<Original Article>

STUDIES ON THE DIMORPHISM AND FERTILITY OF *PERSICARIA JAPONICA* (MEISSNER) GROSS ET NAKAI*

HARN, Chang Yawl
College of Agriculture, Chonnam National University

韓昶烈 : *Persicaria Japonica* (*MISSNER*) *Gross et Nakai*의 二型花와 受精力에 關한 硏究

ABSTRACT

HARN, Chang Yawl : Studies on the dimorphism and Fertility of ***Persicaria japonica*** (MEISSNER) Cross et Nakai. Kor Jour. Bot. 3(I) 1—15 1960 Numerous investigations, since the works of DARWIN, have been made regarding the heterostylous plants by JOST (1907), CORRENS (1924), LAIBACK (1924), LEWIS (1943), and many others.

Studies on the heterostylous Polygonum, however, were not reported except for the buckwheat, ***Fagopyrum esculentum***, which was investigatedy by SCHOCH-BODMER (1930), EAST (1934), FROLOVA & Co-Workers (1946), MORRIS (1947, 1951), TATEBE (1949, 1951, 1953), present author (1957), and others. It is because no heterostylous species, besides buckwheat, have been known to exist in the Polygonum family.

The author, during his studies on both heterostylism and fertility of Polygonaceae, has found that the species, ***persicaria japonica*** (Meissner) Gross et Nakai, is not dioecious as has been known in taxonomy, but in reality heterostylous both morphologically and physiologically. It was found that this plant, regarded by taxonomist, as a male plant setting no seed, actually set seed (botanical fruit) when legitimate combination was made.

Since his brief report on the dimorphic phenomena of this plant in 1956, the author's further research on the manner of fertilization has revealed that this species is a peculiar type whose dimorphism has undergone extreme specialization structurally and physiologically, the short-styled individual behaving in nature as a male plant and the long-styled individual, as female, whereas in controlled pollination the plant shows highly differentiated typical dimorphism.

When compared with the other dimorphous species of this family, *F. esculentum* and *P. senticosa*, it has been clarified that these three species differ in the degree of differentiation of their dimorphism morphologically and physiologically. That is, *P. japonica* has developed such a high specialization as to mislead the taxonomists, while *P. senticosa* shows almost no noticeable difference between long- and shortstyled individuals retaining most of the inherent physiological character common to the genus except for the fact that it has two forms of flowers. *F. esculentum* appears to have taken the intermediate position in every respect.

The results obtained in the present experiment are summarized as follows:

1) *P. japonica* has two kinds of individuals, one long style-short stamened; the other, short style-long stamened. The floral structure of this plants shows typical characteristics of dimorphic heterostylism. The differentiation between the two forms of flower has proceeded so highly both in primary and secondary difference of flower structure that this may be regarded as the most specialized form of dimorphism.

2) The differences of floral structure between the long and short styled individuals are remarkable compared with the other dimorphic species of the family.

3) The stamens of long styled plants show the sign of deteriolation whereas those of the short styled flower are well-developed.

4) When legitimate combinations are made, both L- and S-styled individuals are fertilized well and set seed (fruit), while in the illegitimate combination no fertilization and seed setting occur. Physiologically this species exhibits the typical behavior of dimorphic plants.

5) The self-fertile character, so common in other species of the other non-heterostyle Polygonum familly, has disappeared completely.

6) Under natural conditions, no or few seed setting is observed in short styled individuals that behave as if they were male plants.

7) In hand pollination, the combinations of both L x S and S x L alike yield relatively good fertility and seed-formations, the behavior of short styled individuals in artificial pollination differing remarkably from that in nature.

8) Under controlled pollination, L x S combination sets far more seed than in the combination of S x L. In the S-styled individuals, the fertilized flower has the tendency of its seed more readily falling off in every stage of seed development than in the L-styled individuals.

9) The behaviors of pollen tubes just parallels the results of the fertility test. That is, in the illegitimate combination, L-selfed, L x L, S-selfed, and S x S, the growth of pollen tubes is checked in the style, while in legitimately combined L x S and S x L, the pollen tubes grow well reaching the ovaries within 40-50 minutes after pollination. The response of short styled individuals, known as male plant among taxonomists, is identical, as far as behavior of pollen tube growth and fertilization are concernced, to that of long styled individuals, the so-called female plant.

10) The pollen grains from the short-styled plants are complete and fertile, whereas 70% of those of L-styled are found to be abortive, i. e., empty contents.

11) The remaining 30% of pollen of L-plant shows varied degree of stainability when stained with iron-aceto-carmine……mostly light red, while the pollen grains of S-style individuals are dark brown indicating complete fertility and viability.

12) The abundance of sterile pollen in L-styled and the nature of seed-dropping which occurs in S-styled individuals appear to be the main causes why the short styled individuals bear no seed in nature. Under controlled legitimate union, S x L, the careful and elaborate pollination would give the S-styled flowers the opportunities to receive the fertile pollens, though few in number, from L-styled plant, thus enabling S-plant to bear seed.

13) This species is not dioecious as is regarded by taxonomists, but typical dimorphic plant which has so highly specialized in floral structures and functions that the long-styled plant behaves just like a female individual; and the short-styled, like a male.

