THE CYTOLOGICAL ASPECTS OF THE DETERMINATION OF SEX IN THE DIOECIOUS FORMS OF LYCHNIS.*

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With Two Plates.

I. Introductory.

In examining various groups of plants, in the hope of finding one which would throw fresh light on the question of sex determination, the dioecious forms of Lychnis suggested themselves as being particularly suitable. The genus Lychnis, as a whole, is hermaphrodite, and even the dioecious species may readily exhibit this character. In the female flower rudiments of stamens are always present and physiological disturbances may cause them to develop further.

In the hybrid between the two nearly related forms L. dioica, L. em. Mill (Melandrium rubrum,† Garcke), and L. alba, Mill. (M. album,† Garcke), these stamen initials tend to develop much further than in the normal pure species, and to reach a fair size, although they do not usually develop pollen.

A more marked disturbance, produced by the attack of Ustilago violacea, Fuck., causes the stamens of female flowers to develop completely, but the anthers usually contain only the spores of the smut. However, Doncaster has reported that he once found, on a diseased plant, uninfected flowers in which the stamens were developed but apparently infertile, although usually such flowers are normal females. Infected male plants remain male in character.

Shull worked with a strain of L. dioica‡ which did not correspond to either of the two species recognised in

* Received February 22nd, 1924.
† This name is used in continental works.
‡ Shull follows Linneus in using the name L. dioica to include both the white and the red species. It is now usually limited to the red species.
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European material, but which may possibly have originated from a hybrid between the two. In his cultures two types of hermaphrodites appeared. Experiments with one of these led him to the conclusion that this was a modified male since, when crossed with a normal female, the offspring was female and hermaphrodite. From this he also deduced that the male is heterozygous for sex.

Further work by the same author on a narrow leaved male mutant of *L. alba*, discovered by Baur, confirmed this result, showing the narrow leaf to be a sex-linked character.

The dioecious Lychnids readily form hybrids, not only with other species of *Lychnis* but also with species of *Silene* (Correns, Blaringhem, and others); which makes their investigation a very hopeful field of research.

Strasburger made a detailed cytological investigation of *Melandrium rubrum* in search of differences between the pollen grains of the tetrad. He came to the conclusion that the chromosome complement of all the pollen grains was identical, and that the nuclei of male and female plants were also similar. Sykes also looked in vain for differences between the nuclei of the two sexes.

A short note in *Nature*, by the present author, announced the discovery of an unequal pair of large chromosomes in the male of *L. alba* of which the larger is hooked in shape, with a corresponding pair of chromosomes in the female which were equal in size and shape. These were described as sex chromosomes of the XY and XX type. Very soon afterwards, in a paper on sex chromosomes in plants, Winge also described this unequal pair in the male of *Lychnis alba*.

The present paper is an account of the cytology of *L. alba*, *L. dioica*, and the hybrid between them, with some reference to *L. Flos-cuculi* by way of comparison. It is intended to be followed by further studies on pure and hybrid forms of *Lychnis* and the neighbouring genera, especially those in which there are peculiarities of sex forms.

The chief interest then in these dioecious forms lies in the presence of the XY pair of chromosomes in the male
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plant in all types. This differs from the usual XY pair described in animals in as much as the Y chromosome appears to be larger than the X. However, the figures of *Drosophila melanogaster* possibly suggest the same state of affairs.

2. Material and Methods

The material used in this investigation was all obtained from plants found growing wild in the counties of Northumberland and Durham. Both of the pure species were derived from two localities nearly forty miles apart, and the number of individual plants involved was, in either case, considerable. The hybrid material all came from one source, and since *L. alba* grows in the same place, and *L. dioica* not within fifty yards, it is to be presumed that *L. alba* was the maternal parent. The plants were somewhat variable as to size and tint of flower, and on the whole the characters were somewhat nearer to those of *L. alba*; but the form of the fruit (see Correns, 1913, p. 19), the pink colour of the flower, and other characters distinctly intermediate in type, gave unmistakable proof of the hybrid nature of the material. The plants set a fair amount of seed which was similar to that of *L. alba* but larger in size and possessed larger tubercles, in fact exaggerating the characters serving to differentiate *L. alba* from *L. dioica*. Submitted to germination tests the hybrid proved superior in this respect to either of its parents.

