The Behaviour of the Plastid as a Hereditary Unit: The Theory of the Plastogene

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Introduction

Since plastid originates from plastid by division, a plastid has its own individuality. The characteristics of the plastid are usually controlled by the genotype, or gene complex, by mutation of which a variant character appears and is transmitted as a Mendelian unit. The variant characteristics of the plastid do not depend upon its own trait, so that the property of the plastid, or plasmotype (IMAI 1936b), is normal. Generally speaking, the plasmotype of the plastid is highly constant, as is usually the case with the gene. The plastid, however, spontaneously changes its plasmotype by mutation, which process is called plastid mutation. The mutant plastid may propagate by repeated division and distributes into cells. For example, white mutant plastids form in this way white tissues or patches in the leaves and other chlorophyllous organs, presenting variegation. Such a plastid character is inherited as non-Mendelian.

Analogous to the mutable gene, the plastid sometimes mutates with recurrent frequency. I have divided this case into two classes, auto-mutation and exo-mutation, the former mutating by itself and the latter through the effect of a certain gene (IMAI 1928, 1934b, 1936a). In my opinion, certain variegated forms of *Oryza sativa* studied by Japanese investigators are the result of recurrent automutation of plastids. In *Oryza*, plastid mutation is either from unstable green to stable white and stable green, or from unstable green to stable white. In *Hordeum vulgare*, an automutable yellowish plastid appeared by sporadic mutation from normal (IMAI 1936c). In my view (IMAI 1936a), WINGE's variegated form of *Humulus japonica* may be regarded as due to recurrent automutation of green and white plastids. Presumably, in this case, both green and white plastids are mutable to each other. For exomutable plastids, we can cite a number of cases (IMAI 1928, 1934a, 1936a, 1936b), which are grouped under two classes. In the one the plastid mutates from green to stable white, and in the other to unstable white. In stable white, the white variegation extended sometimes to cause the bud variation to be pure white, and the variegated plants gave albino seedlings, although in some cases, either none or only a few such seedlings resulted through zygotic lethality of the albino embryos. In unstable white, the white variegation has green ticks or lobes, from which originate some green seedlings in the offspring. A large part of the chlorophyll variegation, according to my examination, are due to recurrent exomutation of plastids. Rarely, the mutated plastids do not revert to the prototypic green, but to yellow.

The Plastid and the Plastogene

Since in inheritance, a plastid behaves as a unit, and the mutant plastid maintains its new characteristic from cell to cell and from generation to generation without change or modification, unless new plastid mutation occurs, the plastid has something that is heritable or a factor that manifests the characteristic. A new term "plastogene" is given to this factor. The character of the plastid is manifested by the plastogene under the control of the gene complex. Generally the plastogene is highly constant, and sporadically changes its plasmotype, giving a mutant plastid. The change in the plastogene of the plastid is due to mutation of the plastogene. In the mutable plastids, the plastogenes are unstable and plastid mutation occurs recurrently in one or more directions. Although we cannot see the plastogene under the microscope, it is supposed that plastid division is preceeded by division of the plastogene. Each plastid contains one plastogene, which, divides and enters the daughter plastids after plastid division. Since a plastid contains only one plastogene, the division is simple, that is direct. The plasmotype is also simple, no heterozygous condition of plastogenes occurs, but some cells may have mixed plastids of different plasmotypes, seeing that a cell contains a number of plastids in its cytoplasm. The nucleus is a receptacle for the chromosomes, which are strings of a number of chromomeres. Each chromomere, therefore, is divided at nuclear division in order that the chromosomes may be allocated into equal parts. Mitosis is the total figure of direct divisions of the respective chromomeres.

In bacteria, no nucleus is formed, only scattered chromatins being found. The blue-green algae (Schizophyceae) are a bridge to the more complicated organisms in the evolution of chromatin structure by exhibiting an "incipient nucleus". The chromatin found in the Schizophyta is regarded as a primitive chromosome, probably an aggregate of a certain number of chromomeres. In this connection, I shall make bold to say that the plastid is developed from the common body together with the chromatin. If this is admitted, the unit of chromosome, or chromomere, corresponds to the plastid and the gene to the plastogene. In the course of evolution, some of the common bodies formed chromatins, enclosed in a nucleus, while others developed into plastids, left in the cytoplasm. Under the microscope, the plastid found in a mature cell is relatively a large body, whereas the proplastid found in a meristematic cell is very small in size, so that the plastid undergoes marked growth in its life history. The plastid may be build by a "nucleus" plastogene and the covering matrix plastophore. The growth in size is due to development of the plastophore, which contains pigments in the coloured plastids. In the salivary gland cells of some insects are found giant chromosomes, with lengths as much as from about 100 to 150 times that of the oogonia chromosomes, which indicates the possibility that the matrix of the chromosome swells up greatly in certain media. The relatively large body of the plastids, therefore, does not seem out of reason.

