**Population genetics of cultivated common buckwheat,
Fagopyrum esculentum Moench. IX. Concluding
remarks on worldwide survey of
allozyme variability**

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ABSTRACT

By adding data on allozyme variability in 35 populations from southern and central China, I completed a worldwide survey of allozyme variability in common buckwheat. The following remarks were born out from the survey. (1) At all loci, allele frequency does not vary so much among the populations from a wide range of Asian countries. (2) Most of the evolutionary events which might occur during the spread of buckwheat cultivation are the losses of variant alleles by random drift. (3) Drastic changes of gene frequency have been observed only in marginal populations, in Kumaun, Garwhal hills and Kashmir in India and in southern Europe. (4) Cultivated buckwheat has more genetic variability than the natural populations of the wild ancestor. Large population size and complete panmixis of a population and enough migration between populations may be responsible for observation (1) and may also have led to the accumulation of variant alleles in cultivated populations, i.e. observation (4). Random drift, such as founder effect, took place only in marginal populations, accounting for observations (2) and (3). Buckwheat provides an example that contradicts Vavilov's assertion; it says that the center of genetic diversity of a cultivated plant is the place of its origin. Buckwheat is apparently monocentric, but the pattern of variation suggests domestication over a wide area, instead of progressive decline in diversity from the center of origin to the periphery. The structure of buckwheat populations and their mating system are mainly responsible for this unexpected result.

1. INTRODUCTION

Ten Years ago, when I started a worldwide survey of allozyme variability in common buckwheat (a complete outbreeder due to heterostylous incompatibility), I had hoped to find a center of allozyme diversity in buckwheat, which might represent the original place of buckwheat's domestication as Vavilov (1926)'s dogma asserts. However, as the work progressed this hope is declined (see Ohnishi, 1986, 1988).

For a long time common buckwheat has been thought to have originated in northern China or the area of the Amur river (De Candolle, 1883). My discovery

of the wild ancestor of common buckwheat (*F. esculentum* ssp. *ancestralis* Ohnishi) in Yunnan province of China (Ohnishi, 1991) now resolves a discrepancy between the restricted distribution of wild *Fagopyrum* species in southern China (Steward, 1930; Ohnishi unpublished) and De Candolle's hypothesis.

When I surveyed the allozyme variability of common buckwheat in China (Ohnishi, 1988), only a few samples were available. Now, after five years of searching for wild and cultivated buckwheat in China, many more populations are available for analysis; and the data on allozyme variability in several regions of the world have been published; see Ohnishi (1985) and Ohnishi and Nishimoto (1988) for Nepal and India, Ohnishi (1988) for Japan and Korea, Ohnishi (1992) for Bhutan, Ohnishi (1993a) for Tibet and Ohnishi (1993b) for Europe and the silk road.

By adding the data on the populations from southern China and on the wild ancestor of common buckwheat, I will give several comments on allozyme variability observed in cultivated populations from different parts of the world, and give a clue to understand the genetic processes of domestication and subsequent diffusions of common buckwheat.

2. NEW DATA ON ALLOZYME VARIABILITY OF THE WILD ANCESTOR AND THE POPULATIONS FROM SOUTHERN CHINA

Materials and Methods

The natural populations of the wild ancestor of buckwheat, *F. esculentum* ssp. *ancestralis* Ohnishi, are those found in Yunnan province, one from Yongsheng (see Ohnishi, 1991) and the other is newly found in Jinan village (10 km west from Yongsheng), Lijian xian, Yunnan. Cultivated populations from southern and central China are those collected by the author during his expeditions to China in last five years, 1988–1992.

The method of allozyme assay is by standard horizontal gel electrophoresis; the detailed procedures were described in Ohnishi and Nishimoto (1988). Two hundred individuals from each population were assayed at 19 loci affecting 12 enzymes.

Experimental results

In Table 1, the allele frequencies at nine polymorphic loci (1% criterion for polymorphism) are shown for each population. The cultivated populations from different localities (Shaanxi in north to Yunnan in south) have more or less similar allele frequencies at all loci. Although two wild populations of the ancestral species have almost the same genetic constitutions, their frequencies are quite different from those of the cultivated populations. The cultivated populations have the same alleles as the wild ancestor at many loci, but at some loci the cultivated populations seem to have lost allelic variants. Many such variants are

