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日本における作物近縁野生種の保存：*Vigna* 属 1. Introduction

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Wild relatives of crops conservation in Japan with a focus on *Vigna* spp. 1. Introduction

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摘要

集団動態研究室では，日本における作物近縁野生種の集団構造の解析と集団の遺伝的多様性の効率的保存を目指した研究を行っている。アズキ（*Vigna angularis*）は日本に起源した可能性のある数少ない作物で，その野生型や雑草型に加えその他の近縁野生種も分布していることから，日本における作物近縁野生種研究の対象として最適である。ここでは，これまでに収集した材料を用いた研究結果の要約と，1998年に行った５つの探索およびモニタリング調査の概要を述べる。

Summary

The following 5 collecting and monitoring reports continue the research reported earlier (Tomooka, 1997; Tomooka et al., 1998a,b,c; Vaughan et al., 1998). The object of this introduction is to explain the rationale behind the series of collecting trips reported here and to summarise the results of these missions. In addition a brief summary of the research results obtained from earlier collection missions with respect to *Vigna* is provided.

KEY WORDS: *Vigna*, wild azuki bean, in-situ conservation, genetic resources

The *Vigna angularis* complex （アズキ野生・雑草・栽培複合）

(a) Some historical comments. （歴史的考察）

The Jomon era (縄文時代) of Japan lasted from 10,000 years ago to 2400 years ago. The people of that era had an economy based essentially on hunting, fishing and gathering. It was then replaced by the Yayoi period (弥生時代) based on a wet rice economic system. It has been reported that a few plants were cultivated during the Jomon period (縄文時代) one of which is reported to be green gram (*V. radiata* L.：リョクトウ) (Imamura, 1996). However, remains of beans found at a Jomon (縄文) site, Torihama (鳥浜) in Fukui (福井) prefecture which dates back 5000 BP, are thought unlikely to be *V. radiata* (Maeda, 1987). Umemoto et al. (1983) suggested that seeds from this site in Fukui (福井) were likely to be both *V. radiata* (リョクトウ) and *V. mungo* （ケツルアズキ） based on SEM observations. However, neither of these crops nor their wild relatives are indigenous to Japan. It has not been determined whether the legume seeds found at Torihama (鳥浜), Fukui (福井) are cultivated or wild. If these seeds from an archaeological site represent wild plants and they were annually gathered it seems likely that evolution towards domestication of these legumes occurred. Today wild, weedy and cultivated azuki can commonly be found in Fukui (福井) (Tomooka et al., 1998c). Based on pictures of the archaeological legume seed remains they could be wild or small seeded cultivated azuki (Maeda, 1987).

It has been assumed that rice agriculture arrived late to Korea and Japan from China, because varieties adapted to high latitudes took time to evolve (Imamura, 1996). Once rice agriculture arrived in Japan is spread very quickly suggesting that agricultural technology in itself was not a reason rice based agriculture was late to arrive and spread across Japan. The wild relatives of azuki are indigenous to most of Japan and since cultivation occurred in the Jomon era it is possible indigenous *Vigna angularis* was cultivated in Japan during that era. This hypothesis is supported by the fact that in Japan azuki bean forms a true crop complex having both wild and weedy relatives. The recognition of complex populations in Japan also attests to the dynamic evolutionary status of the azuki bean complex in Japan. If azuki bean was domesticated in Japan these different types of population would be expected. This is the best example of a species complex for a major crop in Japan.

Today members of the azuki bean complex are found in temperate growing conditions in Nepal, Bhutan, China, the Korean peninsular and Japan. We have little data regarding the azuki bean complex in China. Azuki bean has been called hsiao-tou (小豆) in Chinese since the Eastern Chou (2250-2770 BP) (Chang, 1983). The primary center of diversity is reported to be south China (Li, 1970). However, Chang (1983) reports that this is not certain because azuki beans look similar to other indigenous *Vigna cultigens* having red seed coats. While it is generally assumed that most Japanese crops were introduced from China and this is undoubtedly true for rice and soybean, it is possible that azuki bean could have been domesticated independently in Japan. Azuki bean has a special place in Japanese culture and cuisine and the importance of this crop in Japan may reflect the longer association with Japanese culture than previously assumed (Kihara, 1969). In the Japan inland sea close to where the current center of diversity of the azuki bean complex is located there is an island called azuki bean island (Shodoshima 小豆島).

