CYTOLOGICAL OBSERVATIONS ON PSELAPHID BEETLES

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Little information is available on the life cycles and reproductive stages of Pselaphidae although considerable taxonomic and ecological data on the North American fauna have been published (Park, 1953). Consequently a study was initiated some three years ago to determine the condition of the gonads and germ cells of several species collected at different times of the year. The present report deals primarily with two species, *Batrisodes globosus* (LeConte) and *Pselaphus fustifer* Casey, in which male germ cell development and chromosome numbers are described. Some observations are given on the breeding season of three other species, *Reichenbachia peregrinator* Casey, *Reichenbachia subsimilis* Casey and *Pilopius lacustris* Casey.

MATERIALS AND METHODS

Collection of adult pselaphids and their identification were made by Park. The collection site was Peacock Prairie, a locality that has provided two natural population studies reported previously (Park *et al.*, 1949, 1953). The beetles were brought to Cresap Biological Laboratory where they were dissected and prepared for cytological study by Mickey.

The testes were removed in isotonic saline solution, then transferred to a depression slide and immersed in aceto-orcein stain for ten minutes. The glands were placed on a microscope slide in a fresh drop of stain and squashed under a number 1 cover glass. The temporary smear preparation was sealed with melted paraffin-gum mastic mixture and studied at once. Camera lucida drawings and photomicrographs were made from the temporary preparations. Later the slides were made permanent by the method of Conger and Fairchild (1953) which involves freezing the temporary slide on a block of dry ice, removing the cover glass by lifting a corner with a razor blade, transferring the slide to 95 percent ethyl alcohol, absolute alcohol, then mounting in Euparal.

All slides were studied under Spencer apochromatic 90 X oil immersion objective and paired compensating 20 X oculars. The photomicrographs were taken with the same microscope using a monocular tube and 15 X compensating ocular and a Leitz Micro-Ibso attachment with Leica 35 mm. camera. Microfile film was used and the negatives enlarged in printing.

CONDITION OF GERM CELLS AND BREEDING SEASON

Our general ignorance concerning the life cycles of pselaphid beetles is reflected by the fact that no single species has a known life history from copulation to mature adult. This is significant when we realize that the family is broadly distributed over the world, often in high population density, and embraces at least 5,500 described species. Böving and Craighead (1931) described and illustrated presumably mature larvae of *Batrisodes monstrosus* (LeConte) and *Euplectus confluens* LeConte; Besuchet (1952) described and illustrated mature larvae and pupae of *Plectophloeus* taken in deep soil in Switzerland. The meager information has been summarized by Park (1942, 1947) and Jeannel (1950).

At the latitude of Peacock Prairie (northeastern Illinois) the pselaphid population appears to have two seasonal peaks of freshly-pupated adults, one in the spring (May to mid-June) and one in the autumn (mid-September to mid-October). These callow or teneral individuals are easily distinguished as their integuments are light yellow, translucent and thin (Park, *et al.*, 1953).

In this paper we are concerned with the vernal peak chiefly. The copulation period probably fluctuates with the seasonal variation of environmental factors but includes as early as March 31 (1955) when a pair of *Pselaphus fustifer* were observed in copulation, to as late as April 10 (1952) when copulation was noted for a pair of *Reichenbachia subsimilis*.

Additional inferences may be drawn (See Discussion) from the condition of the gonads and the presence of mature sperm in the males. A male of *Pselaphus fustifer* killed on May 10, 1952, revealed only a few dividing cells but many mature sperm in the testes. A male of *Batrisodes globosus* was dissected on October 10, 1953, and mature sperm were present. On November 14, 1953, a male *Reichenbachia subsimilis* was found to have abundant mature sperm in testes and vasa deferentia; only a few dividing cells were observed. Several specimens of *P. fustifer*, *R. subsimilis*, and *Pilopius lacustris* were examined on March 31, 1955, and no mitotic figures were noted in any; mature sperm were present in all. One male of each of *P. fustifer*, *B. globosus*, and *R. peregrinator* were killed on May 26, 1955, and all showed active development of germ cells and presence of mature sperm.