(*The abstracts of this thesis were reported at the 6th annual meeting of Korean Agricultural Society*)

**Polygonum japonica*

INTRODUCTION

Numerous works on the various heterostylous plants were reported, since the classical investigations of DARWIN, by JOST (1907), CORRENS (1924), LAIBACK (1924), LEWIS (1943), and many others. Studies regarding heterostylous Polygonaceae, however, have not been made except for the species *Fagopyrum esculentum (buckwheat) investigated by SCHOCH-BODMER (1930), EAST (1934), FROLOVA & co-workers (1946), MORRIS (1947), TATEBE (1953), present author (1957), and others. The reason of no investigations having been made yet with respect to heterostylous Polygonaceae is due to the fact that except for buckwheat no heterostylous plants have been known to exist in this family. **Polygonum esculentum*

During his studies regarding heterostylism and the fertility of polygonaceae, the author has found that the

Persicaria japonica, regarded in taxonomy as dioecious plant in which, it has been known, female individuals exclusively set seed and not the male plants, is in reality not dioecious, but dimorphism, a phenomenon generally termed as heterostylism. Not only the floral structure of this plant shows typical dimorphism, but its physiological behavior also gives clearly the characteristics of heterostylous plants, the so-called male individuals setting seed well (actually fruit in botanical sense) when legitimately combined in hand pollination.

Since his brief report on the discovery of some new facts about this plant in 1956, the author made further investigations on the morphological features of floral organs and physiological behavior, as the result of which following facts have been made clear:

1) A peculiar type of heterostylism is manifested by *P. japonica*, the organs of which are so highly specialized that the long-styled individuals behave exactly like female plants and the short-styled plants like male.

2) Although the long-styled and short-styled individuals behave so much like the female and male respectively in nature as to mislead the taxonomists identify this species as dioecious, under hand-pollination in the laboratory, this species behaves quite differently and has been proved both structurally and physiologically to be a typical dimorphic plant.

In addition to these facts, it was observed that when this species is compared with the other two heterostyle species of **Persicaria* genus, the *Fagopyrum esculentum* (buckwheat) and **Persicaria senticosa* which has been newly discovered by the author as dimorphic plant, there occurs gradual gradation in the degree of heterostylism among these three heterostylous plants. **Polygonum genus* **Polygonum senticosa*

Through the author's laborious investigations it has been clarified that most of the non-heterostylous species of *Persicaria* genus are homo-styled and normally self-fertilized plants except for the above mentioned three dimorphic species. It is assumed from these facts that some species of Polygonaceae were subjected to changes in floral structure and physiological nature from homo-style and self-fertilization characteristics of this family to heterostyle and cross-fertilizaton, and the three species show the degree and extent of the dimorphic alteration. If this assumption is supposed to be correct, the *P. japonica* appears to be the most highly specialized heterostylous species among them.

Attempt was made in the present experiments to make clearer the following points:

1) Uniqueness of this species as a typical heterostylous plant.

2) Differences of this species from the other heterostylous species of the same family.

3) The extent of change of *P. japonica* toward the dioecious.

MATERIALS AND METHODS

Materials used in the experiments are those collected from various areas and have been kept for several years in pots and in the open field. As the size of the flower of this species is extraordinarily tiny, performing the various experiments including emasculation and pollination works are really laborious. Very often the same experiments have to be repeated several times so that the accurate figures may be obtained. Repetition of research and accurate collection of data was comparatively easy to obtain owing to the perennial nature of this plant and to the long blooming period which ranges from early summer to late autumn.

Most of the experiments were performed in general during the blooming season of July through October except for some cases, when the materials were grown in the green house so that early experiments could be conducted out of season.

Pollinations were carried out usually at about 9,00 A. M. in the summer months and about 11,00 A. M. in the fall. The inflorescences with the flowers to be pollinated were bagged on the previous day and esmasculations were, for the most part, made just two hours before pollination so that the tiny flowers would not be injured by the castration operation.

Fertility was judged commonly 3 to 4 days after pollination, when fertilized flowers were readily recognized by the swollen ovaries. As this species has the property of easily dropping its fertilized flowers in every stage, including even the maturing period, it was found difficult to secure accurate data on the matured seed rate. The fertilized flower tends to drop off more readily in the short-styled individuals.

As the parthenocarpic and parthenogenetic phenomena often cause confusion in the investigations of fertility, prior to conducting this experiment it was carefully determined that these phenomena did not occur in this species. Floral organs were precisely measured with a micrometer. Pollen tube growth and behavior were examined by the following method:

The pistils with ovaries intact were fixed in acetic-alcohol of 3 parts absolute alcohol and 1 part glacial acetic acid for 24 hours. Then they were transferred to the hot lactophenol for 10 minutes for clearing and stained for 30 minutes in hot fuchsin lactophenol. Materials were again moved to lactophenol for the purpose of destaining the overstained tissues and left in it for more than 3 hours. Finally ovaries dissected out, the styles were mounted with lactophenol. Various other staining methods were employed, but the method just mentioned proved most satisfactory not only to this species, but to the examinations of the pollen tubes of all the other species of Polygonaceae as a whole.

RESULTS AND DISCUSSION

Finding out that the *P. japonica*, which has been regarded by taxonomists as dioecious whose female individuals, it has been known, set seed and male individuals do not bear seed, is, in fact, typical dimorphism structurally, the so-called female plant being long-style individual with long-style and short-stamen, the male individual being short-style individual with short-style and long-stamen, the author has made further researches on this species physiologically, and made it clear that this heterostylous species shows a peculiar type of dimorphism of which floral organ and physiological property have undergone so profound alternation that some of its characters resemble those of dioecious plant. When compared with other two dimorphic species of the same Polygonaceae, this species is found to be the most differentiated one among them with the *F. esculentum* and *P. senticosa* coming next in that order.

This plant, considered to stand in transitional position between dimorphism and dioecious, appears to add significance in the discussion of differentiation of sex of sporophytic generation of higher plants.

I FLOWER ORGAN

(1) **Style**

Tab. I

Style size

Length of style(mm)		Style diameter(mm)	
L*	S	L	S
2,24±0,02	1,19±0,01	0,14±0,001	0,13±0,001

*L.....Long-style S.....Short-style

*Some of the figures in the flower organ have been cited from the data reported in 1956 by the author.

