A Preliminary Study on the Variation of *Trapa* in Japan

By Yasuro Kadono

Department of Biology, College of Liberal Arts, Kobe University, Kobe 657

角 野 康 郎 *: 日本産ヒシ属の変異に關する予察的研究

＊神戸大学教養部生物学教室

Translated from the Japanese by V. Chintu Lai, U.S. Geological Survey

English translation edited by Nancy B. Rybicki, U.S. Geological Survey

This English translation is released with the permission of Yasuro Kadono and the publisher of the original article, which was published in final form in Japanese as follows:

Kadono, Y., 1987, A preliminary study on the variation of *Trapa* in Japan: Acta Phytotaxanomica et Geobotanica, v. 38 (September), p. 199–210, https://doi.org/10.18942/bunruichiri.KJ00002992255.

Open-File Report 2018–1075

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
RYAN K. ZINKE, Secretary

U.S. Geological Survey
James F. Reilly II, Director

U.S. Geological Survey, Reston, Virginia: 2018

For more information on the USGS—the Federal source for science about the Earth, its natural and living resources, natural hazards, and the environment—visit https://www.usgs.gov/ or call 1–888–ASK–USGS (1–888–275–8747).

For an overview of USGS information products, including maps, imagery, and publications, visit https://store.usgs.gov/.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Although this information product, for the most part, is in the public domain, it also may contain copyrighted materials as noted in the text. Permission to reproduce copyrighted items must be secured from the copyright owner.

Suggested citation:
Kadono, Yasuro, 2018, A preliminary study of variation of *Trapa* in Japan (translated into English from the Japanese by V. Chintu Lai): U.S. Geological Survey Open-File Report 2018–1075, 15 p., https://doi.org/10.3133/ofr20181075. [The translation was edited by Nancy B. Rybicki. The article was originally published in Japanese with English summary as Kadono, Y., 1987, A preliminary study of variation of *Trapa* in Japan: Acta Phytotaxonomica et Geobotanica, v. 38 (September), p. 199–210, https://doi.org/10.18942/bunruichiri.KJ00002992255.]

ISSN 2331-1258 (online)

A Preliminary Study on the Variation of *Trapa* in Japan

By Yasuro Kadono[1]

角 野 康 郎： 日本産ヒシ属の変異に關する予察的研究

Translated from the Japanese by V. Chintu Lai[2, 3]

English translation edited by Nancy B. Rybicki[2]

Trapa is the genus of annual aquatic plants, growing in lakes, marshes, irrigation ponds, stagnant waters and pools in the river and waterways, throughout Japan. Growing in poorly nourished (mesotrophic) waters to increasingly rich, nourished (eutrophic) waters, it is one of the most common aquatic plants in Japan.

The study about classification of the genus *Trapa* was initiated by Makino (1908), and advanced by Nakano (1913, 1964) and Nakai 中井 (1942). In particular, Nakano's study was made with attempt to clarify the heredity and the phenotypic variation of vegetative morphological characters through cultivation experiments, and is worth a special mention.

When making classification of the genus *Trapa*, one of the important vegetative morphological characters is the morphology of the nut. The genus *Trapa* can be divided into two major groups: after flowering, the first group has all four sepals forming projections, and is called "four-thorn type." The second group has two sepals fallen off and the remaining two protruding, and is called "two-thorn type." The former has big Onibishi (Monster Hishi: *Trapa natans*) and small Himebishi (Little-princess Hishi, *Trapa incisa*), and the latter has Hishi (*Trapa japonica*). In the current Pictorial Books of the Flora or the like (of Japan), although researchers may notice that some discrepancies in adoption of scientific names (cf. table 1) exist, all seem to be basically in agreement of recognizing the above three kinds of *Trapa* species, except in only a few remaining unsettled problems of treating their variations.

However, as investigations of populations in the genus *Trapa* make progress, it has become noticeable that diverse variation forms or in-between forms, with their appropriate belonging classes uncertain, begin to appear in high frequency (Toda 戸田 1979; Sakurai 桜井 1981; Hamajima 浜島 1981; Sakaguchi 坂口 1982, 1983; Takada 高田 1983). Because investigations hitherto depended on a small number of individuals (specimens), the number was insufficient for us to fully understand the diverse variations that exist in the genus *Trapa*. Thus, when faced with handling of the plant group with such diverse variations as that of the genus *Trapa,* it was essential to first explicate fully the variation within the population and the different variation patterns with the population as a whole. Under such problem-consciousness, I promoted investigations of the genus *Trapa* populations at various places. In the present writing, the account of variations in the genus *Trapa* populations, which were based on the data obtained

[1]Currently at Department of Biology, Graduate School of Science, Kobe University, Nada, Kobe 657-8501, Japan. email: kadono@kobe-u.ac.jp
[2]U.S. Geological Survey.
[3]V.C. Lai translated all parts of the journal article that were in Japanese except for the addresses and names of water bodies listed in table 2. The English table titles, figure captions and explanations, and the "Summary" section from the original journal article were reproduced without change.

mostly from Hyogo Prefecture, will be reported. These will hopefully serve as defining a problem-focusing point for the future study.

Table 1. Japanese name and its Scientific names of *Trapa* taxa used in some recent literatures.
( ): literatures

ヒメビシ Himebishi	オニビシ Onibishi
T. incisa Sieb. et Zucc. (1, 2, 3, 4, 5, 6, 7)	*T. natans* L. (4)
ヒ シ Hishi	*T. natans* var. *japonica* Nakai (1, 3)
T. japonica Flerov (2, 5, 6, 7)	*T. natans* var. *quadrispinosa* Makino (2)
T. bispinosa Roxb. var. *Iinumai* Nakano (1, 3)	*T. natans* var. *rubeola* Makino forma *viridis* Sugimoto (7)
T. bicornis L. f. var. *Iinumai* (Nakano) Nakano (4)	*T. bispinosa* Roxb. var. *japonica* Nakai (6)
イボビシ Ibobishi	メ ビ シ Mebishi
T. bispinosa Roxb. var. *Makinoa* Nakano (1, 3)	*T. natans* L. var. *rubeola* Makino (1, 2, 3, 6)
T. bicornis L. f. var. *Makinoa* (Nakano) Nakano (4)	*T. japonica* Flerov var. *rubeola* (Makino) Ohwi (5)
アカモンビシ Akamonbishi	コオニビシ Koonibishi
T. bicornis L. f. var. *Iwasakii* (Nakano) Nakano (4)	*T. natans* L. var. *pumila* Nakano (4)