The best preparations obtained were those preserved in Carnoy’s chloroform-acetic-alcohol mixture, though even then the results were not consistently good. *L. dioica*, in particular, shows a tendency to clumping of the chromosomes, in spite of careful dissecting out of the stamens and peeling off of the ovary wall. Curiously enough, the hybrid material gave the best preparations; in fact, these were almost diagrammatic in their clearness.

The sections were cut at a thickness of 6 μ, stained with Heidenhain’s iron-alum hæmatoxylin and counterstained with orange G. dissolved in clove oil. The counterstain was found particularly valuable in showing up the spindle fibres, as a comparison of figs. 4 and 6 will serve to demonstrate. A very light stain was found useful for distinguishing the
sex chromosomes, which stain more heavily than the rest, and more particularly for showing up the chromatin droplets in the heterotype·telophase (see fig. 28).

3. The Cytological Details.

A most careful examination of the three dicocious forms has failed to reveal any difference in their general cytological features. A first examination suggested that the cells and chromosomes of the hybrid form were larger than those of either parent, as might perhaps have been expected, but a strain of L. alba from another source proved, if anything, larger, so the question of size fails to be of any significance. It will be clearly seen from the illustrations accompanying this paper that there is a considerable difference in size of both nucleus and chromosomes in figures taken from different flowers.

The somatic number of chromosomes in these species is twenty-four, which number may prove to be characteristic of the group, since the writer has also found it in Lychnis Flos-cuculi, L. Flos-Jovis, and Silene pendula.

The close likeness between L. alba and L. dioica, accompanied as it is by a similar chromosome complement, is no doubt responsible for the regular behaviour of their hybrid which, besides showing no obvious evidence of hybridity, also normally sets perfectly good seed as before stated. This makes it somewhat surprising that the pure species remain so distinct.

