On the Special Substance that inhibits Self-fertilization.

Physiological consideration on its nature based on the results of the experiments on the fertility of Petunia violacea.¹

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I. Introduction.

It is often found that the fertilization of the eggs by sperm or pollen of the same individual is very difficult or impossible, though both the male and female gamete are morphologically perfect and functional with the complemental gamete of the other individual. This phenomenon is known as "self-incompatibility".

The first physiological expositor of the cause of self-incompatibility of plants was perhaps Darwin (1876), by whom light was thrown upon this problem.

He says in his concluding remarks; "We may therefore confidently assert that a self sterile plant can be fertilized by the pollen of any one out of a thousand or ten thousand individuals of the same species, but not by its own. Now it is obviously impossible that the sexual organs and elements of every individual can have been specialized with respect to every other individual. But there is no difficulty in believing that the sexual elements of each differ slightly in the same diversified manner as do their external characters; and it has often been remarked that no two individuals are absolutely alike. And unless there be such differentiation fertility fails."

From the chemical point of view, Josr (1907) modified this idea. He supposed that the individuals differed qualitatively in their chemical composition from one another, and the gametes of any plant possed the "individual stuff" of that plant. And he assumed moreover that the pollen tubes grew only in the tissues having a different individual stuff. But Correns (1913, 1916) opposed to this idea. He advocated by the evidence based on his genetical investigations that the substance which inhibited the growth of the pollen tube might not be the individual stuff but the "line stuff".

Setting aside the question whether this substance is individual stuff or line stuff, does such a hypothetic substance really exist? If it exists, what nature has it? In order to solve these problems a number of experiments were made with Petunia violacea in the author's

¹) This is a part of the research which is executed with the aid of The Sanno Gratitude Foundation
labatory since 1924, and the reports on various aspects of the inves-
tigations were published from time to time (Yasuda 1927–31). This paper is an abstract of these experiments together with some discussion. The full paper will be published in a near future.

II. Does the special substance that inhibits self-
fertilization exist?

Some investigators such as Scott (1865), Müller (1868) and Dar-
win (1876) reported that the pollen of some self-incompatible plants could not germinate on the stigma of the same individual. But many other workers observed that the pollen-tubes were produced freely in the style of the self-sterile plants after selfing. The pollen of Petunia violacea can also germinate on the self pollinated stigma, but it is doubtful whether the germinating percentage in this case is equal to that of the cross pollination or not. Thereupon tests on the ger-
mation of the Petunia pollen were made by means of the hanging drop culture with sugar solution containing the stigma secretion. The results of these experiments showed us that the secretion of stigma of self-incompatible individuals contained a special substance which was somewhat unfavourable for the germination of the pollen of their own plants (Yasuda & Arai 1927, Yasuda 1928a).

A slow rate of growth of the pollen tubes through the tissue of self pollinated style was suggested as the cause of the incompatibility by a number of investigators, such as Ferguson (1901), Jost (1907), Osterwalder (1910), Martin (1913), Correns (1913, 1916), Knight (1917), East (1918), Anderson (1924), Crane (1925), Shoemaker (1928), Pearson (1930), Kakizaki (1930), Kostoff (1931), etc. Many of these workers supposed that there was a special substance which might inhibit the growth of the self pollinated pollen tubes. The delay of the growth of the pollen tube was also clearly found in the style of the self pollinated Petunia (Yasuda 1929a). In order to investigate this phenomenon, the velocity of the growth of the pollen tubes in the artificial media containing the tissue juice of the style was measured. And it was found that the tube growth of the pollen of any self-incompatible individual was checked in the media containing the stylar juice of its own plant. From this result we see that the tissue juice of the styles of the self-incompatible individuals surely contains a special substance which inhibits the tube growth of the pollen of their own plants (Yasuda 1929a).

