FREQUENCY OF CHLOROPHYLL-DEFICIENT AND OTHER
DETRIMENTAL GENES IN JAPANESE POPULATIONS
OF BUCKWHEAT, *FAGOPYRUM*
ESCULENTUM MOENCH¹⁾

OHMI OHNISHI

Laboratory of Genetics, Faculty of Agriculture,
Kyoto University, Kyoto 606

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The frequency of chlorophyll-deficient genes and other detrimental per gamete was estimated by conducting more than 1,000 full-sib matings in six Japanese populations of buckwheat. The following types of abnormalities were observed in cotyledons; albinos 0.2%, yellows 3.7%, pale yellows 4.5%, pale green 5.6%, variegateds 3.4% and morphological abnormalities 1.9%. Each type of abnormality was also found in the foliage leaves at about a half the frequency in the cotyledons. The total frequencies were not very different among the populations, they ranged from 22 to 34%, except in the Togakushi population where the frequency was very low, 12%. The frequencies of all the abnormalities except the morphological one were also examined in random mating populations in farmer's fields. The total frequency of homozygotes was very low, around 0.02-0.05% in the cotyledons and not much different in the foliage leaves, probably because the population sizes are large and breeding is by insect pollination.—Based on the frequencies of abnormalities in the inbreeding experiment and those in random mating populations, the mechanism whereby detrimental genes are maintained in buckwheat populations was discussed. It seems that such detrimental genes are maintained by mutation-selection balance.—Information on the contribution of other factors to genetic loads, such as female sterility genes and cytoplasmic factors, were also given.

INTRODUCTION

It is well known that buckwheat is heterostylous, consisting of pin and thrum forms, and each form can be fertilized only by pollen from the opposite form. There-

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fore, cultivated buckwheat populations are completely outcrossing. In Japan, buckwheat has been one of the most important crops and has been cultivated in almost all parts of the country. However, no attempt at systematic breeding has been tried during the history of its cultivation. Farmers cultivate buckwheat every year from the seeds they harvested the previous season. From the view point of population genetics, the buckwheat population in a local village can be considered to be a large Mendelian population, usually consisting of 10^4 – 10^6 individuals.

Because of obligatory outcrossing, a large population size and the absence of systematic artificial selection, we expect that populations of buckwheat cultivated in Japan maintain a large amount of genetic variation, not unlike that found in human, *Drosophila* and other animal populations (see, for example, Dobzhansky 1951, 1970). An assesment of the frequency of recessive lethals and other detrimental genes and the elucidation of their place in the genetic variability of a population have, for most part, been attempted only with *Drosophila* species. However, some information indicates that there are high frequencies of detrimental genes in outbreeding plant populations, such as maize (Woodworth 1929, Wents and Goodsell 1929, Sprague and Schuler 1961), orchard grass (Apirion and Zohary 1961, Curran 1963), perennial ryegrass (Jenkin 1931), timothy (Nielsen and Smith 1959, Wexelsen 1941) and sweet cherry (Williams and Brown 1956) (see also a review by Crumpacker 1967). Although most analysis of concealed genetic variability in plant populations is now accomplished by studying isozyme variation, there is still a need to determine the extent of variability attributable to deleterious recessive genes.

In the present study, the frequency of detrimental genes, primarily chlorophyll-deficiencies, in random mating populations from six villages or towns in Japan was estimated by inbreeding experiments. An analysis of the number of loci involved and the frequency of deleterious alleles at each locus has led to a simple hypothesis in which mutation-selection balance accounts for the maintenance of observed genetic variation.

MATERIALS AND METHODS

The populations investigated in the present study were those from Noheji town, Aomori prefecture, Iwate t., Iwate pref., Miwa village, Ibaragi pref., Togakushi v., Nagano pref., Higashi-Iyayama v., Tokushima pref., Geihoku t., Hiroshima pref., the locations of which are shown in Fig. 1. Buckwheat from Noheji and Togakushi belong to the summer type. Seeds of this type are usually sown in the late spring and harvested in the mid-summer. The other material is of the fall type. With these, the seeds are sown between July and September and harvested during late September through early November. The main difference between the two types lies in the photoperiod, but the variation in this seems to be continuous and there exists no critical morphological difference between them.

