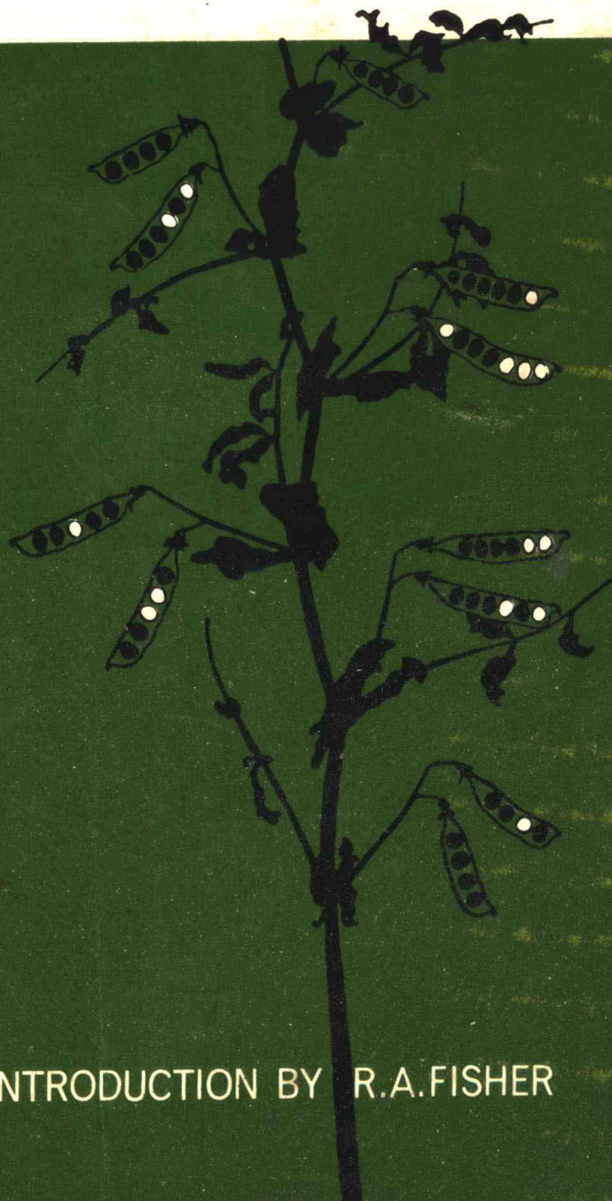
experiments in PLANT HYBRIDISATION

GREGOR MENDEL



INTRODUCTION BY R.A.FISHER

Experiments in Plant Hybridisation

GREGOR MENDEL

Mendel's original paper in English translation
with Commentary and Assessment
by the late

SIR RONALD A. FISHER
M.A., Sc.D., F.R.S.

together with a reprint of
W. Bateson's Biographical Notice of Mendel

J. H. BENNETT

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Editor's Preface

In 1955, Sir Ronald Fisher wrote an introduction and commentary for an English translation of Mendel's paper, "Versuche über Pflanzenhybriden". This was done at the request of the editor of a projected series of publications on source papers in the structure of science. But this series did not come to fruition and Fisher's manuscript has remained unpublished until now. Fortunately, Fisher's literary executors and Oliver & Boyd Ltd readily agreed that the opportunity should not be lost of publishing a translation of Mendel's paper together with this material for the Mendel centenary. In addition, it seemed appropriate to include both an outline of the more important events in Mendel's life, by reprinting the biographical notice from W. Bateson's book *Mendel's Principles of Heredity,* and Fisher's article, "Has Mendel's work been rediscovered?" (*Annals of Science* **1**, 115-137, 1936). It was this characteristically searching and stimulating examination of Mendel's paper which established Fisher's position as leading commentator on Mendel's work.

Fisher's article of 1936 contains two remarkable findings:

(i) statistical tests applied to Mendel's data show that the very close agreement between his observed and expected series is most unlikely to have arisen by chance;

(ii) there is a large discrepancy in one series of results where the observations agree closely with the 2:1 ratio, which Mendel expected, but differ significantly from the expectations corrected so as to allow for the small size of the test families.

