Cytotaxonomy of Passalidae (Coleoptera) [1]

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SUMMARY: Spermatogenesis of 24 species of Neotropical Passalidae, subfamily Passalinae, was studied for chromosomes. Sixteen belonged to the tribe Proculini, 8 species to the tribe Passalini.

All Passalini hace the same chromosome formula: $12^{II}+X$, whereas the tribe Proculini is very variable, the formulas ranging from $8^{II}+$ *neoXY* to $18^{II}+$ *neoXy*. We consider the lowest number the most primitive, because it is closet to the basic karyotype, $9^{II}+ Xy_p$, of Coeloptera Polyphaga. Presumably, the evolution of the Passalid karyotypes started by translocation of X chromosome on an autosome; thereafter, the number of autosomes started increasing by centric dissociation. However, centric dissociation alone cannot explain the range of autosomal number in Proculini, because most chromosomes are metacentric in all species. We have suggested that pericentric inversions have accompanied centric dissociations as a double series of karyotypic othoselection. Localization of chiasmata to the chromosome ends facilitates the former type of rearrangements in the Passalid chromosomes, presence of procentric heterochromatin, the latter type.

Comparison between the Scarabaeoid families shows seemingly controversial fact, that Scarabaeidae, an extremely variable family externally, has almost invariably $9^{II}+ Xy_p$, whereas Passalidae, a very uniform family morphologically, has a great variety of karyotypes. This promoted some discussion on the relationships between karyotype and external morphology.

The number of spermatozoa per bundle is 128 in all Passalids studied. In the genus *Verres*, 25% (32) of the spermatozoa contain a cytoplasmic inclusion.

A tentative phyliogenetic tree of Proculini is presented in page 62.

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[1] Essential parts of this study were presented in the VII National Congress of Entomology of Mexico, México, D. F., october 1970. The Senior Author wishes to express his gratitud to the Universities of El Salvador (Instituto Tropical de Investigaciones Cientificas) and Helsinki, Finland (Rosenberg's Grant) for the support that helped to collect the Salvadorean part of the present material.

Introduction

Of the three Scarabaeoid families, Passalidae is the least known cytologically. Apart from some unpublished data, collected from El Salvador by one of us (Virkki) ten years ago, the only record known to us concerns North American *Odontotaenius disjunctus* (Illiger) [1]. Schaffer (1917) found the chromosome complement of this species to be $12^{II}+$ Xy. Smith (1963) emented this finding as far as the sex chromosomes are concerned: $12^{II}+$ *neo*XY.

Taxonomically, the family Passalidae is a well defined specialized group. Phylogenetically, it is supposed of having arisen a primitive Lucanidae stock (Crowson 1955). Seven species of this latter family have been studied cytologically. Toshioka and Yamamoto (1937) reported $9^{II}+$ X in *Prosopocoilus* (=*Psalidoremus*) *inclinatus* Motschulsky. Virkki (1959, 1967) found $8^{II}+$ *neo*XY in *Dorcus parallelipipedus* (L.), and $8+$ Xy_p in *Sinodendron rugosum* Mann. Abe *et al.* (1969) confirmed the finding of Toshioka and Yamamoto in *Prosopocoilus inclinatus*, and reported four new counts: $12^{II}+$ Xy in *Lucanus maculifemoratus* Motschulsky, $4^{II}+$ Xy in *Nipponodorcus rubrofemoratus* Vollenhoven and $8^{II}+$ Xy in *Macrodorcas rectus* Motschulsky and *M. binervis* Motschulsky. In spite of such a variation in chromosome number, the autosomes seem invariably metacentric. There are, however, notable size differences in the chromosome size within a complement. *Nipponodorcus rubrofemoratus* has one pair of quite large metacentrics.

To elucidate the alleged phylogentic interrelationship, cytological studies of both Lucanidae and Passalidae may prove to be of importance. In the present study, we have checked the cromosomes of 24 Neotropical Passalids belonging to the subfamily Passalinae. This was done in conection with the recent systematical revision of the family by one of us, the results of which have been published elsewhere (Reyes-Castillo, 1970). The data collected earlier in Central America will also be published here.

Material and Methods

Most of the material was fixed in Mexico and sent to Puerto Rico for cytological studies. Adult beetles were opened ventrally with scissors and fixed in *toto* for overnight in 1:3 acetic alcohol, and shipped and stored in 70% ethanol. The storage at + 2° C in refrigerator, had to be prolonged up to 5 years in some cases. This, as well as difficulties in sexing the beetles (the group does not present sexual dimorfism) and the generally late start and/or slow process of spermatogenesis, have limited the material considerably.

In laboratory, the alcohol-stored testes were shortly (20-40 sec.) refixed in Kahle-Smith (96% ethanol 15: formaline 6: glacial acetic acid 2), which no-

[1] This species was known for a long time as *Passalus cornutus* Fab. or as *Popilius disjunctus* (Illiger). In Reyes-Castillo's (1970: 149-50) recent work its taxonomical position is discussed.

tably enhances the contrasts, and squashed in 45% acetic acid. The preparations were studied and photographed with Zeiss Photomicroscope, under phase contrast.

Testes of the specimens from El Salvador were removed in laboratory conditions in 1959-60, fixed in Kahle-Smith for a few minutes, squashed, and studied preliminarly under phase contrast. Selected slides were stained with acetocarmine or basic fuchsin, made permanent and studied and photographed as above.

In a few cases, tips of young ovarioles were studied for female mitoses. They were prepared and studied in the same way as the testes.

The list of the material is as follows:

SPECIES	FIXING DATE	LOCALITY
Tribe Proculini		
1. *Oileus sargi* Kaup	17. V. 67	Lagunas de Montebello, Chiapas, México
2. *Oileus rimator* (Truqui)	24. VII. 65	Omiltemi, Guerrero, México
3. *Spurius bicornis* (Truqui)	11. VII. 64	Villa Juárez, Puebla, México
4. *Proculejus brevis* (Truqui)	29. VII. 66	Huauchinango, Puebla, México
5. *Ogyges politus* (Hincks)	27. VII. 60	Trifinio, El Salvador
6. *Prosoclitus* n. sp.	V. 68	Santa Rosa, Chiapas, México
7. *Petrejoides orizabae* (Kuwert)	17. V. 64	Patoltecoya, Puebla, México
,, ,,	16. X. 64	Villa Juárez, Puebla, México
8. *Petrejoides* n. sp.	9. XI. 68	Huautla de Jiménez, Oaxaca, México
9. *Heliscus tropicus* (Percheron)	12. X. 64	Huatusco, Veracruz, México
10. *Odontotaenius striatopunctatus* (Percheron)	1. V. 67	Huauchinango, Puebla, México
11. *Popilius eclipticus* (Truqui)	20. VIII. 59	La Palma, El Salvador
12. *Verres corticicola* (Truqui)	11. VII. 64	Villa Juárez, Puebla, México
,, ,,	V. 67	Santa Rosa, Chiapas, México
13. *Publius agassizi* (Kaup)	16. VII. 59	Apaneca, El Salvador
14. *Veturius transversus* (Dalman)	24. III. 60	Cacahuatique, El Salvador
15. *Chondrocephalus debilis* (Bates)	V. 67	Santa Rosa, Chiapas, México
16. *Coniger ridiculus* (Kuwert)	V. 67	Santa Rosa, Chiapas, México
Tribe Passalini		
17. *Ptichopus angulatus* (Percheron)	16. IX. 67	Santo Tomás, Estado de México
18. *Paxillus leachi* Mac Leay	17. X. 59	Izalco, El Salvador
,, ,,	5. XII. 59	Izalco, El Salvador
,, ,,	3. III. 60	San Diego, El Salvador
,, ,,	24. XI. 60	Cacahuatique, El Salvador
19. *Passalus suturalis* Burmeister [1]	28. X. 60	Metalío, El Salvador
,, ,,	9. XI. 60	Nancuchiname, El Salvador
20. *Passalus punctatostriatus* Percheron	26. III. 68	Río Coatzacoalcos, Oaxaca, México
21. *Passalus interstitialis* Eschscholtz	7. XII. 59	Izalco, El Salvador
	9. XII. 59	Izalco, El Salvador
	28. X. 60	Metalío, El Salvador
	31. X. 60	Metalío, El Salvador
,, ,,	9. XI. 60	Nancuchiname, El Salvador
22. *Passalus interruptus* (Linneo)	24. X. 59	La Libertad, El Salvador
23. *Passalus punctiger* LePeletier et Serville	26. III. 68	Río Coatzacoalcos, Oaxaca, México
,, ,,	6. IX. 63	El Tajín, Veracruz, México
24. *Passalus mirabilis* (Kuwert)	V. 67	Santa Rosa, Chiapas, México.

[1] Determined by H. W. Hincks.

OBSERVATIONS

The chromosomal behaviour and extrachromosomal features of the spermatogensis are quite similar in all Passalids studied. Therefore we will describe in detail only one Proculini and one Passalini and compare the rest of material briefly with them.

1. Proculini: *Oileus sargi* Kaup.

Judging from the few spermatogonial mitoses we encountered, most or all chromosomes are metacentric. The 2n-number is 18 (Fig. 1). If we arrange these chromosomes in pairs, it appears that two form an inequal pair, being thus obviously the sex chromosomes.

In the early prophase of spermatocyte I, the sex chromosomes appear paired and associated with the nucleolus. All chromosomes show heterochromatic blocks (Fig. 2). As such blocks of the bouquet stages of Coleoptera are usually procentric, it seems, judging from Fig. 2, that two pairs of autosomes are acrocentric, the remainder, metacentric.

There is a tendency of the chiasmata to be localized at the ends of the bivalents. Thus the opening-out of the bivalents immediately leads to ring and rod figures. Interstitial chiasmata do occur, however, because up to 3 cross figures per cell have been seen in diakinesis. The sex bivalent is positively heteropycnotic in diakinesis (Fig. 3).

First metaphase shows 9 bivalents, although by first sight it may seem that there are ten. This is so because both X and Y appear bivalent-like, and there is a space between them. We include two photographs to show this aspect (Figs. 4 and 5). Early anaphases show that the association was arranged by one arm of both X and Y, probably by a chiasma (Fig. 6). Obviously the bivalent is a *neo*XY, the chromosomal formula of the species being thus $8^{II} + neoXY$.

2. Passalini: *Passalus interstitialis* Eschscholtz

Numerous well squashed spermatogonial mitoses were found. They show that all chromosomes are metacentric. The 2n-number is 25, which suggest XO mechanism of sex determination (Fig. 7). Again, all pachytenic chromosomes show a procentric heteropycnotic block (Fig. 8). A typical nucleolus of the early prophase is present. In the diplotene, all chromatin turns diffuse, except for a small bipartite knob. The ordinary nucleolus disappears, instead, a chain of nucleolar droplets is formed (Fig. 9).