<div align="center">SPERMATOGENESIS</div>

The pattern of spermatogenesis in the two species, *B. globosus* and *P. fustifer*, follows that described for most insects. The youngest spermatogonia arise in the apical end of the testicular follicles and become grouped into cysts of synchronously dividing cells. The cysts are progressively older as the vas deferens is approached, thus providing a rough seriation of stages in order of their development.

The metaphase plates of dividing spermatogonia provide accurate counts of the diploid chromosome numbers of the different species. Figure 1 shows a spermatogonial resting stage of *B. globosus* in which the X chromosome and procentric areas of other chromosomes are positively heteropycnotic. The pachytene stage illustrated in figure 2 shows polarization of chromosomes toward the heteropycnotic X which lies against the nuclear membrane. Two spermatogonial metaphases of *B. globosus* are shown in figures 3 and 4 and photographs of the same cells are exhibited in figures 9 and 10. Fourteen pairs or 28 chromosomes are evident. The Y chromosome is identified as the smallest in the group, although the X chromosome is not recognizable at this stage. All chromosomes are metacentric or nearly so, although one pair of the larger and one pair of smaller ones have noticeably unequal arms. Figure 11 depicts another spermatogonial metaphase. Metaphases of the first maturation division showing the fourteen tetrads are illustrated in figures 5, 6 and 7. Most chiasmata are terminalized but the largest chromosome at least retains one interstitial chiasma. The sex chromosomes are obvious; the larger X and the minute Y chromosome, both with median centromere, are associated by means of two terminal points, giving the parachute configuration found in so many beetles by Smith (1949, 1953). They appear to exhibit slight negative heterochromaticity. A photograph of this stage is shown in figure 12. Figure 8 depicts a mature spermatozoön of *B. globosus* and a photograph of a similar stage appears in figure 13. Its structure resembles closely that described for the bruchid, *Bruchus quadrimaculatus* Say, (Mickey, 1935) except that the acrosome is more prominent in *Batrisodes*.

Pselaphus fustifer has fifteen pairs of homologous chromosomes or thirty as the diploid number. Figures 14 and 15 delineate spermatogonial divisions and the same cells are photographed in figures 20 and 21. Here, as in *B. globosus*, the Y chromosome is the smallest in the entire group. Likewise all chromosomes are metacentric with two pairs being clearly heterobrachial. Fifteen tetrads are seen in figures 16 and 17 in which the sex chromosome pair shows particularly well. The longest pair of chromosomes is seen to have at least three chiasmata in the tetrad (fig. 17). The same two cells are photographed in figures 22 and 23.
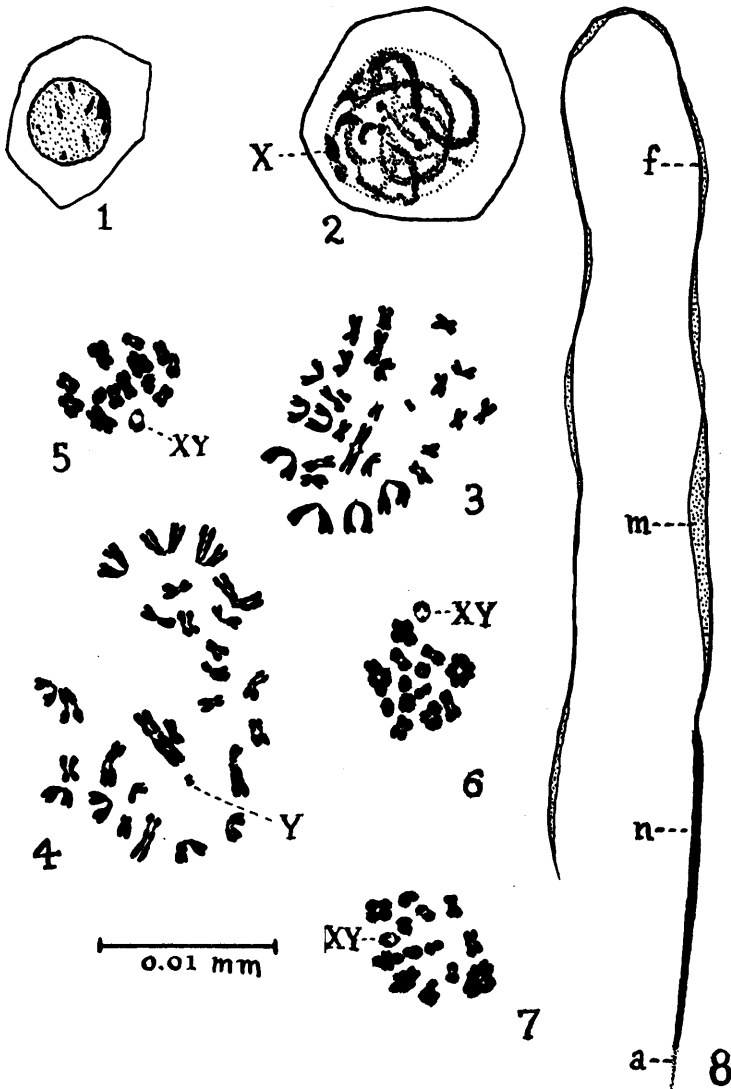
All figures are camera lucida drawings and all are made at the same magnification; scale is indicated.