At the earliest stage examined in the male plants of these three forms the sporogenous tissue of the anthers is closely packed with pollen mother cells. Their nuclei show a faintly granular contents with darker staining areas at the periphery, whereas the centre is occupied by a clear area containing the slightly excentric nucleolus (fig. 12). Rarely, a second smaller nucleolus is present. As the reticulum becomes clearer, it contracts into a very close compact synizetic knot between the nucleolus and the nuclear membrane (fig. 13). It is not until this stage is reached that the mother cells begin to separate from one another. Gradually the tangle becomes slightly looser, and is seen
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to be composed of a tightly intertwined thread from which then loops are thrown out into the nuclear cavity and reach to the nuclear membrane. In this stage the spireme begins to thicken and never opens out into a hollow spireme stage such as that observed in *Rosa* (Blackburn and Harrison, 1921). The thread does not exhibit any traces of a double nature, but gradually becomes shorter and differentiated into thicker and thinner lengths (see fig. 14). The thicker portions correspond to the chromosomes, thus showing clearly an end to end arrangement, though the arrangement is not quite so diagrammatic as in *Cenothera* (Gates, 1908, etc.). This locally thickened thread then becomes closely entwined again producing a second contraction figure (see fig. 15), and at this stage it is seen that the thickening of the spireme does not take place evenly throughout its length, so that frequently one part of the spireme shows short thick chromosomes whereas another reveals loops of varying thickness radiating out from the denser portions. As this knot loosens out, the chromosomes are still end to end, but more obviously arranged in pairs. The linin threads connecting adjacent pairs become pulled out thin and break (see fig. 16), and the pairs of chromosomes become spaced out round the nucleus, though at times some may still be clustered to one side of the nucleolus. At this stage the units of each bivalent may lie over one another in the form of a cross, but more commonly they lie parallel, and careful observation will usually reveal that they are connected at one end by a linin thread. Here also it is often possible to distinguish the chromosomes of one pair as being longer than those of other pairs. As contraction continues the disparity becomes more obvious, and finally the eleven small pairs contract till the individual chromosomes are almost spherical, whereas the large ones remain elongated and are, moreover, unequal in size; the smaller one being about two-thirds the length of the larger (see figs. 1, 2, 18, and 19). The large chromosomes also retain their wavy margin for a very much longer time than the others. Santos has described a similar behaviour in the large chromosome pair of *Elodea gigantea*, but not in the sex chromosomes.
In the earlier stages of the final contraction of the chromosomes many of the pairs strongly resemble in shape the pair of pollinodia of, say, *Epipactis latifolia*, Sw., and in a few cases even bend round at their tips so as to be almost ring-like (see fig. 17). This tendency to the formation of rings is better exemplified by *L. Flos-cuculi*. In that species there are again twelve pairs of chromosomes, all of which, or almost all, form rings at the stage of diakinesis, and these rings are obviously produced in precisely the same way as in the dioecious forms described (see figs. 42 and 43). The difference in this respect between two allied species is exactly paralleled by the difference between *CEnothera grandiflora* and *O. biennis* (see Davis, 1909 and 1910), the former having ring-shaped bivalents, and the latter lacking them. In *L. Flos-cuculi* the bivalents go on to the spindle in the form of rings (fig. 45), but in the other species the behaviour is rather unusual; the elements of the bivalents having contracted to two separate spheres, these move apart so that the diploid number of chromosomes appears again at this stage. Fig. 19, though not quite a complete nucleus, shows twenty-one of them present. This may be compared with the state of affairs in *Lepidosiren* (Agar, 1911), though in the case of *Lychnis* it is usually possible to distinguish the units of a pair; so that they may possibly be still connected by faint linin threads. Before disappearing the nucleolus becomes highly vacuolated (see fig. 19).

When the spindle is formed the chromosomes arrange themselves at the equator remarkably quickly and tidily, and the spindle fibres attached to them are peculiarly strong and well marked. At the metaphase the chromosomes are usually seen to be connected in pairs by a single fine thread (figs. 4 and 21). Very rarely do we find the connection more intimate, as in fig. 20. The frequency with which loculi containing only the heterotype metaphase, or only the late diakinesis, are found suggests that these are resting periods, while the multipolar spindle (if present) and the anaphase stages take place very rapidly. This statement is true for material killed at all times of the day and up to 10 p.m.

The heterotype metaphase is the ideal stage for studying
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the unequal pair of chromosomes. Owing to its rather excentric position in the chromosome group, as shown in figs. 7 and 8, it is possible to observe the pair in a very large proportion of nuclei at this point (see figs. 3 to 6 and 20 to 22). If there is any difference between the forms, in this respect, the larger chromosome may be a trifle more hooked in the pure species than in the hybrid, but I doubt that there is any constant difference. An examination of Strasburger's figures reveals this hook very faintly portrayed in Plate IX. fig. 1, but he missed seeing the very obvious difference in size, owing, no doubt, to the great difficulty in getting well-fixed material in \textit{M. rubrum}, of which difficulty he himself complained. The present author was fortunate enough to begin with the much more easily fixed \textit{L. alba}.

However, in view of the fact that Strasburger was actually searching for some such difference, it seems advisable to examine \textit{M. rubrum} from continental and other sources before one can feel quite confident that the difference is always present and obvious.

The chromosomes continue to be widely spaced as they pass to the poles, as is shown in figs. 23 and 24, and are again easily seen to be eleven small and one large at each pole. There is little difficulty in distinguishing the two unequal ones throughout, especially as the larger one usually exhibits a cross-like form when viewed from above, as shown in fig. 25, though sometimes it appears as a dumb-bell instead.