文献 1. 原 (1954), 2. 牧野 (1961), 3. 北村・村田 (1961), 4. Nakano (1964), 5. 大井 (1965), 6. 大滝・石戸 (1980), 7. 北川 (1982)

Literature 1. Hara (1954), 2. Makino (1961), 3. Kitamura and Murata (1961), 4. Nakano (1964), 5. Ohwi (1965), 6. Ohtaki and Ishido (1980), 7. Kitagawa (1982)

Material and Methods

Because the most notable variation in the genus *Trapa* is manifested in its nuts, the analysis of the variation was carried out by focusing on the nut morphology.

From the populations at 21 locations shown in table 2, 100 pieces of nuts, in principle, were collected from each population by random sampling. (For the U population, only nuts of Himebishi were collected.) For collection, one of the following three methods was appropriately used. (1) Fully ripe nuts were collected during the autumn (referring only to those either already fallen away from the parent plant or, if even not separated from it, a separation layer was formed between the nut and the stem). The outer rind was removed from the nut after allowing it to rot away; the nut was then measured. (2) From spring to early summer, by carefully pulling out the budding plant, the nut attached to it was collected. (3) We collected nuts that drifted to the waterside. More than one-half of those germinated before the previous year, and thus, turned to nutshells, with many of them having been broken. Hence, only those that were still retaining the measurable parts with them were selected. In the following analyses, regardless of what collection method is used, all nuts are treated uniformly.

Parts for measurement and types of lower projections are shown in figure 1. The lower projections include lower spines and pseudohorns. The former is a sharp-headed projection being developed from a sepal, and has, except in the case of Himebishi, fine reverse barbs on its apical part. We classified this as Type IV. The latter is a bulge that forms after the sepal falls off, for which quite a variety of forms and sizes can be identified. For the study conducted this time, Types I–III have been set up according to length of the horn. Also, the nuts of the genus *Trapa* are commonly asymmetrical in front against rear sides (belly vs. back), and thus the projections at the front side and the rear side are often of different lengths, for such cases the classification was made along the longer projection.

Table 2. Location of populations investigated.

Population	Location and name (or type) of water body		
	Prefecture	Address	Name (or type) of water body
A	Hyogo	兵庫県小野市王子町	(Irrigation pond)
B	Hyogo	兵庫県加東郡社町沢部	(Irrigation pond)
C	Hyogo	兵庫県神戸市西区岩岡町古郷	(Irrigation pond)
D	Hyogo	兵庫県加東郡東条町黒谷	(Irrigation pond)
E	Hyogo	兵庫県三木市興治	(Irrigation pond)
F	Hyogo	兵庫県明石市西島	大池
G	Hyogo	兵庫県加西市野田町	(Irrigation pond)
H	Hyogo	兵庫県加西市田原町	(Irrigation pond)
I	Hyogo	兵庫県加古川市平岡町	漬目池
J	Hyogo	兵庫県加古郡稲美町加古	入ケ池
K	Hyogo	兵庫県加西市倉谷町	アシガ池
L	Hyogo	兵庫県明石市大久保町	上池
M	Hyogo	兵庫県加古郡稲美町天満	天満大池
N	Hyogo	兵庫県加古郡稲美町天満	琴池
O	Hyogo	兵庫県明石市中尾	(Irrigation pond)
P	Hyogo	兵庫県加古郡稲美町母里	(Irrigation pond)
Q	Hyogo	兵庫県神戸市西区神出町	和合成池
R	Hyogo	兵庫県小野市高田町	(Irrigation pond)
S	Hyogo	兵庫県神戸市西区岩岡町岩岡	竜ケ池
T	Hyogo	兵庫県加古郡稲美町加古	(Irrigation pond)
U	Fukuoka	福岡県遠賀郡芦屋町山鹿	(Irrigation channel)

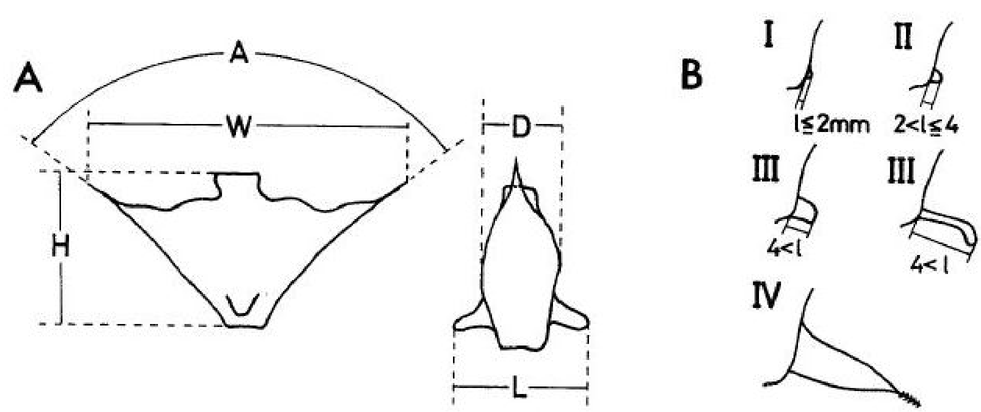

Fig. 1. **A.** Mode of measuring the size of nut. A: angle (°) between upper spines. W: width of the nut across upper spines. H: height of the nut. D: thickness of the nut. L: width across lower projections (pseudohorns or spines).

B. Types of lower projections. I: Pseudohorns shorter than 2 mm. II: Pseudohorns with the length between 2 and 4 mm. III: Pseudohorns longer than 4 mm. IV: Lower spines with acute apex.