When respiralized, the autosomal bivalents are invariably in a ring form (Fig. 10). An interstitial chiasma was never seen. This suggests localization of chiasmata originally to the ends. Procentric heteropycnosis is still recognizable. The X chromosome is first positively, later (Fig. 11) negatively heteropycnotic.

First metaphase (MI) shows 12 autosomal bivalents. Many of them are rod-formed, which shows that only one chiasma exists in many of the diakinetic

rings. The unpaired X chromosome lies tranversally in relation with the bivalents (Fig. 12). To gain an idea of its typical location in the spindle, we collected the following statistics:

TABLE 1. POSITION OF THE X CHROMOSOME IN MI

NUMBER OF OBSERVATIONS

	In equatorial plate	*Laterally of equatorial plate*	*Close to pole*	
Male No. 1	86	129	32	
Male No. 2	6	10	—	
Male No. 3	1	4	17	
Male No. 4	65	72	39	
Male No 5	4	2	4	
Total	162	217	92	= 471
	(35%)	(46%)	(19%)	(100%)

Taking into account that in perhaps up to 50% of cases, the squashin brings a laterally located X in between the autosomes, it seems certain that the position of X has been clearly outside of the autosomal group in about 80% of cases.

Encountering of about every fifth X chromosomes close to a pole in MI cannot be taken as a tendency of precessive heterokinesis of the X. On the contrary, the X tends to be the last to reach the pole in A I (Figs. 13 and 14). The frequent closeness of the metaphasic X to the pole is perhaps due to a movement betwwen the poles, like in the grasshopper *Melanoplus* (Nicklas 1961).

The second metaphases show either 12 chromosomes, or 12 + X (Figs. 15 and 16).

The chormosomal formula of the species is thus $12^{II} + X$.

3. Other species.

Characteristics shared by all species studied are prevailing metacentry, procentric heteropycnosis of all chromosomes in prophase, tendency of chiasmata to be localized to the ends of the chromosomes, and a tendency to a prolonged, diffuse diplotene. The tribe Proculini is more variable than the tribe Passalini, especially in chromosome numbers.

Undoubtedly, the most typical feature of the contents of a Passalid testis is the very abundant diakinesis with its autosomal rings where the procentric heterochromatin is usually very accentuated (Figs. 18 to 26 and 28 to 35; for a specially nice case, see Fig. 33). We call this phase diakinesis, because we have seen in it, in a few cases (like just in *Oileus sargi*), chiasmata in the process of terminalization. In the vast majority of cases, the chiasmata seem terminal as soon as the bivalents reappear from the diffuse stage. As pointed out just when *Passalus interstitialis* was described, the early ring form does not prove that chiasmata are formed in both arms. On the other hand, rod-looking bivalents of

the late diakinesis can be rings, if the distalmost parts of the arms are very soft and stretched. This is best seen comparing early and late diakinesis of *Chondrocephalus debilis* (Figs. 18 and 19). Under Scarabaeoidea, the only beetles having a similar diakinesis are the *Pleocoma* (Virkki 1967).

A. Proculini

Oileus rimator (Truqui) has a cytology very close to *O. sargi*. The chromosome number is the same, but the neoXY looks less clear and could be confunded with a *Xy*. *Chondrocephalus debilis* (Kuwert) has $11^{II} + Xy$ (Figs. 18 and 19). 12^{II} + *neo*XY was encountered in *Odontotaenius striatopunctatus* Percheron. $12^{II} + Xy$ in *Ogyges politus* (Hincks). $13^{II} + Xy$ was found in *Publius agassizi* Kaup, *Verres corticicola* (Truqui) (Fig. 20), and *Petrejoides orizabae* Kuwert. Surprisingly, a new, still unnamed *Petrejoides species* has a number as high as 18^{II} + *neo*XY (Figs. 17 and 23). *Veturius transversus* (Dalman) (Figs. 21 and 40) and *Proculejus brevis* (Truqui) have 13^{II} + *neo*XY (Fig. 42). *Spurius bicornis* (Truqui) *has* $15^{II} + Xy$ (Fig. 22). 16^{II} + *neo*XY was encountered in *Popilus eclipticus* (Truqui) and *Heliscus tropicus* (Percheron) (Fig. 25). *Coniger ridiculus* (Kuwert) has $17^{II} + Xy$ (Fig. 26). $18^{II} + Xy$ was found in *Prosoclitus* n. sp. (Fig. 24).

In some cases the *neo*XY was as obvious as in *Oileus sargi*, in others, the mode of association was less certain. However, it seems possible to us, that a *neo*XY is involved in all these cases, and even in those where had to mark *Xy*, in lack of morphological justification for a *neo*XY. We must emphasize the fact, that the primitive *Xy* bivalent was not encountered.

B. Passalini

Contrary to Proculini, the chromosome number did not vary within the tribe Passalini. All species studied have $12^{II} + X$ (Figs. 27 to 39). *Ptichopus angulatus* (Percheron), *Passalus mirabilis* (Kuwert), *Passalus punctatostriatus* Percheron, and *Paxillus leachi* Mac Leay have a meiotic history well comparable to that of *Passalus interstitialis*. *Passalus suturalis*, *P. interruptus* and *P. punctiger* differ in having two pairs of large chromosome with one arm heterocromatic. The bivalents of these chromosome are arranged by only one chiasma, invariably formed in the euchromatic arms (Figs. 28 to 31 and 36 and 37). In these species, the diplotenic droplet nucleous is formed by these large chromosomes (see Fig. 31). They are probably derived from the normal-sized droplet-formers of the karyotypes like *Passalus interstitialis*.

4. Some remarks on sperm bundles.

As reported by Virkki (1969), the number of spermatozoa per bundle (spz/b) is fairly constant per species in insect orders above Odonata. The spz/b also tends to decrease as a result of specialization.

All Passalids of our material have 128 spz/b, which is the same as in *Aphodius*, and the lowest within Scarabaeoidea at the same time (Fig. 45). The

size of sperm cells is however much smaller than in *Aphodius*. Reduction of spz/b from the typical Scarabaeoid 256 to 128, and reduction of testis follicles to a total of four, are certainly factors limiting the total production of spermatozoa, but this is compensated wholly and even exceedingly by the enormous production of bundles. Despite the fact that the spermatogenesis of the Passalids proceeds in one wave, we were not able to make a satisfactory estimation of the amount of sperm bundles produced. It seems possible that the total amount of spermatozoa is up to 100 times more than in *Pleocoma*, for which an estimate of about 6,6000,000 was given (Virkki, 1967).

A peculiarity reported earlier by Omura (1951) in *Mylabris postulata* (Bruchidae) and by Virkki (1956) in *Tenebrio molitor* (Tenebrionidae) was this time encountered in *Ptichopus angulatus*: the nuclei of spermatids become divided in two groups of equal size, that move apart from one another when the tails start to prolongate (Fig. 46). In *Ptichopus*, this is an exceptional process. Two-headed mature bundles were not seen, nor bundles with spz/b = 64. Thus the abnormal bipolar bundles either turn to normality during the spermiohistogenesis, or are eliminated. A possible explanation for the bipolarity is a competition of two cyst cells to become a cap cell.

Another peculiarity was found in the genus *Verres*. This has a Feulgen-negative cytoplasmic inclusion in spermatocytes (Figs. 20 and 47). In both meiotic anaphases, the inclusion goes undivided to one of the poles. Thus only 1/4 of the spermatozoa will have it. Because it survives the spermiohistogenesis, 32 inclusions can be still counted in young sperm bundles (Fig. 48); soon thereafter they disintegrate.

Discussion

1. Exterior *versus* karyotype variation.

By exterior variation we mean here the exterior *morphological* variation only, being wholly aware that the physiology is as much subjetc to genetic control as is the morphology. If we deem a group monotonous, we do not consider physiology, because we do not know it. This is how concepts of diversity of a systematic group are usually formed, owing to lack of physiological information.

Compared with other Scarabaeoid families, Passalidae is a notably clearcut, uniform group. Within the subfamily Passalinae, the tribe Proculini is externally more variable than the tribe Passalini, at least in the Neotropics. In accordance to this, we found only one chromosome number, with minor variation of karyotype, in Passalini, whereas in Proculini, both the morphology, and especially, the number of the chromosomes vary widely. This variation is so extensive indeed, that is seems to be in a controversy when compared to the exterior uniformity of the family Passalidae. The controversy increases even more if we compare the cytology of all Scarabaeoid families (Table 2).

The family Scarabaeidae, externally extremely variable, is very conservative as chromosomal formula is concerned: most species studied retain the basic Polyphagan $9^{II} + Xy_P$. Most of the few deviations from this are towards lower num-

TABLE 2. CHROMOSOME FORMULAS OF THE THREE SCARABAEOID FAMILIES

5+neoXY 2	6 + Xyp 2	8+Xyp 11	9 + Xyp 118	10+XY 3	SCARABAEIDAE							
4+Xy 1	8+Xy 2	8 + neoXY[1] 1	8+Xyp 1	9+X 1	12 + Xy[2] 1	LUCANIDAE						
PASSALIDAE	8+neoXY 2	11+Xy 1	12+X 8	12+Xy 1	12+neoXY 1	13+XY 4	13+neoXY 2	15+Xy 1	16+neoXY 2	17+Xy 1	18+XY 1	18+neoXY 1

[1] Dorcinae.

[2] Lucaninae.

bers, through autosomal centric fusion and through a *neo*XY formation. Lucanidae, also very variable externally, is little known cytologically, but the seven known karyotypes are rather diverse, promising a wide range of variation. Thus Scarabaeidae and Passalidae remain the main contrasting cases.

When the data of Table 2 were presented in the VII National Congress of Entomology of Mexico, several prominent taxonomists expressed their surprise and even their doubt on the reliability of chromosomal data for systematics. We have heard similar expressions earlier, in connection with other systematical groups. Some groups are really thought-provocative in this respect. Let us mention only the related order pair Trichoptera-Lepidoptera: a group of externally very diverse insects, having 28 to 31 pairs of chromosomes in the vast majority of cases (Suomalainen 1969), and despite of diffuse centromeres which should specially facilitate evolution of karyotipe by fragmentation.

One thing is firmly established: the main part of the genotype is located in the karyotype, which thus deserves all respect as the home of the gene-based variability. If variation of karyotype does not parallel variation of the exterior, the seeming controversy must find a plausible explanation. To understand this, we must discuss the present concept of chromosome and karyotype structure in relation with the genetic effects.

