Response in Genotypic and Breeding Value to a Single Generation of Divergent Selection for Fresh Fruit Color in Strawberry

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Abstract. Four sets of selected strawberry (*Fragaria × ananassa* Duch.) genotypes were generated from within a single breeding population to evaluate the correspondence between predicted and realized selection response for fresh fruit color traits. Genotypes were selected for extreme phenotypes, dark or light, of either internal or external color value (CIELAB L*). Genotypic selection response was evaluated empirically by scoring fruit from the clonal derivatives of these selected genotypes, and response for breeding value was estimated by scoring the offspring of crosses performed among a subset of the genotypes within each selected set. Realized selection response was slightly larger than predicted for internal and external L* when calculated for selected genotypes. Also, more than half of the selected genotypes had genotypic values for L* outside the range of the original parents, providing evidence for transgressive segregation. Realized selection response for breeding value in exterior and interior color was slightly less than predicted. Compared in a different way, genotypic selection response for external color was significantly greater than selection response for breeding value, whereas genotypic and breeding value responses did not differ for internal color. These observations suggest the presence of some nonadditive genetic variance for external color but support the conclusion that the heritabilities predicted previously were reasonably accurate. Estimates of variance components within each of the offspring populations demonstrated that genetic variances were modified substantially by one generation of selection. Selection for dark fruit color reduced genetic variance to nonsignificant levels, with internal color more affected than external color. The total genetic variances within both of the offspring populations from parents selected for light color were changed little by one generation of selection, but substantial dominance variance was detected that had not been found in the original population. The rapid response to selection and large changes in the distribution of genetic variances may indicate the presence of a few genes with comparatively large effect in strawberry color expression. Additional divergent selection response can be expected, but primarily in the direction of light fruit color.

Color is an important component of fresh fruit quality in strawberry and an important factor in most cultivar selection programs (Sistrunk and Morris, 1985). Several studies have been conducted to evaluate the magnitude of genetic variation for color traits within strawberry breeding populations (Lundergan and Moore, 1975; MacLachlan, 1974; Shaw, 1991) and the opportunity for genetic improvement of strawberry color. Although these studies used different experimental populations, evaluated different color traits, and employed different methods for estimating genetic parameters, each identified substantial additive genetic variance for fruit color. Also, significant dominance variance was detected for some of the color traits evaluated in each study listed above, suggesting that prediction of selection response may not follow simply from the parental values (Falconer, 1981).

Together with observations on color variation among and within germplasm sources (Nelson et al., 1972; Scheerens and Brennehan, 1991), the studies cited above suggest that most strawberry breeding populations contain variation sufficient for manipulation of strawberry fruit color by recurrent selection. However, selection responses predicted from genetic variance estimates depend on a number of genetic assumptions, and comparisons of predicted and realized selection response for fresh fruit color traits have not been published. One of the principal unknowns in any single-generation evaluation of genetic variation is the complexity of the traits evaluated. For example, long-term selection response and the magnitude of nonadditive genetic variation depend on allele frequencies in the original population

and will differ depending on the underlying distribution of genetic factors and their mode of expression. Our study was conducted to determine whether divergent selection responses for dark and light fresh fruit color in strawberry genotypes and their progenies were consistent with those predicted from a previous heritability study (Shaw, 1991). A second goal of this study was to provide an evaluation of the consequences of selection for genetic variation within populations resulting from one generation of selection.