In the heterostylous plants, the length of style, along with the filament length, is regarded as the most conspicuous characteristic.

As table 1 shows, there exists great differences between the styles of long and short individuals, the long-styles being approximately two times as long as short-styles. As for the diameter of styles, the differences are slight, but the significance is great.

(2) **Size of stigma**

Tab. 2

Stigma size

Stigma, lengthwise(mm)		Stigma, crosswise(mm)	
L	S	L	S
0,24±0,004	0,30±0,003	0,34±0,003	0,46±0,004

Lengthwise diameter of the stigma of the short-styled individuals is significantly larger than the stigma of long-styled individuals. The difference of crosswise diameter of stigmas between the short and long style individuals is outstanding with the short style stigma being larger than the long style stigma. In general, the stigma of the short styled individuals is far bigger than that of the long styled individuals. The short style's stigma looks rather mushroom-shaped, while, in the longstyle, the stigma is somewhat the shape of a snake-head. The stigmatic outgrowth is conspicuous in short-styled individuals but the long-styled individuals lack it. Both in the long and the short-styled individuals alike, this species has its pistils divided into two in style and stigma. Occassionally there occurs, in only short styled individuals, pistils with three styles and stigmas.

(3) **Ovary size**

Tab. 3

Ovary size

Ovary, lengthwise(mm)		Ovary, crosswise(mm)	
L	S	L	S
0,81±0,002	0,84±0,006	0,54±0,018	0,67±0,006

The lengthwise diameter of the short styled individuals are slightly larger, and the difference is significant. The difference of the crosswise diameter is great with the short styled individuals being larger than the long styled individuals. The ovary of short style is, as a whole, bigger.

(4) **Filament length**

Tab. 4

Filament size

Length of filament(mm)		diameter of filament(mm)	
L	S	L	S
1,32±0,01	3,04±0,02	0,15±0,001	0,16±0,002

Filament length, the most outstanding feature, along with the style length which is termed as a primary difference of heterostylous plants, is remarkably longer in the short styled individuals in any of these kinds of plants and the anthers of the short styled individiuals stand at almost the same level as the stigma of the long styled flowers; likewise the anthers of the long styled flowers are on practically the same level with the stigma of the short styled flowers.

The filament of the short styled individuals of this species is approximately two times as long as that of the long styled individuals and the stigma and anther of the long styled individual stand almost at the same level with those of the short styled individuals respectively, showing typical dimorphism. The diameter of filament is slightly larger in the short styled individuals with statistical significance.

Aside from the fact that the short style filament is longer than that of the long style filament which is the most conspicuous characteristic in heterostylous plants, another significant fact in this species is that the stamen of the long styled individuals shows signs of degeneration, whereas in the short styled individuals it shows the

appearance of perfect development. This speculation will be more apparent with the examinations of the anther, pollen, and the fertility of pollen.

(5) **Size of anther**

Tab. 5

Anther size

Length of anther(mm)		width of anther(mm)	
L	S	L	S
0,57±0,004	0,86±0,008	0,41±0,002	0,48±0,001

The anther of the short styled individuals is far larger than that of the long styled individuals in both length and width. In general appearance the short style's anthers look well-developed, while in the long styled individuals the anthers show signs of degeneration. Such phenemenon has not been observed in other heterostylous plants.

(6) **Size of pollen grain**

Tab. 6

Pollen size(u)

L	S
49,72±0,23	55,90±0,26

Pollen grains are remarkably bigger in short styled individuals. The essential feature in most of heterostylous plants which the author has examined, including the heterostylous *Persicaria* species, is that the short styled individual's pollens are larger than those of the long styled individuals. The difference of pollen grain size is termed in heterostylous plant as the secondary difference. In view of the fact that pollen grain size difference is an outstanding characteristic in any of the heterostylous plants along with the difference in style and filament length, it is considered natural that the pollen grain difference should be named as the primary difference.

The fact that the stamen of the short styled individuals have well-developed filament and anther, and larger and abundant pollen, while, in the long styled individuals the stamen gives the appearance of deterioration with the function of its pollen being reduced to severe abortion, the data of which is to be presented later, reveals that in this species so high a differentiation of floral organ proceeded that the short styled individual seems to function as a pollen supplier. This speculation will be strongly justified with evidences obtained in physiological research.

The morphological observation of floral structure as a whole reveals that this species has the typical characteristics of dimorphic plant with the degree of difference between long and short style flowers exceptionally great.

II FERTILITY

The principal features of heterostylous plants as a whole are the occurrence of two, and sometimes three, kinds of flower forms which give the impression that such a difference of flower forms may have certain ecological significance in effecting cross-pollination rather than the self and like-style pollinations. What is interesting is the physiological property that this kind of plant avoids self-fertilization or fertilization between like-styles physiologically, too. That is, when hand-pollinations are made good fertility is obtained in the legitimate combinations but no or poor fertility is the result when illegitimately combined.

As stated above, morphologically *P. japonica* has the typical dimorphic floral organ with its primary and secondary morphological differences between long and short- styled individuals appearing to have undergone

marked differentiation. In order to make clearer that such heterostylous characteristics also occur physiologically in this species, fertility in various combinations was investigated with the primary attention being focused on the assumption that the physiological behaviors may be as remarkable as the morphological difference which is the outstanding feature of this species.

Some results of brief examination regarding fertility of this species was reported in 1956. For the purpose of obtainng clearer and more extensive data on fertility, experiments were carried out since 1958 through 1959 with various strains, using flowers of different blooming stages.

Tab. 7

Fertility (1) 1958

Combination	Flower pollinated	Flower fertilized	Matured seed
L selfed	182	0	0
L X S	111	105	*abundant
S selfed	61	0	0
S X L	115	abundant	far less than in L X S

* Owing to the severe fertilized-flower (or seed) dropping, the exact figures are not available.