A. Chromosomal formula and karyotype.

First of all, it is necessary to point out that all above comparisons are made between chromosome formulas which are, as we are accustomed to write them in the Coleopteran cytology, records of the situation in the first meiotic metaphase. Although they are more informative than the plain chromosome numbers, giving in a compact form the mode of association of chromosomes, number, kind and size difference of sex chromosomes, and even some infomation on translocations, they cannot express all details of the structure of the karyotype. Teh same is true always when chromosome numbers or formulas are compared. Expressing us in formulas, we say, for instance, that 118 of the 136 cytologically known Scarabaeidae $9^{II} + Xy_p$. This does not mean that the 118 karyotypes are indistinguishable from one another. It is probable —although not necessary— that there are microscopically detectable minor differences between them, sufficiently so for an intelligible taxonomical grouping. Thus the formula does not give the exact karyotype. But it may be —alas— a sufficiently exact unit to express our knowledge on the structure of many a karyotype.

B. *Karyotype invariable, phenotype variable: variation at the gene level.*

Almost all changes in the genes escape the karyotype analysis (with the exception of those genes affecting the chromosomes themselves, like their paring relationships). Only crude aberrations are visually analyzable (except for Diptera, owing to their giant chromosomes). A great part of the rearrangements are eliminated, either because they are genetically intolerable or because they are in-

capable of passing through cell divisions. Obviously, the vast majority of all genetic variability is based on the changes and recombinations at the gene level. Thus it is perfectly possible that one group may show a vast external variability, although the karyotypes look similar. There are numerous good examples of this, for instance among domesticated animals, where the natural selection has been largely replaced by a multifocused artificial selection, that has fixed a great variety of rare or perishable mutants. Let us take the dog, for example. The external variation is such, that if the species were extinct and known as fossils only, it would be hard to believe that it is one species only. All this variability is drawn out of a karyotype of 78 chromosomes, pratically similar in all "races" (Hsu and Benirschke 1967). Similarly, hundreds of mutant lines of *Drosophila* have 4 pairs of similar chromosomes; if there would not be the giant chromosomes which reveal fine details, the karyotype would seem the same in the majority of cases. Actually, will *Drosophila* taxa with so-called homosequential chromosomes also exist; in such cases, even the giant chromosomes look alike (Carson 1970).

C. *Karyotype variable, phenotype invariable.*

These cases are certainly hard to understand if we stick to the classical concept of chromosome structure, which emerged from the crossing over analysis by Morgan and his school, and was completed by cytological analysis of the giant chromosomes of *Drosophila*, begun by Heitz and Bauer (1933). According to this plan, the genes are linearly placed in the axis (chromonema) of the chromosome, one after another, and namely so that the knobs called chromomeres contain genes, the interchromomeral connections not. If the structure would be that simple throughout, and if we think that all genes are indispensable for the developing of a normal phenotype, then any karyotype would indeed be rather intolerant of changes, because loss of a single chromomere would have fatal effects. A later discovery, that the chromomeres are not structural units but just coiled parts of the chromonema (Ris 1945), did not lessen this intouchability of the chromosome, but merely accentuated it by elimination from the view the alleged geneless fibrils between chromomeres.

This oversimplified picture started changing already when Heitz (1928) discovered heterochromatin. A vast literature has appeard since then, concerning this special kind of chromosome consttituent, and several properties are adscribed to it (see review by Hannah 1961, Tschermak-Woes 1963. Mittwoch 1967), among them reversible genetic inactivation, or lack (inerty) or rarity (subinerty) of important genes. These lats properties render the heterochromatin particularly tolerant of breaks, exchanges, and of partial or total elimination. Heterochromatin is rather common. Supernumeray (also called accessory, or B, chromosomes) are often totally heterochromatic, sometimes also regular members of the caryotype can be so (Manna and Mazumber 1967, Virkki 1968). Heterochromatic segments can occur any where in the chromosomes, but the region around centromere is especially susceptible. White (1954, p. 197) thinks that procentric

heterochromatic is probably present in all chromosomes. This is particularly interesting because it explains why whole-arm translocations (centric fusions and dissociations in White's, 1969, sense) are of such an importance in the karyotype evolution: exchanges that involve the procentric heterochromatic region (and also the proper centromere region, according to John and Hewitt, 1966), are apparently well tolerated. Thus remarkable changes of the chromosome number can be arranged by whole-arm rearrangements (like in Mammalia: Ohno 1969) without detrimental genetic effects. Our present series of Proculini is also an example of this; here the centric fragmentation must have been the principal means of increasing the autosomal number.

The extensive heterochromaty of the sex chromosomes facilitates structural changes in them. Expecially the y chromosome, often totally heterochromatic, can be reduced to a tiny particle, the only remaining function of which is to act as an orientation partner for the X chromosome in meiosis. This is probably the case in many Xy_p bivalents of Coleoptera. If X finds its way of segregating without partner, such a y is rendered dispensable and may disappear, as has happened in many insects, including the tribe Passalini of our present material.

The evolutionary reduction of the y chromosome introduces an additional and ample view of chromosomal dispensability: euchromatin (the genetically important chromatin) can turn to heterochromatin. Primitive sex chromosomes are apparently just a pair of conventional homologues where the recently formed sex determining genes are located (Ohno 1967). In order to avoid recombination of these genes it is obligatory to limit the crossing over to "safe" parts of the sex chromosomes. This allows reservation, and subsequent inactivation and heterochromatinization of long segments, and the Y chromosome, confined to the male sex only, tends to deviate more and more from its original role as homolog of the X chromosome. The process of inactivation of Y may be due to accumulation of lethal and nonfunctional genes under sheltering effect of normally functioning genes of X (Muller 1917, Nei 1970).

To recompensate the loss of genes in Y, the corresponding genes of X became boosted by special modificator genes functioning in the male *Drosophila* (Muller's 1950, theory of dosis compensation), so to reach the same functional level as the genes of two X's of the female. In the mammals, the corresponding genes are doubled in the X, to to assure the diploid quanttity of genes in the XY combination. On the other hand, to avoid a quadruple quanttity of functioning genes in mammal females, one of the two X's (or generally, n-1 of n X's) must be inactivated for lifetime in somatic cells. It turns to a compact, heterepycnotic body known as sex chromatin or Barr body (Ohno 1964, 1969; Mittwoch 1967). In other animals, other variations of somatic sex chromosome inactivation are known to occur (see Tschemark-Woess 1963).

Other investigations show that heterochromatinization may affect perhaps any part of karyotype. Manna and Mazumber (1967) report loss of one autosomal pair through heterochromatinization in a grasshopper. In some species of *Chilocorus* (Coccinellidae) with metacentric autosomes, one arm of every autosome is rendered dispensable through heterochromatinization (Smith 1962, 1965).

This would mean approximately a 50% loss of all genes, would not there be any mechanism of compensation. The function of dying genes is supposedly adopted by surviving ones in such cases.

Some systematical groups seem to be especially preconditioned for such losses. Thus Ohno *et al.* (1968), Ohno (1970, *a* and *b*) and Nei (1968) assume that in the early evolution of Vertebrata there was a phase of massive linear and poidic duplication of genes. Later, karyotype rearrangements have distributed originally homologous duplicates to different chromosomes. They may have been also slightly modified in their effects during the evolution, but are still capable of adopting one another's function in the case of losses. Such a situation is by no means limited to Vertebrata (see Stebbins 1966, Britten and Kohne 1968), actually, it could be a widely occurring phenomen.

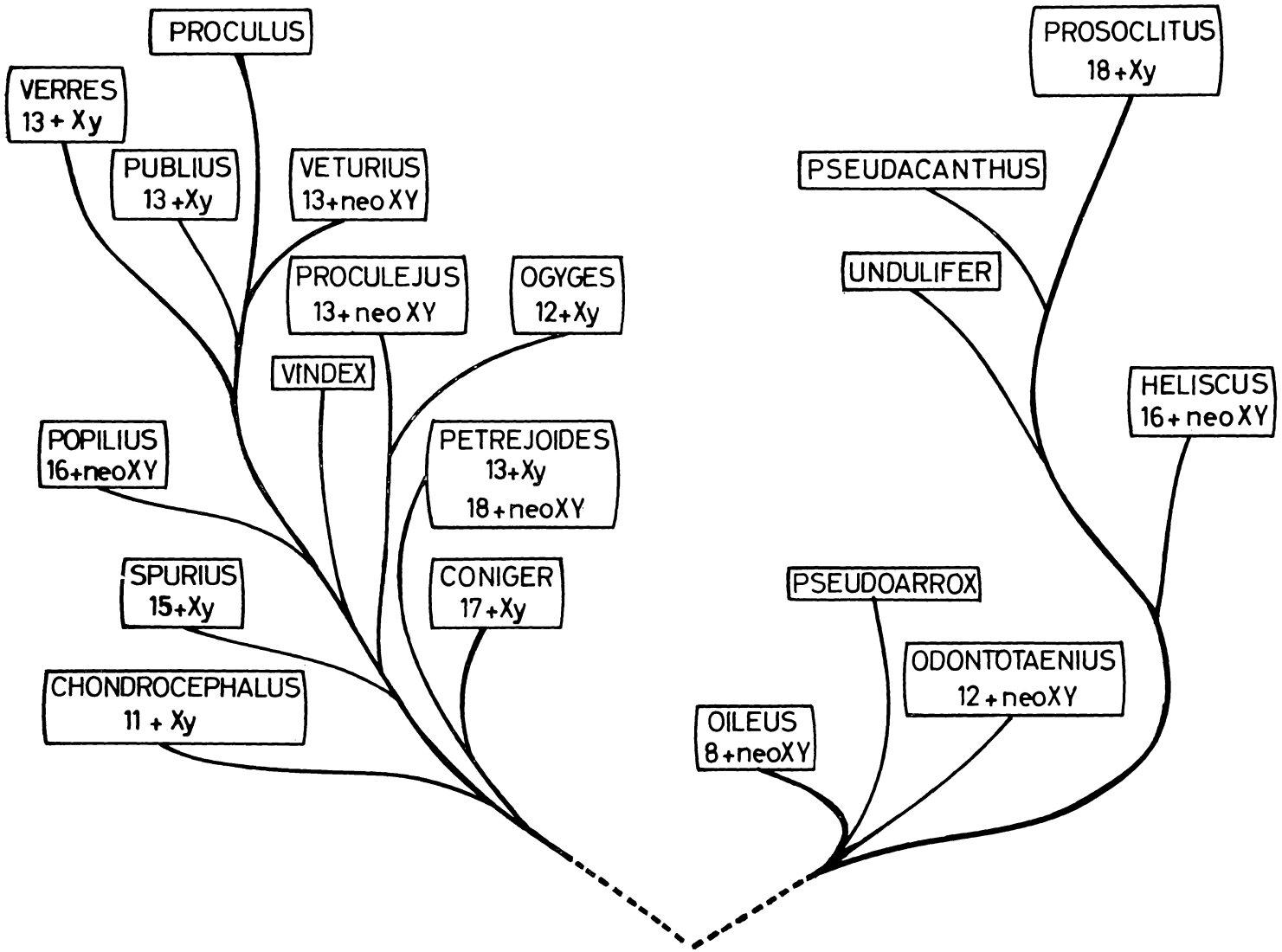
The modern concept of gene action assumes that most genes are pleiotropic in some degree, being responsable for numerous phenotypic effects, and that most phenes are caused by several genes. This means a complex network of interacting processes.