Materials and Methods

Genetic materials. Selected genotypes were retained from an experimental population evaluated for color traits in 1990: descriptive statistics and genetic parameter estimates for this experimental population have been reported previously (Shaw, 1991). Briefly, the original experimental population included 318 genotypes from 20 biparental crosses. This population was evaluated for parameters of CIELAB color space (L*, a*, b*; Commission International de l'Eclairage, 1978) and a subjective color score was derived from visual comparisons of fruit color with a two-dimensional array of Pantone color chips (Shaw, 1991). The nine parents for the genotypes in this original population were a sample of those used in generating the controlled-cross seedlings evaluated for varietal potential by the California strawberry improvement program in 1990. These parents had been selected for a number of traits, including commercial fresh fruit color attributes. Color evaluations were conducted using runner plants from each of the original 318 genotypes propagated at two locations, and genetic × location interactions were determined to be of minimal importance. Internal and external fruit color were evaluated for fruit from individual plants using a Colormet reflectance spectro-

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photometer (Agrtron, Reno, Nev.) with a 1 -cm aperture. Reflectance was expressed as CIELAB L*, a*, and b* calibrated to a white tile (L* = 100, a* =0, b* =0), with three to four fully red fruit evaluated per plant and two measurements per fruit. The nine parental genotypes for this population were also evaluated using fruit from a single plot of 10 runner plants at each location, with 10 randomly sampled fruit per plot.

Although a number of parameters have been proposed for color evaluation (Francis, 1969; McGuire, 1992), the goal here was to modify the total reflectance, or color value, as measured by CIELAB L*. More complex evaluations are possible (hue angle, chroma, or saturation index), but other primary color traits (a*, b*) were genetically correlated with L* in the original experimental population (Shaw, 1991) and, thus, contained largely the same genetic information about color. Strawberries reflect red light, so the intensity of red color and overall reflectance will necessarily be correlated.

Selection procedure. Four sets of selected genotypes were generated from within the original experimental population, with different sets of genotypes chosen for extreme phenotypes (dark or light) of either internal or external color. Genotypes for each of the four selected sets were retained from the 318 in the original experimental population based on the means of their color trait values at the two original test locations. Twelve of the original 318 genotypes were chosen for each selected set. Four of the genotypes chosen for dark internal color were also retained for their dark exterior color and three of the genotypes chosen for light internal color were likewise chosen for light exterior color; thus, 41 genotypes were retained for further evaluation.

Selections were performed using an index on the L* and the subjective color-chip score:

$$I = b_1 v_1 X_1 + b_2 v_2 X_2 \quad [1]$$

In Eq. [1], b_1 and b_2 are index weights for deriving individual-genotype index values, v_1 and v_2 are economic-scaling weights for each trait, and X_1 and X_2 are the phenotypic values for L* and the subjective color score, respectively. Index weights were calculated following Van Vleck (1983), using the genetic and environmental parameters given in Table 1. Color traits have no intrinsic economic value, but the v_i were assigned values equal to the inverse of the phenotypic SD for each X_i to compensate for the differing scales of measurement for the two original variables. The purpose of including the subjective score was to maximize response in L* by utilizing the genetic correlation between the two traits (Van Vleck, 1983). Indexes for selection of internal and external color were calculated as

$$I_{(\text{external})} = 0.145 L^*_{\text{EX}} + 1.16 SC_{\text{EX}} \quad [2]$$

$$I_{(\text{internal})} = 0.082 L^*_{\text{IN}} + 0.47 SC_{\text{IN}} \quad [3]$$

In Eqs. [2] and [3], L^*_{EX} , L^*_{IN} , SC_{EX} , and SC_{IN} are the external (EX) internal (IN) phenotypic values for L* and the subjective color score for individual genotypes.

Correlated response for either internal or external L* from selection using the above indexes was predicted as (Van Weir, 1983)

$$CR(L^*) = i \ b_1 \sigma^2_{g1} + b_2 \sigma^2_{g2} / \sigma_i \quad [4]$$

In Eq. [4], b_1 and b_2 are the index weights described above, σ^2_{g1} and σ^2_{g2} are the genetic components of variance for L* and subjective score values from Table 1, respectively, i is the selection intensity ($i = 2.2$ for 12/318 genotypes retained), and σ_i is the SD of the index values. Genetic variances and covariances used to calculate indexes and indirect selection response for L* (Table 1) were

Table 1. Genetic and environmental parameters for calculation of index weights for selection of internal and external color value (L*).

