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Studies on the Sterility in Garlic, *Allium sativum* L.

Takeomi ETOH
(*Laboratory of Vegetable Crops*)
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I. Introduction

Garlic, *Allium sativum* L., is sterile, and this is regarded as a representative of the obligate apomicts by Fryxell[18]. It has long been known only as a cultivated plant. Its truly wild species has not been confirmed yet, though its native home is presumed to be Central Asia[4,34,68]. It was surely cultivated at ancient Egypt, because the clay models of its bulbs were found in the predynastic cemeteries[28], and since then it has been propagated probably by bulbs or bulbils. It is uncertain whether garlic acquired its sterility after the beginning of its cultivation or not, but the sterility in garlic is no doubt a consequence or a product of the species evolution including domestication.

On account of its sterility, garlic has some problems to be solved. First, it has never been improved by crossbreeding, and its breeding is limited to individual selection and induced mutation. However, the opening of crossbreeding in garlic is desired earnestly by the breeders, because garlic is the most widely used one of the cultivated *Alliums* next to common onion[2]. Moreover, it is believed that the increase of consumption will be the greatest in garlic among the cultivated *Allium* forms because of its importance as a condiment with characteristic flavor. Secondly, the virus infection through bulbs is a serious problem in the practical farming, and the sexual propagation itself is desired by the farmers to avoid this problem. Thirdly, there is difficulty in examining the relationship between garlic and its allied species. In addition to garlic, its allied species, *A. longicuspis* Regel and *A. scorodoprasum* L., are also sterile. The former is regarded as the wild ancestor of garlic by Vvedensky[74]. Because of sterility, the interspecific hybrids between garlic and these species have never been obtained. Without the interspecific hybrids, it is difficult to clarify the relationship between those species cytologically. Lastly, the cause of sterility in garlic, as well as in two other species, has not been clarified yet.

Gustafsson[21] classified the sterile and vegetatively-reproduced plants such as garlic into two types. In the first type, propagules are formed outside the floral region, while those arise instead of or in addition to flowers in the second type (vivipary). In the former, the simplest cause of sterility is to be found in *Lilium bulbiferum*, which is self-incompatible and is propagated by bulbils formed in the axils of leaves or of bracts. In the sterile race of *Fritillaria imperialis*, the female organ develops incompletely. It produces numerous bulblets on the mother bulb. In *Narcissus incomparabilis*, the sterility is probably of species-cross character. In *Acorus calamus*, its seed-sterility is caused by polyploidy. This is propagated with rhizomes. In the second type stated by Gustafsson[21], the viviparous plants can be classified into three groups: (1) The bulbils are accessory formation in the floral branches. (Examples: *Agave* species.) (2) The bulbils are developed instead of flowers or are intermingled with flowers. (Examples: *Allium* species.) (3) The inflorescence or its certain parts are transformed into independent vegetative shoots. (Examples: *Poa*

alpina and *Festuca ovina*.) Garlic belongs to this second group, though it also produces bulbs besides bulbils.

The cause of sterility in garlic was discussed several times. Weber[76] observed the degeneration of embryo mother cells. Takenaka[63] reported multivalent chromosomes in pollen mother cells (PMC), and he acquired an idea that garlic was of hybrid origin. Koul and Gohil[41] observed normal pairing of chromosomes in PMCs, and they presumed that the sterility was caused by nutritional competition between flowers and bulbils. Novak[52] ascribed the sterility in garlic to the abnormal development of the tapetum. Konvicka[37] attributed the sterility to the infection of microorganisms.

The cause of these complicated results appeared to be due to the fact that those workers used different materials, namely, different clones of garlic. Therefore, the present study was conducted to reveal the causal aspects of sterility in various garlic clones and the intraspecific variations of breeding systems in garlic, as a basic study for the cross-breeding in garlic.

II. Aspects of the sterility in garlic

Garlic is one of the minor crops. It was impossible to crossbreed garlic. Moreover, garlic scarcely shows conspicuous properties such as flower colors in the ornamental plants. For these reasons, garlic has been bred insufficiently. Therefore, there are not so many "cultivated varieties" in garlic as in major crops. However, as it is propagated vegetatively, many local varieties are grown in the respective areas. Strictly speaking, these local varieties had better be called local clones, because they have not been established as the "cultivated varieties". Therefore, in this investigation, the term "clone" was used for every material.

Garlic showed some different phenomena on its sterility. The causal aspects of sterility seemed to vary in accordance with the difference in clones. Therefore, "cultivated varieties" such as 'Shanhai-wase' or 'Messidrome' were collected as many as possible. Moreover, the genetical variation among the local clones was previously indicated by Katayama[32], accordingly the local clones were also collected as many as possible.

1. Bolting habit

Garlic showed a wide variation in bolting habit among the clones, from complete- to non-bolting. Complete-bolting clones always bolt, and their flower-stalks fully elongate high above the ground. Their inflorescences come out of the false stems, namely, the leaf-sheaths. On the other hand, non-bolting clones neither bolt nor develop flower-buds. Incomplete-bolting clones do not always bolt, in other words, their inflorescences are partially exserted, remaining within the false stems. Their flower-stalks insufficiently elongate even at the end of the growth, and their bulbils swell within the false stems.

The garlic clones collected were grown in the open field and examined their bolting habits at Kagoshima located in the southern part of Japan.

Materials and Methods

The names of the clones used here are shown in Table 1. Table 1 includes not only the clones used in this examination but also those in the following examinations. The names of the local clones were given according to the habitat or locality where they were collected. Many clones were

Table 1. Garlic clones used in the present study*

No.	Names of clones	No.	Names of clones
1	Hokkaido	51	Taichu-Nankotsu
2	Howaito-Roppen (Miyagi)	52	To-ninniku
3	Tochigi-Roppen	53	Koka (Hinghwa, China)
4	Niigata	54	Fukushu (Foochow, China)
5	Howaito-Roppen (Niigata-Kubiki)	55	Egypt
		56	California Early
6	Niigata-Sado	57	California Late
7	Sado-zairai	58	Thailand
8	Ibaraki	60	Chili
9	Chiba-A	61	Italy
10	Chiba-B	62	U. S. A.
11	Howaito-Roppen (Ishikawa)	63	Saga-zairai
12	Matsumoto	64	Shanhai-wase
13	Shizuoka-zairai	65	Iki-shu
14	Shizuoka-wase	66	Tokushima-zairai
15	Hamamatsu	67	Amami-A
16	Wakayama-Roppen	68	Amami-B
17	Howaito-Roppen (Tottori)	69	Colombia
18	Shimane-Tsunozu	70	Taiwan-A
19	Kochi-shokyu	71	Iki-wase (Tokushima)
20	Kochi	72	Kagawa-Howaito-Roppen (Tokushima)
21	Kochi-daikyu		
22	Kochi-zairai	73	Shanhai-wase (Tokushima)
23	Saga-Ariura	74	Ambon (Indonesia)
24	Saga-ooninniku	75	Kushikino-wase
25	Kagoshima-A	76	Kasari-Sani (Amami)-1
26	Kagoshima-B	77	Agrelo
27	Kagoshima-zairai	78	Tunuyan (red)
28	Okinawa-nanbu	79	Tunuyan (white)
30	Amami-2	80	Okinoerabu
31	Iki-No. 1	81	Morioka-A
32	Iki-No. 3	82	Morioka-B
34	Howaito-Napori	83	Nyu-Howaito-Roppen (Iwate)
35	Toroku-kuroba-Kokotsu	84	Ichinoseki
36	Wase-ninniku	85	Sendai-A
37	Okute-B	86	Sendai-B
38	Red Salmona	87	Iwate-Howaito (Fukushima)
40	Kokotsu	88	Tokyo
41	Roppen-shu	89	Sweden
42	Taiwan-ooninniku-B	90	Kasari-Sani (Amami)-2
43	Taiwan	91	China-Bai Pi
44	Taiwan-daikyu-pinku	93	Mito-A
45	Taiwan-shokyu-pinku	94	Mito-B
46	Taiwan-pinku	95	Mito-C
47	Hong Kong	96	Utsunomiya-A
50	Kanko (Hankow, China)	97	Utsunomiya-B

Table 1 (Continued)

No.	Names of clones	No.	Names of clones
98	Takasaki-A	143	Messidrome (France)
99	Takasaki-B	144	Kabyle (Algeria)
100	Takasaki-C	145	Pag (Yugoslavia)
101	Koriyama	146	Argentina
102	Yonezawa	147	Iberose (Spain)
103	Yamagata-A	148	Rumania
104	Yamagata-B	149	Chinchon(Spain)
105	Shinjo	150	Haiti No. 3
106	Sakata	151	Aurganzinsky (USSR)
107	Kisakata	152	Fructidor (France)
109	Mogami-zairai	153	Ail du Nord No. 6 (France)
110	Hachimantai	154	F. S. Selection No. 2 (Fiji)
111	Howaito	155	Maulitius
112	Ishu-wase (Sakata)	156	Rodriguez
113	Takii	157	Sancti Oriritus
114	Yokohama	158	Suva-1 (Fiji)
115	Matsuwo	159	Suva-2 (Fiji)
116	Howaito (Nagano)	160	Namhae (Korea)
117	Ishu-wase (V. F.)	161	Seosan (Korea)
118	Fukuchi-Howaito	162	Euiseon (Korea)
119	Shichigahama-zairai	163	Towa (Iwate)
120	Iwaki-shu	164	Fukuchi-Howaito (Iwate)
121	Odaresu	165	Hachimantai (Iwate)
122	Yagi	166	Ichinoseki (Iwate)
123	Fukuchi-zairai	167	Kindaichi (Iwate)
124	Kanchi-Howaito	169	Mito-D
125	Manmosu	170	Mito-E
126	Kitami-zairai	171	London-A
127	Furano-zairai	172	London-B
128	Peking	173	London-C
129	Iriomote	174	London-D
130	Moscow	175	Moscow-2
131	Jamaica	176	Gribovo No. 60 (USSR)
132	Meneses (Cuba)	177	Haulien (Taiwan)-A
133	Aegypt	182	Tashkent-2 (USSR)
134	Santi Spiritus (Cuba)	187	Tashkent-7 (USSR)
135	Mexico	188	Tashkent-8 (USSR)
136	Reunion	189	Samarkand-1 (USSR)
137	Peru	190	Samarkand-2 (USSR)
138	Puigcerda 2 (Spain)	191	Samarkand-3 (USSR)
139	Vendeen 14 (France)	197	Dushanbe-4 (USSR)
140	Issoudun No. 2 (France)	202	Frunze-5 (USSR)
141	Germidour (France)	208	Ashkhabad-3 (USSR)
142	Thermidrome (France)	209	Moscow-3 (USSR)

Table 1 (Continued)

No.	Names of clones	No.	Names of clones
226	Mexico-2	231	Ethiopia

* Sources or suppliers of the examined clones

1–65; Saga-ken Hatachi Eino Shidosho (Bureau of Upland Field Crops, Saga)
66; Local variety in Tokushima
67, 68, 90; Local varieties in Amami, Kagoshima
69; Local variety in Colombia presented by Dr. Y. Tashiro of Saga Univ.
70; Local variety in Taiwan presented by Asst. Prof. K. Ishihata of Kagoshima Univ.
71–73; Tokushima Agric. Exper. Sta.
74; Local variety in Ambon, Indonesia, presented by Prof. H. Ogura of Kagoshima Univ.
75; Local variety in Kushikino, Kagoshima
76; Local variety in Amami, Kagoshima, originally introduced from the mainland of Japan
77–79; Local varieties in Argentina presented by Ing. Agr. R. Deromedis of Univ. Nacional de San Luis, Argentina
80; Local variety in Okinoerabu, Kagoshima
81–88, 93–107, 114, 169, 170; Purchased at the local markets in northern Japan
89; Obtained in Sweden by Prof. S. Iwasa of Iwate Univ.
91; Local variety in Peking, China, presented by Dr. Chu Ming Kai of Chinese Academy of Agricultural Science
109–111, 119, 120, 122; Local varieties in northern Japan (See the reference[71])
112; Sakata Seed Co.
113; Takii Seed Co.
115; Matuwo Seed Co., Kagoshima
116; Nanshin Agric. Exper. Sta., Nagano
117; Iki-gun Nogyo-kyodo-kumiai (Iki-gun Agri. Co-op. Ass'n, Nagasaki)
118; San-nohe Agric. Extention Sta., Aomori
121; Purchased at the local market in Kagoshima
123–127; Aomori Agric. Exper. Sta.
128; Local variety in Peking, China, presented by Prof. T. Terashita of Kagoshima Univ.
129; Local variety in Iriomote, Okinawa, presented by Prof. A. Nagatomi of Kagoshima Univ.
130, 175, 176; Moscow Central Botanic Garden, USSR
131–153; Presented by Dr. C. M. Messiaen of INRA, France
154–157; Sigatoka Research Sta. of Agri., Fiji
158–159; Purchased at the local markets in Suva, Fiji
160–162; Korean varieties presented by Prof. Hee-Don Chung of Yeungnam Univ., Korea
163–167; Local varieties in northern Japan presented by Prof. S. Iwasa of Iwate Univ.
171–174; Purchased in London, England (171, 174; imported from France) (172, 173; imported from Italy)
177; Purchased at the local market in Haulien, Taiwan, by Prof. K. Arisumi of Kagoshima Univ.
182–209; Purchased at the local markets in USSR
226; Purchased in Munich, Germany (imported from Mexico)
231; Local variety in Ethiopia presented by Mr. M. Yamamoto
Allium longicuspis; Moscow Central Botanic Garden, USSR
A. ampeloprasum; Prof. S. Iwasa of Iwate Univ.
A. scorodoprasum; Dr. F. Krahulec of Botanical Institute, Pruhonice, Czechoslovakia

collected, but some of those might be included in the same clones, because some of the collected clones were purchased at the local markets. Moreover, the local clones have no certain histories on

their origins. Even the leading varieties have only uncertain histories on their varietal establishments. For example, 'Iki-wase' was originally introduced from Jejudo island in Korea to Iki island in Japan. Probably its origin is of a local clone in Jejudo island. Other Japanese leading varieties such as 'Roppen-shu' were simply selected from the local clones in the respective areas. Therefore, all the materials used here were treated as clones. The cloves were planted in September 1980, 1981 and 1983, and their bolting habits were examined next spring or summer just after harvest. Bolting habit was examined in ten plants of the respective clones.

Results

In relation to the bolting habit, the clones examined here were classified in three types; complete-bolting, incomplete-bolting, and non-bolting clones, though they were somewhat continuous. Table 2 shows the bolting habit in the 154 examined clones. Here in this paper, the clone in which all of the examined plants bolted was defined as a complete-bolting clone, and the clone in which none of the plants bolted was defined as a non-bolting clone. All of the rest were defined as incomplete-bolting clones (Table 3). Of course, these bolting habits are influenced more or less by

Table 2. Bolting habits in the examined clones

Bolting habit	Clone Number
Complete-bolting	1, 3–5, 8–11, 13–15, 17, 18, 20–27, 30–32, 35–38, 40–43, 50, 51, 60–66, 69, 71–73, 85, 94, 95, 97, 99–101, 106, 107, 110, 112, 115, 117, 119, 121, 125–128, 130, 145, 146, 160, 162, 163, 177
Incomplete-bolting	2, 6, 12, 16, 19, 28, 44–47, 52–55, 58, 67, 68, 70, 74–76, 78, 80–84, 86–88, 90, 93, 98, 103–105, 108, 109, 113, 114, 118, 120, 124, 129, 131–137, 144, 147, 154–157
Non-bolting	7, 34, 56, 57, 77, 79, 89, 96, 102, 111, 116, 122, 123, 138–143, 149–153, 158, 159

Table 3. Bolting habits and occurrence of the secondary scapes in the incomplete-bolting clones examined

Constitution of clones	Clone Number
Both incomplete- and non-bolting plants	16, 28, 44, 46, 47, 70, 74, 78, 82–84, 86–88, 90, 93, 98, 103–105, 108, 113, 114, 118, 120, 124, 133, 135, 137, 144, 147, 157
Only incomplete-bolting plants	2, 6, 12, 52–54, 58, 67, 68, 75, 76, 80, 81, 109, 129, 131, 132, 134, 136, 154–156
Both complete- and incomplete-bolting plants	19, 45
Occurrence of the secondary scapes	6, 44, 45, 47, 54, 58, 70, 74–76, 80, 90, 129, 134, 154

the environmental conditions and the clove sizes at planting. Therefore, a few plants have ever bolted even in non-bolting clones. A total of 154 clones were examined, showing 26 non-bolting clones, 57 incomplete-bolting clones, and 71 complete-bolting clones. More than half of the clones examined here were non- or incomplete-bolting. Some incomplete-bolting clones produced secondary scapes which were produced on the inflorescences. These secondary scapes formed secondary inflorescences over the primary inflorescences, and the secondary inflorescences sometimes produced the third scapes. The secondary scapes were formed in 16 incomplete-bolting clones (Table 3).

Discussion

(A) Non-bolting clones

It is clear that non-bolting habit causes sterility in garlic, because non-bolting clones do not differentiate flower-buds. Of the 154 clones examined here, 26 clones differentiated neither flower-stems nor flower-buds. Accordingly, the sterility in those 26 clones is due to their non-bolting habit. These non-bolting clones were classified according to their original homes of cultivation (Table 4). Both of Nos. 158 and 159 collected in the market at Suva in Fiji are non-bolting clones, but their original homes of cultivation are unknown. It is likely that these non-bolting clones collected came from three areas; Europe, the Americas, and northern Japan, though European clones include one from USSR. Furthermore, American clones were probably carried from south Europe so that the five American clones may be classified into European clones. Therefore, the original homes of the non-bolting clones examined here are probably restricted to only two areas; Europe and northern Japan. According to Kazakova[34], Mediterranean area is one of the two secondary centers of the origin of garlic. Garlic has ever been stated to grow wild in south Europe[58].

Table 4. Classification of the non-bolting clones according to their original habitats

Area	Clone Number
Europe	34, 89, 138–143, 149–153
The Americas	56, 57, 77, 79, 150
Northern Japan	7, 96, 102, 111, 116, 122, 123

The climate in the Mediterranean area must be suitable for the growth of garlic. Assuming that the primitive type of garlic bolted, we may reasonably state that garlic must have kept bolting habit in the climate suitable for its growth. Why are there so many non-bolting clones in the Mediterranean area in spite of its suitable climate? A part, at least, of the European clones may have acquired non-bolting habit by artificial selection. In garlic, bulbil formation on the top of the flower-stalk frequently causes decrease of bulb yield, and the bulbils are too small to be used. Therefore, non-bolting might be preferred to complete-bolting by the farmers. The selective pressure by human beings may have resulted in the evolution of non-bolting habit from the complete-bolting habit in south Europe. Non-bolting habit in the clones of northern Japan might be a consequence of adaptation to cold winter with snow. It is probable that other cold areas also have non-bolting clones.

(B) Incomplete-bolting clones

Incomplete-bolting was observed in 57 clones. They were classified in three types. In the

first type, some of ten examined plants in each clones did not bolt, and the rest bolted incompletely. This type was observed in 33 clones. In the second type, all of the plants in each clone bolted incompletely, and 22 clones showed this second type. In the third type, some of the plants in each bolted completely, and the rest bolted incompletely. The third type was found in only two clones, No. 19 and No. 45. The cause of sterility is clear in the non-bolting plants of the incomplete-bolting clones.

Table 5. Classification of the incomplete-bolting clones according to their original habitats

Area	Clone Number
Africa (Mediterranean)	55, 133, 144
The Americas	78, 131, 132, 134, 135, 137
South and S. E. Asia	44–47, 58, 70, 74, 136, 155, 156
China	52, 53, 54
Southern Japan	16, 19, 28, 67, 68, 75, 76, 80, 90, 129
Northern Japan	2, 6, 12, 81–84, 86, 87, 93, 103–105, 108, 109, 118, 120, 124
Unidentified	88, 113, 114, 154, 157

These incomplete-bolting clones were classified according to their original homes of cultivation (Table 5). In addition to the American clones, the African clones may belong to the Mediterranean group, because Egypt and Algeria are their homes of cultivation. The clones from southern Japan, except for Nos. 16 and 19, showed the same growth habit as that of the clones from southern and south-eastern Asia. Most of the clones of the four areas mentioned above came from the tropical or the subtropical areas between 30°N and 30°S. They showed similar growth habit to each other, namely, continuous growth without arrest in winter. Besides, clone Nos. 6, 53, 54, 154 and 157 showed the same growth habit as that of these tropical clones. The secondary scapes shown in Table 3 were seen in only these subtropical or tropical clones. As the climatic conditions in the tropical area are suitable for garlic to continue vegetative growth, these secondary scapes may be formed even after the differentiation of primary inflorescences. Among the 16 clones with the secondary scapes, clone Nos. 47 and 58 formed the third scapes on the secondary scapes. This fact may support the assumption mentioned above. The formation of secondary scapes may probably be a consequence of adaptation to tropical areas, or that of intraspecific evolution. The incomplete-bolting as well as non-bolting was observed in many clones from northern Japan, and the growth habits of clone Nos. 16, 19, 88, 113 and 114 resembled those of the incomplete-bolting clones from northern Japan. Both non- and incomplete-bolting habits among the clones of northern Japan are assumed to be within a continuous variation. Probably some parts of incomplete- and non-bolting clones may be realized to belong to the same group. Non-bolting clones will be found also in subtropical and tropical areas. Accordingly, most of these non- and incomplete-bolting clones are classified almost in three groups; Europe, the tropics, the colder temperate zone. Garlic is frequently called a medium-temperature plant, because it grows well under the medium-temperatures. Both non- and incomplete-bolting habits are presumably the products of adaptation to unfavorable climatic conditions.

