

## SUMMARY

In *Schizophyllum commune*, a common-*B* mycelium, resulting from the mating  $A_{42} B_{42} \textit{arg-6} \times A_{41} B_{42} \textit{ad-2 nic-3}$ , was found to be a permanent diploid rather than a heterokaryon. The evidence for the diploidy is threefold. (a) The mycelium which carries pseudoclamps typical of common-*B* mycelia is very stable and does not yield vegetative homokaryotic segregants as expected if it were a heterokaryon. (b) Only two vegetative segregants with homokaryotic morphology were found: one was a somatic recombinant and the other, an aneuploid or diploid. (c) A cross between the diploid mycelium and a homokaryon (haploid) gave the segregation expected from a diploid  $\times$  haploid cross. Diploid nuclei in Hymenomycetes are therefore not confined to the basidia as was assumed thus far.

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## A CARABID BEETLE WITH ONLY EIGHT CHROMOSOMES

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RECENTLY a diploid chromosome number of eight was found in a chrysomelid beetle (Virkki and Purcell, 1965). This number, which had not been recorded previously, is the lowest known among about a thousand

species of Coleoptera whose chromosomes have been studied. This paper reports the same chromosome number in another beetle, *Graphipterus serrator*, belonging to the Carabidæ.

## 1. MATERIAL AND METHOD

*Graphipterus serrator* FORSKAL (subfamily Graphipterinae) is a fast-running ground beetle, common in sandy habitats. A number of subspecies, differing mainly in dimensions and in the pattern of white spots on the black elytra, have been described from North Africa, Egypt and Israel.

Six individuals were studied. Three, two males and a female, were collected in the Tureibe sand plain, Negev, Israel, on 11th March 1959. One pair was taken in copula. The other three, also two males and a female, were obtained at Bat-Yam, a dune area immediately south of Tel-Aviv, on 23rd April 1959. The two localities are about 115 kilometres, air-distance, apart, and are climatically quite different.

Each testis is a single long tubule twisted into a compact coil. Along it the various stages of spermatogenesis are distributed in a manner somewhat resembling the spermatogenic wave of certain mammals. Under a dissecting microscope it was possible to identify stretches of the tubule in which spermatogonia and meiotic stages were present. Their diameter was about twice as large as that of the segments in which sperm bundles alternated with typical degeneration products of spermiogenesis.

In both sexes, the gonads were dissected out, fixed and stained in acetic-orcein, and squash preparations were made. In females, the large eggs, occupying the proximal parts of the ovarioles, were removed prior to squashing. The ovaries of the female from Bat-Yam were treated in a solution of 0.66 per cent. sodium chloride for 8 minutes, before fixation and staining. This treatment was found to improve the spreading of mitotic chromosomes at metaphase.

## 2. OBSERVATIONS

All stages of spermatogenesis were present in the testes. The ovaries contained egg cells of all sizes, and follicular cells in mitosis.

The diploid chromosome complement comprises 8 submetacentric chromosomes in both sexes (plate, figs. 1 and 2). In favourable mitotic plates the four pairs can be matched. The two largest chromosomes are easily distinguished, while the two smaller ones, which are closely similar in size, are sometimes difficult to tell apart.

The X and Y chromosomes have not been identified and are presumably much alike. Consistent differences in the morphology of the chromosomes have not been detected between either the sexes or the two populations. Differences, if any, in total length or arm ratio, must be small. Unequalities between the two homologues of a cell, and between homologous chromosomes of different cells and individuals have been observed. These may well be due to differences in the coiling of heterochromatic segments and especially to different degrees of condensation of the centromeres and adjacent regions. The longest chromosome shows prominent centromeric granules. The proximal portion of its longer arm is markedly thinner than the distal part (plate, fig. 3). One pair of the two short chromosomes has a "loose" centromere region varying in extent from cell to cell. Sometimes a cell is seen in which the two arms of this chromosome are almost completely separated, apparently under the pressure of squashing.

Early prophase-I was not studied in detail. At diplotene and diakinesis the majority of bivalents are ring-shaped (plate, figs. 4 and 5) and the two

larger ones, at least, preserve this configuration into metaphase-I (plate, figs. 6 and 7). All bivalents are usually symmetrical. Only occasionally one of the two smaller ones appears to be somewhat unequal (plate, fig. 6). The largest bivalent often shows two distinct narrow centromere regions (plate, fig. 7), possibly reflecting the complex structure of this segment described above.

Two cells with interlocking bivalents have been noted in prophase-I, one involving two bivalents (plate, fig. 5), and another three.

At second metaphase four elements were always present. Early spermatids deserve further study as they have a peculiar bifurcated fork-like shape.