The chromosomes next contract into a very tight clump, so tight indeed that it is often very difficult to see their outlines, except at the edge of the cluster, where the large chromosome is usually to be seen projecting beyond the general contour, as in fig. 26.

When the chromosomes begin to separate again, it is to be observed that their clear outlines are lost, and that they are connected with one another by strands, as though their close contact had caused them to adhere to one another (fig. 27). At about this time the nuclear membrane appears once more, and also, not only one nucleolus but several such bodies. These are not all spherical and it requires special light staining of the material to differentiate them from the irregular
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lumps which represent the chromosomes. Careful observation suggests that these nucleoli are in the nature of drops, the spherical shape of which has been disturbed (see fig. 28). The size of the nucleus now gradually increases and, as it does so, the irregularly shaped lumps resolve themselves into pairs which become definite in the form of two almost spherical bodies lying side by side but not touching (see fig. 30). There are thus twenty-four bodies visible, of which two are larger than the rest, as seen in fig. 31. The dissociation of the half chromosomes at this stage is evidently not rare, as Winge (1916) figures a similar condition in *Atriplex hastata*, *A. littoralis*, and *Anthriscus silvestris*. The appearance of the split chromosomes here may also be compared with what is to be found in the somatic divisions of *Lychnis* at the same point, as illustrated in fig. 41.

In *L. Flos-cuculi* the division is obvious at a much earlier period; a polar view of the heterotype metaphase shows twelve four-lobed chromosomes: the radial constriction seems to correspond to the two arms of the chromosome, which is attached to its partner at two points in this case, whereas the tangential split is a precocious separation foreshadowing the homotype division (see fig. 46). The late anaphase shows the appearance even more markedly, as is illustrated in fig. 47.

To return to the dioecious species, it is to be noticed that the half chromosomes in this interkinesis do not separate quite so far as the halves of the bivalents in the heterotype division, and the re-fusion on the homotype spindle is correspondingly more intimate (see fig. 32). The two spindles may be either parallel as in fig. 33 or at right angles as in fig 32. In the former case the conditions are peculiarly favourable for seeing the difference in size and shape between the large chromosomes of the two daughter plates. Here the large member again shows the curious cross-like form so difficult to correlate with its lateral view. It seems probable that the polar hooked end of the chromosome is flattened radially, and the body of the chromosome tangentially, so that by its shape, the body of the chromosome alone would correspond to the other chromosome of the pair. A model in plasticene can be used to test this
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hypothesis. If two almost kidney-bean shaped masses are superimposed on one another in such a way that their shortest axes are at right angles, and their longest at an angle of 135°, a figure can be produced which will give the hooked shape from one side, a cross viewed from above, a dumb-bell if viewed obliquely, and will correspond to all observed forms of the largest chromosome.

The homotype division shows similar phenomena to the heterotype, except that the clumping of the chromosomes at the telophase is less marked, and the nucleus goes into a resting stage with more highly staining areas in a very indefinite reticulum, also, there is only one nucleolus. Whatever the original position of the homotype spindles in relation to one another the tetrad is of the pyramidal shape. So far no difference has been observed between the pollen grains of the tetrad, though it is hoped that some difference may yet be found by which the two bearing the larger bulk of chromatin may be distinguished from the other two.

4. Megaspore Development.

The examination of ovular material is always more difficult than that of the stamens in so far as there is usually but one megaspore per ovule. The difficulties in this case were found particularly great as only a small number of the ovules contain normal megaspore mother cells and, of those which develop further, many fail to produce embryo sacs. This may possibly be due to the fact that much of my material was collected as late as August in a very bad season, for Correns (1917) obtained a maximum of 497 ripe seeds in a capsule, while he states that the number of ovules varies from 300 to 500. A second difficulty that arises is due to the very curved shape of the ovule. At the heterotype division the curve is slight, so that it is possible to get a fair proportion of equatorial views of the spindle from transverse sections and some polar views from longitudinal sections. As the ovule becomes more curved it becomes more and more rare to obtain a median section of the embryo-sac cell.

