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Instructions for use

On Karyo-ecotypes of *Fritillaria camschatcensis* (L.) KER-GAWLER

By

HAJIME MATSUURA

(With Plates XIII and XIV and 10 Text-figures)

Thanks to a series of works on the genetic and ecologic analysis of wild plant populations carried out since 1921 by TURESSON (*cf.* '30), we are now convinced that taxonomic species (*linneons*) comprise smaller units, each ecologically adapted to environment and distinguishable by genetical considerations (*ecotypes*). His works, together with those by others (*e.g.* GREGOR, '31), are making valuable contributions to a newly established branch of biology, 'genecology' (TURESSON, '23).

From the inspection of the nature of ecotypes, it will be *a priori* inferred that they are delimited either by genic constitutions, as suggested from works by these authors, *i.e.*, *geno-ecotypes*, or by chromosomal constitutions, *i.e.*, *karyo-ecotypes*. It is then not surprising to see that HAGERUP ('27, '28, '32, '33) actually found several polyploid ecotypes in wild growing species.

The present study on *Fritillaria camschatcensis* (L.) KER-GAWLER presents another clear instance of karyo-ecotypes. The suggestion for this work was obtained from a fact generally known to Japanese taxonomists that the lowland *Fritillaria camschatcensis* growing in the northern parts of Japan, *i.e.*, in Hokkaido, the Kurile Islands and Saghalien, is more vigorous and larger in general appearance and much easier of cultivation than that to be found in alpine zones in Honshu and Hokkaido. So remarkable as the differences between them are, not only in their morphological characters but also in their ecological nature, no attempt has hitherto been made to deal with them from a karyological point of view. Most probably this may be due to a supposition that the small size of the alpine *Fritillaria* is a direct consequence of the influence of the alpine climate.

In 1933 the writer found that material collected in Sapporo (Hokkaido) is all characterized by auto-triploidy. With a suspicion that the alpine dwarf form may then be of a diploid nature, he collected it in early summer of the following year from the alpine zone of Mt. Gassan,

Yamagata Prefecture (Honshu) and found that his expectation was true. A study was also made on several plants of the alpine form collected by Dr. Akiyama from Mt. Taisetu (Hokkaido). They also proved to be of the same diploid nature.

Though the work along this line is now in progress and the present paper is consequently of preliminary nature, the results obtained up to the present have some interest in this field and so will be briefly given here.

The present study was assisted by Dr. T. Haga and Mr. T. Sutô who helped the writer in collecting material at Mt. Gassan, by Dr. S. Akiyama who supplied the writer fresh material from Mt. Taisetu and by Mr. T. Toyofuku and Dr. T. Akemine who prepared Tables I and II in text respectively. To these gentlemen the writer here wishes to express his cordial thanks.

Comparison between the two Ecotypes

i) *Karyological*

The root-tips of both diploid and triploid plants were fixed in La Cour 2BE and stained with gentian-violet after Newton's method. The anthers of diploids were treated in the same manner, but the result was not satisfactory for critical observations on meiotic metaphase chromosomes. Observations on them were then made with aceto-carmine preparations. The meiotic chromosomes of triploids were studied in permanent smear preparations, Taylor's solution having been employed as the fixative and gentian-violet as the stain. On account of such difference in treatment, direct comparison of meiotic chromosomes between the diploid and the triploid, as represented in Text-figs. 5 and 6 respectively, is not available.

The somatic complement of the diploid plant consists of 24 chromosomes. Morphologically they resemble those described in other species of *Fritillaria* (cf. Newton & Darlington '30, Darlington '29, '30). No fragments, as found in *F. imperialis* by Darlington ('31), however, have been observed in the present case. Four chromosomes of the complement have the attachment constriction sub-median (Type I), the others sub-terminal. Of the latter, four types are distinguishable, according to the presence or absence of the secondary constriction and the size of the shorter arms (Types II–IV). These five chromosome types are represented in Text-fig. 1. On this basis of classification, although necessarily rather rough due to the contorted shape of chromosomes, the diploid complement was found to comprsie: $4(I) + 2(II) + 2(III) + 8(IV) + 8(V)$ (Text-figs. 2 and 3).

