



## Segregation for performance in recombinant inbred populations resulting from inter-gene pool crosses of common bean (*Phaseolus vulgaris* L.)

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### Summary

The germplasm of cultivated common bean exhibits a lower level of genetic diversity within each geographical gene pools (Mesoamerican and Andean) compared to that of the respective wild ancestors. Crosses between these two gene pools potentially provide a source of additional genetic diversity but their progenies have been characterized by phenotypic abnormalities and reduced productivity. In order to gain additional insights into this problem, we examined the segregation for performance in two recombinant inbred populations (RIPs) resulting from Mesoamerican × Andean crosses in three contrasting environments and two years. The two RIPs – ‘California Dark Red Kidney’ (of Andean origin) × ‘Yolano’ (Mesoamerican),  $n = 150$ , and A55 (Mesoamerican) × G122 (Andean),  $n = 67$  – were grown in replicated field tests to assess the agronomic performance of each recombinant inbred line. Both populations exhibited, on average, greater days to maturity (DTM), lower biomass growth rate (above-ground dry weight/DTM), lower economic growth rate (seed yield/DTM), and lesser harvest index. In contrast with the conclusions of earlier experiments, there was no evidence from the field trials for a genetic association (due to linkage or pleiotropy) between seed weight and economic growth rate, but there may be a genetic association between seed weight and life cycle length. We compare the results of these studies with earlier experiments on inter-gene pool recombinant populations of common beans and relate our observations of diminished performance to models of speciation mechanisms.

### Introduction

The majority of cultivated common bean (*Phaseolus vulgaris* L.) genotypes can be easily separated into two distinct gene pools, Andean and Mesoamerican, whose origins predate domestication. Evidence for the existence of these two pools is based on the association of the geographical distribution of genotypes with similar morphological traits, seed storage proteins, isozyme markers, phaseolin seed protein sequences, RFLP markers for single-copy and M13-related sequences, and RAPD markers (reviewed in Gepts, 1993; Kami & Gepts, 1994; Sonnante et al., 1994; Freyre et al., 1996). In addition, the progeny from crosses between these gene pools may suffer from a number of phenotypic abnormalities, beginning with

F<sub>1</sub> hybrid inviability in some genotypes (Shii et al., 1980; Singh & Gutiérrez, 1984; Gepts & Bliss, 1985). In the F<sub>2</sub> and later generations additional abnormal segregants appear, including crippled seedlings exhibiting virus-like symptoms and/or variegated leaves (Singh & Molina, 1996), partial to complete male sterility (Sprecher & Khairallah, 1989), and diminished yield potential (Patiño & Singh, 1989; Singh et al., 1989).

The genetic bottlenecks of at least two independent domestications have limited genetic diversity within the cultivated common bean gene pools relative to their wild progenitors and to each other (Koenig & Gepts, 1989; Singh et al., 1991b; Sonnante et al., 1994). The narrow base of germplasm within gene pools, and particularly within races (Singh et

al., 1991a), suggests that further progress in breeding for quantitative traits such as seed yield (Gepts, 1998) will be limited by a lack of genetic variation. The availability of genetic variation from inter-gene pool hybridizations presents a potential opportunity to breeders of common bean. It may be possible to recombine the desirable traits of each of the gene pools, such as the greater yield capacity of many Mesoamerican derived genotypes with the larger seed size of Andean genotypes, into improved cultivars. Additionally, broadening the genetic base of cultivated common bean may provide opportunities for improved resistance to biotic and abiotic stress factors that limit productivity (Guzmán et al., 1995).

However, despite the opportunities that inter-gene pool recombinants provide for increased levels of genetic variation, dry common bean breeders have been less successful at recovering useful recombinations from inter-gene pool crosses than from intra-gene pool crosses (Singh et al., 1989; Singh, 1995; Welsh et al., 1995). Factors that have discouraged inter-gene pool recombination, in addition to the frequent occurrence of  $F_1$  hybrid inviability, include the difficulty of recovering the agronomic and seed characteristics of accepted cultivars and custom.

Nevertheless, breeders of dry common beans have recently placed increased attention on developing elite germplasm from inter-gene pool cross (Kornegay et al., 1992; Beaver & Kelly, 1994; Singh & Urrea, 1995). Kornegay et al. (1992) suggested, based on poor performance in the progeny of inter-gene pool crosses, that 'the genetic differences may be too divergent, and when crossed a disruption occurs in the genic balance'. Singh & Urrea (1995) also found that the progeny of inter-gene pool crosses performed poorly compared to their parents. However, Beaver & Kelly (1994) successfully employed recurrent selection strategies to improve the yield potential of large-seeded Andean derived genotypes using inter-gene pool crosses. The success of Beaver & Kelly (1994) and snap bean breeders (Weeden, 1984; Skroch & Nienhuis, 1995) suggests that inter-gene pool hybridization could be a valuable resource for dry bean breeders.

We report on the agronomic characterization of two recombinant inbred populations (RIPs) derived from inter-gene pool crosses in which selection during inbreeding generations was minimized. We use the data in an attempt to characterize 1) the segregation of performance in the progeny of crosses between the two major common bean gene pools, and 2) the

negative associations between seed weight and yield potential. Data presented here constitute a first step towards a future quantitative trait locus (QTL) analysis of performance in these wide crosses.