The theory of the plastogene advanced above applies well to the complex behaviour of the plastid mentioned in the beginning of this paper. In the following pages, further observations on the chlorophyll variegation of some plants are described in connection with the application of this theory.

Tropaeolum majus

Variegation in *Tropaeolum majus* is transmitted as simple recessive to green (RASMUSON 1920; CORRENS 1920; BATESON, cited from MOFFETT 1936), the character being regarded as a "type" in inheritance. In my opinion, however, the variegation is not so simple in its manifestation, the mechanism lying in the recurrent mutation of plastids. The exomutation of plastids from green to greenish yellow is accompanied by recurrent reverse automutation of plastids. The exomutation is conditioned by a recessive gene, which produces variegation by bringing about plastogene mutation. The green tissues that occur in the yellowish mesophyll are usually small in size, forming green ticks, so that automutation of the yellowish plastogene seems to take place generally at late cell generation in the ontogeny of the leaf. The yellowish tissues may cover an entire leaf or even a branch, although usually remain to form the mottling in the leaf. Rarely, variegated plants were observed to bear all greenish yellow leaves in the course of developmnt. The yellowish parts have invariably green ticks.

The variegated breeds true to type, giving uniform offspring. The seedlings have cotyledons, which are variegated very slightly. The variegation is not apparent in the early developed leaves, it being gradually marked in the later leaves: the fourth or fifth leaf is fully marked in the matter of variegation, presenting fine mottling (Fig. 1). Pollen and ovules are quite fertile and the production of seeds per capsule is also normal, as shown in Table 1.

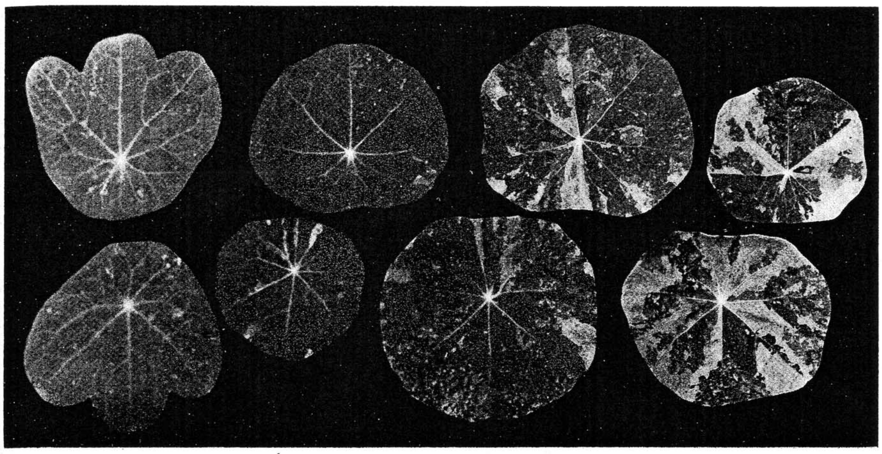

Fig. 1. Cotyledons (left two) and early developed leaves of the variegated *Tropaeolum*, taken from a seedling and arranged in the order of their development.

The germination rate is also substantially the same, showing 92 per cent in the variegated and 93 per cent in normal. These experiments were conducted in a greenhouse. The results gave negative proofs for the lethality of the yellowish gametes and zygotes. In these circumstances, I think that variegation is reduced to the pseudo-green condition at gametogenesis. At this stage, the exomutation

Table 1. Seed production in *Tropaeolum*

Character of plant	Seed number per capsule			Total of seeds	Average seeds per capsule
	1	2	3		
Variegated	29	44	43	246	2.12
Green	16	27	24	142	2.12

of the plastogene from green to yellowish seems to stop, and the yellowish plastids mutate with high frequency to green as the result of their automutability. There is nothing strange in this, seeing that plastid mutation is greatly influenced by environments, especially by the condition of the cell.

The absence of recurrent mutation at gametogenesis has been already pointed out in *Hordeum* and *Oryza* in connection with exomutation in the former and automutation in the latter. Non-mutability at gametogenesis in *Tropaeolum* is therefore not peculiar to this plant alone. The phenomenon of "retroversion" is observed also in the case of pseudo-mutation, as pointed out by IMAI and KANNA (1927) in *Amaranthus*.