### 3. COMMENTS

Focusing attention on this karyotype is chiefly justified by (a) the rarity of the chromosome number  $n = 4$  among beetles in general, and (b) the deviation of this low number from the high chromosome numbers characterizing most Carabidæ.

The only two examples of  $n = 4$  known so far among Coleoptera are *Homoschema nigriventre*, Chrysomelidæ, Halticinæ (Virkki and Purcell, 1965), and the present species *Graphipterus serrator*, Carabidæ, Graphipterinæ. Although neither females nor analysable mitotic metaphases of males of the former species were described, it appears that the karyotypes of the two species are superficially similar and that both lack distinct sex chromosomes. It must, however, be made clear that no close phylogenetic relationship exists between these two species belonging to different suborders of the Coleoptera. Extremely low-numbered karyotypes thus exist independently in the two classic suborders of beetles. No interfamilial comparisons will be attempted, and comments will be confined to the Carabidæ.

A number of classifications of the huge caraboid complex have been proposed assigning different positions to the Graphipterinæ. Although the taxonomic status of the Graphipterinæ is open to discussion it is of some interest to compare the chromosomes of *Graphipterus serrator* with those of other carabids, since its carabid facies are unmistakable. A more penetrating analysis is unfortunately impossible as no other Graphipterinæ have been studied cytologically.

S. G. Smith has assembled critically all data pertaining to beetle chromosomes in two commendable lists (1953, 1960) which incorporate also his own extensive observations. Further information on chromosomes of carabids is given by Agarwal (1960a, 1960b, 1962), Bouix (1962, 1963), Joneja (1960) and Puisségur and Bouix (1963). Altogether, the chromosomes of about 70 species of Carabidæ were examined; but morphological descriptions and figures are lacking in many cases.

The available data allow of the following main conclusions:

(a) In the Adephaga, which include the Carabidæ, the "normal" or "primitive" karyotype of beetles, as defined by Smith (1950), is not represented. No species with a complement of 18 autosomes and  $Xy$ , sex chromosomes (large X and small y attached to a nucleolus in a parachute-like manner) have been reported. It is thus doubtful whether adephagan karyotypes are at all comparable with those of the other suborders of the Coleoptera.

(b) Sex chromosomes are either XO or XY in the male. These two

systems often occur side by side in the same genus. In fact they coexist in 8 genera, including almost all those where more than one or two species have been studied. This appears to indicate that the changes from XY to XO or from XO to neo-XY have occurred repeatedly and independently. At present the exact nature and prevalent direction of the changes cannot be elucidated in this particular group because of incompleteness of most published observations. Nucleolus-mediated segregation of the sex chromosomes, which is common in polyphagan beetles, is not reliably recorded in this family.

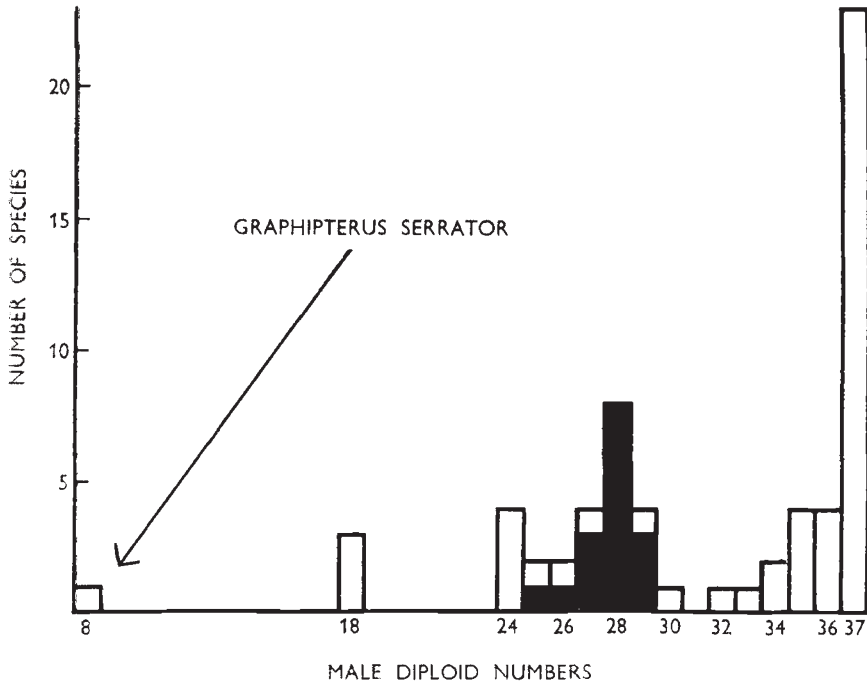


FIG. 1.—Frequency histogram of male diploid chromosome numbers of 64 species of Carabidæ, based on the lists of Smith (1953 and 1960) and subsequent data. Subspecies and doubtful counts were not included. It is possible that one or two numbers determined at the beginning of the century are not correct. All species with even numbers have an XY sex mechanism, while the odd-numbered ones are XO. The black block consists of species of the genus *Carabus sensu lato*.

