The Meiotic Phase of Calandra oryzae[1]

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With Plate 3.

Introduction.

The present work has been undertaken in order to investigate further certain peculiarities observed by Tiegs and Murray (1938) in the oogenesis of Calandra oryzae Fab. incidentally to their study on the embryology of this beetle. They described a complete separation of ex-conjugant chromosomes which takes place between the diplotene and equatorial plate stages, thus necessitating a recoupling of homologues at metaphase I. They also observed that by the end of the first meiotic division the diploid number of elements is reconstituted at each pole, such a condition being due to the precocious temporary separation of sister chromatids during anaphase I; this is followed, in the oocyte nucleus, by an intimate pairing of chromatids prior to metaphase II.

These cytological observations have been fully confirmed on the new material, and the temporary separation of the ex-conjugants has been found to occur in spermatogenesis also.

Material and Methods.

An account of oviposition in Calandra, and the method of extracting the egg from the wheat grain is given by Tiegs and Murray (1938).

The following fixatives have been employed: Bouin, Sanfelice,

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Carl, Flemming, Carnoy, and Lebrun's modification of Carnoy. For eggs, Sanfelice and Carl[1] have given the best results, especially the latter which is used hot at 60° C. for 15 to 20 minutes.

After fixation the eggs are washed in 70 per cent. alcohol for 24 hours, when the delicate chorion may be removed. In a small percentage of eggs the chorion appears to be impermeable to the action of the fixative. Cedarwood oil has proved an excellent clearing fluid, and paraffin an adequate embedding medium.

A closely similar procedure has been adopted in dealing with testes except that, in addition to Sanfelice and Carl, Bouin has been used with advantage.

Sections have been cut at 12 or 14 μ, and stained in most cases with Heidenhain's haematoxylin, eosin being employed as counterstain.

Observations.

1. The Number of Chromosomes.—The somatic chromosome number, as first described by Inkmann for Calandra granaria, and later found by Tiegs and Murray in the female of Calandra oryzae, is twelve. In the male the diploid number is eleven (fig. 23, Pl. 3).

2. The Oogonial Divisions.—The sex organs of the female rice weevil conform to the usual type. Each ovary, consisting of two ovarioles, leads by an oviduct to a median tube that widens into the vagina. The individual ovariole is swollen at its tip to form a germarium containing the oogonia. These give rise to egg and follicle cells which occupy the ovarian tubes, the eggs becoming larger and more mature as they approach the vagina.

On account of the excessively small size of the cells and chromosomes, details of the oogonial divisions are difficult to make out. It is relatively clear, however, that in the resting

[1]

Sanfelice.	
Chromic acid, 1 per cent.	16 parts
Formalin . . .	8 ,,
Glacial acetic acid . .	1 part

Carl.	
Distilled water . .	30 parts
Absolute alcohol . .	15 ,,
Formalin . . .	6 ,,
Glacial acetic acid. .	2 ,,

nucleus (fig. 1, Pl. 3) the chromatin is distributed as scattered particles and coarse blocks, sharply defined chromosomes appearing at the onset of mitosis. In fig. 2, Pl. 3, most of the elements have already split into their daughter halves, so that almost twice the diploid number of chromatids is present.

3. The Meiotic Divisions.—For a description of the structure of the unlaid egg, reference should be made to the paper by Tiegs and Murray (1938).

Throughout the meiotic prophase the nucleus, situated in the centre of the egg, is surrounded by a curiously wrinkled sheath, and lies embedded in a spherical mass of eosinophil and rather granular cytoplasm, enclosed by a definite membrane. This perinuclear cytoplasm is absent from the oogonia and is slowly acquired as the oocytes progress down the ovarioles, forming a prominent feature of the more mature cells. Tiegs and Murray suggest that it may be related to the 'pallial substance' which invests the nucleus in the eggs of certain arthropods. Just before the diplotene stage, while the egg is in the last ovarian chamber, the membrane around the perinuclear cytoplasm ruptures, its substance being invaded by neighbouring yolk-granules (fig. 3, Pl. 3). By the time the egg is ready to be laid, the nucleus, completely divested of its wrinkled sheath and perinuclear cytoplasm, has passed into the periplasm where it lies approximately midway between the two poles.