With the theory of retroversion of the character at gametogenesis, the problem of non-production of the yellowish seedlings is solved. Since retroversion in this case is partly conditioned by recurrent automutation of the yellowish plastids, should they be unable to bring about automutation to green, we ought to expect some yellowish seedlings in the offspring. In the offspring of the white-variegated *Hordeum*, white seedlings appear in a fairly definite proportion, while no albino seedlings are produced in the progeny of the variegated form of *Polygonum orientale*. In both cases, variegation is the result of recessive genes, which change the plasmotype of the plastids from green to white, or in other words, exomutation of the plastogene takes place recurrently. In *Polygonum*, albino embryos, however, fail to germinate because of zygotic lethality. Owing to non-automutability of the chlorophyll deficient plastids to green, retroversion of the character at gametogenesis is hindered, so that the result is albino embryos after fertilization. In the variegated *Pelargonium*, automutation of the plastids from albinotic to green is exhibited in some cases, in which small green ticks occur at times in the creamish tissues (IMAI 1936b). The albino seedlings partially germinated in my experiments. Automutability, however, is not frequent in *Pelargonium*, so that retroversion of the character is not much in evidence, giving albino seedlings. Retroversion therefore seems to be completely accomplished in variegated forms, in which the frequency in the automutation of the plastogene is high.

The embryos of the variegated *Tropaeolum* start from pseudo-green, and by recurrent exomutation of the green plastids, aided by the automutation of yellow plastids, the cotyledons and leaves are variegated. The frequency of exomutation, which is not high during early zygotic development, is increased later, although the extent to which it is done is greatly influenced by environments. The mechanism involved in the variegation of *Tropaeolum* is the same as that shown elsewhere (IMAI 1936a, Figure 7B).

The alternation of yellowish plastids to green is regarded as being due to automutation of the plastogene, and not to exomutation. Since the variant yellowish plastid is conditioned by the change in the plasmotype, its property is already beyond the control of the gene complex, whence mutation should occur automatically. In *Pelargonium*, white plastids with automutability are also held in the green plants, when the plastids are introduced by crossing, which proves that alternation of chlorophyll deficient plastids is not due to the gene that stimulates exomutation of plastids, but to the property of the plastogene itself, that is, automutation.

Polygonum virginianum var. *filiforme*

The variegated form of *Polygonum virginianum* var. *filiforme* has creamish white patches in the leaves, and green ticks occur in the creamish tissues (Fig. 2). The mechanism by which variegation occurs is the same as that of the variegated *Tropaeolum*: the creamish patches are due to recurrent exomutation of the plastogene from green to creamish, and the green ticks to the recurrent automutation of the plastogene from creamish to green.

Fig. 2. Variegated form of *Polygonum virginianum* var. *filiforme*.

The variegated gave only 218 variegated seedlings, no albinos being observed. Retroversion at gametogenesis in connection with variegation seems to be also complete in this case. The seedlings generally start from apparently green, variegation appearing gradually in the leaves. Since the shoots from old stocks also are nearly green when young, retroversion seems to occur also at the meristematic cells of discontinuous growth. However, in 1935, one of the old variegated stocks produced permanent green branches, which, on selfing, revealed their heterozygosity by giving seedlings that segregated for green and variegated, judging from which the variegated form of *Polygonum* seems to tend to revert to the green condition by gene mutation. Mutation here takes place from the gene "variegated", which alters green plastids to creamish ones, to its normal allele.

Pharbitis Nil

The variegated form (Fig. 3) of the Japanese morning glory, *Pharbitis Nil,* is inherited as simple recessive, when the variegation is considered as a whole. The variegated breeds true to type, neither white nor green offspring being found. The cotyledons are sometimes variegated, and a few of the early leaves are usually slightly variegated. Variegation gradually becomes apparent with growth, at the end of which, however, the small ill-developed leaves assume heavy variegation.

Fig. 3. Variegated leaf of *Pharbitis Nil.* (Leaf form : dragonfly retracted).

The range in the degree of variegation is considerable, including even green and white leaves. The green leaves, although very rare, may occur at the axilary branches. When a plant is pinched, the newly developed vigorous branches usually bear slightly variegated leaves by recovering its juvenescent growth. In Figure 4 are shown four leaves which exhibit variation in the extent of variegation, including a pure green. The production of green leaves is due to fluctuation. The white leaves, which also are very rare in occurrence, have usually green ticks. Figure 5 shows white and variegated leaves, the latter of which has large green-ticked white sectors.