It should be possible to resolve the identity of legumes found in Japanese archaeological sites based on

DNA marker methods, as has been done with rice, and use of microscopic methods. Recent and continuing research being done on polymorphism and molecular markers in azuki bean (Xu et al., 1999 a and b, and research in progress) may provide a means to answer this important question.

(b) Population types （集団構造）

One major new findings of the exploration for members of the *Vigna angularis* complex is recognition of a new class of population which we call complex populations. This type of population in the field appears to consist of two or more morphological types. These populations may result from a mixture of wild and weedy plants or be the result of hybridization among plants of the different population types -wild, weedy and the cultigen - and subsequent segregation. Complex populations differ from hybrid swarms since they appear to be quite stable and often of considerable size. During the course of collecting during 1998 we found what appeared to be a hybrid swarm in a small part of one complex population from Tottori (see the following report). Complex populations have more genetic diversity than wild, weedy and cultivated populations (Xu et al., 1999 a and b). Also complex populations are not just the sum of their components. Specific polymorphism in complex populations not found in either wild or weedy populations or the cultigen has been found. Thus it is considered correct to consider this a separate class of population within the *Vigna angularis* complex.

(c) Center of diversity in Japan （多様性中心）

With the reports presented here we have now completed our major survey for populations of the *V. angularis* complex on the three main islands of Japan where it occurs, Honshu (本州), Kyushu (九州) and Shikoku (四国) (Table 1 and 2). The location of collected populations is shown with the status of each population indicated (Figure 1). The azuki bean complex is most diverse and its different members are most abundant is the Kinki region (Figure 1). While some other areas of Japan may have many populations of wild azuki, such as southern Shikoku, weedy and complex populations were most commonly found in the Kinki region. This conclusion is supported by an analysis of seed size and color variation in this complex (Doi et al. 1999).

(d) DNA analysis of intra and inter-population diversity （DNA レベルの集団多様性）

Previously we reported the range of research methodologies which had been applied to studying variation in the *V. angularis* complex (Tomooka et al., 1998a). Most techniques do not reveal much polymorphism in this species complex. We were able to detect 77 polymorphic bands using 30 screened RAPD primers. To improve our resolution of diversity we applied AFLP methodology, which is generally a sensitive method for revealing polymorphism, to the *V. angularis* complex. This technique is not easy to apply and so it is not as widely used as other DNA analysis methodologies. However, we were successful in generating a greater number of clear polymorphic bands both within and between populations using this technique compared to RAPD analysis. A total of 162 polymorphic AFLP bands were detected.

Table 3 and 4 show population parameters estimated from RAPD and AFLP data. Within population diversity reflects the same trend using both techniques. For both techniques wild populations have the most polymorphism. The results of between population diversity analysis are also quite similar and clearly indicate the difference between the three population groups.

Based on both RAPD and AFLP analysis complex populations appear to be a specific population group. Genetic diversity was greater in this population group than wild populations based on RAPD analysis. AFLP analysis of 3 populations - wild, weedy and complex from the same region of Tottori (鳥取) prefecture showed that the complex population consisted of plants which were similar to plants from both weedy and wild populations and that two distinct clusters of the wild like plants were present in the complex population (Xu et al., 1999b).

We are currently developing microsatellite markers for use in our population studies of the *V. angularis* complex. To date no microsatellites have been reported for this species complex.

Summary of pod and seed characters of collections made in 1998 （莢と種子の形態）

Summary tables of the pod length, seeds per pod and seed weight for populations of the *Vigna angularis* complex and *Glycine soja* collected in 1998 are presented (Tables 5 and 6). All three characters measured are larger in the weedy compared to wild azuki bean. The seed size of wild azuki and wild soyabean is very similar. However, wild *V. angularis* has relatively large pods and a greater number of seeds per pod than *Glycine soja*. As a potential food for gathering wild *V. angularis* could be 2 to 3 times more efficiently harvested on a per pod basis than *G. soja*. In addition the pods of wild azuki bean are smooth unlike wild soyabean which are covered in small hairs.

The areas collected during 1998 are shown in Figure 2.