No attempt has been made to describe in detail the very early meiotic phenomena which take place as the oocytes are passing along the ovarian tubes. With regard to those stages previous to the diplotene, it suffices to say that in the female the commencement of prophase is marked by the appearance of a tangle of pale threads which ramify through the nucleus and exhibit no polar orientation. Amongst these filaments one nucleolus is distinguishable (fig. 4, Pl. 3). This is followed by a contraction of the chromatin towards one side of the nucleus, the emerging threads showing bead-like swellings which are linearly arranged (fig. 5, Pl. 3). During the growth period which now intervenes, the chromosomes seem temporarily to lose their identity, and are replaced by a number of chromatic particles which readily take up the basic stains. It is throughout this

stage of development that the investing sheath and perinuclear cytoplasm become increasingly apparent.

Diplotene occurs while the oocyte is in the last ovarian chamber. Fig. 6, Pl. 3, shows a nucleus in this condition, still encased by its sheath and embedded within the remains of the perinuclear cytoplasm. The chromosomes are long, thin, and somewhat beaded; already two pairs (fig. 6, Pl. 3, *a* and *b*) have dissociated into their univalent constituents. An earlier stage of diplotene is figured by Tiegs and Murray (fig. 1, Pl. 3).

From the ovariole the oocyte moves into the oviduct. The chromosomes undergo considerable condensation, and the partial separation of homologues, begun in the long-thread stage, is completed. The ex-conjugants, some of which are constricted, now fall widely apart (fig. 7, Pl. 3). In fig. 8, Pl. 3, the nucleus of a vaginal oocyte is shown; the wrinkled sheath has vanished and the diploid number of univalents, unenclosed by a nuclear membrane, lie free in the periplasm.

By the time the egg is laid, the nucleus has entered the equatorial plate stage, the twelve separate chromosomes having associated once again into six bivalents (fig. 9, Pl. 3).

A study of the corresponding period in spermatogenesis has revealed the fact that a similar dissociation of the homologous chromosomes is intercalated between diplotene and metaphase I. The initial stage of this process is shown in fig. 21, Pl. 3, where the components of two pairs (fig. 21, Pl. 3, *a* and *b*) lie well separated, while in the case of the other three pairs, which are in a less advanced state of contraction, the homologous threads are more closely approximated to one another. The chromosome '*x*' seems to be unpaired. With further condensation, the remaining ex-conjugants dissociate completely so that the full number of separated elements, in the male eleven, is reconstituted. Good preparations make it clear that some of the univalents possess a well-marked constriction (fig. 22, Pl. 3).

In approximately 10 minutes after laying, the egg nucleus is passing through the first meiotic anaphase. At an early stage the separating chromosomes show signs of splitting into their constituent chromatids; this is seen in fig. 11, Pl. 3, where

there are ten chromatids at the polar body, and nine at the oocyte end, with some still aligned upon the equator.

Between 15 and 20 minutes after oviposition the first polar body is beginning to protrude, and the tendency to precocious separation of sister chromatids is even more pronounced. In figs. 12 and 13, Pl. 3, nine or ten elements are recognizable at each pole of the spindle, and the full diploid number is apparently in the process of formation; about half of the chromatids exhibit characteristic shapes which call to mind the constrictions observed in the diplotene univalents.

The first meiotic division is completed in about 30 minutes after laying. The polar body, almost entirely constricted off, is now situated in a depression on the surface of the egg, its chromosomes lying free in the eosinophil cytoplasm; a membrane, however, is formed around the secondary oocyte chromosomes. Fig. 14, Pl. 3, shows the two daughter nuclei, each with practically the full number of separated chromatids. This is from a longitudinally cut egg, where by good fortune, both nuclei are present in the same section. The one lies directly on top of the other, so that on focusing down through one set of chromosomes, the second set is seen immediately beneath. In the two nuclei the chromatids correspond in number, shape, size, and position.

In the case of the female, then, it appears that by the end of the first meiotic division the diploid number of chromosome elements is restored, this being due to an early separation of sister chromatids at anaphase I.

Many preparations of a comparable stage in spermatogenesis have been examined; none show any sign of the precocious dissociation of chromatids, described in the female. Anaphase proceeds normally and the chromosomes clump densely at both ends of the spindle, the nuclei so produced entering a resting phase.

The twelve chromatids of the secondary oocyte nucleus do not clump, but unlike those of the male, prepare without any intervening resting stage for the second meiotic division. By this time there is an accumulation of cytoplasm about the nucleus which has moved away from the periphery and is projecting into the yolk.