In my opinion, variegation of this plant is not simple, being conditioned by recurrent exomutation of green plastids and recurrent automutation of white plastids. Through mutual changes, white variegation with green ticks occurs. Since manifestation of white variegation is due to the exomutation of plastids from green to white, the chances for mutation are greatly controlled by environments. Roughly speaking, rich growth lessens its frequency, while poor growth increases it. In this plant, green-ticked white leaves are only rarely observed, and the white branchlets very rarely, notwithstanding that white variegation is so common that almost every

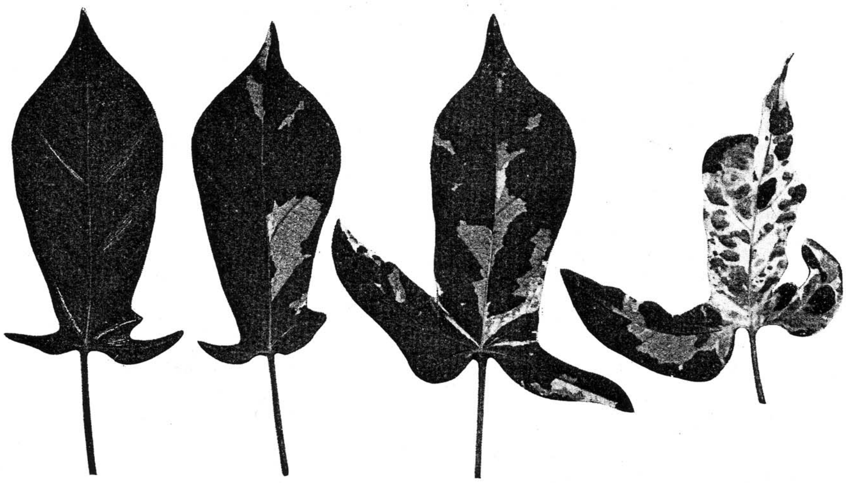

Fig. 4. Leaves picked from a variegated plant of *Pharbitis*, exhibiting fluctuations in the extent of variegation, including a green leaf. (Leaf form : dragonfly).

leaf is mottled with white tissues. This limitation means that the exomutation of plastid does not usually occur at the growing points of the stems and leaves. Since the Japanese morning glory is a

Fig. 5. Leaves of *Pharbitis* ; the left two having large white sectors, the right one being white. Note small green ticks in the white parts. (Leaf form : maple).

trihistogenic plant, white-over-green chimeras may be expected. In the variegated leaves, white patches occur side by side, some in the outer tissues, some in the inner tissues, and others in both tissues. In Figure 3, for example, the dark parts have green mesophyll, the light parts white outer and green inner tissues, and the white parts white mesophyll. In the leaves that have developed at the end of the growth, however, we found some to be white-over-green peri-

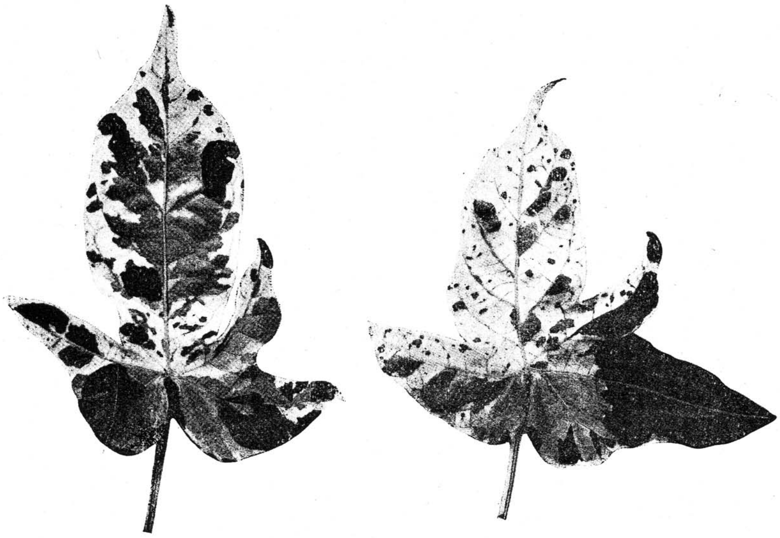

Fig. 6. Periclinal (left) and mosaic (right) leaves of *Pharbitis*. (Leaf form: dragonfly).