(c) Hitherto the known range of male diploid chromosome numbers was between 18 and 37. At least part of the karyotypes, including some with the highest numbers, consist mainly or exclusively of metacentric chromosomes.

Inspection of the frequency distribution of chromosome numbers (fig. 1) reveals a virtually four-modal histogram. About one-third of the species have 36 autosomes plus an X-chromosome in the male; none has been found so far to exceed this number. Adding the species with 32 to 36 chromosomes brings the total number of species in this group to about one-half of the total. The second group clusters around 28 chromosomes, with a range from 24 to 30. Representatives of these two groups are found together in one and the same genus, at least in three instances. The third distinct column of the histogram includes three species with 18 chromosomes,

belonging to two different subfamilies. No species with chromosome numbers between 19 and 23 have been recorded.

A wide discontinuity exists between 18 and 8, the chromosome number of *Graphipterus serrator* reported here. A discussion of the origin of such a karyotype, whether "original" or derived, is of necessity highly speculative, especially in view of the complete lack of information on closely related forms.

There is a widespread tendency to look upon "rare" karyotypes as derived from those which are more "common" in the group under consideration, although the relative frequencies of the reported karyotypes may be partly due to sampling chance.

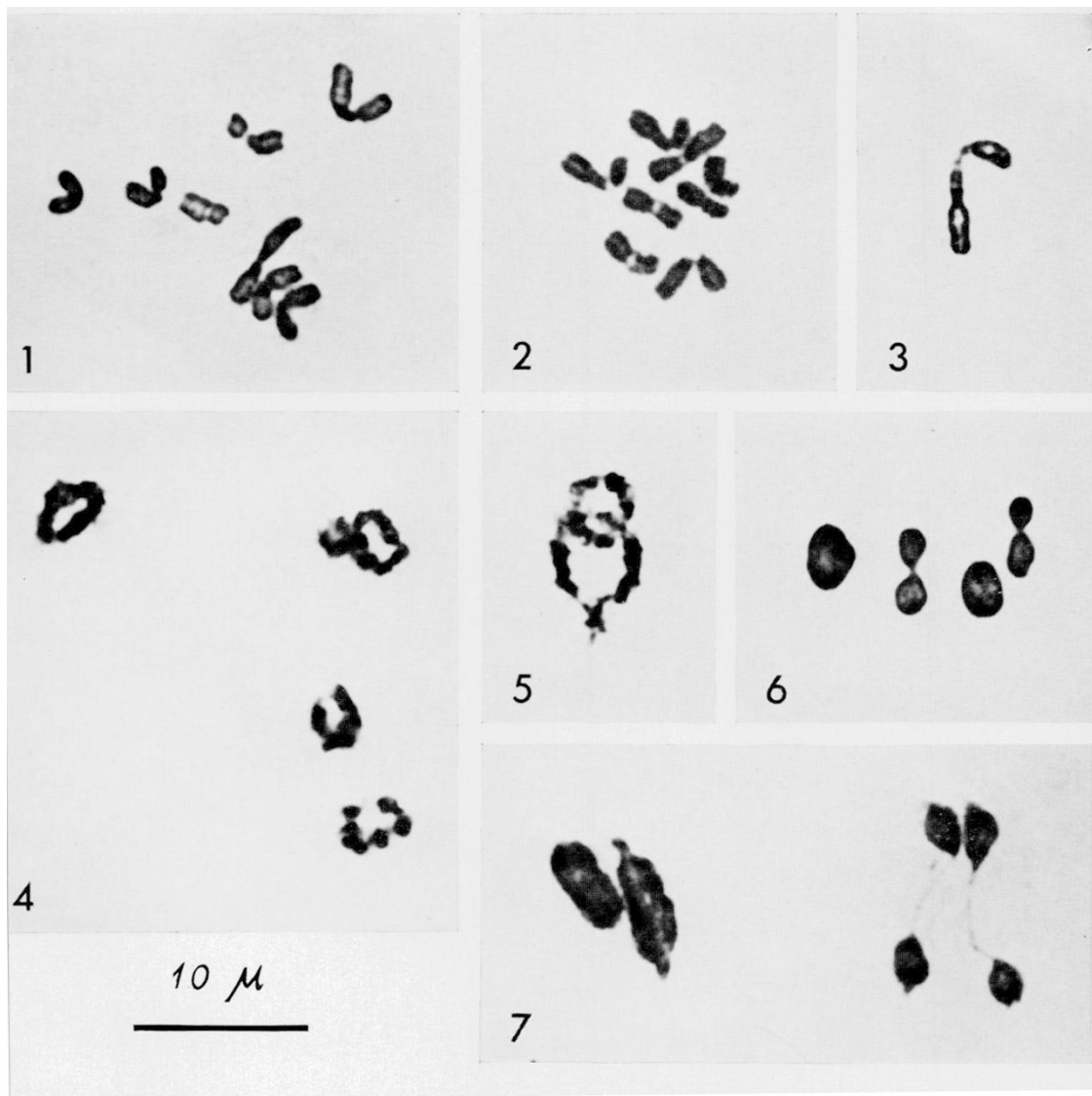
There is in my opinion no compelling reason to assume that the  $n = 4$  karyotype is derived from one with a higher chromosome number by structural rearrangements, loss of heterochromatic segments or otherwise. In fact it is not easy to envisage a mechanism which might have been responsible for such a drastic true reduction or fusion of genetic material.