## Materials and methods

### *Population development*

The California Dark Red Kidney  $\times$  Yolano (CY) RIP was developed from crosses made in a greenhouse in Davis, CA, between the major California cultivars California Dark Red Kidney (CDRK; Andean gene pool, race Nueva Granada) and Yolano (Mesoamerican gene pool, race Durango).  $F_1$  hybrid inviability was not observed in this cross. In the  $F_3$  and  $F_4$  generations, two seeds of each line were planted in a single pot, and one plant was thinned at anthesis. In cases where both seeds failed to germinate or the remaining plant failed to set seed the lines were considered lost. Cross-pollination of the progeny was avoided by growing the populations in the greenhouse. In the  $F_5$  generation three seeds of each line were planted, and seed for the field experiments were bulked to produce  $F_{4:6}$  families. For lines with insufficient seed an additional generation of seed increase was performed, so that the seed for field experiments was a mixture of  $F_{4:6}$  and  $F_{4:7}$  seed. Sufficient seed for 149 out of 151 extant CY recombinant inbred lines (RIL) was available for planting. Of the original 183  $F_2$  lines planted in this population, 32 (17%) were lost due to inviability or sterility during the inbreeding generations.

The A55  $\times$  G122 (AG) RIP was developed in a greenhouse by Steve Magnusson of Harris Moran Seed Company (San Juan Bautista, CA) using single seed descent from the  $F_2$  through the  $F_6$  generations from a cross between the breeding line A55 (a Mesoamerican gene pool, race Mesoamerica, lowland tropical black bean breeding with improved disease resistance characters) and the Andean-derived cultivar G122 (also known as Jatu Rong), an early maturing Andean derived cultivar of race Nueva Granada noted for heat tolerance under California conditions (Shonard & Gepts, 1994). Sufficient  $F_{7:8}$  seed for 58 of the 67 RILs was available for field analyses in 1994. All 67  $F_{7:9}$  lines were included in the 1995 evaluation of this populations. Of the original 77  $F_2$  lines planted in this population, 10 (13%) were lost due to inviability or sterility during the inbreeding generations.

Table 1. Summary of environments assayed and trait data collected

Population	Year	Davis	Salinas	Westside
AG	1994	DTM, SWT, YIE <sup>a</sup>	DTM, SWT, YIE	DTM, SWT, YIE
	1995	ADW, DTM, YIE	ADW, DTM, YIE	–
CY	1995	ADW, DTM, SWT, YIE	ADW, DTM, SWT, YIE	–

<sup>a</sup> ADW, aerial dry weight; DTM, number of days to maturity; YIE, dry seed produced; SWT, 100-seed weight.

### Field trials

The AG population was evaluated in three contrasting field locations: the University of California at Davis, the USDA field station at Salinas CA, and the University of California's Westside Research and Extension Center near Five Points CA. All three locations were used in 1994, and the Davis and Salinas trials were repeated in 1995 (Table 1). The CY population was evaluated in trials at Davis and Salinas in 1995 (Table 1). Salinas represents a cool, dry coastal climate near the Monterey Bay with average July temperature of 19 °C and a Chualar sandy loam (Typic Argixeroll) soil type. Davis, near the mouth of the Sacramento Valley, has a warm summer climate with average July temperature of 26 °C and a Yolo loam (Mollic Xerofluent) soil type. Westside is located in the San Joaquin Valley and has hot summers with an average July temperature of 31 °C and a Panoche clay loam (Thermic Typic Torriorthent) soil type. Although the CY population was planted in a single year, the climatic differences between the two sites (Davis and Salinas) are larger than any year-to-year location that might occur within these two locations. Hence, a comparison of the results in the two locations is an adequate evaluation of environmental effects on QTL expression.

The RIPs were replicated three times in each trial in a randomized complete block design. Ten seeds for each RIL were space planted per 1 m plot, with a two plant-in-row border between plots. The spacing between rows was 0.75 m at Davis and Westside and 0.70 m at Salinas. Standard agronomic practices were maintained at each site. A summary of field trials and trait data collected in each trial is provided in Table 1. Days to maturity (DTM) was measured as the number of days from planting (day 0) to the first day when half of the pods on half of the plants in the plot were dry. Plots were dried and threshed individually and the seeds cleaned and weighed. Replicates with less than 50% plant stand at maturity were not included in the analysis (87 out of 1860 total plots,

< 5%). Economic growth rate (EGR) was calculated as seed yield (YIE) divided by DTM. In 1995, we also measured above-ground dry weight by harvesting the entire plot (sheared at ground level, no attempt was made to collect fallen leaves) 5 to 10 days after maturity, thoroughly drying the plants (10 to 20 days at 50 °C), and weighing. Adjustments for differences in DTM were made by dividing the above-ground total dry weight (ADW) by DTM to obtain the biomass growth rate (BGR). This figure measures the ability of the plant to produce photoassimilate, which was assumed to be independent of reproductive fertility. Harvest Index (HI) was calculated as YIE/ADW.