At meiotic metaphase, they appear as 12 gemini (Text-fig. 5). Ap-

parently there are many chiasmata in each geminus, a condition similar to one described by DARLINGTON (*l. c.*) in *F. imperialis*. The chromosome distribution in the first and the second divisions takes place in regular fashion, resulting in the normal tetrad formation. The pollen grains are nearly all normal ones (Text-fig. 9).
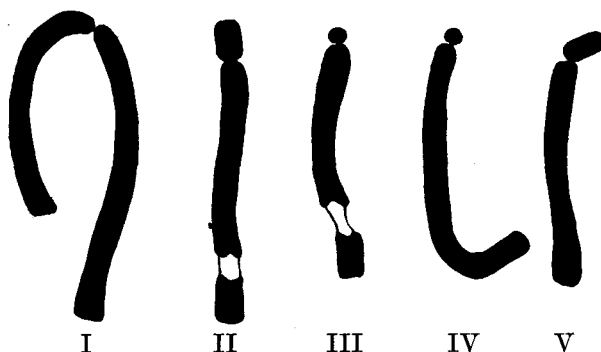

Text-fig. 1.   Five different types of chromosomes distinguishable in the somatic complement of *Fritillaria camschatcensis*.   × 3300.

The triploid plant has 36 chromosomes as the somatic complement. On the same basis of classification as applied to the diploid, the five different types were distinguishable within it. The number of members found in each type was just according to expectation, namely, $6(I) + 3(II) + 3(III) + 12(IV) + 12(V)$ (Text-fig. 4). It will be noted from these figures that the chromosomes of the triploid are distinctly longer—roughly estimated as 50% longer—than those of the diploid. This may be ascribed to differences in climatic conditions at the time of fixation to which the two types are subjected in growth.

