THE PROBLEM OF THE FERTILIZATION AND EVOLUTION OF PHANEROGAMS IN DARWIN'S WORK: A CRITICAL STUDY

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Darwin has written three works on the problem of fecundation of phanerogams. These works have all been translated into Rumanian and published in full in the collection 'The Classics of Universal Science' of the Rumanian S.R. Academy Press between 1964-65. They are: *Various contrivances by which Orchids are fertilized by Insects*,[1] *Cross- and self-fertilization in the Vegetable Kingdom*,[2] and *Different Forms of Flowers on Plants of the same Species*.[3] All these three works constitute a homogeneous aggregate and, according to Darwin himself, each one is complete by itself.

We propose to analyse in these works certain aspects such as the fertility problem in phanerogams and the evolution problem of these higher plants. We also propose to devote one chapter to the problem of the methodology used by Darwin in these three works. At the same time we want to bring into the limelight the actuality of many of the theories formulated by Darwin in these three works, and the considerable interest they called forth. They opened a new pathway in materialistic biology, which was continued and widened by contemporary biology. Many of the theories elaborated by Darwin in these three works belong at present to the treasures of universal science, though Darwin's name is hardly ever mentioned.

There are, however, many hypotheses and theories which still remain unsolved. They are well worth the attention of research workers and should constitute the object of actual studies.

PART I

BIOLOGY OF THE SEXUAL PROCESS IN HIGHER PLANTS IN DARWIN'S WORK: HISTORY OF THE PROBLEM

The problem of the perpetuation of vegetable species by sexual reproduction was very little elucidated in 1839, when Darwin began his studies.[4] This problem had also been dealt with by Camerarius (1694), Kölreuter (1761–66), Knight (1791), Sprengel (1793), and in the nineteenth century by Robert Brown (1833), Gärtner (1844), Hooker (1854), up to the publication of the *Fertilization of Orchids* (1862), and subsequently by Asa Gray (1862), Hildebrandt (1864), Delpino (1867), Müller (1883), among others. The names of these forerunners or contemporaries of Darwin's are often mentioned in the above-mentioned works.

There were, in Darwin's time, very few correct and precise landmarks regarding the problem of plant fertilization. Besides, various erroneous views and dogmatic ideas were circulated freely. An instance of such erroneous views was that plant self-fertilization constituted a general law—an opinion expounded by Camerarius, which persisted unabated till Darwin's time. Others wrongly supported the free fertilization of plants of different species. In such conditions the purity of species was illusory. ' This wrong idea espoused also by Lyell, among many others, likewise circulated as a dogma (p. 257).[5] Sprengel, though he understood that plant self-fertilization is not a necessary phenomenon, failed to understand the role of cross-fertilization (p. 258).[6]

Darwin realized, especially after reading Sprengel's book,[7] that the authors who preceded him succeeded to clear up only some disparate aspects of the plant fertilization problem.[8] They failed to see a connection between the functions and structures of flower parts, and this implication on the visiting insects. They failed to use adequate methods in the study of the plant fertilization process. Therefore, according to Sachs, though a theory on plant fertilization had begun to be elaborated on scientific basis, only at the beginning of the nineteenth century it progressed with extraordinary slowness.[9]

Darwin looks for and finds his own way in elucidating the plant fertilization problem, a problem which he studied methodically, publishing during a period of more than thirty years of study several small works, especially in connection with the fertilization of orchids[10] and of heterostyled plants.[11] He experimented uninterruptedly and offered a sound basis to the facts mentioned in his works, thus succeeding in disposing of the wrong ideas, in eliminating the lack of precision and dogmatism, as well as aprioristic arguments and generalities based on an insufficient number of facts which resulted from experiments sometimes carelessly carried out.

The hazard played its part in the discovery of biomorphological differences in the offspring of self- and cross-fertilized plants with homo- and hetero-styled flowers, as well as of fertilization mechanisms of orchids.

Darwin's son thought that his father was concerned with the study of orchids, as these were the common species around Down. This is the first occurrence. The second would be the fact that Darwin succeeded, in 1860, to discover the way pollinic masses of *Orchids* are adapted and how their transport by insects to other flowers is effected. The solution of the problem of impeding self-fertility by the adaptation of the flower parts which support the stamens, on the one hand, and the manner pollen is transported by insects, on the other, has become 'one of the most familiar and striking facts' of the work on the fertilization of orchids.[12]

The subject of cross- and self-fertilization incidentally asserted itself on Darwin as a result of his direct observations on the effects of the two manners of fertilization, in two beds of *Linaria* *vulgaris* of his garden. The differences astonished him. Darwin had never found in the literature he had read and neither had he thought before that the two modes of fertilization would yield such great differences.

The following year he repeated the experiment on carnations, cross- and self-fertilized, and likewise obtained considerable differences between the seedlings from seeds thus fertilized.[13]

In 1838 or 1839 Darwin incidentally observed the dimorphism of the flowers of *Linum flavum*. He thought at first that it was a case of unimportant variation. 'But on examining the common species of *Primula* I found that the two forms were much too regular and constant to be thus considered . . . I think that nothing has given me such satisfaction in my scientific life as the fact to have explained the structure (of the flowers) of these plants.'[14]

In his *Origin of Species*, Darwin did not deal with the problem of plant fertilization. When he published this work he had already been studying this problem for 15 years, having succeeded to amass valuable data on plant fertilization. Darwin only devotes a single phrase in his *Origin of Species* on cross-fertilization.[15]

Thus, even for Darwin, the problem of plant fertilization represents a new problem, different from those studied in the *Origin of Species*, but closely correlated to it. The connection between these works is made by the application of the law of selection, and proves that the most insignificant structures of the flower are modified by the influence of this law. By the biological aspects they present, the three works constituted a splendid, concrete demonstration of the role of selection, contributing to a large extent to the consolidation and development of the evolutionist conception. The effects of this work was enormous, imparting a creative stimulus to new investigations.[16]

From the theoretical point of view, *Various contrivances by which orchids are fertilized by insects* is the first work published after the *Origin of Species* (1862), thus in the midst of Darwin's struggle for imposing his evolutionist conception. It influenced considerably the mind of biologists, gaining many adherents and reducing the number of his opponents. On the one hand, *Various contrivances* . . . stimulated research works on the problem of flower fertilization. The beginning was made by Darwin's friend, Asa Gray, in 1862 and 1863. Gray was followed by well-known botanists, such as Hildebrandt (1864), Moggdridge (1865), Delpino, Müller among others.

The Effects of Cross-fertilization, which, even according to Darwin, represents a completion to the *Fertilization of Orchids* (Nora Barlow: Autobiography . . . , p. 133, Rumanian P.R. Press, 1962, p. 152), just as does *Various Forms of Flowers*, have had a great impact. In the bibliography of Müller's work *Die Befruchtung* (1883), 814 works on plant fertilization are mentioned, which had appeared in the 20 years since the publication of the *Various contrivances*.[17] Works continued to appear also after 1883 up to the present date.

Darwin's son maintains that by these works Darwin has created a new branch of biology. Though not going so far, we deem it not exaggerated when it is affirmed that the three works of Darwin have had a very considerable influence on the biologists of the second half of last century and of the present one, and that they contributed very much to the theoretical and practical development of materialist biology by the brilliant elucidation of the complex aspects of the sexual process in plants and, within the framework of this process, of numerous problems connected with flower fertilization.

Asa Gray, Bentham, Oliver, Hooker, Wallace, Moggdridge, Delpino, Müller, Hildebrand among others approved of the theories propounded in the three works, though Bentham (the President of the Linnean Society), for instance, was not a supporter of evolutionism. On the other hand, Lyell stated that 'after the *Origin of Species*, the *Various Contrivances* . . . is of the greatest value'. Even reviews of an ecclesiastical character, such as the *Saturday Review*, *Literary Churchman* among others, eulogize the work.[18]

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In the decennia which have passed since the publication of Darwin's three works, biology has achieved a tremendous progress. The present direction of studies concerning fertilization involves inbreeding and sexual hybridization with another line, variety or species; though in the meantime what is now called 'molecular biology', cytogenetics and the genetics of populations developed and very great importance has been given to biostatistics. These are new directions of investigations, particularly important for our present level of knowledge. On the occasion of the centenary of the '*Origin of Species*', Whitehouse has presented a critical exposition of Darwin's conceptions contrasted with the present ones. We consider this multilateral parallel as particularly valuable, and in the course of our study we shall have the opportunity of quoting it several times. Whitehouse's exposition is entitled 'Cross- and Self-Fertilization in Plants'.[19] He has further worked on the evolution of angiosperms as interpreted in the light of formal genetics.[20]

We have the impression that in this first work of synthesis Whitehouse has lent great weight to the conception of formal genetics and only gave a minor place to Darwin's concept. There are, without any doubt, but very few relationships between Darwin's concept and genic theory. There is no question about any interrelationship between the two conceptions, as the theory of pan-genesis has no circulation value in present-day genetics. We are under the impression that the importance imparted to Darwin's conception in present-day biology is much too small. From this absence of a confrontation between the two conceptions present-day biology is but all the poorer. On the other hand, if we were to make a critical parallel between Darwin's biological concepts and the present ones, we should necessarily have to repeat many of the arguments presented by Whitehouse. Or such repetitions would lead us away from the purpose we proposed when we wrote this critical study as an introduction to the Rumanian translation of the three works of Darwin on the fertilization in plants and the evolution of phanerogams.[21] *

As a matter of fact, we undertook a much more modest task, namely to render certain aspects of Darwin's concept evident, which are still actual and which so far have not been studied by other biologists, as far as we can judge

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* In the present translation we have left out certain portions and added, on the other hand, several phases, as well as some new passages. The translation of the *Critical Study* was made by our colleague Eugen Margulius, who translated from English into Rumanian five of Darwin's works, printed by the Academy of the Socialist Republic of Rumania.

from the bibliography at our disposal. They relate to the problem which may be perceived like a red thread* throughout Darwin's entire concept about the developmental directions of his research work in the problem of plant fertilization, about the methodology used in these experimental works, about the taxonomical directions in the evolution of phanerogams, as it results from the three works analysed by us. Often these aspects are passed over in silence. Without underestimating the present progress in biology, we remind the reader, as Whitehouse also shows, that the problems of inbreeding and self-sterility constitute the basic elements of present-day biology. In these problems last century's level was surpassed to a great extent.