Detrimental genes in populations were identified by performing full-sib matings. For each sib-line, two individuals of normal phenotype arbitrarily chosen from a popu-

Fig. 1. Location of the populations studied.

lation, one pin and one thrum, were crossed, and in the next generation one pin and one thrum were again randomly chosen from the F_1 progeny and crossed to give a sufficient number of F_2 progeny. Approximately 1,000 sib-lines were established in the present study. The F_2 individuals which germinated were examined for the abnormalities of the cotyledons at the time when the two cotyledons had just opened. The individuals were re-examined for leaf abnormalities when the two foliage leaves had appeared. The detrimental abnormalities investigated in this study were mostly those of chlorophyll-deficiencies which will be described in more detail in the section on experimental results. To minimize the possibility of missing a recessive homozygote in crosses of two heterozygous sibs, the number of F_2 individuals examined in each line was always greater than 20. If the segregation ratio of normal to abnormal plants conformed to the 3 : 1 ratio as judged by a χ^2 test, the abnormality was considered to be due to a recessive gene. Thus, only recessive abnormalities were scored in these experiments. Since buckwheat is a diploid species, the possibility of a 15 : 1 or a 63 : 1 segregation is extremely low. When abnormals were much less frequent than expected, the case was disregarded. The labor needed to clarify these ambiguous cases was too great to be practical.

The frequency of sib-lines which segregated recessive homozygotes gives an estimate of the frequency of detrimental genes per gamete in a population. Let x be the frequency of detrimental genes per gamete. Performing a cross $+_1/+_2 \times +_3/+_4$ of randomly chosen two normal individuals, we have $+_1/+_3$, $+_1/+_4$, $+_2/+_3$ and $+_2/+_4$ progenies in equal frequency. The probability of detection of recessive genes in the $+_1/+_3 \times +_1/+_3$ type crosses in F_2 is $2x$ by counting two independently segregating abnormalities as twice the occurrence of recessive genes, and that in the $+_1/+_3 \times +_1/+_4$ and $+_1/+_3 \times +_2/+_3$ type crosses is x . We have no chance of detection in the $+_1/+_3 \times$

$+_2/+_4$ crosses. The probability of each type of cross is $1/4$, hence the chance of detection of recessive genes in the full-sib analysis is equal to the frequency of detrimental genes per gamete.

For the populations other than Noheji and Geihoku, the frequency of phenotypically abnormal individuals in random mating populations was studied by examining the individuals in the farmer's field at the cotyledon and foliage leaf stages. On account of the similarity between the recessive homozygotes in the inbreeding experiment and the individuals showing abnormal phenotypes in the field, most of the abnormal individuals in the field were considered to be homozygous for a recessive detrimental gene. Morphological abnormalities were excluded in this survey, since the heritability of those detected in the field was nearly impossible to judge.

RESULTS

The classification of abnormal individuals was arbitrary, but done so that the data were compatible with similar investigations in maize and other species (see the classification in Crumpacker 1967). The classes of abnormalities include (see Fig. 2): 1) Albinos—completely lacking chlorophyll and carotinoids, so that the color of the cotyledons and leaves is white. Individuals of this type were always lethal. 2) Yellows—lacking chlorophyll but having carotinoids, so the color is dark yellow or orange yellow. Plants of this type were also lethal. 3) Pale yellows—not to be confused with yellow, individuals of this type had pale yellow cotyledons or leaves and were usually lethal, but they were able to survive in few cases. 4) Pale greens—this type was indistinguishable in some cases from the pale yellow type. Pale green individuals sometimes survived and occasionally set seeds. 5) Variegated plants—part of the cotyledons or leaves was white or yellow, with white striped or spotted leaves often observed. Individuals in this type were often viable and survived to the stage of seed set, but in many cases they were lethal. 6) Morphological abnormalities—this class included such abnormalities as dwarf, gigantic, curled cotyledons and folded cotyledons. Morphologically abnormal individuals usually died before the mature stage.

