greenhouse conditions.

This backcrossing scheme was devised based on the genetic information available on yield components, their correlations and heritabilities, and linkage between target traits (i.e., yield components) and 15 marker loci (Table 1; Fazio et al. 2003a, b). The BC_1 families were subsequently designated as: (4×8)×8, (6×8)×8, (18×8)×8, (4×15)×15, (6×15)×15, and (18×15)×15.

Many factors were taken into consideration when selecting markers, such as marker type, trait correlations, genetic distance from QTL, and number of QTL in proximity to the marker (Robbins et al. 2002; Robbins and Staub 2004). In general, dominant markers flanking QTL or codominant markers tightly linked to QTL were selected. The desired genotype (Gy-7 allele, H-19 allele or both Gy-7 and H-19 alleles) for each marker was determined depending on the QTL surrounding the marker, in an attempt to create the ideal genotype, or ideotype (Tables 1, 2). To increase marker efficiency, some RAPD markers were converted to SCARs (e.g., OP-M8 was converted to M8SCAR) following Staub et al. (2002). The individuals whose genotype most closely matched the ideotype at the greatest number of marker loci were selected and intercrossed (e.g., $(C_{2S(4)}) \times (C_{2S(8)})$, $[(C_{2S(4)})\times(C_{2S(8)})] \times (C_{2S(18)})$).

Molecular marker analysis

Tissue from parental lines and populations used in this study was harvested and DNA extracted according to Fazio et al. (2003a). Two SSR (CSWCT28, CSWTAAA01), seven SCAR (AJ6SCAR, AW14SCAR, BC523SCAR, AK5SCAR, M8SCAR, L19-1SCAR, and

Table 2 Molecular marker phenotype of cucumber (*Cucumis sativus* L.) parents used in the development of a base population (C_0) for three cycles of PHE selection (C_1–C_3) for yield components, and single plant selections from C_2 used in the production of F_1 progeny for use in MAS

Marker	Parents for base population development				Parents for production of F_1 progeny				
	6632E	6823B	6996A	6995C	C_2 #4	C_2 #6	C_2 #8	C_2 #15	C2 #18
CSWCT28	H[a]	H	G	H	H	H	H	H	H
OP-AG1-1	H	G	G	H	H	H	H	H	G
AJ6SCAR	G	G	G	H	H	H	G	H	G
BC523SCAR	G	G	G	H	G	G	G	G	G
AW14SCAR	G	G	H	G	G	G	G	G	G
CSWTAAA01	G	G	G&H	H	G&H	G&H	G	G	H
OP-AI4	G	G	G	H	G	G	G	G	G
OP-AO12	H	H	G	G	G	H	G	G	G
OP-AI10	H	G	G	H	H	H	H	H	H
AK5SCAR	H	G	G	H	H	H	H	G	H
M8SCAR	G	H	G	H	H	H	H	H	H
OP-W7-1	G	H	H	G	G	G	G	G	H
L19-2SCAR	G	G	H	G	G	G	G	G	G
BC515	G	G	H	H	H	H	H	H	H
L19-1SCAR	G	H	H	H	H	G	H	H	H

[a]G = present in Gy-7 (synom. G421), H = present in H-19, G&H = present in Gy-7 and H-19 (codominant marker)

L19-2SCAR,), and six RAPD (OP-AG1-1, OP-AI4, OP-AO12, OP-AI10, OP-W7-1, and BC515) markers identified by Serquen et al. (1997a), Horejsi et al. (1999), and Fazio et al. (2003a) were employed in the analysis of parents and populations after selection. These markers are associated with QTL for DTF, GYN, MLB, and L:D as designated by Fazio et al. (2003a) and Serquen et al. (1997a). Since cucumber has only two possible alleles per locus, parental lines possess alleles for Gy-7 or H-19 at each marker locus associated with the traits examined herein.

The RAPD, SCAR and SSR polymerase chain reactions (PCR) and electrophoresis were performed according to Fazio et al. (2002). In some cases PCR reactions were multiplexed to improve experimental efficiency (Table 2) with empirical optimization following Staub et al. (2002).

Open field evaluation

The original parents (4), their derived C_0, C_1, C_2 , C_3 populations, selected recurrent parents (selfs of plant #8 and #15), hybrid progeny resulting from C_2 selections (6), backcross families (12), and 'Vlasset' (Seminis Seed Company, Woodland, Calif.; control) were sowed on June 5, 2004 in a greenhouse in Madison, WI, and transplanted on June 24 to UWESH. The design was a randomized completed block design with five replications or on rare occasion (three cases) three replications as seed amounts were limiting. Each plot had eight plants and consisted of single rows (5.2 m long) with plants spaced 13 cm apart in rows on 1.5 m centers including edge borders corresponding to a plant density of approximately 51,000 plants/ha.