Hundred-seed-weights (SWT) for the AG RIP were estimated by averaging the measured 100-seed-weights from each replicate and location of the 58 AG RILs grown in 1994, with extrapolation of data for replicates producing fewer than 100 seeds. For the CY population, 50 RILs from the RILs with data available from all three replications in both environments in 1995 and with non-zero yields in all replications were chosen at random. Average 100-seed-weight was measured for each of the replicates and averaged.

### Statistical analyses

Means over replications were used for correlation analyses of seed weight with other traits using Minitab. Analyses of variance were conducted for each population independently, considering locations, genotypes, and years as random treatments and were performed using SAS PROC GLM (SAS Institute, 1988).

### Results

Analyses of variance showed very highly significant effects ( $p < 0.0001$ ) of Location, Year, and Genotype, as well as first order interactions of these factors for all traits evaluated (data not shown) with the exception of the location  $\times$  year interactions for DTM in the AG

Table 2. Parental and progeny summary data for traits of interest

	SWT	DTM	YIE (g-plot <sup>-1</sup> )	EGR (g-plot <sup>-1</sup> ·day <sup>-1</sup> )	ADW (g-plot <sup>-1</sup> )	BGR (g-plot <sup>-1</sup> ·day <sup>-1</sup> )	HI
Davis 1994							
A55 avg	17	110	92 <sup>a</sup>	0.83	NA	NA	NA
G122 avg	38	89	281	3.15	NA	NA	NA
AG <sup>b</sup> RILs range low/high	16/39	90/142	4/303	0.03/2.67	NA	NA	NA
AG RILs avg	26	117	92	0.81	NA	NA	NA
Avg. std dev of AG RILs	1.50	5.7	32	0.29	NA	NA	NA
Salinas 1994							
A55 avg	25	127	298	2.35	NA	NA	NA
G122 avg	46	119	358	3.02	NA	NA	NA
AG RILs range low/high	24/60	109/153	20/348	0.14/2.99	NA	NA	NA
AG RILs avg	34	124	217	1.78	NA	NA	NA
Avg. std dev of AG RILs	1.82	4.2	35	0.29	NA	NA	NA
Westside 1994							
A55 avg	20	100	195	1.95	NA	NA	NA
G122 avg	35	95	243	2.56	NA	NA	NA
AG RILs range low/high	15/34	92/126	7/196	0.08/1.91	NA	NA	NA
AG RILs avg	23	107	89	0.85	NA	NA	NA
Avg. std dev of AG RILs	1.98	3.5	33	0.31	NA	NA	NA
Davis 1995							
CDRK avg	50	91	161	1.79	323	3.57	0.50
Yolano avg	36	88	227	2.59	506	5.77	0.45
CY <sup>a</sup> RILs range low/high	20/54	87/126	0/254	0.00/2.91	85/815	0.82/7.44	0.00/0.53
CY RILs avg	32	102	63	0.63	343	3.37	0.16
Avg. std dev of CY RILs	1.04	2.9	40	0.22	63	0.64	0.04
A55 avg	NA	99	127	1.28	539	5.44	0.23
G122 avg	NA	90	214	2.40	425	4.76	0.50
AG RILs range low/high	NA	93/123	1/197	0.01/2.12	178/603	1.52/5.91	0.00/0.45
AG RILs avg	NA	104	68	0.68	369	3.56	0.18
Avg. std dev of AG RILs	NA	1.1	18	0.18	48	0.48	0.04
Salinas 1995							
CDRK avg	66	103	310	3.00	565	5.46	0.56
Yolano avg	42	96	370	3.83	584	6.05	0.63
CY RILs range low/high	28/62	95/128	62/483	0.50/4.30	283/995	2.50/8.84	0.12/0.61
CY RILs avg	42	108	263	2.46	542	5.01	0.49
Avg. std dev of CY RILs	0.91	2.78	46	0.43	76	0.71	0.04
A55 avg	NA	110	332	3.01	590	5.36	0.56
G122 avg	NA	103	320	3.09	635	6.15	0.48
AG RILs range low/high	NA	98/123	35/358	0.29/3.26	310/718	2.54/6.69	0.11/0.61
AG RILs avg	NA	111	235	2.14	513	4.63	0.46
Avg. std dev of AG RILs	NA	2.46	35	0.32	36	0.55	0.04

DTM, number of days to maturity; BGR, biomass growth rate; HI, harvest index, EGR, economic growth rate; SWT, 100 seed weight; NA, not available.

<sup>a</sup> To convert YIE, EGR, ADW, and BGR data to results on a m<sup>2</sup> basis, multiply by 1.33 for Davis and Westside and 1.43 for Salinas.

<sup>b</sup> CDRK, California Dark Red Kidney; CY, California Dark Red Kidney × Yolano; AG, A55 × G122.