Locus Allele		Province		Population																	
Prefecture or city		Province		6-Fgdt-1		Pgm-2		Mth-1		Mth-3		Sth-1		Adth		Got-2		Dia-2		Pgm-1	
Town or village		S	F	N	S	F	N	F	N	U	S	F	N	U	S	F	N	U	S	F	N
1. Qingting	Shaanxi	1.0	0.0	99.0	15.0	8.3	76.7	50.8	49.2	1.8	26.3	17.3	54.6	0.5	49.5	50.0	0.0	0.0	1.3	98.7	13.3
2. Huaninpu	"	1.3	0.8	97.9	15.3	7.3	77.4	46.5	53.5	0.0	23.0	17.0	60.0	0.5	51.7	47.8	0.0	0.5	1.0	98.5	12.5
3. Markan #1	Sichuan	12.3	1.8	85.9	10.0	5.3	84.4	39.9	60.5	0.5	36.2	8.3	55.0	0.8	55.5	43.7	0.0	0.0	9.3	90.7	8.8
" #2	"	12.0	2.3	85.7	9.0	6.5	84.5	38.8	61.2	0.8	31.8	6.0	61.4	0.5	54.0	45.5	0.0	0.0	10.0	90.0	9.8
5. Lixian	"	4.3	0.5	96.2	7.8	13.8	78.4	43.3	56.7	0.3	23.0	13.0	63.7	1.5	49.5	49.0	0.0	0.0	5.8	94.2	7.3
6. Guangbao	"	1.3	0.8	97.9	10.0	5.5	84.0	39.9	61.0	0.0	22.5	14.8	62.7	1.8	52.4	45.8	0.0	0.3	4.0	96.7	8.1
7. Taoping	"	2.8	1.8	95.9	12.5	8.3	79.7	36.0	64.0	0.0	23.8	21.3	54.9	3.0	48.2	48.8	0.0	0.0	4.0	96.0	9.0
8. Yenen	"	2.5	1.8	95.7	13.0	7.8	79.2	44.4	55.6	0.3	20.0	15.5	64.2	0.5	51.8	47.7	0.0	0.0	4.0	96.0	9.5
9. Maowen	Mao	0.5	0.7	98.8	10.7	7.4	81.9	39.0	61.0	0.3	24.0	17.2	61.5	1.0	46.0	53.0	0.0	0.0	1.7	98.3	11.5
10. Yamen	Wenchuan	3.5	0.5	98.2	14.5	5.3	80.2	35.5	64.5	0.3	24.3	16.0	59.4	0.5	52.2	47.3	0.0	0.3	3.0	96.7	11.0
11. Wenchuan	"	2.0	0.8	97.2	13.5	7.0	79.5	38.8	61.2	0.0	23.5	16.3	60.2	2.5	52.5	46.5	0.0	0.5	5.0	94.5	10.3
12. Yulong	"	3.0	0.5	96.4	11.8	7.5	80.7	40.5	59.5	1.0	20.0	25.0	53.5	1.3	50.2	45.8	0.0	0.3	2.5	97.2	7.3
13. Yinxing	"	2.8	1.5	95.7	7.3	9.3	83.4	36.0	64.0	0.2	17.8	19.7	62.3	1.0	51.3	47.7	0.0	0.3	2.3	97.4	9.9
14. Wushie	Hanyuan	3.0	0.3	96.7	14.5	9.3	76.2	45.5	54.5	0.3	19.3	15.3	64.6	0.3	45.3	54.4	0.0	0.0	2.0	98.0	11.8
15. Guangho	"	3.5	1.0	95.5	12.5	8.3	79.2	39.8	60.2	0.3	20.8	11.0	67.9	1.0	43.0	56.0	0.0	0.3	3.0	96.7	11.0
16. Zhaojiao	"	4.0	0.3	95.7	13.0	9.3	77.7	38.5	61.5	0.8	22.8	14.0	62.4	1.0	46.0	53.0	0.0	0.0	2.8	97.2	7.0
17. Yanyuan	Yunnan	3.0	0.3	96.7	13.5	8.5	78.0	35.8	64.2	0.8	27.0	14.0	58.2	1.8	50.3	47.9	0.0	0.0	2.8	97.2	7.5
18. Yongsheng	Yunnan	1.3	0.5	98.2	15.8	8.3	75.9	40.8	59.2	0.3	24.0	11.3	64.4	1.0	50.5	48.5	0.0	0.8	1.5	97.7	10.0
19. (west)	"	2.3	0.4	97.3	13.4	8.0	78.6	29.6	70.4	0.3	20.3	8.5	70.9	1.8	57.7	40.5	0.0	0.4	2.1	97.5	11.0
20. Heqing	"	3.0	1.0	96.0	11.5	10.3	78.2	35.8	64.2	1.8	23.5	12.5	62.2	1.8	62.3	35.9	0.0	0.8	3.5	9	

* Natural population of the wild ancestor.