Data were collected on yield component traits including DTF, sex expression, number of lateral branches, fruit L:D, and fruit number. DTF was recorded on an individual plot basis as the number of days from planting until at least one fully expanded flower was present on 10% of the plants in a plot. Sex expression was recorded on individual plants in a plot as the number of pistillate nodes in the first 10 nodes of the main stem. Total number of lateral branches was recorded for each plant in the first ten nodes at or just after anthesis. Mean fruit L:D per plot was obtained by measuring the length and diameter of 5–10 randomly selected fruits (USDA 2B-3A grade; 25–30 mm in diameter) in each plot, then averaging over four harvests. Likewise, the number of fruit per plot was counted at each of four harvests (68, 75, 82 and 89 days after planting), and is presented herein as cumulative four-harvest means. The first harvest interval of each plot occurred as two to three fruit >51 mm in diameter (oversized) were observed within a plot (Wehner 1989), and all immature fruits >20 mm in diameter and >10 cm in length were taken for analysis. The remaining three harvest intervals occurred about every 7 days when 2–3 mature oversized fruits were observed within a plot.

Statistical analysis

Data of all traits were analyzed using a mixed models procedure (PROC Mixed) employed by SAS (Littell et al. 1996). Analysis of variance was followed by least square mean comparison of morphological trait value using SAS (SAS Institute 1999). While entries (lines and populations; designated hereafter as treatments) were considered fixed effects, blocks and interactions were considered random effects. Least square means and *t* test probabilities ($P=0.01$) of mean differences were calculated for all treatments. While *F* tests were used to determine significance of main effects and interactions, mean separation of main effects was performed based on the Waller–Duncan *K*-ratio test ($K=100$). Specific single-degree of freedom contrasts were employed to determine general response to selection for biologically important comparisons (e.g., PHE selection and MAS).

To determine the relationship between the traits under selection, PHE correlations among treatments for

Table 3 Selection response of yield component traits in cucumber (*Cucumis sativus* L.) over three cycles (C_1–C_3; C_0 is the base population) of recurrent mass selection by phenotype

Trait	Cycles of selection						Response to selection			
	C_0	C_1	C_2	C_3	Mean	S.E.	b[f]	S.E[g]	R^2	*P* value
Days to flower[a]	43.8	44.8	44.0	42.8	43.9	0.8	-0.38	1.40	0.09	0.19
Gynoecy (%)[b]	93.2	83.1	90.9	82.0	87.1	5.6	-2.30	9.40	0.08	0.24
Lateral branch no.[c]	1.40	1.54	1.94	2.20	1.76	0.37	0.29	0.36	0.46	0.001
Fruit L:D[d]	2.70	2.72	2.76	2.92	2.78	0.01	0.08	0.11	0.44	0.001
Fruit number[e]	2.05	1.92	2.28	2.04	2.08	0.15	0.01	0.21	0.01	0.77

[a]Days from transplant until at least one fully expanded flower was present on 10% of the plants in a plot
[b]Percentage of pistillate flowers in the first 10 nodes on the main stem
[c]Primary branches in the first 10 nodes
[d]L:D of 5–10 randomly chosen fruit (USDA 2B-3A grade; 25–30 mm in diameter) at each of four harvests
[e]The number of fruits (20–51 mm diameter) per plant averaged over four harvests
[f]Slope of the regression using a linear model
[g]Standard error of the slope b

Table 4 Selection response of yield component traits in cucumber (*Cucumis sativus* L.) over two cycles of MAS in two populations (nos. 8 and15) during backcross-based line extraction

Trait	Generation of selection[f]					Response to selection			
	C_3	F_1	BC_1	Mean	S.E.	b[g]	S.E.[h]	R^2	*P* value
Plant number 8									
Days to flower[a]	42.8	43.3	43.0	43.1	0.26	-0.02	2.49	0.01	0.97
Gynoecy (%)[b]	82.0	94.9	97.3	91.4	8.23	5.6	7.44	0.26	0.001
Lateral branch no.[c]	2.20	2.54	2.78	2.48	0.29	0.30	0.42	0.23	0.001
Fruit L:D[d]	2.92	2.98	3.07	2.99	0.08	0.08	0.15	0.12	0.01
Fruit number[e]	2.04	2.51	2.54	2.36	0.28	0.17	0.56	0.04	0.14
Plant number15									
Days to flower[a]	42.8	43.9	43.1	43.3	0.58	-0.17	2.5	0.01	0.74
Gynoecy (%)[b]	82.0	66.5	89.1	79.2	11.5	9.76	16.3	0.16	0.003
Lateral branch no.[c]	2.20	2.75	2.36	2.44	0.28	-0.11	0.46	0.02	0.25
Fruit L:D[d]	2.92	3.24	3.27	3.15	0.20	0.12	0.20	0.17	0.002
Fruit number[e]	2.04	2.25	2.25	2.18	0.12	0.07	0.20	0.02	0.35

[a]Days from transplant until at least one fully expanded flower was present on 10% of the plants in a plot
[b]Percentage of pistillate flowers in the first 10 nodes on the main stem
[c]Primary branches in the first 10 nodes
[d]L:D of 5–10 randomly chosen fruit (USDA 2B-3A grade; 25–30 mm in diameter) at each of four harvests
[e]The number of fruits (20–51 mm diameter) per plant averaged over four harvests
[f]C_3 = population resulting from three cycles of PHE selection, F_1 = mating among parents identified by marker profiling (see Table 1) for each of four traits (DTF, gynoecy, lateral branch number, and fruit L:D), BC_1 = backcross of F_1 progeny to either recurrent plant #8 or #15 which had been previously selected based on their optimal marker profile for the traits under selection according to Fazio et al. 2003a (see Table 2), where S.E. designates standard error of the mean over selection generations
[g]Slope of the regression using a linear mode.
[h]Standard error of the slope b

each trait were calculated by Pearson correlation using SAS (SAS Institute 1999). Gain from selection was measured by assessing mean treatment differences over selection cycles (C_0–C_3, and C_2 selections, C_3, F_1, and BC families) using regression analysis where best-fit models (linear or quadratic) were identified and are presented based on comparative analyses (Steele and Torrie 1980).