To account for the rather sensational evidence that "the data of most, if not all, of the experiments have been falsified so as to agree closely with Mendel's expectations", Fisher suggested that Mendel was possibly deceived by an assistant "who knew too well what was expected" and that the experimental programme was probably a carefully planned demonstration of the factorial scheme which Mendel had discovered earlier.

It is of interest that H. Iltis in his *Life of Mendel* (translated from the German, Allen & Unwin, 1932, p. 209) writes:

> "I had several opportunities of talking about Mendel with an old man named Josef Maresch, the monastery gardener who died only a few years ago. Unfortunately Maresch was of bibulous inclinations (Mendel, we gather, had rather a poor opinion of the man), and this failing had played havoc with his memory. He told me that he had some of Mendel's notebooks, but was never able to produce them, and no one could find them among his belongings after his death."

On page 105 of the same work, there occurs the following passage:

> "We know from the reports of Hornisch and Nowotny that Mendel used to breed mice in his rooms, grey mice as well as white mice, crossing these varieties. We may plausibly suppose that during these experiments, undertaken perhaps more 'for the fun of the thing' than for any profoundly conceived scientific purpose, the phenomena of dominance and separation forced themselves upon his notice. Mendel himself tells us nothing about this matter, making no reference whatever to his experiments on mice."

Fisher was evidently not familiar with Iltis' biography of Mendel when he wrote the 1936 paper. The German edition of Iltis' work is indeed listed in the bibliography to Fisher's paper but the only reference to it occurs in a footnote* (*p. 76*) written after the paper was completed and apparently supplied by the late Dr J. Rasmussen. Furthermore, if Fisher had been familiar with Iltis' work, it is hardly possible that he would have overlooked the reference (p. 108) to Mendel's letter to von Nageli in which 1856-63 is stated as the period of the experimental work with peas, whereas Fisher (1936) writes (*p. 66*), "if he first grew his experimental peas in 1857, he could then be reporting on eight seasons' work. . . . On this basis, parts of the experiment can be definitely dated".

The circumstances in which Fisher came to make his examination of Mendel's results are of some interest. In a

* Page references in italics refer to the present publication.

letter to Dr Douglas McKie, one of the Editors of the *Annals of Science*, on 8th January 1936, Fisher writes:

> "I am sending enclosed a copy of my paper on Mendel which I wrote in the Christmas holidays, after getting your letter. I started writing the paper with a strong impression that innumerable people who referred to the rediscovery of Mendel's work as the foundation of modern (genetical) knowledge had paid very little attention to the paper itself, and were not aware that it presents some rather remarkable problems. I had not expected to find the strong evidence which has appeared that the data had been cooked. This makes my paper far more sensational than ever I had intended, and adds another mystery to those that have been puzzling me, some of which I think I had made some progress with. As it stands, my title is now more ironical than I had intended it to be, but I cannot help it if circumstances proceed to emphasise so strongly my main point, that Mendel's own work, in spite of the immense publicity it has received, has only been examined superficially, and that the re-discoverers of 1900 were just as incapable as the non-discoverers of 1870 of assimilating from it any idea other than those which they were already prepared to accept.
>
> "I suppose the real mystery is how science manages to make any progress at all."

Fisher's *Introduction* and *Marginal Comments* (Chapters 1 and 3 of this present book) naturally reflect the findings of the 1936 paper but it is of interest to note what, after twenty years, he has added to that account. We see that, in particular, he lays emphasis on the importance of the role of combinatorial mathematics in Mendel's work and he draws attention to the fundamental difference between Mendel's demonstration of what might be called the combinatorial independence of the separate characters and the tests required to show whether or not there is independent assortment.

That Mendel's paper warrants reprinting and so amply repays careful study one hundred years or more after it was written is perhaps sufficient testimony to the outstanding and lasting merit of Mendel's work. With Fisher's analysis and commentary added, there is an abundance of intellectual excitement and still some mystery associated with this fundamental advance in scientific thought. Amongst the many papers which now exist describing basic advances in genetical knowledge, none stands out more than these as essential reading for a proper understanding of the foundations of Genetics. In the History of Science—to which, as Fisher often remarked, more attention should be paid, especially by biologists—this material must have a very special place. Invaluable as a demonstration of the importance of statistics in genetical experimentation, these papers also help us to appreciate how in the twentieth century and in Fisher's hands, Mendel's factorial scheme provided the stimulus for the development of factorial and other experimental designs of still wider consequence. For all readers, the careful study of this material which is needed for its true understanding offers a splendid exercise in critical thinking and evaluation.