From many sources comes evidence for the surprising stability of phenotype despite the diversity of genotype (see Mayr 1969, pp. 219-221 and 279-282). The fittest phenotype tends to be buffered against effects of harmful mutations, recombinations and rearrangements by genic interaction that renders them inefective (by insufficient penetration) or weak (by low degree of expresivity). This conservatism of the phenotype can survive even speciation, as we know from the so-called sibling species.

To conclude: We cannot anymore consider the total length of the karyotype uniformly significant in its genetic effects. On the contrary, each chromosome consists of many different segments, some highly significant genetically, some totally dispensable, others something between the extremes. Furthermore, such segments are subject to evolution on their site, some significant ones losing their genes, the inert ones being eliminated. Karyotype rearrangements change the sequence of such segments, but on the other hand, their sequency and quality the genetic architecture of the karyotype, as White (1969) calls it —must affect the quality and quantity of rearrangements a karyotype can tolerate.

2. On the phylogeny of Passalidae.

Karyotypes marked $9^{II} + Xy_P$ represent the basic condition in Coleoptera Polyphaga. Such karyotype are turning up in almost every Polyphagan family (see Smith 1953, 1960), approximately every fifth of the cytologically studied species has had it. There is no doubt that it is the starting point of the karyotype evolution of Lucanidae and Passalidae as well. *Trox*, considered a primitive scarabaeid akin to the ancestors of Lucanidae (Crowson 1960), still retains $9^{II} + Xy_P$ (Purcell and Virkkii 1966, Virkki 1967). The little we known about the cytology of *Trox* shows that notable changes of karyotype have taken place within the formula: the less specialized group *terrestris* has all autosomes metacentric, the specialized group *suberosus* having them acrocentric. A series of pe-


PROCULUS
VERRES
13 + Xy
PUBLIUS
13 +Xy
VETURIUS
13+neo XY
PROCULEJUS
13+ neo XY
OGYGES
12+Xy
VINDEX
POPILIUS
16+neoXY
PETREJOIDES
13+Xy
18 +neoXY
SPURIUS
15+Xy
CONIGER
17 +Xy
CHONDROCEPHALUS
11 + Xy
PROSOCLITUS
18 +Xy
PSEUDACANTHUS
UNDULIFER
HELISCUS
16 + neoXY
PSEUDOARROX
ODONTOTAENIUS
12 + neoXY
OILEUS
8 +neoXY

ricentric inversions and/or heterochromatinization and loss of second arms seem possible explanations for evolution of the *suberosus* karyotype.

Metacentric autosomes have been reported in Lucanidae (Virkki 1959, Abe *et al.* 1969). Hints of the same directions of variation as seen in Scarabaeidae and in Passalidae, appear in Lucanidae: 1) Lowering of the chromosome number by autosomal fusion and by neoXY formation, and 2) increase of the autosomal number. If we adventure to hypothetize on basis of the few data, it would semm that first the *y* chromosome disappeared (9^{II} + X reported in *Prosopocoilus inclinatus* by Toshioka and Yamamoto, 1937), then X joined with an autosome to form 8^{II} + *neo*XY. From here on, the autosomal number either lowered by centric fusions, or increased by centric dissociations or fissions, including also pericentric inversions and/or whole-arm losses. *Neo*XY was probably reduced by heterochromatinization to *Xy*, which is the sex chromosome system reported in most cases (Table 2). Somewhat outside of this plan stays *Sinodendron rugosum* with its intact Xy_P and autosomal complement reduced to 8 pairs, apparently by fusion.

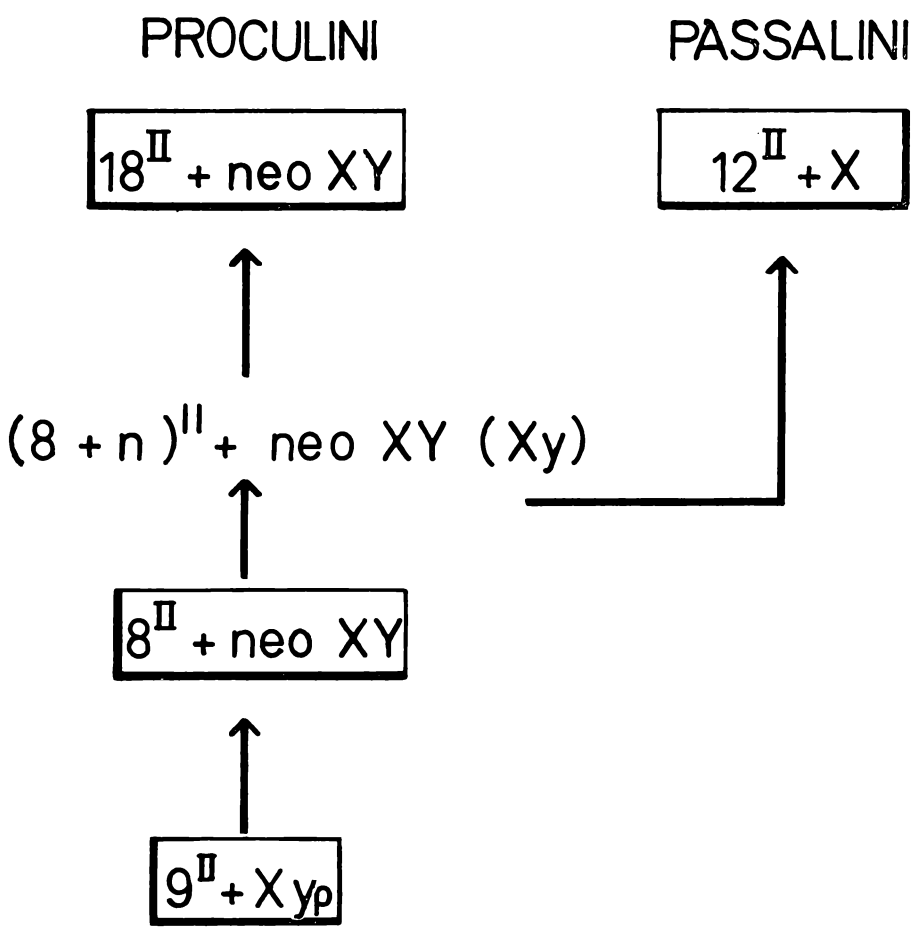
The course of events we suspect to have taken place in Passalidae is much the same. The *Oileus* species, like *Dorcus parallelipipedus* (Virkki 1959), have 8^{II} + *neo*XY. This condition needs to differ only by one step from the basic 9^{II} + Xy_P, because a dispensable *y* chromosome gest easily lost in the process of *neo*XY formation. Lack of Xy_P, and presence of *neo*XY (or *Xy*, which proba bly is a modified *neo*XY), was the first step, the increase of autosomal number starring thereafter. Passilini may have branched off from this course of events at the level of 12 pairs of autosomes, by losing the neoY. This simplest hypothesis of the evolution within Passalinae is given in schematical form in the Fig. 49.

The increase of the autosomal number should have taken place by some sort of recurrent centric fragmentation. We could not decide whether it has been by centric dissociations in the sense of White (1969), or by centric fissions in the sense of John and Hewitt (1966). The faci that we did not practically observe supernumerary chromosomes (donors of centromeres), seems to favorise the latter alternative. It can be, however, that different numbers of donors have been present earlier, but have been totally used for series of centric dissociations. Because we have not seen chromosomal polymorphism either, the rearrangements are hardly of very recent origin.

Whatever the means has been, it must have been accompanied by pericentric inversions, because metacentrics are still seen in karyotype with 16 pairs of autosomes. Also, the presence of higher autosome numbers than 16 pairs has been plainable in terms of centric fragmentation alone, provided that 8 pairs has been the initial number. Both whole-arm translocations and pericentric inversions are facilitated by the characteristics of the Passalinae chromosomes: procentric heterochromatin facilitates the former, localization of chiasmata to ends, the latter.

Adjusting the taxonomical and cytological views together, we have drawn a tentative phylogenetic tree for Proculini (Fig. 50).

At the point of divergence of the two main branches, the chromosome formula



was supposed to be 8^{II} + *neo*XY, below that, 9 + XY_P. The existence of two main branches is based on the external morphology alone. In the branch *Oileus-Prosoclitus*, the increase of the autosomal number corresponds well to the degree of especialization, in the branch *Chondrosephalus-Proculus*, the picture is more complicated. *Petrejoides* is complex taxonomically, as suggested also by the karyotypes.

Resumen

La espermatogénesis de 24 especies de Passalinae Neotropicales, de las cuales dieciséis pertenecen a la tribu Proculini y ocho a la tribu Passalini, fue estudiada para conocer los cromosomas.

Todas las especies de Passalini tienen la misma fórmula cromosómica: 12^{II}+ X. Los Proculini son muy variables, mostrando fórmulas desde 8^{II}+ neoXY hasta 18^{II}+ neoXY. Consideramos el número más bajo como el más primitivo, ya que es el más próximo a la fórmula básica de Coleoptera Polyphaga, 9^{II}+ XY_P. Pro-

blemente, la evolución del cariotipo de los Passalidae comenzó con la translocación del cromosoma X sobre un autosoma; luego, el número autosomal empezó a aumentar mediante disociaciones céntricas. Sin embargo, las disociaciones céntricas no bastan para explicar la variación del número autosómico en Proculini, puesto que en todas las especies, la mayoría de los cromosomas son metacéntricos. Hemos sugerido qué inversiones pericéntricas han acompañado las disociaciones céntricas, aparentemente en una doble serie de ortoselección cariotípica. En Passalidae la localización de los quiásmas en los extremos de los cromosomas facilita los rearreglos del primer tipo, presencia de heterocromatina procéntrica, los del segundo tipo.