Parameters ^a	External color		Internal color	
	L*	Score	L*	Score
σ^2_g	8.78	0.47	28.80	2.17
σ^2_p	5.08	0.32	15.98	1.36
r_{p_y}		-0.74		-0.77
r_g		-0.99		-1.00
$\sigma_{p(xy)}$		1.51		6.06
$\sigma_{g(xy)}$		1.27		4.66

(xy) are the phenotypic and genetic variances, correlations, and covariances, respectively, for pairs of external and internal color traits estimated from Shaw (1991).

Values for r reported here were calculated using genotypic means over two original test occasions and differ slightly for those reported in Shaw (1991).

obtained directly from results given in Shaw (1991). Estimates of σ^2_g used in deriving indexes and gain predictions included additive and dominance genetic variance components, and the response predicted therefore is appropriate for selection of genotypic value.

Standard errors for predicted selection response from an index on multiple traits are not easily obtained. However, Sales and Hill (1976) showed that the variance of predicted selection response for index selection is always somewhat larger than the variance of response predicted for direct selection, which were estimated following Wricke and Weber (1986).

Realized selection response and residual genetic variation. Trials were conducted in 1991 to estimate realized selection response for genotypic value and in 1993 to estimate the response for breeding value. To evaluate genotypic selection response, a random sample of 8 to 11 of the original 12 selected genotypes from each set (and 8 of the 9 original parents) were propagated vegetatively (as runner plants) and planted at the Watsonville Strawberry Research Facility in November 1990. Plants were treated according to recommendations for commercial winter plantings (Welch, 1989) and fruit from these genotypes was scored for L* in 1991. These selected genotypes were part of a larger study aimed at evaluating the consequences of genetic $\times$ harvest date interactions for color traits; experimental design and sampling schemes for these evaluations have been reported previously (Sacks and Shaw, 1994). Fruit from each selected genotype (and parent) were evaluated on three dates throughout the harvest season, with an average of 52 fruit evaluated for each genotype. Estimates of L* for each genotype were obtained as the average for all fruit pooled over the three harvest dates; genetic $\times$ harvest date interactions were ignored as they were found to be a significant but small fraction of the total phenotypic variance (Sacks and Shaw, 1994). Selected-set means were then calculated as the average for all genotypes evaluated within each set, and realized selection response was calculated as the difference between divergent set means for internal and external L*. The significance of realized selection response was evaluated by comparison of divergent set means with Student's t .

Seedling progenies from each of the four sets of selected genotypes were generated in 1992 to provide offspring populations for evaluation of cumulative selection response, or breeding value. Five of the 12 selected genotypes from within each set were crossed in a half-diallel design and 9 to 10 crosses per set provided sufficient seed for further testing. None of the crosses performed resulted in a ΔF greater than 0.125. The selected genotypes used as parents were chosen among the 12 original selections for their extreme color value in the 1991 trials, with the most extreme 5 to

6 genotypes included for each offspring population. Because the genotypes used as parents were chosen using information from two cycles of color evaluation, their predicted response must reflect cumulative gains expected from both selection stages. Predicted response for this second stage of selection was calculated as

$$R = ih^2\sigma_p \quad [5]$$

In Eq. [5], i is the selection intensity based on retaining 5 or 6 of 12 genotypes ($i = 0.88$ or 0.75), h^2 is the heritability of genotypic means with genetic components modified to reflect that the base population had been selected but not intermated (Finney, 1956), and σ_p is the phenotypic SD of genotype means in the base population. Predicted responses for this second stage of selection were added to that predicted for the first stage to provide a combined response prediction for the offspring populations.