Our firm impression is that by his so thorough and vast research works, Darwin has tried to fight generalizations and absolutenesses devoid of a real basis, which his predecessors or contemporaries put forward in the problem of the perpetuation of species by sexual process. It would seem that, in order to avoid perpetrating the same error, he elaborated an experimental method and created a generalization instrument, such as the inductive-deductive method. This method is of great biological and philosophical value likewise for our present-day biological conceptions. We therefore deem that the way Darwin organized his research work, and the way he succeeded in generalizing his results, may constitute even nowadays a model which is not in the least obsolete. This model is worth being used by present-day biologists and philosophers when wishing to give a solid theoretical basis to the experimental results obtained by new biological methods and conceptions. Knowing and applying inductive-deductive methods are essential, as unfounded generalizations and absolutenesses may nowadays still be met with, sometimes in disquieting proportions.

* We shall quote one single instance regarding the importance of the study of interrelations between parts. Nowadays a greater and greater attention is paid to the relationship between nucleus and cytoplasm, under various aspects. In present-day studies, an important place is occupied by the nucleic acids and proteins syntheses, as well as by the passage of RNA and proteins from nucleus into cytoplasm. The forerunners of DNA are synthesized in cytoplasm in the *S*-stage of the interphase (Gelfant), and from there pass into the nucleus. In experimental polyploid condition simultaneously with the increase in the number of chromosomes, grows the volume of the nucleus and often that of cytoplasm. The nucleoplasmatic relationship is modified. At the same time also grows the number of chloroplasts (Dudley, *Agronom Journal*, 1958, V. 50, No. 3), and that of mitochondrial elements. The way the variations of nucleoplasmatic relationship consequent of quantitative growth of cytoplasm and implicitly the consecutive increase in the number of chloroplasts, mitochondria, etc., affect the polyploidization mechanism (and reversely) has been but very little studied. We believe that these interrelations are particularly important for a right understanding of the polyploidization phenomenon; they would deserve a closer study by quantitative biological methods. Or, what is shown above fits well in between the interrelations between parts, a problem which Darwin dealt with most extensively to the great advantage of theoretical and practical investigations in the field of biology.

The directions followed by Darwin in the study of the problem

In the three decennia during which he had studied plant fertilization, Darwin succeeded in going thoroughly, in a complex way, into this problem. The analysis of the three Darwinian works devoted to the problem of fertilization enabled us to see not only the amplitude given by Darwin to the studies concerning sexual multiplication, but gave us also the possibility to concentrate and systematize the theories scattered throughout the three works, and to detect the directions used in the study, and the methodology employed.

By this analysis we will be able to present unitarily Darwin's conception of the problem of biology in the plant sexual process.

The directions of the study in these three works have many new aspects not only for the time when Darwin was elaborating his works, but also for contemporary biology, and might be grouped together, according to us, in the following ways:

1. Darwin has studied: (*a*) the biology of plant *fertilization*; within the framework of this problem, he studied like no one before him, (*b*) the problem of *hermaphroditism* in phanerogams as representing (*c*) a form of the *perpetuation of plant species* by sexual process. In the development of these three aspects of the sexual process in plant fertilization, hermaphroditism and perpetuation of species—Darwin has brought many proofs and has propounded thoroughly new theories, also valid for the contemporary period.

2. Darwin wanted to prove how *natural* and *artificial selection* interfered with functional adaptation correlations of flowers and visiting insects, on the one hand, and with the adaptation of the plant and its flowers to physical medium, on the other. In this way Darwin's works on plant fertilization complete the knowledge of the general and special action modalities of the law of selection.

The entire work regarding the fertilization of orchids may be considered as a brilliant demonstration of the interrelations between visiting insects (as a morphogenous agent) and the different flower parts. The species of orchids adapt themselves to the visits of certain insects belonging to several orders or families (e.g. diptera, coleoptera, hymenoptera, etc.), or become specialized for a certain species of visiting insects. In the latter case, should the fertilizing insect species disappear from the area of the plant, the respective species of orchids would likewise disappear—and vice versa. Thus, for instance, the *Angraecum sesquipedale*, a Madagascar species of orchids, has an extremely long nectary (11 inches) and the nectar is situated only at the bottom (p. 265). Only the large lepidoptera (and such insects are not lacking in that island) can introduce their very long proboscis down to the bottom of the nectary (p. 163).

Among lepidoptera, only the *Sphingidae* seem to correspond to the type of butterfly with an excessively long proboscis. Bourgogne states that the *Sphingidae* are 'particularly well adapted to flowers with deep corollas, whose nectaries cannot be reached by most of the other insects'. He mentions the case of species *Amphimoea walkeri* B., a large neotropical *Sphingida* whose extended proboscis measures 28 cm., 'almost four times the length of its body'.[22]

The interrelations between flowers and visiting insects are very close in heterostyled plants, as it results from numerous passages from these three works. Such interrelationship is not lacking in flowers with irregular corollas either, such as *Lys gloriosa* and *Scrophularia aquatica*. In the former, the curious and irregular form of corolla was differentiated, according to Darwin, under the influence 'into the circular route which follow insects in proceeding from one nectary to the other'. While in the second example the downwards bent pistil 'touches the pollen powdered chest of visiting wasps'.[23]

From the very thorough study of interrelations between flowers and insects, Darwin draws the conclusion that 'in all these cases we see the supreme dominating power of insects on the structures of flowers'.[24]

This is but one aspect of the problem. Especially in *Fertilization of Orchids* . . . Darwin proves that the modification of flower parts is also subjected to another imperative, namely the disfavouring of self-fertilization and favouring of cross-fertilization. In plants with heterostyled flowers this effect is likewise strengthened by the relative or absolute sterility of illegitimate fertilizations.

3. Darwin, in his three works, has allotted much importance to the *morphology of flower parts*, which were adapted according to the nature of the visiting insects and to the possibilities offered by living conditions. In order to prove the morphological modifications, Darwin attentively studied various mechanisms by which cross- and self-fertilization of orchid flowers and of plants with homostyled flowers are realized, as well as the particular aspects presented by illegitimate fertilization of di- and tri-morphous plants.

The morphology of floral parts was described not statistically but functionally. In orchids Darwin has described the structures of column, caudicles, rostellum, pedicles and stigma of viscous disc, of viscous substance and its variations, as depending on the very distance which may separate two plants, likewise of the way this viscous disc fits in and is detached. The structure and peculiarities of pollinia, of the application of the principle of the economy in the elaboration of pollen grains, of the number of seeds in each capsule, in each of the more than 150 species of orchids were studied in this book. He was engaged in determining the insects which fertilize each species; the percentage of orchid flowers fertilized in natural conditions (p. 33). He dealt with the species of visiting insects, which may sometimes be absent or in insufficient numbers. He studied the relationship between the number of pollen grains and fertilization process,[25] the development of self-fertilization mechanisms, secondary mechanisms grafted on the cross-fertilization ones.[26] Discussing the structure of orchid flower parts, he expounds the problem of flower part homologies in this great family (Ch. VI–IX).

In *Various forms of Flowers* Darwin unravels (by studying the functional aspect of the process) the morphological peculiarities of heterostyled flowers; legitimate and illegitimate fertilization modalities; the fertility or sterility percentage in both.

He succeeded to show the peculiarities of this group of hermaphroditic plants. His studies are, even today, a brilliant example of comparative, determinist biomorphology.

The dialectical connection between structure and function is masterfully proved by Darwin in these three books of his.

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4. On the basis of morphology and orchid fertilization mechanisms by means of insects, Darwin establishes new *systematic classifications* for certain species of orchids, which are still valid in our times, e.g. the systematic position of species *Perystilus viridis, Habernaria chlorantha* and *H. bifolia.* Darwin studied critically the tribes *Epidendrae* and *Malaxeae* (ch. V), clears up the problem of genus *Catasetum* (ch. IV); he studied the filiation of *Dendrobiae, Epidendridae* and *Vandeae,* starting from the form with old characters of *Pleurothalidae* (ch. IX). He also studied the filiation of *Neotteae* towards *Ophreae,* etc.

Part II

Methodology employed

One century ago, Darwin employed in the execution of his works a completely new, multilateral methodology, in many respects different from that used in other works. In all his three works, the experimental and the verification as well carry a very great weight. This has given him the possibility of avoiding some hasty conclusions based on insufficient, non-biological, unverified experiments, arrived at by some investigators, who had dealt with plant fertilization before him.

Darwin has studied the morphological, functional and biological aspects of fertilization in phanerogams. He used the comparative methods of logic also to arrive at establishing the real characters of the fertilization process; he used—for the first time in the history of biology—on an exceptionally wide scale the *inductive-deductive method.*

Having in view the great complexity of the studied aspects, Darwin had to create working methods, where they were lacking, or to interpret and substantiate his experimental conclusions, regarding the functional and biological aspects of fertilization by means of statistical methods at a time when biostatistical methods had not yet been applied by any other biologist.

The vastness of this methodology is even at present very impressive. Some of the pathways opened by Darwin are surpassed, but they have nevertheless a great historical value.

We failed to find in literature any analysis of the methodology of Darwin's works. We shall, therefore, try to outline in a somewhat greater detail and—as systematically as possible—the methodology used by Darwin. We draw the reader's attention to the fact that the experimental methods employed by Darwin vary from one work to another, proceeding in fact along two lines: the first is that of *the effects of cross- and self-fertility,* and the second is that of *pollenization mechanisms.*

1. *Interrelationships between function and structure* imply in these three works:

(*a*) The study of the morphology of reproductive flower parts, and occasionally of sepals and petals.

(*b*) The precise determination of fertilization mechanisms in orchids and in plants with heterostyled flowers, as well as the great variety of these mechanisms, particularly in orchids.

The mechanisms of cross-fertilization have been dealt with very complexly in the three of Darwin's works. In his *Effects of Cross-fertilization* . . . Darwin mentions and uses proterogyny and proterandry. In *Different Forms of Flowers* . . . he points out the modalities and transformations, such as the rotation of stamens within corollas (like in the short styled *Faramea* flower), adaptations which facilitate legitimate fertilization in heterostyled flowers.

The most complex fertilization mechanisms, however, were found by Darwin in orchids. Some of these modifications in the male and female flower parts become differentiated, so that they serve as direct or indirect 'guides' to insects towards nectaries (for instance in *Listera*, *Stanhopea*, etc., flowers). But at the same time, the respective flower parts differentiated structures which impede self-fertilization.

Similarly under the influence of selection a viscous secretion of rostellum cells is being differentiated (e.g. in species of genera *Orchids*, *Habenaria*, *Oncidium*, etc.). This viscous matter is of great importance in cross-fertilization. Its hardening occurs quickly in plants which have free nectar in the nectary cavity (e.g. in some species of *Ophreae* studied by Darwin).

In plants with nectar secreted within the intercellular nectary spaces (as in the case of the flower of the leguminous *Edwardsia* and of the flowers of some orchids like *Orchis morio*, *Orchis maculata*, etc.), the insects must perforate the nectary walls for sucking the nectar. In this case the viscous substance hardens very slowly.