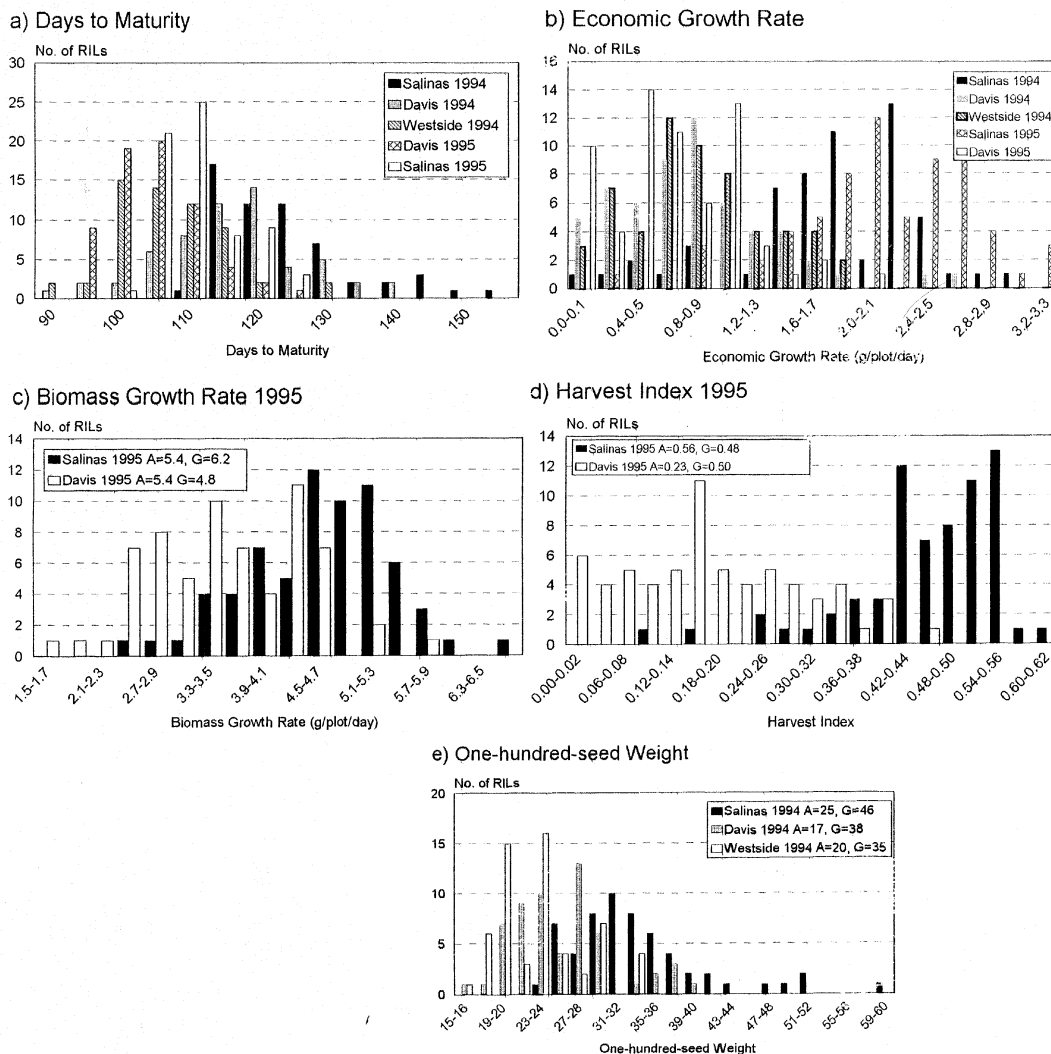


Figure 1. Trait distribution of A55 (A) × G122 (G) recombinant inbred population (AG) at Davis, Salinas, and Westside, CA in 1994 and at Davis and Salinas CA in 1995 (a) DTM: days to maturity; (b) EGR: economic growth rate ( $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ); (c) BGR: 1995 biomass growth rate ( $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ); (d) HI: 1995 harvest index; (e) SWT: 1994 100-seed-weight (g).

RIP which was only significant ( $p < 0.05$ ). Second order interactions (locations × years × genotypes) were very highly significant for DTM in the AG RIP but not significant for EGR. However, compared to the parental genotypes, the average trait values of the inter-gene pool recombinants were consistently higher for DTM, and lower for BGR, EGR, and HI in all environments tested (Table 2, Figures 1, 2). Average seed weight of the RILs, however, was below that of both parents only in the CY population (Table 2, Figures 1, 2). The data for the highest performing lines – those with an EGR RIP mean + 2 standard deviations in one or more locations – are presented in Tables 3 and 4.

#### Days to maturity (DTM)

Earliness is one of the traits of primary importance to dry bean producers because beans are often grown in short seasons or as a relay crop. Delayed maturity of the RILs was observed, such that the average number of days to maturity of the RIPs was higher than that of both parents (with the notable exception that the mean DTM of the AG RIP matched the later parent in Salinas in 1995) (Table 2). The developmental delay exhibited by the RILs was more pronounced in the CY RIP than in the AG RIP, and was more pronounced at Davis than Salinas. Although individual RILs matured earlier than both parents in most en-