As editor, I am greatly indebted to Miss Denise Ryan for her generous assistance in checking the translation of Mendel's paper with the original, to Dr Jean Mayo for her helpful suggestions on the presentation of this material, and to the publishers and editors of the *Annals of Science* for permission to reproduce the article contained in Chapter 4.

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All royalties from this publication are being given to the University of Adelaide for Fisher Memorial Scholarships, etc., in Genetics.

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INTRODUCTORY NOTES ON MENDEL'S PAPER

Ronald A. Fisher

Mendel's celebrated paper on inheritance in the garden pea was read at two successive meetings, 8th February and 8th March 1865, of the Brünn [now Brno] Natural History Society, and was published in the following year in the Proceedings of that Society for 1865. Although Mendel's material was thus laid rather fully before a not undistinguished provincial society, and although the publication was doubtless made available to the leading Academies of Europe (Bateson verified that copies were received in London by the Royal and by the Linnaean Society), it must be supposed that it did not come under the eyes of any scientist capable of appreciating its importance, for it attracted little contemporary notice, and required to be "rediscovered" with some sensational circumstances in 1900, when three European botanists, de Vries in Holland, Correns in Germany, and Tschermak in Austria, had all discovered its existence. It had at this time the triple aspect of a confirmation, an anticipation, and an interpretation of their own researches. Almost instantly, or at least so quickly that it is difficult to discern the order of events, it was recognised that Mendel's discovery was applicable not only to plants, but also to inheritance in animals, including Man, for human pedigrees existed eminently susceptible to a Mendelian explanation.

In 1900, therefore, it was natural that scientific attention should be concentrated on a discovery of blazing importance. This was the interpretation of the phenomena of heredity, bafflingly complex as these had appeared to be, in terms of the *transmission* unchanged from generation to generation of relatively permanent units, for which Johannsen later suggested the convenient name of "genes". It is true that much work was still needed to show how widely this simple concept would be successful; with quantitative characters, for example, or with microorganisms widely different in their reproductive processes from the higher plants and animals at first investigated. The facts available in 1900 were at least sufficient to establish Mendel's contribution as one of the greatest experimental advances in the history of biology. The minority who could not adjust their ideas, only demonstrated in the ensuing controversies how great a revolution in biological thought was in progress.

For the twentieth century, therefore, in order to understand Mendel's paper, it is necessary to set aside very much that to us is obvious. We must come with a fresh mind to such questions as; What was Mendel's purpose in the series of experiments he sets forth? Or, what, at their conclusion, did he think he had demonstrated? Of the phenomena he had discovered how confident was he that they extended beyond the genus *Pisum*? If he was tempted by the notion of generalisation, were his hopes shattered by his experience with *Hieracium*, in which, in the absence of true sexual reproduction he was unable to demonstrate the Mendelian phenomena?

The eight years which he tells us his researches had occupied, are known from his letters to have extended from 1856 to 1863. Mendel had, therefore, more than a year for the preparation of his report, which was evidently a work of studious care. When he started, the evolution of organisms by progressive modification was not a burning question. In 1865 it was the central preoccupation of the biological world. It is therefore misleading to say, as Bateson does (*p. 90*) "with the views of Darwin, which at that time were coming into prominence, Mendel did not find himself in full agreement, and he embarked on his experiments . . ." as though Mendel's primary aim was to enter into the evolutionary controversy. It is improbable indeed that he had even heard of Darwin at the beginning of his work. When he came to write it up, the situation in this respect was greatly changed, and it is interesting to note the references to evolution in his paper. In the first of these he expresses the modest confidence that an understanding of inheritance had an important contribution to make towards the understanding of evolution; a process which he appears to accept quite simply as factual. Towards the close of his paper he makes a contribution, showing quite a deep understanding, in which without emphasis he shows that his views on inheritance would remove one of the difficulties which Darwin, with characteristic candour, had himself discussed. As an amateur, and a newcomer to biological research he felt perhaps that it would be unbecoming for him to drop more that this light hint.