1. Spermatogonial resting stage; X chromosome and procentric areas of other chromosomes positively heteropycnotic.
2. Primary spermatocyte in pachytene showing polarization of chromosomes toward the heteropcynotic X chromosome which lies against the nuclear membrane.
3. Spermatogonial division of *Batrisodes globosus* showing the 28 chromosomes (14 pairs) in the diploid group.
4. Another division figure of *B. globosus* spermatogonium. The minute Y chromosome is indicated.
5. First maturation division metaphase of *B. globosus* showing the 14 tetrads and identifying the Xyp pair (parachute type).
6. Metaphase I of *B. globosus*.
7. Another figure of first maturation division of *B. globosus*.
8. A mature spermatozoön of *B. globosus*: a, acrosome; n, nucleus; f, axial filament of tail; m, undulating membrane of tail.
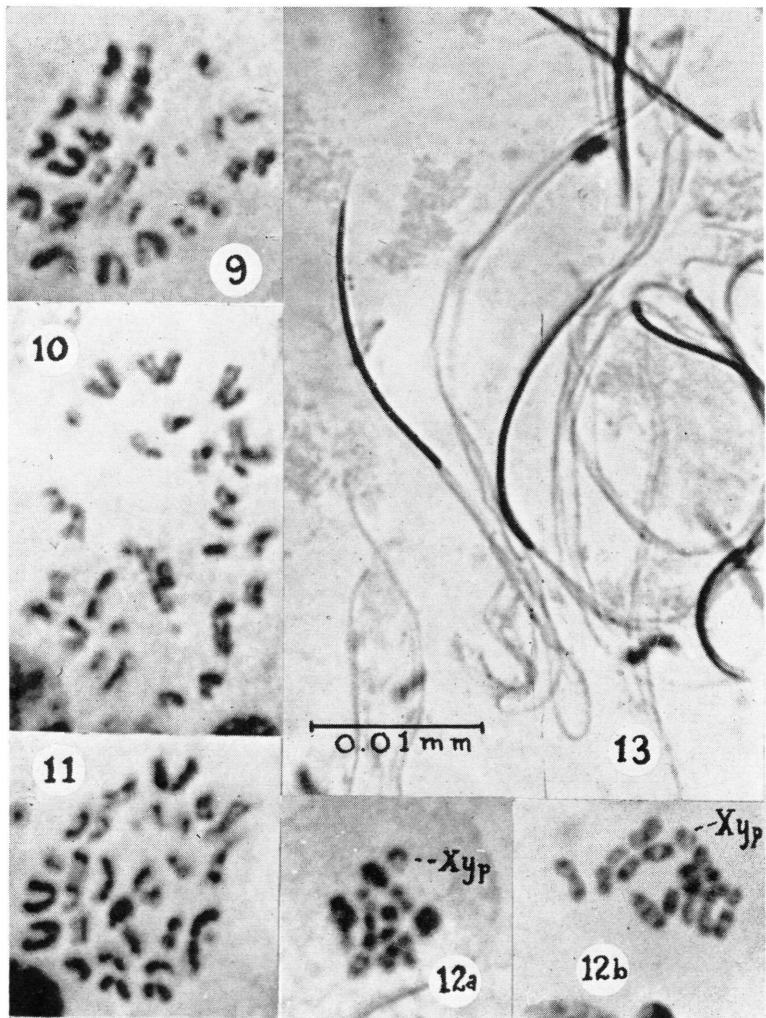
All photomicrographs made on 35 mm. microfile film and all enlarged equally for printing. Scale is indicated.