At meiotic metaphase, most of them appear as trivalents, with occasional formation of bivalents and corresponding univalents in variable number. At anaphase, a few lagging univalent chromosomes or split halves of them are often met with (Text-fig. 7), which would be excluded from the division spindles and give rise to extra micro-nuclei in the tetrads. Behavior of the chromosomes subsequent to the tetrad formation is rather regular (Text-fig. 8). Most of the pollen-grains are apparently normal and larger than those of the diploid, but some are shrunken (Text-fig. 10). The proportion of normal appearing grains to abortive ones was estimated as 20.7% on the average (Table I).
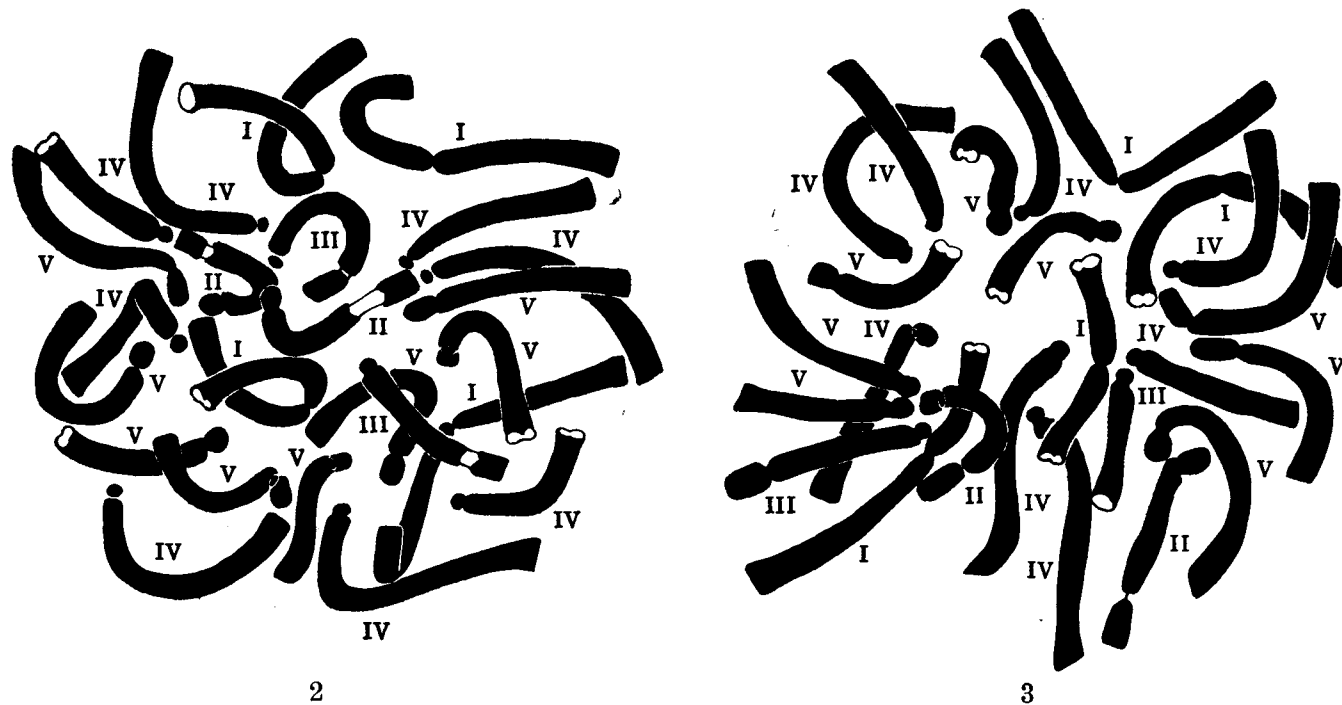
Text-figs. 2 and 3. Somatic mitoses in the root-tips of the diploid *Fritillaria camschatcensis*;
Fig. 2, the material from Mt. Gassan and Fig. 3, the material
from Mt. Taisetu. × 3300.
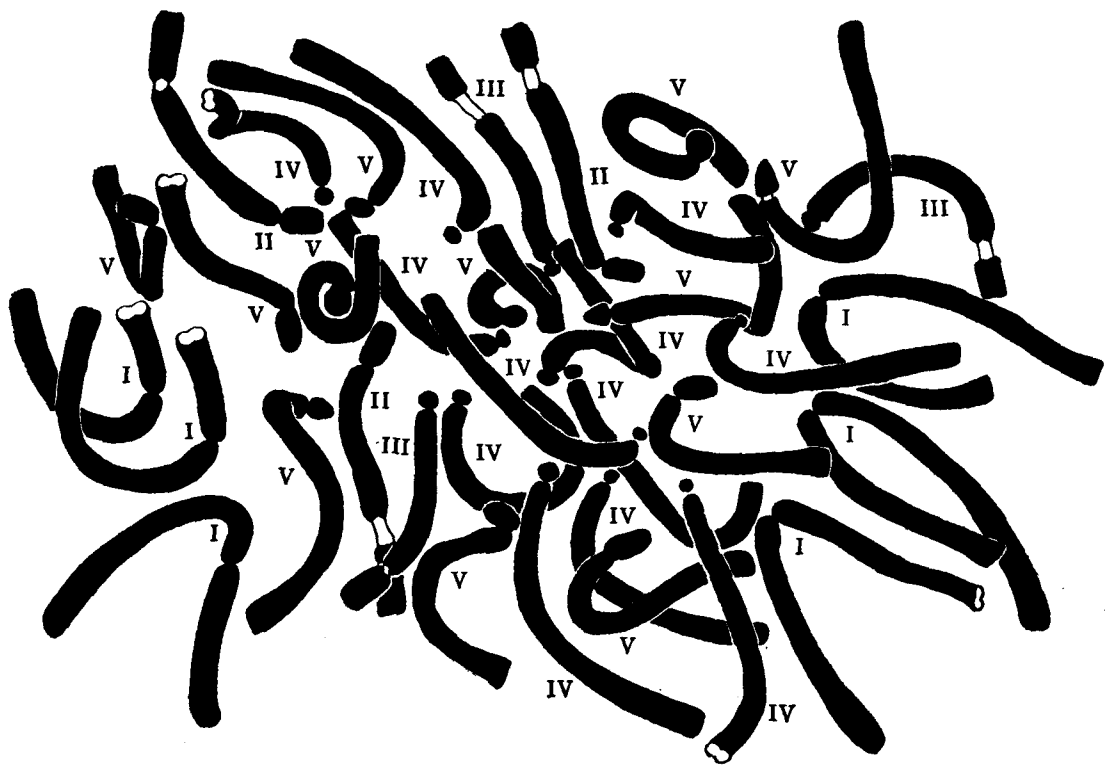
Text-fig. 4.   Mitotic metaphase in the triploid; the material from Sapporo.   The chromosomes have been slightly spaced out in drawing.   Note greater size of the chromosomes, compared with those in the diploid.   × 3300.
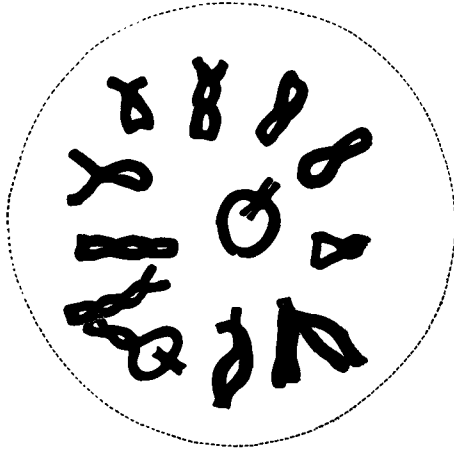
Text-fig. 5.   Meiotic metaphase I of the
pollen mother-cell division in the
diploid.   Aceto-carmine
preparation.   × 1700.
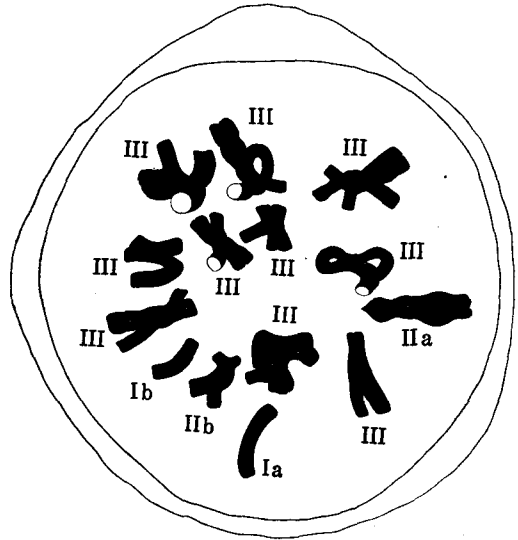
Text-fig. 6.   Meiotic metaphase I of the
pollen mother-cell division in the
triploid, with 10 trivalents, 2 bivalents
and 2 univalents.   Permanent smear
preparation.   × 1700.

