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Comparison of ancestral and current-generation inbreeding in an experimental strawberry breeding population

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Abstract Progenies from first-generation self, half-sib, full-sib, and cross fertilizations were generated to evaluate the magnitude of inbreeding depression for vegetative and production traits in strawberry. Tests were conducted to determine the linearity of trait mean depression with inbreeding rate (ΔF) over this range of inbreeding values, as an indication of the presence of non-additive epistasis. A control population, for which a similar range of coancestry had accumulated over several cycles of breeding and selection, was also generated to compare the consequences of ancestral and current-generation inbreeding. Trait means for crosses among current-generation half-sibs, full-sibs, and selfs were 2–17%, 3–12%, and 14–45% lower than for unrelated crosses among the same set of parents, respectively. Linear regression of progeny means on current-generation ΔF was significantly negative for all traits and explained 17–44% of the variance among progeny means. Mean depression was largely linear over the range of inbreeding rates tested in this population, indicating the absence of epistasis for the traits evaluated. Conversely, regressions of progeny means on pedigree inbreeding coefficients (F), where coancestry had accumulated over several cycles of breeding and selection, were uniformly nonsignificant and explained 0–10% of the variance among cross means. Further, multiple regression of progeny means for current-generation relatives on pedigree F failed to improve fit significantly over regression on current-generation ΔF alone for all traits. Together, these results suggest that pedigree inbreeding coefficients are poor predictors of changes in homozygosity when populations are developed through multiple cycles of breeding and selection. They also imply that inbreeding depression will be of minor importance for strawberry breeding populations managed with adequate population sizes and strong directional selection.

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Introduction

Coancestry develops in closed populations over time, and within-population relatedness is further increased by directional selection (Wright 1931, Robertson 1961). The primary consequences of coancestry in predominantly cross-fertilized crops – inbreeding depression resulting from mating among relatives and the loss or redistribution of genetic variance – have been studied widely using controlled pollinations (Hallauer and Sears 1973; Cornelius and Dudley 1974; Wilcox 1983; Sniezko and Zobel 1988; Woods and Heaman 1989) and pedigree evaluations (Mendoza and Haynes 1974; Hancock and Seifker 1982; Scorza et al. 1985). In general, these studies suggested serious limitations to future selection response when inbreeding is not managed appropriately.

Domestic strawberry (*Fragaria ananassa*) improvement is managed using recurrent selection within largely closed populations, followed by clonal propagation of superior genotypes (Bringhurst and Voth 1984). Concern has been expressed over the potential long-term impact of inbreeding in strawberry populations (Sjulin and Dale 1987), and the consequences of severe inbreeding have been well documented (Morrow and Darrow 1952). Selfing for a single generation has typically resulted in mean depressions for production and horticultural traits of 21–44%; up to 80% trait mean depression was realized after two generations of selfing (Jones and Singleton 1940, Morrow and Darrow 1952, Aalders and Craig 1968, Spangelo et al. 1971). With recurrent self-fertilization and intervening cycles of selection, means for yield were reduced by 57% and 80% for S_2 and S_5 generations, respectively (Aalders and Craig 1974, Melville et al. 1980, Niemirowicz-Szczytt 1989).

Although the negative effects of forced inbreeding at relatively rapid rates can severely limit long-term selection response, the relevance of such observations to the management of coancestry generated in breeding populations may be limited. In the University of California program, which is among the more mature in North America (Sjulin and Dale 1987), breeding and selection for over 50 years with little infusion of germplasm have generated a population with an average pedigree inbreeding coefficient (F) of 0.18 (Shaw 1991). Because most strawberry improvement programs utilize overlapping generations, the rate of pedigree inbreeding per generation (ΔF) is difficult to compute with precision. However, during the past 50 years selection cycles typically have run 8–10 years for the California program, suggesting an advance of 6–7 generations and a ΔF of 0.03–0.04 per cycle over the history of this breeding program. The rates of inbreeding used in the studies cited above exceeded those expected in most programs aimed at genetic improvement of predominantly cross-fertilized crops; inbreeding at lower rates may have different consequences for long-term selection response. The impact of slow rates of inbreeding in breeding populations is further obscured because directional selection and inbreeding have conflicting consequences for the accumulation of deleterious homozygosity. For example, F obtained using analysis of pedigrees in an advanced strawberry breeding population ranged from 0.04 to 0.31 among biparental progenies, but no relationship was found between pedigree F and trait means for yield, fruit size or commercial appearance ratings (Shaw 1991) . This lack of correspondence is not unexpected (Elihou et al. 1989), where coancestry has accumulated over several cycles of breeding and selection (Falconer 1981). Models have been developed to predict the actual rate of inbreeding in populations undergoing selection (Wray et al. 1990, Verrier et al. 1990), but these models depend on discrete generations and random mating within the selected population, and rarely have been tested empirically.