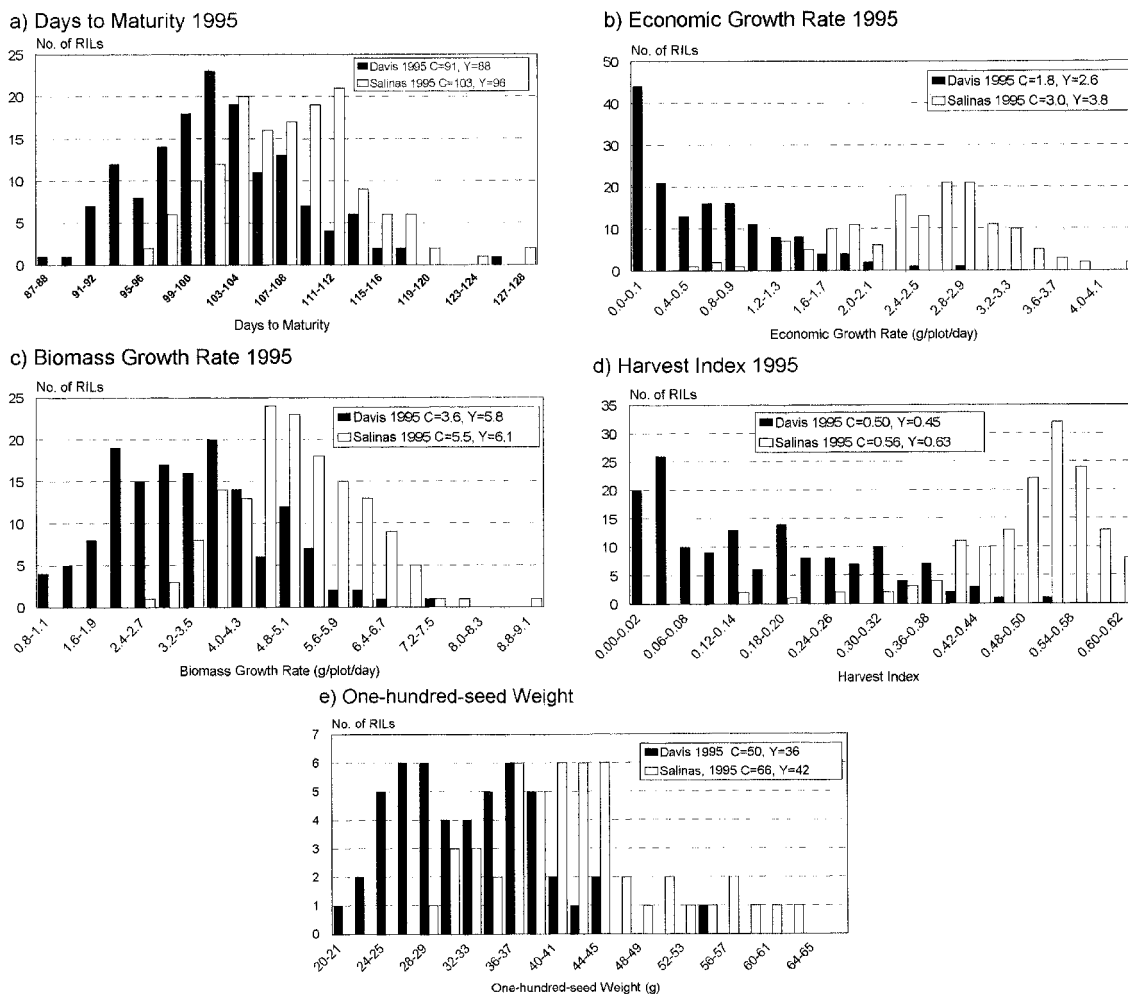


Figure 2. Trait distribution of California Dark Red Kidney (C)  $\times$  Yolano (Y) recombinant inbred population (CY) at Davis and Salinas CA in 1995 (a) DTM: days to maturity; (b) EGR: economic growth rate ( $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ); (c) BGR: 1995 biomass growth rate ( $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ); (d) HI: 1995 harvest index; (e) SWT: 1994 100-seed-weight (g).

vironments and in both populations (Figures 1a and 2a), when averaged across environments none of the RILs matured before the earlier parent in either population. The earliest maturing lines differed by location, though less so across years, suggesting that it may be possible to select for decreased DTM for specific environments (data not shown). Although the parents of both populations differed in growth habit, there was no significant difference (less than two days in all cases) in DTM between determinate and indeterminate types except in the AG population in 1994, where determinate genotypes matured on average 7 days earlier. We attribute this general lack of differences in DTM to the similar phenology of the parents, which suggests that

other maturity time genes distinguish them in addition to determinacy.

#### *Yield (YIE) – Economic growth rate (EGR)*

Common bean cultivars exhibit a positive correlation between yield and days to maturity that confounds the value of raw yield data. In order to estimate productivity per unit of time, we focused on the grain yield accumulation per day (EGR) to measure performance in the AG and CY populations. EGR and yield were significantly correlated in our experiments ( $r \geq 0.99$ ). Average EGR measurements for the RIPs were lower than both parents in both populations and in all locations and years (Table 2). The overall distribution of

Table 3. Comparison of best RIL performance to parental performance in the California Dark Red Kidney × Yolano population in 1995

