VARIATION IN THE FLAKED LINES OF *LATHYRUS ODORATUS*

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(With Plate XVIII)

In our collection of striped flowers certain lines of the sweet pea, *Lathyrus odoratus*, exhibited flaked corollas, "America" showing flaking in crimson red, "Felix" in red, and "Senator" in dark purple. The flaked, however, showed complexities, throwing out some mutants. Regarding this property, Punnett (1936) wrote: "(1) a consistent deficit in the 25% of recessive flakes expected in F_2, (2) a wide range in the grading of the extracted flakes, from the normal light type to an almost self-coloured form, and (3) the fact that certain forms of flake gave rise to some self-coloured individuals which hereafter bred true." The variability in the flaked lines having attracted our attention, we wished to make clear its nature, desiring to contribute something to the genetics of this plant, which has long been Punnett's favourite material.

Before proceeding further, we wish to express our thanks to Prof. K. Miyake for his useful advice and to Prof. R. C. Punnett for his valuable criticisms. The expenses in connexion with the present investigation were defrayed by a grant from the Hattori-Hôkôkai, which financial help is greatly appreciated. Thanks are also due to Mr K. Kasahara for his assistance in these experiments.

The pedigree culture of flaked lines

The flaked flower (Pl. XVIII, fig. 3) is conditioned by fine splashed variegation consisting of minute dots. In 1935, mass cultures of the two flaked strains, America and Felix, were made in order to observe the variability exhibited in the offspring, the data being summarized in Table I.

TABLE I

Mass culture of flaked lines, 1935

Strain	Self-coloured	Dark-flaked	Flaked	Tinged	Total
America	15	20	612*	3	650
Felix	1	5	323	0	329

* Two of them showed bud variation, one to self-coloured and the other to dark-flaked.

The flaked did not breed true to type, throwing out some variants. As to the flaked itself, there were some variations in the extent of

variegation, which, however, were disregarded and placed in the flaked class. The variants comprised self-coloured, dark-flaked, and tinged. To the naked eye the self-coloured (Pl. XVIII, fig. 1) is fully coloured, the

TABLE II

Offspring of flaked lines, 1936

(*a*) *America*

Pedigree no.	Self-coloured	Dark-flaked	Flaked	Tinged	Total
1	5	1	124	—	130
2	—	2	34	—	36
3	1	1	48	—	50
4	1	3	64	—	68
5	7	4	128*	1	140
6	4	2†	83	—	89
7	3	1	45	—	49
8	7	1	57	—	65
9	3	5	87	—	95
10	1	3	81	1	86
11	—	3	34	—	37
12	7	7	79*	—	93
13	—	—	31	—	31
14	—	—	33	—	33
15	1	—	27	—	28
16	1	4	16	—	21
17	—	—	22	—	22
18	3	2	61	—	66
19	—	4	58	—	62
20	—	1	33	—	34
21	1	2	37	—	40
22	2	3	57	—	62
23	4	—	25	—	29
24	1	2	28	1	32
25	3	2	38	—	43
26	3	6	34	—	43
27	3	—	58	3	64
28	2	1	35	—	38
29	3	1	34	—	38
30	2	2	54	—	58
31	3	—	29	1	33
32	5	—	37	—	42
Total	76	63	1611	7	1757

(*b*) *Felix*

Pedigree no.	Self-coloured	Dark-flaked	Flaked	Tinged	Total
1	—	—	21	—	21
2	2	—	22	—	24
3	—	1	72	—	73
Total	2	1	115	0	118

* One sported to dark-flaked in each case. † One sported to flaked.

dark-flaked (Pl. XVIII, fig. 2) is almost self-coloured but mixed with flaked patches, while the tinged (Pl. XVIII, fig. 4) is colourless except the periphery of the petals, which is tinged with red.

Under the microscope the epidermis of the flaked petal has coloured cells that are scattered here and there in the otherwise white or tinged

cells (Pl. XVIII, fig. 7). The coloured minute spots consisted of either one or several cells. The distribution of coloured cells differs with the degree of flaking. The dark-flaked petal has coloured epidermis mixed together with colourless cells, the coloured and white cells occurring like patchwork (Pl. XVIII, fig. 6). Even in the self-coloured petal, the epidermis does not consist exclusively of coloured cells, but contains some white cells (Pl. XVIII, fig. 5), although it is apparently fully coloured to the naked eye. Therefore the self-coloured, dark-flaked, and flaked are three types of grades in a mixture of coloured and white cells in the epidermis of the petal. The epidermis of the tinged petal, however, is almost colourless (Pl. XVIII, fig. 8), although the marginal parts consist of tinged cells.