The frequency of inbred lines which segregated homozygotes of detrimental recessive genes is shown in Table 1. In a few cases, an inbred line had two independently segregating recessive genes and the segregation ratio was $9:3:3:1$ by the χ^2 test. This was counted as twice the occurrence of recessive genes. Chlorophyll-deficient phenotypes appeared independently on cotyledons and on foliage leaves in some cases, meaning that some genes affected only cotyledons and others the foliage leaves. Several recessive genes, however, affected both cotyledons and leaves simultaneously, although the phenotypes on the two were not necessarily the same. The figures in the brackets in the column concerning foliage leaves in Table 1 show the number of such cases. Among the detrimental genes affecting foliage leaves, 46% affected cotyledons as well as leaves. The ratio of the frequency of detrimentals in cotyledons to that in foliage leaves was approximately $2:1$ (19.4% to 9.4%).

The frequency of sib-lines with a detrimental gene, which is equal to the frequency

Fig. 2. Examples of chlorophyll-deficient and other detrimental phenotypes. A) Albinos of the foliage leaves. B) Yellows. C) Pale yellows of the foliage leaves. D) Pale greens of the foliage leaves (marked by a piece of red string). E) Variegated plants both on cotyledons and foliage leaves.

Table 1. The frequency of sib-lines with a detrimental gene

Population	No. of tested lines	No. of sib-lines with a detrimental gene affecting		
		Cotyledons	Foliage leaves	Total
Noheji	160	39 (24.4%)	19 (9) * (12.0%)	49 (30.6%)
Iwate	193	37 (19.2)	17 (11) (8.8)	43 (22.3)
Miwa	128	33 (25.8)	19 (8) (14.8)	44 (34.4)
Togakushi	297	26 (8.8)	13 (3) (4.3)	36 (12.1)
Higashi Iyayama	145	34 (23.5)	19 (6) (13.1)	47 (32.4)
Geihoku	94	28 (29.8)	9 (7) (9.6)	30 (31.9)
Total	1017	197 (19.4)	96 (44) (9.4)	249 (24.5)

* The number of lines with a gene affecting both cotyledons and foliage leaves.

of detrimental genes per gamete, was the highest in the Miwa population (34.4%) and the lowest in the Togakushi population (12.1%). Roughly speaking, the frequencies were rather uniform, with the mean 24.5% within the range 22.3–34.4% throughout all populations, except Togakushi.

Table 2 gives the frequencies of six types of detrimental in each population. The spectrum of relative frequencies was slightly different among the populations from different localities. For example, individuals of variegated type were seldom found in the Iwate population, and the yellow type of cotyledon appeared in a high frequency in the Higashi-Iyayama population. Albinos, either in cotyledons or leaves, and leaf yellows were extremely rare in all populations, although cotyledon yellows were rather common.

Throughout the present study, much attention was given to detrimental genes at the seedling stage, for seedlings are handled with little effort. Additional data on the frequency of female sterility genes were obtained by permitting individuals in some inbred-lines to grow to maturity. The data for several localities are presented in Table 3. The frequency was relatively high in the two summer type populations, Noheji (6.45%) and Togakushi (5.98%). At present, I am not sure whether this is attributable to a characteristic of the summer type or not.

Table 4 presents the number of observed individuals and the number of phenotypically abnormal individuals in random mating populations in farmer's fields. Two populations from Noheji and Geihoku were not included in the field survey. The values in the brackets are the number of the cases in which an individual showed abnormalities both on cotyledons and foliage leaves. The relative frequencies of each type were roughly proportional to those of the inbreeding experiments. The percentage of abnormal individuals in cotyledons was highest in the Higashi Iyayama population (0.051%) and lowest in the Togakushi population (0.024%). This tendency was also observed with respect to the frequency of foliage leaf abnormalities. The ratio of the frequency of cotyledon abnormalities to leaf abnormalities was approximately 1:1, quite different from the 2:1 observed in the inbreeding experiments.