9. Spermatogonial metaphase of *Batrisodes globosus*. Drawing of this same cell is shown in fig. 3.
10. Similar division figure of spermatogonium (*B. globosus*). Drawing of this same cell is shown in fig. 4.
11. Another spermatogonial metaphase of *B. globosus*.
12a. Metaphase of first maturation division of *B. globosus* showing the parachute type sex chromosomes.
12b. Late metaphase or beginning anaphase I.
13. Mature sperm of *B. globosus*.
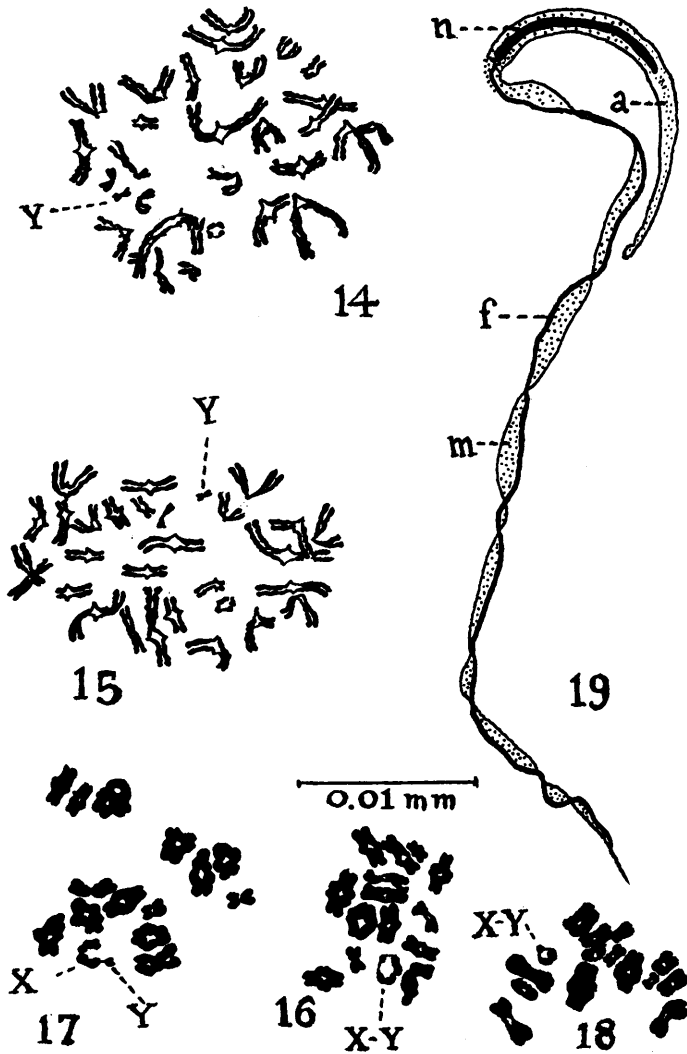
14. Spermatogonial metaphase of *Pselaphus fustifer*. All thirty chromosomes show clearly and the positions of the centromeres are obvious. The small Y chromosome is identified.