Our study was concentrated on the strain America, only a few tests having been made with Felix. As to Senator, only a few plants were cultivated. The type America and its variants that appeared in 1936 were cultivated by pedigree the following year. In the 1936 census of the offspring of the flaked, variability occurred as shown in Table II.

The results were virtually the same in both strains, although the frequency in the occurrence of variants seems to differ. As the 1937 data from America also duplicated the previous observations, they will be summed up in Table III to save space.

TABLE III

Pedigree culture of flaked lines, 1937 (*America*)

No. of pedigrees	Self-coloured	Dark-flaked	Flaked	Tinged	Total
27	20	9	704	0	733

Collecting all the available data for the three years, the figures for America are

Self-coloured	Dark-flaked	Flaked	Tinged	Total
111	92	2927	10	3140
3·54 %	2·93 %	93·22 %	0·32 %	

In our flaked lines marked variability as the result of sexual propagation is thus a constant feature, this property being transmitted from generation to generation. So far as colour distribution is concerned, the variant types that appeared in the offspring of the flaked are restricted to three, viz. self-coloured, dark-flaked, and tinged, in the proportion of 3·54, 2·93, and 0·32 %.

SELF-COLOURED MUTANTS

There were 3·54 % self-coloured variants in the progeny of flaked America. The direct offspring of these variants are collected in Table IV.

Of thirty-four self-coloured variants tested, three bred true to type; the others segregated the flaked and at times also produced dark-flaked. With these data we can safely conclude that the occurrence of self-

TABLE IV

Direct offspring of self-coloured mutants (America)

(a) True-breeding families

No. of families	Self-coloured	Dark-flaked	Flaked	Tinged	Total
3	90	0	0	0	90

(b) Segregating families

Plant No.	Self-coloured	Dark-flaked	Flaked	Tinged	Total
1	35	—	15	—	50
2	29	—	13*	—	42
3	33	2	12	—	47
4	33	—	13	—	46
5	23	1	7	—	31
6	1	—	1	—	2
7	21	—	6	—	27
8	3	—	1	—	4
9	9	—	2	—	11
10	20	—	7	—	27
11	32	—	8	—	40
12	14	—	3	—	17
13	15	—	4	—	19
14	18	1	2	—	21
15	20	5	11	—	36
17	2	1	1	—	4
18	12	1	3	—	16
19	7	1	1	—	9
20	3	—	1	—	4
22	18	3	5	—	26
24	38	2	2	—	42
25	16	3	4	—	23
26	22	4	3	—	29
27	7	—	2	—	9
28	10	2	3	—	15
29	6	—	1	—	7
30	38	2	2	—	42
Total	485	28	133	0	646
	75·08 %	4·33 %	20·59 %	0 %	
16	21	9	4	—	34
21	23	6	—	—	29
23	42	6	2	—	50
31	51	11	3	—	65
Total	137	32	9	0	178
	76·97 %	17·98 %	5·06 %	0 %	

* One sported to dark-flaked.

coloured variants in the flaked lines is due to mutation of the gene flaked to its dominant allele, self-coloured. In the majority of cases the self-coloured mutants, as expected, were heterozygous in constitution.

Of these mutant families two were tested for their further progenies, which, however, were not many on account of the fact that the self-

coloured mother plants bore but few pods. The results are shown in Table V.

TABLE V

The culture of self-coloured mutant families (America)

Pedigree	Self-coloured	Dark-flaked	Flaked	Tinged	Total
1–1	4	—	—	—	4
–2	10	—	—	—	10
–3	8	—	—	—	8
–4	5	1	2	—	8
–5	5	—	3	—	8
–6	33	—	—	—	33
–7	5	—	1	—	6
–8	7	—	4	—	11
–9	14	—	1	—	15
2–1	2	—	—	—	2
–2	2	—	2	—	4
–3	3	—	2	—	5
–4	3	—	—	—	3
–5	1	—	—	—	1
–6	3	—	2	—	5
–7	4	—	—	—	4
–8	3	—	1	—	4
–9	7	—	2	—	9

Some of these families bred true to self-coloured, whereas others segregated out recessive flowers. The gene self-coloured, therefore, acts normally, with full constancy.