A brief general account of the development of the reproductive cells will suffice in this connection. A single
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hypodermal archesporial cell is differentiated at an early date. This divides by a transverse wall into a parietal and a sporogenous cell, the latter frequently divides once more in the same direction giving two superimposed spore mother cells. In one case, in the hybrid form, a row of three cells in the resting stage suggested three mother cells, but I think none of these would have been functional, as neighbouring ovules were at a considerably later stage. On the other hand in *L. Flos-cuculi* the sporogenous cell normally divides to form a group of three or four cells not, however, in a row, but arranged somewhat in the manner of a pollen tetrad.

In *L. dioica* the lower spore mother cell lags behind the upper one in preparation for the heterotype division and usually fails to divide at all. The upper one may form a linear tetrad or may only produce three cells. In either case the basal cell alone develops but, when there are only two disorganised cells, the presence of the lower megaspore mother cell, which does not break down, may give the appearance of a penultimate embryo-sac cell (see Strasburger, *loc. cit.*, fig. 25a). The development of the embryo-sac seems to be typical though its narrow and curved shape makes it difficult to observe.

The prophase stages of the megaspore mother cell show little divergence from what has been described in the male. The spireme seems to loosen out more and the spindle is more elongated, but there is no significant difference except in relation to the large pair of chromosomes. At diakinesis these are apparent as in the male, but less markedly so, and they are equal in size. Careful comparison indicates that the units of this pair correspond in size and shape more nearly to the smaller member of the pair in the male plant. Fig. 37 shows a polar view of the metaphase plate, and, owing to a precocious separation of the large pair, it is obvious that they are equal. One plate at this stage showed the XY pair towards the inside of the group; this is the only time it was observed other than at the periphery of the group, in either sex. In one case I was fortunate enough to obtain an ovule in which the knife had cut neatly between two anaphase plates, only displacing one autosome. This
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is illustrated in fig. 38. This, in conjunction with equatorial views such as figs. 10, 35, and 36, established the equality of size and shape without possibility of dispute.

5. Conclusions.

Although heterochromosomes are well known in animals, and their relation to sex established in many forms, they have as yet only been recorded in a few cases in plants, and most of these during the last year.

In the liverwort *Spharocarpos*, Douin showed that there were two kinds of spores in the tetrad, producing two male gametophytes and two female respectively. Allen showed that in *S. Donnellii* the female gametophyte possessed seven autosomes of different sizes and one quite large chromosome which corresponded to a very small one in the male plant. These were evidently sex chromosomes. Schacke obtained similar results in *S. texanus*.

The flowering plants give a much closer parallel to animals, in so far as the plant body is the diploid phase, the haploid being reduced to a few cell divisions at most. Among these, several cases of sex chromosomes are now known.

An unequal pair of chromosomes has been described in the reduction division of the male plant of *Populus tremula* (Blackburn and Harrison, 1922 and 1924), but full details are not yet published.

The first full account of sex chromosomes was that published by Santos for the male plant of *Elodea gigantea*. In this plant he describes an unequal pair of chromosomes accompanied by twenty-three equal pairs, including one conspicuously large one, but he has not, so far, given any details of the female plant.

Kihara and Ono (1923) have investigated both sexes in *Rumex acetosa*. The conditions there are somewhat unexpected. The male plant has fifteen chromosomes and the female fourteen. The sex chromosomes in the male consist of a group of three, one larger and two smaller, comparable to those found in such a form as *Fitchia* (Payne). In a similar way the two small ones constitute the one member of the heterochromosome
pair and the larger one the other, but in the case of *Rumex*, it is the two small ones that constitute the Y, thus giving the male plant one more chromosome than the female.