15. Another dividing spermatogonium of *P. fustifer*. These same cells are photographed in figs. 20 and 21.

16. Metaphase of first meiotic division of *P. fustifer* showing the 15 tetrads, among which the XY pair stand out distinctly.

17. Similar figure of another spermatocyte (*P. fustifer*). The Y is detached from the X on one end. These two cells are shown in photomicrographs in figs. 22 and 23.

18. Another metaphase I of *P. fustifer* showing the parachute arrangement of the X and Y chromosomes.

19. Nearly mature spermatozoön of *P. fustifer*: a, acrosome; n, nucleus; f, axial filament; m, undulating membrane.

Figure 18 illustrates another metaphase of the first meiotic division. In the same field with the tetrads in fig. 23 is a resting nucleus of a spermatogonium in which the heterochromatic X chromosome is seen lying at one margin of the nucleus. Figure 24 is another metaphase of the first meiotic division in *P. fustifer*.

A mature spermatozoön of *P. fustifer* is shown in figure 19 and a photograph of a similar stage in figure 25. The prominent acrosome, compact nucleus and tail with axial filament and undulating membrane are visible in both drawing and photograph.

POLYPLOID GERM CELLS

Several polyploid spermatogonia and spermatids were noted in both *B. globosus* and *P. fustifer*. Figure 26 shows two early spermatid nuclei of the former species lying side by side, the one having the normal haploid number of fourteen chromosomes and the other about twice as many. A tetraploid spermatogonium of *P. fustifer* containing two heterochromatic X chromosomes and surrounded by normal diploid nuclei is seen in figure 27. Figure 28 demonstrates a group of early spermatid nuclei, most of which are normal haploid but two of which are diploid, and one tetraploid. Later stages of metamorphosis of spermatids may be highly polyploid as seen in figures 29 and 30. These large nuclei should be compared with the normal haploid nuclei in the same field of view. Presence of polyploid germ cells in these pselaphid beetles resembles the situation in grasshoppers (Mickey 1942a, 1942b, 1944, 1945, 1947 and Gremillion, 1947).

DISCUSSION

Judging from the condition of male gonads reported here, the number of dividing germ cells and presence of mature sperm in testes and vasa deferentia, it appears that there is a mating season for five species of Pselaphidae under discussion during the spring and early summer. The greatest mitotic activity of germ cells has been observed in May. The copulating pair of *P. fustifer* observed on March 31, 1955, in which the male had abundant sperm, demonstrates that mating can occur in the Chicago area in early spring; copulation of a pair of *R. subsimilis* on April 10, 1952, tends to suggest an extended mating season, or a seasonal adjustment with local conditions. It would be desirable to check specimens several times each month throughout several years, in parallel with data on the microenvironmental meterology to be certain of the length and onset of the breeding season.

All observations on spermatogenesis have been made on adults, some of which appeared to be rather old as judged by the state of their reproductive systems. This fact may help to explain the relative paucity of dividing germ cells in most of the specimens. In order to obtain the maximum number of mitotic and meiotic figures, probably just-pupated adults (callows or tenerals) are needed. It would be interesting to examine pupae in this regard were they to be discovered.

