EVOLUTION OF EXTREMELY COMPLICATED SEX CHROMOSOME SYSTEMS IN **BLAPS** *(COLEOPTERA)*[1]

Once turned to sex chromosomes, a pair of homologues is immediately on the way to mutual alienation: the Y chromosome is confined to male, and crossing over must be excluded from the segments containing sex determinators. Heterochromatinisation, especially of Y, conducts the differentiation to such an extent that a conventional meiotic pairing of X and Y becomes impossible and must be substituted by some novel method. This is what has happened in the beetles, where the X and y associate by means of a nucleolus.[2] The method is very ancient in Coleoptera, dating back to times preceding the division into Adephaga and Polyphaga.[3] As a rule the far heterochromatinised y is much smaller then the X, which causes profile views of the bivalent to resemble a parachute; hence the labelling Xy_p[4] (p from *parachute*). A competent and thus conservative device, Xy_p has survived innumerable processes of speciation during some 200 millions of years, and still is found in most families of beetles.

Any common nucleolus would not serve as a pairing device because it disintegrates during prophase. The essential feature of Xy_p nucleolus is its unusual persistence, which ensures the association until first anaphase of meiosis. There are indications of the persisting nucleolar component being deposited on the periphery of the sex nucleolus (blister hypothesis of Xy_p by Suomalainen, see Smith and Virkki[5]).

Blaps and *Caenoblaps* are tenebrionid genera in which the archaic Xy_p has not been detected. Instead, there are mixed association modes, both nucleolar and chiasmate association, usually in one and the same multivalent. This evolutionary series culminates in *B. cribrosa*, in which 50 percent of all chromosomes are sex chromosomes:

$8^{II} + X^1X^2Y$	: *B. judaeorum*, southern race[6]
	B. lusitanica

[1] Manuscript submitted to Editorial Board September 28, 1973.

[2] John B., and Lewis, K. R., Nucleolar controlled segregation of the sex chromosomes in beetles, Heredity 15: 431–439, 1960. (Note that a small letter is used when emphasizing the small size of the sex chromosome; capitals are always used when speaking in general terms of the sex chromosomes.)

[3] Smith, S. G., The cytology of *Sitophilus* (*Calandra*) *oryzae* (L.), *S. granarius* (L.), and some other Rhynchophora (Coleoptera), Cytologia 17: 50–70, 1952.

[4] Smith, S. G., The cyto-taxonomy of Coleoptera, Canad. Entom. 82: 58–68, 1950.

[5] Smith, S. G., and Virkki, N., Coleoptera, in: B. John (Editor): Animal Cytogenetics, Borntraeger, Berlin (in preparation).

[6] Read: "8 autosomal bivalents plus sex multiple X^1X^2Y", and so on (Coleopteran karyotypes are usually written as metaphase I condition of male).—The list is taken from Smith, S. G., Chromosome numbers of Coleoptera, Heredity 7: 31–48, 1953;

$9^{II} + X^1X^2Y$	: *B. judaeorum*, northern race
$15^{II} + X^1X^2X^3Y$	: *B. waltli* *B. sulcata*
$15^{II} + X^1X^2X^3X^4Y$	: *B. gigas* *B. "lusitanica"* [7] *B. wiedemanni*
$15^{II} + X^1X^2X^3X^4Y^1Y^2$	: *B. tenuicollis*?
$16^{II} + X^1X^2Y$	: *Caenoblaps nitida*
$16^{II} + X^1X^2X^3Y$	: *B. mucronata* *B. mortisaga* *B. tenuicollis*?
$17^{II} + X^1X^2Y$	: *B. lethifera*
$9^{II} + X^{1\text{-}12}Y^{1\text{-}6}$	: *B. cribrosa*

Attempts at explaining the evolution of such karyotypes meet serious difficulties. There is no doubt that the nucleolar component of these multiple associations is of parachute origin. A particular problem is that the number of Y's is always one (except for *cribrosa* and probably *tenuicollis*). Lewis and John[8] published an ingenious hypothesis which copes successfully with this problem (fig. 1 (1–2). They proposed: 1, An interchange of short segments between X_p and an autosome, which ensures a chiasmate chain association X_p—intact autosome (now *neo*Y)—rearranged autosome (now *neo*X), the tiny y_p was supposed to disappear as it does normally in derivative sex chromosome systems; 2, a progressive heterochromatinization rendering the neochromosomes capable of nucleolar association, thus both *neo*X and *neo*Y will enter into the parachute leaving chiasmata obsolete; 3, a repeated incorporation of an autosome pair by similar translocation, this time with *neo*X_p, whereby the former *neo*Y gets lost. This gives the sex multivalent of *B. mucronata* they analysed. Mechanically speaking, this process could be repeated as long as autosomes are left. Each incorporation of an autosomal pair adds one X to the sex system but does not increase the number of Y's.