Table 5 Frequencies of molecular marker loci associated with yield components over three cycles (C) of recurrent mass selection by phenotype in cucumber (*Cucumis sativus* L.)

Marker[a]	Expected frequency[b]	Marker frequencies over cycles of selection[d]						Regression analysis		
		C_0	C_1	C_2	C_3	Mean	S.E.	Equation[e]	R^2	*P* value
CSWCT28	< 1.00	0.09 [c]	0.11	0.09	0.00	0.07	0.05	$y = -0.036x + 0.153$	0.32	0.045
OP-AG1-1	> 0.00	0.51	0.13	0.42	0.39	0.36	0.16	$y = -0.025x + 0.42$	0.02	0.671
AJ6SCAR	0.00	0.60	0.69	0.64	0.44	0.59	0.11	$y = -0.077x^2 + 0.345x + 0.312$	0.58	0.013
BC523SCAR	0.00	0.78	0.80	0.96	1.00	0.88	0.11	$y = 0.103x + 0.622$	0.71	0.001
AW14SCAR	1.00	0.76	0.96	0.76	0.89	0.84	0.10	$y = 0.029x + 0.747$	0.03	0.545
CSWTAAA01	0.00	0.27	0.33	0.29	0.33	0.30	0.03	$y = 0.005x + 0.28$	0.01	0.904
OP-AI4	1.00	0.64	0.84	0.96	0.83	0.82	0.13	$y = 0.115x + 0.582$	0.47	0.010
OP-AO12	1.00	0.27	0.36	0.82	0.83	0.57	0.30	$y = 0.2497x - 0.051$	0.64	0.001
OP-AI10	1.00	0.47	0.07	0.04	0.00	0.15	0.22	$y = 0.089x^2 - 0.571x + 0.918$	0.78	0.001
AK5SCAR	0.00	0.51	0.24	0.22	0.50	0.37	0.16	$y = 0.112x^2 - 0.574x + 0.962$	0.62	0.007
M8SCAR	0.00	0.40	0.24	0.40	0.11	0.29	0.14	$y = -0.032x + 0.422$	0.05	0.452
OP-W7-1	1.00	0.53	0.80	0.69	0.78	0.70	0.12	$y = 0.087x + 0.466$	0.33	0.039
L19-2SCAR	1.00	0.69	0.89	0.89	0.94	0.85	0.11	$y = 0.106x + 0.576$	0.44	0.013
BC515	0.00	0.18	0.29	0.20	0.17	0.21	0.06	$y = -0.004x + 0.173$	0.01	0.853
L19-1SCAR	0.00	0.64	0.85	0.38	0.28	0.54	0.26	$y = -0.097x^2 + 0.305 + 0.458$	0.56	0.016

[a]Marker type, map location, and association with yield components given in Table 2
[b]Expected frequencies of the Gy-7 marker phenotype (G; Table) based on marker QTL associations (Fazio et al. 2003a). The values of < 1.00 and > 0.00 indicate that the marker is associated with multiple QTL from both parents and therefore the optimal frequency is unknown, but fixation is unlikely. The value of < 1.00 indicates the Gy-7 marker phenotype frequency is expected to be greater than the H-19 marker phenotype frequency (> 0.00 indicates Gy-7 < H-19) at this marker
[c]Frequencies of the Gy-7 (synom. G421) marker phenotype at marker loci linked to selected traits (Table 2) according to QTL mapping by Fazio et al. (2003a)
[d]Selection for days to 10% flowering from transplanting, pistillate flowers in the first 10 nodes on the main stem, primary branches in the first 10 nodes, and L:D of 5–10 randomly chosen fruit at each of four harvests
[e]Statistical significance of change in marker frequencies was tested using both linear and quadratic models. The equation, R^2 and *P* value of the best fitting model is reported

Changes in the genetic structure of the populations examined were assessed by calculating marker frequencies in C_0–C_2 (45 plants each), C_3 (20 plants), C_2 plant 8 self, C_2 plant 15 self, and hybrid progeny [at least 10 plants each (~92% chance of detecting a heterozygote in the population; Widrlechner et al. 1992)] derived from the mating of selected C_2 plants (i.e., plant nos. 4, 6, 8, 15, and 18). Marker and not allelic frequencies are reported since it is not possible to ascribe allelic frequency to dominant marker loci. The marker constitution of the parents [lines H-19 and Gy-7 (synom. G421); hereafter designated as H and G, respectively] used for mapping metric traits (QTL) by Serquen et al. (1997a) and Fazio et al. (2003a) were employed as the basis for comparative analysis of ΔG between selected populations (Tables 1, 2). Marker frequencies were calculated as the proportion of individuals having the same marker phenotype as that of G. Marker frequencies of BC_1 progeny were obtained from the marker phenotypes of the F_1 individual and the recurrent parent used in backcrossing. The four original parents (i.e., differing in genetic constitution of G and H alleles at the QTL under selection) contributing to C_0 were intermated to recombine favorable alleles to achieve an optimum ideotype (described above), and thus resulting populations (C_1–C_3, and C_2-derived F_1 hybrid and BC progeny) possessed selection-dependent allelic frequencies (i.e., G and H). Significant directional shifts in marker frequency are defined herein as either positive (toward the expected or desired frequency; Table 5) or negative (away from the expected frequency). The ideal type (ideotype) possesses the desirable H or G marker phenotype at all the marker loci examined.