Alternatively, the low-number karyotype may be considered as ancestral having given rise to the more common caraboid karyotypes by repeated polyploidisation. If a process of this kind did indeed operate, it is partly obscured by chromosome repatterning through structural changes in which the sex chromosomes are frequently involved. Inherent in a hypothesis such as this are well-known and often-discussed difficulties (but see, for example, Goldschmidt, 1953 and Lewis and John, 1957). The idea that polyploidy has played a role in the evolution of the Adephaga can be tested through the accumulation of further data.

Both low and high numbered carabid karyotypes often comprise chiefly metacentric chromosomes. It appears that simple Robertsonian changes, which are of decisive importance in the chromosome evolution of many groups of animals, had only a secondary function here. DNA measurements in the caraboid series, may throw some light on their evolution. Length measurements of chromosomes are a poor substitute for a number of reasons. We have however measured the length of 5 male and 7 female chromosome sets of *Graphipterus serrator* on photographs of metaphase plates obtained by squashing. The average total length was  $32 \mu$  in both male and female cells, with a range from  $22 \mu$  to  $39 \mu$ . This is somewhat less than the shortest complement measured by Bouix (1963) in sectioned material of four species with 28 chromosomes.

In looking for analogous situations of highly deviant karyotypes, one should beware of taxa where sampling may have been much biased. Only in groups where a fair proportion of species have been investigated is the danger of observing merely a sampling effect minimised. A most impressive example of a "foreign" karyotype among classical and well-known objects of cytological study, is provided by a grasshopper of the genus *Dichroplus* (Saez, 1956 and 1957). Most species of this genus possess standard acridiid karyotypes, with 19 or 23 chromosome arms. However, *D. silveiraguidoi* has the amazing number of 12 arms, combined into only 8 chromosomes. No satisfactory explanation has been proposed for the origin of this discordant karyotype, unique in the Acridiidae.

This note may direct the interest of cytologists to the study of Graphipterinae. Sixty-four species, and many subspecies, were recognised by Burgeon (1929), in his monograph of the genus *Graphipterus*. Nearly all are distributed in Africa, mainly in regions devoid of forests. Only a few



Mitosis and meiosis in *Graphipterus serrator*. Acetic-orcein squash preparations. Fig. 2 is from an animal which has been hypotonically pretreated before fixation.  $\times 2500$ .

FIG. 1.—Male from Tureibe. Spermatogonial metaphase.

FIG. 2.—Female from Bat-Yam. Somatic metaphase.

FIG. 3.—Female from Tureibe. The largest chromosome in early mitotic metaphase. Note complex centromere region and the adjacent thinner segment of the longer chromosome arm.

FIG. 4.—Male from Tureibe. Diakinesis.

FIG. 5.—Male from Tureibe. Two interlocking bivalents in diplotene.

FIG. 6.—Male from Tureibe. First meiotic metaphase. Note slight inequality of the bivalent at the right.

FIG. 7.—Male from Bat-Yam. First meiotic metaphase; the two smaller bivalents have already separated. Note narrow centromere region of the largest bivalent.

forms are found in similar habitats of Arabia, Iraq, Sinai and Israel. It is to be hoped that a chromosome survey will contribute to a better understanding of the singular karyotype here described.

#### 4. SUMMARY

1. Males and females of two Israeli populations of *Graphipterus serrator* (Coleoptera:Carabidæ) possess  $2n = 8$  chromosomes. All chromosomes are submetacentric. Sex chromosomes were not identified.

2. Eight is the lowest chromosome number so far recorded in adaphagan beetles. On the basis of an analysis of chromosome numbers reported in the Carabidæ, it is tentatively suggested that polyploidy may have been involved in the evolution of this group.

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