clinal, although they are frequently found with more or less non-periclinal parts (Fig. 6). A close observation of leaves of variegated plants shows these periclinal leaves, although not frequent—a fact strongly supporting the view that white variegation is not a simple "type" as formerly supposed, but is conditioned by recurrent exomutation of the plastogene, induced by a recessive gene "variegated". The white leaves or white parts of leaves have green ticks, which are regarded as the result of recurrent

Fig. 7. Variegated-reduced leaf of *Pharbitis*. (Leaf form: normal).

automutation of the plastogene. Thus, variegation is conditioned by two factors of reversible plastid mutations. The fact that the green tissues form generally small ticks indicates that the automutation occurs late in the cell generation of the leaf.

Under these circumstances, we should expect white seedlings among the variegated offspring, but my experience during the last twenty years denies it. The pollen and ovules are quite fertile as in normal green, no lethal gametes having been found. Neither zygotic lethality is detected; the average proportion of seeds per capsule is substantially alike for green and variegated plants; the germination rate also does not differ. These observations force me to conclude that the absence of white seedlings is due to retroversion of the character at gametogenesis, whence the mechanism involved in *Pharbitis* is exactly the same as that in *Tropaeolum*.

For variegation in *Pharbitis*, a modifier, variegated-reduced was found (IMAI 1925), by means of which very finely mottled variegation (Fig. 7) occurs with the aid of the gene "variegated". In the leaves, recurrent automutation of plastogene from whitish to green occurs very frequently during late cell generation of the leaves, forming fine green speckles in the white tissues. The gene variegated-reduced modifies the rate of mutation and the time at which mutation occurs. With this exception, the mechanism of variegation is the same as that of ordinary variegation of this plant.

Polygonum Blumei

A variegated form of *Polygonum Blumei* exhibits white mottling in the leaves. In the leaves of the variegated plant, a wide range of variegation occurs from pure green to almost white with green ticks (Fig. 8). Although the variation is concerned with the extent of variegation, it is confined to rather limited ranges. The significance in its variation is in the distribution of variegation, in other words, the degree of mosaic of the green and white parts. Roughly speaking, the leaves or the sectors of the leaves are grouped under two classes, green (genetically pseudo-green) and variegated. Generally the borders of the mosaics are abrupt. The variegated form gives graded offspring according to the extent of variegation, as shown in Table 2.

Besides variegated individuals, the offspring included apparently green ones which, however, were unstable. These plants, when cultivated with ample space will show variegation in their leaves. They, however, give equally variegated offspring as well. In the variegated offspring, a series graded with respect to the amount of alternate distribution of green and variegated parts occurred, including whites with green ticks, which, however, grew poorly owing to the smaller amount of photosynthesis.

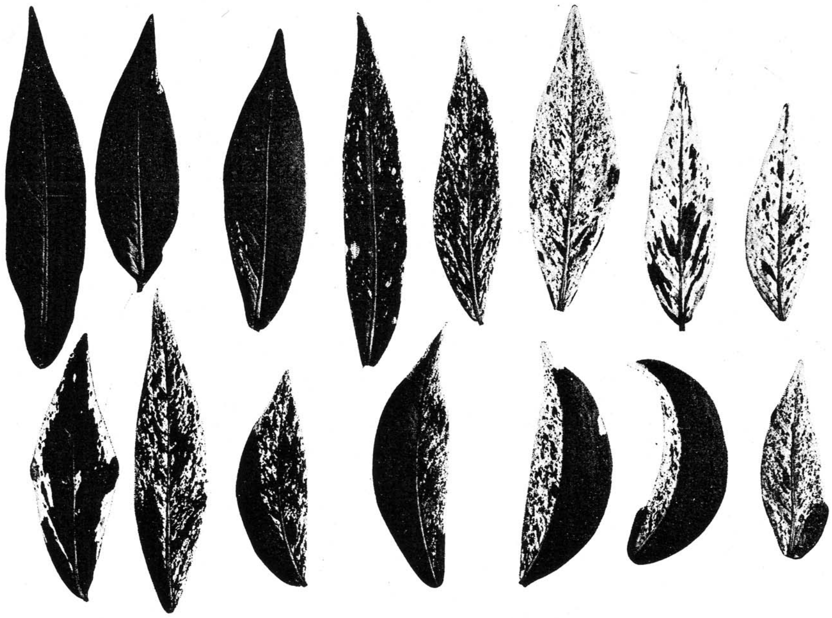

Fig. 8. Variation in the variegated pedigree of *Polygonum Blumei*, including mosaics.

