Genetic Analysis of Fruit Ripening Time in Japanese Persimmon

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Abstract. Three individuals in progeny from each of 39 crosses and their parents in Japanese persimmon (*Diospyros kaki* Thunb.) were evaluated for fruit ripening time. Analysis of variance for the progeny, which estimated between- and within-cross variance, and the regression of the mean value in a full-sib family on the mid-parental value (MP) revealed that the genetic differences among crosses could be explained solely by MP. Genotypic values of individuals in progeny from a cross were assumed to be normally distributed around the regression line with within-cross genetic variance. Based on the parental mean performance of 3.5 fruit on a single tree for three years, the coefficient of regression of mean values in a full-sib family on MP was 0.99 ± 0.10 , and the proportion of individuals in progeny having genotypic values ripening earlier than early October was estimated as 52%, 24%, and 7% for three sets of mid-parents differing in their ripening time, i.e., early, middle, and late October, respectively. On the basis of the parental mean performance in 10 fruit on a single tree without yearly repetition, the regression coefficient was estimated as 0.91 and the proportion was estimated as 44%, 20%, and 6% for the three sets of mid-parents, respectively.

The estimation of narrow-sense heritability by coefficient of regression of progeny means on mid-parental values, is frequently used in animal breeding (Falconer, 1989), and has been one of the major subjects in genetic analysis of quantitative characters in fruit breeding (Bell and Janick, 1990; Caldwell and Moore, 1982; Fanizza and Raddi, 1973; Firoozabady and Olmo, 1982; Hansche et al., 1968, 1972a, 1972b; Kester et al., 1977; Machida and Kozaki, 1975). Narrow-sense heritability is, by definition, a proportion due to average effects of genes, or additive gene effects among total variation, and a measure of the degree to which we can predict the genotypic mean of progeny from their parental phenotypes.

However, the heritability does not provide any information on the variance in individuals of progeny in a cross due to segregation. In fruit breeding, if only one superior individual is selected, it can be vegetatively propagated and released as a cultivar. The selection is based exclusively on individual selection in the F_1 generation. Although it is generally accepted that a character with a low narrow-sense heritability is difficult to improve, even with low narrow-sense heritability, segregation with large variance in a progeny may increase the probability of individuals of progeny exceeding a critical value in selection. Therefore, narrow-sense heritability is not the only index for genetic improvement; the variance in individuals in progeny is also important. Selection in fruit breeding is intrinsically different from selection in animal breeding and many annual crops. The information that fruit breeders wish to know is the expected proportion of individuals in progeny, without environmental bias, superior to a given critical value in a cross.

From this point of view, we have already reported on the expected proportion of individuals in progeny over a given critical value for fruit weight in Japanese persimmon (Yamada et al., 1994b).

Japanese persimmon breeding at the Fruit Tree Research Station (FTRS), Japan, started in 1938 from crosses among a relatively small number of late ripening pollination constant-nonastringent (PCNA) cultivars (Yamada, 1993). Breeding has been carried out with the aim of releasing early ripening PCNA cultivars (Yamada, 1993), because there is a shortage of that type of cultivar.

The purpose of this study was to elucidate the relation of mid-parental value of a cross and the mean value in the progeny in fruit ripening time (FRT), and to estimate the proportion of individuals in progeny exceeding a critical value in a cross, without environmental bias.

Materials and Methods

The population. Our study was based on the records of the FTRS Japanese persimmon breeding program at Akitsu and used data on three individuals in progeny from each of 39 crosses; this population was analyzed for fruit weight in our previous report (Yamada et al., 1994b). The crosses were made solely for the purpose of genetic improvement without any regards to a specific mating design. One scion was taken from each seedling and top-grafted on branches of mature 'Fuyu' trees (one scion per branch) to promote early fruiting. This population was slightly inbred and although Japanese persimmon is hexaploid (Zhuang et al., 1990), the genomic composition has not been elucidated. The inbreeding coefficients (F) tentatively calculated as allopolyploid, were 0 in 25 crosses, 0-0.2 in 4 crosses, 0.2-0.3 in 9 crosses and 0.5 in one cross.

The parents consisted of cultivars and selections of pollination constant-nonastringent type with the exception of 'Nishimurawase', a pollination variant nonastringent (PVNA) type (Kitagawa and Glucina, 1984; Yamada, 1993).

Fruit ripening time evaluation. Data on individuals in progeny were collected for a single year during the period from 1982 to 1985, while

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parents were evaluated for three years from 1984 through 1986 based on 35 fruit per year on a single tree. Fruit samples from progeny and parent were evaluated for fruit ripening time based on the edibility which was mainly judged by flesh firmness. Ten fruit were usually evaluated for each individual in progeny. Ripening time was rated for each individual in progeny and for each fruit of parents on a 1 to 8 scale; 1 = late September, 2 = early October, 3 = mid-October, 4 = late October, 5 = early November, 6 = mid-November, 7 = late November, and 8 = early December.