2. Flower-bud formation in the incomplete-bolting clones

As mentioned above, garlic contains a number of incomplete-bolting clones. Their inflorescences remain within the false stems even at the end of growth, so that their flower-buds may be useless for pollination even if they are functional. Here in this experiment, the number of flower-buds in 43 incomplete-bolting clones was counted at harvest in 1981.

Results

The numbers of flower-buds were shown in Table 6. Most of the incomplete-bolting clones lacked flower-buds in their inflorescences and bore only bulbils. Flower-buds were scarcely differentiated in four clones, counting one and less flower-bud per inflorescence, and they degenerated before meiosis. Two clones, No. 19 and No. 45, developed several flower-buds per inflorescence, and a part of their plants bolted completely.

Table 6. Mean number of flower-buds per plant in the incomplete-bolting clones

Clone No.	No. of plants examined	Number of flower-buds per plant	Clone No.	No. of plants examined	Number of flower-buds per plant
2	10	0	80	10	0
6	10	0	81	2	0
12	10	0	82	3	0
16	10	0	83	3	1.0
19	10	3.5	84	10	0.9
28	3	0	86	3	0
44	10	0	87	10	0
45	10	4.0	88	8	0
46	10	0	90	10	0
47	10	0	93	10	0
52	2	0	98	4	0
53	9	0	103	10	0
54	10	0	104	10	0
55	10	0	105	10	0
58	10	0	108	9	0.7
67	10	0	109	7	0
68	10	0	113	10	0
70	10	0	114	10	0
74	10	0	118	10	0
75	10	0	120	10	0
76	10	0	124	10	0.3
78	10	0			

Discussion

From the viewpoint of species evolution, these incomplete-bolting clones may be on the way from the complete-bolting clones with flower-buds to the non-bolting clones without flower-buds. However, these incomplete-bolting clones already lost the ability to differentiate flower-buds.

Probably these clones evolved from the complete-bolting clones after they lost the capability of differentiating flower-buds, because the majority of the incomplete-bolting clones produce only bulbils without flower-buds in their inflorescences. It is clear that the cause of sterility is the absence of flower-buds not only in non-bolting clones but also in most of the incomplete-bolting clones.

3. Flower-bud formation in the complete-bolting clones

Complete-bolting was observed in 71 of the 154 examined clones, and the number of flower-buds was examined in 61 of these 71 clones as in the incomplete-bolting clones at harvest in 1981.

Results

Table 7 shows mean numbers of flower-buds per inflorescence and the number of the plants bearing no flower-buds in the complete-bolting clones. Of the 61 clones, 21 clones bore no flower-buds in several plants. However, all of the 61 clones developed flower-buds, though some plants of the 21 clones bore only bulbils in the inflorescences. In Table 8, the examined complete-bolting clones are divided according to the amount of flower-buds, derived from Table 7. A total of 55 clones produced less than 30 flower-buds per inflorescence, and only six clones produced more than 30 flower-buds. The clones with less than five flower-buds were observed most frequently, namely, the peak of distribution of the examined clones occurred under five flower-buds of number. And the number of clones decreased toward 30 flower-buds gradually.

Table 7. Mean number of flower-buds per inflorescence and the number of the plants bearing no flower-bud in the complete-bolting clones

Clone No.	No. of plants examined	No. of flower-buds	Plants without flower-bud	Clone No.	No. of plants examined	No. of flower-buds	Plants without flower-bud
1	10	4.0	0	26	10	4.9	0
3	10	4.3	0	27	9	4.2	0
4	10	5.1	0	30	5	0.5	4
5	10	15.4	0	31	10	5.4	0
8	10	4.5	1	32	10	5.3	0
9	10	2.5	1	35	8	0.5	6
10	10	4.9	0	36	10	14.4	0
11	10	3.2	1	37	10	3.1	0
13	10	4.0	0	38	10	0.8	3
14	10	1.6	3	40	8	3.4	2
15	10	0.9	4	41	10	31.8	0
17	10	10.4	0	42	7	2.9	2
18	10	3.3	0	43	10	10.0	0
20	10	7.4	0	50	10	3.6	0
21	10	20.4	0	51	10	2.4	1
22	10	3.7	1	60	10	2.5	2
23	10	6.5	0	62	10	12.1	0
24	10	4.0	1	63	10	11.2	0
25	10	5.3	0	64	10	30.6	0

Table 7 (continued)

Clone No.	No. of plants examined	No. of flower-buds	Plants without flower-bud	Clone No.	No. of plants examined	No. of flower-buds	Plants without flower-bud
65	10	12.5	0	101	10	6.2	0
66	10	3.1	1	106	10	7.8	0
69	10	3.0	2	110	10	146.5	0
71	10	7.1	2	112	10	5.1	1
72	10	17.0	1	115	10	5.5	0
73	10	29.3	0	117	10	3.9	1
85	10	3.4	0	119	10	3.6	0
94	10	18.9	0	121	10	2.9	0
95	10	4.7	0	125	10	76.5	0
99	10	0.6	4	126	10	30.6	0
100	10	24.1	0	127	10	22.8	0
				130	9	79.9	0

Table 8. Distribution mode of the complete-bolting clones producing different amounts of flower-buds per inflorescence

Average number of flower-buds	0.1–5.0	5.1–10.0	10.1–15.0	15.1–20.0	20.1–25.0	25.1–30.0	30.1–
Number of clones	31	12	5	3	3	1	6

Discussion

Both of bolting habit and number of flower-buds in garlic clones are affected more or less by size of cloves and by the environmental conditions under which they and their mother bulbs were grown. Consequently, both of those somewhat vary with the year, but fundamentally each clone has its own bolting habit. This examination revealed the properties of the clones on the flower number and the proportion of the plants without flower-bud formation.

Even in the complete-bolting clones, a part of plants did not differentiate flower-buds. No flower-bud formation was observed in 42 of 596 plants examined (7.1 %). It means that plants of no flower-bud formation were quite few in the complete-bolting clones, as compared with those in the incomplete-bolting clones. The cause of sterility in these plants of no flower formation is apparent.

In this complete-bolting group, all of the clones differentiated flower-buds, but most of them developed only several flower-buds. A half of the examined clones differentiated less than five flower-buds. The existence of the clones without flower-formation was expected from the result of the examination in the incomplete-bolting clones, and from the fact that the peak of clonal distribution in Table 8 occurred at flower-buds counting less than flve. However, such a clone was absent. Therefore, if garlic should be shifting from complete- to non-bolting through incomplete-bolting in its intraspecific differentiation, those facts mentioned above may support the following hypothesis; a complete-bolting clone may be converted into an incomplete-bolting clone whenever it loses the ability to differentiate flower-buds. The fact that the clones provided with quite few flower-buds as average frequently produced the plants without flower-bud formation may also support this hypothesis.

4. Abortion of flower-buds

As mentioned before, two of the incomplete-bolting clones and most of the complete-bolting clones could develop flower-buds beyond the meiotic stage. Some of the flower-buds were malformed[14]. Malformation of garlic flowers was reported also in the previous papers[21,61,78,79], and this is perpetuated as in case of fasciation in *Celosia cristala*[19]. This malformation is presumably one of the causes of sterility in garlic.

In addition to the malformation, even the morphologically-normal flowers withered to death before anthesis under the natural condition. Therefore, the development of flower-buds was examined anatomically to clarify the internal development of flower.

Materials and Methods

The cloves of clone 'Shanhai-wase' were planted at Kagoshima in September of 1973, and the growing points were observed periodically. After the initiation of flower-buds, the inflorescences or the flower-buds were fixed in Belling's modified Navashin fluid without saponin for paraffin sections. The embedded materials were sectioned at a thickness of nine microns and stained with Heidenhain's iron hematoxylin solution. The flower-buds were picked up to their death for the observation of the development of the female organ.

Results

The flower of garlic is fundamentally trimerous and consists of five whorls. Diagram of a normal garlic flower was shown in Fig. 1. A total of six perianth lobes are arranged in two outer whorls, and the three perianth lobes in the outermost whorl are keeled and thick, while the inner three are flat, being thinner and shorter than the outer three. In the third and fourth whorls, three outer and three inner stamens are contained respectively. The outer and inner stamens are situated in front of the respective outer and inner perianth lobes. The inner stamens are trifid, bearing one or two

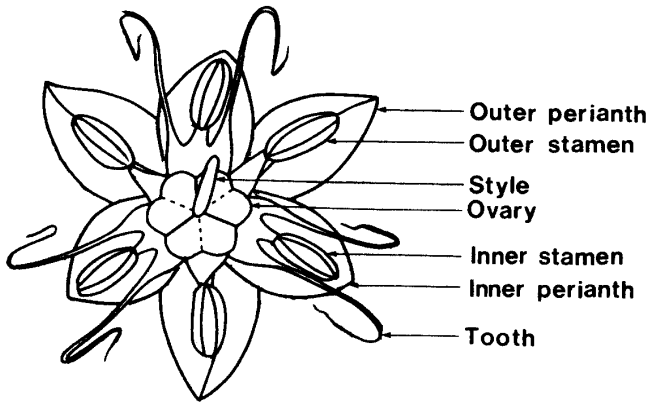
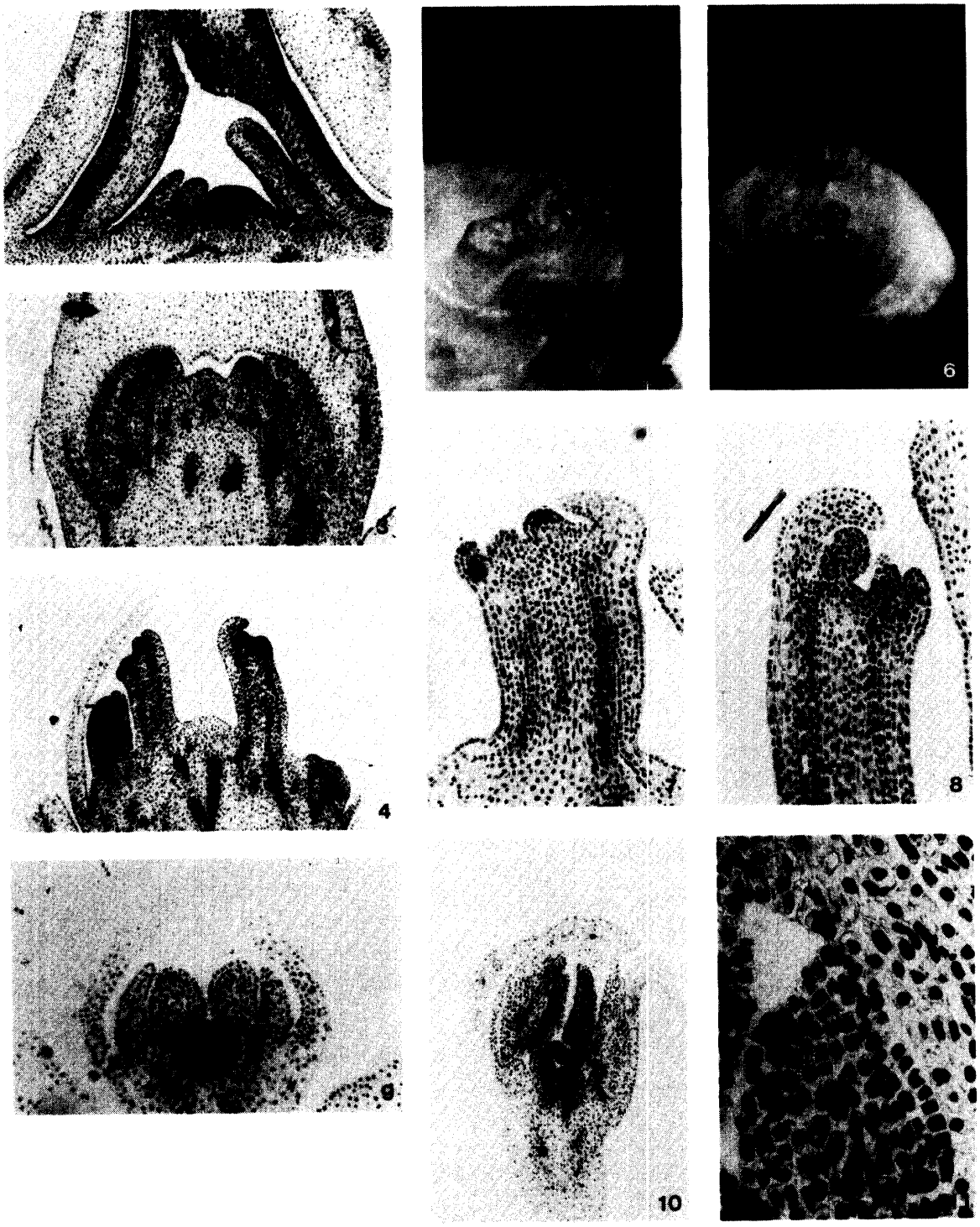


Fig. 1. Diagram of a garlic flower.

Fig. 2. Longitudinal section through the growing point of a garlic plant, showing a primordium of an inflorescence (×22). Fig. 3. Longitudinal section through the developing inflorescence axis. The involucral bract is shown closely covering the inflorescence (×22). Fig. 4. Longitudinal section through the developing inflorescence, showing three elongating flower-buds and one primordium of bulbil. Outer perianths and outer stamens are differentiating. The spathe is removed (×22). Fig. 5. Flower primordia



at the different stages of their development. Fig. 6. Several flower primordia and a bulbil primordium. The first-differentiated perianth lobe is overgrown in the most-developed flower primordium. Fig. 7. Longitudinal section through a flower primordium. The outer perianth lobe and the outer stamen are developing, and the pedicel is elongating, though still short (×55). Fig. 8. Longitudinal section through a flower primordium. The outer perianth lobe is beginning to cover the stamen. The pedicel is considerably elongated (×55). Fig. 9. Longitudinal section through a flower-bud. The inner perianth lobes are developing, and the ovary is just differentiated (×55). Fig. 10. Longitudinal section through a flower-bud. The anthers are elongated, and the ovules are beginning to differentiate in the ovary (×31). Fig. 11. Longitudinal section through an ovule, showing EMC. The inner integument begins to differentiate (×220).

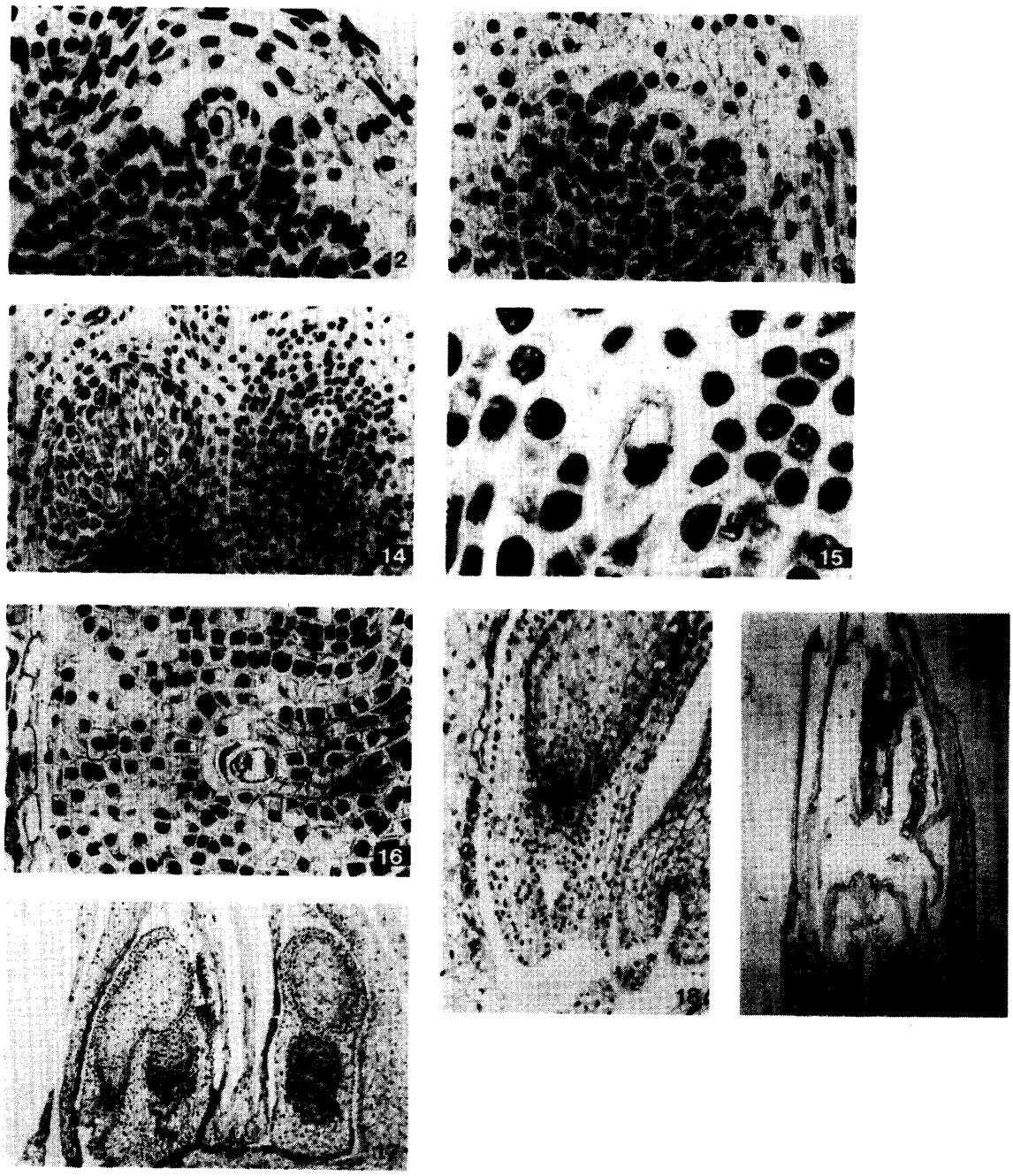


Fig. 12. Longitudinal section through an embryo-sac mother cell at the prophase. The outer integument is about to begin to differentiate (×220). Fig. 13. Longitudinal section through an embryo-sac mother cell at the prophase. Both of the outer and inner integuments are developing (×220). Fig. 14. Longitudinal section through the ovules, showing an EMC at the first metaphase (right) and the abnormal nucellar cells (left) (×110). Fig. 15. Longitudinal section through an EMC, showing the first metaphase (×550). Fig. 16. Longitudinal section through the degenerating binucleate embryo sac from the chalazal daughter cell. The micropylar daughter cell has already been degenerated, and only its remnant is left on the embryo sac (×220). Fig. 17. Longitudinal section through the ovules with abnormal nucellar cells (×22). Fig. 18. Longitudinal section through the micropyle in the ovule with abnormal nucellar cells, showing no embryo sac. The outer integument is longer than the inner one (×55). Fig. 19. Longitudinal section through a degenerated flower-bud without opening (×22).

long teeth at the base in both sides of the filaments[23,47]. A tricarpellary pistil situates at the innermost whorl.

The inflorescences and the bulbs of clone 'Shanhai-wase' initiated at Kagoshima late in January. The first stage in the development of the inflorescences appeared to be very similar to that of the leaves (Fig. 2). However, the beginning of scape elongation made it easy to distinguish them. A thick involucral bract covered the meristematic region for flower development (Fig. 3). Within the involucre, one or two primordia appeared first and the primordia increased in number. The flower primordium developed at first as a slight projection and then became globose. The primordia with convex summits elongated to make flower-buds. On the other hand, the primordia with the central domes and the marginal rises began to make bulbils without elongation. The summits of the flower primordia became somewhat flatted and triangular. At each angle of the triangle, an outer perianth lobe was differentiated first (Figs. 4, 5). In front of the outer perianth lobe, an outer stamen was differentiated next (Fig. 5). This first outer perianth lobe was frequently overgrown, and consequently it covered most of the other floral parts (Fig. 6). This overgrowth of the first outer perianth lobe must cause some kinds of malformation, for example, lack of other perianth lobes.

Subsequently, other outer perianth lobes and outer stamens were differentiated (Figs. 4, 5). The inner whorls of perianth lobes and stamens were differentiated later than the outer. The first member of the inner perianth lobes appeared with its subtended anther between the oldest and the second oldest lobe of the outer whorl (Fig. 5). The next member appeared between the oldest and the youngest lobe of the outer whorl. Occasionally this sequence was reversed. As a rule, the last lobes appeared opposite the oldest. The perianth lobes grew up, covering the stamens (Figs. 5, 7). With the growth of perianths and stamens, the pedicels elongated (Figs. 4, 8). The carpels were differentiated at about the time the outer perianth lobes overarched the stamens (Fig. 9). Along with the growth and elongation of perianths and stamens, the differentiated carpels grew upward and toward the center. After the growth of carpels, the primordia of the ovules were differentiated and grew on the inner edges of the carpels (Fig. 10).