Results

There were significant differences ($P = 0.05$–0.001) detected among cycles of PHE selection (C_0–C_3) for all traits, among cycles of MAS (F_1, BC_1) for GYN, MLB, and L:D, and between PHE selection and MAS for MLB and L:D (data not presented). Interactions were detected for MLB between families developed through MAS backcrossing of plant nos. 8 and 15 derived over selection cycles. Numerous single degree of freedom contrasts were significant ($P = 0.05$–0.01), most notably C_0 versus C_3 (GYN, MLB, and L:D), C_3 versus $BC_{1,8}$ (DTF, GYN, MLB, and fruit number), and C_3 versus $BC_{1,15}$ (DTF and L:D).

Genetic gain from PHE selection and MAS

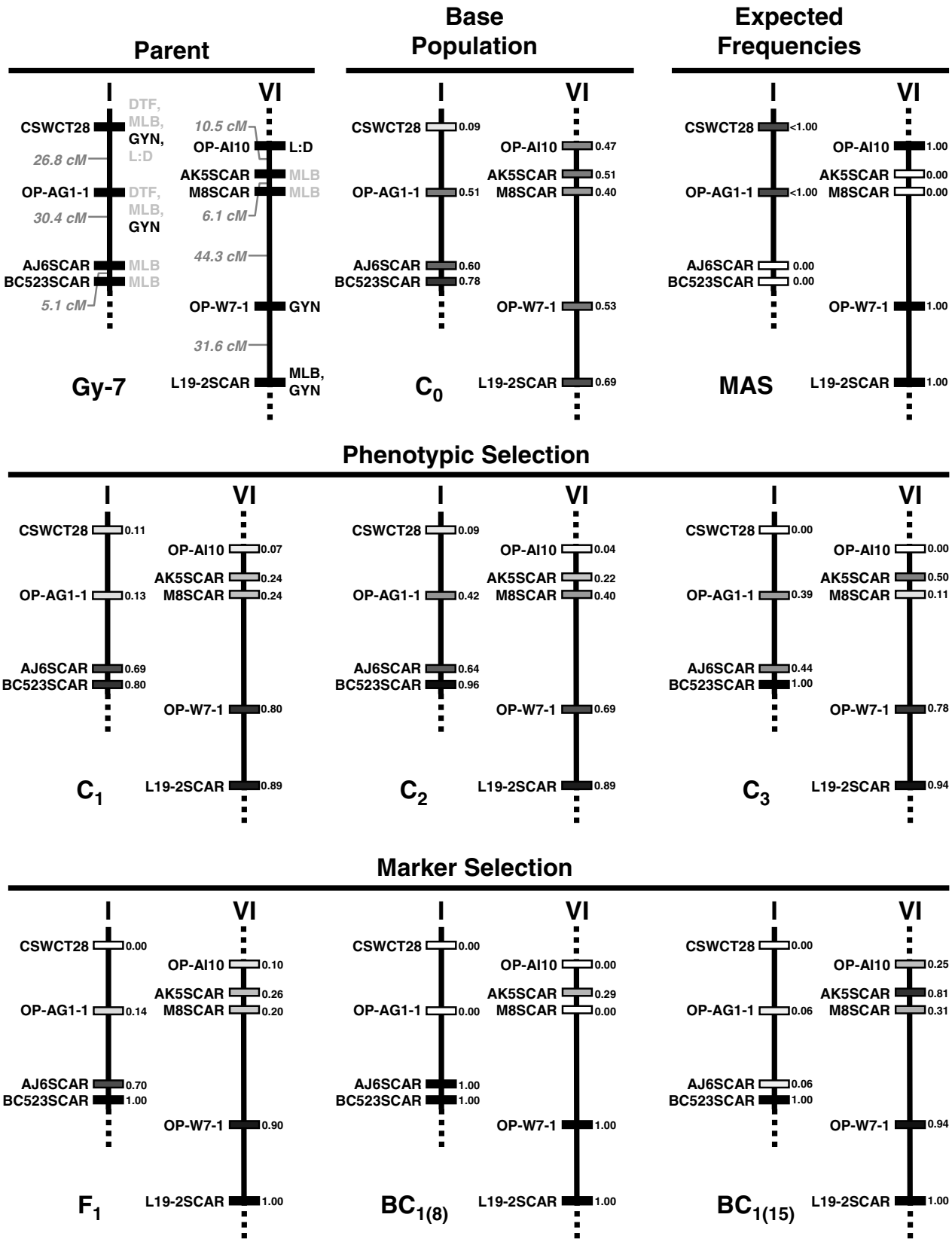
A positive response to three cycles of PHE selection was detected for MLB and L:D (Table 3). While average gain for MLB was about 0.3 branches per cycle of selection, average gain from selection for L:D was approximately 0.1 units. Concomitant gain in fruit number (not directly selected for) was not detected.