If we read his introduction literally we do not find him expressing the purpose of solving a great problem or reporting a resounding discovery. He represents his work rather as a contribution to the *methodology* of research into plant inheritance. He had studied the earlier writers and tells us just in what three respects he thinks their work should be improved upon. If proper care were given, he suggests, to the distinction between generations, to the identification of genotypes, and, to this end, to the frequency ratios exhibited by their progeny, when based on an adequate statistical enumeration, studies in the inheritance of other organisms would yield an understanding of the hereditary process as clear as that which he here exhibits for the varieties of the garden pea. There is no hint of a tendency to premature generalisation, but an unmistakable emphasis on the question of method.

"The friendly decision of the reader" to which he appeals on this fundamental issue, was, it cannot be doubted, to be aided by his unmistakable success in explaining the phenomena he had demonstrated in *Pisum*. This, however, is very different from asking the reader to extend to other organisms the operative rules, or the notation which he had found effective in his own studies. In Mendel's view it would seem that the laws of inheritance in other plants were to be elucidated one by one by the application of the same painstaking and thorough method of which his work with *Pisum* had exhibited an example.

The two concluding sections of the paper, namely "Experiments with hybrids of other species of plants" and "Concluding remarks" are of interest in that while reaffirming his methodological principle, Mendel points out that the type of inheritance discovered in *Pisum* receives some confirmation in the preliminary and incomplete trials he had made with *Phaseolus*, and that some steps can be taken to reinterpret some of the published results of Kölreuter and Gärtner on the same principles. The sections are especially interesting in revealing Mendel's clear realisation that, especially with wide crosses, a large number of genetic factors may be expected to segregate, and that these, so far as the analogy of *Pisum* is to be relied on, must yield an enormous number of different genotypes, so that the comparatively small number of individuals that can be bred will be quite insufficient to include them all, and still less to exhibit the true proportions of their occurrence. In this respect Mendel's thought is in advance of genetic opinion in the earlier decades of the twentieth century, in which there are many examples of two- or three-factor hypotheses being set up on no stronger basis than an approximately continuous distribution of second generation hybrids, and for which only a minority of geneticists were sufficiently thorough to attempt to demonstrate, by further tests, the real existence of the diverse genotypes postulated by their theories.

The fact that Mendel was principally concerned to justify a method of investigation, and not primarily to exhibit particular results, is at least a partial explanation of another group of peculiarities of his paper, which might flow from the fact that he is reporting a carefully planned demonstration, rather than the protocol of the first observations which led to the formation of his ideas. The simplicity of his plan, and the adequacy of the numbers of the first crosses reported, are indications that he knew in advance very much what he intended to do, and what he ought to expect. He constantly omits reference to the confirmation of his first conclusions, which the later generations and other experiments reported must have supplied in abundance. Only once is he led to repeat a test. He seems never to be unsure of the sufficiency of the first evidence reported, even when it is not really so strong as might be wished, as in reporting on the independence in inheritance of his seven factors. He is acting as it were on principle, and without the opportunism with which research workers usually seek vigilantly for supplementary information.

In the bifactorial and trifactorial experiments reported in the eighth section, the only new *experimental* evidence Mendel was supplying was that on the genetic or statistical independence of the two or three factors used. Mendel's interest in these experiments was not, however, instigated by this question of statistical independence, for he seems scarcely to have considered the possibility of linked inheritance. His interest, as his exposition shows, lay in the purely mathematical or combinatorial properties of the set of genotypes made possible by two or three factors. The independence he was concerned to demonstrate was, so far as the distinction can be made, closer to a logical than to a statistical independence. His summary statement on this section is in fact (*p. 27*)

> "Thereby is simultaneously given the practical proof *that the constant characters which appear in the several varieties of a group of plants may be obtained in all the associations which are possible according to the laws of combination, by means of repeated artificial fertilisation.*"

This stress on combinatorial mathematics, which has been almost constantly overlooked by commentators, very well deserves the attention of all who teach the subject; for it is common experience that young workers are at first quite unprepared even for the task of enumerating the genotypes to be expected when several factors are segregating, and this is an obvious first step towards exploring the possibilities of a cross. More recently, indeed, in the exploration of the complexities to be expected in tetrasomic and hexasomic inheritance, combinatorial mathematics, of by no means an elementary character, has provided the only possible means of clarification.