The archesporial cell for the embryo sac arises from a hypodermal cell and it functions directly as the embryo-sac mother cell (EMC). This cell could not be distinguished from other nucellar cells at the earlier stage, namely, before the beginning of the integument formation. However, when the inner integument was differentiated, the embryo-sac mother cell could be easily distinguished among the nucellar cells by its size of cell and nucleus (Fig. 11). At this stage, the prophase had already begun in EMC, and the first or the second divisions were almost finished in pollen mother cells (PMC) in the same flower-buds. The inner integument developed and the outer integument began to differentiate, but EMC remained at the prophase (Fig. 12). Both of the integuments developed to enclose EMC, but EMC appeared to remain still at the prophase (Fig. 13). At these stages of the developing integuments, PMC almost finished meiosis, producing pollen tetrads and uninucleate microspores. After EMC was wholly enclosed by the integuments, it showed the first metaphase but the chromosomes were so concentrated and complicated that their pairing was not distinguishable (Figs. 14, 15). At this metaphase-I of EMC, the uninucleate pollen grains were degenerating in the same flower-buds. This first division of EMC produced two cells, namely, a micropylar daughter cell and a chalazal daughter cell, but the micropylar daughter cell disintegrated immediately after the division. Some of EMCs degenerated during or just after this first division. However, other EMCs underwent one more division, thus forming two megaspores (Fig. 16). At this stage showing two megaspores and a remnant of the disintegrated micropylar daughter cell, almost all the degenerated pollen grains became empty. At the latest, the megaspores stopped their

development by this stage and degenerated after all. There were no advanced megaspores observed. In some ovules, nucellar cells developed abnormally without the development of megaspores (Fig. 14). These abnormal nucellar cells differed from the normal ones in density of protoplasm like EMC and in obscure cell wall. These abnormal nucellar cells could develop well even in the anatropous ovules, but there was no embryo sac in the nucellus (Figs. 17, 18). Both of the integuments also developed well around the nucellus (Fig. 18). However, these nucelli degenerated after all, and all of the flower-buds withered to death without opening (Fig. 19). Consequently, the clone used here did not produce female gametophytes at all. The cause of sterility is clear in this clone.

Discussion

The development of the flower and the macrogametophyte in *Allium cepa* was stated in detail by Jones and Emsweller[27], and it was similar to that of *A. sativum* observed here. However, in garlic, the flower-buds and the ovaries were frequently malformed as described before. And the embryo sac of garlic did not develop in contrast with that of *A. cepa*.

In *A. cepa*, the archesporial cell of EMC was distinguishable in the earlier stage, even before the integuments began to form. Nevertheless, it was not distinguishable before the differentiation of integuments in garlic as in *A. mutabile*[57]. Weber[76] reported on the development of inflorescences and macrogametophytes in many *Allium* species. She described that in *A. sativum* var. *vulgare* the development ceased at the first division stage of EMCs, and that in *A. sativum* var. *ophioscorodon* the development of the ovule proceeded till the first nuclear division stage of the embyro sac. She also observed that in both of them the flower-buds shrivelled without opening. Therefore, at least three clones of garlic do not form any normal embryo sacs, and most of garlic clones may probably show the similar procedure. Besides these results, Gvaladze[22] observed certain aposporic embryo sacs in some Russian clones. He stated that apospory was a manifestation of the disruption of normal reproduction. He also stated that even such clones of garlic in which female gametogenesis proceeded normally were in reality apomicts as well. As a conclusion, flower-buds developed normally, except for the malformed ones, but no functional embryo sac was formed in the clone treated here.

It was clarified that garlic failed to develop female gametophytes. This must be a cause of sterility in garlic. However, it is well known that garlic also fails to develop male gametophytes. Male sterility is frequently discussed in relation to the development of tapetum, as all the food materials nourishing the sporogenous cells must be supplied through it[46]. In *Allium*, the stage of tapetal degeneration or its abnormal development was reported previously[50,51,59,66,73]. In a garlic clone, hypertrophy of tapetum was observed[52], but in another clone, such an anatomical abnormality was not noticed[11]. It is presumable that the abnormal development of tapetum does not always cause the pollen sterility in garlic.

5. Abnormality in microsporogenesis

As mentioned above, the female gametophytes in garlic failed to develop. Its male gametophytes also fail to develop. The meiosis of PMCs was examined in the garlic clones as many as possible, because different meiotic pairings of chromosomes were observed previously[32,43,63]. Most of the results were reported in 1978[15], 1979[9], and 1983[12].

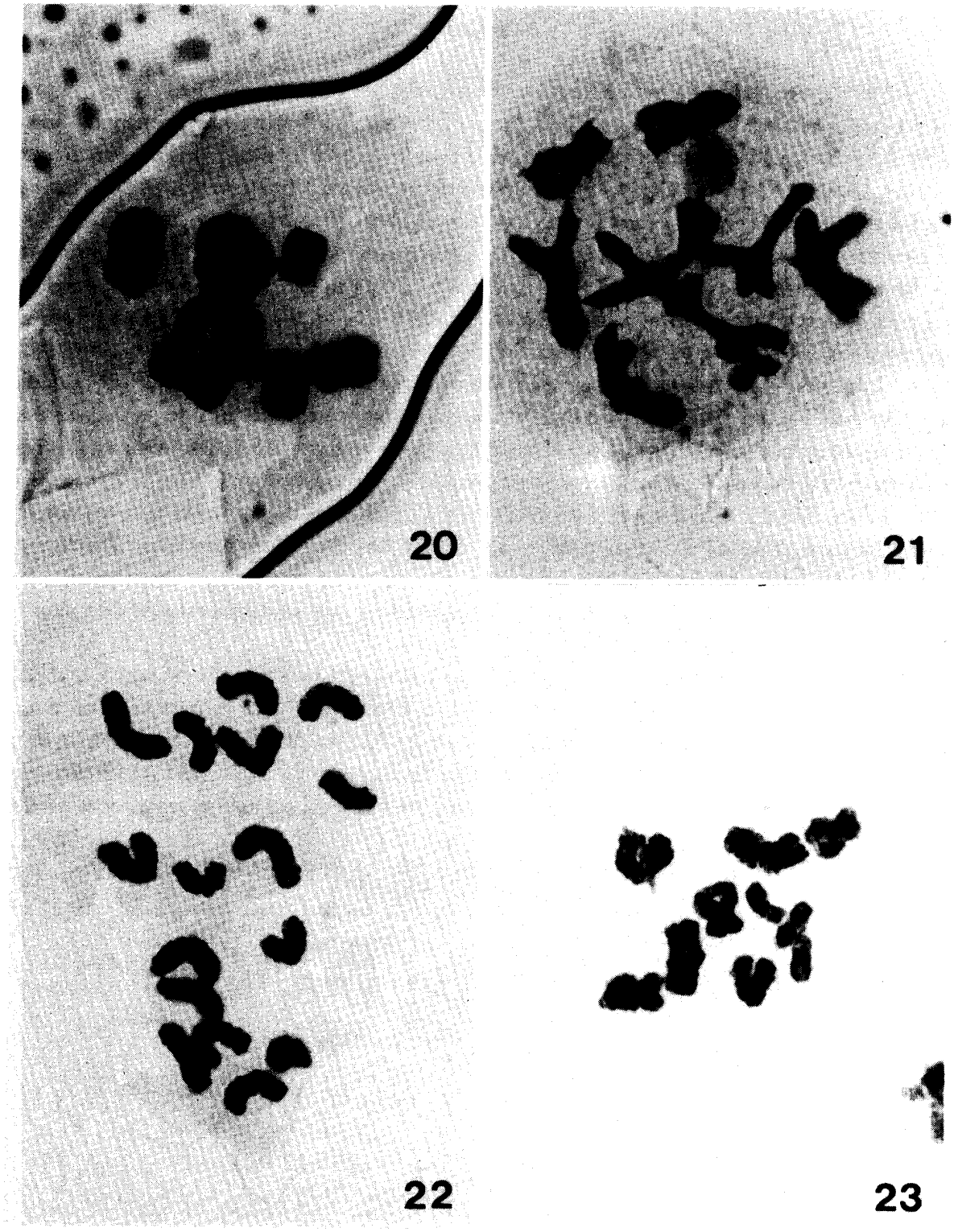
Results

Table 9 shows chromosome pairings at meiosis of PMCs in the examined clones. Irregular meiosis was observed in 74 clones. Their chromosome pairings varied with the clone. In some parts of the examined clones, the pairing also varied within a clone, but the pairing proper to each of the clones could be found. Garlic has 16 somatic chromosomes. In clone No. 64 'Shanhai-wase', a total of 128 PMCs were observed, and 104 cells of them showed $1_{VIII}+4_{II}$ as the chromosome configuration. Therefore, it was presumed that every garlic clone has its proper chromosome-pairing at meiosis. The chromosomes mostly formed $1_{X}+3_{II}$ in one clone of the 74 clones showing irregular meiosis, $1_{VIII}+4_{II}$ in 37 clones (Fig. 20), and $1_{VI}+5_{II}$ in 34 clones (Fig. 21). Moreover, desynapsis or asynapsis was seen in two clones (Fig. 22). Except for these two desynaptic or asynaptic clones, 72 clones showing irregular meiosis frequently formed ring multivalent chromosomes in their PMCs. After meiosis, their microspores degenerated at the pollen-tetrad stage or at the advanced uninucleate pollen stage. The two desynaptic or asynaptic clones produced malformed microspores which resulted from desynapsis or asynapsis. The malformed microspores degenerated before pollen mitosis. Malformed microspores which resulted in degeneration were also observed in another clone, No. 146, in which meiosis was not seen because of few materials. Therefore, it is most probable that clone No. 146 shows desynapsis or asynapsis at meiosis. Consequently, it was confirmed that all the microspores produced through irregular meiosis degenerated at the uninucleate pollen stage or at the pollen-tetrad stage.

On the contrary, the regular pairing of chromosomes (8_{II}) at meiosis was found in two clones, Nos. 130 and 148 (Fig. 23). After meiosis, normal pollen tetrads were formed in clone No. 130, while the pollen tetrads degenerated in clone No. 148. The microspores of clone No. 130 were released in the anther locule and developed into uninucleate pollen grains. The first pollen mitosis began in a number of pollen grains. After the first pollen mitosis, the pollen grains developed into

Table 9. Chromosome pairings most frequently observed at meiosis of PMCs in the respective clones[8, 11]

Chromosome configuration at meiosis of PMC	Clone Number
$1_{X}+3_{II}$	40
$1_{VIII}+4_{II}$	1, 5, 11, 17, 21, 30, 32, 34, 35, 36, 41, 42, 43, 51, 62, 63, 64, 65, 71, 72, 73, 91, 94, 97, 100, 103, 106, 112, 115, 117, 121, 128, 160, 161, 162, 170, 177
$1_{VI}+5_{II}$	3, 4, 8, 9, 10, 13, 14, 16, 18, 19, 20, 22, 23, 24, 25, 26, 27, 31, 37, 45, 50, 60, 66, 69, 85, 95, 99, 101, 107, 110, 119, 125, 126, 127
Desynapsis or asynapsis	61, 145
8_{II}	130, 148



Figs. 20–23. Metaphase-I or diakinesis at the meiosis of PMCs (×1,100).
Fig. 20. $1_{VIII}+4_{II}$ in clone No. 161. Fig. 21. $1_{VI}+5_{II}$ in clone No. 119. Fig. 22. Desynapsis or asynapsis in clone No. 61. Fig. 23. 8_{II} in clone No. 130.

normal binucleate ones, similar to those of other fertile *Allium* forms. A lot of grayish pollen grains were observed at the dehiscence of anther. Thus, viable pollen grains were produced in clone No. 130 introduced from USSR. Accordingly, it was confirmed that garlic has also a pollen-fertile clone.

Discussion

In garlic, chromosome behavior at meiosis was first observed by Takenaka[63]. He found multivalent chromosomes, but he could not identify the chromosome configuration. Later he made further observations of meiosis and reported ring-shaped hexavalent, octovalent, or decavalent chromosomes[64,65]. His materials were obtained in Korea, Japan and Formosa. Katayama[32] observed multivalent chromosomes in two clones, one from Korea and the other from Japan. However, he also observed normal meiosis of 8_{II} in one Korean clone. After their observations, both of normal meiosis and abnormal meiosis were reported. Normal meiosis was found in a Turkestan clone of USSR[43], Indian clones[41,42], Czechoslovak clones[37,38,39,52], and German clones[40]. On the other hand, quadrivalent chromosomes were found in one clone from USSR[39], and desynapsis and multivalent chromosomes were observed in India[20] and in England[8], respectively. Besides those, a lot of multivalent chromosomes and desynapsis or asynapsis were found in the present investigation.

After all, it was clarified that garlic includes a series of chromosome-pairing as in the following: 8_{II}, $1_{IV}+6_{II}$, $1_{VI}+5_{II}$, $1_{VIII}+4_{II}$, $1_{X}+3_{II}$, and desynapsis or asynapsis. This is a great variation in the chromosome pairing at meiosis within a species. All of the garlic clones showing various chromosome configurations are sterile, except for one clone showing 8_{II}. This fact is considerably suggestive.

First, garlic contains the clones with normal meiosis, so that garlic is not probably hybrid origin, though Takenaka[64] suggested hybrid origin because of the presence of multivalent chromosomes. Moreover, as the multivalent chromosomes accompanied few univalent chromosomes in the present investigation, they might have resulted from reciprocal translocations, not from hybrid origin. The basic chromosome number of garlic is eight ($2n=16$), and the genus *Allium* has only three basic chromosome numbers, 7, 8, and 9[17]. Therefore, garlic is not polyploid obviously. Consequently, the multivalent chromosomes of garlic have not resulted from polyploidy.

Secondly, it is most probable that the chromosome constitution of garlic was originally regular. Structural changes of chromosomes may be apt to occur in garlic. The multivalent chromosomes in question may have been successively derived from the normal chromosome pairing through the reciprocal translocations mentioned above. Once the multivalent chromosomes occurred, they must have been easily kept through the vegetative reproduction.

Thirdly, garlic was probably fertile in its original type. The fertile clone found here may keep the original characters of garlic. Some more fertile clones may be discovered among the clones showing normal meiosis, and they also may keep the original characters.

Fourthly, it is obvious that fundamental behaviors of the chromosomes of the EMCs are similar to those of PMCs in the respective clones, even if meiotic chromosomes of EMCs behave differently with those of PMCs as Ved Brat[70] stated.

Finally, one of the causes of sterility in garlic must be the abnormal constitution of chromosomes. The extreme example is desynapsis, though it is possible that the case observed here is asynapsis, because the zygotene or pachytene was not observed. Three desynaptic or asynaptic clones in this examination came from Italy, Yugoslavia, and Argentina. Another desynaptic clone of the previous report was found in India. It is presumable that these four desynaptic or asynaptic clones originated independently. According to Katayama[31], in the most cases so far reported, the desynaptic plants appeared spontaneously in the natural population. They also appeared in the progenies of hybridization. Most of desynaptic genes are recessive. In garlic, desynapsis

induced spontaneously must have been perpetuated by its vegetative reproduction, because desynapsis usually accompanies sterility.

The relationship between the fertility and the chromosome ring by the reciprocal translocation has ever been studied in many species, especially in *Oenothera*[5,6], *Isotoma*[25], *Triticum*[80], and *Tradescantia*[75]. As a rule, the pollen-fertility decreases with the increase of chromosome number composing a ring multivalent. However, in garlic, every clone with multivalent chromosomes shows complete pollen-sterility. If the formation of multivalent chromosomes is the only one cause of pollen-sterility, at least a part of microspores would survive surely to maturity.

According to Konvicka and Levan[39], even the clone named OH with eight bivalent chromosomes showed complete pollen-sterility in garlic besides the clones with multivalent chromosomes. The OH clone of Konvicka and Levan[39] showed chromatic bodies outside the spindle during the first and the second division of meiosis, though it showed eight bivalent chromosomes. The sterile clone with eight bivalent chromosomes may have suffered cryptostructural changes of chromosomes leading to sterility. As mentioned before, EMCs degenerated at the first division or at the second division in clone 'Shanhai-wase', which showed 1Ⅷ+4Ⅱ at meiosis of PMCs. As well as the degeneration of the EMCs, all the microspores in the sterile clones degenerated in the early stage after meiosis. The gametophytes of the halved chromosome number may consist of the deficient unit of the genome in the sterile clones, for structural changes of chromosomes including cryptostructural ones which lead to sterility may be apt to occur and to persist in garlic. At the metaphase-I of meiosis (M-I), the chromosome pairing was hard to be distinguished in the clone with multivalent chromosomes here in the present examination. The chromosomes at M-I were quite sticky, as compared with those of M-II. This may be somewhat related with microspore degeneration after meiosis.

On the structural chromosome changes in the genus *Allium*, Traub[67] stated, "The main value of the structural chromosome changes lies in the production of rapid evolutionary changes. The chief drawback is that it may lead to sterility. In those cases where it has a high survival value, species evolution is rapid and marked." Chromosome changes are supposed to occur so frequently in garlic, and the variants which result from the chromosomal changes have a tendency to be well preserved by vegetative propagation. Some of the rapid evolutionary changes may result from the accumulation of chromosome mutation. As garlic has been cultivated and vegetatively propagated for a long time, the accumulation may be great. These may have produced such a great variation of chromosome constitution within a species.

In conclusion, a wide variation of meiotic irregularity was found in garlic, and it was clarified that the meiotic irregularity leads to pollen-sterility at least in some clones. Normal meiosis was also found in two clones, one of which produced fertile pollen grains. Some more fertile clones may be discovered, and normal meiosis may be indispensable to the fertile clones of garlic.

6. Meiosis in the incomplete- or non-bolting clones

Irregular meiosis was found in PMCs of many garlic clones bearing flower-buds, and regular meiosis was also found as mentioned above. For the examination of meiosis in other clones bearing no flower-buds, the cold storage of mother bulbs was attempted to induce flower-buds in the non- or incomplete-bolting clones.

Materials and Methods

The mother bulbs of the clones shown in Table 10 were stored in the chambers of the low temperature before planting. In 1979, they were stored at 10°C from July 17 to September 16, and in 1980 at 7°C from October 1 to December 3. In most of the clones, 20 cloves were used as materials in both years, but 2 to 19 cloves were used in 15 clones in 1979. After the cold storage, the cloves were planted in the field, and the meiosis of PMCs was examined in the following spring or summer. Some of the examined clones had ever differentiated flower-buds without cold storage. However, as their flower-buds had never been developed up to meiosis, the cold storage of mother bulbs was also attempted in those clones.

Table 10. Clones stored at low temperature before planting in 1979 and 1980

Year	Clone Number
1979	2, 6, 7, 12, 16, 28, 38, 44, 47, 52, 53, 54, 55, 57, 58, 67, 68, 70, 74, 75, 76, 80, 81, 82, 83, 84, 86, 87, 88, 89, 90, 93, 96, 98
1980	2, 6, 7, 12, 16, 38, 44, 46, 47, 52, 53, 54, 55, 57, 58, 67, 68, 70, 74, 75, 80, 82, 84, 89, 90, 104, 105, 108, 109, 111, 113, 114, 116, 118, 120, 122

Results

Only one clone of the 47 examined clones developed flower-buds up to meiosis. The clone, No. 12, is one of the 38 incomplete-bolting clones examined here. In Kagoshima, clone No. 12 fails to develop flower-buds under the natural condition. However, in both years, it developed flower-buds up to meiosis, and it revealed the chromosome pairing as $1_{VI}+5_{II}$ in ten PMCs. After meiosis, the microspores of No. 12 degenerated as well as those of other clones mentioned before. Other incomplete-bolting clones examined failed to develop the flower-buds so that their meiosis was not observed at all. A total of eight non-bolting clones were examined here, and clone No. 89 developed a few scapes with the early degeneration of flower-buds. Clone No. 38 developed flower-buds every year, but the flower-buds were extremely malformed or withered to death before meiosis as well as in the case without the cold storage of mother bulbs.

Discussion

Meiosis was observed only in one clone, though the mother bulbs of 47 clones were stored at low temperature. To induce flower-buds in those clones, a lower temperature may be necessary. However, as one clone showed irregular meiosis similar to those of many other complete-bolting clones, it is presumable that most of other incomplete- or non-bolting clones would show irregular meiosis, even if they developed flower-buds. Besides this No. 12 clone, two other incomplete-bolting clones, Nos. 19 and 45, already showed multivalent chromosomes as mentioned before. Consequently, there may be a great intraspecific variation of the chromosome constitution at meiosis

throughout various garlic clones including incomplete- and non-bolting clones, not only in the complete-bolting clones.