As the results of Table 7 show, in long and short styled individuals alike, when selfed, no fertility and seed formation resulted. When the legitimate combinations, L x S and S x L, were made, however, high fertility and seed maturity were obtained. The result apparently indicates that this species shows even physiologically the characteristics of the heterostylous plants. As was pointed out in the author's former report, this species has a peculiar physiological nature, not yet observed in other heterostylous plants. In the legitimate combinations of S x L, fertility appears to be relatively normal although not so high as in the case of L x S, but the rate of seed maturation is exceedingly poor compared with that of legitimately combined long styled plants. This is due to the fact that in the short styled individuals the fertilized flowers tend to drop off readily in every stage of their ovary swelling until the maturity is well advanced. The peculiar property of this species in which the short styled individuals have the tendency of their fertilized flowers or maturing seeds drop more readily than the long styled individuals, suggests, together with floral structure and other physiological peculiarity which will be given later, very interesting speculation with regard to this species.

Tab. 8

Fertility (II) 1958

Date pollinated		L selfed		L x L		L x S		S selfed		S x S		S x L	
		pol.	mat.	pol.	mat.	pol.	mat.	pol.	mat.	pol.	mat.	pol.	mat.
Aug.	21	-	-	-	-	-	-	-	-	-	-	6	2
	22	-	-	-	-	-	-	-	-	-	-	-	-
	23	-	-	-	-	-	-	-	-	-	-	9	4
	24	-	-	-	-	-	-	-	-	-	-	29	10
	25	50	0	-	-	4	4	-	-	-	-	18	8
	26	6	0	-	-	26	22	-	-	-	-	15	6
	27	12	0	-	-	-	-	-	-	-	-	9	2
Sept.	1	-	-	2	0	-	-	4	0	13	0	19	5
	2	-	-	4	0	-	-	4	0	8	0	25	12
	3	-	-	4	0	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	8	0	6	0	19	8

5	-	-	-	-	-	-	6	0	18	0	-	-
7	-	-	-	-	-	-	5	0	14	0	-	-
8	-	-	35	0	-	-	-	-	3	0	-	-

In Tab. 8, also, the combinations of S-selfed, L-selfed, S x S, and L x L set no seed at all, whereas legitimately combined Lx S and S x L shows good fertility although the seed-bearing in the S x L is markedly less than that in the L x S.

The result shown in the Tab. 9, carried out in the early stage of flower-blooming, clearly indicates the dominant physiological feature of dimorphic flower plant in which good seed-formation is obtained exclusively in legitimate union.

The experiments shown in Tab. 10 and 11 as well as Tab. 9 are the ones performed with various strains at different locations, the results of which are just the same as the previous ones.

One of the conspicuous features in the experiments of fertility of this species is that this plant has compleletly lost the ability of fertilization in the illegitimate pollination. In *F. esculentum*, the well-known dimorphic species of Polygonaceae, the fertility in the illegitimate pollination of selfing or between like-styles, was approximately 2-6%. In other words, the buckwheat still retains the self-fertilizing power although it is slight. The author, in his researches regarding *Persicaria Senticosa*, another heterostylous plant of Polygonaceae together with the *P. japonica* and *F. esculentum*, has observed that P. *senticosa* has showed good fertility regardless of its mode of combinations, that is, this species fertilizes well not only in the legitimate combination, but also in the illegitimate union, thus still retaining completely the self-fertilizing ability. In most of the heterostylous plants of other families so far investigated no cases were reported where illegitimate union completely failed to produce some seed-setting. It has been reported, in most cases, that the fertility of seed-setting is far better in the legitimate combination than in the illegitimately combined pollination. The behavior of *P. japonica* is a particularly peculiar one in which absolutely no fertility and seed formation is the result in the selfed flowers or flowers pollinated with the pollen from the same forms of flowers. In other words, this plant has completely lost the self-fertilizing ability.

When compared the behavior of *P. japonica* in fertility with those of other two species of heterostylous Polygonaceae, *F. esculentum* and *P, senticosa*, it has been found that there exists definite gradation in the self-fertile nature. The *P. senticosa* still maintains complete self-fertilizing power, and the *F. esculentum* has almost lost it, retaining slightly (2-6%), whereas *P. japonica* lost it completely, producing no seed in illegitimate combinations. From the results of this experiment, it will be stated with assurance that if *P. senticosa* is assumed to be non-differentiated type, *P. japonica* may be regarded as the most advanced type as far as fertility is concerned.

Tab. 9

Fertility (III) (July 15-20, 1959)

Combination	Flower Pollinated	Flower fertilized	%	Seed matured	%	Remarks
L1 bagged	28	0	0	0	0	L3, in pot L1, L2, in open field
L2 〃	25	0	0	0	0	
L3 〃	14	0	0	0	0	L1, L2, L4, are in early stages of blooming.
L1 selfed	39	0	0	0	0	L3, middle stage of flower-blooming
L2 〃	28	0	0	0	0	
L3 〃	25	0	0	0	0	
L1 x L4	24	0	0	0	0	
L2 x L4	23	0	0	0	0	
L3 x L4	17	0	0	0	0	

L1 x S	34	34	100	30	88,2	
L2 x S	23	23	100	22	95,7	
L3 x S	29	29	100	25	86,2	
S1 bagged	17	0	0	0	0	S1, S2, S3, S4, middle stages of flowerblooming
S2 〃	14	0	0	0	0	
S3 〃	11	0	0	0	0	S1, S2, in pots S3, S4, in open field
S1 selfed	27	0	0	0	0	
S2 〃	23	0	0	0	0	
S3 〃	15	0	0	0	0	
S1 x S4	14	0	0	0	0	
S2 x S4	18	0	0	0	0	
S3 x S4	25	0	0	0	0	
S1 x L	12	12	100	7	58,3	
S2 x L	15	13	80	4	26,7	
S3 x L	13	10	76,9	5	38,5	