Another mechanism, which appears often at the same time with those described above, is that of the opening of the anthers and the projecting of pollinia. In these cases, Darwin has discovered the existence of some very susceptible points on the column or on other parts of the flower, which, once touched, set off the projecting mechanism of pollinia on to the proboscis, head or chest of the insect to which they adhere strongly. This increased susceptibility has all the attributes of the reactivity of animals, though, differing from it. Such mechanisms, for instance, have been described in *Mormodes ignea* and in many other species of orchids.

In other species of orchids the detaching of pollinia is effected by other mechanisms. Once detached and fixed to the proboscis or head of the insect, pollinia could not fertilize another flower unless it modifies its initial position. Only in this way can they be placed correctly on the stigma of another flower. Also by selection some modification mechanisms of the initial position of the pollinia caudicles were differentiated. Some mechanisms are exhibited by the elasticity of the pedicel (e.g. in certain species of *Vandeae*). In other orchids the same effect is induced by their dehydration in contact with the air (e.g. in *Gymnadenia conopsea*), and at their re-expansion to their initial position in the case of their rehydration.

Fertilization mechanisms and their effects are thoroughly verified in relation to number, weight and germinating power of seeds,* and to the growth of the offspring resulting from cross- and self-fertilization in the *Effects of Cross-fertilization* . . . and in *Different Forms of Flowers*.

* For instance in *Mimulus luteus* the weight of the seeds resulting from cross- and self-fertilization in the second year of experiment is of 100/34. In *Nolana prostata* the same relationship is of 100/2, etc. The greater weight of the seeds resulted from cross-fertilization represents, besides, other elements favourable to the development of the plant, is the possibility of its growing more rapidly in the first development period and in overcoming in vital competition plants poorer in reserves or with poorer vitality resulting from the self-fertilization of the same species.

TABLE

List of orchids and of visiting insects

Species of orchids	Species of visiting insects and Author
Gymnadenia odoratissima	Moth (H. Müller, p. 68)
Habenaria chlorantha	Moth, large butterflies and bees (p. 30)
Gymnadenia conopsea	Night lepidoptera: *Anaites plagiata*, *Triphaena pronuba*, *Plusia chrystis* (G. Darwin, p. 67)
Disa polygonoides	*Bombylius* (Barber, p. 78)
Disa macrantha	Moth (?) (Veale, p. 290)
Pterostylis trullifolia	Diptera (Cheessman, p. 88)
Acianthus sinclairii	Diptera (Cheessman, p. 90)
Vanilla aromatica	Undermined insects of the tropical region of South America (pp. 90-91)
Epipactis palustris	Bees (Fr. Darwin)
Epipactis latifolia	Diptera (Sprengel); *Vespa sylvestris*; wasps (Darwin, pp. 101-102 and 282)
Epipactis purpurata	Wasps (Oxenden, p. 102)
Epipogium gmelini	*Bombus lucorum* (Rohrbach, p. 103)
Goodyera repens	*Bombus pratorum* (Thomson, p. 105)
Spiranthes autumnalis	Humble-bees (Darwin, p. 114)
Listera ovata	Small hymenoptera (Sprengel); *Haemiteles* and *Cryptus* (Darwin, p. 120)
Listera cordata	Minute diptera and hymenoptera (Dickie, p. 125)
Neottia nidus-avis	Diptera (H. Müller, p. 125)
Vandeae with large flowers	Large insects (Darwin, p. 190)
Oncidium	Humble-bees (Wright, p. 156)
Angraecum sesquipedale	Moths (*Sphingidae* ?) with long proboscis (Darwin's hypothesis, p. 282)
Stanhopea	Bee *Euglossa* (Crüger, p. 171)
Coryanthes macrantha	Bee *Euglossa* (Crüger, p. 175)
Catasetum tridentatum	Large insects (p. 188)
Cypripedium pubescens	Small bees (Asa Gray), *Andrena parvula* (Darwin, p. 282)
Cypripedium calceolus	Species of *Andrena* (H. Müller, p. 231)
Orchis pyramidalis	27 species of lepidoptera (Bond, pp. 30-31)
Orchis morio and Orchis maculata	Bees (Darwin, p. 41)
Platanthera chloranta	Night lepidoptera (*Nisoniades*) (Asa Gray, p. 70)

(*c*) The determination of visiting insects (*see* table on p. 80) and of the modalities of getting hold of pollen of having it adhered to the proboscis, head or body of the insect and of its transport on to the stigmas of other flowers on the same plant or on another plant of the same species or from one heterostyled form to another.

(*d*) Establishing the density of visiting insects in the respective area, estimated by the number of fertilized orchid flowers, and the consequences of the absence of the insects, when unfavourable climatic conditions appear or when the species of visiting insects are absent or do not fertilize the flowers or orchids transplanted into a new area.

In the final part of Ch. IX (pp. 291 and ff.), Darwin describes the phenomenon of self-fertilization in a fairly large number of orchid species. Self-fertile species appear isolately in genera which multiply by cross-fertilization. Only in genus *Thelymitra* two species had been observed; in all the others, one single species (e.g. in *Cephalanthera, Neotinea, Listera, Ophrys, Disa, Orchis, Epipactis, Gymnadenia, Platanthera, Epidendrum, Spiranthes,* and *Dendrobium,* etc.), Darwin comes to the conclusion that previously these species were fertilized by insects and that because of their absence their flowers failed to produce seeds and were heading towards extinction. In these conditions—under the influence of selection—the species were gradually modified until they became more or less self-fertile (p. 292).

2. *The detailed study of the entire group of plants.*—In each of the three works, Darwin studied another aspect of plant fertilization problem, though applying the same method to all three:*

(*a*) The study of the entire orchid family.

(*b*) The study of several phanerogamous families for determining the effects of cross- and self-fertilization.

(*c*) The study of numerous species of phanerogams known at his time as heterostyled.[27]

It may be considered that Darwin tried to present a very strong and convincing documentation in each of these three works.

(*d*) Often, the study of the flowers of certain exotic plants, not to be found in Great Britain, appears necessary. In order to complete the study of the entire group, Darwin carried on a vast correspondence[28] and obtained numerous informations and fresh (especially orchids) or dried flowers (mainly heterostyled). He requested his voluntary collaborators to verify locally certain suppositions.[29] By these means, Darwin succeeded in completing his informations regarding the respective problem.

3. (*a*) *Statistico-mathematical methods.*—He sets individual results in comparable series and determines absolute and percentual averages for each group of experiments, in order to establish, on the basis of this partial average, the behaviour of the entire experimental group.

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* Darwin has studied, completely or partially, eight tribes and nearly 150 species.

Galton was one of those who established modern methods of biostatistics. Darwin asked him to determine the validity of his conclusions by statistical methods, the averages obtained from cross- and self-fertilization in plants of various families, such as *Ipomoea*, *Digitalis*, *Reseda lutea*, *Viola*, *Limnanthes*, *Petunia* and *Zea*. The self- and cross-fertilized plants were of the same age, descended from common parents, which had been subjected, from the beginning to the end of the experiments, to the same living conditions. Experiments were carried out on small series. Galton renders a favourable view on the kind of experiments carried out by Darwin (*Effects of Fertilization . . .*, pp. 15–18). In Tables A, B, C (pp. 240–252 of the same work), the number of experiments carried out on cross-fertilized plants (inbreeding and crossing with a new line), amount in all to 1,101, of the self-fertilized ones to 1,076, carried out on 57 species and traced sometimes for several generations. These figures are large enough to form the basis of valid and significant conditions.

Darwin groups his results systematically on categories; then draws conclusions for each category and then proceeds to generalizations.

In his work regarding heterostyled plants, Darwin determines the number of modalities of legitimate and illegitimate fertilization in the case of di- and tri-morphic plants, the minimum number of flowers which have to be fertilized for obtaining valid results (Ch. II.).

Indexes taken into consideration are very numerous and complex. Of them we mention: rate of growth and vigour of plants, number of capsules, average number of seeds per capsule, weight and sometimes their size, germination power and some others. The number of tables with results is particularly large in the *Effects of Cross-fertilization* and in *Different Forms of Flowers . . .*

(*b*) *The comparative methods* (methods of simultaneous concordance, difference and variations), which are foreseen by logic whenever the establishing of causal connection between phenomena of whatever nature is necessary. Darwin permanently employed these methods in estimating the effects of cross- and self-fertilization in *The Effects of Cross-fertilization . . .* and in *Various Forms of Flowers* and very seldom in *Various Contrivances.*

For establishing comparative data, the tables from *The Effects of Cross-fertilization . . .*, as well as from *Different Forms of Flowers*, are grouped together in small categories. The average values of the respective group are always established and the absolute and percentage values of one group are compared with the analogous values of the other group. In no table does Darwin deviate from this method. On account of this, some passages in these two works become wearisome by the presentation of certain analogous experiments. Though Darwin was conscious of this effect, he never deviated from the method he set down as his working rule and which helped him to the comparative interpretation of results.

(*c*) *He determines on a statistical basis the growth or decrease coefficient of the vitality of descendants* issued from cross- and self-fertilization—descendants which he keeps under observation for several generations—grown in hot house, and sometimes, comparatively, also in open field. Darwin thus determines their comparative vigour, their behaviour in moderate and hard vital competitions, their resistance to climatic conditions (such as their resistance to frost, etc.).

Seedlings of *Mimulus, Digitalis, Brassica, Viola, Ipomoea, Iberis, Petunia, Sarothamnus, Zea*, etc., resulting from cross- and self-fertilization have been subjected to weak and severe competition (a small or a large number of plants shown on the same side of the pot or after another plant which had exhausted to a great extent the soil, etc.).

At other times, parts of the plants were kept in the hot house, while the rest were sown in beds and kept in the open during winter. In the case of the *Mimulus* seedlings of the eighth generation, as well as in that of other plants, Darwin compared in the above conditions the effects of fertilization with a new line. The seedlings rssulting from seeds of a cross with a new line resisted better to the severe frost of the winter; the self- and cross-fertilized (by inbreeding) perished.

He obtained similar results, though less numerous, by comparing legitimate and illegitimate fertilization. In all these circumstances self-fertilized plants proved weaker and less resistant than the cross-fertilized ones. In these cases, Darwin has actually reproduced the conditions met with by plants in nature, in severe biological competition, or when exposed to climatic, telluric factors, and so on. Darwin's conclusion is that seedlings resulting from cross-fertilization are more resistant than those resulting from self-fertilization. 'Considering all the cases (in the Table C) there can be no doubt that plants profit immensely, though in different ways, by a cross with a fresh stock or with a distinct sub-variety' (*The Effects of Cross- and Self-fertilization*, p. 269). Also he says: 'In my volume *On the Origin of Species* I gave only general reasons that it is an almost universal law of nature that the higher organic beings require an occasional cross with another individual, . . . Having been blamed for propounding this doctrine without giving ample facts, for which I had not sufficient space in that work, I wish here to show that I have not spoken without having gone into details' (On the *Fertilization of Orchids . . .*, Introduction, p.1).