No members of the Pselaphidae are cited in the most complete and recent list of chromosome numbers of Coleoptera by Smith (1953) who gives information on 340 species including 179 genera and 33 families. He considers the typical formula

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EXPLANATION OF FIGURES

20. Spermatogonial metaphase of *P. fustifer*. Same cell drawn in fig. 14.
21. Similar division of *P. fustifer* spermatogonium. Drawing of this cell shown in fig. 15.
22. First meiotic metaphase of *P. fustifer*. Drawing of this cell shown in fig. 16.
23. The 15 tetrads in metaphase I of *P. fustifer*. Drawing of this cell shown in fig. 17. The heterochromatic X chromosome is visible in an adjacent resting nucleus.
24. Another metaphase I (*P. fustifer*).
25. Nearly mature spermatozoön of *P. fustifer*. A young spermatid nucleus lies between the spermatozoön nucleus and its recurved tail.
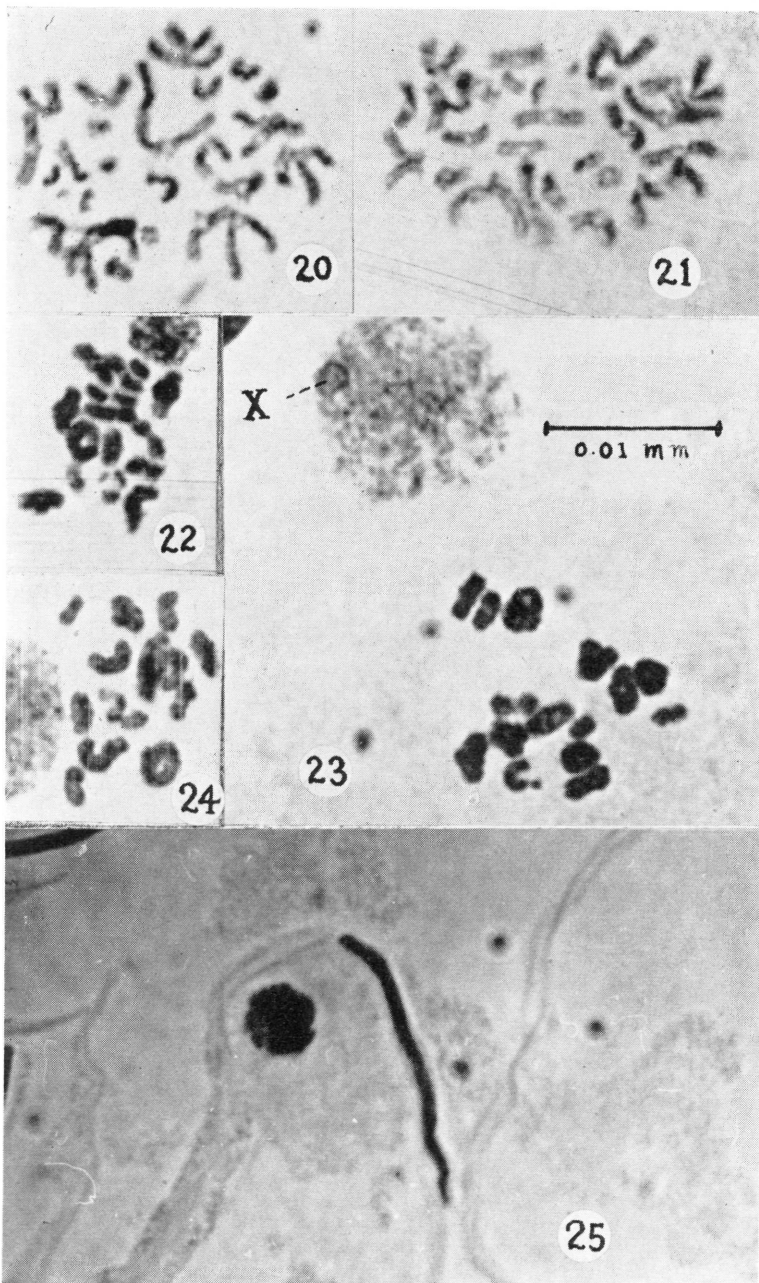
for beetles to be 9 $AA+Xy_p$ (1950a, 1950b and 1952a) or $2n = 20$. The psela-phids dealt with here have 28 and 30, and in the most closely related family of beetles, the Staphylinidae, the known numbers range from 18 to 44. Smith (1952b) discusses the types of changes which lead to diversity of chromosome numbers among beetles. An increase in number of chromosomes may arise by "fragmentation", by which is meant misdivision or break of the centromere thus producing telocentric chromosomes. Since telocentrics are probably unstable, they may become metacentric by inversion or transposition.