Comparando los cariotipos de las distintas familias de Scarabaeoidea encontramos un hecho aparentemente contradictorio. Los Scarabaeidae, una familia con enorme variabilidad morfológica tiene como fórmula cromosómica casi exclusivamente $9^{II} + Xy_P$, mientras los Passalidae, una familia morfológica muy uniforme, tiene una gran variación de cariotipos. Esta contradicción, entre la relación del cariotipo y la morfología externa, es objeto de discusión en este trabajo.

El número de espermatozoides por fascículo es 128 en todos los pasálidos que hemos estudiado. En el género *Verres*, el 25% (32 del total) de los espermatozoides contienen una inclusión citoplásmica.

Un árbol filogenético tentativo de la tribu Proculini se presenta en la página 14.

REFERENCES

ABE, A., K. KUDOH, I. KUDOH, & K. SAITOH, 1969. A chromosome study in five species of Lucanidae (Insecta: Coleoptera). *Kontyu* **37**(2): 179-186.

BRITTEN, R., & D. KOHNE, 1968. Repeated sequences in DNA. *Science* **161**: 529-540.

CARSON, H. L., 1970. Chromosomal tracers of founder events. *Biotropica* **2**(1): 3-6.

CROWSON, R. A., 1955. The natural classification of the families of Coleoptera. London, 187 pp.

CROWSON, R. A., 1960. The phylogeny of Coleoptera. *Ann. Rev. Entomol.* **5**: 111-134.

HANNAH, A., 1961. Localization and function of heterochromatin in *Drosophila melanogaster*. *Adv. Genet.* **4**: 87-125.

HEITZ, E., 1928. Das Heterochromatin der Moose I. *Jahrb. wiss. Botan.* **69**: 762-818.

HEITZ, E. & H. BAUER, 1933. Beweise für die Chromosomennatur der Kernschleifen in den Knäuelkernen von *Bibio hortulanus*. *Z. Zellf. m. Anat.* **17**: 67-82.

HSU, T. C. & K. BENIRSCHKE, 1967. Canis familiaris (Dog). *An Atlas of Mammalian Chromosomes* **1**: 20.

JOHN, B. & G. M. HEWITT, 1966. Karyotype stability and DNA variability in the Acrididae. *Chromosoma* **20**: 155-172.

MANNA, G. & S. MAZUMBER, 1967. Evolution of caryotype in an interesting species of grasshopper, *Tristria pulvinata* Uvarov *Cytologia*. **23**: 236-247.

MAYR, E., 1969. Animal Species and Evolution. Fourth Printing. Cambridge. 797 pp.

MITTWOCH, U., 1967. Sex Chromosomes. New York-London. 306 pp.

MULLER, H. J., 1914. A gene for the fourth chromosome of *Drosophila*. *J. Exp. Zool.* **17**: 325-336.

MULLER, H. J., 1950. Evidence of the precision of genetic adaptation. *Harvey Lect. Ser.* **43**: 165-229.

NEI, M., 1969. Gene duplication and nucleotide substitution in evolution. *Nature* **221**: 40-42.

NEI, M., 1970. Accumulation of nonfunctional genes on sheltered chromosomes. *Amer. Nat.* **104**: 311-322.

NICKLAS, R. B., 1961. Recurrent pole-to-pole movement of the sex chromosomes during prometaphase I in *Melanoplus differentialis* spermatocytes. *Chromosoma* **12**: 97-115.

OHNO, S., 1964. The sex chromatin: its origin and nature. *Proc. Symp. Mamm. Cytogenetics and Rel. Probl. Radiobiol.*: 253-267.

OHNO, S., 1967. Sex Chromosomes and Sex-linked Genes. Berlin-Heidelberg-New York 192 pp.

OHNO, S., 1969. Evolution of sex chromosomes in Mammals. *Ann. Rev. Gen.* **3**: 495-524.

OHNO, S., 1970a. The enormous diversity in genome sizes of fish as a reflection of nature's extensive experiments with gene duplication. *Trans. Amer. Fisheries Soc.* **99**: 120-130.

OHNO, S., 1970b. Evolution by Gene Duplication. New York. 280 pp.

OHNO, S., U. WOLF & N. ATKIN, 1968. Evolution from fish to mammals by gene duplication. *Hereditas* **59**: 169-187.

OMURA, T., 1951. On the behavior of the developing spermatozoa in the follicles of *Mylabris pustulata* (a coleopteran) and *Labidula riparia* (a dermapteran). *Pap. Coord. Comit. Res. Genet.* **2**: 101-106.

PURCELL, C. & N. VIRKKI, 1966. Two *Trox* karyotypes differing radically in the location of centromeres. *J. Agric. Univ. Puerto Rico* **50**(2): 158-160.

REYES-CASTILLO, P., 1970. Coleoptera, Passalidae:: Morfologia y división en grandes grupos; Géneros Amercianos. *Folia Ent. Mex.* **20-22**: 1-240.

RIS, H., 1945. The structure of meiotic chromosomes in the grasshopper and its bearing on the nature of "chromomeres" and "lamp-brush chromosomes". *Biol. Bull.* **89**: 242-257.

SHAFFER, E. L., 1917. Mitochondria and other cytoplasmic structures in the spermatogenesis of *Passalus cornutus*. *Biol. Bull.* **32**: 83-102.

SMITH, S. G., 1953. Chromosome numbers of Coleoptera. *Heredity* **7**(1): 31-48.

SMITH, S. G., 1960. Chromosome numbers of Coleoptera. II. *Canad. J. Genet. Cytol.* **2**(1): 66-88.

SMITH, S. G., 1962. Temporo-spatial sequentiality of chromosomal polymorphism in *Chilocorus stigma* Say (Coleoptera: Coccinellidae). *Nature* **193**: 1210-1211.

SMITH, S. G., 1965. Heterochromatin, Colchicine, and karyotype. *Chromosoma* **16**: 162-165.

STEBBINS, G. L., 1966. Chromosomal variation and evolution. *Science* **152**: 1463-1469.

SUOMALAINEN, E., 1969. Chromosome evolution in Lepidoptera. *Chromosomes Today* **2**: 132-138.

TOSHIOKA & YAMAMOTO, 1937. *Bot. & Zool.* 5. (The publication has not been available and is cited from S. Makino, 1956: A Review of Chromosome Numbers in Animals. Tokyo. 300 pp.).

VIRKKI, N., 1956. Zur Kenntnis der postemeiotischen Ereignisse der Samenentwicklung bei den Skarabäiden (Coleoptera). *Z. Zellf. mikr. Anat.* **44**: 644-665.

VIRKKI, N., 1959. Neo-XY mechanism in two Scarabaeoid beetles, *Phanaeus vindex* MacL. (Scarabaeidae), and *Dorcus parallelipipedus* L. (Lucanidae). *Hereditas* **45**: 481-494.

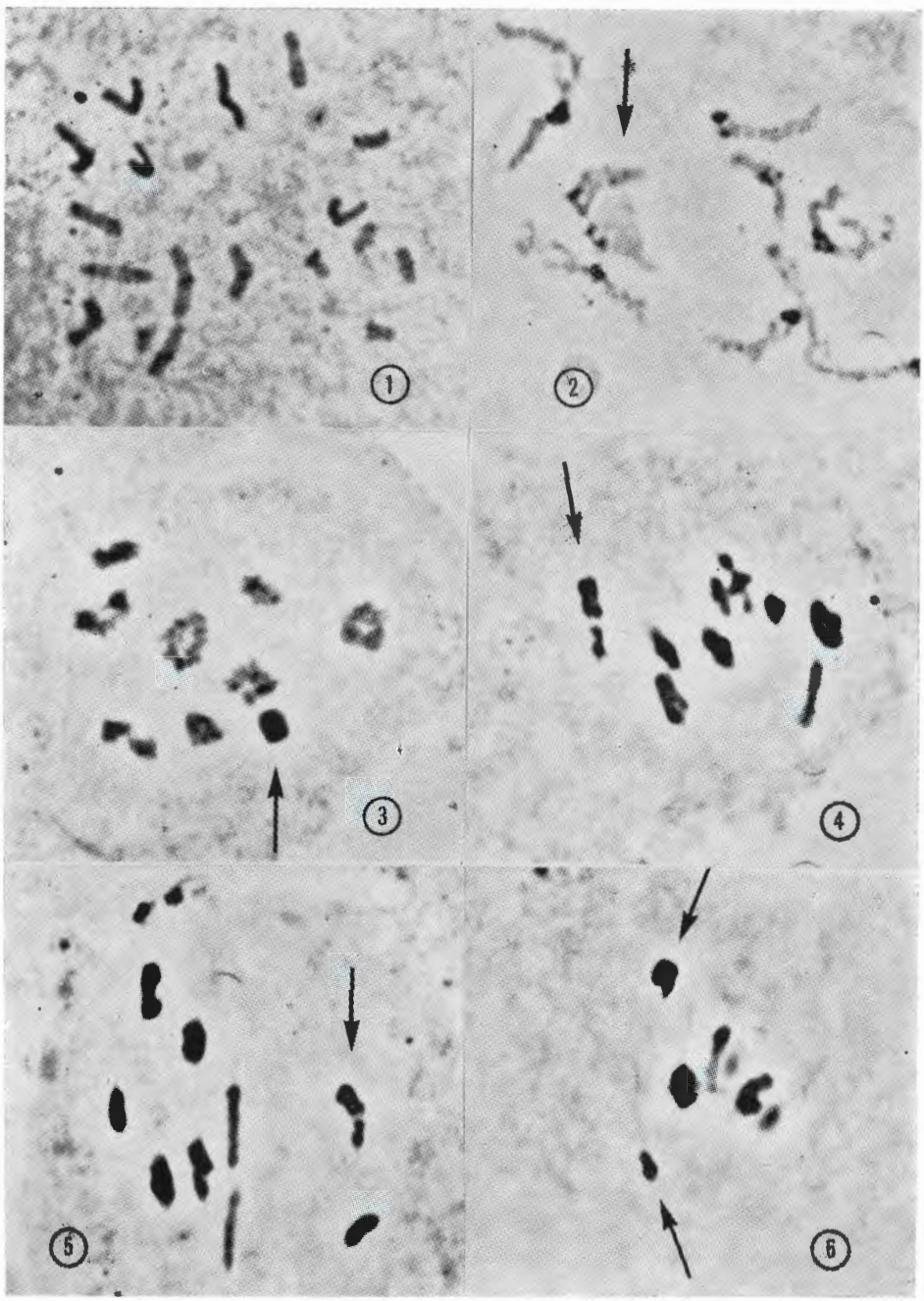
VIRKKI, N., 1967. Chromosome relationships in some North American Scarabaeoid beetles, with special reference to *Pleocoma* and *Trox*. *Canad. J. Genet. Cytol.* **9**: 107-125.