In addition, such features as the relative height of top crest to nut shoulder, the degree of bulging of "trace of petal"[4] and, in the case the lower spine exists, its extending direction (horizontally, or downward), were recorded. Also, for the name of each part and its morphological origin, the reader is referred to Miki (1952a) (In this writing, "upper horns" and "lower horns" were each renamed as upper spines and lower spines.)

For morphology of other parts than the nuts, attention was paid to the form of leaves, the conditions of hair growing on the leaf-back and on the petiole. For observation of these, besides the materials of 21 populations shown in table 2, the specimens of Kyoto University as well as those in possession of Kobe University, Liberal Arts School were extensively examined.

Results

1. Variation in the Lower Projections

Figure 2 shows through the investigated populations the frequency with which each type (I–IV) of lower projections had appeared. Population U was supposed to be a mixed group of Hishi and Himebishi, but only the Himebishi nuts were actually collected, and for this reason U was omitted in this figure.

Populations A–D were supposed to be the populations consisting of only two-thorn-type nuts, yet, in any case (population), there were mixings of nuts with undeveloped pseudohorns (I) and that with developed pseudohorns (II, III). This trend was also noticed among the populations that appeared together with nuts of four-thorn type. There were also populations like L, R, and S consisting only of nuts with not well-developed pseudohorns. On the other hand, there were examples like K and M, in which well-developed pseudohorns prevail over the greater part of two-thorn type nuts.

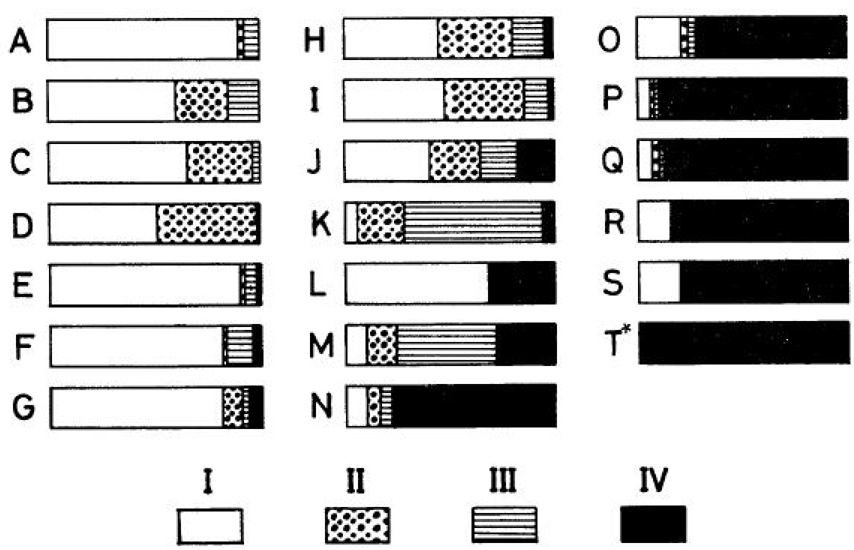

Fig. 2. Proportion of nuts with different types of lower projections in each population. *2-spined nuts were also found on another occasion.

[4]In Miki (1959), this part is considered to be a swelling of an attached piece lying between sepals.

Among populations that include nuts of four-thorn type, it was also found, though small in number, nuts of two-thorn type. As for T, the nuts collected during one autumn were all of four-thorn type, but then in the following spring nuts of two-thorn type were found being dashed against the waterside. This fact allowed us to judge clearly that the population of interest was mixed. This results in a finding that as far as the investigation conducted for this time, there were no nut populations consisting purely of four-thorn type alone.

2. Variation of Size

As the value of the measure of each part is proportional to the size of the whole body of nut, there should have existed, more or less, mutual correlations. Among these, although the width (W: width of the nut across the upper spines) varied by the angle between upper spines (A), it was deemed as the best parameter for indicating the size of the nut itself in the mode of measurement employed this time.

Figure 3 shows the mean widths and the standard deviations of the nuts, based on the types of lower projections. Nuts of two-thorn type will fall into the size class of approximately 30–50 mm (to be called middle-size hereafter) except the case of big nuts that are to be stated later. No difference was found between those with the pseudohorns well developed and those with not well developed (I–III) within the same populations.

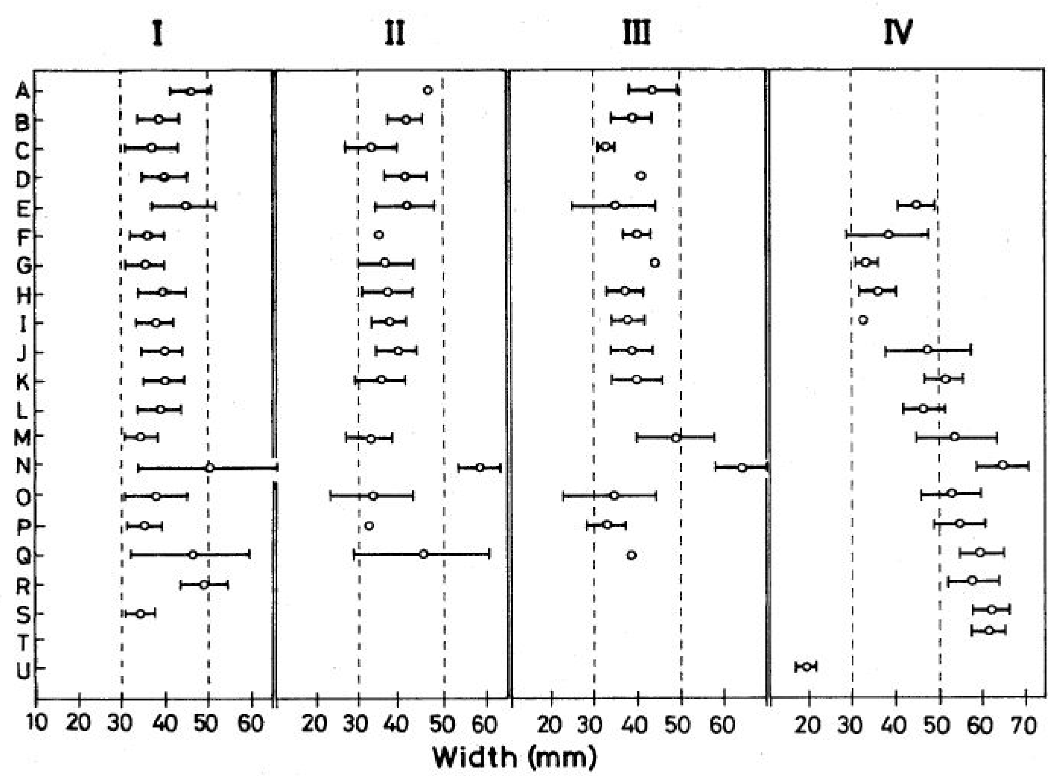

Fig. 3. Width of the nuts (W) with different types of lower projections (I–IV). Means and standard deviations are shown regarding each population (A–U).