In the genus *Oenothera*, the species have 4, 6, 8, 10, 12, or all 14 of their chromosomes linked in a chain or ring at meiosis as a consequence of reciprocal translocations. It is well known that this type is based on hybrid origin and that their translocated chromosomes usually show alternative distribution at the segregation of chromosomes. According to Cleland[5,6], even the plants with multivalent chromosomes higher than octovalent do not show extremely high sterility of pollen.

The multivalent chromosomes of garlic may not be based on hybrid origin as mentioned before, and they show both of alternative and adjacent distributions almost equally[9,15]. Furthermore, all the pollen grains degenerate after meiosis without going to the pollen mitosis. The mechanism of sterility of garlic obviously differs from that of *Oenothera*.

Besides the trouble of formation of multivalent chromosomes, garlic has another trouble of non- or incomplete-bolting which will result in sterility.

According to Darlington[7], the breeding system changes from the normal sexual reproduction to the complex heterozygote or to the asexual reproduction, directly or through subsexual reproduction. In garlic, its breeding system was probably changed from sexual to asexual reproduction, which must have accelerated the irregularity at meiosis.

III. Possibility of crossbreeding in garlic

As mentioned above, the aspect of sterility in garlic was almost clarified, and the fertile clone was discovered. Here in this investigation, the possibility of crossbreeding in garlic was explored.

The pollen grains of clone No. 130 were fully matured at anthesis, though partial sterility of pollen grains was also noticed[12]. The pollen germination of clone No. 130 was ascertained[12]. This fertile clone was self-pollinated and produced seeds[13]. The viability of the produced seeds was also ascertained[13].

Clone No. 130 produced viable pollen grains and seeds in 1982 and 1983.

Basing on the confirmed fertility of the clone No. 130, crossing between the sterile garlic clones and the fertile clone was attempted here. Three allied species, *A. longicuspis* Regel, *A. ampeloprasum* L., and *A. scorodoprasum* L., were also used for crossing, though they are sterile. *A. longicuspis* is presumed to be the wild ancestor of garlic as mentioned before.

Materials and Methods

In July of 1982, the anthers of the fertile clone were picked at anthesis, and they were stored in the freezer at about −20°C for the next season. The reason is that the flower-buds already withered to death in all the sterile garlic clones before the anthesis of the fertile clone, because the fertile clone developed flower-buds latest among the garlic clones.

In June of 1983, 197 flowers of 29 plants of clone No. 64 were cross-pollinated with the stored pollen grains of the fertile clone, and 104 flowers of 10 plants of clone No. 160 were cross-pollinated with the stored pollen grains. Bulbils of both clones were removed before pollination. Two plants of great-headed garlic cv. Elephant, *A. ampeloprasum*, and four plants of *A. scorodoprasum* were also cross-pollinated with the stored pollen grains of the fertile garlic clone. The great-headed garlic does not produce any bulbils in the inflorescences.

For *A. longicuspis*, the fresh pollen without storing was used, because *A. longicuspis* flowered at

almost the same time as the fertile garlic clone flowered. More than 2,300 flowers of 72 plants of *A. longicuspis* were cross-pollinated with the pollen grains of the fertile garlic clones after the removal of bulbils in July of 1983. The seeds were harvested late in August.

Results

All of the two sterile garlic clones, the great-headed garlic, and *A. scorodoprasum* did not produce seeds at all, though many of the pollinated ovaries developed morphologically, increasing in size.

On the other hand, 200 seeds were harvested in pollinated *A. longicuspis*, and 85 seeds of them seemed to be matured. The seeds were put on the wet filter paper in the petri dishes at 20°C. Of those 85 seeds, 33 seeds germinated, and most of them required scarification before germination. However, only two seedlings survived after the germinated seeds were transplanted into the vermiculite medium. The two seedlings grew more vigorously than any of the garlic seedlings, and one of them developed flowers in summer of 1984.

Discussion

Besides the fertile garlic clone found here, two other fertile garlic clones were reported by Kononkov[36], and Katarzhin and Katarzhin[29], respectively. However, Kononkov[36] and Katarzhin and Katarzhin[29] neither observed meiosis nor compared the clones with other sterile clones. None of them referred to the cause of sterility in garlic at all. Consequently, it is difficult to compare their fertile clones with that found in the present investigation. However, it is obvious that garlic has fertile clones besides sterile ones. All of these three fertile clones were originally produced in USSR. Soviet Central Asia is presumed to be a primary center of origin of garlic. According to Kazakova[34], the Caucasus is one of the secondary centers of garlic. Gvaladze[22] reported aposporous embryo sacs and partially-fertile pollen grains of garlic at the Caucasus. He must have used one of the Caucasus clones, which was partially fertile. Probably some more fertile garlic clones will be found in USSR.

In the present study, all of the two sterile garlic clones and the allied species of garlic, except for *A. longicuspis*, failed to produce seeds. The cause of no seed-production in clone No. 64 must be the degeneration of EMCs as mentioned before. Clone No. 160 may have failed to develop their EMCs as well as clone No. 64. The great-headed garlic and *A. scorodoprasum* may not be able to develop EMCs as well as mature pollen grains. The former did not develop any PMCs up to the first metaphase, and the latter showed degeneration of pollen grains at the binucleate stage. Of course, the relationship of these two species and garlic may be too far to fertilize, and the pollinated flowers may have been too few to produce seeds. However, the stored pollen grains were probably viable enough to fertilize, because the pollinated flowers considerably developed not only in the sterile garlic clones but also in these two species.

Katarzhin and Katarzhin[29] obtained two variants of garlic seedlings by the open-pollination, and they presumed that those two might be mutants or hybrids between garlic and common onion, because the mother plants were grown beside the common onion. The seedlings obtained here by artificial cross-pollination must be hybrids between *A. longicuspis* and garlic.

A. longicuspis was expected to produce more viable seeds, because it was presumed to be closely related to garlic. However, it also showed less productivity of seeds. The fertile garlic clone also produced seeds only in some parts of the pollinated flowers, and only a part of the havested seeds

were mature. A part of the mature seeds germinated, and a small part of them survived. In Kononkov's fertile clone, the situation appeared to be same as that in this clone. In the case of the self-pollinated garlic, less seed-productivity may not probably result from self-incompatibility, for very few are known to be self-incompatible in the majority of *Allium* species according to Ved Brat[69]. It is most probable that female gametophytes develop only in a small part of the ovules in both *A. sativum* L. and *A. longicuspis* Regel. As mentioned before, embryo sacs failed to develop in a sterile clone. Therefore, most of female gametophytes may not develop well even in these fertile clones. As the mature seeds were usually harvested one per flower in the present examination, there may be also nutritional competition among the six ovules in a flower or among the flowers in an inflorescence even if the female gametophytes develop completely in some parts of the ovules. The inflorescence provided with bulbils neither developed fruits nor produced viable seeds in the present investigation. There may be also the nutritional competition between fruits and bulbils. According to Katarzhin and Katarzhin[30], their fertile garlic produced seeds without preliminary removal of bulbils. Their clone must have been more fertile than that in the present investigation.

For success of crossbreeding in garlic, it is necessary to find a lot of fertile clones, especially those developing female organs well. Those clones which can develop female organs completely may exist also among the clones showing pollen sterility. To restore the fertility in the sterile clones, somatic cell fusion between the fertile and the sterile clones may be useful in the future, in addition to the crossing between the sterile clones and the fertile clone. In conclusion, the way to cross-breeding in garlic was opened here.

IV. Comparison of the fertile garlic clone with the sterile clones and the allied species of garlic

The fertile clone found in the present investigation was compared with other sterile clones in some respects. One of the reasons is the assumption that the fertile clone may show the primitive type of garlic. Garlic shows a great variation[28], though the varietal differentiation in the strict sense may not be so great. However, its intraspecific evolution has never been discussed because of the absence of the exact wild ancestor. Its comparison with other sterile clones may be useful in discussing the intraspecific evolution, especially, on the breeding system.

Another reason is the fact that the morphological and genetical differences between the fertile clone and the sterile clones make it easy to find other fertile clones.

The fertile garlic clone, No. 130, was also compared with some allied species of garlic, *A. longicuspis* Regel, *A. scorodoprasum* L., and *A. ampeloprasum* L. (great-headed garlic). *A. longicuspis* Regel is sterile and presumed to be the wild ancestor of garlic[49], but it has been insufficiently compared with garlic. According to Vvedensky[74], *A. scorodoprasum* L. is closely related to garlic, next to *A. longicuspis* Regel. This species also shows sterility. According to Jones and Mann[28], *A. ampeloprasum* L. contains three groups, one of which is great-headed garlic. This great-headed garlic is frequently confused with garlic, because its large bulb resembles that of garlic. Most clones of great-headed garlic are relatively-sterile hexaploids[28].

1. Growth habit

Plant height and number of leaves were examined periodically during the growing period in 156 clones including the fertile clone. The cloves were planted in the open field at Kagoshima on September 28, 1982. The plant height and number of leaves were examined at intervals of two weeks

in five plants of the respective clones. They were examined up to the respective harvest time in the following spring or summer. The leaves of garlic usually droop. Therefore, the plant height is that from the ground level to the tip of the straightened longest leaves. The number of leaves always includes the sprout leaf.

Results

Fig. 24 shows the increases of plant height and the number of leaves in the fertile garlic clone, No. 130. The fertile garlic clone grew slowly in autumn, and its growth was arrested during the winter for a long time. In spring, it started to grow again and continued its growth up to summer. This fertile clone matured latest among the examined garlic clones.

Besides this fertile clone, many clones arrested their growth in winter, though some of them grew rapidly in autumn as shown in Fig. 24. On the other hand, some parts of the examined clones did not arrest their growth in winter as shown in Fig. 24. Therefore, all the examined clones were classified into two groups according to their growth habits in winter (Table 11).

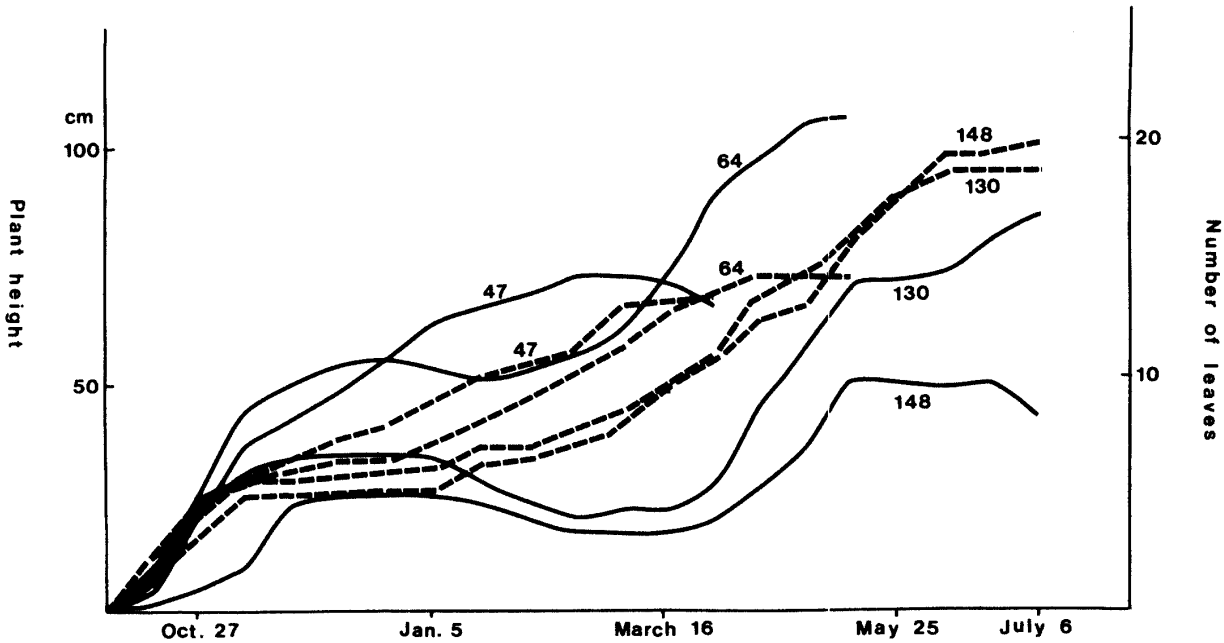


Fig. 24. Comparison of the fertile garlic clone (No. 130) and the three sterile clones (Nos. 47, 64, 148) concerning plant height (—) and number of leaves (---).

Table 11. Classification of clones according to the growth habits in winter

Growth habit	Clone Number
Continuous growth in winter	6, 28, 30, 35, 38, 40, 42, 44, 46, 47, 51, 53–55, 58, 67, 68, 70, 74–76, 80, 90, 129, 131–137, 154–157, 171
Arrested growth in winter	1–5, 7–25, 27, 31, 34, 36, 37, 41, 43, 45, 50, 52, 56, 57, 60, 62–65, 69, 71–73, 77–79, 84–89, 93–109, 111–113, 115–128, 130, 138–153, 158–167, 169, 170, 173, 174

The slow growth in autumn as in the fertile clone was seen in clones Nos. 11, 12, 17, 98, 102, 103, 106, 140, 145, 148, 151, 152, 161, and 162. Most of these showed long rest of growth in winter and slow growth after winter. Of these, clone No. 148 finally differentiated the most numerous leaves among the examined clones (Table 12). However, its early-differentiated leaves withered to death with its growth as well as those of other clones. Clone No. 148 matured as late as the fertile clone but did not grow so high as the fertile clone (Fig. 24).

In contrast with these clones of slow growth in autumn, the following clones grew rapidly in autumn; Nos. 21, 64, 94, 100, 112, 115, 121, 123, 128, 139, 141, 160, 163, and 170.

Table 12. Total number of the differentiated leaves per plant in the examined clones

Total number of the differentiated leaves per plant	Clone Number
9.0–9:9	7, 12, 55, 102
10.0–10.9	3, 19, 20, 35, 38, 73, 131, 132, 152, 157
11.0–11.9	11, 14, 22, 25, 28, 30, 34, 40, 42, 45, 51, 52, 61, 79, 103, 145, 155
12.0–12.9	6, 10, 15–17, 46, 57, 68, 74, 86, 99, 133, 140, 154, 172
13.0–13.9	2, 4, 13, 18, 23, 24, 31, 32, 37, 43, 44, 47, 54, 56, 58, 60, 62, 67, 70, 84, 87, 97, 113, 117, 127, 135, 137, 146, 156, 160
14.0–14.9	5, 8, 9, 27, 36, 41, 53, 63, 64, 69, 71, 75, 76, 80, 93–95, 98, 100, 107, 108, 116, 120, 134, 136, 144, 163, 170, 171
15.0–15.9	21, 50, 72, 78, 85, 88, 90, 104–106, 111, 112, 128, 129, 138, 142, 149, 162, 175
16.0–16.9	1, 77, 89, 101, 109, 110, 115, 118, 121, 122, 124–126, 143, 147, 151, 161, 164, 167, 169, 173, 174
17.0–17.9	65, 119, 139, 141, 150, 158, 159, 165, 166
18.0–18.9	123
19.0–19.9	130, 153
20.0–20.9	148

Table 13. Harvesting times of the examined clones

Harvest time	Clone Number
March 31 — April 13	6, 47, 131, 132
April 14 — April 27	28, 38, 55, 58, 67, 68, 70, 75, 80, 96
April 28 — May 11	20, 30, 35, 40, 42, 44–46, 51, 53, 54, 76, 90, 129, 134–137, 154–157
May 12 — May 25	1–5, 7, 8, 12, 14–19, 21–25, 27, 31, 32, 34, 36, 37, 41, 43, 50, 52, 56, 57, 60, 62–65, 69, 71–74, 87, 88, 94, 97, 99, 100, 102–105, 112, 115, 117, 121, 133, 141, 170, 171, 174
May 26 — June 8	9–11, 13, 79, 84–86, 93, 95, 98, 101, 106, 107–109, 111, 113, 116, 118–120, 122–124, 138–140, 159, 163, 164, 173
June 9 — June 22	77, 78, 125, 142–150, 165–167, 169
June 23 — July 6	89, 126–128, 147, 152, 160–162
July 7 — July 21	130, 148, 151, 153, 158

As shown in Table 11, a lot of clones arrested their growth in winter, but the resting period of growth varied with the clones. Clones Nos. 14, 15, 45, 97, and 99 showed slight rest of growth in winter. On the contrary, the long rest of growth in winter was noticed in clones Nos. 7, 84, 86, 102, 103, 106, 122, 124, 125, 127, 148, 151, 153, 158, 161, 162, and 163. Nos. 153 and 158 survived longer than the others (Table 13), and the former differentiated numerous leaves (Table 12).

After the long rest of growth in winter, the fertile clone elongated gradually. However, many clones elongated quite rapidly in spring, such as Nos. 21, 41, 64, 65, 72, 94, 100, 112, 115, 121, 123, 128, 139, 141–143, 147, 160, 163–167, 169, 170, and 173. Gradual growth after winter as in the fertile clone was seen in a lot of clones, but most of them matured much earlier than the fertile clone or could not survive so long up to summer as the fertile clone (Table 13).

Discussion

The fertile clone revealed its characteristic growth habit different from other sterile clones in some respects. Early in autumn, the fertile clone grew moderately. As mentioned before, some clones grew so slowly, and most of them came from the cool areas obviously. Several clones of slow growth in autumn came from Europe where garlic is frequently planted late in autumn. Therefore, these clones must be very sensitive to cold.

In contrast with these clones of slow growth, some clones grew quite rapidly in autumn. Most of them came from the warm areas, and they were classified into two groups. One group arrested the growth in winter and recovered rapid growth in spring. Clones Nos. 123 and 128 of this group came from the cool areas, and Nos. 139 and 141 came from Mediterranean area. This group may require cool winter for the rapid growth in spring. Another group with rapid growth in autumn continued growing even in winter, for example, clones Nos. 44, 47, 136, and 137. All of them came from tropical or subtropical areas. Probably they may not need cool winter.

In addition to the latter clones, many clones continued to grow in winter as shown in Table 11, and they were also the tropical or subtropical clones. On the other hand, most of the clones with a long rest on elongating came from northern Japan and Korea. However, they quitted their growth much earlier than the fertile clone, and the clones from northern Japan frequently finished their growth before full maturity. It is probable that these northern clones of Japan have already lost heat-resistance. One clone from Korea, No. 162, survived long, but the clone could not catch up with the fertile clone, withering to death late in June. Besides those clones, Nos. 153 and 158 showed a long rest of growth in winter, as well as long survival, and differentiation of numerous leaves as in the case of the fertile clone. However, both of the two did not bolt at all.

A lot of clones began to grow again in spring, and some parts of them elongated more rapidly than the fertile clone. However, they matured much earlier than the fertile clone. Four European clones, Nos. 142, 143, 147, and 173, grew high and survived considerably long, but none of them bolted completely. Many other clones grew moderately in spring. Most of them also matured much earlier than the fertile clone, and the rest of them probably interrupted their growth by the heat of summer in Kagoshima. According to Jones[26], some of the garlic clones reached a height of six feet, but such a high clone was not found among the clones collected by the author. Probably it may be necessary to examine far more clones, especially those in USSR and China which are expected to offer a great variation of garlic.

After all, this fertile clone showed a few characteristic properties on growth habit; a long rest of growth in winter, survival up to summer, and differentiation of a large number of leaves. The

long rest of growth means that the home of this fertile clone has a long severe winter. The survival up to summer may suggest that the habitat of this fertile clone has also a hot summer. The primary center of garlic is assumed to be Central Asia, where both of winter and summer are severe. This fertile clone may be considered to be a primitive type of garlic. This fertile clone has the ability to differentiate a large number of leaves. Clones Nos. 148 and 153 differentiated a little more leaves than this fertile clone, but the latter did not bolt, and the former proceeded towards normal meiosis. This fact is somewhat suggestive when we consider about the intraspecific evolution in garlic. As garlic is a cultigen, or as it has a long history of cultivation, it may be quite proper to think that the evolution and the diversity of garlic should have been connected with the selection by cultivation.

As mentioned before, it is possible to assume that this fertile clone may keep a primitive type because of its fertility. The late maturity as in this fertile clone is probably a primitive character, because it is believed that primary crops usually had long growing periods[7]. The late maturity was also observed in the clone provided with normal meiosis and also in *A. longicuspis* Regel which is a wild form, not yet cultivated. Most of the early mature clones bolted incompletely, and this incomplete-bolting is probably one of the types derived from the primitive type. Therefore, the late maturity of this fertile clone may support the assumption that it is still keeping the primitive type. The differentiation of numerous leaves can be taken as another primitive character of garlic, because the main purpose of cultivation in garlic is the production of bulbs, not of leaves[2].

From the viewpoint of the intraspecific evolution in garlic, it is possible to say that early maturity and continuous growth in winter, in other words, loss of cold hardiness, may be taken as reliable evidences of its evolution.

Kumazawa[44] classified the Chinese garlic clones into two major groups; Kokotsu and Nankotsu. He stated that Kokotsu group was matured early and grown easily in both north and south parts of China, while Nankotsu was grown as seed-bulbs only in the middle China because of its late maturity. It may be necessary to examine the fertility of far more clones of Nankotsu group, though clone No. 51 Taichu-Nankotsu showed sterility in the present study.