VIRKKI, N., 1968. A chiasmate sex quadrivalent in the Male of an Alticid Beetle. *Cyrsylus volkameriae* (F.). *Canad. J. Genet. Cytol.* **10**(4): 898-907.

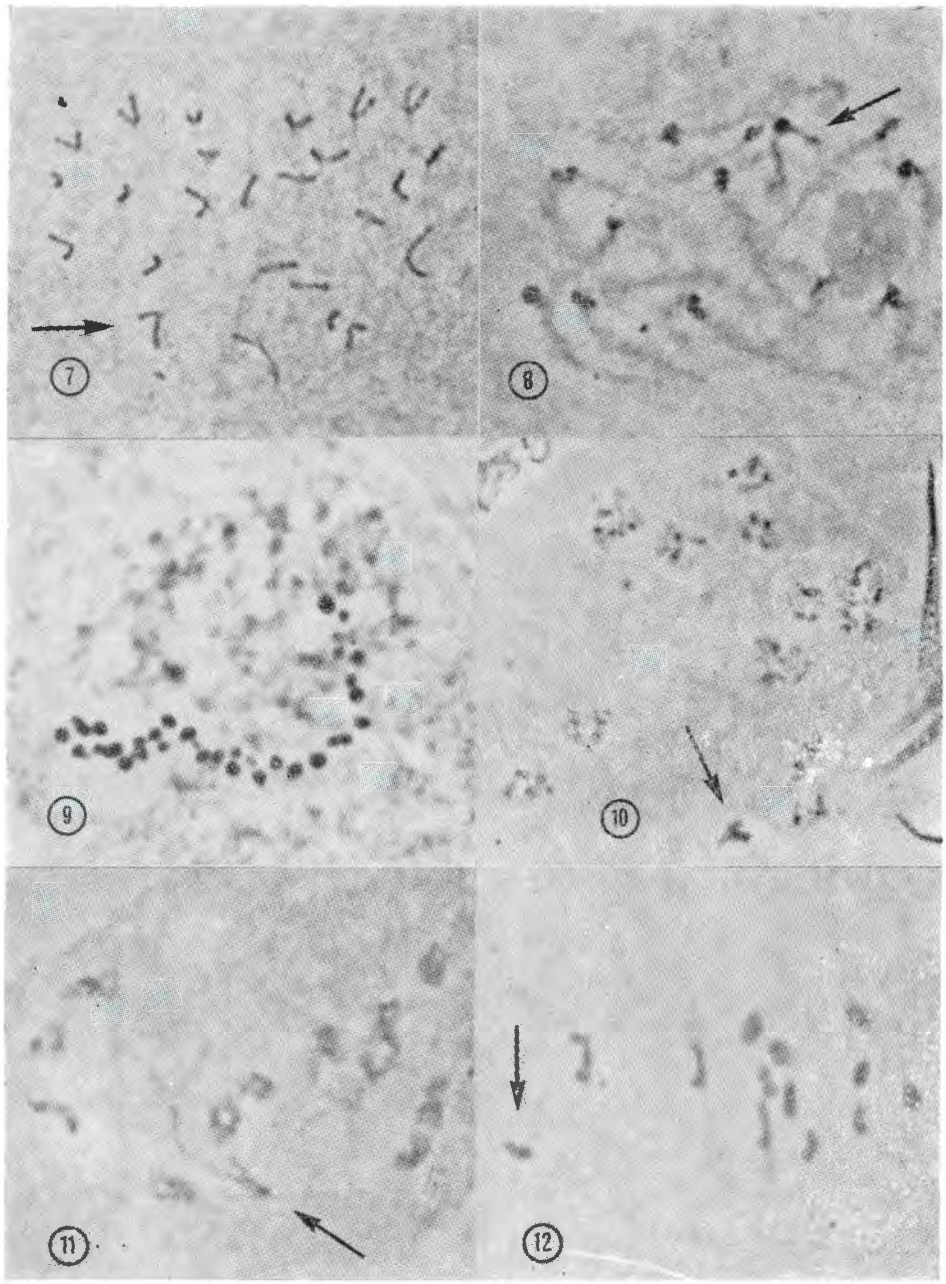
VIRKKI, N., 1970. Sperm bundles and phylogenesis. *Z. Zellf. mikr. Anat.* **101**: 13-27.

WHITE, M. J. D., 1954. Animal Cytology and Evolution. Cambridge. 454 pp.

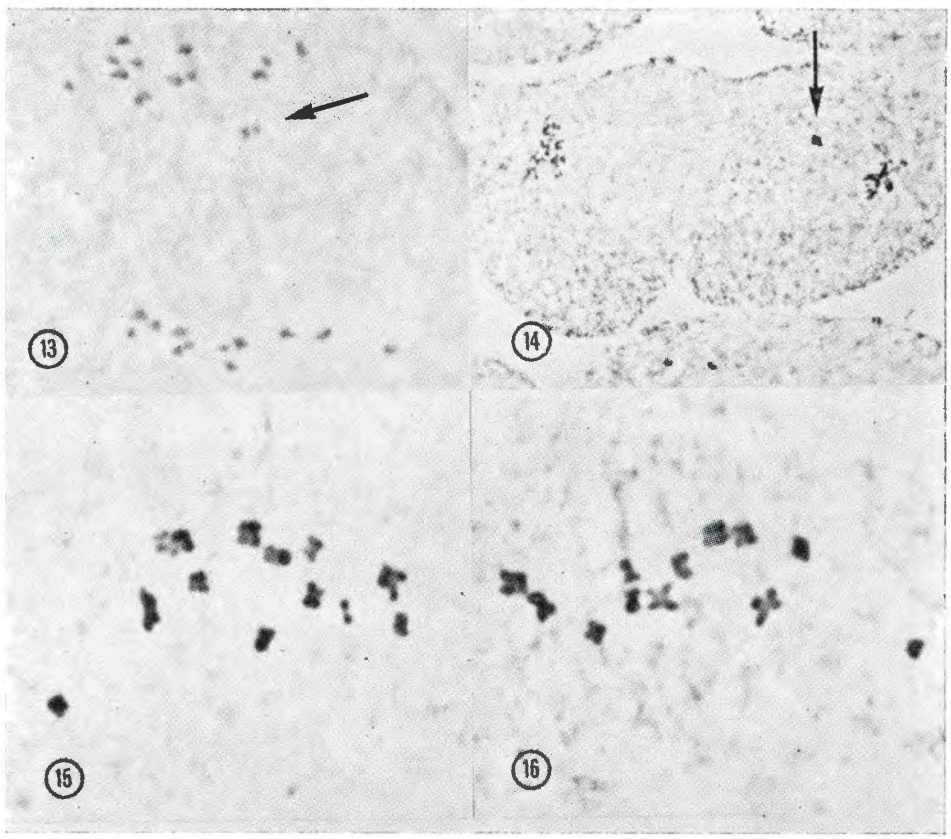
WHITE, M. J. D., 1969. Chromosomal rearrangements and speciation in animals. *Ann. Rev. Genet.* **3**: 75-98.



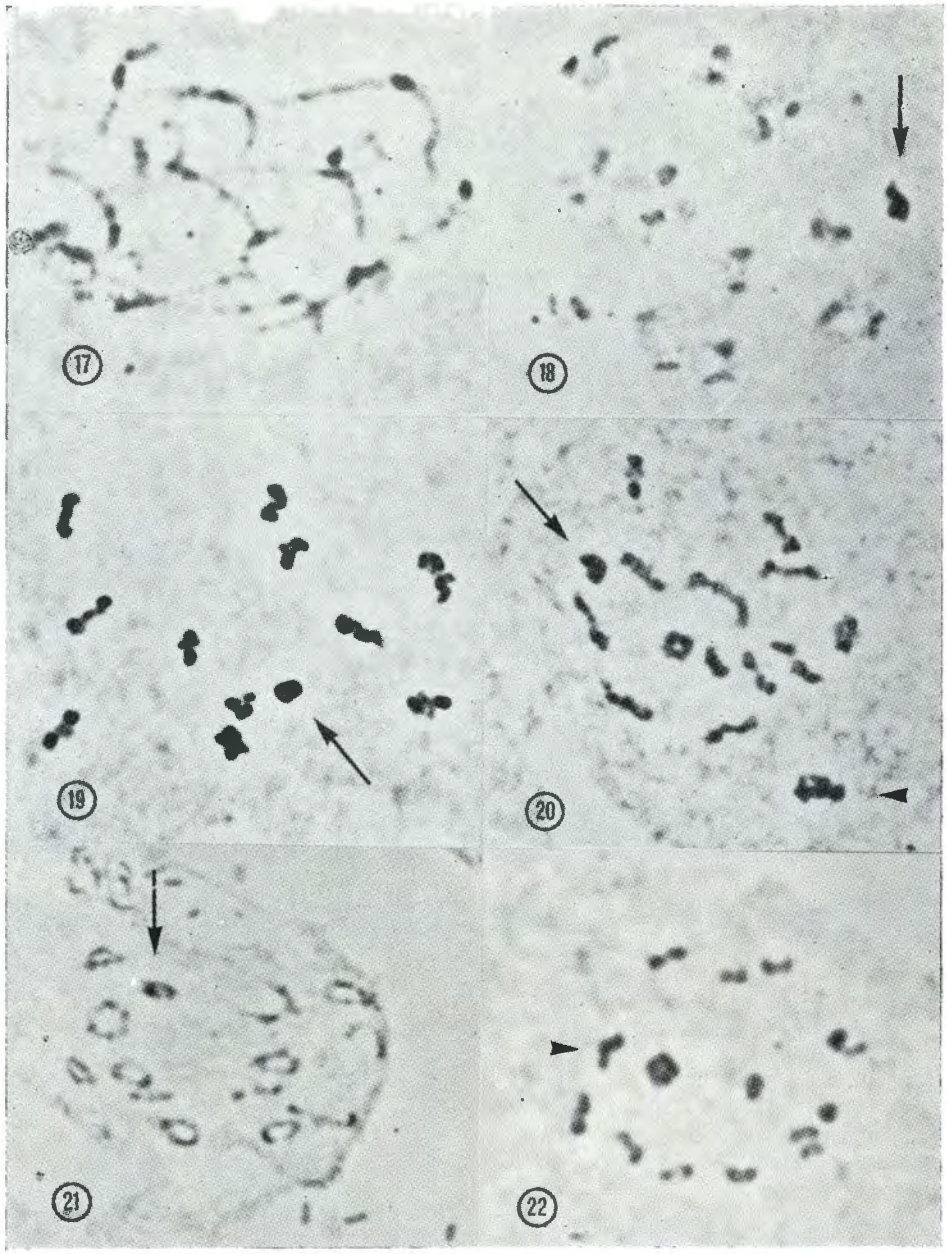
FIGS. 1-6. *Oileus rimator.* $8^{II}+$ *neoXY*. Arrows point to *neoXY* bivalent. All pictures *2300x. 1. Mitosis of spermatogonium, 2n = 18. 2. Pachytene.* Sex chromosomes in contact with nucleolus. All chromosomes show heterochromatic block. 3. Diakinesis. 4. M. I. One cross bivalent still seen. 5. M. I. 6. Early A. I, with *neoXY* in disjunction. A thin thread joins one arm of *neoX* with one arm of *neoY*.