(*d*) *The introduction* of quantitative methods in biology (*Quantitative Biology*), Whitehouse asserts that Darwin's studies regarding the effects of fertilization of *Ipomoea* and *Mimulus* represent 'the first quantitative studies on self-fertilization and cross-fertilization which have ever been made' (p. 228). And further on that 'by his quantitative studies, Darwin has taken the first step towards solving the problems of the effects of inbreeding, and out-breeding' (p. 234). Whitehouse's assertions are correct, and we agree particularly, with the latter one. Statistical method, employed by Darwin on a large scale, is met with in 'Variation of Animals and Plants under Domestication', in 'Insectivorous Plants', in 'Cross- and Self-fertilization in the Vegetable Kingdom', in 'Different Forms of Flowers' and likewise in other works. Based on this we may affirm that it was Darwin who has laid the foundation of what is called nowadays 'Quantitative Biology', by showing us, by fairly simple methods, the necessity of computing quantitative biological indexes in the study of the action of selection in that of the effects of the different crossing modalities, as well as in other cases. No doubt that nowadays the methods of measuring different variations used by Darwin are obsolete, that considerable progress has been achieved in the field of mathematical statistics, and that quantitative biology has enormously improved, as well as the use of most perfect quantitative methods. Darwin's role as a pioneer in the field of biostatistics and quantitative biology should, we deem, be remembered, and that role should not be allotted to others, when the history of the present tendencies in biology will be written.

4. *Interpretation of results.*—In view of the interpretation and generalization of the huge mass of data, Darwin employs the following methods:

(*a*) The comparative method presented above.

(*b*) Results grouped by him in what we know are called 'experiment classes' (categories) and interprets them by the results obtained. Thus, for instance, from grouping there results:

In *Effects of Cross-fertilization.*—We may conclude that Darwin establishes the following classes (categories) of results:

—cross-fertilization with a new line, with the best effects from the biological and morphofunctional points of view;

—inbred cross-fertilization, with much weaker effects than in the first category, but generally better than the following one;

—self-fertilization with different degrees of self-sterility;

—self-fertilization with a poor effect but without self-sterility or with the preservation of a satisfactory biological effect in the case of cleistogamic flowers;[30]

—self-fertilization with recovery of a vitality higher to that of inbred cross-fertilization, but also without self-sterility, approaching the effects of crossing (though self-fertilization with vitalizant effect requires now and again a cross-fertilization).

In *Different Forms of Flowers.*—Darwin establishes the following form classes (categories):

—dimorphic heterostyled forms;

—trimorphic heterostyled forms;

—various transitional forms in different senses.

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The above categories resulted from his numerous experimental data.

Darwin continued his experiments for many years—sometimes up to 11 generations—in some of the cases presented in *The Effects of Cross-fertilization . . .* In these cases experiments were made on the direct descendants, similar and well selected, with equal germinating power. The offsprings resulting from cross- and self-fertilization were grown in exactly similar conditions as regards soil, humidity, heat, light, alike their parents.

Darwin has known the *summing up* of effects from one generation to another. This summing up, however, is not uniform. The initial depressive effect may suffer a relative stabilization in certain experimental cases of self-fertilization, and in some normal cases of prolonged self-fertilization as, for instance, in *Ophrys apifera*, as well as in the cases of inbred cross-fertilization. This stabilizing effect is likewise observed in cases if inbreeding repeated for many generations as in the case of *Bos primigenius*, maintained for several centuries in a half-wild state in the Chillingham Park of England. Though much degenerated in size as against the primitive neolithic species (*Variation of animals and plants* . . . , p. 99).

Self-fertilization may also lead to the reappearance of vigour by the formation of such varieties as the '*Hero*' (in *Ipomoea*) and so on. Bad, depressive effects are obtained not only by self-fertilization, but also by cross-fertilization, as well as by illegitimate fertilization between heterostyled flowers. Sometimes cross-fertilized inbreeding and illegitimate fertilization may lead to effects of absolute sterility or 'Inzucht', much quicker than self-fertilization.

The only constant effects with the preservation of an increase in vigour are produced by cross-fertilization with a new line.

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The problem of self-sterility occupies an important place in Darwin's three works which are analysed in the present study. Since these works had been published, the problem of self-sterility has considerably evolved in many directions up to the present day, therefore it may be stated that, as a problem, self-sterility has an exceptional theoretical and practical importance for contemporary biology. Whitehouse shows clearly the new developmental directions of the self-sterility problem. We shall, therefore, only point out a few points regarding the developmental directions of the self-sterility problem, for instance the problem of pollen incompatibility and of the self-incompatibility in cases within the *Parthenium-Crepis* and *Nicotiana-Veronica* systems. Likewise, the graduation of the phenomenon by establishing certain classes of self-sterility is particularly useful in practice. Whitehouse stresses the relationship between selection, dioicity, gynodioicity, self-sterility and cross-fertilization as well as the interrelationship between self-sterility and poly-ploidicity (p. 251), between homo- and hetero-zygosis—on the one hand, and the weakness or the vigour of offsprings, going as far as heterozis—on the other (p. 230).

The heterozygosis[31] which represents a new direction in biology appeared long after the publication of the three works of Darwin on plant fertilization. We agree entirely with the views of biologists who underline the exceptional theoretical and practical importance of heterozygosis. Our views differ, however, from those of Whitehouse and other biologists, who following Shull's line seem to identify heterosis with heterozygosis. Practice shows that the spheres of these two notions are scant and not concentric.

In very many cases the equilibrium between dominant and recessive characters is fluctuating from one generation to another of the same hybrids, even when one observes the self-fertilization or inbreeding conditions required by such experiments.

The production of heterosis, though fairly general by hybridization, does not constitute, however, in our opinion, an absolute law, as there are many cases in which the production of heterozygosis does not coincide with the occurrence of the heterosis phenomenon. We therefore agree with Whitehouse's view, according to which 'today, 80 years later, despite the enormous advances in our knowledge on inheritance and variation, the problem still remains' (*work quoted*, p. 234). The open problem is that of the effects of in- and out-breeding.

5. *Generalization of results.*—Reverting to the problem of categories, we may state that the establishing of these vast classes (categories), described above, gave Darwin the possibility first to systematize, in a few groups, the entire material collected by him—a huge mass of data and facts. Then within each category, Darwin passes from *individual* to *species*, namely from 'singular' to 'particular', from 'species' to 'groups of species', i.e. to partial generalizations[32] and finally to law-generalizations regarding the entire group of studied plants.[33]

For the summary exemplification of these statements, we shall give below a few instances of particular phenomena, of phenomena with restricted generalization, of very general phenomena, and of absolute generalizations:

(*a*) *Particular phenomena* form a few cases, often limited to a single category. Within this group varieties may be inserted which appear during self-fertilization repeated for several generations, like the '*Hero*' variety (*Ipomoea purpurea*).

Another example is the secretion of nectar by the cells of flower bracts of *Oncidium* 'and from the *outside* of the upper sepal of a *Notylia* . . . and from the base of the flower-peduncles of *Vanilla*' (p. 266). In *Acropera* and *Gongora* flowers the nectar is secreted after the fertilization of flowers. Their secretion does not serve any more for attraction of insects (*Various contrivances* . . . , p. 266).

(*b*) *Phenomena with reduced generality* are fairly numerous. They may be met with in one or several categories. An example of it may be the cleistogamic flowers. They appear in a number of phanerogamic families (Darwin mentions 55 genera and 23 families). But cleistogamic flowers are far from constituting a very extensive character in the huge group of phanerogams.

Another example is the illegitimate fertilization of mid-styled flowers, which yield good results in comparison with the autosterility of illegitimate fertilization of brevi- and longi-styled flowers.

(*c*) *Phenomenon with wide generality* are met with in several of the previously described categories. We shall begin with the nectar secretion mentioned above. The secretion is not general in phanerogams. Wherever it appears, in most cases, stamens and pistils are differentiated towards nectaries in the way covered by the proboscis of the visiting insects.

Unlike the phenomena described at (*a*), in this case the nectar is to be found in the walls or in the nectary cavity (*Various Contrivances*, p. 40). Thus in a fairly well-protected place when 'the excretion happened to occur within the envelopes of a flower, it was utilized for the important object of cross-fertilization' (*Effects of Cross-fertilization* . . . , p. 404).

Another and more general example is the uniformity of somatic characters in female and male individuals of dioid plants, or in the individuals of every form of species with dimorphic and trimorphic flowers. Likewise in this latter group may be placed the effect of illegitimate fertilization in brevi- and longi-styled flowers, a phenomenon which represents a fairly general character by the higher or lower degree of sterility, which accompanies such unions.

Illegitimate fertilization has some characteristics of fertilization between distinct species. Hybridization between distinct species does not yield obligatory sterile products, as Darwin himself repeatedly states (see also *Various Forms of Flowers* . . . , Ch. V, *concluding remarks on the illegitimate offspring*). Sterility of illegitimate fertilizations, however, is a fairly widespread character in hetero-styled plants, regardless of the fact that such unions are homologated by us with hybridizations between distinct species.

Another example of this group is the generalization of the sexual process in certain plants which have lost the capacity of perpetuating themselves by vegetative multiplication. There are still, however, many plant species which multiply by parthenogenesis, by vegetative multiplication (though they produce flowers as well), or by both means of perpetuating the species. Therefore the group of plants which multiply *solely* by fertilization—with the help of insects—does not comprise all the species of higher plants, and consequently does not represent a completely general character.

(*d*) *Phenomena of total, absolute generalization.* During the analysis of these categories within the framework of sexual process, Darwin describes few instances of absolute laws in plants. Still, such laws exist. In our opinion, in this category is included, for instance, the cytoidal structure of bacteria. Likewise in this category is comprised the cellular structure of metazoa and metaphytes.

In the category of absolute generalities phenomena are comprised of both the assimilatory processes, as well as the perpetuation of the species. Sometimes this generality does not comprise the entire group of plants or animals, but only some divisions, classes or orders. Thus, for instance, in vertebrates, the entire division, without any exception, is only perpetuated by sexual process. In this large division sexual process becomes an absolute law of the perpetuation of their species.

From the examples taken from the three works of Darwin's cross-fertilization with a new line, of the same species, yield absolutely good results.