DARK-FLAKED MUTANTS

The dark-flaked variants amounted to 2·93 % in the offspring of flaked America, their direct progenies being shown in Table VI.

None of the dark-flaked mutants bred true to type. Of the families of dark-flaked America, three were placed under observation by raising their offspring, as shown in Table VII.

The dark-flaked plants did not breed true to form, their offspring consisting of forty-three self-coloured, seventy-five dark-flaked, and forty-one flaked, without any tinged. The final available data for the dark-flaked families are as follows:

Self-coloured	Dark-flaked	Flaked	Tinged	Total
287	443	259	3	992
28·93 %	44·66 %	26·11 %	0·30 %	

Thus, the dark-flaked invariably threw out other types, which reached 55·34 %, as a result of their heterozygosity, aided by recurrent gene mutation.

TINGED MUTANTS

A few tinged variants appeared, their proportion being 0·32 % in the flaked America pedigrees. The type bred true for generations.

Bud variation

The two types, self-coloured and tinged, are constant; homozygous individuals of both bred true to their respective types. The flaked and dark-flaked, however, are inconstant. The mutants generally appeared

TABLE VI

Direct offspring of dark-flaked mutants (America)

Plant No.	Self-coloured	Dark-flaked	Flaked	Tinged	Total
1	15	15	10	—	40
2	3	2	3	—	8
3	4	5	1	—	10
4	2	5	1	—	8
5	7	6	1	—	14
6	10	13	11	—	34
7	9	18	11	—	38
8	12	16	9	—	37
9	4	5	1	—	10
10	7	1	1	—	9
11	15	16	9	—	40
12	7	2	6	—	15
13	15	13	1	—	29
14	15	27	15	—	57
15	11	12	5	—	28
16	3	3	3	—	9
17	5	5	7	—	17
18	4	9	2	1	16
19	2	3	1	—	6
20	6	6	7	—	19
21	8	6	2	2	18
22	5	2	2	—	9
23	6	6	3	—	15
24	7	13	3	—	23
25	1	7	4	—	12
26	3	4	1	—	8
27	1	1	—	—	2
28	2	1	2	—	5
29	2	2	—	—	4
30	9	13	10	—	32
31	2	2	3	—	7
32	4	25	10	—	39
33	3	12	3	—	18
34	6	9	5	—	20
35	14	31	19	—	64
36	3	20	15	—	38
37	—	6	4	—	10
38	2	6	7	—	15
39	10	20	20	—	50
Total	244	368	218	3	833

individually in the offspring. Somatic variation was also found in these forms. Flaked flowers bore at times dark-flaked sectors; the dark-flaked frequently had flaked parts. Rarely, bud variation occurred in the same way by somatic mutation at the early stages of bud rudiments. More rarely the sport arose in the direction of self-coloured. We observed six clear cases of sporting, and the progenies of five mosaic plants were raised

in order to ascertain the genetic nature of bud variation, the data obtained being shown in Table VIII.

TABLE VII

The culture of dark-flaked mutant families (America)

Family	Mother plant	Self-coloured	Dark-flaked	Flaked	Tinged	Total
11–1	Self-coloured	7	—	—	—	7
–2	,,	2	—	—	—	2
–3	,,	5	—	—	—	5
–4	,,	4	—	—	—	4
–5	,,	1	—	1	—	2
–6	,,	9	—	—	—	9
–7	Dark-flaked	—	3	2	—	5
–8	,,	1	1	—	—	2
–9	,,	2	4	2	—	8
–10	,,	—	4	—	—	4
–11	,,	3	6	4	—	13
–12	,,	2	2	2	—	6
–13	,,	1	—	—	—	1
–14	,,	—	—	1	—	1
–15	,,	1	4	—	—	5
–16	,,	—	1	1	—	2
–17	,,	1	1	1	—	3
–18	,,	1	1	—	—	2
–19	,,	1	—	1	—	2
–20	,,	6	4	2	—	12
–21	,,	2	8	2	—	12
30–1	Self-coloured	9	1	—	—	10
–2	,,	11	—	—	—	11
–3	,,	19	3	—	—	22
–4	Dark-flaked	2	4	4	—	10
–5	,,	2	1	—	—	3
–6	,,	5	2	3	—	10
–7	,,	12	23	7	—	42
36–1	Dark-flaked	—	2	4	—	6
–2	,,	1	2	3	—	6
–3	,,	—	2	2	—	4