TABLE I.

Percentage of normal appearing pollen-grains to abortive ones
in two triploid individuals[1]

Individuals	I	II
Normal grains	948	821
Abortive grains	294	172
Total	1242	993
Percentage of abortion	24.0	17.4
Average	20.7%	

[1]   One of the two individuals under observation was an adult
plant bearing normal hermaphroditic flowers, whereas the other was an
immature plant, its flowers entirely lacking the female organ.
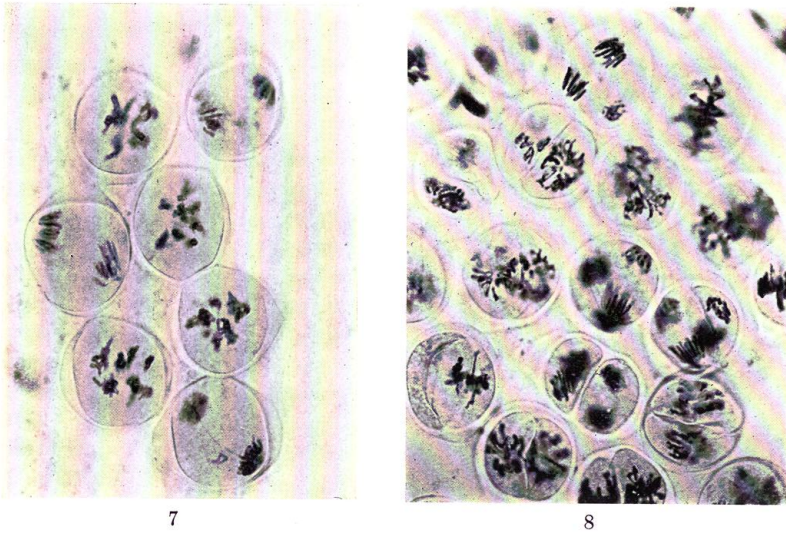
Text-figs. 7 and 8.   Divisions I (Fig. 7) and II (Fig. 8) in pollen mother-cells of the triploid.   Microphotos from permanent smear preparations.   × 330.