Offspring test plantations were established in September 1992 using a randomized complete block design with a single plot of 6 to 10 seedlings from each biparental cross in each of two blocks. Fruit from individual plants were scored for L^* in May 1993 using the sampling strategy described above for the original experimental population (Shaw, 1991). As above, estimates of L^* for each offspring population were obtained as the average for individuals in the population; realized selection response was calculated as the difference between divergent offspring populations for internal and external L^* . Differences among cross means within each offspring population were tested using analyses of variance (ANOVAs), with blocks treated as fixed effects and crosses as random effects. Genetic components of variance were further resolved using the restricted maximum likelihood method (REML) of Geisbrecht (1983) and computer software provided by Huber (1993). Estimates of variance components attributable to general combining ability, specific combining ability, and a within-cross component were obtained and used to calculate narrow- and broad-sense heritabilities (h^2 and H^2) following Hallauer and Miranda (1981).

Results and Discussion

Large and highly significant differences ($P < 0.01$) were detected between paired sets of genotypes selected divergently for internal anti external L^* (Table 2). The differences were similarly significant for divergent offspring populations, suggesting the importance of additive genetic effects in the original population. Although this result demonstrates significant selection response, it indicates little about the symmetry of response. As an additional comparison, original parent genotypes evaluated simultaneously with the selected genotypes in 1991 had mean values for external and internal L^* of 26.3 (SE = 1.0) and 48.9 (SE = 1.8) respectively. The midpoint of divergent-set means, external and internal L^* of 27.6 (SE = 0.72), and 51.3 (SE = 1.28) were slightly larger than the average of the parental genotypic values, suggesting that selection was nearly symmetrical, with some skew toward response for light color.

Color data for the original parent genotypes were not available in the 1993 scoring year. However, the midpoint of the divergent offspring population means, external and internal L^* of 25.6 (SE = 0.56) and 42.8 (SE = 0.75), for scores obtained in 1993 suggest that all of the fruit evaluated from these seedling populations were darker than for previous trials. Variations in external fruit color value of similar magnitude have been reported over years and locations (Nelson et al., 1972; Shaw, 1991), and the difference between genotypic and offspring results is most likely the consequence of yearly environmental effects. Because neither the original parents nor the selected genotypes used as parents for these offspring populations were available in 1993, symmetry of selec-

tion response for breeding value cannot be assessed.

Estimated selection responses for the paired divergent sets of selected genotypes and offspring populations are given together with their predicted responses in Table 3. Because the predicted responses were calculated using indexes and equations that included dominance genetic variance, they can be compared directly only with the realized selection response for genotypic value. However, no significant dominance variance was detected for L^* in the original population, and good correspondence was expected between genotypic and breeding value responses (Shaw, 1991).

Realized selection response for selected genotypes was slightly larger than predicted for internal and external L^* . In addition, more than half of the genotypes in these selected sets had values for L^* outside the range of the original parents. This latter comparison is somewhat subjective but, because individual genotypic means were estimated with considerable precision, it provides evidence for transgressive segregation.

Divergent selection response in L^* for breeding value was slightly less than predicted for exterior and interior color (Table 3). However, the confidence intervals for the predicted responses were rather large, and all observed responses were within 1 SE of their predicted values. Compared in a different way, genotypic selection response for external L^* was significantly greater than selection response for breeding value ($t = 3.4$, $P < 0.01$), whereas genotypic and breeding value responses did not differ for internal color ($t = 0.05$, $P > 0.90$). Together, these observations suggest the presence of some nonadditive genetic variance in the original experimental population for external but not for internal L^* . The nonadditive genetic factors expressed in the genotypic trials in-

Table 2. Means, standard deviations, and sample sizes for internal or external color value (L^*) in four sets of selected strawberry genotypes and the offspring populations obtained from a subset of these selections.

		Selection criterion ^a			
		Light external value	Dark external value	Light internal value	Dark internal value
Selected genotypes	Mean	33.1	22.1	59.7	42.8
	SD	2.4	2.1	2.5	5.1
	N	11	8	8	10
		$t = 10.9^{**}$		$t = 9.6^{**}$	
Offspring populations	Mean	29.2	22.0	51.5	34.1
	SD	5.3	3.7	7.0	4.7
	N	128	147	130	120
		$t = 12.9^{**}$		$t = 23.2^{**}$	

^aParameters are reported either for external or internal L^* , consistent with the selection criterion for the set or population.