TABLE VIII

Offspring of mosaic plants (America)

Plant No.	Branch	Self-coloured	Dark-flaked	Flaked	Tinged	Total
1	Flaked	—	—	6	—	6
	Dark-flaked	—	—	1	—	1
2	Flaked	—	—	5	—	5
	Dark-flaked	6	—	11	—	17
3	Flaked	—	—	11	—	11
	Dark-flaked	3	—	6	—	9
4	Flaked	—	—	15	—	15
	Dark-flaked	1	—	—	—	1
5	Flaked	1	—	4	—	5
	Self-coloured	9	—	4	—	13

All the mosaic plants tested were originally flaked and sported to the dark-flaked in plants Nos. 1–4, and to the self-coloured in plant No. 5.

Discussion and conclusion

According to Punnett's hypothesis (1936), the genetics of the flaked sweet pea is as follows: "Flaking depends upon a factor belonging to a series of multiple allelomorphs. The series is self-colour (G_1), flaking (G_1') and white (g_1), the white being the so-called *C*-white of earlier papers. The flaked character is recessive to self-colour but dominant to white. In its expression the flaked character is influenced by a modifying factor, D_3. A flaked plant ($G_1'G_1'$) becomes much darker (i.e. nearer to self-colour) when heterozygous for D_3. When homozygous for D_3 it develops self-coloured flowers indistinguishable from those of a normal self-coloured plant." Our data conform to his hypothesis, our flaked apparently having the constitution $G_1'G_1'd_3d_3$, whence it follows that both genes, G_1' and d_3, may be regarded as being mutable in the following directions:

$$G_1 \longleftarrow G_1' \longrightarrow g_1'$$
$$d_3 \rightleftarrows D_3$$

The self-coloured mutants that appeared in the flaked families are the result of recurrent mutation from G_1' to G_1, sometimes with simultaneous changes from d_3 to D_3, which, however, is hypostatic to G_1, so that we may have self-coloured mutants with such constitutions as $G_1G_1d_3d_3$, $G_1G_1'd_3d_3$, $G_1G_1'D_3d_3$, etc. The first-named type, although it rarely occurs, should breed true to self-coloured, and the second-named segregate flaked as a recessive. Since the third-named type is heterozygous for D_3, it should invariably segregate digenically to self-coloured, dark-flaked, and flaked. Out of thirty-four tests with the self-coloured mutants, three showed the first type, the remainder either the second or third type. Plants Nos. 16, 21, 23 and 31 seem to belong to the third type, while the others belong to the second. The families that are regarded as having been derived from mother plants carrying $G_1G_1'd_3d_3$ contained at times a few dark-flaked; their occurrence is due to recurrent mutation from d_3 to D_3, giving $G_1'G_1'D_3d_3$ mutants.

The dark-flaked mutants invariably segregated other forms, giving 28·93 % self-coloured, 44·66 % dark-flaked, and 26·41 % flaked (including tinged), which, however, show certain deviations from monogenic segregation, due to the recurrent changes schematized above.

As the tinged seems to be distinct from Punnett's g_1-white, we have designated it by g_1'. Since the occurrence of tinged in the flaked and dark-flaked families is regarded as being due to the change $G_1' \rightarrow g_1'$, the genes G_1, G_1', g_1', and g_1 form multiple alleles, as proved by Punnett,

except g_1'. Both genes, G_1 and g_1', are fully constant, but G_1' is mutable. The d_3 gene is also mutable, changing at times to D_3, which also is inconstant. Three self-coloured families of Table VII gave a few dark-flaked and one flaked, their appearance being believed to be due to the reverse changes from D_3 to d_3 rather than to contamination. The recurrent changes in connexion with the related genes give individual mutants as well as bud variation.