Another case of the self-incompatibility was pointed out by Namikawa (1923), Asami (1926), and Sasaoka (1928). They obtained the
evidence to show that the growth of the tubes of the self pollinated pollen was not delayed in the stylar tissue, but they noticed that these pollen tubes could not enter into the locule. The results of the germinating tests in the artificial media containing the tissue juice of the ovary of Petunia appear to us to show that the inhibiting substance exists also in the ovary of the self-incompatible individual (Yasuda 1931 a).

At any rate, we see from these data, that there exists without doubt a special substance which inhibits self fertilization in the pistils of the self incompatible plants.

III. Where does such a substance come from?

The inhibiting substance, as above mentioned, was found in every part of the pistil of the self-incompatible individual of Petunia. Where is this substance produced? Is it in every part of the pistil or in a special part of it? To answer this question, the pollen of the self-incompatible individual of Petunia was pollinated upon the grafted pistils, the styles of which were glued with gelatine upon the various kinds of the ovaries of the same species. The inspection of the results reveals us clearly the fact that the growth of the pollen tubes is affected exclusively by the character of the ovary but not by that of the style. Namely, only when both the ovaries and the pollen belonged to the same vegetative line, the inhibition was strikingly active. Thus we see the inhibiting substance is secreted mainly in the ovary (Yasuda 1931 b). And the results of the germinating tests in the artificial media containing the juice of the various parts of an ovary showed us that the inhibiting substance is abundant at the part of the placenta of the self-incompatible individual.

The data obtained by a number of workers indicates that the grade of the inhibiting action of this substance may be divided into the following three classes: 1) The pollen germination is inhibited on the stigma. 2) The growth of the pollen tubes is inhibited in the style. 3) The growth of the tubes is checked at the ovary. These differences of the inhibiting action not only appear among the different kinds of the materials, but also occur in the same kind of plant (Osterwalder 1910, Namikawa 1923, Cooper 1928). Why? The results of the

1) Osterwalder stated that the pollen tubes of the self-incompatible apple pollen grew very slowly through the self pollinated pistils, but Namikawa found, in the same plant, that the tubes of the self pollinated pollen grew as fast as those of the cross pollinated ones, and recently the slow rate of growth of the self pollinated pollen tubes in the same plant was reported again by Cooper.
experiments on the nature of *Petunia*, as above mentioned (Yasuda 1931 a), may answer this question. If the inhibiting substance which is produced in the ovary goes up and reaches to the stigma before it will be pollinated, the substance will inhibit the germination of the self pollinated pollen; if the substance arrives at the style and does not yet reach to the stigma, the tube growth will be inhibited at the part of the style, though the pollen can germinate on the stigma; and if the substance stays at the ovary, the pollen tubes of self pollinated pollen will grow as fast as those of the crossed ones and the inhibiting phenomenon will be observed only in the ovary. The experimental results of East & Park (1918) may confirm this idea. They measured the pollen tube growth in the artificial media containing the crushed tissues of the various parts of the pistil. The crushed tissue of the stigma affected on the tube growth in some cases but not in the other at all, while the affection of the crushed tissue of the ovary was always found noticeably. This may depend on the fact whether the inhibiting substance had arrived at the stigma or not.

IV. When or in what condition is this substance produced?

It was found by many investigators such as Williams (1925), East (1925, 1926), Smith (1926), Kakizaki (1930), Pearson (1930), etc., that in the case of the incompatible self pollination, the "bud pollination" increased fertility to a considerable degree. The similar result was obtained from the self pollination in *Petunia* (Yasuda 1929 b, 1930 b). The rate of the growth of the pollen tubes produced after self pollination was higher in the case of the bud pollination than in the case of the pollination in the open flowers (Yasuda 1930 b). And moreover, when the pollen was sown in the artificial media containing the stylar juice of its own individual, the growth of the tubes was faster in the juice of the bud than in that of the open flower (Yasuda 1930 b). These results show that the inhibiting substance is produced shortly before or after the opening of the flower, lacking in the young pistils of the flower buds.

It was observed and studied by several investigators, such as Darwin (1876), East (1917), and Stout (1920), that some self-incompatible plants often showed some self fertility towards the close of their flowering season. Some experiments were made on the nature of the old individual of *Petunia* to make the cause of this phenomenon clear.