Sister chromatids, some of which still show a constriction, now approximate to one another in pairs (fig. 15, Pl. 3, 55 minutes after laying). An intranuclear spindle is formed inside the oocyte nucleus (fig. 16, Pl. 3); no spindle fibres, however, are visible within the polar body, and although its sister chromatids show a marked tendency to reunite, this body never divides but commences forthwith to degenerate, losing its sharp outline and becoming gradually reabsorbed into the periplasm (figs. 16, 17, 18, Pl. 3). At the equatorial plate stage (fig. 17, Pl. 3) the oocyte chromatids may be so intimately associated that it is usually difficult to distinguish the components of a pair.

Anaphase II is initiated by the pulling apart of daughter chromosomes, six of which move to each pole (see Tiegs and Murray, figs. 13, 14, Pl. 21). The nuclear membrane breaks down at late anaphase (although occasionally it may disappear at a rather earlier stage, fig. 17, Pl. 3) and ceases to be recognizable at telophase when the chromosomes become aggregated at opposite ends of the spindle (fig. 18, Pl. 3, 90 minutes after laying). Finally, each nucleus acquires a membrane and enters the resting condition. The second polar body, unlike the first, does not protrude beyond the surface of the egg but remains in the periplasm (figs. 19, 20, Pl. 3).

The subsequent processes involved in fusion of the gamete nuclei and degeneration of the polar bodies have been discussed by Tiegs and Murray, and present no unusual characteristics.

Discussion.

1. The Temporary Dissociation of Ex-conjugants in the Diplotene Stage.

One of the most striking features of the meiotic divisions in *Calandra* is the complete separation of ex-conjugant chromosomes which takes place during the late diplotene stage. This peculiar phenomenon was first described by Agar (1911) in the spermatogenesis of *Lepidosiren paradoxa*, and has since been recorded by Hogben (1920) for the oogenesis of *Cynips*, *Rhodites*, and *Orthopelma*, three genera of parasitic Hymenoptera.

The restoration of the diploid number of separate univalents by the end of the first meiotic prophase is of particular interest with regard to Darlington's hypothesis that the function of chiasmata is to hold homologous chromosomes together from pachytene to metaphase I. He maintains that throughout prophase there is a strong affinity between chromosome threads in pairs, which in mitosis is satisfied by the chromosomes splitting into their daughter halves. In the case of meiosis the leptotene threads, unlike the prophase elements of a somatic mitosis, are unsplit and therefore homologous chromosomes attract each other. Immediately the threads divide, the cohesion between homologues is lost, and they separate except at the points of crossing-over, namely, chiasmata; attractions now operate between pairs of chromatids instead of between whole chromosomes.

Of recent years Huskins has disputed that part of Darlington's theory which attributes universality to the chiasma mechanism; he also holds that the principle of attraction between single threads, and repulsion between pairs of pairs, actually applies not only at prophase but at all stages of both mitosis and meiosis.

2. The Complete Separation of Sister Chromatids during Anaphase I in the Female.

In 1933 Inkmann, working upon the oogenesis of Calandra granaria, described the first meiotic division as equational. A careful examination of the meiotic phase in the allied species, Calandra oryzae, reveals the fact that the first division is undoubtedly the true reduction division, although at first sight it appears equational owing to the precocious temporary separation of sister chromatids during late anaphase I; an account of this process has already been given in the descriptive section.

In most organisms, not only the ex-conjugants at diplotene, but also the sister chromatids of the first anaphase remain in close association. In Calandra the diplotene homologues in both sexes, and the anaphase chromatids in the female, become completely separated.

There is general agreement that the partial dissociation of ex-conjugants in the diplotene stage is due to each chromosome having split (see Tiegs and Murray, fig. 2, Pl. 21, where many of the univalents exhibit a longitudinal constriction). In addition to this 'secondary' split into chromatids, believed to occur at pachytene, Huskins and Nebel, working independently, have described a 'tertiary' split which develops before metaphase I and separates half chromatids. If the concept of the 'tertiary' split is accepted (see fig. 14, Pl. 3, for evidence of a longitudinal cleft in some of the chromatids) then the complete separation of sister chromatids at the first anaphase is brought into line with the temporary dissociation of ex-conjugants during diplotene; both phenomena may be regarded as unusually pronounced manifestations of a repulsion between pairs of paired chromosome elements.

Summary.

1. The somatic chromosome number for the female of Calandra oryzae is twelve; for the male, eleven. The latter is presumably of *XO* composition.