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Table 1. Collecting missions undertaken in autumn 1998
1998年に行った探索収集チームの日程と地域

Dates	Region	Team members
4th, 6th -8th October	Tochigi (栃木), Ibaraki (茨城), Shiga (滋賀), Kyoto (京都), Tottori (鳥取)	N. Tomooka, D. A. Vaughan, K. Hammer, R. Q. Xu, M. Nakagahra*, Y. Tsubokura
9th-16th October	Tottori to Nakasaki (鳥取から長崎まで), Goto Islands (五島列島, 中通島)	N. Tomooka, K. Doi*, Y. Tsubokura
16th-21st October	Kyushu (九州)	D. A. Vaughan, T. Yokoyama, T. Komatsuzaki
21st-28th October	Fukuoka to Tsukuba (福岡からつくばまで)	D. A. Vaughan*, T. Chibana, M. Akiba,S. Hattori
23rd-30th October	Shikoku (四国)	D. A. Vaughan, K. Kashiwaba

* Only joined for part of the trip

Table 2. Samples collected during collecting missions in autumn 1998
1998年の探索における収集品の内訳

Species	Kanto to Tottori 関東～鳥取	Tottori To Nagasaki and Goto Islands, 鳥取～長崎（五島含む）	Kyushu 九州	Yamaguchi to Shizuoka (southern) 山口～静岡	Shikoku 四国	Total 合計
V. angularis var. *nipponensis* (wild)	4 (23)	10 (78)	10 (9)	3 (27)	9 (56)	36 (283)
V. angularis var. *nipponensis* (weedy)	4 (19)	4 (38)	1 (10)	1 (1)		10 (68)
V. angularis (cultigen)	4 (12)	2	6	1	1	14 (12)
V. angularis (complex)	1 (18)					1 (18)
Glycine soja	2 (7)	7	16 (35)	9 (49)	14 (40)	34 (131)
Vigna minima subsp. *nakashimae*		1 (8)				1 (8)
Vigna unguiculata (cultigen)			2		1	3
V. unguiculata (escape)			7		1 (10)	8 (10)
V. umbellata				1 (11)		1 (11)
Echinochloa utilis				1		1
Oryza sativa					1	1
Other legumes			2 (4)	1		3 (4)

Table 3. Intra-population variation based on RAPD and AFLP polymorphism detected in population groups in the azuki bean complex
RAPDおよびAFLP多型からみた野生アズキ，雑草アズキ，栽培アズキの多様性

Population group	Within population diversity based on RAPD polymorphism [1]	Within population diversity based on AFLP polymorphism [1]
Cultigen	0.079	0.353
Weedy	0.124	0.561
Wild	0.132	1.191

[1] Based on Shannon s diversity index

Table 4. Inter-population variation based on RAPD and AFLP polymorphism detected in population groups in the azuki bean complex.
RAPDおよびAFLP多型からみた野生アズキ，雑草アズキ，栽培アズキの遺伝的距離

Population comparison	RAPD analysis	AFLP analysis
	Genetic distance [1]	Phenotypic diversity between groups [2]
Cultigen vs. Weedy	0.348	0.505
Cultigen vs. Wild	0.452	0.561
Weedy vs. Wild	0.428	0.503

[1] Based on Jaccard's dissimilarity index
[2] Shannon's diversity index

Table 5. Summary data on three characters of field collected samples of wild and weedy populations of the *Vigna angularis* complex.
野生アズキと雑草アズキにおける莢長，1莢内種子数，100粒重の地理的変異

Location	Pod length (mm)		Seeds / pod		100 seed weight (g)	
	Mean	Range	Mean	Range	Mean	Range
Vigna angularis var. *nipponensis* (wild) 野生アズキ						
Southern Honshu 本州南部	63.4 (80)	41.5-81.4	8.98 (80)	6.5-11.4	2.78 (80)	1.45-4.55
Shikoku 四国	58.3 (61)	38.4-81.8	8.73 (61)	3.8-13	2.34 (61)	1.1-3.05
Kyushu 九州	60.6 (162)	39.2-90.6	8.51 (162)	2.6-12	2.32 (162)	0.94-4.9
Vigna angularis var. *angularis* (weedy) 雑草アズキ						
Southern Honshu 本州南部	72.6 (50)	58.4-92.2	9.16 (50)	6.8-11.6	4.18 (50)	1.2-9.3
Kyushu 九州	73.8 (10)	63.4-81.4	9.04 (10)	7.2-10.8	3.87 (10)	2.95-4.85