Strikingly big-size nuts with two-thorn type were found mixed in populations N and Q. For the nuts in these populations, it was found that there existed frequency distribution of two-crest shape in regard to width. That is, these can be divided into two groups, the middle-size class and the greater-than-50-mm-size class (to be called big-size class hereafter). These two groups are clearly distinguishable by the height of an ovary projection or by the shape of a crest-top. For the big-size nuts, pseudohorns of not-well-developed form (I–II) and broad-width pseudohorns of well-developed form (III) were found.

In case of the nuts of four-thorn type, there existed large variations in size by population. The big-size nuts with width of greater than (45 ~) 50 mm and those belonging to the middle-size class with width 30–50 mm were each found; and there was a population consisting of in-between size nuts (L), also for those populations within each of which the middle-size nuts and the big-size nuts were mixed, the in-between size nuts too were found (such as J and M). The U samples had around 20 mm of width, of exceedingly small and regular shape (with little variation in size).

Shown in figure 4 are the measurement results of body thickness (D) of the nuts. According to the size-class that is divided by the width, the thickness of the small-size nuts was about 5 mm, that of middle-size nuts was 8–13 mm, and that of big-size nuts was 13–23 mm; the classification by thickness into three size classes of small, middle, and big, can be equally made.

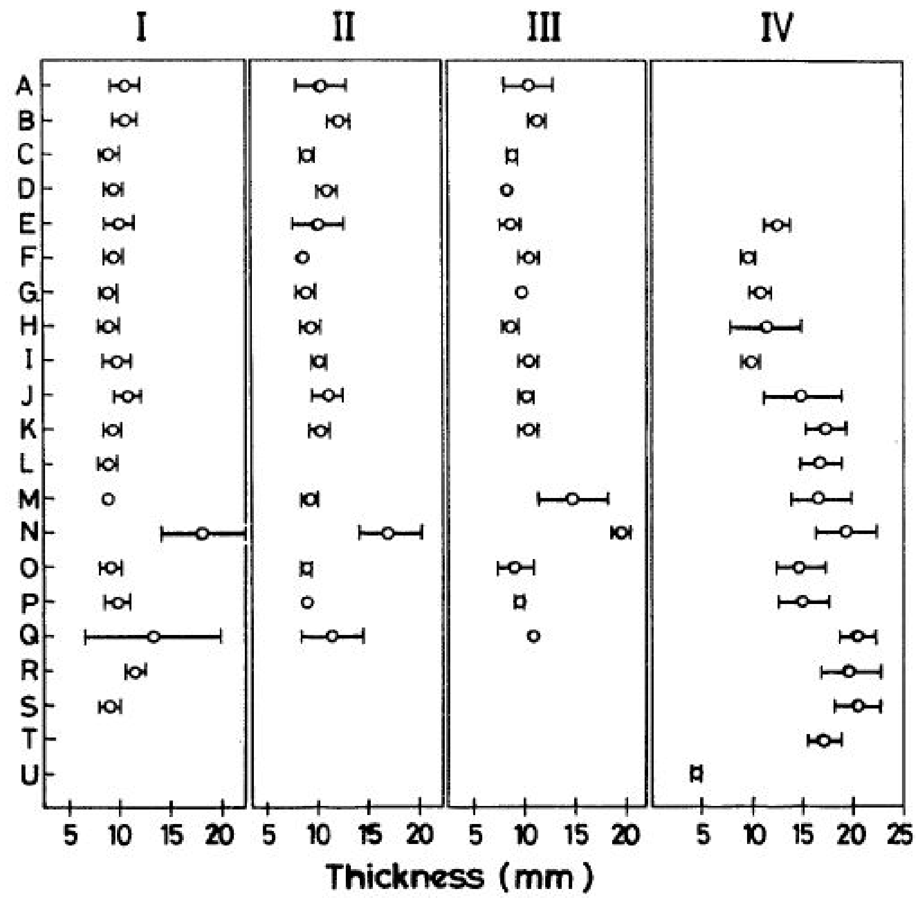

Fig. 4. Thickness of the nut (D). Means and standard deviations are the same as in Fig. 3.

Concerning the height (H, fig. 5), in contrast to the middle-size nuts for which the ovary projection grows quite well, for the big-size nuts the ovary projection will not grow so well and the central part of the nut body is depressed. The difference in its value is not as great as the difference of the body sizes of nuts. For the middle-size nut it gets up to 20 mm, and for the big-size nut it could commonly exceed 20 mm.

The width across lower projections (L) made a large difference in its value depending on the existence of lower spines or the extent of pseudohorn development, and also on the direction in which they extend, horizontal or downward. For those types in which pseudohorns do not develop well, L turned out to be about the same value as that of the thickness; for big-size nuts with lower spines, this value reached to 45–60 mm (data not shown).