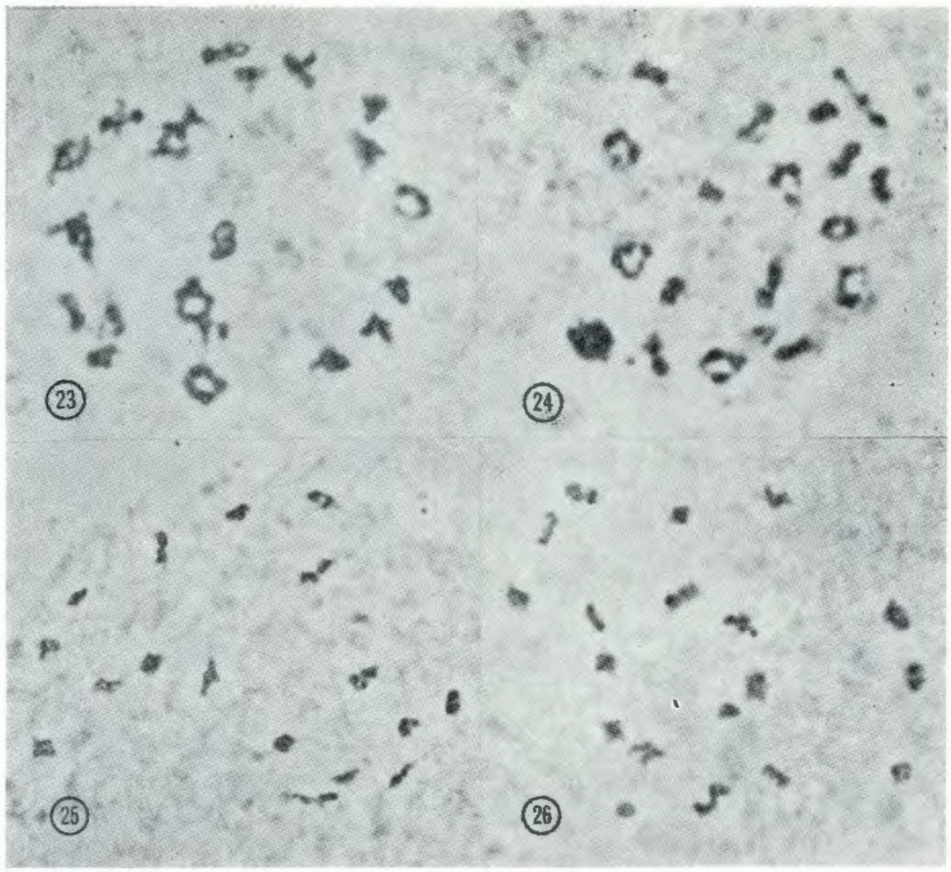
Figs. 7-12. *Passalus interstitialis*. $12^{II}+X$. Arrows point to X chromosome. 7. Mitosis of spermatogonium, 2n = 25. 1800x. 8. Pachytene, $12^{II}+X$. All chromosomes show procentric heterochromatic blocks. A typical prophasic nucleolus is present. 2600x. 9. Begin of the diffuse phase. Cromatin and nucleolus disappear, a chain of nuclear droplets forms. 1800x. 10. Diakinesis. Opening-out of bivalents, procentric heterochromatin still recognizable. 1800x. 11. Later diakinesis. Negative heteropycnosis of the sex chromosome. 2000x. 12. M I. 2600x.



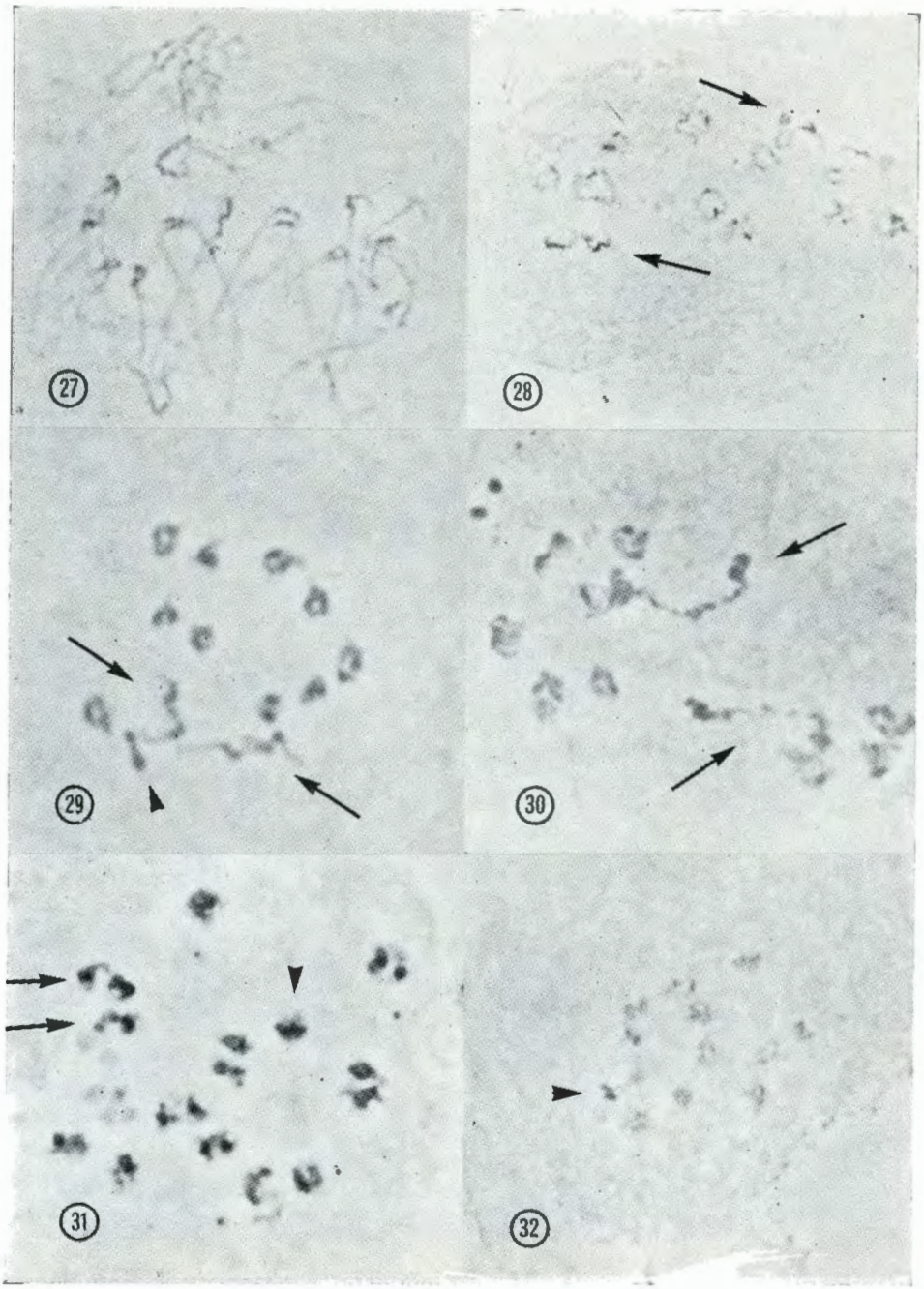
FIGS. 13-16. *Passalus interstitialis, A I—M II.* Arrows point to X chromosomes. 13. Early A I, 12—12 + X. 2000x. 14 Late *A I.* 1600x. 15. M II, 12 + X. 3400x. 16. *M II,* 12 autosomes. 3400x.



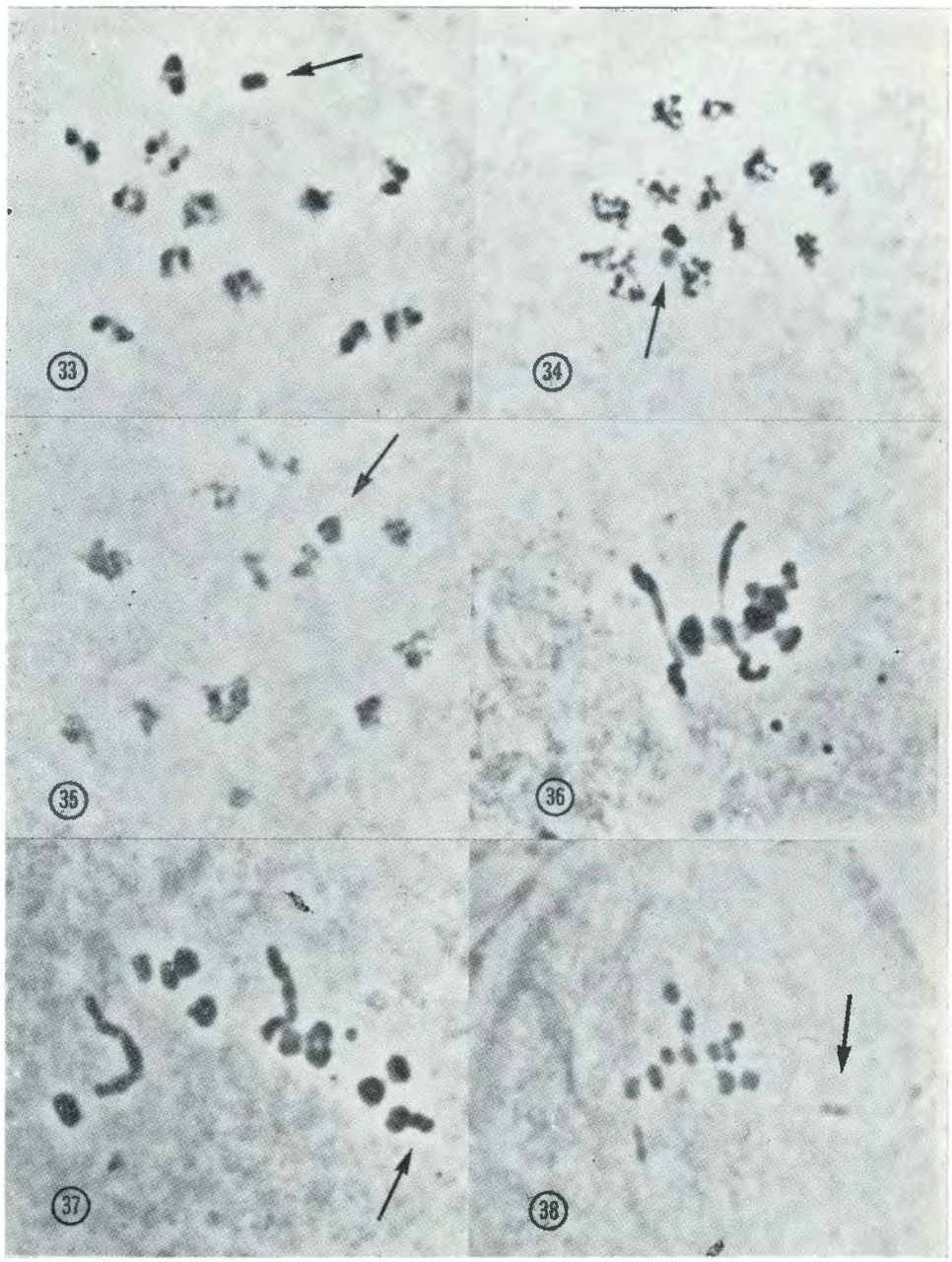
FIGS. 17-22. Proculini, pachytene to diakinesis. Arrow point to sex bivalents. All pictures 2800x. *Petrejoides* n. sp. Pachytene. 18. *Chondrocephalus debilis.* Early diakinesis. $11+^{II}Xy$. 19. The same, late diakinesis. 20. *Verres corticicola* $13^{II}+Xy$. *Arrowhead* points to a cytoplasmic inclusion. 21. *Veturius tranversus.* $13^{II}+neoXY$. 22. *Spurius bicornis.* Arrowhead: two bivalents.