In a private communication Prof. Punnett writes: "My flake was derived from Senator. In 1922 I grew both Senator and America in long rows. There were several selfs in America but none in Senator. Possibly the flake may be less stable in the reds than in the purples. I have found that in my material, which is all in the purples, the flakes are very stable." Our main material was America, which, as Prof. Punnett says, is inconstant. Felix, another flaked line, probably stands between the two in the degree of mutability.

Anthocyanin pigment is present only in the epidermis, all the inner tissues being colourless. This condition restricts the clues by which the nature of the sport is identified. The sweet pea is probably a trihistogenic plant. According to Imai's view (1934, 1935 *a*, *b*), bud variation from flaked to self-coloured or dark-flaked should occur in two ways, namely:

(1) The sport has mutated ectohistogen, the inner histogens being unchanged.

(2) The sport has three mutated histogens.

Should sports arise during embryonic development, the two foregoing conditions may be expected. When dark-flaked sports occur on flaked plants, case 1 should give rise to flaked offspring, and case 2 to mixed dark-flaked offspring. Plants Nos. 2, 3 and 4 of Table VIII might be the latter kind of sports, giving self-coloured and flaked individuals. No dark-flaked was observed in these cases, which, however, from the fact of recurrent mutation, probably does not affect the above view. Owing to scanty data it is difficult to say what are the tissue components of the dark-flaked sport of mosaic plant No. 1. The self-coloured sport of mosaic plant No. 5 gave nine self-coloured and four flaked offspring, indicating that the branch had mutated mesohistogen, or that it is a sport of case 2. Thus we met with no sports of case 1, although we may do so on further examination.

SUMMARY

1. According to Punnett, the constitution of our flaked sweet pea seems to be $G_1'G_1'd_3d_3$. In the flaked America the offspring consisted

of 3·54% self-coloured, 2·93% dark-flaked, 93·22% flaked, and 0·32% tinged, showing the rate of inconstancy to be high.

2. The inconstancy of the flaked is attributed to the following recurrent gene mutations:

$$G_1 \longleftarrow G_1' \longrightarrow g_1'$$
$$d_3 \rightleftarrows D_3$$

The self-coloured is due to the change from G_1' to G_1, the dark-flaked to that from d_3 to D_3, and the tinged to that from G_1' to g_1'.

3. Punnett's hypothesis agrees well in the matter of genotypes of the mutant forms that appeared in our culture. His material was derived from Senator, which is a flaked line with high constancy. Our flaked America and Felix, however, showed marked inconstancy in the related genes. Somatic variation with genic changes also occurred.

REFERENCES

Imai, Y. (1934). "On the mutable genes of *Pharbitis*, with special reference to their bearing on the mechanism of bud variation." *J. Coll. Agric. Tokyo*, **12**, 479–523.

—— (1935 *a*). "Variegated flowers and their derivatives by bud variation." *J. Genet.* **30**, 1–13.

—— (1935 *b*). "The mechanism of bud variation." *Amer. Nat.* **69**, 587–95.

Punnett, R. C. (1936). "The flaked sweet pea." *J. Genet.* **32**, 171–7.

EXPLANATION OF PLATE XVIII

Fig. 1. Self-coloured flowers.
Fig. 2. Dark-flaked flowers.
Fig. 3. Flaked flowers.
Fig. 4. Tinged flowers.

Figs. 5–8. Epidermis peeled from the outer surface of the wings of flowers (×120).

Fig. 5. Self-coloured epidermis.
Fig. 6. Dark-flaked epidermis.
Fig. 7. Flaked epidermis.
Fig. 8. Tinged epidermis.

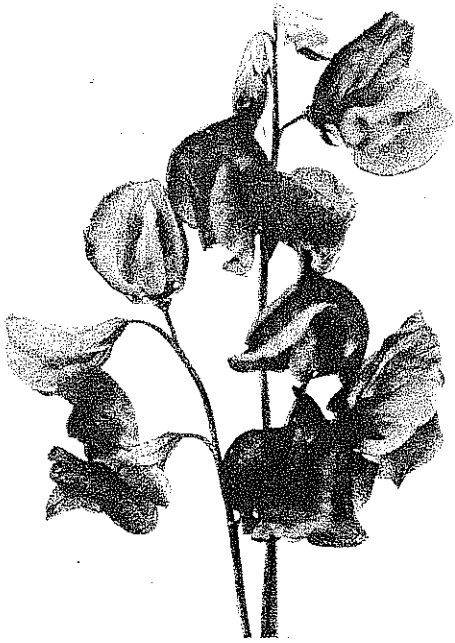

Fig. 1.