2. Morphological characters

In addition to growth habit, some morphological characters were compared between the fertile garlic clone and the sterile clones. These morphological characters were examined in 1981, 1982, and 1983. The numbers of bulbs and bulbils, the length of scapes, and bulb weight were examined after harvest.

Results

A) Number of cloves per bulb and leaf width

The number of cloves per bulb was examined in ten plants, and the leaf width was examined in five plants. Fig. 25 shows the distribution of the examined clones in relation to the mean number of cloves per bulb and the mean leaf width. The clones were classified by their bolting habits mentioned before. A bulb of this fertile clone consisted of ten cloves, and its leaf was 1.6 cm wide as average. The fertile clone was situated almost in the center of the frequency distribution in all the complete-bolting clones. Many of the incomplete-bolting clones were distributed around the complete-bolting ones, though some of them were situated in the frequency distribution in the complete-bolting ones. Of the incomplete-bolting clones, northern clones usually showed fewer cloves per bulb than the complete-bolting clones. On the other hand, southern clones of the

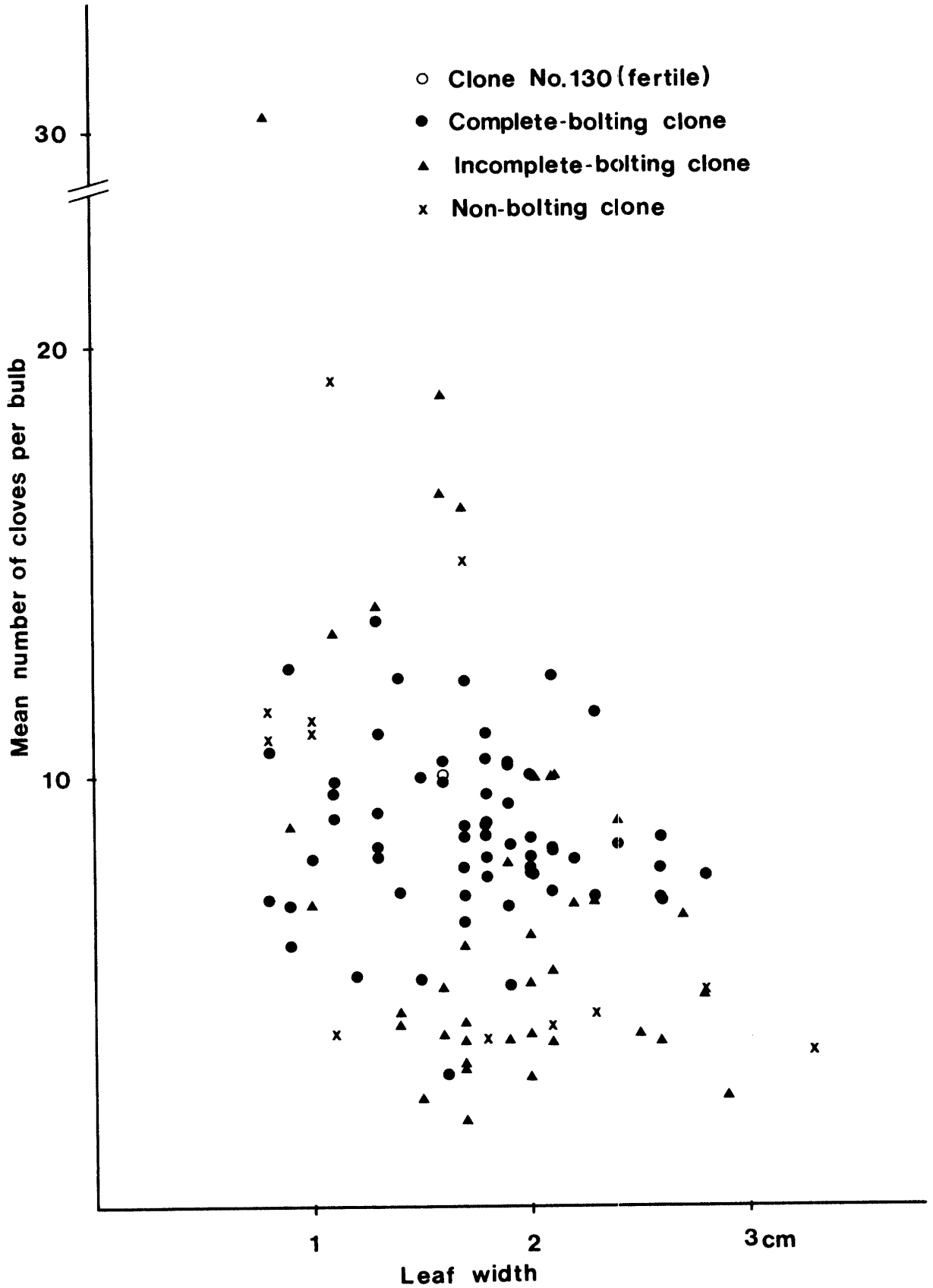


Fig. 25. Distribution of the clones in relation to the mean leaf width and the mean number of cloves per bulb.

incomplete-bolting clones showed either fewer or more cloves per bulb than the complete-bolting clones. Non-bolting clones were situated exclusively outside the variation range of the complete-bolting clones. In other words, non-bolting clones showed either fewer or more cloves per bulb than the complete-bolting clones as in case of the incomplete-bolting clones. As a rule, these non- or incomplete-bolting clones having numerous cloves per bulb were provided with narrower leaves, while those having fewer cloves with wider leaves.

B) Numbers of bulbils and flower-buds per inflorescence

Numbers of bulbils and flower-buds per inflorescence were examined in ten plants of the respective clones in 1981. Fig. 26 shows the frequency distribution of sterile clones in relation to the

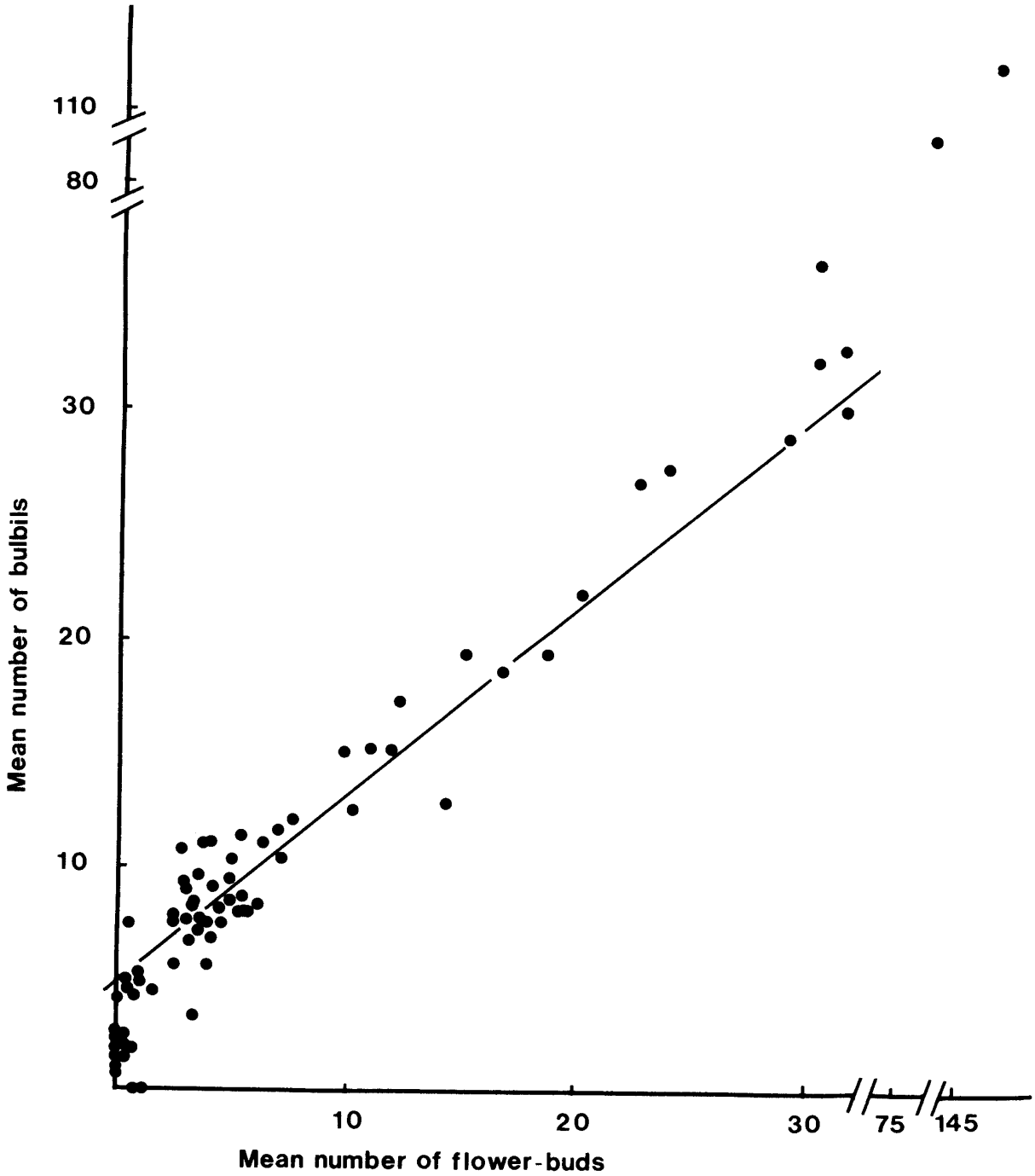


Fig. 26. Distribution of the sterile garlic clones in relation to the mean numbers of bulbils and flower-buds.

numbers of bulbils and flower-buds per inflorescence. The number of bulbils was positively correlated with the number of flower-buds. Their relationship was shown as the following expression.

$$Y=0.81X+4.74$$

Y: number of bulbils, X: number of flower-buds

The incomplete-bolting clones usually bore only bulbils in the inflorescences as mentioned before. Most of the complete-bolting clones differentiated less than 30 bulbils and flower-buds. The fertile garlic clone differentiated 79.9 flower-buds as average of ten plants, coming next to the clone bearing the most numerous flower-buds throughout all the clones examined. This fertile clone may theoretically differentiate 69.5 bulbils as average.

C) Lengths of scape, beak, and the longest leaf-blade

Length of scape was examined in ten plants of the respective clones. Fig. 27 shows average length of scape in relation to the number of flower-buds and the chromosome configuration at meiosis in various garlic clones and *A. longicuspis*. The chromosome configuration $1_{VI}+5_{II}$ was always observed in the clones with short scapes below 60 cm, while the chromosome configuration $1_{VIII}+4_{II}$ was mainly observed in the clones with long scapes beyond 60 cm. Most of the clones with $1_{VI}+5_{II}$ bore only several flower-buds, while many of the clones with $1_{VIII}+4_{II}$ bore more than ten flower-buds. In the clone with $1_{X}+3_{II}$, the scape was as short as those of the clones with $1_{VI}+5_{II}$, but it was a little longer than those of the most clones with $1_{VI}+5_{II}$. The flower-buds of the clones with $1_{X}+3_{II}$ were as few as those of the most clones with $1_{VI}+5_{II}$.

On the other hand, the fertile garlic clone produced long scapes as in *A. longicuspis*. The scape of the fertile clone was one of the longest among those of garlic clones examined. *A. longicuspis*

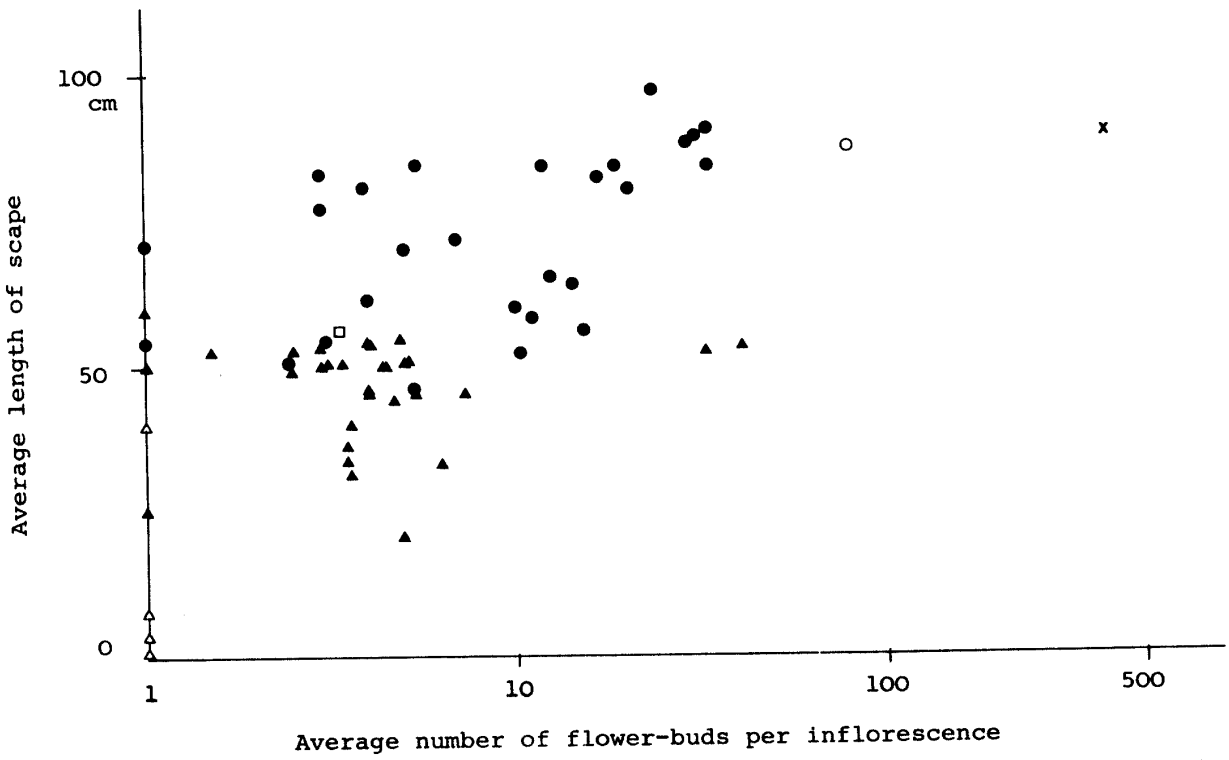


Fig. 27. Distribution of garlic clones and *A. longicuspis* Regel in relation to the chromosome configuration at meiosis, average length of scape, and average number of flower-buds. □; $1_{X}+3_{II}$, ●; $1_{VIII}+4_{II}$, ▲; $1_{VI}+5_{II}$, ○; 8_{II} (fertile), △; degeneration of flower-buds before meiosis, ×; *A. longicuspis* Regel

bore numerous flower-buds, and none of the garlic clones bore such a lot of flower-buds. The clones with very short scapes were limited to the incomplete-bolting clones, and they did not develop flower-buds up to meiosis as mentioned before. Table 14 shows mean length of the longest leaf blade and beak in several garlic clones including the fertile clone and *A. longicuspis*. The fertile clone produced shorter leaf blades and shorter beaks in comparison with other garlic clones. The leaf blade of *A. longicuspis* was longer than those of garlic clones, but it showed shorter beaks like those of the fertile clone.

Table 14. Mean lengths of the longest leaf-blade and the beak of spathe in the fertile garlic (No. 130) and the sterile clones

Clone No.	Mean length of leaf blade	No. of plants examined	Clone No.	Mean length of beak of spathe	No. of plants examined
130	43.8 cm	5	130	13.2 cm	5
56	41.8	5	44	32.0	5
64	47.2	5	64	28.0	6
78	48.4	5	126	23.6	5
141	58.2	5	127	23.6	5
161	60.0	5	145	10.3	3
164	55.4	5	202	12.0	5
A. longicuspis	61.2	5	*A. longicuspis*	15.8	6

D) Weights of bulb and clove

Weights of bulb and clove were examined in ten plants of the sterile clones, and Fig. 28 shows the frequency distribution of the sterile clones in relation to weight of bulb and clove and bolting habit. In the complete-bolting clones, mean bulb weight was almost positively correlated with mean clove weight. Their relationship is shown as the following expression.

$$Y=6.33X+4.07$$

Y: mean bulb weight, X: mean clove weight

This expression is drawn in Fig. 28. In those complete-bolting clones, both of bulb weight and clove weight were restricted almost within 40 g and 5 g, respectively.

On the other hand, incomplete- and non-bolting clones were mostly distributed around the complete-bolting clones, being scattered. However, several clones produced much heavier bulbs and cloves than the complete-bolting clones, for example, clones Nos. 96, 109 and 118. These three clones came from northern Japan, and No. 96 is a non-bolting clones, the other two being incomplete-bolting clones. A part of non- and incomplete-bolting clones produced relatively lighter cloves and heavier bulbs than the complete-bolting clones. About a half of them came from the warm areas. Some clones from warm areas were situated within the variation range of the complete-bolting clones, and they did not produce relatively heavy cloves.

In contrast with these clones which came from warm areas, the non- or incomplete-bolting clones from cool areas produced relatively heavy cloves. A part of them also produced heavy bulbs, but some of them could not.

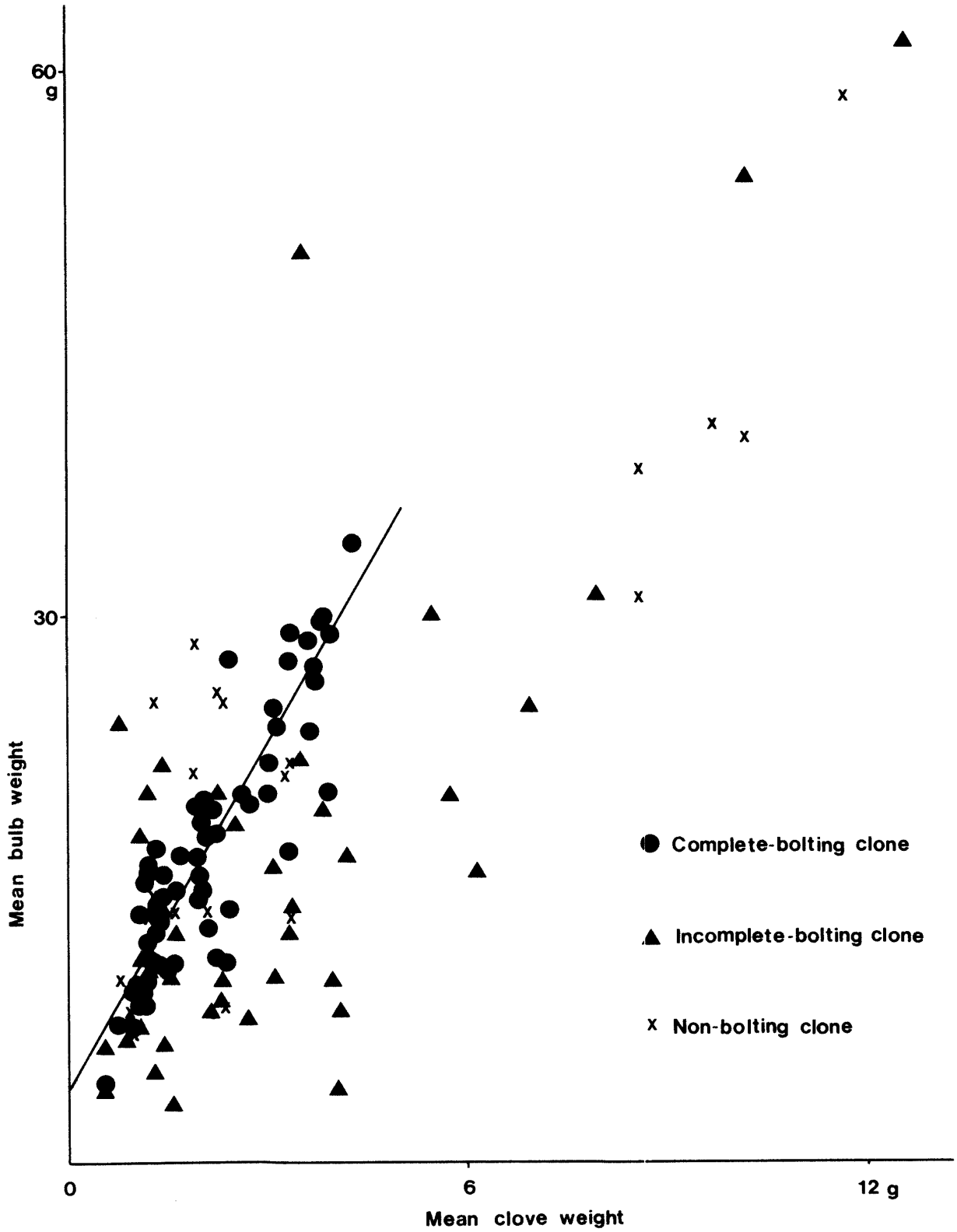


Fig. 28. Distribution of garlic clones in relation to mean bulb weight and clove weight under the classification of their bolting habits.