Genotype	Davis			Salinas		
	EGR	BGR	HI	EGR	BGR	HI
CDRK	1.79 <sup>a</sup>	3.57	0.50	3.00	5.50	0.56
Yolano	2.59	5.77	0.45	3.70	6.10	0.61
CY14	1.44	3.92	0.17	3.82	7.12	0.54
CY28	1.96	4.98	0.39	2.83	5.86	0.49
CY36	2.01	5.65	0.36	2.98	5.62	0.53
CY50	2.91	5.46	0.53	3.65	6.02	0.60
CY60	1.18	5.29	0.22	4.30	8.81	0.49
CY62	2.47	6.13	0.40	3.00	5.29	0.57
CY67	0.08	2.68	0.03	4.16	7.76	0.54
CY82	1.93	6.00	0.32	3.60	6.88	0.52
CY89	0.75	4.01	0.19	3.84	7.30	0.53
CY146	0.97	4.66	0.20	3.61	6.03	0.60
CY160	1.88	4.35	0.43	2.85	4.77	0.60
RIL	0.63	3.37	0.16	2.47	5.01	0.49
RIL std dev	0.59	1.26	0.13	0.70	1.06	0.09

BGR, biomass growth rate in  $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ; EGR, economic growth rate in  $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ; HI, harvest index.

<sup>a</sup> To convert BGR and EGR data to results on a  $\text{m}^2$  basis, multiply by 1.33 for Davis and Westside and 1.43 for Salinas.

Table 4. Comparison of best RIL performance to parental performance in the A55 × G122 population

Genotype	1994						1995					
	Davis		Salinas		Westside		Davis			Salinas		
	SWT	EGR	SWT	EGR	SWT	EGR	EGR	BGR	HI	EGR	BGR	HI
A55	17.0	0.83 <sup>a</sup>	25.3	2.35	20.2	1.95	1.28	5.40	0.24	2.83	4.87	0.58
G122	37.5	3.15	45.7	3.02	34.5	2.56	2.40	4.80	0.50	3.10	5.71	0.52
AG8	23.3	0.69	29.3	2.19	23.7	1.64	0.65	4.20	0.15	2.53	4.49	0.56
AG25	27.7	0.58	34.0	2.79	28.5	0.94	0.48	3.30	0.14	2.26	4.85	0.45
AG38	26.7	1.63	31.0	1.77	19.7	0.43	0.28	2.96	0.09	1.97	4.44	0.44
AG49	23.3	1.42	28.0	1.89	23.7	1.84	2.12	4.75	0.45	2.89	5.21	0.55
AG54	34.7	2.45	35.0	0.66	32.7	1.91	1.56	4.55	0.34	1.30	4.78	0.27
AG63	22.0	0.29	31.0	2.99	25.0	0.87	0.53	3.86	0.14	3.19	6.69	0.48
AG65	NA	1.23	NA	NA	22.5	1.88	1.00	3.72	0.27	1.79	3.48	0.51
AG66	25.7	1.30	33.3	0.87	23.0	1.70	1.11	3.53	0.31	1.66	4.57	0.37
AG72	21.3	1.29	25.3	2.29	20.3	1.06	1.57	3.87	0.41	2.66	5.35	0.50
AG73	27.3	1.88	32.0	2.58	22.7	1.40	1.01	5.90	0.17	2.43	5.20	0.47
AG75	28.7	2.67	34.3	2.42	23.0	1.70	1.37	4.58	0.30	3.26	5.88	0.55
Best RIL avg	25.8	1.40	31.3	2.04	24.1	1.40	1.06	4.11	0.25	2.36	4.99	0.47
RIP Avg	25.6	0.81	33.7	1.78	23.4	0.85	0.68	3.56	0.18	2.14	4.63	0.46
St. Dev	5.24	0.53	7.37	0.61	4.92	0.48	0.44	0.87	0.11	0.61	0.80	0.10

BGR, biomass growth rate in  $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ; EGR, economic growth rate in  $\text{g}\cdot\text{plot}^{-1}\cdot\text{day}^{-1}$ ; HI, harvest index; SWT, 100-seed weight (g).

<sup>a</sup> To convert BGR and EGR data to results on a  $\text{m}^2$  basis, multiply by 1.33 for Davis and Westside and 1.43 for Salinas.

EGR was skewed towards smaller values (Figures 1b and 2b). Nevertheless, individual RILs outperformed both parents in the CY population at both locations, and in the AG population at Salinas only. None of the RILs, however, significantly outperformed the parents in both populations (Figures 1b and 2b).

#### *Above-ground dry weight – Biomass growth rate (BGR)*

Common bean cultivars exhibit a positive correlation between above-ground dry weight and days to maturity. In order to estimate biomass production per unit of time, we focused on the biomass accumulation per day (BGR) in the AG and CY populations. BGR and ADW showed high levels of correlation in our experiments ( $r \geq 0.98$ ). BGR values were below the parental values in most four population/location combinations analyzed, except for the CY RIP in Davis, where the distribution of progeny values for the trait was only slightly skewed below the parental values (Figures 1c and 2c).