My own announcement of the discovery of an unequal pair of chromosomes in the male plant of *Lychnis alba*, with a corresponding equal pair in the female was followed, almost immediately, by a paper by Winge describing heterochromosomes in the male plants of that and three other forms. *Humulus Lupulus* and *H. japonicus* both show an unequal pair of chromosomes in the reduction division. Particularly during diakinesis the larger of these two was frequently observed to be divided by a deep constriction, so that it looked like two; an arrangement very suggestive of the metaphase figures of *Thyanta calceata*, Say. (Wilson, 1911). Similar features were described for *Melandrium album*.

*Vallisneria spiralis*, on the other hand, has a single unpaired sex chromosome which Winge likened to that of *Protenor*. This chromosome has only been observed in the mitoses in the pollen grain where it appears deeply though unevenly constricted. Since the other chromosomes show a tendency to adhere in pairs it is thought that perhaps this chromosome may appear as two in the somatic mitoses.

Winge assumes that the large chromosomes in *Humulus* and *Melandrium* and the single one in *Vallisneria* are respectively the X element. Santo's makes the same assumption for *Elodea*.

In the present description of *Lychnis dioica* (agg.) it has been shown that the male plant has an unequal pair of large chromosomes of which the larger suggests by its shape that it consists of two fused elements. The female plant also shows a pair of large chromosomes, but they are not very much larger than the autosomes, so that it is often difficult to identify them. They are thus comparable in size to the smaller member of the unequal pair in the male. They also show no traces of any double structure, so that one is forced to the conclusion that the larger chromosome is, unexpectedly, the male determiner or Y element. Considering that the only other angiospermous form in which the female has been described is *Rumex*, in which a double Y element is reported, it seems
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just possible that the apparently double chromosome in *Humulus* and *Vallisneria* might also be the Y and not the X as supposed.

These cytological details seem quite compatible with the results of the large amount of breeding and other experimental work which has been carried out, particularly with *Lychnis dioica*.

The discovery by Baur (1912) and by Shull (1914) of the sex-linked character of narrow leaves in *L. dioica* (*Melandrium album*) immediately suggested the possession of sex chromosomes, as it showed clearly that the male plant was heterozygous for sex. The sex ratios cited by Strasburger, Blaringhem, and many others are somewhat unexpected in that usually there is a great preponderance of female plants, whereas equal numbers might have been expected from pollen grains half of which were male producing and half female producing. Correns has, however, investigated this problem with some very interesting results which show that sex determination and sex ratios are regulated by very different factors. He has shown that by using a large quantity of pollen a great preponderance of females is generally obtained; if, on the other hand, a small quantity is used the proportion of males in the progeny is increased. It would seem from this that when the quantity of pollen is such that every grain can function the sexes are more equal. These results depend upon the discovery that pollen tubes are produced more quickly by the female producing pollen and hence reach the ovules first. Winge puts this extra speed down to the extra amount of chromatin assumed to be in the pollen grain, but in view of the cytological facts described some other explanation is needed.

In dealing with a hermaphrodite mutant of *Lychnis dioica* which showed the variation to be heritable, Shull came to the conclusion that the form was a modified male, since, when used as the male parent, hermaphrodites appeared in the progeny in the place of males. Arguing on these and other facts he came to the conclusion that in *L. dioica* a genetical formula of "mm" for the female and "Mm" for the male corresponded better with the facts than the more usual
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“FF” female and “Ff” male used for animals with a heterozygous male. Curiously enough, the large Y chromosome here described might appear to correspond to such a formula.

Since the dioecious condition in flowering plants, or at least in *Lychnis*, would seem to be derived from a hermaphrodite one, it is perhaps not very surprising if the method of sex determination produced to meet the condition is not quite identical with that in animals. To me, the surprising part is that they should be so similar; after all, the condition seen here is less different from say *Lygaeus* (Wilson) than that form is different from *Tateoporia tubulosa* (Seiler, 1917).