Tab. 10

Fertility (4) **Aug.** 25-28, 1959

Combination	Flower pollinated	Flower fertilized	Seed matured
L10 selfed	48	0	0
L10 x S	35	35	28
S8 selfed	29	0	0
S8 x L	19	18	5

Tab. 11

Fertility (5) **Sept.** 2-4, 1959

Combination	Flower pollinated	Flower fertilized	Seed matured
L11 seled	55	0	0
L12 〃	20	0	0
L11 x S	57	54	45
L12 x S	17	14	12
S10 selfed	30	0	0
S11 x L	10	10	3

Another outstanding result made clear in the present experiment is the fact that while in the cross of L x S good seed setting is obtained, the reciprocal cross of S x L produced poorer seed formation. That is, in the short styled individuals many of the fertilized flowers tend to fall off in various stages.

The cause of dropping after fertilization and the explanation as to its physiological mechanism can be given in various ways. What is significant, however, in the present investigation is not the account on the seed dropping, but rather the fact that seed dropping or fertilized flower dropping occurs far more abundantly in the short style individuals. It is supposed that this peculiar behavior of short styled plants, together with the occurrence of abundant abortive pollen grain in the long styled individuals, the experimental result of which will be given later, seems to be the primary cause that makes the short styled individuals bear no, or few, seed in nature, thus misleading the taxonomists to regard the short styled plant as a male individual.

These facts mentioned above give the impression that the short styled individual has changed or is changing in its role as the pollen supplier than the seed bearer. In nature the aggregate of short styled plants with no

seed on their raceme gives the same appearance as male individuals.

Another fact revealed in this experiment is that although the short styled individuals of this species, conspicuous entomophilous plant with well-developed nectary gland, bear, no seed in nature, comparatively good seed setting, though not so much as compared with that of long style plant, is secured when hand pollinated. The detailed account concerning this question will be given in experiment IV.

What should be added here is the author's observation that the short styled individuals, usually not seed-bearers, often set seed in the end-season especially in late autumn under natural condition. This problem is not solved yet, but the causes may be supposed as follows: it may be caused by the increase of self-fertilizing ability stimulated by low temperature; the decrease of dropping of legitimately formed seed; or parthenocarpic phenomena caused by low temperature.

Through this experiment it has been made clear that *P. japonica* is typically a dimorphic plant not only from the morphological structure of its flower organ, but from its physiological behavior. This species possess peculiarities in its dimorphism which appears to be remarkably different from other heterostylous plants. So different are the degree of differentiation between the long and short styled individuals and so proceeded are the behavior and specialization that this plant shows the sign that it has changed or is changing toward dioecious. This assumption has been proved to be correct by performing subsequent experiments including the comparision of pollen fertility of long and short styled individuals.

III POLLEN TUBE GROWTH

In heterostylous plants, generally speaking, the floral structure in which occurs two or three kinds of flower forms, appears to suggest that it is helpful to carry out mostly the legitimate pollination and avoid the illegitimate union in nature. The flowers of these plants are not only so constructed as to aviod self-pollination, but there have occurred in most cases physiological changes so that the self-fertilization or fertilization between like-styles tends to be inhibited and cross-fertilization is mostly preferred. The author has termed these phenomena of heterostylous plants as "Structural cross pollination-physiological self sterility."

P. japonica is assumed to be the specific kind of plant in which the structural differentiation between long and short styled flowers has undergone greatly and at the same time the physiological specialization has proceeded so remarkably that it has completely lost the power of self-fertilization. Such complete loss of self-fertility has not been observed in any of the other heterostylous species of Polygonaceae or other families. In order to clarify further the physiological peculiarity as to the fertilization of this plant, experiments on the detailed examination of pollen tube behavior in various modes of pollination have been made.

When the pollen tube growth was examined 33 hours after pollination, the average pollen tube length in the combinations of L selfed and L x L was approximately 1.8 mm, the growth of pollen tubes having been checked in most cases in the areas about four-fifths of the stgle in long-styled individuals. No pollen tubes which reached the ovary were observed. On the other hand, in the combinations of L x S, most of the pollen tubes reached the ovaries.

Tab. 12

Pollen tube grwth(1) 33 hrs after pollination

Combination	L selfed	L x L	L x S	S selfed	S x S	S x L
Region of style, tube tip cheched	4/5	4/5	Ovary	4/5	4/5	Ovary
Average tube length	Approximately 1,800mm	〃 1,800mm	Ovary	Approximately 0,960mm	〃 0,960	Ovary

In the illegitimate combinations of S-selfed and S x S, when examined 33 hours after pollination, the aver-

age pollen tube length was approximately 0,960mm. They stopped their growth at the regions nearly four-fifths of the length of the shortstyle. There has not been observed even a single pollen tube which reached the ovary. When long-style pollens, however, were pollinated on to the stigmas of short-styled flowers, there observed in almost all the ovaries examined some of the long-style pollen tubes reaching the ovaries.

The result of pollen tube behavior completely agrees with the data obtained in the experiment of fertility in which only legitimate pollinations were found to be fertile.

In the styles of long-styled flowers, the pollen tube of long-styled flowers, regardlesss of whether it is its own or of other long-styled individuals, cannot penetrate down to the ovary, being checked on the way, whereas the short style pollen tubes have no trouble in growing down the entire length of style to the ovary. In the styles of short styled flowers, the short style pollen ceases its penetration at certain regions of the style, when long-style pollen tubes were able to grow down the ovary.