ii) *Morphological*

As in other similar cases, the triploid *Fritillaria camschatcensis* is of a gigas type in the general character expression as compared with the diploid.   The stature is distinctly taller in the former, most vigorous plants attaining more than 50 cm. in height, whereas the diploid in its natural conditions does not usually attain even half of this height.   Since the stem length appears very variable according to the age of the plants (*cf.* Plate XIII) and conditions of growth, it was impossible to deal with the matter statistically.   Some of the triploid grow to a lower stature than the diploid.

The most remarkable difference between the two types is in the size and number of leaves.   This is clearly illustrated in Plate XIV, in which the whole leaves of an individual of the diploid form from Mt. Taisetu are compared with those of a triploid plant from Sapporo.   Usually the diploid has two nodes, bearing 3 leaves in each (the upper node sometimes bearing only 2 leaves), whereas the triploid is characterized by more than three nodes and more numerous leaves in each.   In Table II are given the results of some measurements made on both the types (the diploid plants being those from two different localities) under natural environment as to the number, size and thickness of whole whorled leaves on the lowest node. It will be noted from this table that in spite of the greater area of the laminae of the triploid leaf, its thickness remains almost nearly invariable
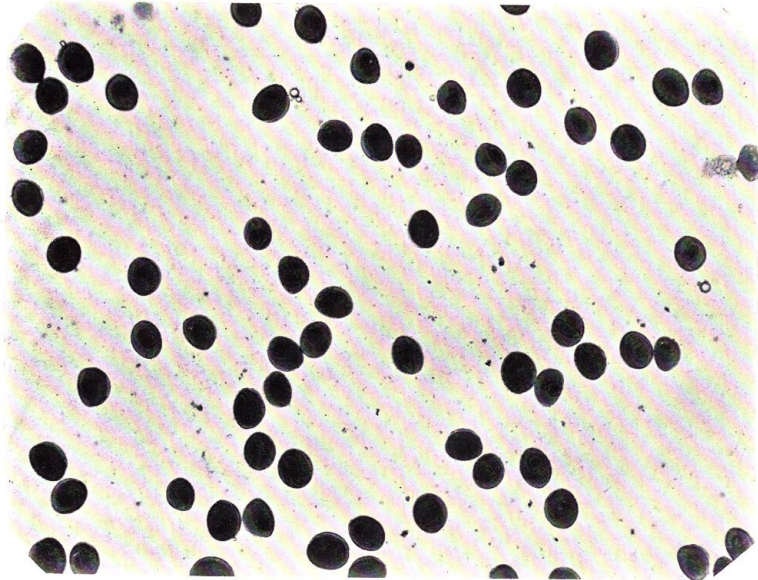
Text-fig. 9. Pollen grains of the diploid. Aceto-carmine. ×90.
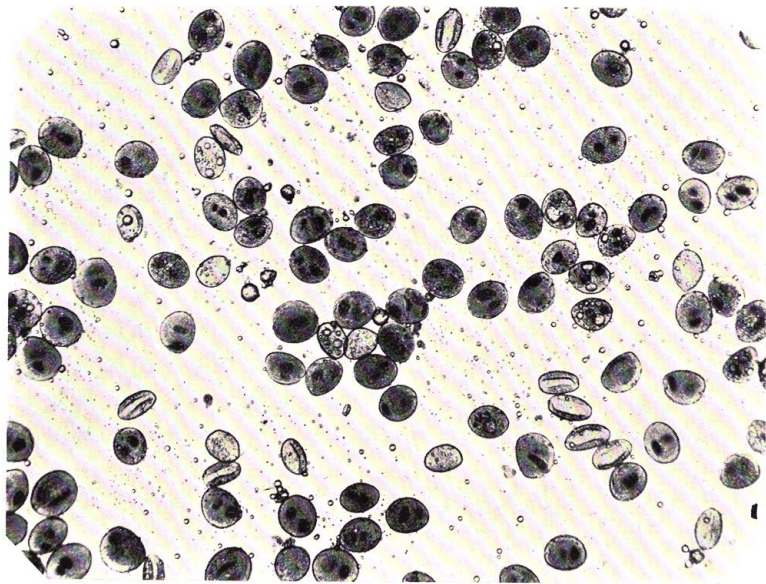
Text-fig. 10. Pollen grains of the triploid. Aceto-carmine. ×90.