The first objective of the study presented here was to evaluate the magnitude of mean depression for vegetative and production traits in strawberry at levels of inbreeding likely to be encountered in a strawberry breeding program. A second objective was to determine the linearity of mean depression with respect to F, as an indication of the presence of non-additive epistasis (Falconer 1981). Domestic strawberries are diplodized octoploids (Bringhurst and Voth 1984), and duplicate epistasis might be expected (Comstock et al. 1958). Because the rates of inbreeding imposed in most programs are small, the importance of F accumulated to date is difficult to interpret, and the third objective of this study was to compare the consequences of current-generation inbreeding and ancestral inbreeding where coancestry had accumulated over several cycles of breeding and selection.

Table 1 Expected mean squares for six traits at four rates of inbreeding (ΔF)

Source	*df*	Expected mean squared		
Inbreeding rate (R)	3	σ^2	$+ 19.2\ \sigma_{2C/R}$	$+ 331.8\ \sigma^2_R$
Cross/(R)	68	σ^2	$+ 19.2\ \sigma_{2C/R}$	
Error	1305	σ^2		

Materials and methods

Twenty genotypes were chosen from the University of California breeding population and used as parents to generate an experimental population of 72 biparental progenies with four rates of inbreeding (ΔF). The genotypes used as parents were chosen from the 1990 seedling population based only on their coancestry; they were unselected otherwise and represented the range of germplasm available within the California breeding population at present. The four sets of progenies included 16 unrelated combinations, 19 crosses among half-sibs, 19 crosses among full-sibs, and 18 self-fertilizations, with ΔF of 0, 0.125, 0.250, and 0.500, respectively. Sets of progenies in this population with differing ΔF were generated using the same 20 parents, but in different cross combinations. Differences in trait means among the sets thus result from their differing levels of current-generation inbreeding and not from genetic sampling variation. Because genetic improvement has proceeded in the California population for over 50 years, no completely unrelated crosses were possible. The average pedigree F for progenies treated as unrelated was 0.17, but the ΔF due to inbreeding other than that imposed in this study was 0.

A fifth set of 18 biparental progenies was chosen from among those generated for the cultivar improvement program in 1991. This set was treated as a control population and used to infer the relationship between ancestral inbreeding and trait values when pedigree inbreeding coefficients had accumulated over several cycles of breeding and selection. Progenies were chosen to represent the range of F in the current breeding population and rates of inbreeding realistic to the California program. Pedigree inbreeding coefficients for progenies of this control population ranged from 0.07 to 0.36, and none of the progenies in this set resulted from a cross with ΔF greater than 0.07 in the current generation. Furthermore, at no time during the last 3 cycles of breeding and selection had ΔF been larger than that expected due to mating of half sibs (ΔF = 0.125). Direct comparison of trait values between this control set and the four sets generated with controlled ΔF is inappropriate, because their respective parents differ substantially in origin and selection history. Parents for the control population had been selected and tested extensively for a number of productivity and quality characters, whereas parents used to generate progenies with forced inbreeding were chosen based on their coancestry only.

Twenty seedlings (occasionally fewer) from each of the 90 biparental progenies described above were established in field trials on September 10, 1991, at the Wolfskill Experimental Orchard, near Davis, California and cultured as described previously (Shaw et al. 1987). A randomized complete block design was used, with a single plot of 10 seedlings from each cross in each of two blocks. Data for growth and productivity traits were collected for individual plants throughout the annual season. Plant cross-sectional diameters were obtained on February 27, 1992 to describe vegetative growth from planting to mid-winter, and on June 26, 1992 to evaluate sustained growth throughout the production season (Shaw 1993). Weekly yields, fruit numbers, and a commercial appearance score (Shaw et al. 1989) were recorded for each plant for 15 consecutive weeks, beginning the second week of April and ending in late July of the year following planting. Fruit size was calculated using weekly yields and fruit numbers; both seasonal fruit sizes and appearance scores were expressed as weighted averages, with weighting performed using weekly yield values.

Preliminary analyses detected no significant block effects, and these are not considered in subsequent analyses. ANOVAs were conducted using the four sets of current-generation inbred progenies, with rate of inbreeding (ΔF) and progenies nested in rate as sources of variation. Variance components were estimated for inbreeding rate

Table 2 Means and standard deviations (in parentheses) for six traits, for sets of bi-parental progenies generated using four rates of inbreeding (ΔF)