#### *Harvest index (HI)*

HI measures the fraction of the photoassimilate partitioned to the seed. The production of mostly parthenocarpic pods was associated with a low harvest index ( $< 0.35$ ) in the RILs. The difference in harvest index between the parents and the average of the RIPs was notably skewed downward, particularly when measured in Davis (Table 2, Figures 1d and 2d). Compared to Salinas, the Davis environment is a harsher environment because of the high summer temperatures, which fall above the normal growing temperature range for common bean (Laing et al., 1984). A few exceptional genotypes, however, outperformed the parents for HI. In the AG population, one RIL (AG 77) exhibited a higher HI (0.61) than the parental genotypes (A55: 0.58; G122: 0.52) at Salinas, but apparently at the expense of vegetative growth (BGR and EGR values lower than RIP averages). In the CY population, however, one RIL (CY 50) exhibited a higher HI (0.57) than the parents (CDRK and Yolano: 0.53) when averaged across both locations while maintaining a high BGR (137% of RIP average) and EGR (212% of RIP average).

#### *100-seed-weight*

Most of the RIL seed weights were intermediate to the values of the two parents in the AG population

in all environments, but larger and smaller transgressive segregants were present (Figure 1e). In the CY population, however, the distribution of trait values was skewed toward lighter seeds (Figure 2e), though larger and smaller transgressive segregants were also observed at one location in this population.

#### *Correlation of SWT with DTM, BGR, HI, EGR*

Phenotypic correlation analyses were performed to determine if there were significant associations between SWT and the other quantitative traits measured in this study (Table 5). Few significant associations were observed between SWT and BGR, EGR, or HI for any site  $\times$  year  $\times$  population combination. Some significant, positive associations, however, were noted between SWT and DTM in several site  $\times$  year  $\times$  population combinations (Table 5).

## **Discussion**

Development of the recombinant inbred populations was hampered by the expected occurrence of phenotypically anomalous plants beginning in the F<sub>2</sub>. Plants exhibiting virus-like symptoms, variegation, and apparent sterility caused the loss of 30 out of 181 original CY F<sub>2</sub> seedlings. These characteristics resembled those described earlier work with inter-gene pool crosses (see Introduction) and are under genetic control (Singh & Molina, 1996). Two additional lines proved inviable, and we were unable to produce sufficient seed for a field analysis. Variegated, crippled, and apparently sterile segregants were also observed in the field at low frequencies.

Poor fertility was evidenced by downward skewing of progeny distributions for HI and large numbers of RILs exhibiting anomalous phenotypes (acute chlorosis, variegation and virus-like symptoms on leaves and parthenocarpic pods). This suggested that the low yield and late maturity of the RILs were due to factors affecting both vegetative and reproductive development. Comparisons of the distribution of parental and progeny values for the CY population when measured at Davis also supported this assertion (Figure 2, Table 2). The CY parental genotypes are well-adapted and extensively cultivated in the Central Valley of California. The similar levels of skewing for BGR, EGR, and HI in this population suggest that the factors which recombine poorly between the gene pools tend to be associated with both vegetative and reproductive growth.



Table 5. Phenotypic correlations of SWT with other traits<sup>a</sup>

Genotype	No. of lines	Location and year	DTM	EGR	BGR	HI
California Dark Red Kidney × Yolano						
1995 site-specific correlations	50	Salinas '95	0.06	0.20	0.06	0.19
		Davis '95	0.48**	-0.13	0.24	0.01
		Average '95	0.31*	-0.02	0.15	0.11
A55 × G122						
1994 site-specific correlations	58	Salinas '94	0.41**	-0.28*	NA <sup>b</sup>	NA
		Davis '94	0.09	0.15	NA	NA
		West Side '94	-0.01	0.19	NA	NA
		Average '94	0.23	-0.12	NA	NA
RILs with 1994 Salinas EGR > 0.50	54	Salinas '94	0.27*	-0.03	NA	NA
		Davis '94	0.04	-0.26	NA	NA
		West Side '94	-0.01	-0.12	NA	NA
		Average '94	0.13	0.07	NA	NA

<sup>a</sup> BGR, biological growth rate; DTM, number of days to maturity; EGR, economic growth rate; HI, harvest index; SWT, 100-seed weight.

<sup>b</sup> NA, not available.

\* Significant at the 0.05 level.

\*\* Significant at the 0.01 level.

Differences in seed weight are one of the morphological traits distinguishing the Andean and Mesoamerican gene pools (Singh et al., 1991a). Debouck et al. (1993) presented evidence that the larger seeded Andean common bean germplasm was better adapted to cooler, higher elevations than Mesoamerican germplasm. In certain environments, White & González (1990), White et al. (1992), and Sexton et al. (1994) found significant negative associations between the yield of common bean and its seed weight in certain environments with the small-seeded, Middle American materials achieving higher yields than their large-seeded, Andean counterparts. The negative association between SWT and productivity was not observed in the cooler environment tested by Sexton et al. (1994), and a significant positive association between yield and seed weight was observed in the coolest experimental environment of White & González (1990). All of these studies used non-segregating germplasm accessions and therefore it was not possible to determine whether the lower yields of the large-seeded accessions was an effect of large seed size *per se* or of other factors characteristic of the Andean gene pool. Our present results, based on the use of segregating populations in higher temperature environments, show that with the exception of the AG population in Salinas 1994, there was no apparent

relationship between EGR (and therefore yield) and SWT.