TABLE II

Quantitative comparison of leaf and stoma characters between the diploid and the triploid of *Fritillaria camschatcensis*.

i)   Number and size of basal leaves

Material	Number of indi-viduals	Number of basal leaves per plant		Length		Breadth		Breadth-length-ratio		Correlation-coefficient between breadth and length
		Mean	σ	Mean (in cm.)	σ(in cm.)	Mean(in cm.)	σ(in cm.)	Mean	σ	
Triploids (from Sapporo)	22	6.45±0.1967	±1.3047	6.94±0.1103	±0.7772	2.27±0.0339	±0.2258	0.330±0.0042	±0.0294	+0.6931±0.0747
Diploids (from Mt. Gassan)	10	3.20±0.1214	±0.5692	5.44±0.1771	±0.9135	1.85±0.0608	±0.2898	0.344±0.0041	±0.0195	+0.9386±0.0254
Diploids (from Mt. Taisetu)	10	3.00±0	±0	4.50±0.1417	±0.5566	1.70±0.0643	±0.2538	0.379±0.0074	±0.0304	+0.8338±0.0776

ii)   Thickness of basal leaves[1]

Material	Apical		Basal		Middle		Both sides of Middle	
	Mean(in mm.)	σ(in mm.)	Mean(in mm.)	σ(in mm.)	Mean(in mm.)	σ(in mm.)	Mean(in mm.)	σ(in mm.)
Triploids (from Sapporo)	0.36±0.0035	±0.0245	0.70±0.0045	±0.0304	0.50±0.0056	±0.0400	0.35±0.0043	±0.0301
Diploids(from Mt. Gassan)	0.37±0.0051	±0.0237	0.67±0.0166	±0.0777	0.50±0.0106	±0.0508	0.34±0.0034	±0.0224
Diploids (from Mt. Taisetu)	—		—		—		—	

[1]   Measured with the aid of Ames dial micrometer.

iii)   Length and mumber of stomata[2]

Material	Length of stomata		Number of stomata on the under surface of leaves in a circle of the radius of 1.16 mm.	
	Mean (in μ)	σ (in μ)	Mean	σ
Triploids (from Sapporo)	90.08±0.4096	±3.8418	73.06±1.1733	± 6.7358
Diploids (from Mt. Gassan)	80.84±0.4133	±3.8760	144 50±2.2045	±12.6583
Diploids (from Mt. Taisetu)	79.98±0.4328	±4.0599	191.00±4.6255	±17.9145

[2]   The length of stomata was measured in distilled water.

in both the types. The ratio of the breadth to the length of the laminae of the triploid is not so much greater than that in the diploid as found by Stow ('34) in *Convallaria majaris*.

Comparison was also made of the length and number of stomata on the under surface of basal leaves. It will be of some interest in view of cell-constancy or eutely (*cf.* Heilborn, '33) to notice that the number of stomata in the same area is much smaller (less than half) in the triploid than in the diploid.

Increase of the number of flowers per plant is another characteristic feature of the triploid *Fritillaria*. Vigorous triploid plants usually bear 3, in extreme cases, 7 flowers, making a marked contrast to the diploid form which is usually characterized by a single flower, less frequently by two. In the survey at Mt. Gassan, the writer very rarely met with 3-flowered plants, but none has been found with more flowers.

The floral construction does not vary in the two types. It may be noteworthy, *en passant*, that *F. camschatcensis* appears to be 'preandrously' bisexual in development, young slender plants from small bulbs usually lacking the female organ, whereas vigorous ones bear normal hermaphroditic flowers. This peculiar characteristic is common in both the diploid and triploid forms.