2. In both sexes the haploid number of loosely paired threads is present at early diplotene. Upon condensation, the ex-conjugant chromosomes undergo a temporary complete dissociation into their univalent constituents, so that the diploid number of separate elements is reconstituted.

3. At the equatorial plate stage the twelve univalent chromosomes associate once again into six bivalents.

4. In the female this is followed by a precocious separation of the anaphase chromatids, so that by the conclusion of the first meiotic division the diploid number of chromosome elements is evident at both ends of the spindle. There is no comparable process during the course of spermatogenesis; at anaphase I the haploid set of chromosomes passes to each pole, the nuclei so produced entering a resting condition.

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EXPLANATION OF PLATE 3.

All drawings are of Calandra oryzae, and have been made with the help of a Zeiss camera lucida. The final magnification of the figures, as reproduced, is mentioned in each case.

Lettering.

a and *b*, bivalents dissociating into univalent components; *n*, nucleus; *pb1*, first polar body; *pb2*, second polar body; *x*, unpaired chromosome.

Fig. 1.—Resting oogonial nucleus, showing chromatin distributed as fine particles and coarse blocks. ×1,500.

Fig. 2.—Oogonium, very early anaphase in polar view; almost twice the diploid number of chromatids present. ×1,500.

Fig. 3.—Longitudinal section of primary oocyte; note cytoplasmic 'plug' at anterior end (below) and nucleus (*n*) surrounded by sheath; perinuclear cytoplasm invaded by yolk-globules. ×140.

Fig. 4.—Primary oocyte, early prophase; chromatin spread out into a tangle of threads; nucleolus also visible. (Only the chromatin lying immediately under the nuclear membrane is shown.) From oocyte at anterior end of ovariole. ×1,500.

Fig. 5.—Contraction of chromatin towards one side of nucleus; emerging threads show bead-like swellings. Oocyte at anterior end of ovariole. ×1,500.

Fig. 6.—Diplotene stage; nucleus invested by wrinkled sheath; all that remains of perinuclear cytoplasm has been drawn; two pairs of homologous chromosomes (*a* and *b*) separated into their univalent constituents. Oocyte in last ovarian chamber. ×1,500.

Fig. 7.—Pre-metaphase stage, showing ex-conjugant chromosomes much condensed and completely dissociated; a constriction developed in some of the univalents; note investing sheath still intact. Oocyte in oviduct. ×1,500.

Fig. 8.—Nuclear membrane and sheath disappeared; univalents lying free in periplasm. Vaginal oocyte. ×1,500.

Fig. 9.—Equatorial plate of first meiotic division; spindle fibres well developed, and haploid number of bivalents present. Freshly laid oocyte. ×1,500.

Fig. 10.—Late metaphase I; chromosomes still confined within perinuclear sheath, a structure which usually vanishes at the end of prophase. Vaginal oocyte. ×1,500.

Fig. 11.—First meiotic anaphase; many of the chromosomes split into their component chromatids; ten at polar body, nine at oocyte end of spindle. Ten minutes after laying. ×1,500.

Figs. 12–13.—Late anaphase I; first polar body beginning to protrude; nine or ten chromatids at each pole. Fifteen to 20 minutes after laying. ×1,500.

Fig. 14.—Very late anaphase; nuclei seen in polar view; uppermost nucleus displaced in drawing. At both poles eleven chromosome elements are distinguishable; approximately half of the chromatids in each figure exhibit a constriction. Thirty minutes after laying. ×1,500.

Fig. 15.—Early stage in reuniting of secondary oocyte chromatids; first polar body practically constricted off; membrane investing oocyte nucleus. Fifty-five minutes after laying. ×1,500.

Fig. 16.—Advanced stage of same; note intranuclear spindle, and polar body in process of reabsorption into periplasm. Seventy minutes after laying. ×1,500.

Fig. 17.—Equatorial plate of second meiotic division. Eighty minutes after laying. ×1,500.

Fig. 18.—Telophase II; chromosomes clumped at both ends of spindle. Around equator note 'mid-body' (see Wilson, 1925, p. 144). Ninety minutes after laying. ×1,200.