Table 2. Frequency of sib-lines with a detrimental gene in each type

Population	Type of abnormality						
	Albinos	Yellows	Pale yellows	Pale greens	Variegateds	Morphologicals	Total
Cotyledons							
Noheji	1* (0.6%)	6 (3.8%)	8 (5.0%)	13 (8.1%)	10 (6.3%)	1 (0.6%)	39 (24.4%)
Iwate	0	6 (3.2)	14 (7.3)	11 (5.7)	1 (0.5)	5 (2.6)	37 (19.2)
Miwa	0	8 (6.3)	8 (6.3)	8 (6.3)	7 (5.5)	2 (1.6)	33 (25.8)
Togakushi	0	4 (1.3)	5 (1.7)	8 (2.7)	6 (2.0)	3 (1.0)	26 (8.8)
Higashi Iyayama	1 (0.7)	11 (7.6)	5 (3.4)	6 (4.1)	6 (4.1)	5 (3.4)	34 (23.5)
Geihoku	0	3 (3.2)	6 (6.4)	11 (11.7)	5 (5.3)	3 (3.2)	28 (29.8)
Total	2 (0.2)	38 (3.7)	46 (4.5)	57 (5.6)	34 (3.4)	19 (1.9)	197 (19.4)
Foliage leaves							
Noheji	0	0	9 (7)** (5.6%)	7 (1) (4.3%)	1 (0.6%)	2 (1) (1.3%)	19 (9) (12.0%)
Iwate	1 (1) (0.5)	1 (1) (0.5)	8 (5) (4.1)	3 (1) (1.6)	0	4 (3) (2.1)	17 (11) (8.8)
Miwa	0	0	10 (4) (7.8)	6 (4) (4.7)	1 (0.8)	2 (1.6)	19 (8) (14.8)
Togakushi	1 (1) (0.3)	0	3 (2) (1.0)	2 (0.7)	2 (0.7)	5 (1.7)	13 (3) (4.3)
Higashi Iyayama	1 (1) (0.7)	0	4 (2) (2.8)	8 (5.5)	3 (1) (2.1)	3 (2) (2.1)	19 (6) (13.1)
Geihoku	0	0	2 (2) (2.1)	3 (1) (3.2)	1 (1) (1.1)	3 (3) (3.2)	9 (7) (9.6)
Total	3 (3) (0.3)	1 (1) (0.1)	36 (22) (3.5)	29 (7) (2.9)	8 (2) (0.8)	19 (9) (1.9)	96 (44) (9.4)

* For the number of tested lines, see Table 1.

** The number of lines with a gene affecting both cotyledons and foliage leaves.

Table 3. The frequency of sib-lines with a female sterility gene

Population	No. of lines tested	No. of female sterile lines
Noheji	62	4 (6.45%)
Iwate	88	0 (0.00)
Miwa	71	1 (1.41)
Togakushi	117	7 (5.98)
Higashi Iyayama	56	1 (1.79)
Total	394	13 (3.30)

DISCUSSION

Before discussing the maintenance of chlorophyll-deficient and other detrimental genes in buckwheat populations, I would like to mention some genetic characteristics of the plant species itself.

According to the summary of Crumpacker (1967), the frequencies of various classes of chlorophyll-deficient genes in maize are: white 5.5%, yellow 3.9%, yellow green 0.3%, pale green 3.3%, virescent 2.6% and striped 3.1%. In other monocotyledon species, as with maize, the most frequently appearing type is the albino. Although the classification criteria used in the present study were not the same as those used for maize, the low frequency of albino (only 0.2%) and the predominance of yellows and pale yellows (3.7 and 4.5% respectively) indicate that buckwheat populations have a characteristic assemblage of deleterious genes. This might be attributed to the difference between monocotyledons and dicotyledons.

Furthermore, in contrast to monocotyledons such as maize, some recessive genes in buckwheat affect both cotyledons and foliage leaves. Among 249 sib-lines identified as segregating detrimental genes, 153 carried genes affecting only the cotyledons, and 52 had genes affecting only the foliage leaves; the remaining 44 had genes affecting both.

Only two summer type populations were investigated in the present study, but after taking the data of the Noheji population into the account, there exists no critical difference between summer and fall types. One might speculate that populations of the summer type have less genetic variation because there has been a sharp decline in the cultivation of this type in the last several decades, causing, of course, a reduction in population size. This is not born out by the data.