Fig. 2 Graphical representation of marker frequencies during PHE selection and MAS in cucumber. Markers (names on the left side of the linkage groups) from portions of Linkage Groups I and VI from Fazio et al (2003a) are shown (*dotted lines* on linkage groups indicate where linkage groups continue). Genetic distances (cM) between markers are shown in *gray italics* on the left side of the linkage groups in the Gy-7 panel. The QTL (*DTF* earliness; *MLB* multiple lateral branching; *GYN* gynoecious; *L:D* length to diameter ratio) associated with each marker are shown on the right of the linkage groups in the Gy-7 panel (QTL from the Gy-7 parent and H-19 parent are in black and gray text, respectively). The shading of each marker (a continuum from *white* = 0.00 to *black* = 1.00) depicts the frequency of the Gy-7 marker phenotype in the population (the value is also given to the immediate right of each marker). The top portion of the figure contains explanatory notes on the linkage groups of Gy-7, one of the original parents (H-19 not shown) used in map construction (Fazio et al. 2003a), and marker frequencies of the base population before PHE selection (C_0), and the expected frequencies after selection by molecular markers (MAS). The remaining panels depict marker frequencies in populations that underwent PHE selection (C_1–C_3) and selection by markers (F_1, $BC_{1,8}$, and $BC_{1,15}$). The marker frequencies of the F_1 panel are averaged over all hybrid crosses

Significant differences ($P = 0.05$) were detected in response to MAS between the two backcross populations examined. Therefore, results are presented separately for populations derived from recurrent parent plant nos. 8 and 15 (Table 4). A positive response to selection was detected for gynoecy, MLB, and L:D when populations derived from backcrossing to recurrent plant no. 8 (F_1 and BC_1) were assessed. Examination of populations derived from recurrent plant no. 15 (F_1 and BC_1) indicated that a positive response to MAS occurred only for gynoecy and L:D. These positive responses were more dramatic when these MAS populations (i.e., F_1 derived from unique C_{2S} selections, BC_1 derived from F_1 progeny and two C_2 selections used as recurrent parents) were compared to C_0, C_1, and C_3 (i.e., derived from all C_2 selections). Although an increase in mean fruit number was not observed in PHE or MAS derived populations alone, significant ($P < 0.05$) increases in fruit number and other associated yield components (i.e., GYN, C_0 vs. $BC_{1,8}$; MLB, C_0 vs. $BC_{1,8}$ and C_0 vs. $BC_{1,15}$; L:D, C_0 vs. $BC_{1,8}$ and C_0 vs. $BC_{1,15}$) were detected after a combination of PHE selection and MAS, when compared to C_0 (data not presented).

Marker frequency changes during PHE selection and MAS

Marker frequency changes were detected at some of the marker loci associated with yield components after PHE (Table 5; Fig. 2) and MAS (Table 6; Fig. 2) selection. In several instances (e.g., phenotypic selection; Table 5; OP-AG1-1, AW14SCAR, CSWTAAA01, M8SCAR, and BC515) fluctuations in marker frequency (both increases and decreases) were detected over cycles of selection, but these were not adequately modeled by either linear or quadratic regression. Nevertheless,



higher mean frequencies of favorable marker phenotypes were detected in C_3 for OP-AG1-1, AW14SCAR, and M8SCAR than in C_0. Significant ($P \leq 0.05$) negative responses (i.e., opposite of expected direction) to PHE selection were detected at CSWCT28 (linked to DTF, MLB, GYN, L:D), BC523SCAR (MLB), and

Table 6 Significant shifts in the frequency of markers associated with three yield components in cucumber (*Cucumis sativus* L.) in response to marker-assisted backcross selection using recurrent parent nos. 8 and 15

Trait	Marker[e]	Mean marker frequency [f]			Regression analysis for response to selection		
		C_3 [g]	F_1	BC_1	Linear model equation	R^2	P value
Recurrent parent no. 8							
Gynoecy (%)[a]							
Marker frequency	OP-AI4	0.88	1	1	$y=0.038x+0.727$	0.56	0.008
	AW14SCAR	0.89	1	1	$y=0.036x+0.789$	0.56	0.008
	OP-AO12	0.83	0.92	1	$y=0.083x+0.050$	0.99	0.001
	OP-W7-1	0.78	0.89	1	$y=0.11x+0.333$	0.44	0.025
Trait mean[b]		82.0^1	94.9^2	97.3^2			
Lateral branch number[c]							
Marker frequency	AJ6SCAR	0.44	1	1	$y=0.182x-0.059$	0.56	0.008
	M8SCAR	0.11	0.06	0.00	$y=-0.056x+0.333$	0.44	0.026
Trait mean[b]		2.20^1	2.54^2	2.78^3			
Fruit L:D[d]							
Marker frequency	BC515	0.17	0.11	0	$y=-0.093x+0.561$	0.55	0.009
Trait mean[b]		2.92^1	$2.98^{1,2}$	3.07^2			
Recurrent parent no. 15							
Gynoecy (%)[a]							
Marker frequency	OP-AI4	0.88	1	1	$y=0.037x+0.780$	0.56	0.005
	AW14SCAR	0.89	1	1	$y=0.036x+0.791$	0.56	0.005
Trait mean[b]		82.0^2	66.5^1	89.1^2			
Fruit L:D[d]							
Marker frequency	BC515	0.17	0.25	0	$y=-0.143x+0.873$	0.37	0.036
	L19-1SCAR	0.28	0.08	0	$y=-0.119x+0.710$	0.60	0.003
Trait means[b]		2.92^1	3.24^2	3.27^2			