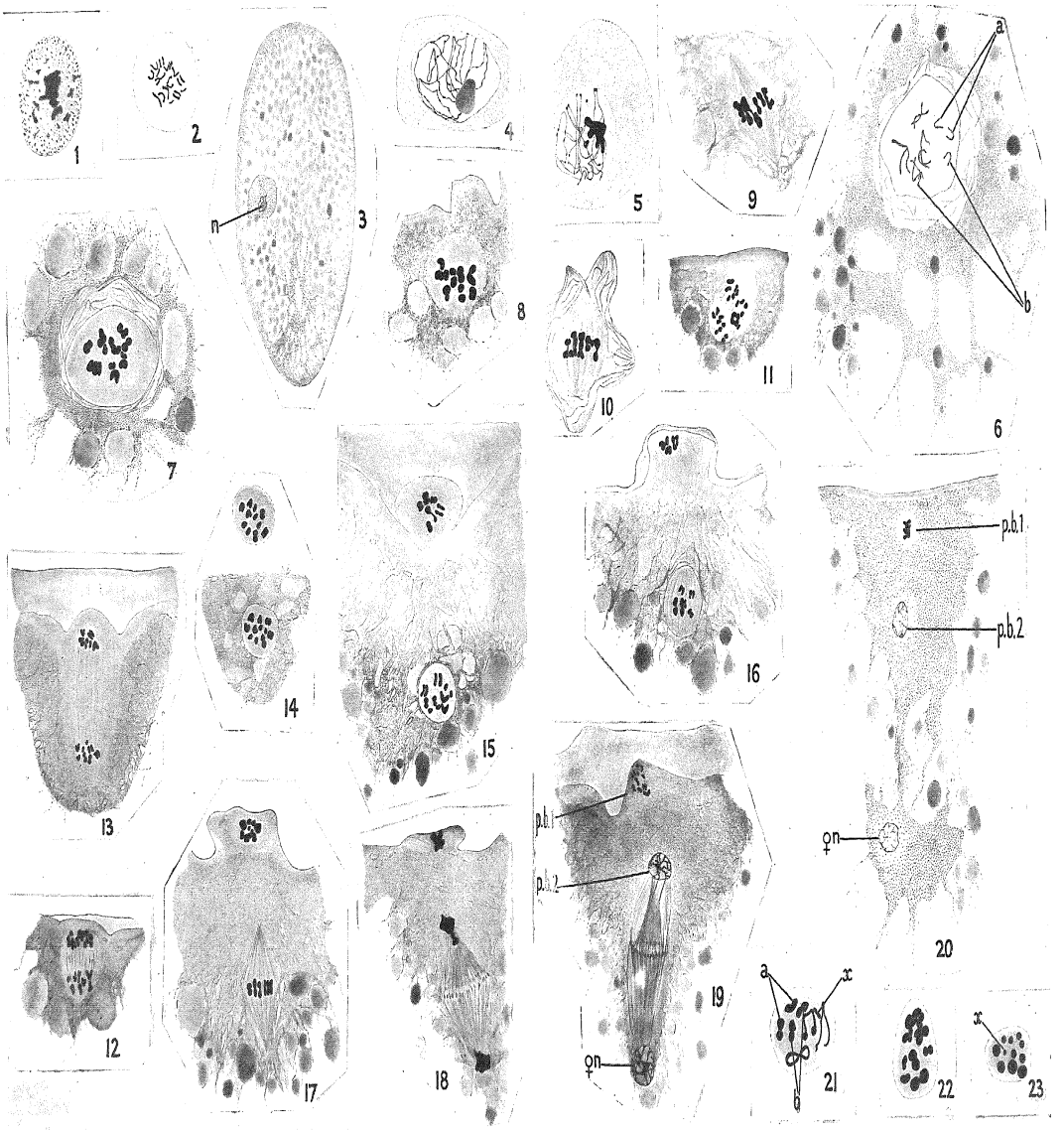
Fig. 19.—Reconstitution of nuclear membranes; nucleus of egg (♀ *n*) and second polar body (*pb2*) in resting state; first polar body (*pb1*) withdrawn into periplasm. Note 'mid-body'. One hundred minutes after laying. ×1,200.

Fig. 20.—Female nucleus lodged deeply in yolk. Reconstructed from three sections. One hundred and twenty minutes after laying. ×1,200.

Fig. 21.—Primary spermatocyte nucleus, diplotene stage; two pairs of homologous chromosomes (*a* and *b*) separated into their constituent univalents; note the single, unpaired chromosome (*x*). ×2,400.

Fig. 22.—Advanced stage of above; homologues more condensed and completely dissociated into their components. ×2,400.

Fig. 23.—Primary spermatocyte, nucleus in polar view. Early stage in pairing of homologues; note eleven elements including unpaired chromosome. ×2,400.



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