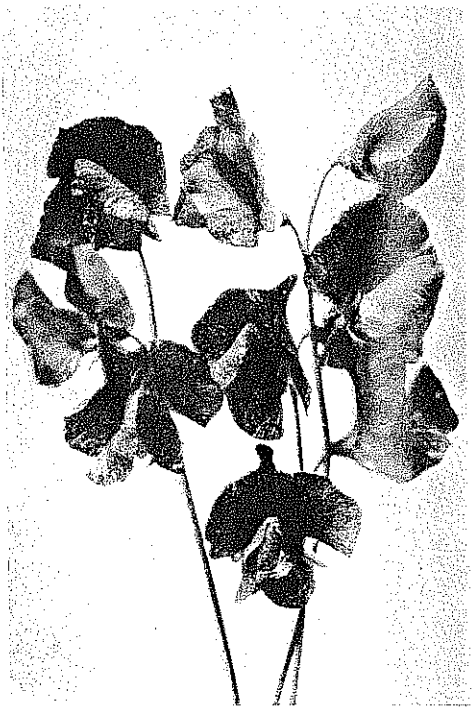

Fig. 2.

Fig. 3.

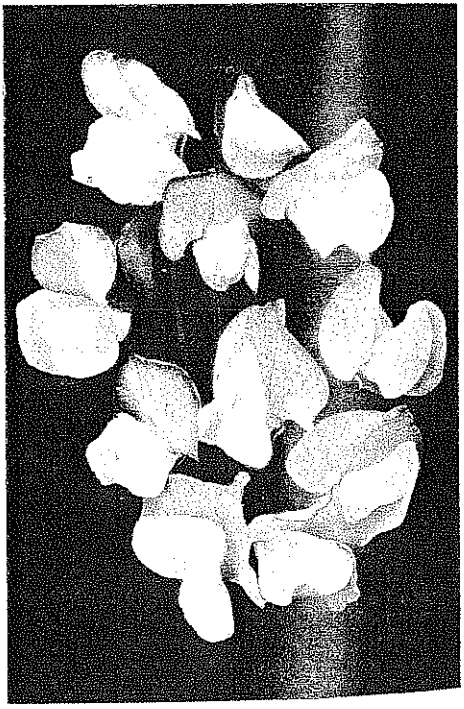

Fig. 4.

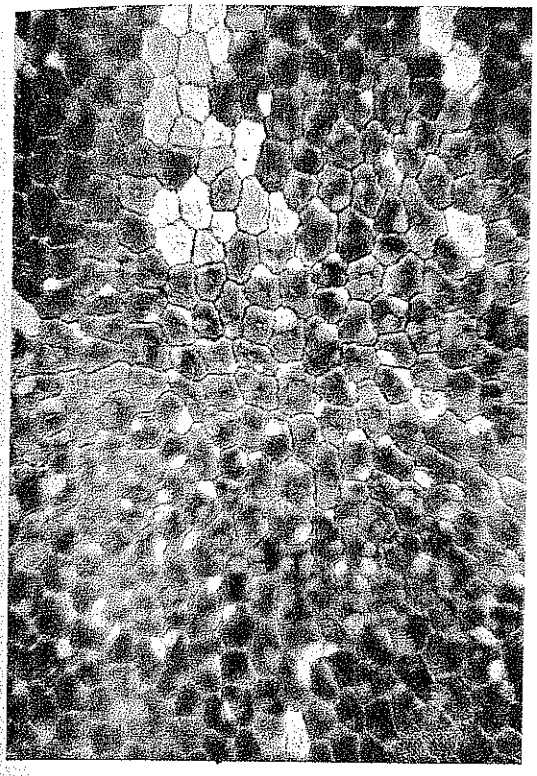

Fig. 5.