The general result justified Darwin in stating that sexual process constitutes the 'grand law of Nature'. But, if he passes beyond cross-fertilization, with an unrelated individual towards crossing with a related individual or towards self-fertilization, the effects of these crossings may sometimes be good, but more often they are depressive, as we have seen above. In these cases the law of good effects of sexed crossings no longer has an absolute value.

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The grouping in large classes (categories) and the use of the inductive-deductive method in the study of the dynamics of phenomena grouped in these categories give us the possibility of judging objectively the value of certain biological phenomena.

The conclusions which he draws on each comparative step are verified by him within the framework of the entire class. The conclusions of one class—let us call it 'A'—are verified by him, applying them to another class 'B'—and conversely—until he reaches entirely general conclusions. In this way Darwin succeeds in rising to law generalizations such as:

—regarding the role of selection in the development and perfecting of fertilization mechanisms;

—of the differentiation of plants with heterostyled flowers;

—of the transition from anemophilous to entomophilous plants (and sometimes to the return to anemophily), by the transition from dioic to hermaphroditic plants, and from the latter to heteromorphic or polygamic plants and so on.

The knowledge of the sense and diversity of evolution, as well as the laws of selection in the process of the perpetuation of species by sexual process are the result of the judicious use of inductive-deductive method in the study of the biology in the fertilization process in plants.

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We have expounded this methodology and, systematizing only part of the vast material of these three works, we deemed that the study of this methodology may constitute a school for young and even for elderly biologists.*

* It should be remembered that the studies on phenomena, occurring at molecular or cellular level, constitute one of the fundamental characteristics of the present directions in biological investigations. We have shown above that we deem these directions as having a particularly great importance in the knowledge of those levels of organisms which Darwin failed to study. On the other hand, we consider, however, that in the present-day directions of study, it would be worth while to integrate also such directions which have remained actual from Darwin's conception; for instance the mutual functional interrelations and conditionings between parts of organism have been left, in our opinion unjustly, on a secondary plane. Only in the field of medicine the integration of organism through the nervous system and hormones, the co-ordination and

No doubt that methodology, as we expounded it, will not be found discussed or interpreted by Darwin. But in the whole of his scientific material, as well as in his letters, not once does Darwin speak about the knowledge and use of comparative analysis method in logic—more exactly of the method of the concordance, difference and concomitant variations—he likewise employs only incidentally the terms 'inductive' and 'deductive' and almost never the term of inductive-deductive method. And yet, Darwin knew and commanded masterfully comparative analysis methods, as well as the inductive-deductive method.

Reading attentively Darwin's *Autobiography*, the reason which induced him to adopt this pathway can be grasped. Returning from the voyage on the 'Beagle', Darwin strove to unravel the laws of variability and succeeded to discover the law of natural selection. Using the fertilization method in plants, Darwin tried to unravel in the sexual process the laws of the perpetuation of species by the sexual process and discovered the almost universal law of cross-fertilization as a source of vigour and of unbridling variability.

We consider not to be too far on the wrong side when we assume that the complex methodology, used by Darwin, constituted a model of setting up of some vast experiments, worth following. This manner of studying a biological process is one of the secrets of covering the thorny pathway on which very often we have to go and namely the pathway from effect to cause, from concrete to abstract. This is real in which experiment can render but some of the particular aspects of the pathway from cause to effect. Only thus the existence of materialistic and dialectic elements in Darwin's work may be explained. Or, these elements abound in the three works of Darwin which theoretically and practically develop his conception exposed in the *Origin of Species*.

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We shall analyse now some of the similarities and differences of the methodology used by Darwin in his *Variation of Animals and Plants under Domestication* and in his three works on the fertilization of flowers in phanerogams. In both groups of works, the purpose of measurements, weighings, etc.,

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the mutual-functional conditionings through the nervous and endocrinic system on the one hand and the rest of the organism on the other, constitute one of the great conquests of contemporary medicine, a tribute to Darwin's genius. We consider that it would be particularly advantageous that the problem of the interrelations between the parts which make up an animal or vegetable organism be raised also in other fields than the medical one, to the level of the attention given to 'molecular' biology, to cytogenetics, to the genetics of populations. The methodology in the study of interrelations is now well-established. It only has to be systematically applied. The results, fairly numerous but often disparated, could, if grouped in classes, in accordance with logical methods, be examined from the angle of inductive-deductive method, and generalized.

was to show the manner in which species varied in the course of time under the influence of artificial selection, and how they may also vary nowadays.

The main idea which runs throughout the *Variation of Animals and Plants* is to prove that *past and present variability of species is a property inherent to all animal and vegetable organisms.* In this work we find arguments which fight metaphysical stagnation. The movement of live matter and the role of selection in the development of this movement are concretely proved.

The three works of Darwin on fertilization prove—sometimes with the same methods as in his previous work, at other times with new methods—the interrelations between medium, organism and selection, without trying to assert the identity between the effects of natural and artificial selection.

In the later three works on fertilization, the interrelations between function and structure are similar, as general laws, with those described in the *Variation of Animals and Plants* . . . But the object to which these interrelations are applied—the reproductive parts of the flower—and the effects obtained enter the sphere of the fundamental phenomenon of the perpetuation of the species.

Adaptation to medium is evident in both cases. But adaptation phenomenon has another content in the works of fertilization. In the case of phanerogamous flowers—and by extension in that of other lower plants—the main and common idea in the three works is that cross-fertilization is *the basic law in the perpetuation of species by sexual processes.* It favours survival in conditions in which vital competition in nature adopts harsh and very harsh forms.

Nevertheless cross-fertilization, particularly with a new line, is not an absolute law. Self-fertilization produces much more numerous effects (categories) than cross-fertilization, as it likewise results from the present-day studies on self-sterility, directions of studies well underlined by Whitehouse. By self-fertilization we may obtain graduated effects of self-sterility, which we can group in classes; we may likewise obtain, most frequently, feeble effects which may persist for many generations without decreasing; or they may lead to inbreeding (Inzucht), namely to effects incapable of protecting the species from extinction. By self-fertilization we may obtain fairly good effects in the case of cleistogamic flowers, as we may obtain a vitality recovery with effects superior to inbred cross-fertilization in the case of experimental polyploidization.

In conclusion, Darwin proposed the studying of sexual processes in plants, and for solving this problem he used complex methods, so far unapplied in the domain of biology; where methods were lacking, Darwin elaborated his own methodology, adequate to the studied problem.

PART III

EVOLUTION OF SEXUAL PROCESS IN PLANTS, REFLECTED IN THEIR CLASSIFICATION, ACCORDING TO DATA TAKEN FROM THE THREE WORKS OF DARWIN

In these works Darwin expounds fragmentarily both by working hypotheses regarding the problem of the sexual differentiation generally and the stages of phylogenetic evolution of sexual process in plants.

Hypotheses regarding sex differentiation.—The premises with present values of these hypotheses are:

(*a*) sexual differentiation in plants represents an evolutionary, biological process. The starting point of this process is

(*b*) the existence of a difference between two individuals (respectively between their gametes);

(*c*) conjugation (gamete amphimixis) has good effects when the two genitor individuals differ (metabolically) between themselves—differences are efficient only within certain limits;

(*d*) in these conditions biological advantages of gametes amphimixis are growth in vitality, resistance to unfavourable medium agents, survival capacity in harsh biological conditions of offsprings resulting from this process (as compared to the same properties of individuals resulting from inbred cross-fertilization and from self-fertilization).

We give hereunder these hypotheses, presented disparatedly in the three works.

The problem of the difference between the two individuals (of the two respective gametes) appears in Darwin's letter to Dyer,[34] in which he states that sexes must have arisen from individuals (it is the question of unicellular organisms—author's note), differing slightly among themselves by constitution,[35] individuals which conjugated, while in the *Effects of Cross-fertilization* (Ch. XII) the working hypothesis likewise finds its biological justification.

Darwin states: 'There are many other methods by which organisms can be propagated asexually. Why, then, have the two sexes been developed, and why do males exist which cannot themselves produce offspring? The answer lies, as I can hardly doubt, in the great good which is derived from the fusion of two somewhat differentiated individuals; and with the exception of the lowest organisms this is possible only by means of the sexual elements, these consisting of cells separated from the body, containing the germs of every part, and capable of being fused completely together' (*Cross- and Self-fertilization*, p. 466).

'It has been shown that offspring from the union of two distinct individuals, especially if their progenitors have been subjected to very different living conditions, have an immense advantage in height, weight, constitutional vigour and fertility,[36] an advantage not offered by reproduction, if these were entirely asexual[37] and rarely by self-fertilization.' [38]

The problem of morphofunctional differences between the gametes elaborated in the same flower is worth bringing up to date. Some biologists dispute it, though wrongly, we believe. Formal genetics admits, however, that among the sets of chromosomes of the male and female gametes constituent differences may exist in connection with sexual unpaired chromosomes. But Darwin's opinion on (metabolic) differences was based on the problem of morphological differences between the pollen of certain short- and long-styled plants, and particularly on differences in their behaviour in the case of legitimate and illegitimate fertilizations. The study of polyploidy in heterostyled plants (such as *Primula*) has likewise the value of a document.

We take the liberty to stress a few arguments out of Whitehouse's valuable work in support of the metabolic differences between the gametes established on the same flower. The first group of facts was the one discovered by Lewis (1952) in *Oenothera organensis.* Lewis ascertained that the pollen of this plant produces antigens. Under the influence of these antigens, antibodies are produced in the stigma-style. The phenomenon varies according to temperature (Whitehouse, the above quoted work, p. 240). Lewis too ascertains in *Linum grandiflorum* differences between the pollen from stamens of short- and long-styled flowers. The differences concern the suction power measured by Lewis by means of arbitrary units scale. We quote from Whitehouse's work: 'In consequence, when a long-styled plant is self-pollinated, the pollen fails to germinate, because the suction pressure of the pollen is insufficient (being only twice that of the style) and when a short-styled plant is self-pollinated, the pollen grains burst because the suction power of the pollen is so great (about eight times that of the style)' (p. 243).

According to Whitehouse, the death of young embryos is due to differences in the genetic constitution of partners (thus in fact of the gametes, added by us), differences which do not allow a normal development.

Many other instances might be mentioned, though they would bring no new elements. We shall, therefore, refrain from mentioning any. All these examples come to the support of Darwin's hypothesis regarding the differences between the two gametes, if they are limited to their chromosomal sets, or if they amplify and become visible also by other investigation methods.

Thus, metabolic and chromosomal differences between organisms which yield gametes explain the good biological effects of cross-fertilization—as compared with the effects of vegetative multiplication and of self-fertilization —which at the same time explain the differentiation of di- and tri-morphic forms. This law has also directed the intervention of natural selection, which developed, in the first place, cross-fertilization mechanisms, and only in the second place those of self-fertilization.