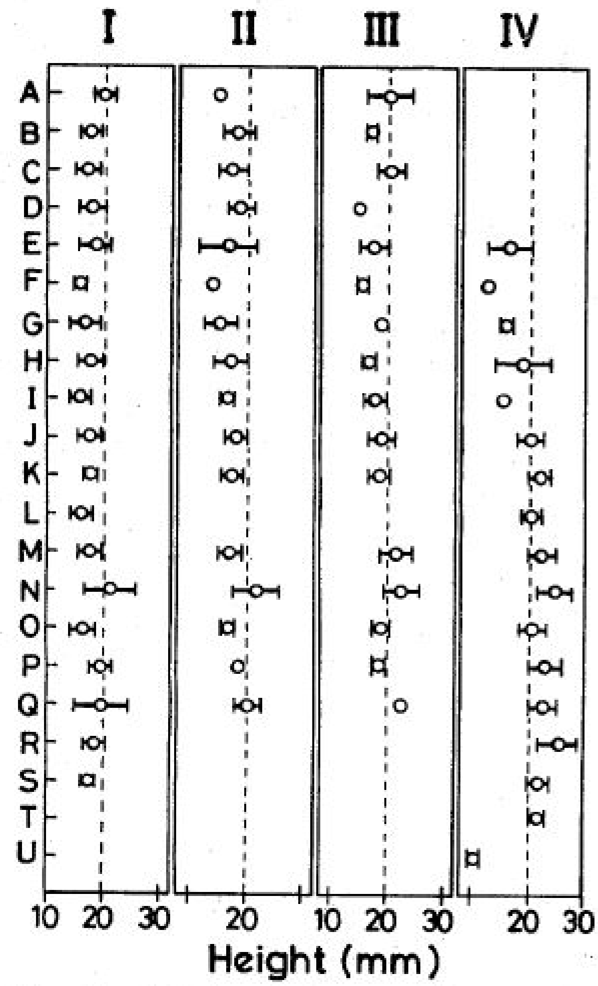

Fig. 5. Height of the nut (H). Means and standard deviations are the same as in Fig. 3.

3. Variation of Open-Angle of Upper Spines; Others

The angle between upper spines (table 3) varies from -30° (oriented inwardly) to 190° for the nuts of two-thorn type, and does so in a range of 60° to 220° for the nuts of four-thorn type. The ranges of variation were rather large whether within or between populations.

When the nuts of two-thorn type and that of four-thorn type (excluding Himebishi of U) were compared, it was found that the latter exhibited a tendency of larger angle between upper spines in most of the populations, yet there was also found a case of reverse situation in L.

The ovary projections of the middle-size nuts of two-thorn type generally grow fairly well and their top-crown often reaches higher than the shoulder of the body of nut. On the other hand, in the case of the nuts of four-thorn type, it has become clear that a tendency has existed such that projections of small-size nuts protrude high, and that of big-size nuts has been depressed around the ovary projection, making the top-crown lower than the shoulder. For the middle-size nuts, there were ovary projections that would grow and that would not.

Table 3. Variation of angle between upper spines (A). The means and standard deviations are shown regarding each population. In parentheses the number of nuts.

Population	Type of Lower projections			
	I	II	III	IV
A	119±19(62)	150±14(2)	112±25(5)	—
B	99±17(60)	104±20(25)	109± 8(15)	—
C	93±22(52)	64±31(26)	50±14(2)	—
D	116±19(51)	113±16(48)	130 (1)	—
E	119±30(91)	118± 4(2)	78±13(5)	120±14(2)
F	112±23(63)	95 (1)	134±13(10)	138±32(2)
G	101±17(82)	108±24(11)	130 (1)	103±17(6)
H	119±23(46)	108±25(35)	110±21(15)	138±36(4)
I	124±18(49)	118±13(38)	117± 8(11)	108± 4(2)
J	116±21(41)	109±15(24)	110±14(18)	139±24(17)
K	103±42(6)	68±24(24)	87±31(70)	141±14(5)
L	154±29(68)	—	—	128±12(32)
M	107±19(16)	95±24(23)	113±22(77)	161±32(45)
N	140±46(8)	167±26(5)	173±13(4)	171±25(62)
O	127±30(20)	103±40(3)	105±21(2)	127±22(75)
P	91± 8(6)	70 (1)	80±14(2)	150±26(91)
Q	134±30(7)	105±26(3)	100 (1)	187±24(88)
R	117±18(15)	—	—	148±24(85)
S	95±21(18)	—	—	187±20(81)
T	—	—	—	153±18(100)
U	—	—	—	83±11(100)

Although there were mixes of some number of nuts on which surfaces exhibited distinctly different forms of projections and protuberances, none of these would make their appearance in any specific populations as a group.

4. Variation of Leaf

Concerning the form and size of leaves, no quantitative analysis has so far been made, only those points that were noticed during qualitative observations will be written down herewith. Moreover, these observations were limited to the plants which were determinable to be Himebishi, Hishi, or Onibishi based on the morphology of nuts.

The width of a Himebishi leaf reaches up to about 20 mm, but its length often grows longer than its width, and teeth at the edge of the leaf looked conspicuous. However, as Hishi also sometimes has the same shape of small leaf, it was rather risky to identify Himebishi only by the shape of leaf. The leaves of both Hishi and Onibishi have extremely rich variety of shapes and sizes, and it was thought impossible to distinguish one kind from the other.

About the hairs on leaf-back or on leafstalk (petiole), those of Himebishi, except the undeveloped leaves, were of mostly inconspicuous kinds, but specimens of leaves with hair were also found. As to those of Hishi and Onibishi, considerable variations in growing conditions of hair were noticed. As against the Hishi grown in the West Japan, for which many leaves grow thick hair on leaf-back or leafstalks, it was noteworthy to mention that very little hair was found for specimens of Hokkaido (northern Japan).

Discussion

1. How to Comprehend the Variations

It has been clarified through the study made this time that there exists a great breadth of variation among the populations of the genus *Trapa*, and that the mode (or pattern) of variation differs from population to population. Hence, the examination into the background of such variations will be made first herein.

Among the nuts of the genus *Trapa*, the factors that make striking variations and cause confusion in taxonomy are the size of the nuts and the form of or the difference in degree of development of pseudohorns. For the size of the nuts, it was pointed out by Staszkiewicz and Wojcicki (1981) that the size could be influenced by the environmental conditions of the place in which the nuts grew. With this in mind, the author would like to advance his study further in future. Not only an incidental factor attendant with the falling of calyxes foster the development of pseudohorns, but, it is certain, the heredity background does affect the development of pseudohorns also. The trend in pseudohorn development shows clear difference by population.