Ujihara and Matano (1974) observed a cline in quantitative characters in Japanese populations of buckwheat. They studied several quantitative characters, mostly those associated with photoperiod such as flowering time, and identified a north-south cline. As far as detrimental genes are concerned, there is no indication of a cline.

Now, I shall consider the maintenance of detrimental genes in buckwheat population. From the inbreeding data, it appears that the frequency of detrimental genes per gamete in cultivated populations is around 22-34%, except in the Togakushi population where the frequency is much lower (12.1%). Although we are largely ignorant

Table 4. Frequency of phenotypically abnormal individuals in random mating populations

Population	No. of observed individuals	Type of abnormality					
		Albino	Yellow	Pale yellow	Pale green	Variegated	Total
Cotyledons							
Iwate	139411	0	9 (.006%)	8 (.006%)	6 (.004%)	10 (.007%)	33 (.024%)
Miwa	99746	0	14 (.014)	7 (.007)	3 (.003)	2 (.002)	26 (.026)
Togakushi	473076	0	30 (.006)	16 (.003)	9 (.002)	57 (.012)	112 (.024)
Higashi Iyayama	152183	1 (.001)	22 (.014)	16 (.011)	23 (.015)	16 (.011)	78 (.051)
Total	864416	1 (.000)	75 (.009)	47 (.005)	41 (.005)	85 (.010)	249 (.029)
Foliage leaves							
Iwate	139411	0	3 (1)* (.002)	14 (.001)	13 (.009)	11 (7) (.008)	41 (8) (.029)
Miwa	99746	0	2 (1) (.002)	6 (1) (.006)	8 (.008)	3 (.003)	19 (2) (.019)
Togakushi	473076	6 (.001)	4 (1) (.001)	23 (7) (.005)	31 (3) (.007)	68 (19) (.014)	132 (30) (.027)
Higashi Iyayama	152183	0	2 (.001)	17 (1) (.011)	32 (2) (.021)	17 (4) (.011)	68 (7) (.045)
Total	864416	6 (.001)	11 (3) (.001)	60 (9) (.007)	84 (5) (.010)	99 (30) (.011)	260 (47) (.030)

* The number of individuals showing abnormalities both in cotyledons and foliage leaves.

about genetic deaths in other developmental stages, my unpublished data suggest that the genetic deaths caused by major genes affecting germination are negligible compared with those occurring in the seedling stage. Therefore, the frequency of detrimental genes obtained in the present study may be compared with the frequency of chlorophyll-deficient genes in other plant species and with the frequencies of lethal genes in *Drosophila*. The heterozygosity for chlorophyll-deficient genes in cross-pollinating species other than maize is 22% (12.5–67%) and 24% (0–92%) in maize (Crumpacker 1967). The estimated value for buckwheat is between 39 and 56%, assuming the random occurrence of mutant genes in gametes. This is higher than that for other plants studied. Only the heterozygosity in Togakushi, being 23%, lies near the mean of the other species.

In many respects, e.g., the low frequency of detrimental, the relatively high frequency of the variegated type and the unexpectedly high frequency of abnormalities in the field, the Togakushi population is quite different from others. At present, I hesitate to conclude that the Togakushi population has a low frequency of detrimental genes because of the past history of cultivation in this village. This is, however, the most promising working hypothesis.

Chlorophyll-deficient and other detrimental abnormalities were observed in cotyledons in 0.02–0.05% of the field plants and in foliage leaves at about the same frequencies. These values are extremely low compared with the frequencies of genetic abnormalities in other outbreeding species, such as *Drosophila*, humans and maize. In these, the frequency in random mating populations is on the order of 0.1 to 1% (see Kennedy 1967 for human populations, for *Drosophila* see the review by Spencer 1947 and Dobzhansky 1970, and for maize see Tavcar 1955). Only Eiche's observation (1955) on Scotch pine trees indicates the same order of frequency of abnormals in a random mating population. There is a large difference between the frequency of abnormals in maize and buckwheat populations, and this presumably has an explanation. Tavcar (1955) observed high frequencies of abnormals in maize where the size of the population was small and some amount of inbreeding was inevitable. The low frequency in buckwheat probably due to the virtual absence of inbreeding; populations are large—usually consisting of more than 10^5 individuals—and breeding is by insect pollination: Maize has relatively small population sizes, perhaps as small as several hundred or less (see Crumpacker 1967), and some amount of inbreeding.