Inbreeding rate[a]	Traits					
	Plant diameter increment (cm)		Yield (g/plant)	Appearance score	Fruit size (g/fruit)	Fruit number
	Winter	Spring				
Crosses ($\Delta F = 0$)	22.2 (2.6)	48.2 (4.6)	912 (389)	2.44 (0.39)	12.2 (3.42)	78.0 (34.3)
Half-sibs ($\Delta F = 0.125$)	21.1 (2.8)	47.4 (5.1)	756 (305)	2.37 (0.39)	11.9 (2.9)	65.8 (26.9)
Full-sibs ($\Delta F = 0.250$)	21.0 (3.4)	47.0 (5.3)	794 (391)	2.31 (0.38)	11.9 (3.1)	69.6 (37.0)
Selfs ($\Delta F = 0.500$)	19.0 (2.8)	43.1 (5.2)	501 (259)	2.10 (0.41)	10.3 (3.4)	52.0 (28.9)

[a] n = 312, 361, 361, and 343 for progenies of crosses, half-sibs, full-sibs, and selfs, respectively

and progeny-within-rate to evaluate the relative importance of these sources by equating SAS Type III mean squares to the expected mean squares in Table 1 (SAS 1988). Progeny means for the population generated with four rates of inbreeding were regressed on ΔF, and means for progenies from the ancestral control set were regressed on F, as explanatory variables. As an additional test for non-linearity with ΔF in progenies created by forced inbreeding, analyses were performed for all traits by forcing linear regression on ΔF, and then entering ΔF^2 in a stepwise fashion. As a supplemental test of the explanatory value of pedigree F in the control population, multiple regressions were performed using the population of current-generation relatives by first forcing a fit to ΔF, then entering the pedigree F in a stepwise fashion.

Results and discussion

Trait means for progenies from crosses among current-generation relatives were lower than for the progenies of unrelated crosses among the same 20 parents (Table 2). Means for progenies from crosses among half-sibs were from 2–17% lower than those from progenies from unrelated crosses, full-sib trait means were reduced by 3–12%, and reductions were 14–45% for the offspring of selfs. Yield suffered the largest depression, with a 45% reduction for selfed progenies compared with the progenies of unrelated crosses.Most of the inbreeding depression for yield was associated with reduced fruit numbers rather than reduced individual fruit size (34% vs. 16% reduction for selfs). Yield and fruit number were the only traits to express substantial inbreeding depression at rates of inbreeding other than selfing (maximum of 17% and 16%, respectively, Table 2). Vegetative growth was slower for inbred offspring (Table 2), especially during the fall and winter. Percent reductions in plant diameters were modest for selfed progenies (11–15%) and very small for progenies with lower rates of inbreeding (maximum of 5%). However, percent reductions in plant growth would be substantially greater when expressed in units of cross-sectional area, which might be a better indicator of photosynthetic capacity. Significant genetic correlations between early vegetative growth and productivity have been detected (Shaw 1993), and much of the inbreeding depression observed for yield may be expressed early as reduced plant vigor.

Highly significant variances were detected by ANOVAs, both among sets with differing inbreeding rates and among progenies within inbreeding level, for all traits scored (Table 3). The proportion of the phenotypic variance attributed to the four rates of forced inbreeding differed among the traits scored (5.6–19.1%, Table 3), but was usually equal to or less than the variance attributed to progenies within inbreeding level (10.3–25.5%, Table 3). Interpretation of the genetic components of variance in a population with such heterogeneous rates of inbreeding is not possible (Falconer 1981), and direct comparison of the importance of genetic versus inbreeding effects using variance components is not valid. Although most of the differences among individuals and/or progenies in partially inbred populations will be due to genetic effects rather than non-random combinations generated by matings among relatives (Wright and Cockerham 1986), caution is required when using phenotypic values for selection. Genetic values are confounded with inbreeding effects in populations that are heterogeneous for F, and progeny means used without correction for inbreeding level may be poor predictors of an individual's breeding value.

Mean depression for all traits appeared to be linear over the range of inbreeding rates tested in this study. Linear regression of progeny means on current-generation ΔF was significantly negative for all traits and explained 17–44% of the variance among progeny means (Table 4). Although the difference between cross and full-sib progeny means was greater than between full-sib and selfed progenies for some traits (Table 2), visual inspection of residuals suggested no non-linear trends. ANOVAs detected large among-progeny variance components for these traits, and previous studies have demonstrated dominance variance for several of the traits studied here (Shaw et al. 1989). Lack of fit that does not follow any clear trend could be explained by specific combining ability rather than by non-linear response to inbreeding. Analyses performed by forcing linear regression on ΔF, and then entering ΔF^2 in a step-

Table 3 Mean squares and variance components (as a percentage of the phenotypic variance) for six traits for sets of bi-parental progenies generated using four rates of inbreeding

Source	Traits					
	Plant diameter increment		Yield[a]	Appearance score	Fruit size	Fruiter number[a]
	Winter	Spring				
Inbreeding Level (R)	595.5**	1755.4**	9741.5**	9.45**	231.0**	391.0**
Cross/R	47.2**	115.7**	579.1**	0.50**	52.9**	62.7**
Error	6.6	21.0	91.5	0.14	7.9	7.5
σ^s_R	15.9	16.0	19.1	14.8	5.6	8.5
$\sigma^2_{C/R}$	20.5	16.0	17.6	10.3	24.7	25.5
σ^2	63.6	68.0	63.3	74.9	69.7	66.0