Thereupon, what becomes the problem is the origin of such hereditary variations. Plants that bear the nuts with well-developed pseudohorns were considered to be the in-between forms that intervene the four-thorned species (of *Trapa*) (Onibishi) and the two-thorned one (Hishi), and to be regarded as the results of hybridization (Sakurai 桜井 1981; Sakaguchi 坂口 1982). However, Nakano (1913) maintains a negative opinion to the theory of hybrid origin of intermediate forms. Among those populations investigated this time, the fact of witnessing complete absence of the in-between form in populations such as L, R, and S, where in spite of mixed growing of Hishi with poorly developed pseudohorns and Onibishi with four spines, might suggest the limited chance of crossing between Hishi and Onibishi.

In order to explore the possibility of hybrid formation, it is necessary to clarify the breeding system of the genus *Trapa*. Kadono and Schneider (1986) state that for Onibishi the main mode of pollination is by self-pollination, and cross pollination occurs only contingently. They also show that bearing nuts by apomixis is attainable. By this matter, their statement throws a doubt upon the viewpoint expressed by Staszkiewicz and Wojcicki (1979) that frequent hybridization is taking place in the genus *Trapa*.

It also looks necessary here to examine whether or not an entity yielding nuts of well-developed pseudohorns possesses the character of crossbred species. A preliminary investigation has been made at the K population hitherto as to the fruit set and the situation of pollen grains. As a result, it was found that there was no such thing as to the “in-between form” generating inferior nuts, and also that the generated pollen grains were quite normal (Kadono, unpub. data). These facts do not by themselves negate the possibility of the “in-between form” to be of mixed breed, it could show the limitation in an approach from such a side.

On the other hand, there exists a fact that suggests a possibility of the "in-between form." When Toda 戸田 (1979) sowed nuts having well-developed pseudohorns, the second-generation plants yielded a great variant of nuts, ranging from those having no pseudohorns to those with well-developed pseudohorns. This fact may allow us to infer that for those populations in which the pseudohorns may develop, the introduction of the gene from the plant of four-thorn type takes place first, after which character separation may occur. In such case, it could be interpreted that the populations like L, R, and S have not had a chance for cross breeding. In any case, it is necessary to give a rigorous examination on this matter from the viewpoint of cytogenetics. It is also of help to conduct artificial cross breeding experiments.

The fact that every population has different pattern of variation may be explained by the dominance of self-pollination, which might facilitate preservation, within that population, of an entity variation resulting from some key factor or other. Furthermore, the irrigation pond or the waterway, in which *Trapa* plants grow, each becomes an island isolated from each other. In addition, the seeds of the genus *Trapa* will adapt themselves by sinking down and then sprouting at the same location where the parent plants had grown up. Because the nuts may be unsuitable for extensive seed scattering (Miki, 1952b), it is understandable that the chance for exchanging genes with other populations will be much limited.

Although presently it cannot be argued as to when the genus *Trapa* changed its mode of pollination to self-pollination, it is shown, according to a study of fossil by Miki (1952b), that hereditary isolation has played an important role in the evolution of the genus *Trapa*. Miki has reported from Japan 16 fossil species of the genus *Trapa*, where each individual species ceased to exist in a relatively short period and is replaced by a new species. He also has pointed out finding of numerous "local species." For the genus *Trapa*, new variation forms continue to appear both temporally and spatially, and these (variation forms) each speciate to new "species" on one hand, yet, many other populations were exterminated because of the poor migratory nature: such a history of differentiation is repeated and today's complex variation patterns were considered to be brought about in this way.

2. The Type and Classification of the Nuts

One of the reasons for confusion in classification of the genus *Trapa* is, as stated earlier, in the variation of nut morphology. However, by considering together its size and configuration of the lower projections, it is possible to recognize some entities for the shape of nuts. An attempt is made herein to examine the present results with the previous way of classification.

Among the middle-size nuts of two-thorn type, there were the nuts without well-developed pseudohorns (fig. 6-B) and those that had pseudohorns well developed (fig. 6-C, D). According to the degree of pseudohorn development, Nakano (1964) has divided these into three varieties, called Akamonbishi (which was called as Akababishi in (Nakano (1913)), Hishi, and Ibobishi. However, not only do many of these "variants" grow mingled within one population, but they could also coexist often on one same plant shoot. Nakano (1913) maintained the degree of pseudohorn development to be hereditary, but it is clear from the cultivation experiments of Toda 戸田 (1979), as mentioned earlier, this form would not necessarily be considered to be a stable character. (Furthermore, although it was not included in the report this time, there were also some populations in which all nuts had extremely stable, well-developed pseudohorns; thus, before we make any conclusion on the matter of pseudohorns it seemed necessary to conduct a broader investigation.) I think that this type of variations is, as stated by Toda 戸田 (1979) or Takada 高田 (1983) also, better treated as the polymorphic variation within the species Hishi (*Trapa japonica*).