Amphimixis remains inoperative if between fertilizing gametes no differences exist. The effect of such mating is self-sterility in the case of self-fertilization, a process which is thoroughly dealt with by Darwin in his three works.

Metabolic differences between the two gametes—an idea generally accepted nowadays—constitute the keystone of Darwin's entire conception regarding the role of the sexual process in the perpetuation of species.

It is true that Darwin did not study the cytological nature of differences between gametes and neither did he deal with the problem of amphimixis or of the starting off of the ontogenetic process consequent to this phenomenon. At present, however, we are more advanced in the last two problems than in Darwin's time as regards the biochemistry, histochemistry and genetic constitution of the gametes, on the self-fertilization, on polyploidy, on the manner in which the egg-activation is realized, as well as on some of the induction mechanisms of embryonic differentiation. At the present time we also know that differences between gametes are of a metabolic and genetic nature, and that gametes cover a stage-cycle which may likewise be a source of differences.

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The Premises of Hypotheses Regarding Phylogenetic Evolution of the Sexual Process in Plants

In the three works, Darwin made a multilateral biological analysis of the hermaphroditism of phanerogams. In this respect Darwin's hypotheses seem to have as essential premises:

(*a*) In the three works on plant fertilization Darwin concentrates his attention on the most complex forms of sexual processes in phanerogams, the most evolved plants.

(*b*) The hermaphroditism of the present entomophilous phanerogams is the result of a gradual phylogenetic evolution.

(*c*) This evolution has its starting point in the separation of sexes and its final point in hermaphroditism.

(*d*) In phanerogams with hermaphroditic flowers the separation of sexes may take different morpho-functional aspects.

(*e*) Natural selection has favoured the development of particular adaptation mechanisms of flowers in cross-fertilization-adaptation, which presents very varied morpho-functional aspects; or, when medium conditions were not always favourable to this kind of fertilization, selection has favoured the development of certain secondary adaptations which permitted the effecting of both cross-fertilization and self-fertilization.

(*f*) Sexual process represents a much more perfected and complex form of the perpetuation of species than vegetative multiplication.

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Darwin merely outlines the graded, phylogenetic evolution of the sexual process in lower plants, without entering the morphological details when describing the stages prior to the differentiation of phanerogams. Likewise, Darwin is but casually preoccupied by the comparison between vegetative and sexual multiplication. Thus, Darwin does not deviate from the subject which he developed masterfully, namely from the biomorphological study of the hermaphroditism of phanerogams.

The principle of graduation.—In describing the stages of the sexual process, Darwin uses the graduation principle which, with a great competence, he had employed for demonstrating in *The Origin of Species* the increase in complexity of structures and functions.

We describe, for readers who are less acquainted with this subject, the manner in which Darwin applied the principle of graduation. He first described the initial stage, the older and simpler one, of the studied processes. Very often evolution proceeds from a simple form and becomes diversified in more or less numerous forms, according to existing living conditions. He likewise tries to study the causes of the differentiation towards the new direction, then the intermediary and the final stages, namely the most evolved form of the process. In the case of the sexual process in plants, Darwin insists upon the increase in complexity, upon the diversification of forms, as well as upon the functional mechanisms in this last stage, realized by means of selection, like in the previous stages.

As this valuable theoretical material is likewise dispersed in the three works, we have endeavoured to concentrate on it and synthesize it in the following paragraph, without attempting to interpret them.[39]

Stages of Phylogenetic evolution of the sexual process in plants

The hypotheses formulated by Darwin regarding the factors which have set off sexual differentiation, as well as the problem of genic and metabolic difference between the two gametes, have been described in the previous paragraph. We shall no longer revert to them.

The initial and at the same time the simplest stage of the sexual process is described by Darwin in protista and algae. During these stages sexes are separated.*

Separated sexes, also to be met with in transition forms from aquatic to terrestrial plants, are to be mentioned. Darwin merely outlines this evolution step without entering into details. Separated sexes are also met with in

* Protista and many algae were considered in Darwin's time as representing clearly expressed forms of the sexual process. A recent acquisition in the domain of microbiology shows that sexual differentiation begins to appear from bacteria and not from protista in forms which approach simple vegetative hybridization.

higher plants, for instance, in anemophilous plants,[40] and more seldom in entomophilous ones. Accordingly, we consider that the stage of separate sexes represents an old feature which may be found again as such in the most evolved plants with flowers.

The existence of separate sexes in anemophilous plants presents the following inconveniences according to Darwin:

(*a*) Cross-fertilization is obtained at the cost of the formation of a huge quantity of pollen, pollen grains not adhering among themselves.[41] In spite of this waste of pollen, there is a risk of female flowers remaining unfertilized.

(*b*) Half the population of the species, namely the male specimens, does not produce seeds.

(*c*) According to Delpino, dioic species do not spread as freely as the monoic and hermaphroditic species, a view which is only partially accepted by Darwin.

(*d*) Anemophilous plants with separate sexes have appeared before the differentiation of visiting insects. But even before the appearance of the latter, phylogenetic evolution of plants takes place towards diclinic forms.

The above presented ideas retain their validity even nowadays. Anemophilous, diclinic forms represent the intermediate stage between plants with separate sexes and those with hermaphroditic flowers.

In anemophilous plants male and female flowers are completely separate, but may be found on the same plant.[42] Diclinic forms present an advantage over plants with separate sexes; in diclinic ones fertilization possibilities grow and alongside with them the perspectives of species perpetuation.* He likewise succeeds in showing the sense of evolution which is diversified at this stage. Certain species and genera remain anemophilous, while others become entomophilous,[43] according to living conditions in their area.

The next stage is that of hermaphroditic plants. According to Darwin, transitional forms existed between dioic or diclinic[44] plants and hermaphroditic ones.

The offspring of plants, initially dioic, have always profited by crossing mutually with another individual and were transformed into hermaphroditic plants.[45] The evolution process from diclinic, anemophilous to hermaphroditic, entomophilous plants would have also been rendered manifest by the proportion of the genera of anemo- and entomo-philous plants. In Linnean monoecian class there are 28 anemophilous and 17 entomophilous genera. With the transition towards the Linnean dioic class, the proportion is modified: ten genera are anemophilous and 19 entomophilous. In the great class of hermaphroditic plants, the proportion of

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* Darwin pays great attention, unusual for his time, to the number of flowers and percentage of seeds resulting from fertilization in a great number of orchids and other homo- and hetero-styled flowers.

anemophilous genera is extremely small as against entomophilous ones.[46] There is no doubt that the estimation of evolution by the number of genera is relative, as certain genera are much richer in species than others, and some species are more widespread than others of the same genera. In the X chapter of *Effects of Cross-fertilization*, even Darwin gives instances in the same sense, showing that some anemophilous genera with a few species, yield compact populations in mountainous medium, in which visiting insects become rarefied because of unfavourable climatic conditions. Nevertheless, even with these corrections, the sense of phylogenetic evolution in plants seems to be the one shown by Darwin.

Darwin distinguishes two moments: the first shows up when plants, still anemophilous, have become hermaphroditic. This transformation had taken place after the appearance of diclinic anemophilous plants. The second moment occurs simultaneously with the transition from hermaphroditic to entomophilous plants. This moment would have occurred at the appearance of visiting insects. The above stated graduated evolution makes us understand the relationship between hermaphroditism and fertilization by means of winged insects.[47] The interrelationships which, in their turn, are being established between hermaphroditic plants and insects have had a historical evolution. At first there appeared pollen-consuming insects.[48] In the next stage of evolution it would have proceeded towards pollen- and nectar-searching insects.[49]

The evolution of flying insects have influenced the evolution of hermaphroditic flowers and vice versa.

Paleobotanical and paleoentomological data have shown concordances between the appearance of hermaphroditic plants and visiting insects.[50] But in Darwin's time these data were relatively scarce. Darwin's arguments strengthen considerably the theory of interrelations between the evolution of phanerogamic flowers and insects, a theory emitted by Saporta.[51]

In the next stage, two directions are followed: on the one hand, a number of visiting insects collect the nectar from the flowers of some very different species of phanerogams; on the other hand, a second line leads to the narrowing of interrelations between flowers and insects. In this case the narrow specialization begins between flowers and insects in the sense of certain mutual conditioning and mutual advantage.

For instance, the flowers of some species of orchids may be fertilized by insects belonging to several families or orders (e.g. diptera, hymenoptera, etc.).[52] There are, however, orchids which can only be fertilized by a certain insect, in which the specialization of mutual relationships has gone so far that, should the fertilizing insect disappear from the area of the orchid, this species of orchids would disappear as well, and vice versa.[53] Specialization gone to an extreme constitutes one of the directions of interrelation development between flowers and insects.

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Directions of fertilization development: Self- and cross-fertilization

Another aspect of the problem is the realization modality of cross-fertilization and the avoidance of self-fertilization. This appears in plants with hermaphroditic flowers, in which the male and female reproductive parts are situated in close proximity to one another. The above problem does not refer to plants with separate sexes or to diclinic ones.[54]

1. The solving of the cross-fertilization problem in entomophilous, hermaphroditic phanerogams has given rise to varied forms of flowers and to different cross-fertilization mechanisms, which reflect the diversity of mutual conditionings between insects and visited flowers. In each separate case, the most adequate mechanisms for the realization of cross-fertilization developed under the influence of selection.

Darwin attended to cross-fertilization: (*a*) between flowers on the same plant,[55] and on this occasion he discovered the phases of development of flowers which, in many orchids, are being formed on the same stalk;[56] (*b*) cross-fertilization with flowers from another plant. Logically, in this case, Darwin distinguishes two categories of effects: the first is that produced by inbreedizing plants (issued out of seeds from the same capsule and crossed amongst themselves), the second category is produced by cross-fertilization with a new line; (*c*) cross-fertilization between di- and tri-morphic flowers, likewise produces two categories of effects: one concerning legitimate crossing of flowers, the second is produced by illegitimate crossings.

2. Another direction in the evolution of hermaphroditic flowers is the possibility of self-fertilization. It is evident that self-fertilization cannot manifest itself in species with separate sexes. It only appears, as Darwin also underlines, in phanerogamous species with hermaphroditic flowers.