Table 2. Average heterozygosity and average number of alleles per locus in each population**

Population	Average heterozygosity	Average No. of alleles
1. Qingling	0.1556	1.74
2. Huanniupu	0.1511	1.63
3. Markan #1	0.1592	1.68
4. " #2	0.1554	1.68
5. Lixian	0.1513	1.68
6. Guanbao	0.1484	1.68
7. Taoping	0.1552	1.74
8. Yemen	0.1540	1.68
9. Maowen	0.1418	1.63
10. Yanmen	0.1498	1.63
11. Wenchuan	0.1499	1.68
12. Yulong	0.1487	1.74
13. Yinxing	0.1433	1.74
14. Wusihe	0.1513	1.63
15. Guanluo	0.1491	1.79
16. Zhaojiao	0.1545	1.68
17. Yanyuan	0.1564	1.68
18. Yongsheng	0.1487	1.68
19. " (west)	0.1431	1.68
20. Heqing	0.1555	1.84
21. Niujie	0.1453	1.79
22. Huaguanxiang	0.1521	1.79
23. Xiaguan	0.1427	1.63
24. Shalang	0.1529	1.74
25. Liujiaguan	0.1473	1.79
26. Songmin	0.1464	1.79
27. Malong	0.1472	1.68
28. Quijin	0.1385	1.63
29. Xianwei	0.1562	1.74
30. Tianba	0.1552	1.63
31. Puer	0.1507	1.74
32. Simao	0.1533	1.74
33. Liupanshui	0.1436	1.74
34. Mawei north	0.1464	1.68
35. " south	0.1514	1.63
36*. Yongsheng	0.1088	1.58
37*. Jinan	0.1049	1.42

* Natural populations of the wild ancestor

** Percentage of polymorphic loci is 47.4% for cultivated populations and 26.3% for the wild ancestral populations

maintained only in cultivated populations and not in the wild populations. These alleles must have arisen by mutation after domestication.

The average heterozygosity and other variability indices are shown in Table 2. Cultivated populations have more variability than the wild populations. Nei's genetic distance between populations (Nei, 1972) shown in Table 3 is negligible for pairs of cultivated populations in China (below 20×10^{-4}); it is large only between cultivated populations and the wild ancestor (more than 300×10^{-4}).

Table 3. Average genetic distance between the distinct groups of populations ($\times 10^{-4}$)*

Group		3-13	14-17	18-23	24-30	31-32	33-35	36-37
1- 2	5	19	14	25	20	17	17	405
3-13		14	17	15	15	12	13	343
14-17			9	20	21	12	10	380
18-23				9	13	10	14	311
24-30					15	13	13	336
31-32						10	6	322
33-35							6	342
36-37								129

* see Table 1 for identification of the population numbers.

3. COMPARISON OF ALLOZYME VARIABILITY IN DIFFERENT PARTS OF THE WORLD AND CONCLUSIONS DERIVED FROM IT

Allozyme variability in cultivated common buckwheat populations has already been reported for many parts of the world (see references mentioned above). The following remarks are based on this worldwide survey; they confirm and extend the preliminary discussion found in Ohnishi (1988).

- (1) Allele frequencies do not vary so much among populations from Asian countries, ranging from Japan to Nepal (see Fig. 1).
- (2) Most of the evolutionary events that might have occurred during the spread of buckwheat cultivation are losses of variant alleles, probably by random genetic drift.
- (3) Drastic changes of allele frequency, including losses, have been observed only in marginal populations, that is in Kumaun, Garwhal hills and Kashmir in India and in southern Europe (Ohnishi and Nishimoto, 1988; Ohnishi, 1993b; see Fig. 1).
- (4) Cultivated populations have more genetic variability than their wild ancestor (Table 2), although in Tartary buckwheat, a self-fertilizing species, wild populations have more genetic variability than the cultivated populations.
- (5) The average heterozygosity in cultivated populations seems a little bit higher than the average of other annual plant species (cf. Gottlieb, 1981).

Fig. 1. Allele distribution in Asia at three polymorphic loci, A: *Mdh-1*, B: *Pgm-2* and C: *Got-2*.

In Ohnishi (1988), I gave a reason for (1). Migration pressure nullifies the local differentiation of cultivated populations as proposed by the theory of Kimura and Maruyama (1971) and Maruyama (1971). Although we do not have any estimate of migration rate between populations, I occasionally encountered examples of long distance migration of buckwheat seeds following human migration or other human activity in Japan. Besides these facts, a large population size (10^6 or more) and panmixis of each population by insect pollination and harvest-sowing processes by man have probably led to the uniformity of gene frequency among cultivated populations of buckwheat in wide areas in Asia.

The basis for (2) and (3) is genetic random drift, including founder effects during the spread of buckwheat cultivation; these events are more likely in marginal populations, where the colonization to new areas most frequently takes place, and where the population size is relatively small.