[a]Percentage of pistillate flowers in the first 10 nodes on the main stem
[b]Mean separation within rows at $P=0.05$ according to Waller–Duncan's K-ratio test, $K=100$ (values with the same superscript number are not significantly different)
[c]Primary branches in the first 10 nodes
[d]L:D of 5–10 randomly chosen fruit (USDA 2B-3A grade; 25–30 mm in diameter) at each of four harvests
[e]Marker type, map location, and association with yield components given in Table 2. according to Fazio et al. (2003a)
[f]Frequencies of the Gy-7 (synom. G421) marker phenotype linked to selected traits (Table 2) according to QTL mapping by Fazio et al. (2003a).
[g]C_3 = the mean of the population after three cycles of PHE selection. derived from mass selection for four traits after the original intercrossing of four inbred lines (Table 1). The value of the F_1 is the average cumulative frequency of progeny derived from the mating of four sets of crosses, and the BC_1 is the average value of two populations derived from F_1 selections

OP-AI10 (L:D), and significant positive responses during selection were identified for AJ6SCAR (MLB), OP-A14 (GYN), OP-AO12 (GYN), OP-W7-1 (GYN), L19-1SCAR (L:D), and L19-2SCAR (MLB, GYN) (Table 5). Although the frequency of the undesirable G phenotype at AK5SCAR (MLB) initially decreased (C_0–C_2), there was no absolute change in frequency detected between C_0 and C_3. Similarly, positive (expected direction) and significant ($P<0.05$) changes in marker frequency were detected during backcross introgression at marker loci associated with gynoecy (OP-AI4, AW14SCAR, OP-AO12, and OP-W7-1), lateral branch number (AJ6SCAR and M8SCAR), and L:D (BC515 and L19-1SCAR) after MAS (Table 6).

Trait correlations during PHE selection and MAS

Correlation coefficients were low (0.02–0.17) to moderate (0.34–0.56) between the pairs of characters examined (data not presented). After three cycles of PHE selection (C_3), significant correlations were detected between lateral branch number and gynoecy (−0.51, $P=0.05$), and L:D and lateral branch number (0.47, $P=0.05$). Likewise, significant correlations were detected between gynoecy and L:D (−0.56, $P=0.01$), gynoecy and fruit number (0.40, $P=0.01$), and lateral branch number and fruit number (0.34, $P=0.05$) among MAS progeny taken collectively. When traits from MAS cycles (F_1 and BC_1) among backcross progeny derived from recurrent parent plant no. 8 were compared, correlations were detected between gynoecy and fruit number (0.31, $P=0.05$) and fruit number and lateral branch number (0.51, $P=0.001$). Similarly, comparisons among backcross progeny obtained from recurrent parent plant no. 15 indicated that significant correlations exited between gynoecy and lateral branch number (−0.54, $P=0.001$), L:D and lateral branch number (0.36, $P=0.01$), and fruit number and lateral branch number (0.38; $P=0.01$).

Discussion

The practical application of MAS can only be justified when predicted benefits (long- or short-term gain from selection) outweigh the additional cost of MAS above

traditional breeding methodologies (Gu et al. 1995). MAS has been found to be more (Yousef and Juvik 2001, 2002; Fazio et al. 2003b), equivalent (Wilcox et al. 2002), or less (Hoeck et al. 2003; Lu et al. 2003) efficient and/or effective for increasing gain from selection when compared to PHE selection in various plant species.

In crops such as cucumber (*C. sativus* L.) with a low chromosome number ($n = 7$), a small genome (genetic map length = ~750–1,000 cM; DNA content approaching *Arabadopsis thaliana* (L.) Heynh.; Staub and Meglic 1993), a rapid life cycle (four cycles per year), and many economically important, simply inherited traits, MAS could be a valuable tool for crop improvement. Nevertheless, the narrow genetic base of this species may preclude the rigorous application of MAS, and the implementation of MAS for multi-trait selection requires careful examination. We used previously characterized markers linked to QTL associated with yield components in cucumber (Serquen et al. 1997a; Fazio et al. 2003a), and provide herein the first report of successful multi-trait MAS for QTL associated with plant architecture in a vegetable crop species through backcross introgression.

Genetic gain from PHE selection in this cucumber population was detected for only lateral branch number and L:D ratio (Table 3). Concomitantly, significant positive (with respect to preferred ideotype) shifts in marker frequencies for gynoecy (OP-AI4, OP-AO12, OP-W7-1, and L19-2SCAR), fruit length (L19-1SCAR), and MLB (L19-2SCAR, AJ6SCAR), as well as significant negative responses [i.e., CSWCT28 (DTF, MLB, GYN, and L:D), OP-AI10 (L:D) and BC523SCAR (MLB)] were identified (Table 5; Fig. 2). However, such positive shifts were not universal, and MAS (i.e., C_2–F_1, F_1–BC_1) for favorable phenotypes at some marker loci for these traits did not result in gain from selection (Fig. 2).