The triploid *Fritillaria* is sterile, as far as the present observations go. Though the writer could not find any diploid individual bearing fruits at Mt. Gassan, its fructibility is undoubted, as several botanists have actually met with cases.

iii) *Ecological and Plant-geographical*

The most remarkable difference between the diploid and the triploid *Fritillaria camschatcensis* is that in their ecological nature. While the triploid form restricts its distribution to lowland meadows, the diploid one is of pure alpine nature. At Mt. Gassan, the latter is found at a region about 1,800 *m. sup. m.*, where *Geum pentapetalum* Makino and *Anemone narcissiflora* L. are dominating, together with other alpine plants, such as *Sanguisorba albiflora* Makino, *Aletris foliata* Franch., *Maianthemum bifolium* DC., *Pedicularis apodochila* Maxim., *P. yezoensis* Maxim., *Shortia soldanelloides* Makino f. *typica* Makino, etc. At Mt. Taisetu, the diploid form is also found at a region of nearly the same altitude, and the same is probably true of other places where the dwarf type of this plant is known to occur. In this connection it must be mentioned that there are found no intermediate forms as would be expected

in regions of intermediate altitudes. They are either of the alpine or of the lowland type, no connecting forms existing between them. It is then quite clear that the triploid form has completely changed the original habit of the initial form and chosen quite different external conditions under which to live. One may be surprised to find the gigas form of *Fritillaria* growing even on the sea-shore in Hokkaido (*e.g.* at Daikoku-jima near Muroran).

It should be added however that no lowland plants of *Fritillaria* have been found in Honshu, where the alpine form is distributed in small areas of its northern middle part; their localities are restricted to regions of higher latitude, beginning from Hokkaido. Thus the southern limit of the triploid form lies more to the north than does that of the diploid form. This fact seems to favor a view that the present triploid form may be one derived from diploid plants of northern localities, such as those found in Mt. Taisetu. In this connection, some minor morphological differences observed between the diploid form from Mt. Gassan and that from Mt. Taisetu are of some significance; namely, (i) in the former the inside of the corollas is characterized by marked yellow patches at the margin, whereas the latter lacks them, (ii) in the former the anther-filament is colorless, while in the latter it is dark purple as in the corolla, and (iii) the bulb-scales of the former are usually thin but numerous, while those of the latter are more fleshy and less in number. These characteristics of the diploid plant from Mt. Taisetu correspond well with those of the present triploid form.

Discussion

As described on previous pages, the lowland form of *F. camschatcensis* is characterized by auto-triploidy, a condition directly derived from the alpine diploid form. It seems most probable that this triploid form has resulted from the union of the normal gamete with the unreduced diploid one which probably originated from certain irregularities in environmental factors under which the diploid was subjected to grow, *e.g.* unusual temperature conditions at the time of meiosis, as previously substantiated by experimental investigations in several instances. It is then naturally inferred that the triploid form has entered into a new territory on account of (i) change in its biological properties (*cf.* NAVASHIN, '29) and (ii) the capacity of asexual reproduction (by means of bulbs).

How the present status of the diploid and the triploid plant of this species has been brought about is a problem of rather speculative nature,

but will be significant to the considerations of evolution of plants in general. Two possibilities may be considered for the processes of evolution within the species involved. One of them is the view of polygenesis, that is, an assumption that the triploid was and is being thrown off from time to time by the diploid. This hypothesis demands however the actual occurrence of the triploid in *regio alpina* as the consequence and also at regions of intermediate altitudes as the inevitable route of its invasion into a new area, but these appear not to be the case. Furtheremore it seems to be untenable for the reason that there is no *a priori* reasoning why the diploid in Honshu, which is at the climatic frontier, does not throw off the triploid, in spite of the fact that the latter is known to be very easily cultivated there.

The other alternate possibility is the view of monogenesis and may be stated as follows: (i) in the Diluvial epoch the diploid *Fritillaria* was growing on lowland meadows, as the present triploid does, on account of the similarity of the atmospheric temperature at that time with that of *regia alpina* at the present; (ii) on the occasions of profound changes in climate (such as intergracial stages) the triploid happened to appear probably at the climatic frontier and at the same time the diploid made its way to regions of higher altitudes; and (iii) with the coming of milder climate the diploid has sought a place for living in the alpine region and the original area has been occupied by the triploid. If such an interpretation for the evolutionary process in this species is correct, the consequence will be that the alpine type is a residual type caused by the selective effect of climate. To what extent this idea may be applied to other alpine and lowland plants will provide a further interesting field of research. And it will be mentioned here that some preliminary work has been published from a similar view-point by SAKAI ('34) on the chromosome number in a number of alpine plants. Such a line of research has been also undertaken by the writer and is now in progress. In this connection it is noteworthy that HAGERUP('33) found in *Vaccinium uliginosum* two ecotypes, the arctic dwarf form which is diploid and the southern tall form which is tetraploid, a condition to be considered as similar to the present case.