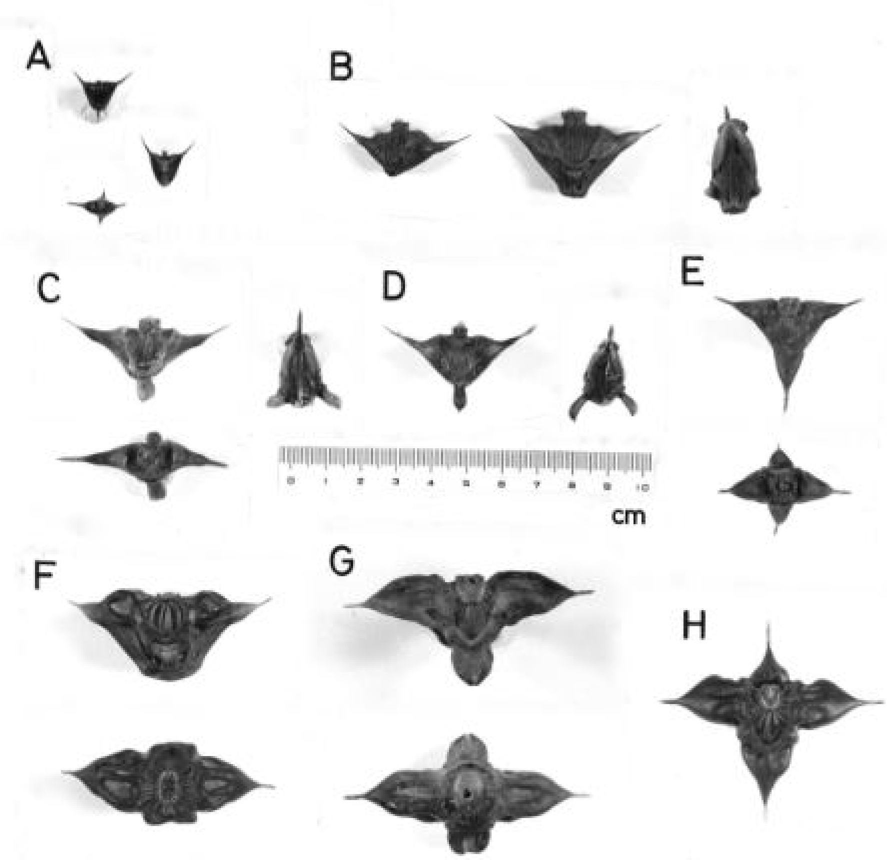

Fig. 6. Representative specimens of the nuts. A. 4-spined nut of small size. B–D: 2-spined nuts of middle size without pseudohorns (B) and with developed pseudohorns (C, D). E: 4-spined nut of middle size. F–G: 2-spined nuts of big size without pronounced pseudohorns (F) and with pseudohorns (G). H: 4-spined nut of big size.

Among the big-size nuts of the two-thorn type, one type having little developed pseudohorns (fig. 6-F) and another type having well-developed broad pseudohorns (fig. 6-G) can be found. The former grows in India or China, and is possibly imported to Japan by the name, *T. bispinosa* Roxb. (トウビシ). This matter will be left as a study subject hereafter, but because of its low frequency in the fields, it is conjectured not to have a particularly great fecundity, or competitive power. The latter type found in the M population showed especially little variations. This type of nut is said to grow also in irrigation ponds of Kagawa Prefecture (Sakaguchi 坂口, private communication). Whether or not to recognize the said type as a new taxon is also a task awaiting decision.

Among the nuts of four-thorn type, the small-size Himebishi (fig. 6-A) has a 16- to 23-mm-wide gap between the upper spines, and about 10-mm-tall height; the nut itself is rather long in the vertical direction, and is clearly identifiable as a distinct species. According to the specimen, the Himebishi has been widely collected from everywhere in Japan, yet it has become exceedingly rare species presently. [It is to be called attention herein that there are often examples of misidentifying Koonibishi (コオニビシ) —to be mentioned later—with Himebishi (ヒメビシ).]

The big-size nut (fig. 6-H) corresponds to Onibishi (オニビシ). However, for the lower spines, there are those having broad width with thick spine and those with thin one, which show rich variations.

As to the orientation of the lower spines, there are those directing downwards, or those growing horizontally, or even those bending upwards, and all these can be found within the same population. The difference in forms of the lower spines may probably be treated as the variation within a species.

Among the big-size nuts bearing four thorns, there are those having petioles tinged with red. Makino (1913) classified these as a Mebishi (メビシ var. *rubeola*), being a variety of the Onibishi. However, the fact that the petioles, or sometimes the laminas themselves, become tinged with red in the early autumn is more or less a common feature for the genus *Trapa*, and is not a phenomenon seen in a particular population or for some limited entities. Because there is no difference in the morphology of nuts between the "Onibishi" whose leafstalk does not turn to red, and the "Mebishi" that does turn to red, the writer cannot agree to regard the Mebishi as an independent taxon.

The middle-size nuts of the four-thorn type (fig. 6-E) were made as an independent variety named Koonibishi コオニビシ (a variant of Onibishi) by Nakano (1964). Among the data collected this time, the nuts considered to be corresponding to Koonibishi had made quite a frequent appearance. Yet, they always mingle with Onibishi and, moreover, are continually shifting their sizes. Looking at this fact, a question might be thrown in as to if the Koonibishi is nothing but the ill-developed nut of the Onibishi nuts.

When this question is considered, it is important to examine the germination ability of immature seeds. In groups of the genus *Trapa*, even during the summer to early-autumn growing season, Hishihamushi (*Galerucella nipponensis* Labois) bites the leaves of plants, and sometimes the whole population would get a destructive blow. In such a case, most of the still small, premature nuts will fall away and die in the water. Had these nuts grown up to a certain point, could they have survived to the next year and functioned rightly as seeds? Thus, in population M, in which many Koonibishi nuts were found, the nuts of various mature stages were collected, and they were left submerged in water till the next spring in order to observe their germination ability. The results were as follows: The nuts taken out of individuals identified as the Onibishi have the minimum size of 32-mm width (which amounts to 47.6% that of the largest Onibishi nuts in the M population), the inner pericarp became hard, and the sprouting has been observed. In the study made this time, the true characteristics of what we thought to be the nut of Koonibishi could be questionable. They may be the immature seeds of Onibishi. This doubt is further intensified by the fact that the writer had not yet observed the state of the Koonibishi nut attaching to a parent plant. It probably demands our reexamination of the true identity of Koonibishi.

The Onibishi has also large variations between populations as the Hishi does. The variations are particularly notable in nut size. There are populations (such as S, T) with average width exceeding 60 mm on the one hand, there found a population (L) with that being less than 50 mm on the other. For the dry weight, which is not affected by the shape of nut, it has been confirmed that there exist statistically significant differences between populations (Kadono, unpub. data). The writer thinks that in the Onibishi case, it is best at this point to comprehensively treat the Onibishi as a species with considerable degree of variations.

The writer hopes to deepen understanding of the identity of varied types and to reexamine classification ranks and (valid) scientific names by collecting additional data throughout the country in the future.