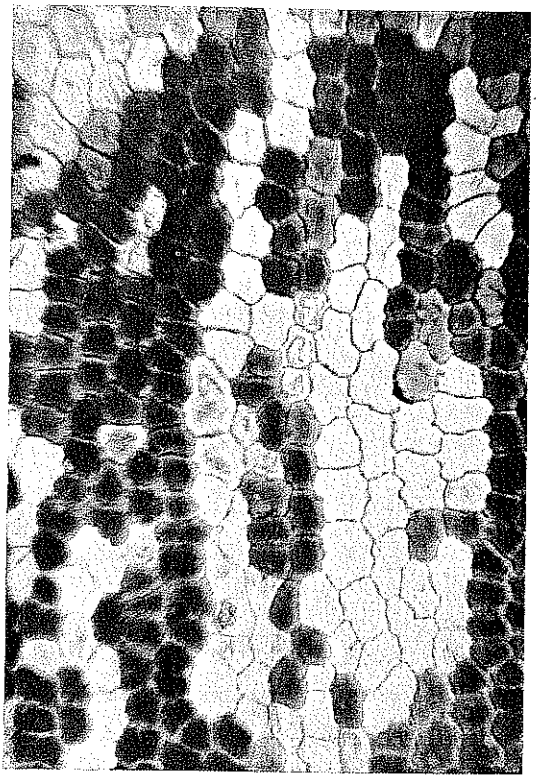

Fig. 6.

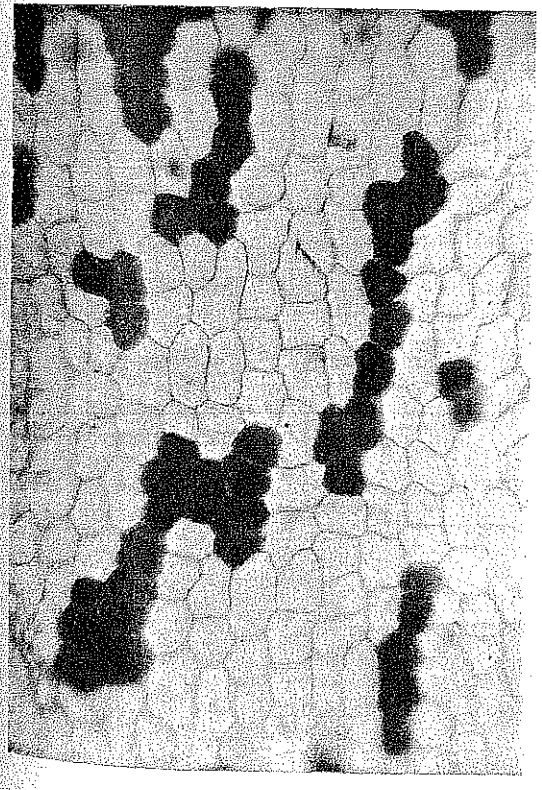

Fig. 7.

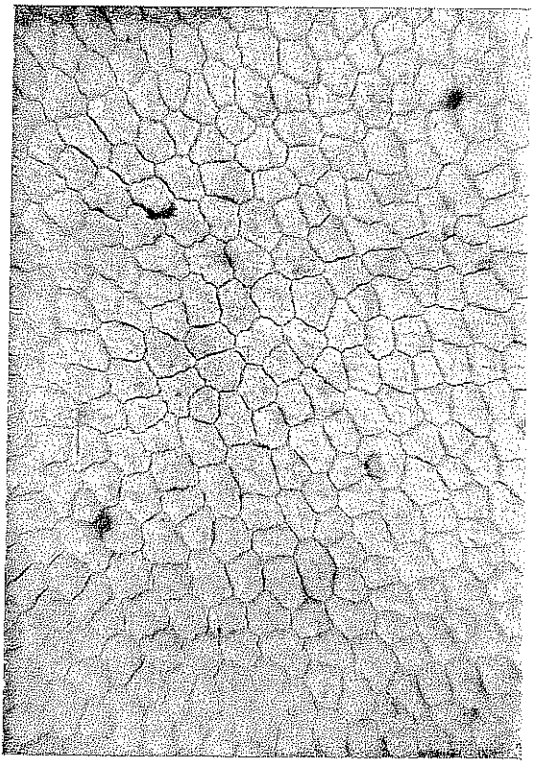

Fig. 8.