In the case of *F. esculentum* the illegitimately pollinated pollen tubes, i.e., L-selfed, L x L, S-selfed, S x S, were occasionally found penetrating to the ovary, thus bringing about 2-6% of self-fertility or "like-style" fertility. Present microscopic examination clearly shows that in *P. japonia* the incompatibility existing between the pollen and style length in the incompatible combination is such a strong one as not to allow even a single pollen tube to grow down the style of its own or same forms of flowers.

For the purpoes of further verification of pollen tube behavior, the rapidity of pollen tube growth was examined both in legitimate and illegitimate combinations. When long style pollens were pollinated onto the stigma of the same flower or flowers of other long style individual, that is, L-selfed or L x L, the pollen tube were measured growing down about two-fifths of style in 30 minutes, three-fifths in 40 minutes, and approximately four-fifths of style length in 50 minutes. The pollen tubes were mostly checked at the area four-fifths of entire style length. (Tab. 13)

The result of present experiment is somewhat slightly different from the data the author reported in 1956 in which the pollen tubes penetrated a little more than half of the style length in one hour. Both results, however, have shown marked similarity in that the pollen tubes have grown down over half of the style length within an hour and their growth has been checked at certain region.

In the compatible combination of L x S, most of the short style pollen tubes have crossed the four-fifth point of style in 30 minutes and reached the ovaries when examined 40 minutes after pollination was made.

Tab. 13

Pollen tube growth (II) 1959

Combination	Intervals after pollination					
	30 min.	40	50	1 hr.	9,00″	33″
L selfed	0,900mm 2/5 of style	1,300 3/5	1,600-1,800 3/5-4/5	*	1,800 4/5	1,800 4/5
L x L	0,900 1/3-2/5	1,600 3/5-4/5	1,600-1,800 3/5-4/5	1,600-1,800 3/5-4/5	1,800 4/5	1,800 4/5
L x S	1,800 4/5	2,400 Ovary	Ovary	Ovary	Ovary	Ovary
S selfed	0,600 1/2 of style	0,650 1/2-4/7	0,650 3/5-4/7	*	0,960 4/5	0,960 4/5
S x S	0,600 1/2	0,600 1/2	0,650 3/5	0,800 3/5	0,960 4/5	0,960 4/5
S x L	0,600 1/2	0,960 4/5	1,300 Ovary	Ovary	Ovary	Ovary

* ..... means not examined.

1 Growth of L-pollen tube is extremely irregular.

2 L-pollens their tubes checked at the stigmatic tissue or at the upper part of style are excluded from consideration.

In the styles of long-styled individuals, the short-style pollen seems to grow much faster than the long-style pollen; while the long-style pollen grows only 1.6mm in 40 minutes down the long-style, the short-styles pollens have made complete penetration down through the entire length of 2.4mm style to the ovary.

When pollens of short-styled flowers were placed onto the stigmas of the same flowers or the other flowers of the same individuals or different individuals of short style types, that is, S-selfed or S x S, the pollen tubes were observed growing down the style at the rapidity of one half of style length in 30 minutes, and three-fifths of the entire length in 50 minutes, the pollen tubes mosty being checked thereafter at this region or a little beyond. When legitimate combination of S x L was made some of the pollens completed their growth down to the ovary in 50 minutes. It has been found that in the style of short-style flower, the pollen tubes of long-style appears to be slightly faster than the short-style pollens. What is noteworthy is the phenomena that the growth of long-style pollen was extremely irregular, their growth being checked in various areas of the style including stigmatic tissue, while the short-style pollen was found uniform in their growth.

In short, in the illegitimate combinations regardless of whether the mother plant is long or short-styled, the pollen tubes were checked at the definite regions of the respective styles allowing not a single pollen tube penetrate down to the ovary, while in the legitimately combined pollination the pollen tubes have reached the ovaries in 40 to 50 minutes.

In the studies of heterostylism including the study of Polygonaceae it has not been known the instances where the incompatible pollen tubes are checked so completely in the illegitimate combinations as in *P. japonica*, bringing about absolute sterility. Even in the *F. esculentum* or buckwheat, a well-known dimorphic plant and noted for its complete self-sterile property, the pollen tubes in the illegitimate combination were found occasionally growing down to the ovary resulting in seed-setting. In the case of *P. japonica* the incompatibility existing between the stigma and pollen of the same (or like style) flowers is proved far more serious than in the *F. esculentum*.

The result concerning the rapidity of pollen tube penetration and the region where pollen tubes are checked in the style is slightly different from the observations of author's previous report. This kind of slight difference is considered to be brought about by the variations in environmental conditions and other factors at the time when the experiments were carried out.

III ABORTIVE POLLEN OF LONG-STYLE INDIVIDUAL

P. japonica is regarded in taxonomy as dioecious plant, the male individuals of which bear no seed. Actually the short styled individuals grown wild in clusters in natural habitats give the same appearance as male plants with no seed in any of the racemes, while the long-styled individuals growing nearby the short styled plants bear abundant seed. Since this species is perennial and propergates by underground roots, both long-styled individuals and short-styled plants used to grow in aggregate in definite places respectively, giving good contrast between the seed-bearing long-styled plant group and the non-seed-setting short-styled group growing next to the long-styled individuals.

When precisely examined in nature, however, in the so-called non-seed-forming short styled individuals, there often occurs in the racemes matured seeds with their contents complete. The series of these experiments were motivated by the author's discovery that this species has two kinds of flower forms and the male plants known as non-seed bearing occasionally bear seed in nature though it is of very rare occurrence.

It has been found out in the experiment of fertility that the short-styled individual, the so-called male, when legitimate hand pollinations were made with the pollen of long styled individuals, are fertilized well and set a

relatively large numbers of seed. In his subsequent researches carried out in the successive years, the author has made extensive experiments using several strains collected from various parts of this country; and performing these experiments in different places and at different blooming stages, he has confirmed that this species is a typical dimorphic plant with its short-styled individuals setting seed well in artificial legitimate pollination as described in Experiment II.