Discussion

According to Yakuwa[77], the number of cloves per bulb is almost constant in a clone regardless of the number of cloves in its mother bulb. Shimada and Shozaki[61] stated that Japanese bolting clones usually differentiated ten cloves in two axils as average, six cloves in the axil nearest to the scape and four in the second nearest axil. Yamada[79] reported five and five cloves differentiating in those two axils of the bolting clones. Aoba[1] stated that the clones differentiating cloves in those two axils always bolted, and that the clones differentiating cloves in three axils showed low bolting rate. He also stated that the clones differentiating cloves in more than three axils rarely bolted. Moreover, he stated that one non-bolting clone differentiated 18–22 cloves in 11–12 axils, and another non-bolting clone differentiated 12–24 cloves in 6–12 axils. According to Mann[48], non-bolting American clones differentiated cloves in six to eight axils, producing one to five cloves per axil.

The results mentioned above almost coincide with those in the present investigation. Complete-bolting clones usually produced 6 to 12 cloves, because the complete-bolting clones differentiated cloves in the restricted axils. Moreover, one axil can differentiate only a limited number of cloves in any clones. Northern clones of incomplete-bolting usually produced fewer cloves than the complete-bolting clones.

As shown in Fig. 25, the fertile clone is situated almost in the center of the distribution range of the complete-bolting clones on the leaf width and in the number of cloves per bulb. Those complete-bolting clones are surrounded by non- or incomplete-bolting clones. These clonal variations could be realized in connection with the intraspecific evolution of garlic, as mentioned repeatedly. Non-bolting clones are always situated outside the distribution range of complete-bolting clones, though the incomplete-bolting clones are sometimes located within the distribution range of the complete-bolting clones. This may support the hypothesis that garlic has evolved from complete-bolting to non-bolting through incomplete-bolting.

The northern clones of Japan with non- or incomplete-bolting habit usually differentiated fewer cloves than the complete-bolting clones. Some of them produced heavy bulbs. One of those clones is No. 118 which produced the heaviest bulb among the clones. On the contrary, several southern clones of incomplete-bolting produced small numerous cloves, for example, No. 55 from Egypt and No. 58 from Thailand. The clones bearing numerous cloves did not produce wider than 2 cm leaves. All of these may be the consequences of specialization of the characters in the complete-bolting clones. The fertile clone in question is located at the center of the diversity on the two characters as if it is located at the center of the adaptive radiation in a small scale or at the center of the expansion of the characters by the selection pressure. The fertile clone found here may keep a primitive type of garlic on the leaf width and on the number of cloves per bulb.

The number of bulbils in the inflorescence was positively correlated with number of flower-buds. Besides garlic, the genus *Allium* contains many viviparous species, and *A. grayi* Regel is one of the typical species. According to Kawano[33], *A. grayi* Regel consists of (i) obligate amphimictic individuals bearing only flowers, (ii) obligate apomictic individuals bearing only bulbils, (iii) intermediate individuals bearing both flowers and bulbils. He found that the proportion of those three types in the respective areas depended on the environmental conditions including human activities.

In garlic, the numbers of bulbils and flower-buds per inflorescence as well as the leaf-width and the number of cloves per bulb depend on the clone. Garlic usually bears both flowers and bulbils

in the inflorescence, though two of the incomplete-bolting clones bore negligible number of flower-buds without bulbils as mentioned before. There was no garlic plant bearing a lot of flower-buds without bulbils such as the obligate amphimictic plants of *A. grayi*. On the other hand, a lot of incomplete-bolting clones bore only bulbils, and many plants of the complete-bolting clones bore only bulbils in their inflorescences as mentioned before. Possibly these facts may be related with the sterility in garlic, because *A. grayi* usually produces fertile pollen grains in spite of its vivipary.

The fertile clone differentiated almost 80 flower-buds. However, most of sterile clones produced less than 30 flower-buds, and not only the non-bolting clones but also most of the incomplete-bolting clones produced no flower-buds. This variation of the flower-number may allow us to fix the following hypothesis on the evolution of the breeding system in garlic.

It is presumed that garlic originally produced a lot of flowers when it was fertile. With its evolution, garlic decreased the flowers in number, attending sterility. When garlic lost the ability of flower-bud differentiation, it may have started to bolt incompletely. Through those processes, garlic also decreased the bulbils in number consistently. Finally garlic lost even the ability to bolt. Abundance of cloves in some parts of non-bolting clones may be caused by early differentiation of cloves at low axils.

Of course, this hypothesis mentioned above is based on the assumption that the fertile clone found here may keep the primitive characters of garlic. From a different point of view, the selection of the clones provided with fewer bulbils may have been attended with the selection towards fewer flower-buds formation.

The chromosome configuration at meiosis showed a wide variation in the examined clones as mentioned before. However, the majority of the clones were divided into two groups, showing either $1_{VIII}+4_{II}$ or $1_{VI}+5_{II}$ as the chromosome pairing. It was clarified here that those two groups of clones also differ from one another on the length of scapes. The chromosome configuration $1_{VI}+5_{II}$ was observed in the clones with short scapes, while $1_{VIII}+4_{II}$ was observed mostly in the clones with long scapes. However, any other phenotypical difference was not noticed in the present investigation. It is unlikely that all the clones showing the same chromosome configuration at meiosis belong to the same clone group. As mentioned before, several clones differentiated less than one flower-bud as average, and their flower-buds degenerated at the young stage. The existence of those clones may support the hypothesis on the evolution of the breeding system; from numerous flower-buds to few flower-buds and from complete-bolting to incomplete-bolting. The fertile clone with 8_{II} produced long scapes as well as *A. longicuspis* Regel. The long scape may be a primitive character, though Jones[26] stated that the six-foot-high garlic clones also failed to produce seeds. Therefore, it is difficult to say that the clones with long scapes and $1_{VIII}+4_{II}$ evolved from the clones with short scapes and $1_{VI}+5_{II}$. However, it is possible to assume that the primitive type with the long scape and the numerous flower-buds evolved towards the long scape with a few flower-buds or the short scapes with numerous flower-buds, and that both of the evolved types are going to form short scapes with few flower-buds. Many clones with short scapes and $1_{VI}+5_{II}$ produced less than five flower-buds. For them, to get shorter scapes may possibly mean to get fewer flower-buds and finally to get incomplete-bolting. *A. longicuspis* differentiated abundant flower-buds, and the difference in the number of flower-buds may be the most distinguishable one between *A. longicuspis* and garlic. However, according to Jones[26], some of the garlic clones bore a thousand or more flowers. Therefore, garlic may have a much greater variation in the number of flowers than that shown in this examination.

Both of the longest leaf blade and the beak of spathe in the fertile clone were rather short among

those of the examined clones. The longest leaf-blade of the fertile clone was much shorter than that of *A. longicuspis*, though the longest leaf-blades of some other garlic clones were as long as that of *A. longicuspis*. On the other hand, the beak of the spathe of the fertile clone was as short as that of *A. longicuspis*. However, the short beaks of both were not so short as that of *A. scorodoprasum*. These morphological characters of the fertile clone may be useful to find other fertile clones.

In the complete-bolting clones, the bulb weight was correlated with the clove weight. Many of the incomplete- and non-bolting clones produced heavier cloves than the complete-bolting clones, and some of them also produced heavier bulbs than the complete-bolting clones. It is clear that human beings would prefer the big and heavy bulbs to the small and light ones, because the bulb is usually the edible part of garlic. The selection of heavy bulbs by human beings might be one of the causes which introduced incomplete- and non-bolting habits into garlic, because the maximum weight of bulb was limited in the complete-bolting clones as shown in Fig. 28. Anyway, from the view point of intraspecific evolution, the clonal distribution in Fig. 28 seems to tell us that the incomplete- and non-bolting clones derived or evolved from the complete-bolters.

3. Karyotype

The fertile garlic clone was already compared with the sterile clones on the growth habit and in some morphological respects. This comparison cleared the phenotypic variation in garlic. Moreover, the observation of meiosis revealed that garlic has a variation on the mode of chromosome pairings, as mentioned before. These suggested that garlic may also have a karyotypic variation among the clones. Therefore, the somatic chromosomes in various clones were examined here. A part of this examination was reported in 1983[13].

Materials and Methods

The root tips of 60 garlic clones, *A. longicuspis* Regel, and *A. ampeloprasum* L. cv. Elephant were pretreated in 0.002 mol 8-hydroxyquinoline solution, and the squashed materials were observed after being stained by means of Feulgen reaction.

Results

The somatic chromosomes of the fertile garlic clone were observed in ten cells. Their lengths were measured, and each relative length was obtained. The average idiogram is shown in Fig. 29.

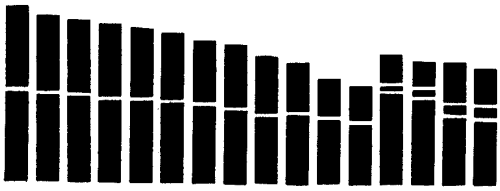


Fig. 29. Average idiograms in the fertile garlic clone, No. 130[13].

The idiogram of the fertile clone consisted of ten long median chromosomes, two pairs of SAT-chromosomes, and one pair of short submedian chromosomes. The ten median chromosomes showed gradual difference in length. Both pairs of SAT-chromosomes had big satellites and short proximal segments. The longer pair of the SAT-chromosomes had smaller satellites than the

shorter pair. Long secondary constrictions were often observed in the SAT-chromosomes. Consequently, the karyotype of this fertile clone was expressed as follows;

$$K(2n)=10m+2sm_1^{sc}+2sm_2^{sc}+2sm$$

The chromosomes of sm_1^{sc} are longer than those of sm_2^{sc}. This karyotype was most frequently observed not only in the garlic clones examined here but also in the materials of the previous reports[3]. Therefore, this type was regarded as the basic karyotype of garlic here in this investigation.

Table 15 shows the karyotypes of the examined garlic clones. Many of these clones frequently showed a karyotypic variation within the respective clone. Among the somatic chromosomes of garlic, the SAT-chromosomes were the most distinguishable. The shortest pair was the next. Two pairs of SAT-chromosomes were distinguished from each other owing to the chromosome length and the satellite size. However, when they were not distinguishable from each other, they were

Table 15. Karyotypes in various garlic clones

Clone No.	Number of cells		
	Karyotype		Total
	Basic*	Other	
1	0	2 ($11m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	2
4	0	3 ($10m+1sm_1^{sc}+2sm_2^{sc}+3sm$)	3
9	1	2 ($11m+1sm_1^{sc}+1sm_2^{sc}+3sm$), 1 ($10m+1sm_1^{sc}+1sm_2^{sc}+4sm$)	7
		1 ($12m+1sm_1^{sc}+1sm_2^{sc}+2sm$), 1 ($11m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	
		1 ($9m+2sm_1^{sc}+2sm_2^{sc}+3sm$)	
10	0	1 ($10m+2sm_1^{sc}+1sm_2^{sc}+3sm$), 1 ($10m+1sm_1^{sc}+2sm_2^{sc}+3sm$)	2
13	0	1 ($10m+1sm_1^{sc}+2sm_2^{sc}+3sm$)	1
17	0	3 ($11m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	3
21	0	1 ($11m+1sm_1^{sc}+2sm_2^{sc}+2sm$), 1 ($11m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	2
38	3		3
40	13		13
41	3		3
45	0	2 ($8m+2sm_1^{sc}+2sm_2^{sc}+4sm$)	2
50	2	1 ($11m+1sm_1^{sc}+1sm_2^{sc}+3sm$), 1 ($10m+1sm_1^{sc}+1sm_2^{sc}+4sm$)	5
		1 ($10m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	
54	0	3 ($9m+2sm^{sc}+5sm$), 4 ($10m+2sm^{sc}+4sm$)	9
		2 ($9m+3sm^{sc}+4sm$)	
56	3		3
58	3		3
60	2		2
62	2		2
64	3	3 ($10m+2sm_1^{sc}+1sm_2^{sc}+3sm$), 5 ($11m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	15
		3 ($11m+1sm_1^{sc}+2sm_2^{sc}+2sm$), 1 ($10m+1sm_1^{sc}+2sm_2^{sc}+3sm$)	
69	0	2 ($11m+2sm_1^{sc}+1sm_2^{sc}+2sm$)	2
71	2		2
72	1		1
79	1	1 ($8m+2sm_1^{sc}+2sm_2^{sc}+4sm$)	2
89	2		2
105	0	2 ($11m+1sm_1^{sc}+2sm_2^{sc}+2sm$)	2

Table 15 (Continued)

Clone No.	Number of cells		
	Karyotype		Total
	Basic*	Other	
107	1		1
115	4		4
121	3		3
122	0	1 (11m+2sm$_1^{sc}$+1sm$_2^{sc}$+2sm), 3 (11m+1sm$_1^{sc}$+2sm$_2^{sc}$+2sm)	4
127	0	1 (10m+2sm$_1^{sc}$+1sm$_2^{sc}$+3sm), 3 (11m+1sm$_1^{sc}$+2sm$_2^{sc}$+2sm)	4
128	3		3
131	2		2
134	1		1
138	3		3
140	2		2
141	3		3
142	3		3
143	3	1 (11m+2sm$_1^{sc}$+1sm$_2^{sc}$+2sm), 1 (11m+1sm$_1^{sc}$+2sm$_2^{sc}$+2sm)	5
144	0	1 (10m+2sm$_1^{sc}$+1sm$_2^{sc}$+3sm), 2 (10m+1sm$_1^{sc}$+2sm$_2^{sc}$+3sm) 1 (11m+1sm$_1^{sc}$+2sm$_2^{sc}$+2sm)	4
145	4		4
147	0	1 (10m+2sm$_1^{sc}$+1sm$_2^{sc}$+3sm), 1 (8m+2sm$_1^{sc}$+1sm$_2^{sc}$+5sm) 1 (11m+2sm$_1^{sc}$+1sm$_2^{sc}$+2sm)	3
148	8	1 (10m+2sm$_1^{sc}$+1sm$_2^{sc}$+3sm), 1 (11m+2sm$_1^{sc}$+1sm$_2^{sc}$+2sm)	10
150	2	1 (9m+2sm$_1^{sc}$+2sm$_2^{sc}$+3sm)	3
151	3		3
152	2	1 (8m+2sm$_1^{sc}$+2sm$_2^{sc}$+4sm)	3
160	4	1 (8m+2sm$_1^{sc}$+2sm$_2^{sc}$+4sm)	5
161	3		3
167	3		3
182	3	1 (10m+1sm$_1^{sc}$+2sm$_2^{sc}$+3sm), 1 (9m+1sm$_1^{sc}$+2sm$_2^{sc}$+4sm), 1 (9m+1sm$_1^{sc}$+2sm$_2^{sc}$+3sm)** 1 (8m+2sm$_1^{sc}$+2sm$_2^{sc}$+3sm)**, 1 (9m+2sm$_1^{sc}$+2sm$_2^{sc}$+2sm)**	8
187	2	1 (10m+2sm$_1^{sc}$+1sm$_2^{sc}$+3sm)	3
188	2		2
189	2		2
190	2		2
191	1		1
197	2		2
202	3		3
208	4	3 (11m+1sm$_1^{sc}$+2sm$_2^{sc}$+2sm)	7
209	4	1 (11m+2sm$_1^{sc}$+1sm$_2^{sc}$+2sm)	5
226	1		1
231	0	1 (12m+2sm^{sc}+2sm), 1 (9m+2sm^{sc}+5sm) 3 (9m+2sm$_1^{sc}$+1sm$_2^{sc}$+4sm)	5

* Basic karyotype; K(2n)=10m+2sm$_1^{sc}$+2sm$_2^{sc}$+2sm

** 2n=15

expressed as sm^{sc} instead of $sm_1^{sc}+sm_2^{sc}$. The chromosomes of "sm" in the karyotype formula of Table 15 include a wide range of chromosomes. The cells of the basic karyotype contained one pair of "sm", submedian chromosomes, but the cells of the karyotypes having more than three "sm" chromosomes contained submedian chromosomes of various types besides the pair. Some of them

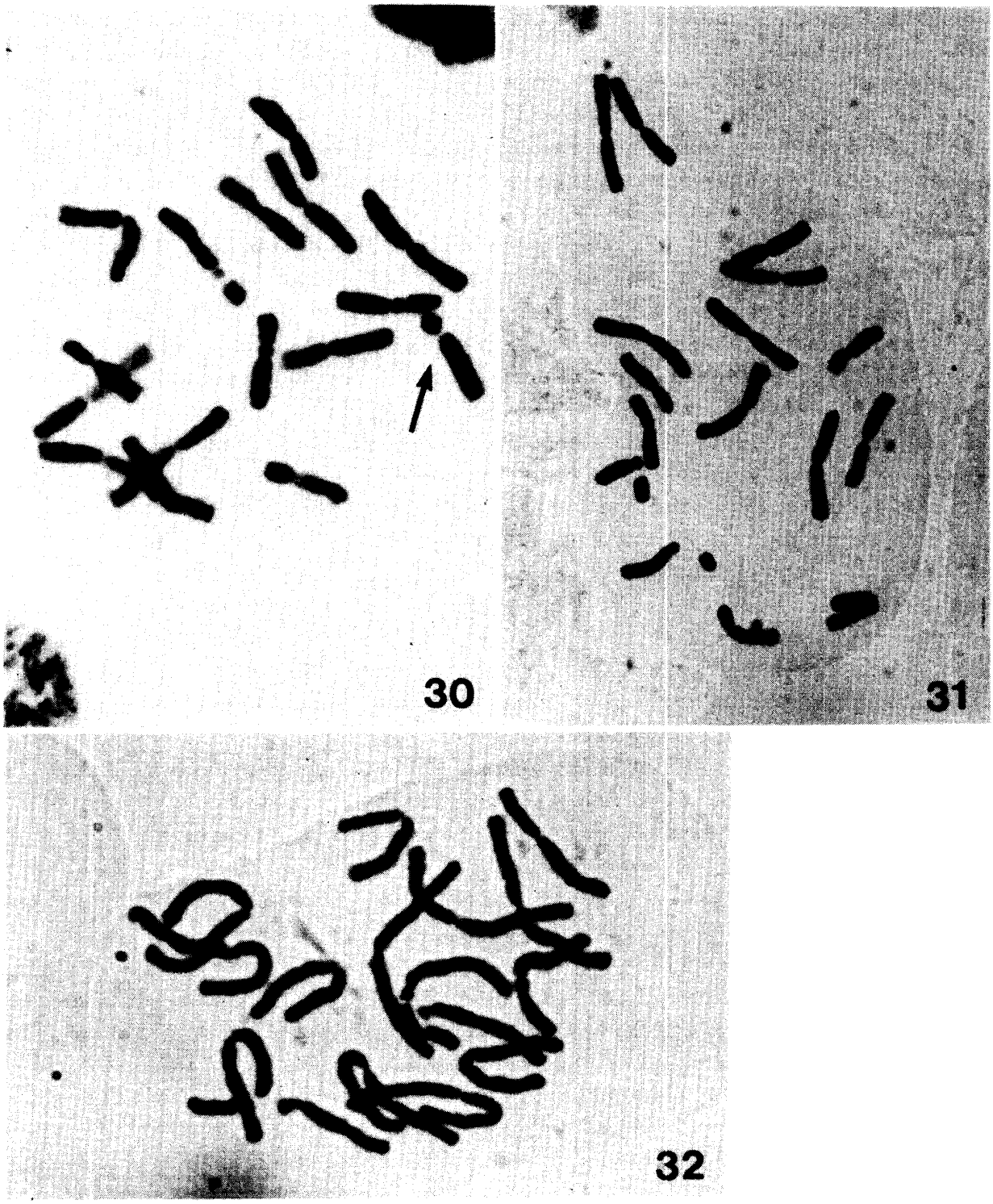


Fig. 30. A root tip cell of clone No. 144 showing a submedian chromosome which was not observed in the basic karyotype (×1,540). Fig. 31. A root tip cell of clone No. 9 showing only two SAT-chromosomes (×1,100). Fig. 32. A root tip cell of *A. longicuspis* Regel (×1,540).

were nearly subterminal chromosomes (Fig. 30), but obviously they were far from being terminal or median chromosomes. Owing to easy distinction of the SAT-chromosomes and of the short submedian chromosomes, they were used as indicators at the karyotype analysis of the examined clones.

Besides the fertile clone, 31 clones showed only the basic karyotype, namely, $K(2n)=10m+2sm_1^{sc}+2sm_2^{sc}+2sm$. Both of the basic and other karyotypes were observed in 13 clones, of which eight clones showed the basic karyotype more frequently than the other karyotypes. The basic karyotype was not observed in 15 of 60 examined clones. In 12 of the 15 clones, only three SAT-chromosomes were observed, instead of four in the basic karyotype. In two of the 15 clones, both two and three SAT-chromosomes were observed. In one of the 15 clones, four short submedian chromosomes instead of two in the basic karyotype were observed in addition to the two pairs of SAT-chromosomes.

In four clones, the basic karyotype was observed fewer times than other karyotypes. In clone No. 9, four of seven examined cells showed only two SAT-chromosomes (Fig. 31). Also in clone No. 50, two of five examined cells showed only two SAT-chromosomes. In clone No. 64, 12 cells of 15 examined cells showed three SAT-chromosomes. In clone No. 182, three of the eight examined cells showed three SAT-chromosomes, and three cells showed only 15 chromosomes which mean 2x–1.