Mendel's summary statement is also to be read as a hint to plant breeders. Practical plant improvement in this century might be described as an extensive commentary on this text.

With the understanding that Mendel's interest in the simultaneous segregation of characters was not concerned with the exclusion of linkage, several statements become intelligible.

> "In addition, further experiments were made with a smaller number of experimental plants in which the remaining characters by twos and threes were united as hybrids; all yielded approximately the same results" (*p. 27*).

If there had been serious tests of statistical independence, they would have to have been as large as the two trials reported, the latter of which obviously strained Mendel's resources to the utmost. In reality they must on the contrary have been small trials merely to check that all recombinant phenotypes were produced; as such they are quite compatible with the fact that two of the seven factors which Mendel had declared to be independent, have in later tests been shown to be linked.*

It is noticeable too that of the two tests reported on gametic ratios the first involves simultaneous segregation, and, therefore, so far as it goes, tests the possibility of linkage between the two seed characters. The second, between two plant characters, involves segregation of one factor in the pollen, and of the other in the ovules of the parents, and therefore involves no test of linkage at all. Mendel calls no attention to this difference, and, for him, it must be supposed that they were equivalent demonstrations.

The History of Science has suffered greatly from the use by teachers of second-hand material, and the consequent obliteration of the circumstances and the intellectual atmosphere in which the great discoveries of the past were made. A first-hand study is always instructive, and often, as in this case, full of surprises.

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* H. Lamprecht, who has made an extensive study of linkage relations in *Pisum*, advises me that the seven genes used by Mendel are, in present gene symbolism, 1. R; 2. I; 3. A; 4. V; 5. Gp; 6. Fa; 7. Le (cf. Mendel's list of character differences (*p. 11*)) and that these are located in linkage groups I-VII as follows:

I. A-I	V. Gp
IV. Fa-Le-V	VII. R

Only Le and V show linkage, the recombination frequency being about 13 per cent (cf. H. Lamprecht (1961): Die Genenkarte von *Pisum* bei normaler Struktur der Chromosomen, *Agric. Hortique Genetica*, **19**, 360-401).—J.H.B.

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EXPERIMENTS IN PLANT HYBRIDISATION

Gregor Mendel

1. *INTRODUCTORY REMARKS*

Experience of artificial fertilisation, such as is effected with ornamental plants in order to obtain new variations in colour, has led to the experiments which will here be discussed. The striking regularity with which the same hybrid forms always reappeared whenever fertilisation took place between the same species induced further experiments to be undertaken, the object of which was to follow up the developments of the hybrids in their progeny.

Editor's Note. Mendel's paper, "Versuche über Pflanzenhybriden", was read at meetings of the Brunn Natural History Society on 8th February and 8th March 1865, and was published in the *Verhandlungen des Naturforschenden Vereins in Brünn,* 4, 1865, which appeared in 1866. An English translation was made by the Royal Horticultural Society of London and published in volume 26 of the Society's Journal in 1901. It is the modified version of this translation as given by W. Bateson in his book, *Mendel's Principles of Heredity* (C.U.P., 1909) that is reprinted here. The footnotes and changes due to Bateson are shown within square brackets whilst the few small changes introduced with this reprinting are enclosed within double square brackets. Attention should perhaps be drawn to one of these changes. Some confusion has arisen in the past from the use of the same word " trial " for Mendel's preliminary two-year tests and for his later experimental breeding work. Here, "trial" is used to translate "Probe" with which Mendel describes his tests with the 34 varieties of peas in 1854-55; "Versuch" and "Experiment", which Mendel uses in referring to the rest of his programme, are translated throughout as "experiment".

As suggested by Fisher in his *Marginal Comments*, the sections of Mendel's paper have been numbered from 1 to 11 for ease of reference.

To this object numerous careful observers, such as Kölreuter, Gärtner, Herbert, Lecoq, Wichura and others, have devoted a part of their lives with inexhaustible perseverance. Gärtner especially in his work "Die Bastarderzeugung im Pflanzenreiche" [The Production of Hybrids in the Vegetable Kingdom], has recorded very valuable observations; and quite recently Wichura published the results of some profound investigations into the hybrids of the Willow. That, so far, no generally applicable law governing the formation and development of hybrids has been successfully formulated can hardly be wondered at by anyone who is acquainted with the extent of the task, and can appreciate the difficulties with which experiments of this class have to contend. A final decision can only be arrived at when we shall have before us the results of detailed experiments made on plants belonging to the most diverse orders.