The significant negative association between SWT and EGR observed in the AG population in 1994 was apparently due to a small number of large seeded outliers with extremely low fertility and low harvest indices. These lines, because of the small number of seeds produced per plant, appeared to exhibit source-sink imbalances resulting in larger seeds and delayed maturity. Removal of these few lines resulted in the absence of significant associations between SWT and EGR (Table 5). The lack of significant correlation between SWT and EGR in our RIPs and the existence of RILs with very high values of SWT, HIX, and EGR (AG33 and CY22, CY52, and CY58) suggest that the associations between SWT and EGR observed by White et al. (1992) are not due to pleiotropy (i.e. a common physiological mechanism) or tight linkage between the two traits. These results substantiate the conclusion of Beaver and Kelly (1994) that it is possible to recombine the genes conditioning seed weight and yield.

Welsh et al. (1995) presented a study comparing the inbred progeny of intra- and inter-gene pool crosses. However, the authors used F<sub>2:6</sub> inbred lines, allowing ample opportunity for inadvertent selection of performance-related traits during the inbreeding generations. Nevertheless, they also found that for the

traits of yield and biomass, all three inter-gene pool RIPs had mean values lower than the lower parent. In contrast, the intra-gene pool RIPs had mean values intermediate between those of the two parents for yield, biomass, HI and DTM. The best performing line in their intra-gene pool population yielded 119% of the best parent, while out of three inter-gene pool populations only one produced a line that significantly outyielded (126%) the best parent.

The frequency of inter-gene pool recombination in dry common bean cultivars is in sharp contrast to more intensively bred snap bean cultivars. Although early snap bean cultivars were almost all uniformly the product of germplasm from the Andean gene pool, molecular marker based phenetic analyses typically do not group modern varieties as clearly within the Andean or Mesoamerican gene pools (Weeden, 1984; Skroch & Nienhuis, 1995). The intermediate nature between the Middle American and Andean gene pools may be due to introgression of disease resistance genes from the Middle American into snap beans. The identification of a line marginally superior (higher EGR) to the best parent in the CY RIP and significantly superior to the best parent in the Welsh et al. (1995) study suggests that inter-gene pool crosses may be useful for improving productivity of dry common bean.

Genetic distance has been positively associated with  $F_1$  heterosis in many crops, including common bean (Foolad & Bassiri, 1983; Ghaderi et al., 1984; Nienhuis & Singh, 1986), lima bean (Schoneweis & Erickson, 1989), rice (Xiao et al., 1996), faba bean (Link et al., 1996), mung bean (Ramanujam et al., 1974), winter canola (Ali et al., 1995), flax (Murty & Anand, 1965), and maize (Moll et al., 1965). However, at very high levels of genetic divergence 'genic unbalance in population hybrids... may be manifest by poor  $F_1$  viability (Moll et al., 1965)'. As mentioned earlier,  $F_1$  inviability is observed in some inter-gene pool crosses of common beans (Shii et al., 1980; Singh & Gutiérrez, 1984; Gepts & Bliss, 1985).

A simple model relating the interactions of dominance and epistatic variance to genetic distance has been presented by Lynch (1991). According to this model  $F_1$  hybrid vigor increases with increasing genetic distance to a point and then decreases. But the fitness of successive generations after the initial hybridization begins to decrease at much lower levels of genetic distance than in the  $F_1$  generation. The observation of increased  $F_1$  fitness (Gutiérrez & Singh, 1985; Nienhuis & Singh, 1986) and decreased RIL fitness in inter-gene pool crosses of common bean

conforms to this model. The classic allopatric models of speciation by Dobzhansky (1936) and Muller (1940) stated that two geographically isolated populations could eventually, through processes of random mutation and drift or selection, diverge to the point that they produce only sterile or inviable hybrids. Orr (1995) recently generalized the model to describe how this divergence could be caused by any number of genes and result in any level of decreased fitness of the progeny from hybridization.

Common beans from the Andean and Mesoamerican gene pools clearly fit the conditions necessary for the allopatric model of speciation. Even prior to domestication, the two gene pools have been isolated reproductively by their autogamous mating system, geographically by their lack of a method for long-distance seed dispersal, and genetically by the presence of genes conditioning hybrid weakness and crippling (Koinange & Gepts, 1992; Singh & Molina, 1996). These observations suggest that common bean has been domesticated at a point of incipient speciation in its evolutionary history. Using Orr's model of speciation, the hybrid weakness and crippling gene system (conditioned by the *D11*, *D12*, and *Lcr* loci, Singh & Molina, 1996) can be described as a three-gene system with extreme phenotypic effects that result in reproductive isolation. The number of genes conditioning the increased performance of RILs in the present study population cannot be determined from the present data, and further speculation as to their genetic basis and mode of action (through decreased fertility, diminished photosynthetic capacity, and/or changes in photoassimilate partitioning) will require a QTL mapping analysis (W.C. Johnson & P. Gepts, unpubl. results).

The number of genes or genomic regions involved in the observed performance of the inter-gene pool cross progenies and their linkage relationships will have an important effect on the success of breeding programs that attempt to use inter-gene pool crosses. The number of genes and the complexity of their interactions is directly proportional to the difficulty of recovering novel complementary gene assemblies. If further analyses reveal that a significant portion of the variation in these traits can be accounted for by a small number of genomic regions, genomic analysis using molecular markers may facilitate rapid identification of promising recombinant genotypes.

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