Now the critical questions which must be answered are how many loci are involved in the segregation of the buckwheat abnormalities and what is the frequency of detrimental genes at each locus. Let n be the number of loci segregating detrimental genes and p_i be the frequency at the i -th locus. Then, the frequency of detrimental genes per gamete is $A = \sum_{i=1}^n p_i$ and the frequency of homozygotes in random mating population is $B = \sum_{i=1}^n p_i^2$. A and B were estimated in the present study. The observed frequency in random mating populations was extremely low. This implies that the observed data can be explained by taking n large and p_i small. Therefore, it may be more sensible to consider the minimum number of loci. Since $\sum_{i=1}^n p_i^2$ is minimum when all the p_i 's are equal under the condition of constancy of $\sum_{i=1}^n p_i$, we can estimate the minimum number of loci, N , by A^2/B and the maximum frequency of p_i , P , by

B/A. Although the data, particularly in random mating populations, are still too scanty to give very satisfactory values for N and P , we can apply the formulae given above to the data for each class of abnormality in Togakushi and Higashi-Iyayama where the sizes of the field samples were relatively large. For Togakushi, P lies between 1.1×10^{-3} (for pale greens) and 10.3×10^{-3} (for variegated leaf abnormalities) and the corresponding N 's are 17.1 and 1.4. By summing up all classes, we have the total number of loci, which is 38, and the average frequency of a detrimental gene, which is 2.5×10^{-3} . For the Higashi Iyayama population, P lies between 1.0×10^{-3} for albinos and 5.1×10^{-3} for variegated leaf abnormalities, and the number of loci is in the range of 4.3 for variegated leaf abnormalities to 36.8 for yellow cotyledons. The total number of segregating loci is 134 and the average maximum detrimental gene frequency is 2.2×10^{-3} . Similar figures were obtained in other populations. Therefore, in all cases the frequency of detrimental genes per locus is less than 10^{-2} and the number of segregating loci is several tens to more than a hundred.

The discrepancy due to a high frequency of detrimental homozygotes in Togakushi field plants as compared with the frequency seen in the inbreeding experiment can now be interpreted on the basis of the relatively high frequencies of detrimentals at a small number of loci. A question yet remains: Why are detrimental genes maintained at relatively high frequencies there?

So far, I have not found a case where a chlorophyll-deficient gene is maintained by balanced polymorphism, as was observed in orchard grass (Apirion and Zohary 1961; Curran 1963) and probably in maize (Sprague and Schuler 1961). Thus, most of the detrimental genes in buckwheat populations, and probably all of those observed in the present study, are likely to be maintained by mutation-selection balance, as envisioned by the so-called classical theory of genetic variation.

In the present report, the frequency of chlorophyll-deficient genes and others with detrimental effects in the seedling stage were investigated; female sterility genes were also studied. There appear to be many factors which contribute to the genetic load in buckwheat populations. Some of these factors are polygenic, but others are major genes. The frequency of female sterility genes was 0-6.5% (Table 3) and male sterility genes are likely to be equally frequent, judging from *Drosophila* data (Oshima and Watanabe 1973). In a brief survey of detrimental polygenes affecting germination and seed production in Iwate and Togakushi buckwheat populations, the loads attributable to these (in terms of lethal equivalents) were 0.30 and 0.31 for germination and 1.07 and 1.19 for seed production, in the two respective populations (Ohnishi 1978). Another factor contributing to the load and revealed in this investigation is a cytoplasmic one, although the data were not described in the section on experimental results. In the F_2 tests, some sib-lines segregated abnormals in a 3:1 ratio in only one of the two reciprocal crosses. The frequencies were 0.78% in Miwa, 0.79% in Togakushi, 2.07% in Higashi-Iyayama and 1.06% in Geihoku. No such case was found in Noheji and Iwate. Local differentiation of cytoplasmic factors may have occurred, although further studies are needed to confirm this. More detailed pursuit of this and other issues concerning genetic loads in buckwheat populations are now under investigation.

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