Those who survey the work done in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations.*

It requires indeed some courage to undertake a labour of such far-reaching extent; this appears, however, to be the only right way by which we can finally reach the solution of a question the importance of which cannot be overestimated in connection with the history of the evolution of organic forms.

The paper now presented records the results of such a detailed experiment. This experiment was practically confined to a small plant group, and is now, after eight years' pursuit, concluded in all essentials. Whether the plan upon which the separate experiments were conducted and carried out was the best suited to attain the desired end is left to the friendly decision of the reader.

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* [It is to the clear conception of these three primary necessities that the whole success of Mendel's work is due. So far as I know this conception was absolutely new in his day.]

2. SELECTION OF THE EXPERIMENTAL PLANTS

The value and utility of any experiment are determined by the fitness of the material to the purpose for which it is used, and thus in the case before us it cannot be immaterial what plants are subjected to experiment and in what manner such experiments are conducted.

The selection of the plant group which shall serve for experiments of this kind must be made with all possible care if it be desired to avoid from the outset every risk of questionable results.

The experimental plants must necessarily—

1. Possess constant differentiating characters.
2. The hybrids of such plants must, during the flowering period, be protected from the influence of all foreign pollen, or be easily capable of such protection.

The hybrids and their offspring should suffer no marked disturbance in their fertility in the successive generations.

Accidental impregnation by foreign pollen, if it occurred during the experiments and were not recognised, would lead to entirely erroneous conclusions. Reduced fertility or entire sterility of certain forms, such as occurs in the offspring of many hybrids, would render the experiments very difficult or entirely frustrate them. In order to discover the relations in which the hybrid forms stand towards each other and also towards their progenitors it appears to be necessary that all members of the series developed in each successive generation should be, *without exception*, subjected to observation.

At the very outset special attention was devoted to the *Leguminosae* on account of their peculiar floral structure. Experiments which were made with several members of this family led to the result that the genus *Pisum* was found to possess the necessary qualifications.

Some thoroughly distinct forms of this genus possess characters which are constant, and easily and certainly recognisable, and when their hybrids are mutually crossed they yield perfectly fertile progeny. Furthermore, a disturbance through foreign pollen cannot easily occur, since the fertilising organs are closely packed inside the keel and the anthers burst within the bud, so that the stigma becomes covered with pollen even before the flower opens. This circumstance is of especial importance. As additional advantages worth mentioning, there may be cited the easy culture of these plants in the open ground and in pots, and also their relatively short period of growth. Artificial fertilisation is certainly a somewhat elaborate process, but nearly always succeeds. For this purpose the bud is opened before it is perfectly developed, the keel is removed, and each stamen carefully extracted by means of forceps, after which the stigma can at once be dusted over with the foreign pollen.

In all, thirty-four more or less distinct varieties of Peas were obtained from several seedsmen and subjected to a two years' trial. In the case of one variety there were noticed, among a larger ⟦considerable⟧ number of plants all alike, a few forms which were markedly different. These, however, did not vary in the following year, and agreed entirely with another variety obtained from the same seedsman; the seeds were therefore doubtless merely accidentally mixed. All the other varieties yielded perfectly constant and similar offspring; at any rate, no essential difference was observed during ⟦the⟧ two trial years. For fertilisation twenty-two of these were selected and cultivated during the whole period of the experiments. They remained constant without any exception.

Their systematic classification is difficult and uncertain. If we adopt the strictest definition of a species, according to which only those individuals belong to a species which under precisely the same circumstances display precisely similar characters, no two of these varieties could be referred to one species. According to the opinion of experts, however, the majority belong to the species *Pisum sativum*; while the rest are regarded and classed, some as sub-species of *P. sativum*, and some as independent species, such as *P. quadratum*, *P. saccharatum*, and *P. umbellatum*. The positions, however, which may be assigned to them in a classificatory system are quite immaterial for the purposes of the experiments in question. It has so far been found to be just as impossible to draw a sharp line between the hybrids of species and varieties as between species and varieties themselves.