Data were adjusted for yearly variation. The yearly deviations from the base year's value were computed annually on the average performance in 19 control genotypes which were evaluated every year using 15 fruit on a single tree. The average performance in 19 control genotypes in 1988 was used as the base year's value. The yearly deviations were uniformly subtracted from all values of progeny and parent measured within each year. The efficiency and error in the adjustment was analyzed by Yamada et al. (1994a).

Estimation for genetic parameters. Progeny mean was calculated as the mean value of individuals in the full-sib family derived from a cross. The regression analysis was carried out, using progeny mean in a cross as a dependent variable, and mid-parental value (MP) as an independent variable.

An analysis of variance for data of each progeny individual was made to estimate between-cross variance (σ_{bi}^2) and within-cross variance (σ_w^2). The σ_{bi}^2 was assumed to consist of the variance due to the regression (σ_r^2) and the variance that cannot be explained by the regression (σ_{b2}^2); σ_r^2 was calculated by subtracting σ_{b2}^2 from σ_{bi}^2 . The σ_w^2 consisted of genetic and environmental variance (σ_e^2). Within-cross genetic variance (σ_{wg}^2) was calculated by subtracting σ_e^2 from σ_w^2 . Taken together, (σ_{wg}^2 and σ_{b2}^2 constitute the genetic variance derived from the deviation from the regression line towards mid-parental values.

The homogeneity of within-cross variance over crosses was tested by Bartlett's test (Snedecor and Cochran, 1972). It was not significantly rejected at $P = 0.05$.

The deviation of the progeny mean value from the regression line was tested for the normality of the distribution by Kolmogorov-Smirnov's one sample test (Campbell, 1974), and the normality was not rejected at $P = 0.05$.

Estimation for environmental variance. The σ_e^2 was the environmental variance in measurements of 10 fruit on a single tree in a single year after the yearly adjustment. Generally, the environmental variance of mean performance in the individual in progeny (σ_e^2) may be expressed as $\sigma_e^2 = \sigma_f^2/(ytf) + \sigma_t^2/t + \sigma_{ty}^2/(ty) + \sigma_y^2/y$, where σ_f^2 is the variance among fruit within a tree, σ_t^2 is the variance among trees, σ_{ty}^2 is the variance due to interaction between genotype and year, σ_y^2 is the variance due to interaction between tree and year, y is the number of years repeated, t is the number of trees, and f is the number of fruit per tree (Yamada et al., 1993). The σ_e^2 was estimated as $(\sigma_f^2/10) + \sigma_t^2 + \sigma_{ty}^2 + \sigma_y^2 + \sigma_e^2$ ($f = 10$, $t = 1$ and $y = 1$). The σ_e^2 is an estimate of the error variance due to the adjustment for yearly variation. It was estimated as $(\sigma_f^2/15 + \sigma_{ty}^2 + \sigma_y^2)/19$, which is the variance of the yearly mean of 19 control genotypes derived as previously described by Yamada et al. (1994a). Estimates of environmental variance components were previously obtained (Yamada et al., 1993).

The environmental variance included in mid-parental values (σ_{MPF1}^2) was estimated similarly as $\{(\sigma_f^2/35 + \sigma_{ty}^2 + \sigma_y^2 + \sigma_e^2)/3 + (f = 35, t = 1 \text{ and } y = 3)\}$, and here the variance among mid-parents is half of the variance among individual parents. The adjustment for yearly variation was conducted for the data obtained each year.

In practical breeding, selection of parents for the next generation out of the progeny population, is often based on performance in small samples with a small number of yearly repetitions. A

longer period for evaluating the genetic property of parental candidates would delay the genetic improvement, although it may improve the heritability. Therefore, the expected proportion of individuals in progeny exceeding a given critical value was also estimated from the mid-parental value based on the determination of each of 10 fruit on a single tree without yearly repetition. The environmental variance in mid-parents was calculated as $\{(\sigma_f^2/10) + \sigma_{ty}^2 + \sigma_y^2 + \sigma_e^2 + \sigma_t^2\}/2$ (σ_{MPE2}^2 , $f = 10$, $t = 1$ and $y = 1$), following the previous equation.