Table 3. Predicted and realized divergent selection response (standard errors in parentheses) for internal and external color value.

Set or population	External value selections		Internal value selections	
	Predicted response	Realized response	Predicted response	Realized response
Selected genotypes	9.6 (3.5)	11.1 (0.72)	14.0 (4.8)	17.2 (1.28)
Offspring population	11.6 (4.2)	7.2 (0.58)	18.2 (5.5)	17.3 (0.86)

^aParameters are reported either for external or internal L^* , consistent with the selection criterion for the set or population.

Table 4. Results for analyses of variance (ANOVA) and heritabilities of internal and external color value for offspring populations created after divergent selection of parents for color value.

Source	Selection criterion ^a							
	Light external value		Dark external value		Light internal value		Dark internal value	
	df	MS	df	MS	df	MS	df	MS
Blocks	1	5.1	1	29.1	1	4.8	1	8.2
Crosses	9	84.7**	9	20.7	9	130.1**	8	18.5
Within-cross	117	24.0	137	13.1	120	44.2	110	11.8
ANOVA								
h^2 (S.E.)	0.33 (0.35)		0.14 (0.16)		0.19 (0.27)		0.09 (0.14)	
H^2 (S.E.)	0.51 (0.46)		0.14 (0.16)		0.37 (0.41)		0.09 (0.14)	

^aParameters are reported either for external or internal L^* , consistent with the selection criterion for the population.

elude epistatic components, which would not have been detected in the original trial. Despite some evidence for nonadditive genetic effects, these results support the assertion that heritabilities estimated previously (Shaw, 1991) were reasonably accurate.

Comparisons of genetic parameter values estimated for the four offspring populations suggest that the genetic variances for color have been modified substantially by one generation of selection. First, ANOVAs showed significant differences among crosses within offspring populations selected for external and internal light color, but no significant among-cross variance was detected within populations selected for dark color (Table 4). Further resolution of genetic variances with REML showed distinct patterns for offspring populations selected for light and dark color. Selection for dark color had reduced genetic variance compared with the original population: $H^2 = 0.14$ and 0.09 for internal and external L^* in the selected offspring populations compared with 0.41 and 0.39 in the original population (Shaw, 1991). Selection for light color had not reduced the total genetic variance, but substantial dominance variance was detected for light-color offspring populations that had not been found in the original experimental population: $H^2/h^2 = 1.55$ and 1.95 for external and internal L^* in the offspring populations compared with 1.00 and 1.08 for the original population. The magnitude and distribution of genetic variances are expected to change with successful selection (Falconer, 1981). Our results are consistent with observations that suggest some directional dominance for genes conferring dark color in crosses among divergent types (Lundergan and Moore, 1975).

Long-term selection response depends on the number of loci affecting a trait, the frequency of alleles at these loci, and their mode of inheritance (Falconer, 1981). Rapid selection response coupled with substantial changes in genetic variance parameters may imply that a continuously varying trait is affected by relatively few genes, or at least signal the presence of a few genes with large effect among those conditioning the trait of interest (Eisen et al., 1991). Tests for continued divergent selection response together with eventual crosses among extreme genotypes would provide a more critical test of this hypothesis.

The California breeding population may be near fixation for the variation useful in deriving cultivars with dark fruit color. Additional divergent selection response can be expected within this population, but primarily in the direction of light fruit color. Infusion of germplasm from other sources might break this apparent plateau, but in fact the practical utility of genetic materials darker than those obtained to date is questionable. External and internal color of the darkest selections were substantially greater than for the parent genotypes and may exceed the commercially

acceptable range for fresh fruit already. It appears that color value can be modified rapidly within commercially acceptable limits and that research effort might be better applied to manipulation of other color traits, such as hue or sheen, within acceptable limits for L^* .

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