A variety of situations with regard to the sex chromosomes exist in Coleoptera (Dutt, 1953, and Smith, 1953): (1) multiple sex chromosomes, (2) X and Y indistinguishable or unidentified, (3) relatively small sex chromosomes, (4) large X chromosome with the Y missing, (5) neo-XY, derivative or complex sex-determining mechanism, (6) rod-type association between X and Y at metaphase I, (7) centric association between the two, and (8) "parachute-type" association of X and small Y chromosome. Smith (1949) thinks that the parachute type is the primitive system in the order, whereas the XO and X- large Y are derivative systems.

The sex chromosome mechanism appears to be alike in *B. globosus* and *P. fustifer;* that is, of the Xy_p or parachute type. In this connection it is of interest to note that a Bahaman species, *Dalmomelba cazieri* Park, was thought to have the XY type of sex determination on the basis of genetic behavior of eye and wing characters in the two sexes (Park, 1954).

The significance of the rather common occurrence of polyploid spermatozoa in insects is problematical. Diploid eggs of *Drosophila melanogaster*, when fer-tilized by haploid sperm, give rise to triploid individuals. It is not known whether diploid (or higher ploid) sperm function or not. In a study of the Louisiana lubber grasshopper, *Romalea microptera*, (Mickey, unpublished) hundreds of young nymphs were examined to determine if any were polyploid. None was found. It may be postulated that such sperm seldom if ever fertilize an egg even though the polyploid spermatids undergo metamorphosis and assume the structure of mature sperm. Several possible reasons may be cited, among which are (1) unfavorable competition of the polyploid sperm with normal haploids and (2) inability to enter the egg through the micropyle because of their large size. Perhaps the micropyle of a diploid egg would be large enough to accept a diploid spermatozoön, and such an event could occur among the enormous numbers of germ cells produced by a species over long periods of time, and thus give rise to a tetraploid individual. However, diploid gametes from such an individual, when united with normal haploid gametes, would give rise to triploids which would be highly sterile. Segregation of chromosomes in the germ cells of an autotetraploid is notoriously irregular resulting in much sterility because of deficiency-duplication gametes. Furthermore, as pointed out by Muller (1925) imbalance of sex chromosomes militates against establishment of polyploid strains among animals.

EXPLANATION OF FIGURES

26. Two young spermatid nuclei of *B. globosus*; the one on the left containing the haploid number of 14, the one on the right approximately twice as many chromosomes.

27. Tetraploid spermatogonium of *P. fustifer* containing two heterochromatic X chromo-somes. The other nuclei in this figure are diploid and contain only one heterochroma-tic X chromosome.

28. A group of metamorphosing spermatids of *P. fustifer*, most of which are normal haploid, but two of which are diploid and one tetraploid.