However, it is well not to lose sight of the fact that the chromosome mechanism is only a small part of the story, since sex intergrades occur naturally in various species of *Lychnis* and *Silene*. For example, in *Silene dichotoma* hermaphrodite and female flowers occur, with a varying stamen development in the female flowers; *Arenaria peploides* may be either hermaphrodite or dioecious, in the latter case having fairly well-developed rudiments of the other sex in the flowers; in *Silene inflata* different plants may be male, andromonecious, hermaphrodite, gynomonecious or female (Correns). These examples, taken in conjunction with the effect of infection by *Ustilago*, and the spontaneous occurrence of hermaphrodites in Shull’s races of *Lychnis dioica*, are enough to show that the order Caryophyllaceae, though in the main hermaphrodite, is in a somewhat unstable state as regards its sex relations. We now have some details in the matter of sex ratios and sex determination in the definitely dioecious forms of *Lychnis*, but much remains to be done in relation to the intermediate* and hybrid forms before we can attempt to give any definite picture of the underlying causes.

* Since writing the above, the abstract of an interesting paper by Collins on “Sex Conditions in *Silene nutans*, L.” has come to hand. Starting with a hermaphrodite plant, a female plant and also some seed he found that all the families he reared contained individuals of the following four types: hermaphrodite plants, plants with both female and hermaphrodite flowers, female plants, and unfertile female plants. What seems particularly interesting in this connection is that the fertile females showed a strong tendency to become hermaphrodite in course of time.
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The present research is part of a more general inquiry into the conditions of sex determination in the genera *Lychnis* and *Silene*.

This paper consists of an account of the cytology of the dioecious forms of *Lychnis* with special reference to sex chromosomes. *Lychnis Flos-cuculi* is referred to at times for the sake of comparison.

The somatic number of chromosomes in *L. dioica* (agg.), as well as in *L. Flos-cuculi L. Flos-Jovis*, and *Silene pendula*, is twenty-four.

The hybrid *L. alba* × *L. dioica* behaves in a perfectly regular manner and is practically indistinguishable cytologically from its parents.

In *Lychnis dioica* (agg.) the pollen mother cells show a particularly close synizetic knot from which the spireme never opens out fully.

Just before the second contraction the spireme becomes differentiated into thicker and thinner portions of which the thicker clearly represent the chromosomes.

The thread becomes much shorter and stouter and, as the contraction figure opens out, the chromosomes separate off in pairs forming typical bivalents lying either crossed or parallel.

The reduction is thus typically telosynaptic and there is no trace of a split thread at any stage.

As the bivalents contract, one pair is seen to be larger than the rest and to consist of two unequal portions, both of which are larger than the other chromosomes.

At diakinesis, the bivalents become completely dissociated, once more giving twenty-four separate chromosomes.

*L. Flos-cuculi* differs in having ring-shaped chromosomes, both at diakinesis and on the equator of the heterotype spindle.

In *L. dioica* (agg.), the large pair of chromosomes lies at the periphery of the metaphase plate and is seen to consist of one very large hooked member and one smaller, more pear-shaped, chromosome.

Anaphase figures show the larger chromosome in the form of a cross, as seen from above, suggesting a double structure. There is no difficulty in distinguishing which member of the unequal pair is present in any given daughter plate.
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In the interkinesis there are two points worthy of note. (1) Several nucleoli are present, in place of the usual single one typical of all other stages. (2) The twelve chromosomes separate completely into halves, thus giving twenty-four bodies lying within the membrane, just as in diakinesis.

At the ensuing metaphase the X and Y chromosomes are again most distinct, especially when the two spindles lie parallel.

Megaspore development is briefly described.

Meiosis is similar to that found in the male plant, except in relation to the large chromosomes.

The large chromosomes are equal in size, and by careful comparison of size and shape, are shown to correspond to the small one of the unequal pair in the male. The Y chromosome is thus larger than the X, quite contrary to expectations based on the condition in animals. It is also probably double in nature though it does not separate into two parts, as far as has been observed.

A brief review of the literature shows that in Rumex there is a pair of Y chromosomes; in Humulus and Vallisneria a double structure is presumed to be the X element, but on the basis of Lychnis may possibly turn out to be the Y instead.