Acknowledgment: Ms. Mutsuko Ohno (大野睦子) of Kita-kyushu City kindly collected nuts of Himebishi from Fukuoka Prefecture, and also guided the writer through his field visit. The writer would like to express his most sincere appreciation. This study is a part of the research grant by the Japan Society of the Promotion of Science, No. 60740381.

Summary

Frequent occurrence of intermediate forms and poor knowledge on the variability of characters have caused some difficulties in the taxonomy of *Trapa* in Japan. Thus I made a preliminary analysis on the variation of nuts collected from 21 populations in Southwestern Japan. Attention was paid to some morphometrical characters of the nut and development of lower spines or "pseudohorns."

Each population usually contained different forms of nuts. Among them, however, several entities could be recognized based on the shape of nut as follows.

1) Two-spined form: This included nuts of middle size (width 30–50 mm) and ones of big size (width over 45 mm). In case of the former ones (fig. 6-B, C, D) the nuts with pseudohorns of varying degree of development usually occurred together within one population and even on a single plant. I propose to treat them as one taxon, *Trapa japonica,* sensu OHWI (1965), without inventing varieties. But at the same time it was remarkable that the tendency of development of pseudohorns was apparently different from population to population.

The bigger ones included two types, that is, one without pseudohorns (fig. 6-F) and the other with pseudohorns (fig. 6-G). The former one may be identified as *T. bispinosa* ROXB., but the latter one has not been described in literature.

2) Four-spined form: The nuts of small size (width of about 20 mm, fig. 6-A) were well definable and thought to be *T. incisa* SIEB. et ZUCC. The nuts of bigger size showed some variations with respect to their size and/or stoutness of lower spines. The big ones (width over 45 mm, fig. 6-H) may be treated as one taxon, *T. natans* or its variety. The nuts of middle size (fig. 6-E) have been named *T. natans* var. *pumila* NAKANO. But so far as present materials were concerned, its entity seemed dubious. They might be immature nuts of bigger ones.

The different patterns of variation among populations were ascribed to genetic differentiation. Predominance of self-pollination and isolation of habitats were thought to promote genetic isolation and preservation of genetic variations which occurred in each population. But the possibility of hybridization cannot be excluded.

References

Hamajima, S. 1981. Investigation of water plants in irrigation ponds. 3. Irrigation ponds in Mie and Gifu. *Plants and Nature* 15 (13): 27–30 (in Japanese).

Hara, H. 1954. Enumeratio Spermatophytarium Japonicarum. Vol. 3. Iwanami Shoten, Tokyo.

Kadono,Y. and E.L. Schneider, 1986. Floral biology of *Trapa natans* var. *japonica*. Bot. Mag., Tokyo 99: 435–439.

Kitagawa, M. 1982. Trapaceae (Hishi Family) *in* "Wild Plants of Japan II" edited by Y. Satake 佐竹義輔, J. Ohwi 大井次三郎, S. Kitamura 北村四郎, S. Watari 亘里俊次, and T. Tominari 富成忠夫: 262, Heibonsha, Tokyo (in Japanese).

Kitamura, S. and G. Murata, 1961. Colored illustrations of herbaceous plants of Japan. Vol. 2 (Choripetalae). Hoikusha, Osaka (in Japanese).

Makino, T. 1961. Makino's new illustrated flora of Japan. Hokuryukan, Tokyo (in Japanese).

Makino, T. 1908. Observations on the flora of Japan. Bot. Mag., Tokyo 22: 171–172.

-------------, 1913. Observations on the flora of Japan. Bot. Mag., Tokyo 27: 251–252.

Miki, S. 1952a. Morphological character of remains of *Trapa* and adaption to water life. Bull. Soc. Pl. Ecol. 2: 111–116 (in Japanese).

Miki, S. 1952b. *Trapa* of Japan with special reference to its remains. J. Inst. Polytech. Osaka City Univ., Ser. D 3: 1–29, pls. 2
---------, 1959. Evolution of *Trapa* from ancestral *Lythrum* through *Hemitrapa*. Proc. Jap. Acad. 35: 289–294.
Nakai, T. 1942. Notulae ad Plantas Aseie Orientalis (XXI). Jour. Jap. Bot. 18: 421–437 (in Japanese).
Nakano, H. 1913. Beitrage zur Kenntnis der Variationen von *Trapa* in Japan (Contributions to the cognizance of the variations of *Trapa* in Japan). Bot. Jahrb. 50: 440–458 (in German).
---------, 1964. Further studies on *Trapa* from Japan and its adjacent countries. Bot. Mag. Tokyo 77: 159–167.
Ohwi, J. 1965. Flora of Japan. Revised Edition. Shibundo, Tokyo (in Japanese).
Ohtaki, S. and T. Ishido, 1980. Illustrated Japanese water plants. Hokuryukan, Tokyo (in Japanese).
Sakaguchi, S. 1982. A report of the genus *Trapa* in Kagawa Prefecture. Japan (1). Kagawa Seibutsu, 10: 13–17 (in Japanese).
----------------, 1983. A report of the genus *Trapa* in Kagawa Prefecture, Japan (2) Kagawa Seibutsu, 11: 35–39 (in Japanese).
Sakurai, Y. 1981. Changes of flora, vegetation area and biomass of aquatic plants in the recent progress of eutrophication in Lake Kasumigaura—Research Report from the National Institute for Environmental Studies 22: 229–279 (in Japanese).
Staszkiewicz, J. and J.J. Wojcicki, 1979. Biometrical analysis of *Trapa* L. nuts from Poland. Fragmenta Floristica et Geobotanica 25 (1): 33–59 (in Polish with English summary).
--------------------- and ---------------, 1981, Variability of the metrical characters of nuts of the genus *Trapa* L. from central Europe. Fragmenta Floristica et Geobotanica 27 (3): 415–431.
Takada, J. 1983. Taxonomic notes of the plants in Akita Prefecture (1) Tohoku Shokubutsu Kenkyu (Plant Research in Tohoku Region) 1: 7–12 (in Japanese).
Toda, H. 1979. On the Hishi (*Trapa*) of western Shizuoka Prefecture. Biology of Shizuoka Prefecture: 330–333 (in Japanese).