Then what is the reason of this species' peculiar behavior in fertility where the short styled flower acts differently in the laboratory from in nature? This species is a typical entomophilous plant with its well-developed nectary gland and abundant insect-visitors, and seems even rather better adapted as insect-pollinated plant than the buckwheat of the same family.

In view of the fact that in hand pollination the short-styled individuals are fertilized well and set a considerable number of seeds; and judged from the degree of seed formation in the long style by insect-pollination, the short styled plant should produce in nature a considerable amount of seeds effected by long-style pollens.

It will be recalled that in the experiment of fertility the fertilized flower or seed tends to drop more readily in the short-styled plants than in the long-styled ones. Even with the tendency of seed-dropping in the short-styled individuals taken into consideration, the short-styled ones should bear a certain amount of seed in nature. It is inconceivable that the short-styled plants seldom bears seed in nature as it is recognized by taxonomists as "Male plant."

In order to make further clarification of these problems in question, the author made the examination of the fertility of pollen grains.

Investigating the pollen grains of both long and short styled flowers fixed and stained with iron-aceto-carmine, it has been found that while the short-style pollens (S-pollens) are stained well with filled cytoplasmic contents and look uniform, in the long-style pollen (L-pollen) approximately 70% of them are empty contents having numerous micropollen, giving a rather irregular appearance.

Tab. 14

Abortive pollen

L			S		
Pollen exam.	Normal pollen	Abort. pollen	Pollen exam.	Normal pollen	Abort. pollen
561	211	350	305	283	22

The remaining 30% of L-pollen grains with contents so that stained with dye are extremely variable in the stainability. That is, the colour of the stained pollens ranges from dark brown to light pink, of which the number of pollen with dark brown color is not so numerous, while the pollens of the short-styled flower (S-pollen) stained with iron-acetocarmine and examined under microscope were uniformly dark brown, suggesting that S-pollens are filled with cytoplasm and organic substances such as starch grains.

If it is assumed that the dark-brown pollens are due to the sufficient cytoplasm and cytoplasmic substances and these pollens are exclusively viable and fertile, then it would be safely stated that the S-pollens are mostly fertile whereas in the long-styled flower approximately 70% of the pollen grain with empty contents are sterile and even remaining 30% of stainable pollen are not complete except for the few dark brown pollens.

In addition, toward the late autumn or end-season of the plant body, there occur in long and short styled flowers alike numerous giant pollen or micropollens supposed to be derived from the meiotic irregularity due to the low temperature or deteriolating physiological conditions of plant body. This tendency of abnormality is observed to be far more severe in the long style pollens than in the short style ones though detailed data is lacking due to the failure of accurate calculations.

From the above mentioned results it is concluded with assurance that in the long styled individuals there,

occurs relatively few fertile pollen grains which are able to ensure fertilization and there will be rare cases when the fertile L-pollen alight on the stigma of short style flower in nature. In addition to it, even if the fertile complete L-pollen has an opportunity to be transferred onto the stigma of short style and the short styled flower gets fertilized, the seed-dropping tendency characteristic of short styled individual will give the short styled plant little chance to bear mature seed. This appears to be the primary cause that in nature the short styled individual sets no seed thus behaving like a male plant. The better fertility of short styled individual in the laboratory experiment than in nature is, it is supposed, due to the fact that nearly every stigma of short styled individuals would have the opportunity to receive the fertile L-pollen, although few in number, because of the careful hand pollination in which usually abundant pollen grains are placed on the stigma.

The long styled individuals with its deteriorated anther and nearly sterile pollen grains has lost, as a whole, its function as the supplier of pollen, while the short styled individual has well-developed filament and anther as well as the numerous functional pollens.

Merely the sole fact that, L-pollen has been reduced to sterility and S-pollen, on the contrary, is uniformly fertile gives the impression that long and short styled individuals have become, as a matter or fact, in some parts, female and male respectively. No cases like this were observed in the *F. esculentum* and *P. senticosa*.

In view of the results obtained in the present experiment described above, *P. japonica* is not a dioecious plant as has been known by taxonomists, but a heterostylous species with the typical dimorphic characteristics in its physiological behavior and floral structure. This species, however, as compared with other dimorphic species, *F. esculentum* and *P. senticosa* of Polygonum genus and other heterostylous plants of the other families, is a peculiar type of heterostyle plant in which both the long styled and short styled individuals have undergone striking differentiation. Not only is there marked difference between long and short styles, but also the result of fertility and the behavior of pollen tube shows evidence of severe changes to such an extent as not to be seen in any other heterostyle plants. Particularly, the difference observed in the function of pollen grains of the two forms of flowers appears to show the transitional tendency from the dimorophism to dioecious.

The common feature of *Polygonum* (or *Persicaria*) genus as a whole, except the heterostylous species, is partially allogamous autogamy. That is, the plants of this genus for the most part are naturally self-fertilized. On the other hand, in the three species of heterostylous Polygonacae there occurs distinct gradation in the mode of fertilization. *P. senticosa*, although typically dimorphic in its floral structure, behave exactly like non-heterostyle Polygonum relatives. That is, this species retains still nearly equal fertility both in legitimate and illegitimate combinations alike, still preserving perfectly the self-fertile property so characteristic of all Polygonum genus. In *F. esculentum*, on the other hand, there exists marked difference between the fertility of legitimate and illegitimate combinations, bringing about extreme sterility in the illegitimate pollinations. This species, however, still retains, though slight, the self-fertile property common to the plants of the genus. As for the *P. japonica*, the complete loss of self-fertilizing property as well as the sharp distinction of fertility between the two forms of combinations and the tendency of transition toward dioecious is the outstanding trait.