According to Snedecor and Cochran (1972), the regression coefficient (b) of progeny mean on mid-parent based on the repetition of 35 fruit per year on a single tree for three years may be expressed as $b = \beta / \{1 + (\sigma_{MPE1}^2 / \sigma_{MPG}^2)\}$, where β is the regression coefficient of progeny mean on the genotypic value of mid-parent, and σ_{MPG}^2 is the variance among the genotypic values of mid-parent. The regression coefficient (b') of progeny mean on mid-parent based on the repetition of 10 fruit on a single tree without yearly repetition may be expressed as $b' = \beta / \{1 + (\sigma_{MPE2}^2 / \sigma_{MPG}^2)\}$, and $b' = b \{1 + (\sigma_{MPE1}^2 / \sigma_{MPG}^2)\} / \{1 + (\sigma_{MPE2}^2 / \sigma_{MPG}^2)\}$.

Results

Mean FRT for mid-parental value and progeny mean were 5.18 and 5.08, respectively. According to the regression analysis (Fig. 1), the estimated progeny mean was given by $Y = 0.99 MP - 0.04$ and the SE of the coefficient was 0.10, where Y is the estimated progeny mean in the cross.

The regression was significant at $P = 0.01$ (Table 1). The σ_{wg}^2 and σ_{b2}^2 were estimated as 1.68 and 0, respectively (Table 2). The genetic difference among crosses was hardly influenced by factors other than MP-value. Thus, σ_{wg}^2 was considered solely as the genetic variance derived from the deviation from the regression line. The deviation of the value in each progeny individual from the regression line was tested for the normality of the distribution by Kolmogorov-Smirnov's one sample test, and the normality was not rejected at $P = 0.05$. The genotypic values of progeny individuals from a cross were therefore assumed to be normally distributed around the estimated value on the regression line with the variance of σ_{wg}^2 .

Genetic variances were estimated as 1.13 for the mid-parents and 2.62 for the progeny, respectively. The environmental variance of the mid-parental value (σ_{MPE1}^2) was estimated to be 0.041, which was only 2.4 % of σ_{wg}^2 . This is almost negligible in relation to the expected proportion of genetic values of individuals in progeny. The environmental variance for within-cross variance (σ_e^2) was also small (1.4 % of σ_{wg}^2).

Figure 2A indicates the expected proportion of individuals in progeny with a genotypic value earlier than a critical value predicted from the mean performance in parents which were determined using 35 fruit samples on a single tree for three years. The probabilities for individuals ripening before early October decreased in relation to the mid-parental values, 52% for MP = 2 (early October), 24% for MP = 3 (mid-October) and 7% for MP = 4 (late October). The equivalent probabilities for progeny individuals ripening earlier than mid-October, 79% (MP = 2), 52% (MP = 3) and 24% (MP = 4).

The expected proportion of individuals in progeny predicted from the performance of parents based on 10 fruit on a single tree without yearly repetition was calculated (Fig. 2B). In this case, the environmental variance of mid-parent (σ_{MPE2}^2) was also small (0.141), compared to σ_{wg}^2 . The regression coefficient (b') was 0.91. The probabilities of individuals in progeny ripening earlier than early October were 44% (MP = 2), 20% (MP = 3), and 6% (MP = 4).

Fig. 1. Relationship between mid-parent and progeny mean in a cross for fruit ripening time index. Fruit samples in progeny and parents were evaluated for fruit ripening time rated as the index on a 1 to 8 scale; 1 = late September, 2 = early October, 3 = mid-October, 4 = late October, 5 = early November, 6 = mid-November, 7 = late November, and 8 = early December. Mid-parental values were obtained from mean parental performance measured for three years, using 35 fruit on a single tree. Each point represents the mean of three progeny individuals in each cross.

Discussion

The population analyzed here is a slightly inbred one. Fruit weight was markedly affected by the inbreeding coefficient for the same population (Yamada et al, 1994b).

Inbreeding depression and heterosis occur when the dominance of the genes concerned is preponderant in one direction (Falconer, 1989). If no dominance effects are found, the regression coefficient of progeny mean on mid-parent corresponds to the narrow-sense heritability even in an inbred population. In a random mating population, the overall mean of progenies in the population equals the mean of parental population, but in an inbred population these are different, to a degree which is determined by dominance effect and inbreeding coefficient (Wricke and Weber, 1986).

In the present study, means of mid-parents and progeny means were nearly equal, i.e., 5.18 and 5.08, respectively. Moreover, the differences among crosses in progeny could be solely explained by

the regression on mid-parent. These results suggest that little or no dominance effects are involved in FRT. Therefore, it was suggested that the regression coefficient of progeny mean in a cross on MP represented the narrow-sense heritability (Falconer, 1989), although this population was slightly inbred. The heritability was suggested to be as high as 0.99.

Since the environmental variation included in mid-parental value was very small, precise determination of genetic value of mid-parent was available for 35 fruit from a single tree over 3 years.