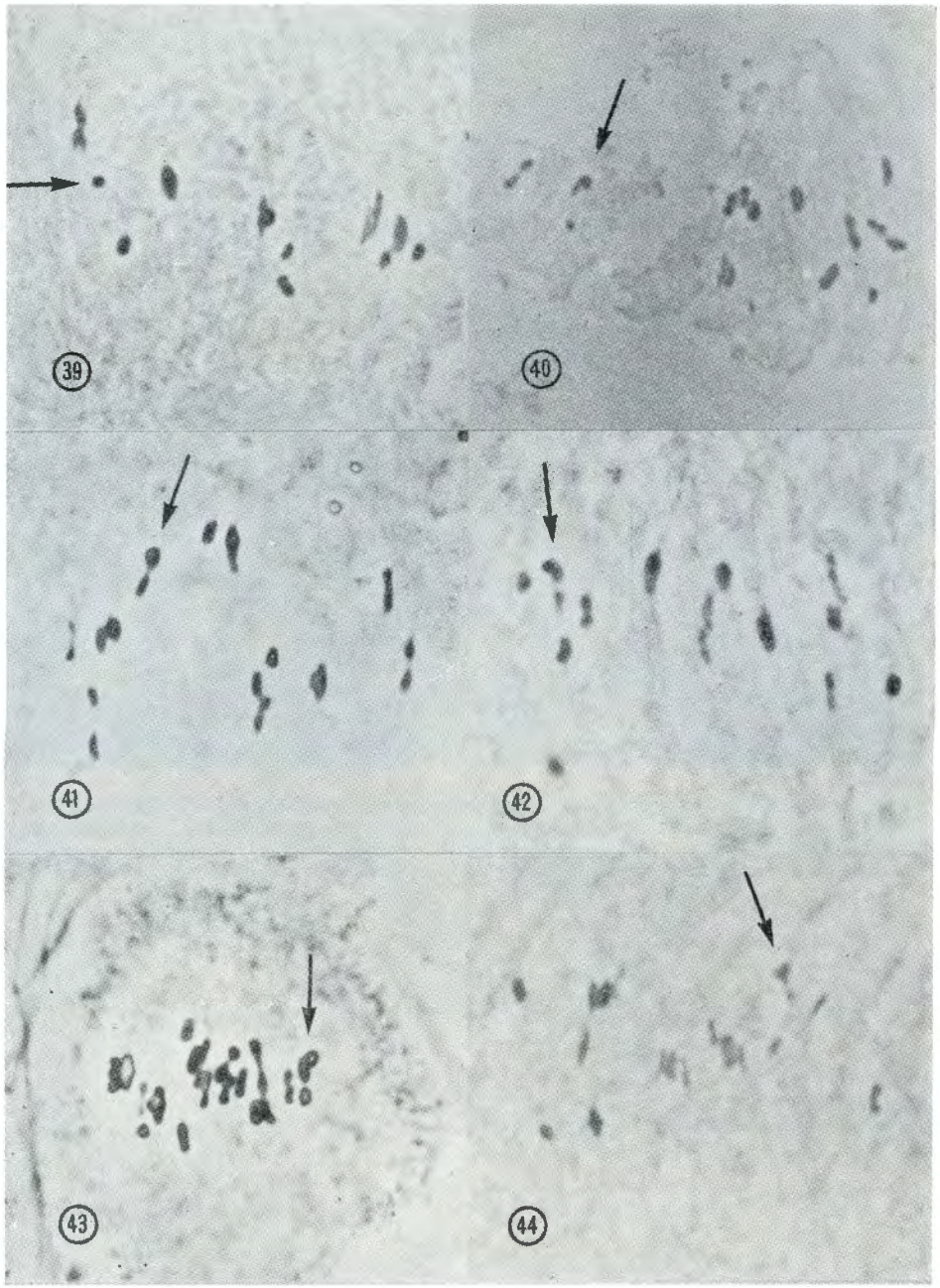
FIGS. 23-26. Proculini, diakinesis. All pictures 2800x. 23. and 24. *Petrejoides n. sp.* $18^{II}+Xy$. 25. *Heliscus tropicus.* 16^{II} + *neoXY*. 26. *Coniger ridiculus,* 17^{II} + *Xy*.



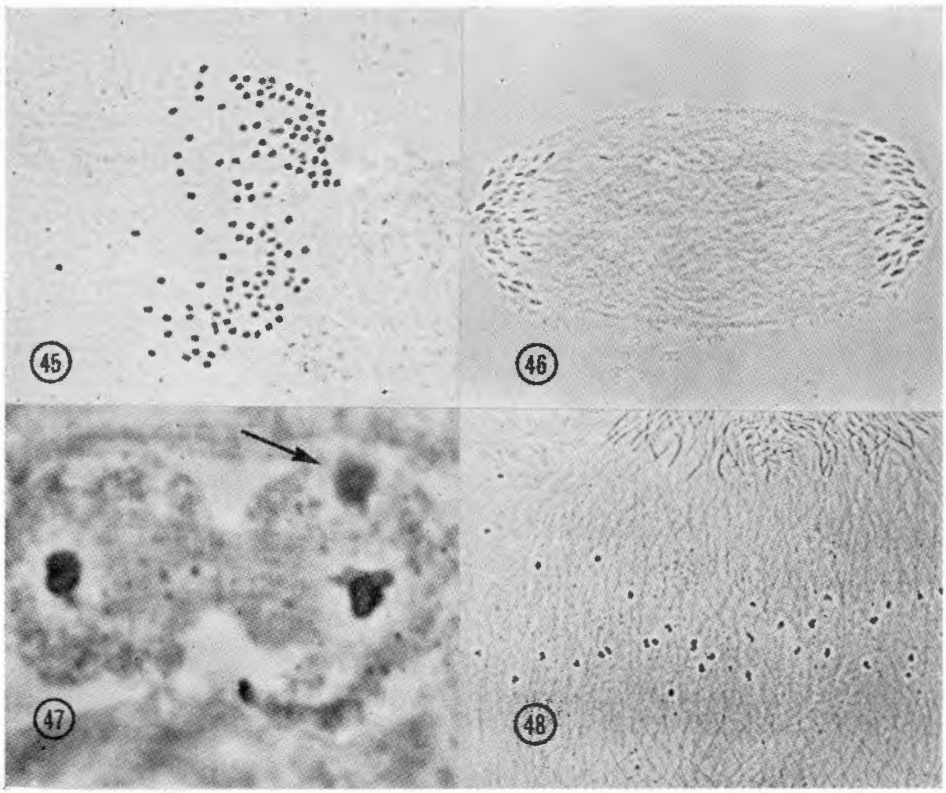
FIGS. 27-32. Passalini, diplotene to diakinesis. $12^{II}+X$. Arrows point to large allocyclic bivalents arrowheads, to X chromosomes. 27. *Passalus suturalis*. Schizotene. Opening-out of bivalents begins at the heteropycnotic centric regions. 2200x. 28. *Passalus suturalis*. 1600x. 29. *Passalus interruptus* 2600x. 30. *Passalus punctiger*. 2000x. 31. *Passalus punctiger*. Nucleolar droplets immediately below large bivalents. 2800x. 32. *Paxillus leachi*. 2200x.



FIGS. 33-38. Passalini, diakinesis to M I. $12^{II} + X$. Arrows point to X chromosomes. 33. *Passalus mirabilis*. 2800x. 34. *Ptichopus angulatus*. 2800x 35. *Passalus punctatostraitus*. 2800x. 36. *Passalus punctiger*. Heteropycnosis of the large bivalents. 3200x. 37. *Passalus punctiger*. Total number of bivalents seen. 3200x. 38. *Paxillus leachi*. 2800x.



FIGS. 39-44. M I. Arrows point to the sex chromosome or bivalent. 39. *Passalus punctatostriatus.* 12^{II} + X. 2200x. 40. *Veturius tranversus.* 13^{II} + *neoXY*. 2200x. 41. *Heliscus tropicus.* 16^{II} + *neoXY* (all autosomes not delimited). 2000x. 42. *Proculejus brevis.* 13^{II}+*neoXY*. 2500x. 43. *Petrejoides* n. sp. 18^{II}+ *neoXY*. 2800x. 44. *Popilus eclipticus.* 16^{II}+ *neoXY*. 2500x.



FIGS. 45-48. 45. *Coniger ridiculus*. spermiohistogenesis. 128 nuclei per bundle. 1400x. 46. *Ptichopus angulatus*, spermiohistogenesis. Bipolar arrangement of nuclei of a bundle. 1100x. 47. *Verres corticicola*. A II. Cytoplasmic inclusion (arrow) goes undivided to one of the poles. 2900x. 48. *Verres corticicola* Sperm bundle with 32 cytoplasmic inclusions. 1300x.