[a] Mean square for yield and fruit number have been multiplied by 10^{-3} and 10^{-2} to shorten the table
* and ** indicate statistical significance at the 0.05 and 0.01 probability levels, respectively

Table 4 Results for analysis of regression of trait family means on their pedigree inbreeding coefficients for six traits for sets of bi-parental progenies generated using four rates of inbreeding

Source	*df*	Traits					
		Plant diameter increment		Yield[a]	Appearance score	Fruit size	Fruit number[a]
		Winter	Spring				
Regression	1	90.5**	256.1**	1390.6**	1.47**	31.0**	52.3**
Error	70	2.5	6.2	32.2	0.03	2.8	3.4
b		−6.17	−10.4	−765.5	−0.79	−3.6	−47.0
R^2		0.33	0.36	0.37	0.44	0.14	0.17

[a] Mean square for yield and fruit number have been multiplied by 10^{-3} and 10^{-2} to shorten the table
* and ** indicate statistical significance at the 0.05 and 0.01 probability levels, respectively

Table 5 Results for analysis of regression of family means on their pedigree inbreeding coefficients for six traits using bi-parental progenies chosen with random levels of ancestral inbreeding

Source	*df*	Traits					
		Plant diameter increment		Yield[a]	Appearance score	Fruit size	Fruit number[a]
		Winter	Spring				
Regression	1	2.2	0.6	5.4	0.012	3.78	0.2
Error	16	3.4	6.9	23.5	0.019	1.26	2.2
b		−4.64	−2.49	−230.8	−0.34	−6.2	4.55
R^2		0.0	0.0	0.0	0.0	0.10	0.0

[a] Mean square for yield and fruit number have been multiplied by 10^{-3} and 10^{-2} to shorten the table

wise fashion, detected no significant added fit due to regression on the quadratic of ΔF, and the results are not presented. Although the absence of significant regression on ΔF^2 supports the linearity of mean depression with F, conclusive evidence for the absence of non-additive epistasis is lacking. The form of the non-linearity expected with inbreeding and non-additive epistasis is difficult to predict and depends on the type and strength of epistasis (Namkoong 1979). In some cases, non-linearity might be expressed only at rates exceeding those tested here.

Consistent with previous results (Shaw 1991), no relationship was found between ancestral pedigree F and progeny means for traits evaluated in the control population. Coefficients for regression of progeny means on pedigree inbreeding coefficients were all negative, but were uniformly non-significant and explained 0–10% of the variance among progeny means (Table 5). Regression coefficients estimated for the control population were generally less negative than corresponding estimates for current-generation inbreeding (compare Tables 4 and 5), but direct comparisons are suspect because none of the coefficients for the control population were significant. Multiple regressions performed using the population of current-generation relatives, with ΔF forced into the model first, did not improve fit significantly for any trait by including pedigree F as an added explanatory variable. Together these results suggest that inbreeding coefficients calculated from pedigrees provide little information about the accumulation of deleterious homozygosity in strawberry breeding populations.

Conclusions

The levels of trait mean depression found here due to selfing are similar to those published for production and horticultural traits in other strawberry populations. Also, substantial mean depression for yield and fruit number was detected at lower rates of inbreeding, suggesting that even ΔF due to matings among half-sibs can impact genetic evaluations in strawberry improvement programs. Most strawberry breeding populations contain a mixture of progenies with differing current rates of inbreeding: inbreeding equivalent to that of half-sibs is somewhat frequent, that of full-sib or backcross progenies somewhat uncommon, and selfs are rarely used. Generally, related crosses are made using highly select parents, and the consequences of low rates of inbreeding where strong selection has been effected are likely to be less severe than detected here. However, in breeding populations that are heterogeneous for ΔF, family values might be substantially altered by inbreeding depression, and breeding values calculated on the basis of progeny performance should be interpreted cautiously.

Although this and other studies demonstrate the negative consequences of close inbreeding in strawberry, the absence of associations between trait means and pedigree F accumulated over multiple cycles suggests that inbreeding depression will be of minor importance for strawberry breeding populations managed with adequate population sizes and intensive directional selection. This study did not address the consequences of coancestry and inbreeding for the quantity and distribution of genetic variance (Robertson 1961). However, separate studies designed to estimate variance components have demonstrated that the combined effects of breeding, testing, and selection accumulated during the past 50 years has had little effect on genetic variances within the California population (Shaw et al. 1989). In the long term, questions relating to the appropriate level of genetic diversity within strawberry breeding populations should address the issue of selection efficiency and sustained selection response rather than concerns about trait mean depression from inbreeding.

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