The positive responses to PHE selection and associated marker frequency changes might have been predicted if PHE selection was imposed on traits which are controlled by relatively few genes with additive effects. Gynoecy in cucumber is controlled by a major locus, *F* (Linkage Group 1; $R^2 = 68$–74%), whose action can be influenced by at least five modifying genes (Serquen et al. 1997a). GYN sex expression can be dramatically altered by biotic and abiotic stress (Cantliffe 1981), where modifiers act to increase the number of staminate flowers on plants (Serquen et al. 1997a, b; Fazio et al. 2003b). Alleles for female sex expression were contributed by parental lines 6996A and 6995C at OP-AO12 (Linkage Group 5), all but 6995C at OP-AI4 (Linkage Group 5), 6632E and 6995C at OP-W7-1 (Linkage Group 6), and 6632E, 6823B and 6995C at L19-2SCAR (Linkage Group 6). These operationally important sex expression QTL (LOD > 3.0) are conditioned not only by additive and dominant, but also to some extent by epistatic genetic factors (Serquen et al. 1997b; Fazio 2001). These loci contribute relatively small effects to sex expresssion ($R^2 = 2$–10% for each QTL). It is clear that PHE selection for gynoecy in this population favorably changed marker frequencies at four of the seven marker loci associated with this trait without increasing femaleness. The fact that other modifiers having significant, but relatively small effects on GYN sex expression exist on Linkage Groups 1, 3, and 4 (Serquen et al. 1997b) suggests that fixation of alleles for gynoecy at these loci, along with the four identified herein, are required to realize gain from selection for this trait. Moreover, it is clear that environment is a significant contributing factor in sex expression and that response to selection for gynoecy is environmentally dependent.

MLB is controlled by as few as four genes whose effects are primarily additive (Serquen et al. 1997a; Fazio et al. 2003a). In contrast to gynoecy, QTL associated with MLB were contributed primarily by 6823B and 6995C, and PHE selection for MLB resulted in a dramatic response (~1.5 branch increase C_0–C_3) (Tables 1, 2). Moreover, as with gynoecy, PHE selection for this trait resulted in allelic changes in two distinct genomic regions. The marker loci CSWCT28, AJ6SCAR, and BC523SCAR on Linkage Group 1, as well as AK5SCAR and L19-2SCAR on Linkage Group 6 that exhibited changes in marker frequencies during PHE selection are associated with QTL for high branch number (Table 1).

Our data indicate that changes in frequency at marker loci brought about by PHE selection can be complex (Table 3). The expected frequency of the G marker phenotype of CSWCT28 after PHE selection is close to 1.00 (Table 5) since CSWCT28 is closely linked (5.0 cM) to the *F* locus (from G), the major locus controlling sex expression. By C_3, however, the H marker phenotype of CSWCT28 was fixed, despite a fairly high level of gynoecy (82.0%). Although AJ6SCAR and BC523SCAR are located in close proximity to each other (~5 cM) and their frequencies were both expected to decrease during selection, the frequency of the former decreased while that of the latter increased. Selection complexity was also allied with a genomic region on Linkage Group 6 containing three linked markers (OP-AI10-*10.5 cM*-AK5SCAR-*6.1 cM*-M8SCAR; Table 1). PHE selection resulted in a positive change in frequency at M8SCAR but not AK5SCAR even though these markers flank a QTL for MLB. In contrast, a negative change was realized in the frequency of OP-AI10, which is associated with a QTL for L:D (LOD = 7.3). The most probable explanation for this disparity in response during PHE selection is that linkages between such markers (i.e., those linked to traits [CSWCT28 to *F*] or to each other [AJ6SCAR to BC532SCAR]) were broken as a result of recombination.

The strength and direction of the correlations between yield-related traits in cucumber have been documented in a wide range of genetic backgrounds (Kupper and Staub 1988; Serquen et al. 1997b; Fazio et al. 2003a). A consideration of these associations, as exemplified in the three-locus linkage block given above, is integral to managing MAS during crop improvement.

Likewise, the heritability of traits under selection is also an important consideration for the optimal deployment of MAS. The narrow-sense heritabilities for yield component traits in our population range from 0.14 (sex expression modifiers) to 0.48 (number of lateral branches), depending on genetic background and growing environment (Serquen et al. 1997b; Fazio et al. 2003a). It is clear that physiological factors, genetic variance, and the genomic location of QTL associated with these yield-related traits are important for the creation and management of selection strategies in cucumber.

Negative correlations exist between fruit L:D and number in cucumber (Kupper and Staub 1988; Fazio et al. 2003a). In our study, both positive (L19-1SCAR; LOD 4.2; $R^2 = 5.4\%$) and negative (OP-AI10; LOD 7.3; $R^2 = 6.3\%$) changes in marker frequency during PHE selection were detected at marker loci associated with L:D (Table 5) (Serquen et al. 1997a; Fazio et al. 2003b). It is likely that the negative correlation between these traits precluded genetic gain for one of these traits during PHE selection in this population.