P. japonica, as compared with the other two dimorphic species of Polygonaceae, is a highly specialized peculiar type exhibiting dimorphism, and showing in some of its functions and behavior clear signs of dioecious features.

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to Dr. Tai Hyun, Chung, Sunggyunkwan University for taxonomic assistance. The author is indebted also to the members, including his assistant Dong Chul, Kim, of Department of Plant Breeding and Genetics of this university for the laborious experimental works during the course of the studies.

摘 要

*P. japonica*가 分類學에서 말하는거와 같이 雌雄異株가 않이고 異型蕊植物이라는 것을 發見하고 數年間 이 植物의 長柱花個體, 短柱花個體間의 花器의 形態, 受精力, 花粉管의 行動 및 L, S株의 花粉의 稔, 不稔等을 調査해 보았드니 이 植物은 異型蕊植物中에서도 L, S株間의 分化가 가장 甚하게 되어 있어 一部器官이나 機能은 雌雄異株와 같이 變해버린 特殊한 異型蕊植物이라는 것이 明白히 되었고 이 植物은 植物造胞體世代의 性의 分化가 生기는데 異型蕊現象을 거쳐서 되는 境遇도 있지 않나 하는 것을 假定케하는 한 證據가 될수 있다고 生覺된다. 또한 여뀌科의 다른 異型蕊植物인 *F. esculentum*이나 *P. senticosa*와 比較해 보면 이 植物이 가장 L, S 株間의 分化가 甚하고 *F. esculentum* 은 中間, *P. senticosa* 는 가장 未分化狀態에 있어 三種의 여뀌科 異型蕊植物間에 그 分化의 程度에 gradation이 生겨 있다. 이제 實驗結果를 要約하면 다음과 같다.

1) *P. japonica*는 花柱長, 花絲長, 等의 第一次의 形態的 差異뿐만 않이라 第二次的 差異도 大端히 甚한 Dimorphism을 나타내고 있다.

2) L, S株의 花器의 差異는 同科의 他異型蕊植物에 比해 極度로 分化되어 있다.

3) L, S株의 雄蕊를 보면 S株는 大端히 發達되어 있는 反面에 L株의 雄蕊는 衰萎된것 같은 느낌을 주고 있다.

4) 適法授粉에 依하면 L, S株가 다 結實이 잘 되지만 不適法授粉에서는 受精結實이 全혀 안된다. 受精生理上으로도 뚜렷한 異型蕊植物이다.

5) 自家可稔의 性質 또는 同型花間의 受精力은 完全히 喪失하고 있다. 여뀌科의 他異型蕊植物과는 이 點에 差가 甚하다.

6) 自然狀態下에 있어서는 S株는 結實이 거이 안되어 마치 雄株와 같은 外觀을 나타내고 있다.

7) 人工授粉에서는 L×S나 S×L 株 受精結實이 다 되어 S株의 行動은 自然狀態下에서 와는 大端히 相異하다.

8) L株에 比해 S株는 受精後 落果가 더 甚하다.

9) 花粉管의 行動은 受精力과 完全히 一致된다. 即 L−selfing, L×L, S−selfing, S×S 等의 不適法 授粉에서는 花粉管은 花柱의 途中에서 伸長이 停止되지만 L×S, S×L,에서는 授粉 約 40−50分 後이면 花粉管은 子房까지 到達된다.

10) S株는 雄木으로 誤認되어 있지만 人爲適法授粉을 하면 受精力이나 花粉管의 行動은 L株에서와 同一하다.

11) S花粉은 完全하지만 L花粉은 約 70%가 內容空虛한 Adortive pollen 이다.

12) L花粉中 나머지 30%도 S花粉에 比해서 染色度가 낮은것이 많고 S花粉 같이 濃染되는 것은 極히 少數이다.

13) L花粉의 無能化와 S株의 落果容易性 等이 自然狀態下의 S株가 不結實되는 重要한 原因이 되는 것같다.

14) 本植物은 分化가 高度로 進行된 典型的인 異型蕊植物이며 마치 Dimorphism 에서 Dioecious 에로 移行되는 수가 있다는 것을 表示하는 證據가 되는 것같다.

REFERENCES

1) ATTIA, M.S., and H.M. MUNGER, (1950) Pro. Amer. Soc. Hort. Sci., 56:363-368. 2) CHUNG, T.H., (1956) Korean Flora (II), Seoul. 3) DAWIN, C., (1877) The different forms of flowers on plants of the same species. Ed. 2. 4) EAST, E.M., (1934) Proc. Nat. Acad. Sci. Vol. 20:225-230. 5) FROLOVA, S.L., (1946) Nature: 158 pp. 520. 6) HARN, C., (1957) Bull. Chonnam Univ. Nat. Sci. Vol. 1: 122-128. 7) (1957) Bull. Chonnam Univ. Nat. Sci. Vol. 1: 80-110. 8) (1959) Res. Bull. Korean Agr. Soc. Vol. 5: 66-69. 9) (1960) Studies on the dimorphism of Persicaria senticosa Nakai (Unpublished). 10) MAKINO, T.,(1942) Illust. Flora of Nippon. Ed. 2, Tokyo. 11) MORRIS, M.R., (1949) Cornell Univ. Abst. Thesis: 356-359. 12) NAKAYAMA, H., (1954) Breeding 3; 88. 13) SEARS, E.R., (1937) Genetics 22: 130-181. 14) STEVENS, N.E., (1912) Bot. Gaz. 13: 59-66. 15) TATEBE, T., (1947) Jour. Hort Assoc. Japan, 16: 106-125. 16) (1949) Res. Breeding 3: 91-95. 17) (1950) Breeding 4: 71-74. 18) (1953) Breeding Vol. 2, No. 4: 240-244. 19) YASUDA, S., (1950) Reproduction of Higher plants Ed. 5, Tokyo.