The basic karyotype was observed more frequently in eight clones than the others, and five of the eight clones showed three SAT-chromosomes. Three clones of the eight showed either three or four short submedian chromosomes instead of two in the basic karyotype.

The chromosome constitution of *A. longicuspis* Regel was similar to that of the fertile clone of garlic (Fig. 32). A total of six cells were examined, and all of them showed the same karyotype; ten long median chromosomes, two pairs of SAT-chromosomes, and one pair of short submedian chromosomes. One of the two pairs of SAT-chromosomes were longer than the other, and their satellites were smaller than those of the shorter SAT-chromosomes. Therefore, the karyotype of *A. longicuspis* presented was the same as that of the basic karyotype in garlic. It was shown as follows; $K(2n)=10m+2sm_1^{sc}+2sm_2^{sc}+2sm$.

A. ampeloprasum L. cv. Elephant (great-headed garlic) showed 48 chromosomes including 9 SAT-chromosomes. These 48 chromosomes mean hexaploidy, undoubtedly. Therefore, cv. Elephant is widely different from not only the fertile garlic clone but also from other garlic clones of diploid.

Discussion

About a half of the examined garlic clones showed the basic karyotype, including the fertile clone. The basic karyotype was observed most frequently in the clones from USSR, including the fertile clone. It was observed frequently in Japanese clones, next to the Russian. However, in Japanese clones, many other karyotypes were also observed. As all the Japanese bolting clones showed the multivalent chromosomes in PMC, their chromosomes of the basic karyotype may have been translocated reciprocally. The ten long median chromosomes showed gradual change in length and were quite indistinguishable, so that the modification in those ten chromosomes may not have been noticed, even if they were modified to some extent.

Besides the fertile clone, seven Russian clones showed exclusively the basic karyotype. In these Russian clones, all of the indicator chromosomes mentioned before were clearly distinguished.

Of the seven Russian clones, six clones were collected in Central Asia which is presumed to be original home of garlic, though they are not strictly identified as *A. sativum* L. yet. This basic karyotype was considered to be the most primitive among the various karyotypes observed here. Many more clones of the primitive type may be left in Soviet Central Asia. Of the clones with the basic karyotype, several clones showed non- or incomplete-bolting. It is likely that the modification of bolting habit was not attended with the definite modification of karyotype in garlic.

Mixoploidy is well known among the plants, especially among the vegetatively-propagated plants. Garden chrysanthemum is one of the examples[24], and it is fundamentally hexaploid. Probably mixoploid could exist in these polyploid plants. However, it is possible that the diploid plants propagated vegetatively may sometimes keep various karyotypes as chimera, even if their chromosome numbers remain constant.

A quarter of the examined clones always showed the karyotypes different from the basic. Of the 15 clones without the basic karyotype, nine clones were Japanese garlic, and all of them showed three SAT-chromosomes. At meiosis, their chromosomes mostly formed either $1_{VII}+4_{II}$ or $1_{VI}+5_{II}$, and the clones with $1_{VII}+4_{II}$ showed $2sm_1^{sc}+1sm_2^{sc}$ in their karyotypes, while the clones with $1_{VI}+5_{II}$ showed $1sm_1^{sc}+2sm_2^{sc}$. Furthermore, both $1sm_1^{sc}+2sm_2^{sc}$ and $2sm_1^{sc}+1sm_2^{sc}$ were observed in a few clones with $1_{VI}+5_{II}$ or $1_{VII}+4_{II}$. It is assumed that the octovalent of these Japanese clones contains the sm_2^{sc} chromosome, the hexavalent containing the sm_1^{sc} chromosome. In this case, a small proximal segment and a nucleolar organizing region (NOR) may have been lost through deletion from one of the SAT-chromosomes, because there were three respectively in each cell of those clones. On the assumption mentioned above, some of the reciprocal translocations have possibly occurred between the median and the SAT-chromosomes. Besides this type, some of the reciprocal translocations may have occurred between the sm_1^{sc} and sm_2^{sc} chromosomes, because it was considerably difficult to distinguish them in a lot of cells as in clone No. 54.

Two Japanese clones showed non- or incomplete-bolting and also three SAT-chromosomes. They would form the multivalent even if they produced flower-buds after bolting.

It was revealed that garlic includes various clones with different karyotypes. Not only in this examination but also in some previous papers by other workers, the heterokaryotypes have been reported besides the homokaryotype. The examples of Saini and Kohli[60], Konvicka and Levan[39] and Verma and Mittal[72] clearly show the heterokaryotypes. None of them, except for Konvicka and Levan[39], observed the meiosis of their clones, but at least some of those heterokaryotypes may result from reciprocal translocations as in the present examination. In fact, Konvicka and Levan[39] observed quadrivalent chromosomes. Moreover, Konvicka and Levan[39] reported different karyotypes in the same clone as well as in the present investigation. It is possible to assume that in garlic the basic karyotype is changing to various heterokaryotypes. The formation of multivalent chromosomes at meiosis may support this. The clones including both the basic and the heterokaryotype may be on the way to a modification of karyotype. It is probable that the modification of karyotype is accelerated by the vegetative propagation of garlic.

From the observation of meiosis, it is easily understood that the chromosomes may have been translocated reciprocally, as mentioned above. Besides this reciprocal translocations, deletion may have frequently occurred, because many of the clones with only three SAT-chromosomes showed the submedian chromosomes which were presumably derived from the SAT-chromosomes (Fig. 30). Some of these clones may have lost the small proximal segment from one SAT-chromosome in the basic karyotype. The shapes of those submedian chromosomes were a little different from that of

the short submedian chromosome in the basic karyotype. In other words, the long arm of the former was longer than that of the latter as shown in Fig. 30.

On the occurrence of these long submedian chromosomes, the following hypothesis may be fixed.

The clones with these long submedian chromosomes originally possessed two pairs of SAT-chromosomes as in the basic karyotype. Each of the four SAT-chromosomes had a small proximal segment and a big satellite in the short arm. One small segment was lost with NOR from the SAT-chromosome, and consequently one big satellite turned into one short arm without its secondary constriction. For instance, Kurita[45] also showed a characteristic pair of subterminal chromosomes which might probably be formed by the loss of two small proximal segments of the SAT-chromosomes. The deletion of the small segment may have occurred at or after the inversion of the arm in some cases. As the result, some of the SAT-chromosomes might have turned into the long submedian chromosomes mentioned above. Besides this deletion of the small proximal segment from the SAT-chromosomes, many more deletions may have occurred, though they could not be detected clearly. Accumulation of these deletions may have produced sterility in some of garlic clones, because the microspores in most of the sterile clones degenerated a little after they attained the haploid phase.

This hypothesis mentioned above is still uncertain, but it is possible to assume that each of the haploid gametophytes may hardly contain the entire constitution of a single genome because of the accumulation of deletions by vegetative propagation. In clone No. 182, three cells showed 15 chromosomes instead of 16. This was an extreme example of the heterokaryotypes. In the examined garlic, there was no polyploid clone.

A. longicuspis Regel showed a karyotype similar to that of the basic karyotype of garlic. Obviously it is a closely related species of garlic. *A. longicuspis* and garlic should have at least a common ancestor.

In conclusion, it was revealed that the fertile clone and many other clones have the basic karyotype mentioned before. Besides, some parts of garlic clones were found to have heterokaryotypes which must have been derived from the basic karyotype. Deletion and reciprocal translocations were pointed out as the causes of those heterokaryotypes. The fertile clone might be found among the clones with the basic karyotype as shown in this examination. It was ascertained that *A. longicuspis* is closely related to garlic on account of karyotypical similarity between both species.

Great-headed garlic cv. Elephant is a hexaploid form. Great-headed garlic is frequently confused with common garlic. However, as the basic chromosome set(2x) of 'Elephant' has only three SAT-chromosomes, it is different from that of common garlic which includes four. Therefore, it is clear that the great-headed garlic was not directly derived from the primitive type of the common garlic, except for the case in which it was originated by the hybridization between common garlic and other species.

4. Peroxidase isozymes of the leaves

Some phenotypical and karyotypical characters were compared between the fertile and sterile clones in addition to the meiotic characters mentioned before. Here in this examination, the peroxidase isozymes of the leaves were analyzed in these clones and the related species to compare the zymogram pattern of the fertile garlic clone with those of others. The isozymes were analyzed twice, in 1980 and 1983. The first analysis was reported in 1981[16].

Materials and Methods

The materials used here were 93 garlic clones in the first analysis, and 72 other garlic clones including the fertile clone, *A. longicuspis*, *A. ampeloprasum* cv. Elephant, and *A. scorodoprasum* in the second analysis. The cloves were planted at Kagoshima in autumn of 1979 and 1982. The newly expanding leaves, approximately 10 cm long from the tips, were collected as materials for electrophoresis in February of the next year. Those materials, just after sampling, were stored in the freezer at about −20°C. After weighed, one gram of the frozen leaves were ground in a mortar with one ml of 0.2% $NaNO_3$–0.8% NaCl (1: 1) solution. Paste-like crude extract obtained was kept for about two hours at 5°C in a centrifuge tube, and it was centrifuged at 12,000 rpm for 20 minutes at 5°C. The supernatant, just after centrifugation, was used for horizontal polyacrylamide gel electrophoresis to separate peroxidase isozymes. The thin layer electrophoresis was carried out without a cellophane sheet in the manner described by Ogita *et al.*[54], except for a continuous buffer system. Gel buffer contained 0.2 g of NaOH and 1.8 g of boric acid in one liter of water (pH 8.52), and electrode buffer contained 3.4 g of NaOH and 18.5 g of boric acid in one liter of water (pH 8.60). Catalyst-monomer solution was as follows: A (9.5 g acrylamide, 0.5 g BIS, 100 ml gel buffer), B (1 ml TEMED, 100 ml water), C (120 mg ammonium persulfate, 100 ml water). Three kinds of the solution, that is A, B and C, were mixed in the ratio 2: 1: 1 in volume.

The thin layer gel was 14 cm long, 5 cm wide, and 0.8 mm thick, and had six specimen slots. Into each slot, 1.3 micro liter of the supernatant mentioned above was poured. Both ends of the gel plate were connected with the filter paper to the gel buffer solution.

Electrophoresis was carried out by a constant current of 0.5 mA per cm for three hours and forty minutes at 5°C. To make a staining solution, 0.3 g of 4-chloro-1-naphthol was stirred for thirty minutes in 150 ml of the buffer solution (pH 4.0) which contained 2.4 g of sodium acetate and 4.5 ml of acetic acid. To the naphthol solution, 1.5 ml of 3% solution of hydrogen peroxide was added. After electrophoresis, the plate was dipped and stained in the staining solution, that is, the naphthol solution containing hydrogen peroxide. The isozyme bands of peroxidase were observed after the staining executed for half an hour.

Results

A) The first analysis

The peroxidase isozyme bands in the garlic leaves analyzed in 1981[16] are diagrammatically represented in Fig. 33. The zymograms were classified according to the bands and the light-stained zones in both sides of P2-band. The examined clones and their zymogram types are shown in Table 16.

Among the clones examined in this experiment, the clones included in the zymogram types (A–J) having no light-stained zone between origin and P2-band were much fewer than those in the types (K–P) having the light-stained zone. Furthermore, P6- or P7-band, or both, were absent only in some of the types (A–J) as compared with the types (K–P). As far as the present experiment concerned, most of the clones were included in one of the three types of O, K and P, all of which contained, without exception, P3, 5, 6, 7-bands, lacking P1-band. Zymogram type (O) including the largest number of clones showed also P2- and P4-bands, but the other two types showed either P2- or P4-band.

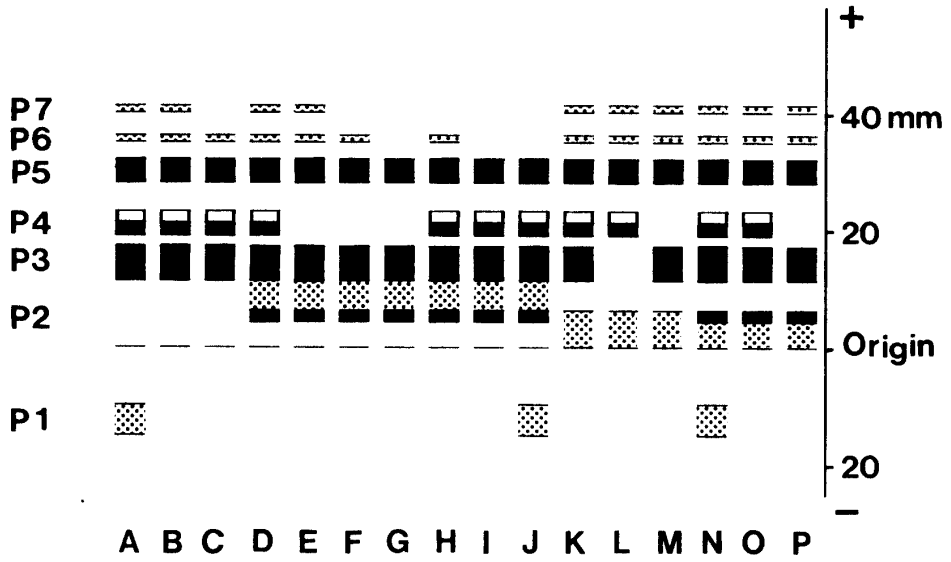


Fig. 33. Diagrammatic zymograms of the peroxidase isozymes in the leaves of garlic clones shown in Table 16[16].

Table 16. The garlic clones examined in the first analysis and their zymogram types[16]

Zymogram type	Clone Number
A	34, 56, 57
B	7, 38, 55, 61, 89
C	70
D	6
E	46
F	44, 53, 67, 68, 75
G	74
H	54, 90
I	47, 80
J	58
K	5, 9, 22, 25, 42, 43, 50, 51, 64, 71, 73, 94, 97, 100, 103, 104, 105, 108
L	106
M	1, 11, 62, 99, 102
N	63, 85, 95
O	2–4, 10, 13, 14, 18–21, 23, 24, 26, 30, 31, 35, 36, 40, 65, 69, 72, 81–84, 86, 93, 96, 98, 101
P	8, 12, 15–17, 27, 32, 37, 41, 45, 60, 66, 87, 88

B) The second analysis

The peroxidase isozyme bands in the leaves of garlic clones and the three related species analyzed in 1983 are diagrammatically represented in Fig. 34. A total of eight bands were observed. Of these, seven (P1–P7) were the same bands as in the first analysis. Besides these seven, P8-band was observed between P2- and P3-bands in one clone, which was the fertile clone in question. The zymograms observed in the second analysis were classified into 14 types (a–n), as shown in Fig. 34. Of these types, two (m, n) were observed only in the related species, not in garlic.

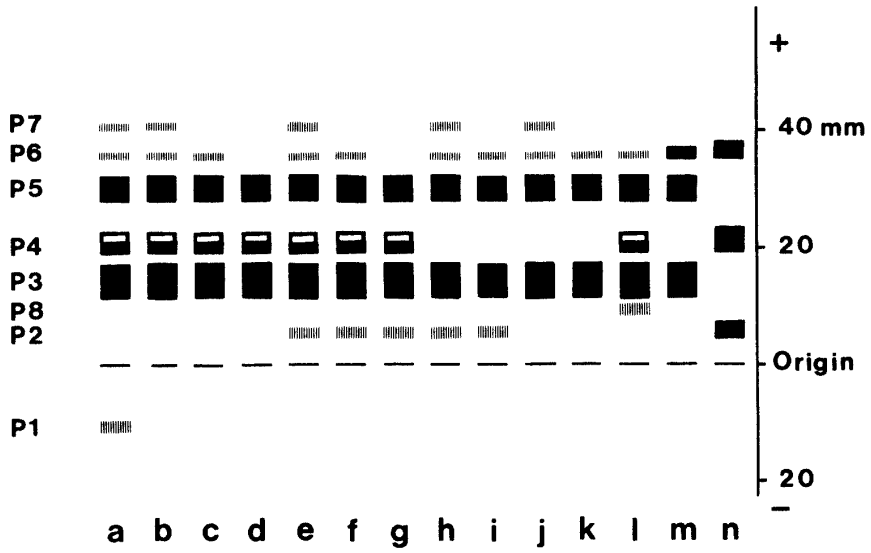


Fig. 34. Diagrammatic zymograms of the peroxidase isozymes in the leaves of garlic clones shown in Table 17, including the fertile clone (1), and *Allium longicuspis* (e), great-headed garlic of *A. ampeloprasum* (m), and *A. scorodoprasum* (n).

P1-band was observed only in one clone. P2-band was seen in six zymograms, one of which was seen only in *A. scorodoprasum*. This band was stained dark in the zymogram (n) of *A. scorodoprasum* and it was stained faint in other zymograms. Neither *A. ampeloprasum* nor the fertile garlic clone showed P2-band, but *A. longicuspis* as well as many garlic clones showed it. P3-band was shown in all the garlic clones and two related species, *A. longicuspis* and *A. ampeloprasum*, but it was not detected in *A. scorodoprasum*. P4-band showed a colorless zone in its anodic side as in the first analysis, and it was seen also both in the fertile garlic clone and in

Table 17. The garlic clones and the related species examined in the second analysis and their zymogram types

Zymogram type	Species or clone No. of garlic
a	158
b	77, 132, 139, 141, 143, 147, 149–151, 159
c	131, 138
d	133
e	76, 109–115, 117, 119–122, 124, 125–128, 142, 145, 146, 161, 162, 165, 174, *A. longicuspis*
f	116, 118, 123, 129, 134, 135, 160, 164
g	79, 107, 137, 175
h	144, 166, 167, 170
i	154–157, 163, 169, 171
j	28, 78, 140, 152, 153, 172, 173
k	136
l	130
m	*A. ampeloprasum* (great-headed garlic)
n	*A. scorodoprasum*

A. longicuspis. In *A. scorodoprasum*, P4-band was stained dark without the colorless zone. P5-band as well as P3-band was observed in all the garlic clones, *A. longicuspis*, and *A. ampeloprasum*, but it was not seen in *A. scorodoprasum*. P6-band was seen in garlic including the fertile clone and the three related species, but it was not detected in several garlic clones. Moreover, it was stained dark both in *A. ampeloprasum* and in *A. scorodoprasum*. P7-band was never observed without P6-band. Both P6- and P7-bands were always faint in garlic clones and *A. longicuspis*. Table 17 shows the examined garlic clones and their zymogram types. It also shows the zymogram types of the three related species.

As mentioned first, P8-band was detected only in the fertile garlic clone, and consequently this clone showed a characteristic zymogram type among those of garlic clones, though it lacked two bands (P2, P7) which were observed in many garlic clones.

Neither the zymogram (n) of *A. scorodoprasum* nor (m) of *A. ampeloprasum* was seen in any garlic clone. However, *A. ampeloprasum* and garlic had two major bands (P3, P5) in common, while *A. scorodoprasum* had neither. On the other hand, *A. longicuspis* showed a typical zymogram of garlic. The zymogram type (e) included *A. longicuspis* and the most numerous garlic clones. The zymogram (b) included many garlic clones, next to the zymogram (e). Three zymogram types (a, d, k) included only one garlic clone, respectively. All of these three clones came from the tropical areas.

Discussion

In the first analysis, the zymograms detected were classified into 16 types (A–P). Some of them were classified according to the light stained areas, not particular bands, on both sides of P2-bands as mentioned above. However, those light stained areas were not observed in the second analysis. This difference of stain might have resulted from the different meteorological conditions between the growing periods of the materials, though they were collected at the same season in both years. In the zymograms (K, L, M) of the first analysis, P2-band was hardly observed, and instead of it the light-stained area was noticed at the same location. Therefore, it is proper to think that those three zymograms should have had a trace of P2-band. Consequently, the zymogram (e) corresponds to (D, K, O), and the zymogram (h) corresponds to (E, M, P). Therefore, for convenience in the present discussion, the zymograms (D, K, O, e) and (E, M, P, h) were classified here as one group respectively.

The zymogram (1), namely, P8-band was observed only in the fertile garlic clone. It is uncertain whether P8-band is directly connected with the fertility of garlic, or whether other fertile clones show this band or not. However, this band may be the most useful to find other fertile clones.

Among the three related species, *A. scorodoprasum* showed a zymogram considerably different from that of garlic, and in the zymogram each of the three bands was stained dark and wide. This species produces bulbils in the inflorescence as well as garlic, while *A. ampeloprasum* does not. According to Vvedensky[74], *A. scorodoprasum* is closely related with garlic next to *A. longicuspis*. However, it is probable that *A. scorodoprasum* is not so closely related with garilc as *A. ampeloprasum*.

On the other hand, *A. ampeloprasum* and garlic showed two major bands (P3, P5) in common. Therefore, from the result of an isozyme analysis, *A. ampeloprasum* is considerably related with garlic. Both of the morphological comparison and the isozyme analysis tell us that the fertile garlic clone does not belong to these two related species obviously.