3. One of the main mechanisms of the realization of self-fertilization is an old feature inherited by hermaphroditic plants from plants with separate sexes, and from diclinic ones, namely the *simultaneous maturation* of male and female elements. Self-fertilization—and also the polyploidy—*could not take* place even in cleistogamic flowers. But, besides this feature, possibilities of placing its own pollen on the stigma have likewise to be developed. These possibilities may appear, more seldom, as a primary mechanism. It mostly appears as a secondary mechanism in plants with cross-fertilization. This mechanism adopts various forms, such as: (*a*) coexistence on the same flower of mechanisms of cross- and self-fertilization;[57] (*b*) differentiation in the same inflorescence of perfect flowers with cross-fertilization, and of imperfect flowers—cleistogamic self-fertilized ones;[58] (*c*) the co-differentiation may go up to the elimination of cross-fertilization mechanisms.[59]

Synchronic maturation of the two sexual elements is a *sine qua non* condition for self-fertilization. The synchronism of the maturascent process may likewise be met with in the case of the cross-fertilization of orchids, in plants with heterostyled flowers and in other plants. In these cases, however, mechanisms and structures which impede self-fertilization are first differentiated.

The entire work on the fertilization of orchids abound in instances regarding synchronic maturation of male and female parts and in descriptions of differentiated modalities of natural selection by which cross-fertilization is favoured and self-fertilization impeded. Therefore, *synchronism in the maturation of sexual elements* may constitute a basic character in phanerogams, which leads to *the development of cross- and self-fertilization mechanisms.* Between these two directions there are numerous intermediary forms.

When reaching this development stage, phanerogams presented the following characters: entomophily as external morphogenetic factor, while as internal factors: homostyly, or heterostyly simultaneous maturation of the two kinds of gametes and cross-fertilization achieved by the development of numerous mechanisms related both to reproductory flower parts and, to a smaller extent, petals and sepals.

4. As might result from Darwin's expounding, it might be maintained that subsequent evolution of *homostyled plants* has proceeded on three lines :

—One of them of maintaining the regular forms of flowers and of the organization level previously reached.

—The second line comprises phanerogams whose corollas have become irregular.

Out of these, the great families of the *Leguminosae, Labiatae, Scrophulariaceae, Orchideae,* etc., would have been formed. In all these, the common above enumerated characters were preserved.

—A third development line would be that of plants with *heterostyled flowers.* These do not constitute compact groups, as do the homostyled ones, with irregular corollas, but appear within other groups of homostyled plants, particularly in the group of the first development line—indicated above —and almost never in the homostyled group with irregular corollas (among the heterostyled plants only *Pontederia* has an irregular corolla).

In chapter VI of *Various Forms of Flowers* . . . from which we have taken the above data, there are yet other elements which show that Darwin was concerned with the place the heterostyled plants occupy among phanerogams and with some of their formation modalities. In heterostyled plants the sexual process reaches a degree of evolution not met with in either the higher Metaphytes or in Metazoa.

Darwin mentions the characters which, by variation, might have led to the differentiation of heterostyled plants:

(*a*) Differences (which, at present, we would name metabolic or chromosomal) between male and female gametes.

(*b*) These differences become accentuated in heterostyled plants. They concern not merely the pollen and the oosphere of one single form, but even the pollen of the two or three forms of anthers which the plant may differentiate.

(*c*) The two or three kinds of stamens or pistils might have been obtained by the great variability of these organs (a phenomenon observed by Darwin in certain plants with homostyled flowers, which he first considered as dimorphic). Natural selection might have intervened, stabilizing the evolution at two or three lots of stamens or pistils.

(*d*) The problem of biological differences between the pollen of the two or three forms of flowers remains open, differences which Darwin records but does not explain.

(*e*) The transition towards heterostylism would have been favoured to a certain extent by constitutional sterility when the flowers were self-fertilizing themselves.

5. Another development line—towards or from hermaphroditic entomophilous and homostyled phanerogams—would be the polygamous plants; Darwin is but relatively very little concerned about them.

6. From the above, it results that homostyled plants, with simultaneous maturation of the two gametes on one plant, have evolved in a great number of directions. But the evolution of phanerogams has not been exhausted with these new development directions. Other groups of phanerogams have proceeded towards proterandric or proterogyny (maturation at different intervals of their male and female elements). The *asynchronism of maturation rhythm* has induced a radical modification in fertilization mechanisms: the old character of cross-fertilization was preserved and even strengthened.

In the case of asynchronic maturation of sexual elements, self-fertilization is completely excluded.—In this case no special complex mechanisms are necessary, like those in orchids, for hindering self-fertilization. Both wind and insects may facilitate fertilization.

7. An aspect of fertilization efficiency studied by Darwin is that of the quantity of pollen necessary to a good fertilization.[60] To this phenomenon is connected the problem of polyspermia, a subject merely touched upon by Darwin.[61]

The summary description of the stages in the sexual process in plants enabled Darwin to show that hermaphroditic phanerogams have covered several steps in their differentiation (steps which some groups of plants have not exceeded).

Other groups of phanerogamic species have exceeded the initial stage, raising gradually towards higher forms. The evolution process of phanerogams, on the other hand, developed divergently in a great number of directions.

Our systematization does not claim to bring new elements in the analysis of Darwin's work. We only proposed to prove the complex and multilateral manner used by Darwin in his studies on fertilization, functional unity of the three works and the actuality of the problems relating to hermaphroditism in phanerogams.

The tendency towards cross-fertilization which appears in lower aquatic plants as the single law of the perpetuation of species by sexual process is also preserved in higher anemophilous, dioic, diclinic or hermaphroditic plants.

Simultaneously with the appearance of entomophilous, hermaphroditic phanerogams, cross-fertilization may be found alongside with self-fertilization. Cross-fertilization is no longer the general, absolute law of the perpetuation of species by sexual process, at least in certain species, but it remains a very law in entomophilous, hermaphroditic phanerogams.

In all these categories, the old characteristic—the cross-fertilization—is maintained as a strongly dominant character—also in entomophilous phanerogams which have reached the highest development step in plants. Cross-fertilization mechanisms became very considerably diversified.

References and Notes

1 First edition (1862) got quickly out of print. Second edition (1887) revised on the basis of facts and new personal observations, as well as of literature. (Fr. Darwin, *Life and Letters of Ch. Darwin*, edited by John Murray, London, 1888, Vol. III, pp. 286 and 363). All quotations are made after the second edition.

2 Published in 1876 (First edition) in 1,500 copies, which were soon out of print. Reprinted in 1878 in 3,000 copies. The second edition was not wholly revised, but only partially. This work took Darwin 11 years of efforts and difficult experiments. (Fr. Darwin, *ibid.*, *idem*, I, pp. 96, 97 and III, p. 364). All quotations are made after second edition re-reprinted in 1888.

3 Published in two editions—the first in 1877, and the second in 1888 in 3,000 copies. (Fr. Darwin, *ibid.*, *idem*, I, p. 97 and III, p. 364). The interest it aroused will be described further on. Quotations are made after second edition re-reprinted in 1888.

4 Barlow Nora, *The Autobiography of Ch. Darwin*, Collins St. James's Place, London, 1958, pp. 127-28 and *Autobiografia lui Ch. Darwin*, Rumanian R.S. Academy Press, 1962, pp. 147-48.

5 Fr. Darwin, *Life and Letters of Ch. Darwin*, Ed. G. J. Murray, London, 1888, Vol. III, p. 257.

6 *Ibid.*, p. 258.

7 Sprengel's book entitled *Das entdeckte Geheimnis der Natur* (1793) had been recommended to Darwin by Robert Brown (*ibid.*, *idem*, p. 258).

8 Brown, Robert (1833) dealt with the fertilization of Orchids by insects. Darwin realized that Brown failed to see the problem clearly (*The various contrivances* . . . , p. 3).

9 Sachs, *Histoire de la Botanique* (1875), quoted from Fr. Darwin, *Life and Letters on Ch. Darwin*, Vol. III, p. 256.

10 Mainly published in '*Gardner's Chronicle*' beginning from 1857. In that year he published a study on species *Lobelia fulga* in 1861 on the flower of *Orchis apifera* and *Vinca major* in which he explains the influence insects had in adaptive modifications of flower parts in these orchids. From 1860 he begins to deal with *Leschenaultia* (leguminous), but published his observations only in 1871 (quoted from Fr. Darwin, *Life and Letters*, Vol. III, p. 261).

[11] Darwin has written five articles on this problem, between 1862 and 1868. All these articles were presented to the London Linnean Society (Fr. Darwin, *Life and Letters*, Vol. III, pp. 296-97).

[12] Fr. Darwin, *Life and Letters*, Vol. III, p. 262.

[13] *Ibid.*, III, p. 290.

[14] Nora Barlow, *The Autobiography of Ch. Darwin*, p. 128; *see also* the Rumanian translation of this work, 1962, p. 148.

[15] Speaking about the rules and effects of cross-fertilization, Darwin asserts: 'I was surprised to discover how generally these rules apply to both kingdoms.' (*Origin of Species*, R.P.R. Academy Press, 1959, p. 238), while in the Introduction to *Various Contrivances* Darwin shows: 'In my volume on the *Origin of Species* I only presented general arguments in support of the hypothesis that there might be an almost universal law of nature, according to which higher organisms sometimes need a crossing with another individual, or what comes to the same thing, that no hermaphrodite self-fertilizes itself over an uninterrupted series of generations', p. 1.

[16] *Various Contrivances*, IX, pp. 284-85.

[17] Fr. Darwin, *Life and Letters*, Vol. III, p. 275.

[18] Fr. Darwin, *Life and Letters*, III, p. 274.

[19] Published in the collection '*Darwin's Biological Work*', *Some Aspects reconsidered*, Edited by P. R. Bell, in Science editions, John Wiley & Sons, Inc., New York, 1964, pp. 207–261.

[20] Whitehouse, H. L. K.: (*a*) Multiple-allelomorph. 'Incompatibility of Pollen and Style in the Evolution of Angiosperms. *Annals* of *Botany*, N.S., 1950, XIV, Nr. 54, pp. 199–216; (*b*) 'Origin of Angiosperms', *Nature*, 1960, V, 188, Nr. 4754, pp. 957-58.

[21] Ch. Darwin, *Diferite forme de flori de pe plante de aceeasi specie, Plantele insectivore.* In the collection 'The Classics of Universal Science', Ed. Acad. R. S. România, 1965, *Critical Study*, written by Vasile D. Mârza and I. T. Tarnavschi.

[22] Bourgogne, J., *l'ordre des lepidopteres*, in P. P. Grasse's *Traité de Zoologie: Les insectes*, Vol. IX/I, Ed. Masson, Paris, 1951, p. 424.

[23] Ch. Darwin, *Different Forms of Flowers* (Ch. IV, p. 147).

[24] *Ibid.*, *idem*, p. 147.

[25] *Cross- and Self-fertilization*, p. 24.

[26] *Various Contrivances...*, p. 291.

[27] In his *Effects of Cross-fertilization* . . . Darwin studied 13 families, 52 genera and 57 species. And almost an equal number of species (72) were studied directly (39) or indirectly (33) in *Various Forms of Flowers* . . . So that, the number of species studied in three works exceeds 250.