A problem still remaining is the unexpectedly high level of allozyme variability observed in cultivated buckwheat populations: average heterozygosity = 0.15 and 50% of the loci are polymorphic (see Table 2). Table 2 apparently indicates that the cultivated populations have much more variability than the wild populations. In some plant species such as tomato, wild species have more genetic variation than their cultivated counterpart, but in other species such as barley, cultivated populations have more genetic variability (Brown, 1978). Thus, whether, the

wild or cultivated populations have more genetic variability depends on the nature of the plant's geographical distribution, its history and recent breeding practices.

Ohnishi (1991) pointed out that the high variability observed for buckwheat lies in the range expected from the neutral theory of allozyme variants; the large size of cultivated buckwheat populations is mainly responsible for this variability. Allozyme variants in wild populations of the ancestral species may also have contributed to the high level of variability in cultivated populations, since they are intercrossable. However, as we can see in Table 1, high variability in cultivated populations cannot be explained solely by the inheritance of variants from the ancestral wild species. Also the appearance of more than 10 newly arisen variants in buckwheat populations after domestication seems unrealistic. Only two mutations were fixed in cultivated Tartary buckwheat after its domestication (Ohnishi, unpublished); this species has a very similar history to that of common buckwheat.

What is a reasonable interpretation for the observed high level of genetic variability in cultivated buckwheat populations? Perhaps wild populations (or larger populations) were highly variable at the beginning of buckwheat domestication, endowing the cultivated populations with a rich supply of genetic variation. However, this is merely speculation. Alternatively, migration that annihilated the local differentiation of allele frequencies may also have brought together allozyme variants that arose in different populations. According to this hypothesis, the high variability in buckwheat is essentially due to a large population size (probably more than 10^9 as a species) and migration among subpopulations.

In buckwheat, wild populations have less genetic variability compared to cultivated ones. So far, only two such populations have been discovered and analyzed; several reports by Chinese scientists documenting other natural populations have not yet been confirmed. Of course, a small size reduces genetic variation in natural populations.

The allozyme survey did not contribute to the theory of the origin of cultivated buckwheat as one might have expected. The discovery of the wild ancestral species of common buckwheat in southern China (Ohnishi, 1991) and its narrow endemic distribution indicated that the birth place of cultivated common buckwheat was in the north-eastern part of Yunnan province. A philological survey in Yunnan province by Li and Yang (1992) supports this conclusion. Combining the worldwide survey of allozyme variability with the discovery of buckwheat's wild ancestor has made it possible to discuss the diffusion of buckwheat cultivation.

Buckwheat diffused along the southern slope of the Great Himalayas in one direction accompanied by two wild species, *F. cymosum* and *F. gracilipes*; this was the issue which I discussed in more detail in Ohnishi (1992) and Ohnishi (1993a). In another direction, buckwheat cultivation spread first to northern China, then to Europe probably through the silk road, accompanying Tartary

buckwheat as a weed. This was discussed in more detail in Ohnishi (1993b). The arguments for the diffusion of buckwheat cultivation are still incomplete, mainly because of a lack of archeological evidence and written historical documents in southern China, particularly in such minority tribes as Yi (Lolo), Naxi and Qiang. We do not know where Japanese buckwheat came from, even though we do have considerable archeological evidence bearing on the question; the history of buckwheat in Japan goes back to the Jomon era (ca. 1000BC, see for example Yasuda, 1986).

Harlan (1992) discussed the origin of *Sorghum*, a noncentric crop. Buckwheat is apparently a monocentric crop in Harlan's sense, that is, a crop with a definable center of origin and wide dispersal, without a secondary center. Yet, in Harlan's terminology, buckwheat is also noncentric because it has a pattern of variation that suggests domestication over a wide area. Harlan's terminology is therefore inadequate. Otherwise, we would have to abandon Vavilov (1926)'s dogma which asserts a strict relationship between the original place of domestication and geographical genetic variability. I believe that the main issue to be considered carefully is the species' botanical nature, primarily the structure of its populations and the breeding system. Both Harlan's argument and Vavilov's dogma are primarily for crops from predominately self-fertilizing species. For those outbreeders with a high migration rate, their principle or dogma no longer holds. This was previously pointed out by Hutchinson (1965). He stated (p. 173); "It now appears that the classical Vavilov type of distribution of variability in a crop plant, with a progressive decline in diversity from center of origin to periphery, is associated with the development of self-pollination. —Cross-pollinated plants such as maize and cotton do not exhibit the Vavilov effect, but on the contrary develop new centers of diversity,—." Buckwheat, a complete outcrossing species, did not form a new center, but rather, became widely dispersed without any local differentiation due to strong migration pressure between populations, and between distinct regions.

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