Summary

Fritillaria camschatcensis (L.) KER-GAWLER comprises two distinct karyo-ecotypes:

i) the alpine type, its distribution being exclusively restricted to *regio*

alpina, and characterized by a dwarfish appearance and by the diploid chromosome complement (2n=24) ;

ii) the lowland type, its distribution being restricted to lowland meadows, even to the sea-shore, and characterized by a gigas-like appearance and by the triploid chromosome complement (2n=36).

Consequently the triploid is a form directly derived from the diploid, and with the change in the chromosome organization, the former has acquired the ability of withstanding the different climatic conditions, thus resulting in complete isolation of the two types from each other.

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Explanation of Plates

Plate XIII

Left, the diploid *Fritillaria camschatcensis* in the natural habitat. Photo. July 4, 1934 at Mt. Gassan. Middle, the triploid form. Photo. June 5, 1934 at Sapporo. The scales indicated are in cm. Right, two individuals of the triploid, showing difference in stature according to difference in the age of plants; the taller plant bears normal flowers, whereas the other lacks the female organ in its flower.

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Plate XIV

Camparison of number and size of leaves between the diploid and the triploid. Upper, the whole leaves in one individual of the diploid from Mt. Taisetu. Lower, those in one individual of the triploid from Sapporo. Reduced to 1/3.5 natural size.
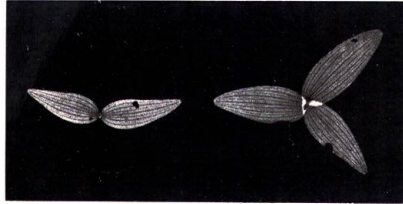
Pl. XIII

H. Matsaura photo.

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Pl. XIV

Diploid
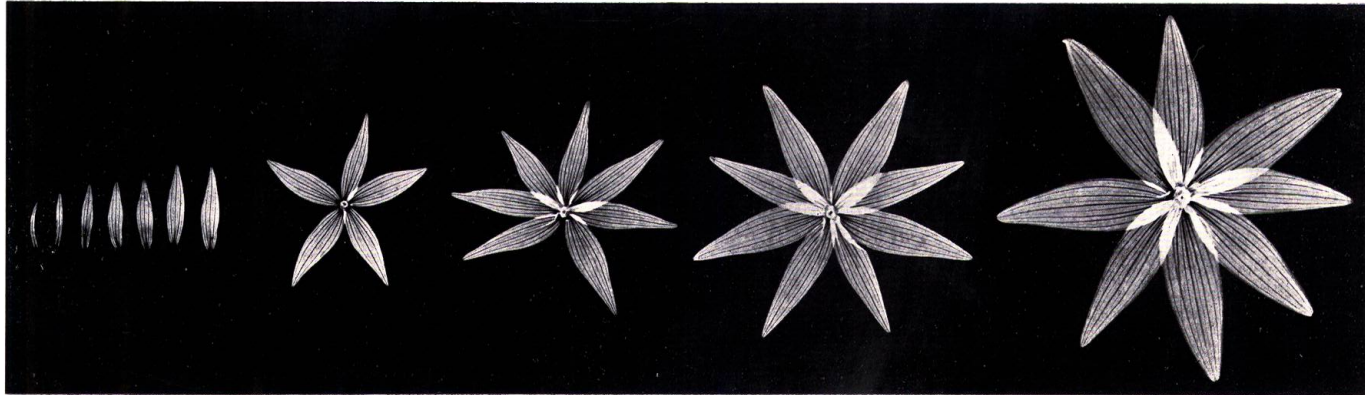
Triploid

T. Akemine photo.

H. MATSUURA: On Karyo-ecotypes of *Fritillaria camschatcensis* (L.) KER-GAWLER.