Generally, there are two factors causing reduction in a narrow-sense heritability, the parental environmental variance, and the dominance variance. The breeder can raise the heritability for the environmental effect in parent by increasing the replication and repetition of measurement, but not for the dominance effect. Heritability alone does not provide the information on factors causing reduction in the heritability. Little or no dominance effect was found for FRT and the heritability can be raised up to a very high value.

Between-cross variance in progeny, which is the variance among progeny means with an infinite number of individuals in each cross, could be largely explained by the regression of progeny mean on mid-parent. Therefore, the progeny mean in a cross can be precisely estimated. The expected proportion of individuals in progeny with fruit ripening time equal or superior to a given critical value, without environmental bias, can be easily computed when the assumption of homogeneity of within-cross variance and the normality of within-cross distribution of individuals in progeny hold.

Our results showed that we should grow twice as many individuals for MP = 3 than for MP = 2, and seven times as many for MP = 4, to get individuals in progeny earlier than early October. And, we can hardly expect such type of individuals in progeny for MP > 5. The within-cross variance in progeny was not large so that only early ripening mid-parents yielded early ripening individuals in progeny.

Pollination constant-nonastringent cultivars are desirable, but rare among native cultivars and nearly all late ripening (Yamada, 1993). The trait is qualitatively inherited and PCNA is recessive to non-PCNA (Ikeda et al., 1985). Only a small percentage of PCNA individuals in progeny could be obtained even in the backcrossing of non-PCNA F₁ hybrids (PCNA x non-PCNA), to PCNA. At the beginning of the breeding program at FTRS, crosses had been made mostly among late ripening PCNA cultivars. Little progress towards early ripeness was made; several cycles of selection have produced early ripening PCNA individuals (Yamada, 1993). The difficulty in the task is explained by a high narrow-sense heritability and fairly small within-cross variance.

In fruit breeding, selections from progeny or newly introduced

Table 1. Analysis of variance table for regression of progeny mean on mid-parental value for fruit ripening time of Japanese persimmon, using 117 progeny individuals from 39 crosses.

Sources of variation	df	SS	MS	F	Expected MS ²
Between-cross	38	181.25	4.77	2.43**	$\sigma_B^2 = \sigma^2 + 1/38 \sigma_R^2 = \sigma_w^2 + 3 \sigma_{bl}^2$
Regression ¹	1	130.67	130.67	95.38**	$\sigma^2 + \sigma_R^2$
Residual	37	50.58	1.37		σ^2
Within-cross	78	153.01	1.96		σ_w^2
Total	116	334.26			

² $\sigma^2 = \sigma_w^2 + 3\sigma_{bl}^2$, $\sigma_{bl}^2 = \sigma^2 + \sigma_{b2}^2$.

¹Regression of progeny mean in a cross on mid-parent.

**Significant at $P = 0.01$.

Fig. 2. Proportion of individuals in progeny having genotypic values for fruit ripening time earlier than a critical value, estimated from mean parental performance in measurements (A) repeated for three years, using 35 fruit every year on a single tree, (B) without yearly repetition, using 10 fruit on a single tree. See Fig. 1 for rating for fruit ripening time. Lower case letters indicate critical values in selection, (a) earlier than early October, (b) mid-October, (c) late October, (d) early November, (e) mid-November and (f) late November. Vertical bars in A indicate the range of the expected proportion in $\pm$ SE points of progeny mean in a cross predicted by the regression, assuming the estimates of diverse variance components as valid.

Table 2. Estimates of variance components obtained from the analysis of variance, using 117 progeny individuals in 39 crosses.

Variance component	Estimate
Between-cross (σ_{bl}^2)	0.94
Regression ^a (σ_r^2)	1.13
Residual (σ_{b2}^2)	-0.19 (0)
Within-cross (σ_w^2)	1.96
Genotype (σ_{wg}^2)	1.68
Environment ^y (σ_e^2)	0.28
Total	2.90

^aRegression of progeny mean in a cross on mid-parent.

^yVariance computed from estimates of variance components in the previous report (Yamada et al., 1993). Performance of individual in progeny was based on the measurement for 10 fruit on a single tree without yearly repetition.

genotypes for which the characteristics are not well known are often used as parents. Breeding efficiency depends on selecting parents soon after introduction based on performance of a small sample for a short period, as opposed to evaluation over several years.

Our results show that the expected proportion of individuals in progeny exceeding a given critical value can easily be predicted from the performance of parents measured with 10 fruit from a single tree in a given year. The regression coefficient was as high as 0.9 1, even for such a limited measurement.

The expected proportion of individuals in progeny was not markedly different from that calculated by parental performance with 35 fruit on a single tree for three years, because the heritability in a broad sense is high for the trait (Yamada et al., 1993). It is concluded that early ripening selections, even if based on the performance of a small sample as adopted here, can be effectively used as parents for advancing fruit ripening time.

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