[28] Darwin continued in the case of flower fertilization, too, the method of the exchange of material and views by letters as he did during his elaboration of the *Variation of Animals and Plants*. Darwin's most faithful collaborators to these works were Asa Gray, Firtz Muller, Hooker and others. *Various Contrivances*, footnote, pp. 128 and 129.

[29] At Darwin's demand, Asa Gray examined flowers of *Spiranthes gracilis* and *S. cernua* which grow in the U.S.A. (*Various Contrivances . . .*, footnote, p. 111). Such examples abound in the three works analysed here.

[30] Darwin's point of view regarding cleistogamic flowers was subsequently confirmed in very many cases. Cleistogamic self-fertilization is considered as a less satisfactory means of perpetuating the species than cross-fertilization, though much better than the absence of seed formation (P. Knuth, *Handbuch der Blütenbiologie*, Leipzig, 1898–1905). Some comparisons were made, of which we mention a single one: in Strasburger's *Treatise of Botany* is shown that *Viola odorata* forms simple flowers in the short-day season (spring), while in the long-day (summer) it bears cleistogamic flowers (p. 306). In the case of cleistogamic flowers it was shown that pollen grains germinate already in anthers. The anther wall is penetrated by pollinic tubes, these grow towards the stigma. The seeds resulting from cleistogamic flowers germinate very well (p. 558), (Strasburger, *Lehrbuch der Botanik*, 1962, 28th edition, Fischer Press, Jena).

[31] The term *heterozygosis* first used in *Cardamine pratensis* by W. Bateson (1902) and not by Mendel (R. Rieger and A. Michaelis, *Genetisches und Cytogenetisches Wörterbuch*, II, Aufl., 1950, Springer Press, pp. 235–254).

[32] As are those expounded in Ch. VII–IX of 'Cross-fertilization . . .'.

[33] As those expounded in the final chapters of each of the three works analysed.

[34] Written, February 16, 1877 (quoted from Fr. Darwin, *Life and Letters*, Vol. III, p. 294).

[35] *The Effects of Cross-fertilization*, Ch. XII, p. 467. In the letter addressed to T. Dyer (16-2-1877) Darwin said: 'I cannot drive out of my head that sexes must have originated from two individuals, slightly different, which conjugated.' (Fr. Darwin, *Life and Letters* . . . , III, p. 294).

[36] *The effects of Cross-fertilization*, pp. 347, 449 and 459.

[37] Fr. Darwin, *Life and Letters* . . . , Vol. III, p. 290.

[38] *The Effects of Cross-fertilization*, Ch. IX, as shown above, present-day opinions about self-fecundity have been much modified.

[39] With great satisfaction we find that on certain points Whitehouse's works are in agreement with the present paper, though our methods of analysing the problem differ. We proposed to show in this chapter only the directions of the evolution of phanerogams, as this phenomenon results from Darwin's three works which are analysed in the present study. Whitehouse has published (1950 and 1960) two particularly interesting theoretical works in which he tries to account for the sudden occurrence of phanerogams from gymnosperms by his hypothesis on the multiple-allele incompatibility between the pollen and the diploid tissue surrounding the ovules (1960, p. 957). Whitehouse's hypothesis is supported by the wide distribution of multiple-allelomorphous incompatibility in living angiosperms (1950, p. 215).

[40] Darwin devotes an entire paragraph of Ch. X of the *Effects of Cross- and Self-fertilization in the Vegetable Kingdom* to anemophilous plants. The oldest terrestrial plants, the *Coniferae* and *Cycadaceae*, were undoubtedly anemophilous like the present species of these groups (Ch. X, Anemophilous plants, p. 402). At present, this viewpoint is wholly confirmed.

[41] *Effects of Cross-fertilization* . . . , p. 403; for the amount of pollen produced by anemophilous plants, *see* pp. 407–409.

[42] According to Darwin, there is a connection between dioic and diclinic anemophilous plants, though at first sight no connection seems to exist between them (*Effects of Cross-fertilization*, pp. 410-11).

[43] Darwin mentions at the same time anemophilous and entomophilous species, as is the common rhubarb (*Rheum rhaponticum*), Delpino and Müller describe species of *Plantago* which present, like rhubarb, an intermediary stage. *Effects of Cross-fertilization*, p. 403.

[44] *Effects of Cross-fertilization* . . . , p. 412.

[45] *Ibid.*, *idem*, Ch. X, Anemophilous plants, p. 412.

[46] *Ibid.*, *idem*, p. 411.

[47] Anemophilous plants presented the risk of not being always fertilized and thus of not having offspring. This inconvenience is smaller in hermaphroditic plants, in spite of the eventual disadvantage of frequent self-fertilization (*Effects of Cross-fertilization* . . . , pp. 412 and 413).

[48] '. . . it is probable that pollen was aboriginally the sole attraction to insects and although many plants now exist whose flowers are frequented exclusively by pollen devouring insects, yet the great majority secrete nectar, which is the main attraction for insects' (*ibid.*, *idem*, p. 403). This view still holds good.

[49] Darwin presents a series of facts from which it results that the nectar represents a secretion (outside the cells, as it is said today), (*ibid.*, *idem*, Ch. X, pp. 403 and following).

[50] Ch. Darwin, *Effects of Cross-fertilization* . . . , p. 402.

[51] In *Origin of Species*, reprinted from the sixth edition, Oxford University Press, 1956, Darwin raises the problem which he expounds at length much later in a letter to J. D. Hooker, 6th August, 1881. In this letter he quotes a work by Saporta, sent to him the manuscript in which this Italian paleobotanist maintained that 'as soon as flower-frequenting insects

were developed, during the latter part of the secondary period, and enormous impulse was given to the development of the higher plants by cross-fertilization, being thus suddenly formed'. (Fr. Darwin, *Life and Letters,* III, p. 248).

52 E.g. *Listera cordata* (small diptera and hymenoptera), *Catasetum tridentatum* and such others (*see* Table).

53 Specializations led so far are not rare. Darwin mentions several instances in *Various Contrivances* (pp. 163 and 282). We content ourselves to recall only the Madagascar orchid named *Angraecum sesquipedale* which has an extremely long nectary and the nectar is only to be found at the bottom of it.

54 *See* part III of the development directions of dioic and gynodioic self-sterility after Whitehouse.

55 The flowers which grow on the opposite sides of 'a tree will have been exposed to somewhat different conditions and a cross between them might be to a certain extent beneficial; but it is not probable that it would be nearly so beneficial as a cross between flowers on distinct trees . . .' (*Effects of Cross-fertilization,* p. 400). Many of the self-fertilized flowers from big tree fall if other neighbouring flowers had been cross-fertilized.

56 We only mention two examples of geitonogamy. In orchid *Goodyera repens* in young flowers, the passage between rostellum and labellum is contracted and insects cannot penetrate into the flower. When the flower becomes mature, the column withdraws and moves away from the labellum. In this case insects can enter the flower easily (p. 104). In *Spiranthes autumnalis* Darwin describes such differences between young and mature flowers (pp. 110-11). In young flowers pollinia are in a good state to be detached. They cannot be fertilized until they are a little older, when the column moves away from the labellum. On the other hand, the stigma of older flowers is more viscous than that of younger flowers (pp. 112-13). Darwin shows incidentally that the stage variations also occur in plants with dichogamous flowers.

57 For instance, in *Neotinia* (*Orchis intacta*) Darwin was convinced by experiments verified by himself that the flowers of this orchid presented self- and cross-fertilization mechanisms. The self-fertilization mechanism consists of the possibility of the pollen falling on the stigma of the same flower. The cross-fertilization mechanism is made manifest by the way the nectary, viscous discs of pollinia and of other formations of the reproductive portions of the flower are constituted. The portion of the reproducing apparatus of the flower is in a certain regression: the pollen is very little adherent, an aspect not met with in entomophilous phanerogams and particularly in orchids. The nectary is short, the remaining reproducing portions are with cross-fertilization like in orchids (pp. 27 and 291). Thus the *Neotinia* flower succeeded in differentiating the two fertilizing possibilities, without differentiating two kinds of flowers (perfect and imperfect ones). The flower of orchid species (*Epipactis viridiflora*), *Gymnadenia tridentata, Platanthera hyperborea* (Asa Gray), *Disa macrantha* (Neale) and others may likewise be self-fertilized (pp. 291-92).

58 Darwin devotes a whole paragraph to plants with perfect and cleistogamic flowers in his *Different Forms of Flowers* (Ch. VIII). He also gives a table with phanerogamous genera (a total of 55 genera known at that time) which create perfect and cleistogamic flowers. Darwin studies certain climatic, seasonal or geographical conditions which, in species of the same genus, may or may not incite to differentiations of cleistogamic flowers. He also observes the result of the germination of cleistogamic flowers and finds that seedlings issued from the seeds of these flowers develop almost as vigorously as those resulting from perfect flowers. Darwin establishes gradations between perfect and cleistogamic flowers, the structure of their floral parts, their variant functions in the perpetuation of species in cases when the visits of insects cannot take place on account of being too early or too late in the season.

59 *Ophris apifera* modifies its structure and adapts itself perfectly to self-fertilization, as a permanent modality for the perpetuation of the species (pp. 279 and 291).

60 Darwin has studied the quantity of pollen requisite for fertilization, starting in this case from Kölreuter's, Gärtner's and Naudin's investigations. On the basis of his experiments on

orchids and heterostyled plants (e.g. *Neottia* and *Lythrum salicaria*), Darwin reaches the valid and verified conclusion that even nowadays fertilization is not achieved or fails to yield good results, below a number of pollen grains. Darwin's experiments on *Ipomoea purpurea* are, however, not conclusive. Darwin's conclusions have been verified nowadays on animals and were found quite correct as regards the minimum of spermatozoons per cubic mm. required for animal fecundation.

61 Darwin has realized from observations on *Neotteae* that considerable pollen masses are placed on every stigma. Orchid pollen grains bound together by feeble threads avoid waste and at the same time favour the placing of a great number of grains on every stigma they reach (p. 288). Thus, in this and similar cases, polyspermia constitutes a law. The pollen of one flower is in a sufficient quantity to fertilize several flowers. Yet, Darwin mentions the case of other orchids of the *Malaxeae* group which only produce a pollen mass, sufficient for fertilizing 1-2 flowers. Darwin believes that such cases are met with in no other plant groups (p. 288). But even in the single pollen mass of the *Malaxeae*, the number of pollen grains is sufficiently large, to enable us to speak about polyspermia. Polyspermia in plants—presumed by Gärtner (1884)—is met with more and more both in plants and animals.