The variegated individuals frequently bear mosaic leaves and branches, and green and variegated branches occur as bud variation.

Table 2. Offspring of the variegated *Polygonum*

Plant or branch	Pseudo-green or almost green	Slightly variegated	Variegated	Heavily variegated	Total
Pseudo-green	20 18.87%	66 62.26%	16 15.09%	4 3.77%	106
Slightly variegated	4 7.27%	38 69.09%	9 16.36%	4 7.27%	55
Variegated	56 24.78%	120 53.10%	40 17.70%	10 4.42%	226
Heavily variegated	14 10.14%	83 60.14%	29 21.01%	12 8.70%	138

A separate observation of the offsprings from the green and variegated branches of the same plant are given in Table 3.

Although the results show some differences, they do not seem important. As to the mechanism of variegation in this plant, my opinion is as follows: Since crossing experiments have not been made, it cannot be decided whether alternation of the plastogene involved in the present case is due to automutation or to exomuta-

Table 3. Offspring of green and variegated branches

Branch	Pseudo-green or almost green	Slightly variegated	Variegated	Heavily variegated	Total
Pseudo-green	18 25.00%	43 59.72%	7 9.72%	4 5.56%	72
Heavily variegated	2 5.13%	24 61.54%	11 28.21%	2 5.13%	39

tion. Most probably, there are three plastogenes to account for the mechanism of variegation, namely,

1. Green-alpha: a plastogene is responsible for the green plastid, and having relatively low mutability, produces pseudo-green leaves.
2. Green-beta: a plastogene is responsible for the green plastid, and having high mutability, produces white leaves with green ticks.
3. White: a plastogene is responsible for the white plastid, and having high mutability, produces white leaves with green ticks.

As stated above, it is not clear whether the cause of the change is due to the property of the plastogene itself (automutation) or to a special gene (exomutation). These plastogenes, however, seem to mutate reversibly with respect to each other, resulting in variation in the variegated pedigree, sometimes also exhibiting mosaics. The reversible mutation is influenced considerably by environments. Owing to the characteristics and the mutability of the respective plastogenes, three kinds of leaves result. The pseudo-green leaves have plastids containing green-alpha, which may change into either green-beta or white, the former mutation resulting in green and variegated mosaics and the latter in green and white (with green ticks) mosaics. The variegated leaves have plastids of both green and white as the result of high reverse mutation. The plastogene green-beta may also transform to green-alpha, by which mutation variegated and green mosaics are produced.

The young seedlings are generally green or slightly variegated, showing that they start from the pseudo-green condition. In this case, retroversion of the character in connection with variegation seems to occur also at gametogenesis, due to absence of mutation from pseudo-green to variegated or white and to their frequent reverse mutation. Consequently, no albino or heavily variegated seedlings occur, and the heavily variegated plants or branches give substantially the same offspring as the pseudo-green ones.

In *Polygonum Blumei*, no periclinal leaves occur. I examined the species of *Polygonum*, together with *P. orientale* and *P. virginianum* var. *filiforme*, but no periclinals for chlorophyll deficiency were observed. In *Tropaeolum*, the absence of periclinal leaves was also noted, so that among dicotyledons, these species may be regarded as dihistogenic plants.

Conclusion

Plastid mutation is regarded as a change in the plastogene contained in the plastid. The plastid propagates by direct division, preceded, presumably, by division of the plastogene. A plastid may correspond to the chromomere that is located in the chromosome. The mechanism of some chlorophyll variegations lies in the recurrent auto- and exomutation of the plastogenes. Through plastogene mutation, variant plastids appear among otherwise homogeneous plastids of a cell, variegation being exhibited when they propagate into the cells. Anatomical examination of the variegated leaves reveals the fact that some cells contain mixed plastids of different plasmotypes.

In the variegated forms, in which the rate of automutation of the plastid from white (or yellowish) to green is high, retroversion of the character in connection with variegation takes place at gametogenesis, as the result of which no albino seedlings appear. All zygotes start from an apparently green condition, variegation gradually appearing in the leaves of the seedlings. Retroversion is conditioned by hindrance of plastogene mutation from green to white and by increase in the frequency of its reverse mutation. Therefore the rate of plastogene mutation is greatly influenced by the condition of the cell. In meristematic cells, plastogene mutation is frequently hindered. Generally speaking, rich nourishment or rejuvenescence of tissues decreases the rate of such mutation.

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