Response to MAS differed in the two backcross cucumber populations derived from recurrent parent nos. 8 and 15 (Table 4). When mean trait values were compared over selection generations (C_3, F_1, and BC_1), positive responses to selection were detected for gynoecy and fruit L:D in both populations. Marker frequencies at OP-AO12 ($C_3 = 0.83$) and L19-2SCAR ($C_3 = 0.94$) (both associated with gynoecy) moved towards fixation during PHE selection (Table 5; Fig. 2). However, GYN sex expression increased dramatically during backcrossing when compared to PHE selection ($BC_{1,8}$ from 82 to 97%; $BC_{1,15}$ from 82 to 89%), indicating that MAS for increased gynoecy at OP-A14, AW14SCAR, OP-AO12, and OP-W7-1 enhanced population characteristics (Table 6). Although allelic frequency shifts can result from genetic drift alone, this is likely not the case in this study. Fazio et al. (2003b), in fact, reported that genetic drift was not a significant factor contributing to gain from selection during backcross introgression of MLB in populations derived from Gy-7 and H-19.

The dramatic increase in the frequency of favorable marker phenotypes (i.e., for increased gynoecy) at these loci during MAS with a concomitant increase in the level of gynoecy indicates that MAS can be more effective for increasing the level of gynoecy than mass PHE selection when selecting for multiple traits in this population. Moreover, given the fact that three to four reproductive cycles are possible in cucumber per year and that the identification of sex stable GYN plants is difficult due to large genotype by environment interactions (Cantliffe 1981), MAS has potential for increasing gynoecy when compared to PHE selection. This potential for increased efficiency and effectiveness is advantageous for once-over machine harvest hybrid breeding programs where the release of sex stable GYN cultivars is critical.

An emphasis in some cucumber breeding programs has been to increase yield while maintaining commercially acceptable fruit size. Achievement of such objectives depends, in large part, on trait heritabilities and source/sink relationships (Staub 1989; Cramer and Wehner 2000). Heritabilities (broad sense; H^2_B) for lateral branching typically range between 0.33 and 0.60 in germplasm of a similar genetic background as that examined herein (Serquen et al. 1997b). However, heritabilities for L:D ($H^2_B = 0.09$–0.11) and fruit number ($H^2_B = 0.00$–0.08) are remarkably low. Therefore, the simultaneous positive response to PHE selection and MAS of lateral branching and fruit L:D in $BC_{1,8}$ is encouraging. Both selection methods realized the same gain per generation of selection (i.e., ~0.3 and 0.1 units per cycle for branch number and L:D, respectively). For PHE selection, changes in marker frequency were detected at five loci (CSWCT28, L19-2SCAR, BC523SCAR, AJ6SCAR, and AK5SCAR) associated with lateral branch number. An additional response to MAS for MLB and gynoecy was simultaneously characterized by positive directional changes in the frequency of M8SCAR and AJ6SCAR, and OP-A14, AW14SCAR, OP-AO12, and OP-W7-1, respectively (Table 6). Likewise, gain from PHE selection for L:D was augmented by positive directional changes at BC515 and L19-1SCAR. Thus, MAS operated to fix favorable alleles that were not exploited by PHE selection in this population.

Given that the QTL-trait associations described herein are consistent over environments (Serquen et al. 1997b; Fazio et al. 2003) and across populations (F_3 and BC_1; Dijkhuizen and Staub 2003), it is likely that MAS for gynoecy, lateral branching, and fruit L:D could be broadly applied in cucumber breeding programs for altering plant architecture. There were, in fact, MAS backcross derived lines—(6×15)×15, (6×8)×8, and (18×15)×15—recovered in this study that were early flowering (39 DTF), GYN, highly branched (3.1 branches) with long fruit (L:D = ~3.2) and higher yielding (~2.7 fruit per plant per harvest) than the monoecious commercial standard 'Vlasset' (46 DTF; 2.4 fruit per plant per harvest; L:D = ~2.6). Nevertheless, given the complex genetic and environmental interactions governing the expression of these traits, further progress for refinement of these lines will likely require their rigorous evaluation in multiple environments.

The severe economic challenges that currently face plant-breeding programs have prompted broader searches for ways to increase breeding efficiency and effectiveness. In vegetable crop species, marker-assisted backcrossing has most recently been used to introgress wild chromosomal regions (*Lactuca saligna* L.) into cultivated lettuce (*L. sativa* L.) (Jeuken and Lindout 2004), as well as for the improvement of sweet corn (Yousef and Juvik 2002), pepper (Thabuis et al. 2004), and fruit quality traits (Lecomte et al. 2004) and early blight resistance (Foolad et al. 2002) in tomato (*Solanum lycopersicum* L.). The confirmation of marker-QTL associations by this study and their successful use in changing the plant architecture of cucumber through backcross MAS (three cycles per year) is indicative of its potential benefits for

cucumber breeding. Nevertheless, although MAS has proven effective for selection of architectural traits in cucumber, the strategic use of both PHE selection and MAS will likely enhance breeding strategies, as evidenced by our finding that yield increased only after PHE then MAS. It may now be possible, for instance, to effectively use MAS and PHE selection for altering cucumber plant architecture and fruit quality while using MAS to incorporate single gene disease resistance such as down mildew (causal agent: *Pseudoperonospora cubensis* (Berk. & Curt) Rostow) for which marker-trait associations are also known (Horesji et al. 2000).

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