A. longicuspis showed the zymogram (e) which was the most typical zymogram of garlic. In

other words, the zymograms (D, K, O, e) contained the most numerous garlic clones among the detected zymograms. Therefore, it was difficult to classify *A. longicuspis* and garlic clones by means of peroxidase isozyme analysis. Probably *A. longicuspis* is so closely related with garlic, or both species have a common ancestor. However, the zymogram of the fertile garlic clone was somewhat different from that of *A. longicuspis*. It is an interesting problem whether a fertile clone of *A. longicuspis* may show P8-band or not.

The zymogram (N) showed seven bands (P1–7), and all the three clones with (N) bolted completely, developing flower-buds. The zymograms (D, K, O, e) included six bands (P2–7) and they were observed in 74 garlic clones, of which 50 clones developed flowers up to meiosis. The zymograms (E, M, P, h) lacked P4 of the P2–7 bands and were observed in 24 clones, of which 16 clones developed flowers and PMCs. The zymograms (F, i) lacked P4 and P7 of the P2–7 bands, and all the clones with these zymograms could neither bolt completely nor develop PMCs. Moreover, the zymograms (G, d, k) showed the fewest bands, only three, though their bands were different from each other. All the clones with these zymograms neither bolted completely nor developed PMCs. It is likely that the decreasing of isozyme bands attends the loss of ability to bolt or to develop flower-buds and PMCs up to meiosis.

The zymograms (A, B, a, b) lacked only P2 of the P2–7 bands. Excepting one, all the clones with these zymograms bolted incompletely or did not at all. Most of those clones hardly developed PMCs. Besides (A, B, a, b), the zymograms (C, c, d, j, k) also lacked P2-band, and the clones with these zymograms have never developed PMCs up to meiosis. It is probable that garlic may be losing P2-band with its intraspecific differentiation towards complete sterility. Besides P2-band, garlic may be losing other bands, too. Of 45 garlic clones without P4-band, 30 clones bolted incompletely or did not at all. The P6-band was not detected in nine clones, eight of which could not develop PMCs up to meiosis. The zymograms (H, f) lacked only P7-band except for P1, and nine of the ten clones with those zymograms scarcely developed the scapes and PMCs respectively. Consequently, bigger loss of peroxidase isozyme is presumably attended by deeper sterility, though there are some exceptions. For example, the zymogram (L) lacked P3, one of the two major bands in garlic. However, clone No. 106 with (L) bolted completely and developed PMCs, showing only $1_{VIII}+4_{II}$ exclusively.

Zymogram (1) of the fertile clone showed neither P2- nor P7-band in comparison with the zymograms (D, K, O, e) which include P2–7 bands and the most numerous clones of complete-bolting. However, instead of P2, the fertile clone has P8-band. Possibly P2-band may be related with P8 because of the shorter distance between the both bands.

Neither the habitats nor the chromosome constitutions of the respective clones were tightly connected with the zymogram patterns. Frequently southern and northern clones showed the same zymogram and the bolting habits similar to each other. For example, the zymograms (I, g) were shown in the clones from Hong Kong, a southern island of Japan, Argentina, northern Japan, Peru, and USSR. Of these, two clones have ever bolted and differentiated flower-buds.

In conclusion, this isozyme analysis revealed the followings. Neither *A. scorodoprasum* nor *A. ampeloprasum* (great-headed garlic) showed any zymogram of garlic including the fertile clone. However, the zymogram of *A. ampeloprasum* was more closely related with those of garlic than that of *A. scorodoprasum*. On the other hand, *A. longicuspis* showed one of the typical zymograms of garlic. Karyotype analysis, isozyme analysis, and success in crossbreeding between *A. longicuspis* and garlic suggest that both of the species are closely related with each other or that both have a common ancestor. The fertile garlic clone showed a zymogram band which has never been noticed

in other sterile clones. In general, decreasing of zymogram bands may increase the degree of sterility concerning bolting habit or ability to differentiate flolwer-buds in garlic.

V. General discussion

Sterility of garlic has hitherto been studied by several workers.

Weber[76] observed the early degeneration of EMCs in two varieties. The author's observation coincides with Weber's, though only one clone was used.

Takenaka[65] who observed the meiotic irregularities assumed that garlic was a species of hybrid origin, but his assumption may conflict with the reports that garlic contains also the clones showing normal meiosis. He also assumed that garlic had been changing its chromosomal structure since its establishment, and this assumption is consistent with the results obtained here.

Krivenko[43] who observed normal meiosis of garlic stated "The climatic conditions can noticeably displace or hinder the development of either of the sexual elements". However, the climatic condition may not presumably be the principal cause of sterility, because both of fertile and sterile clones were observed simultaneously here in this investigation. Krivenko[43] also observed the early degeneration of EMCs. It is likely that most of the garlic clones are unable to develop not only male gametophytes but also female gametophytes.

Gvaladze[22] who observed the development of aposporic embryo sacs from the chalazal tissue in some Russian clones stated that even such garlic forms where female gametogenesis proceeded normally remained as apomicts in reality. Malformed flower-buds were also observed in the author's investigation. These abnormal developments of the floral organs in garlic may be taken as the products of its vegetative propagation performed since ancient times.

Koul and Gohil[41] who also observed normal meiosis in garlic attributed the sterility of garlic to the nutritional competition between the flower-buds and bulbils during their developments. However, Shimada and Shozaki[61] could not obtain fertile pollen grains in the sterile garlic by the removal of bulbils, though it developed flower-buds considerably. The fact that the fertile pollen grains were produced even in the flowers without the removal of bulbils[12] does not prove the nutritional competition as the cause of pollen sterility. Moreover, the existence of the clone such as reported by Katarzhin and Katarzhin[30] who could obtain viable seeds without the removal of bulbils strongly proves that the nutritional competition is not the principal cause of sterility in garlic.

Konvicka *et al.*[40] succeeded in obtaining the fertile pollen grains in the clones showing normal meiosis by means of antibiotic treatment. In some garlic clones, the pollen development was possibly disturbed by infection of virus or rickettsialike organisms as they stated. The author also has ever seen sterile flowers probably infected with the aster yellows virus in a garlic clone and in some plants of *A. longicuspis*. These flowers had characteristically elongated pedicels and distorted floral parts as in the onion flowers shown by Jones and Mann[28]. However, those sterile flowers infected by aster yellows virus are rarely observed among the garlic plants in the field. Moreover, the antibiotic treatment by Novak and Havranek[53] failed to produce the fertile pollen grains in the clones showing normal meiosis, and the author's attempt[10] failed to obtain fertile pollen in the clone showing irregular meiosis. Konvicka *et al.*[40] presumed that the rickettsialike organisms caused certain nutritional disturbances from the tapetum layer to microspores. However, it seems rather difficult to clear up the problems of non- or incomplete-bolting and early degeneration of EMCs by their presumption. Therefore, the sterility caused by the infection with microorganisms may not

probably be common to all the garlic clones, though all the garlic clones might be infected with some kinds of microorganisms.

Novak[52] concluded that the hypertrophy of the tapetal cells caused sterility in both of garlic and *A. longicuspis*. However, the author's observation[11] revealed the existence of a garlic clone without showing such a hypertrophy in the tapetal development. Furthermore, the degeneration

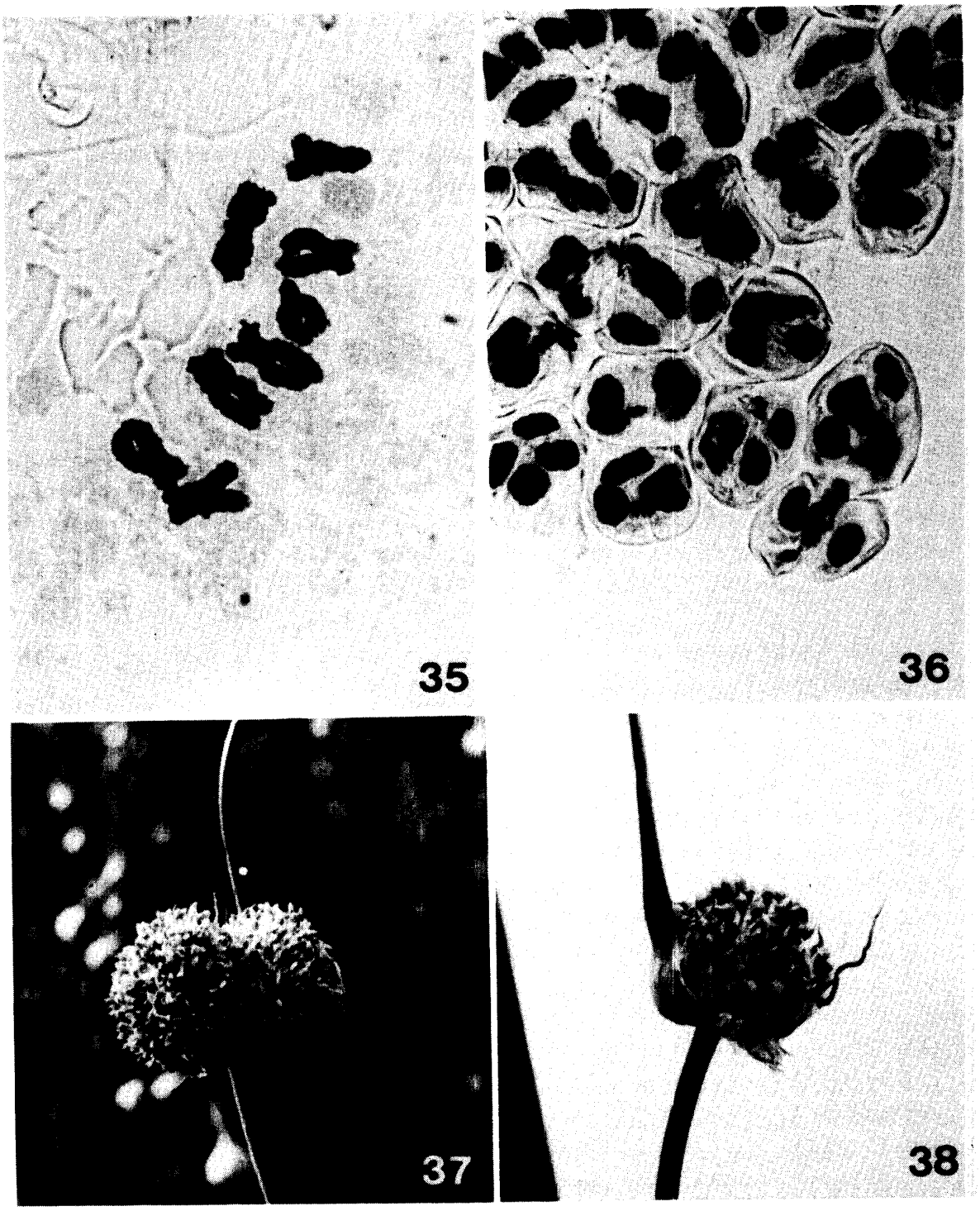


Fig. 35. Metaphase-I of PMC in *A. longicuspis* Regel, showing normal eight bivalents (×1,100). Fig. 36. Degeneration of the pollen-tetrads in *A. longicuspis* Regel (×440). Fig. 37. An inflorescence with two hemispheres of *A. longicuspis* Regel. Fig. 38. An inflorescence of the fertile garlic clone, No. 130.

of EMCs observed in several clones makes us imagine more general or rather fundamental causes of sterility. Besides, according to the present author's preliminary observation, the microspores degenerated at the tetrad stage in *A. longicuspis*, after showing normal meiosis (Figs. 35, 36). On the other hand, the microspores or the uninucleate pollen grains degenerated after being released from the tetrads in most of garlic clones showing irregular meiosis. Consequently, it is more likely that at least the cause of sterility of most of the Asian garlic clones differs from that of *A. longicuspis*.

The irregular meiosis reported by Takenaka[63] was ascertained by Katayama[32], Gohil and Koul[20], Konvicka and Levan[39], Etoh and Ogura[15], and Etoh[9,12]. Therefore, it was confirmed that garlic had a large number of clones showing irregular meiosis, and that even an incomplete-bolting clone which usually failed to develop flower-buds showed irregular meiosis. Besides, the heterozygous karyotypes of garlic were reported in many previous works. Probably most of those reported clones were of the non- or incomplete-bolting types as mentioned here. For example, both UH clone of Konvicka and Levan[39] and A type clone of Verma and Mittal[72] have non- or incomplete-bolting habit and show heterozygous karyotypes. In the present investigation, at least five non- or incomplete-bolting clones showed heterozygous karyotypes. These clones would produce only sterile flowers even if they bolted completely. For the discussion concerning the causes of sterility, it is necessary to pay attention to these non- or incomplete-bolting clones.

In conclusion, the sterility of garlic possibly lies on the piled causes. The most fundamental cause of the sterility may probably be the structural heterozygosity observed in a large number of clones. This is supported by the fact that the fertile clone observed here showed normal meiosis. The structural heterozygosity must have been caused by reciprocal translocations or deletions as mentioned before. It is obvious that these chromosomal changes have been accumulated by the vegetative propagation. The desynapsis in garlic may be a product of these chromosomal changes, though its cause is uncertain. Several minor causes of sterility mentioned before may have been piled up on to the fundamental one. In addition to these, both the disturbance of seed formation by the bulbils and the trouble in seed germination may increase the difficulty for us to find fertility in garlic[13].

Thus, it is likely that the sterility in garlic lies on the piled causes. Such a pile as this could be interpreted as an accumulation through the species evolution. Usually garlic is referred as an obligate apomict[18]. According to Khokhlov[35], the evolutionary significance of apomixis in plants is to be evaluated from various view points. For instance, some workers consider apomixis as an abnormal chance deviation from sexual reproduction, while others consider it as a sort of legitimate and progressive phenomenon. However, nobody doubts that the change from sexual reproduction to apomixis is irreversible[7]. One of the reasons why it is irreversible may be that the barriers to fertility-restoration are piled throughout the processes of sexual reproduction once a plant species has taken a change in an apomictic direction as in the case of garlic. As was cleared in the present investigation, garlic contains both fertile and sterile clones. Furthermore, garlic is composed of complete-bolting, incomplete-bolting, and non-bolting clones. These can easily be realized as the products of intraspecific evolution, and its direction is obvious; from fertility to sterility, from complete-bolting to non-bolting through incomplete-bolting.

Stebbins[62] who investigated the relationship between breeding systems and habitats of various species in Compositae indicated that many perennial and cross-fertilizing species were found in the "stable habitats", while many annual and self-fertilizing species with greater reproductive efficiency were found in the "unstable habitats". Garlic is not a cross-fertilizing species, but it is perennial. Domestication followed by cultivation presumably gave garlic the stable habitat, and garlic may

have lost the ability for sexual reproduction, increasing perenniality or apomixis in consequence. According to Pernes[56], "When selective pressures are missing, apomixis rules out sexuality". This may support the hypothesis that garlic is evolving from sexual to asexual reproduction, and the fact that in garlic almost all the clones are sterile may support his suggestion.

Fryxell[18] reviewed the breeding system in many plant species, and he made the "reproductive triangle", showing garlic as a typical example of apomictic species. However, the variation in the breeding system within a species has scarcely been reported. The variation within a speices, *Oryza perennis* Moench, was surveyed by Oka and Morishima[55]. This species includes annual, perennial, and rhizomatous types. Several characters of each type were measured, but their variations were hardly discussed from the viewpoint of intraspecific evolution.

In the genus *Allium*, variation on the breeding system is frequently observed. *A. fistulosum* L. includes the non-bolting varieties such as 'Bozu-shirazu' and the bolting varieties with a high tillering capacity such as 'Shiki-negi', besides the common type.

In the case of garlic, the fertile clone treated here seems to present us with certain informations on the intraspecific evolution. As mentioned above, the fertile clone is regarded as the most primitive type among the examined clones because of its several characters. From these characters of this fertile clone, the intraspecific evolution of garlic could be traced.

Originally, garlic may have been fertile and have developed numerous flowers and bulbils on the long scape, showing normal meiosis. Its long scape is presumable from the fact that *A. longicuspis* also produces a long scape. *A. longicuspis* and garlic may have a common ancestor because of their similarity in karyotypes and zymogram patterns of peroxidase isozymes. However, today's *A. longicuspis* seems to bear too many flower-buds to produce fertile pollen grains or seeds, because it frequently forms two hemispheres in an inflorescence for numerous bulbils and flowers as shown in Fig. 37. The fertile clone found here has never formed such an inflorescence with two hemispheres (Fig. 38). The primitive garlic may have shown the basic karyotype mentioned before, cold hardiness, heat tolerance, late maturity, and differentiation of many leaves. It is uncertain whether garlic obtained sterility before or after domestication, but mutations including chromosomal ones which resulted in sterility might have been accumulated gradually during its long vegetative-propagation period. After the occurrence of sterility, garlic may have been evolving towards shorter scapes and decreasing the flower-buds and bulbils in number. When garlic lost the ability to differentiate flower-buds, the incomplete-bolting began, showing very short scapes with a few bulbils. Finally garlic lost the ability to bolt and became non-bolting. On the process of its evolution, the karyotype has also changed from the basic to the modified ones, mainly by reciprocal translocations and deletions in certain chromosomes. The peroxidase isozymes may be decreasing in number. The evolutionary changes might have given the reduction of its genotype and the restriction of its phenotype. Malformation of flower-buds may have been induced as an evolutionary product after the occurrence of sterility. Hypertrophy in the tapetum reported by Novak[52] and infection with microorganisms reported by Konvicka *et al.*[40] may have been acquired in some garlic clones through the evolutionary processes, giving them sterility in effect. In the evolution of garlic, domestication followed by cultivation would have probably accelerated its sterility. One of the reasons for this may be that the garlic bulbs have been usually harvested far before flowering in ordinary culture.

As mentioned above, garlic may be evolving from sexual to asexual reproduction, but some fertile clones producing functional female organs must still survive besides this fertile clone treated here. In garlic, the evolution on the breeding system could be also realized as an adaptation to

various habitats. Some more fertile clones may survive in the primary center of origin, namely, Soviet Central Asia. These fertile clones may be proper materials for the sterile clones to restore fertility by means of somatic cell, or by the usual hybridization between them.

VI. Summary

Garlic, *Allium sativum* L., is sterile and the cause of its sterility is still indistinct. In order to clarify the causal aspects of its sterility, various clones were collected and examined. One fertile clone was found among the collected ones. Therefore, this fertile clone was compared with the other sterile ones. The results were summarized as follows.

1. Garlic showed a wide variation in the bolting habit among the clones. Complete-, incomplete-, and non-bolting were seen in 71, 57, and 26 clones respectively.

2. Both non- and incomplete-bolting habits were presumed to be continuous among the clones. The original habitats of these non- and incomplete-bolting clones examined were the tropics, the colder temperate zone, and Europe.

3. Most of the incomplete-bolting clones produced no flower-buds, developing only bulbils in their inflorescences. Moreover, some parts of the plants in the complete-bolting clones examined differentiated no flower-buds.

4. At the early stage of the flower-bud formation, anatomical abnormality was not noticed, but the embryo-sac mother cells degenerated after the first or the second division. In some ovules, nucellar cells developed abnormally without full development of macrogametophytes.

5. Irregular meiosis of pollen mother cells was observed in various garlic clones; $1_{\mathrm{X}}+3_{\mathrm{II}}$ in one clone, $1_{\mathrm{VIII}}+4_{\mathrm{II}}$ in 37 clones, $1_{\mathrm{VI}}+5_{\mathrm{II}}$ in 34 clones, and desynapsis or asynapsis in two clones. Another clone produced malformed pollen grains presumably caused by desynapsis or asynapsis. The regular meiosis of eight bivalents was observed in two clones, and one of them produced fertile pollen grains and viable seeds after self-pollination.

6. By means of cold storage of mother bulbs, one of the incomplete-bolting clones which failed to differentiate flower-buds every year formed flower-buds, showing $1_{\mathrm{VI}}+5_{\mathrm{II}}$ at meiosis.

7. *A. longicuspis* Regel which is sterile and presumed to be the wild ancestor of garlic was cross-pollinated with the fertile garlic clone, and it produced viable seeds.

8. Of the examined clones, the fertile garlic clone showed later maturity, stronger cold-hardiness and heat-tolerance.

9. The fertile clone was also compared with other sterile clones concerning some morphological characters such as number of cloves per bulb, leaf width, number of flower-buds per inflorescence, or length of scape. In garlic clones, the number of bulbils per inflorescence was positively correlated with the number of flower-buds per inflorescence. From the results on the morphological characters, it was presumed that non- or incomplete-bolting clones were of the evolved forms of the complete-bolting ones.

10. The fertile clone showed the karyotype as follows. $K(2n)=10m+2sm_1^{sc}+2sm_2^{sc}+2sm$. This karyotype was observed in a half of the examined garlic clones and *A. longicuspis*. A quarter of the clones showed other karyotypes, which were presumed to have been modified from this karyotype.

11. The fertile clone showed a particular band of peroxidase isozymes. Most of the complete-bolting clones and *A. longicuspis* showed six bands of peroxidase isozymes. Incomplete- or non-bolting clones frequently lacked some of the isozyme bands.

Basing on these results, the intraspecific evolution of garlic concerning the breeding system was discussed.

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