29. Spermatid nucleus of *P. fustifer*, much further along in metamorphosis, which is obviously highly polyploid.

30. Another highly polyploid nucleus of *P. fustifer* spermatid. A (probably) diploid nucleus lies to the left and a normal haploid nucleus to the right.
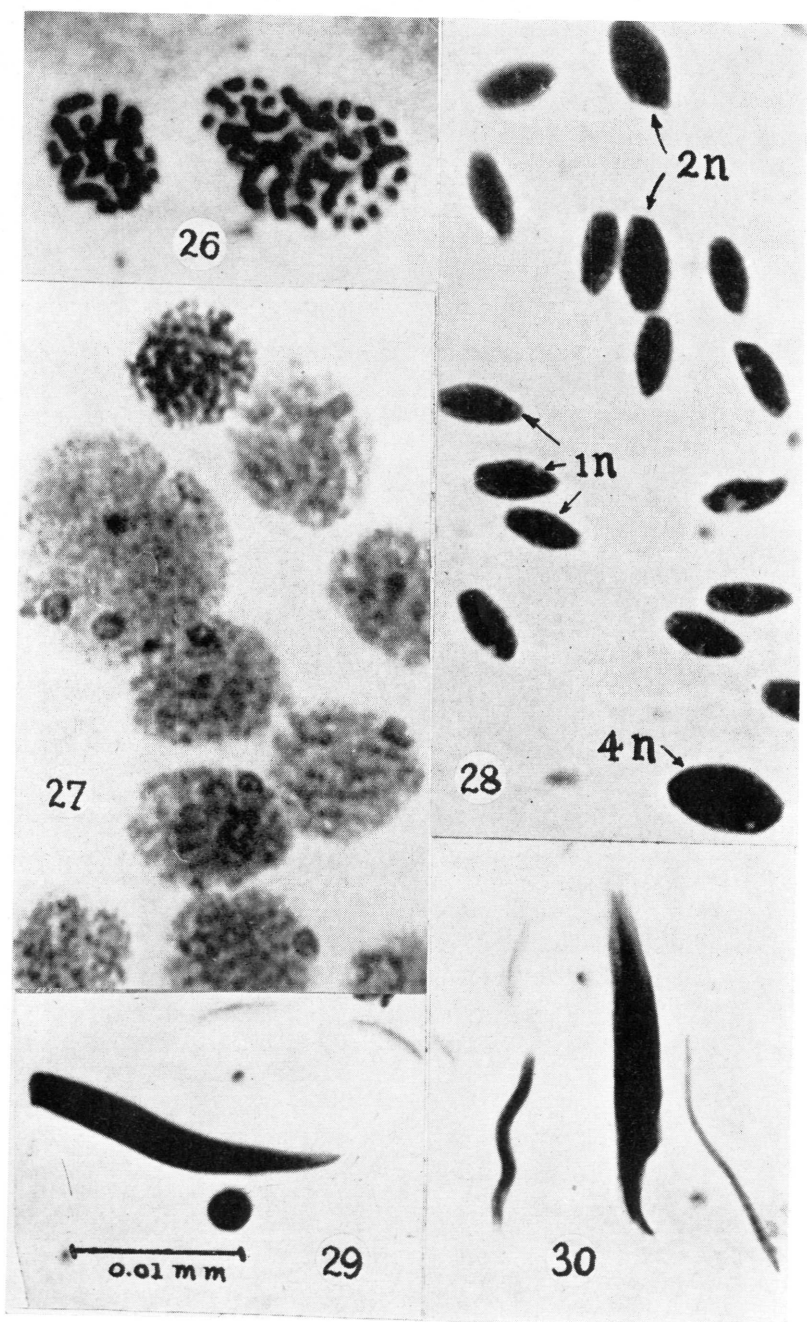
SUMMARY

1. A breeding period during early spring for the five species of pselaphid beetles, at the latitude of Northern Illinois, is established on the basis of condition of gonads and presence of mature sperm in the males, copulation dates, and the appearance of callow individuals in late spring and early summer.

2. The diploid chromosome numbers are determined for *Batrisodes globosus* as 28 and for *Pselaphus fustifer* as 30.

3. An Xy_p chromosome mechanism of sex determination in these species is identified; the minute Y is associated with the X at metaphase I by two terminal contact points, thus resembling a parachute.

4. The structure of mature sperm in *B. globosus* and *P. fustifer* is described.

5. The occurrence of polyploid germ cells in these two species is noted.

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