Table 6. Summary data on 3 characters of wild soyabean (*Glycine soja*) based on field collected individuals and population samples.
ダイズ野生種における莢長，1莢内種子数，100粒重の地理的変異

Location	Pod length(mm)		Seeds/pod		100 seed weight(g)	
	Mean (sample size)	Range	Mean (sample size)	Range	Average (sample size)	Range
Central Honshu 本州中部	27.1 30.8 (38)	21.6-	3.00 3.2 (38)	2.6-	2.9 (36)	1.65-3.7
Southern Honshu 本州南部	26.0 30.2 (25)	19.6-	2.82 3.4 (25)	2.0-	3.49 (25)	1.8-6.35
Shikoku 四国	25.4 29.4 (51)	20.2-	2.98 3.4 (51)	2.8-	2.74 (50)	1.4-4.35
Kyushu 九州	25.8 30.8 (61)	20.2-	2.99 3.2 (61)	2.2-	2.79 (61)	1.55-4.25

Figure 1. Distribution of wild(○), weedy (●) and complex (◑) populations collected between 1996-1998.
1996年から1998年に収集した野生アズキ集団（○）雑草アズキ集団（●）野生・雑草・栽培アズキ混生集団（◑）の分布

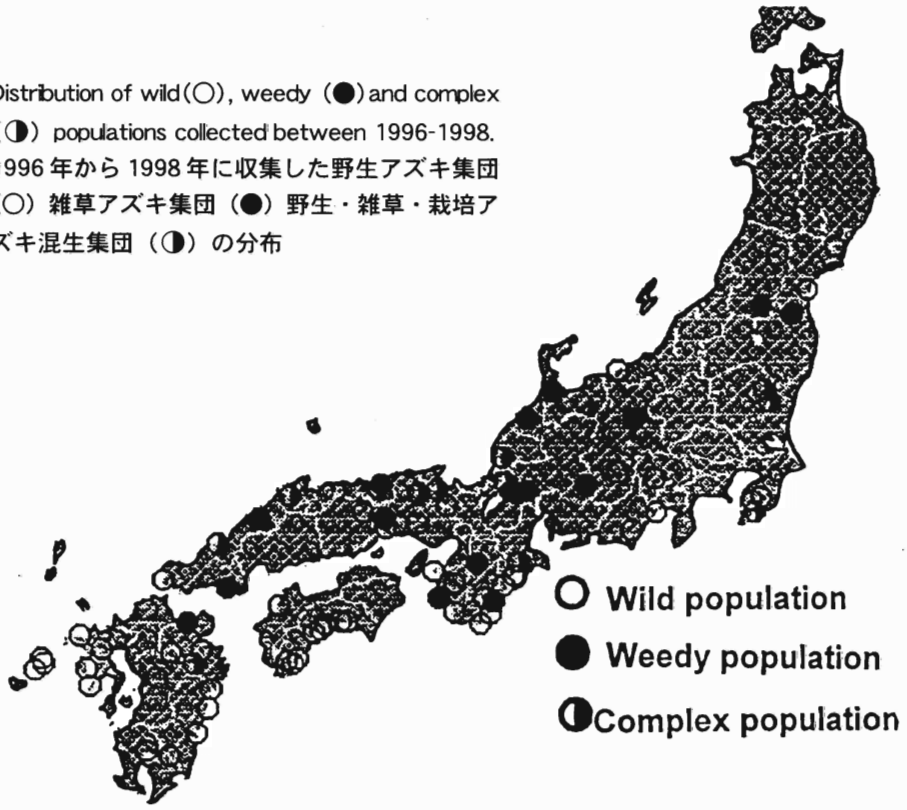

Figure2. Collecting areas in 1998

1a. Ibaraki（茨城県）, Tochigi（栃木県）, and Yamanashi（山梨県);

1b. Kyoto（京都府）, Shiga（滋賀県）, and Tottori（鳥取県);

2. San-in（山陰）, Honshu（本州）, and northwestern Kyushu（九州);

3. Kyushu（九州);

4. Yamaguchi（山口県）, Hiroshima（広島県）, Okayama（岡山県）, Aichi（愛知県）, and Shizuoka（静岡県);

5. Shizuoka（静岡県);