1) The flowers in buds are pollinated with pollen of the open flowers of the same individual.
And it was discovered that the inhibiting substance became less or its inhibiting action became weak at the end of the flowering season. The so called "end-season self fertility" will be explained from the fact above stated (YASUDA 1930 a).

East (1917) obtained some evidence to show that the self-incompatibility was fully expressed while the plant was vigorously growing. This was demonstrated by the experiments on the self fertility of Petunia which was vigorously growing under wet-soil-condition. And it was also noted that the inhibiting action of the stigma secretion and that of the tissue juice of the pistil became powerful when the plants were vigorous (YASUDA 1928 b).

It was noted by Scott (1865), Müller (1868), Darwin (1876), Pashkevich (1925) and others that the self-incompatible plants at high temperature tended towards fertile as the temperature became low. Such a fact was often observed in Petunia in the author's laboratory. Although the failure of the germination of the pollen at high temperature may be one of the causes of this phenomenon (YASUDA & Satow 1928), it may not be overlooked that the action of the inhibiting substance is powerful at high temperature. This is proved by the germinating tests of the pollen of Petunia with the artificial media containing the juice of several kinds of the pistils of Petunia cultivated at low and high temperature.

V. What nature has this special substance?

It is proved by the experiments with Petunia, as above stated, that there is a special substance which inhibits self fertilization. But East and Park (1918) say that the special substance does not inhibit self fertilization but accelerates cross seed bearing. These two ideas seem to be quite different from each other. Which will be in the right? In order to answer this question, the relation between the pollen grains of some strains of Petunia and the special substance of the other lines was investigated, and it was discovered that the special substance of Petunia accelerated cross fertilization on one hand inhibiting self seed setting on the other (YASUDA 1931 a).

Next, we see the special substance can diffuse out through gelatine. In the grafting experiments with Petunia, as above mentioned, the style was glued with gelatine upon the ovary, and it was observed that the special substance which was produced in the ovary could go up to the style through this gelatine membrane.

The activity of this substance was found, as above stated, by means of adding the stigma secretion or the stylar tissue juice of Petunia to
the aquatic solution of sugar. This means that the special substance is water soluble.

This substance is still active in the dry matter of the water extract of the pistils and also in the dry powder of them. The materials were dried up, in this case, at low temperature. The germination and the tube growth of the pollen of self-incompatible Petunia were inhibited in the media containing these dried materials of their own vegetative line (Yasuda 1931a).

VI. Summary

The following general conclusion may be drawn from the results of the experiments done with Petunia in the author's laboratory.

1. The special substance, which is supposed by many investigators to inhibit self fertilization, can be surely found in the pistils of the self-incompatible line of Petunia violacea.

2. This substance accelerates compatible cross fertilization at least in the author's material.

3. It is secreted in the ovary and goes up to the upper parts of the pistil.

4. The part of the placenta is especially abundant in this substance.

5. When the plant is week or becomes old the activity of this substance is powerless.

6. When the plant is cultivated at high temperature, the activity of this substance becomes powerful.

7. This substance is water soluble and can diffuse out through gelatine.

8. This substance is still active in the dry powder of the pistils and in the dry matter of the water extract of them.

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安田貞雄： 自家受精ヲ妨ゲル特異物質ニ就イテ一覧はねあさかほノ受精力ニ関スル実験ノ結果ヨリ見タル此物質ノ性質ヲ生理学的考察

自家不和合ノ起ル理由ハ、自家ノ花粉ノ発芽及ビ其花粉管ノ生長ヲ抑圧スル物質ガ雛夢内＝存在スルノデアルウトハ、多クノ学者ノ想像スル所デアル。果シテスル物質ガ存在スルカ、若シ存在スレバ其性質如何。此問題ヲ解決スルガ此研究ノ目的デアル。本報ハ数年來つくはねあさかほ材料トシテ行ク実験ノ結果ノ要點ヲ記シ、多少ノ考察ヲ試ミテモデアタテ、大體ノ入ク要約スル事ヲ出来ズ。