The cytological results above are shown to corroborate the results of the experimental work of Shull and Correns on the same forms.

In conclusion, I should like to thank Professor M. C. Potter, in whose Department this research was carried out, and to whom I am indebted for the facilities for carrying out the work.

7. References.

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8. Description of Plates.

PLATE I.

The illustrations are untouched microphotographs taken with a Leitz 2 mm. apochromatic objective and No. 6 ocular. Magnification about 1500. Nos. 4-6 and 9-11 from Lychnis alba. Nos. 1-3, 7 and 8 from L. alba x L. dioica.
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Figs. 1 and 2.—Diakinesis in the pollen mother cell, showing unequal pair at k.
Figs. 3-6.—Equatorial view of the heterotype metaphase showing the XY pair.
Figs. 7-8.—Polar view of the same showing twelve haploid chromosomes. Note the external position of "A" in the plate.
Fig. 9.—Somatic metaphase plate from the female showing twenty-four chromosomes.
Fig. 10.—Equatorial view of the heterotype division in the female. Observe the equal pair of large chromosomes at x.
Fig. 11.—Heterotype plate in the female showing large pair at x.

Plate II.

Camera lucida drawings made with a Leitz 2 mm. apochromat objective, 18 eye-piece and 200 mm. tube length. Reduced to one-half in reproduction giving a magnification of 2200 in the figure.

The figures are from the following plants, from the anthers unless otherwise stated.

Lychnis alba, Nos. 12-14, 22, 25-26, 30, 32-33, 35-39.
L. dioica, Nos. 20 and 37.
L. Flos-cuculi, Nos. 42-47.

Fig. 12.—Resting stage previous to meiosis.
Fig. 13.—Characteristic tight symietic knot.
Fig. 14.—Partial loosening of the knot at a late stage. A typical hollow spireme never appears. Note the local thickening of the thread.
Fig. 15.—Second contraction.
Fig. 16.—Twisted bivalents, a stage immediately following the second contraction.
Figs. 17-19.—Stages of diakinesis. XY pair very clear in figs. 18 and 19. Note the vacuolated nucleolus in fig. 19.
Figs. 20-22.—Equatorial view of heterotype metaphase.
Figs. 23-24.—Anaphase stages.
Fig. 25.—End view of polar plate.
Fig. 26.—Telophase of heterotype division.
Fig. 27.—Chromosomes beginning to separate again.
Fig. 28.—Appearance of nuclear membrane and several nucleoli.
Figs. 29-30.—Show more clearly the precocious splitting for the homotype division.
Fig. 31.—Shows a group of split chromosomes, including a large one.
Figs. 32-33.—Homotype metaphase. X and Y chromosomes clearly distinguished.
Fig. 34.—Tetrad from the hybrid. No inequality or irregularity observable.
Figs. 35-36.—Equatorial view of the heterotype spindle in the female showing the equal or XX pair of large chromosomes.
Fig. 37.—Polar view of same showing XX pair already separated.
Fig. 38.—Two plates of one anaphase, showing equality of XX pair. The dotted chromosome came from next section.
Fig. 39.—Somatic metaphase plate from female, large pair to left.
Fig. 40.—Somatic anaphase showing large pair of chromosomes.
Fig. 41.—Diakinesis-like stage in somatic nucleus. Cf. fig. 30.
Fig. 42.—Diakinesis in L. Flos-cuculi. Notice the ring-like bivalents.
Fig. 43.—Next stage of same.
Figs. 44-45.—Heterotype metaphase, equatorial view.
Fig. 46.—Same polar view, showing twelve chromosomes.
Fig. 47.—Anaphase of same, showing tetrad form of chromosome.
THE CYTOLOGICAL ASPECTS OF THE DETERMINATION OF SEX IN
THE DIOECIOUS FORMS OF LYNCHIS.—KATHLEEN B. BLACKBURN

PLATE II