イ 自家受精ヲ妨ゲル物質ハ明＝自家不和合ノつくはねあさかほノ雛夢＝存在スルノヲ通過スル他ノ花粉＝対シテハ刺戟作用ヲ呈スル事ヲ認メラレタ。
ハ 此物質ハ子房内＝出ラルヲ特＝胎座ノ部分＝ハ多量＝アル。而シテ斯カガカラ雛夢ノ上方＝異ツテ行ク。
ニ 此物質ハ樹勢ノ弱イ時＝老衰シタ時＝ハ其力バ弱イ。
ホ 植物體ガ高温＝栽培スラレルト此物質ノ力ガ強マル。
ヘ 此物質ハ水＝溶ケル。而シテゼラチンヲ浸透スル事ヲ出来ル。
福田八十楠：植物細胞ノ完全凍結狀態ニ関スル研究特ニ其ノ耐寒性トノ関係ニ就イテ

植物細胞ノ凍死＝関スル説ハ種々アルガ Maximov 及 Iljin イ説ト Walter イ説トノ比較スル=五=低溫スル處ガアル。Walter イ説ハ氷結或ハ乾燥ノ為メヲ起ル＝滲透圧増加ガ害シ及ポスト云フノデアルカラ、其レガ解決ヲ成ス＝メヲハ、氷結＝際シテ=如何ナル物理化学的現象ガ起ルカト云フ點ノ考察カラ出發シテケレバナラス。相律＝依ル氷点以下＝於テハ最初溶液カラ氷ガ出来ルガ、共融點デハ氷ト

溶質トノ一定混合物トシテ凝固シテ終ワウト＝ナル。其レ故細胞デハ最初ノ氷ハ

原形質退ノ外ハ出来ルガ共融混合物ハ空胞内＝出来ル。斯ウシテ完全＝凍ラテ結ツタモノデハ最早＝滲透圧等考ヘル要ハ無イノデ、之ハ恰モ種子ノ中デハガ安ラカ＝休息シテ居ル状態＝似デ居ル。種子ノ中ノ胚＝就イテハ原形質ガ完全ナル乾燥＝モ耐エ得ルト云フ例＝ Iljin ガ採用シテ居ルノデ、私ハ共融點＝於テハ完全＝凍結シクル状態細胞ノ寒害＝抵抗シ得ル凍結ノ状態トシテ種子ノ中細胞ノ状態ト並ラスペキ賞例＝デアルト提言スル。細胞液ノ共融點＝非常＝低温度＝位スルランサガ、大體=20℃ 乃至=30℃ 迄＝アリト見テ器＝ケラ良カラウト思フ。我ハ染料デハ植物細胞ハ越冬中上述ノ状態＝過スルモ有り得ルモノト思ハレル。此ノ状態ハ原形質膜外ハ水ヲハ氷ヲ余シラテ居ルシ、内＝ハリクトモ共融混合物ヲ作ル＝足ヲ欠ケノ水分

分ヲ保存シラテ居ル＝ウケデ、何時＝デモ暖クナレバ共融混合物ハ先＝液＝細胞細胞ノ水分吸収ガ可能＝ナル。足＝立派＝生き細胞ガ冬ノ越セル事＝ナル。若シ共レ如何ナル乾燥＝モ耐＝得ル植物＝ハ斯ノ様ノ説明ハ不要デアラガ、此＝＝デウ所ノモノハ乾燥＝サケシルハ死＝デガ＝而＝デサケシム＝耐エテキル普通＝高等植物＝就テイテアリ。然ラ＝如何ナル状態＝於ケルモノガ寒害ヲ受ケ死スル＝ト云フ問題＝就イテハ、＝＝＝モノ場合＝詳説シテ居ナイ＝根本條件＝ハ本文中＝＝シテク論及シテ置イタ。詳細＝＝＝モノ資料ト共＝＝＝将来発表スル＝テデアル。

（在奉天満洲医科大学＝於テ、昭和六年十一月）