This hypothesis is debatable on two grounds. First, the initial association requires two chiasmata in *neo*Y, but typical beetle chromosomes associate by only one chiasma, even if they are metacentric (due to diphaseness of

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Smith, S. G., Chromosome numbers of Coleoptera. II, Canad. J. Genet. Cytol. 2: 66–68, 1960; and Wahrman, J., R. Nezer, and O. Freund, Multiple sex chromosome mechanisms with "segregation bodies", Chromosomes Today 4 (in press).—Formerly, *B. polychresta* was supposed to have a similar karyotype as *cribrosa*, but according to the above Israeli workers, the species was confounded with *cribrosa*.

[7] Certainly not the same *lusitanica* above.

[8] Lewis, K. R., and John, B., The organization and evolution of the sex multiple in *Blaps mucronata*, Chromosoma 9: 69–80, 1957.

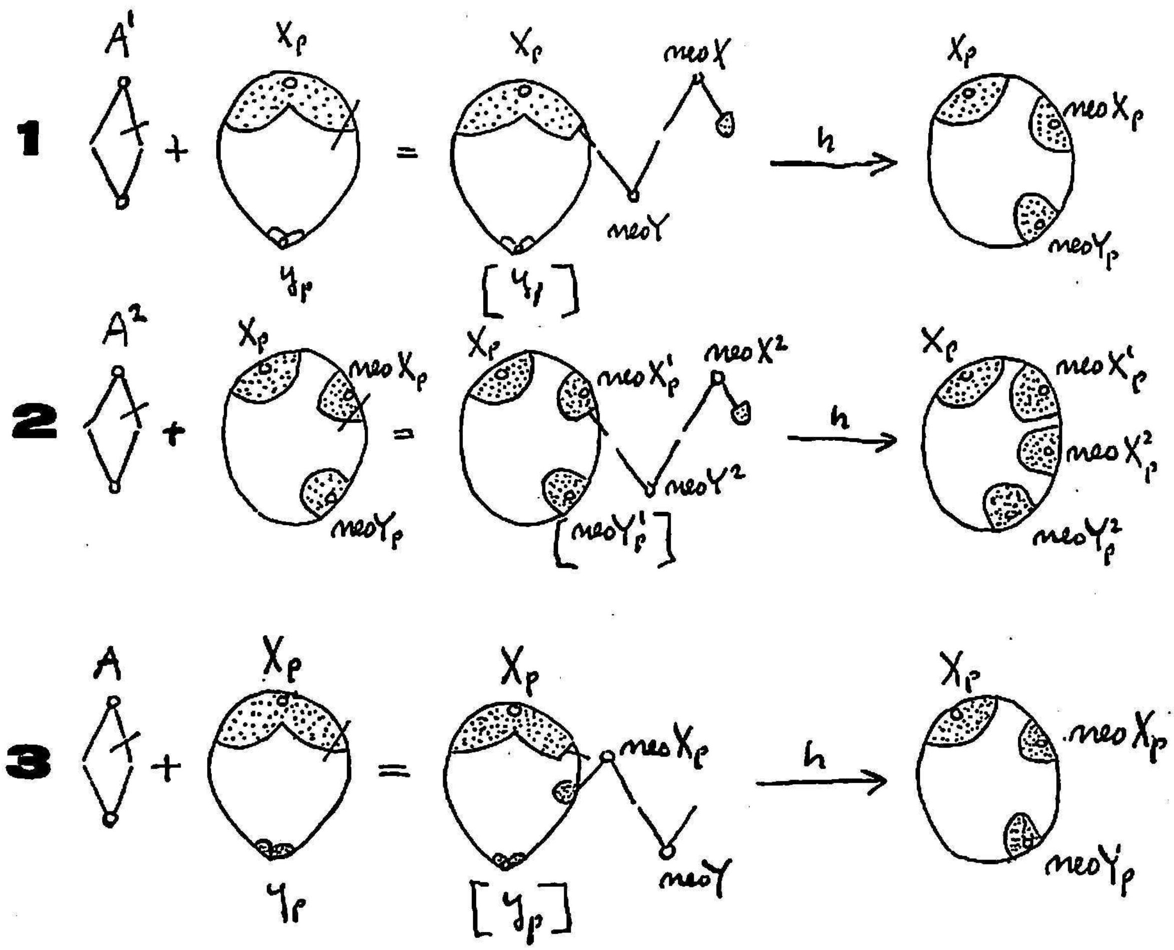

FIG. 1.—Hypothesis of Lewis and John. 1, First autosomal incorporation and subsequent heterochromatinisation: Metacentric autosomes (A′) + Xy_p produce an $X_pneoXneoY$, which, after heterochromatinisation (*h*) and switch from chiasmate to nucleolar association produces an $X_pneoX_pneoY_p$; y_p is supposed to disappear (brackets). 2, Second autosomal incorporation, this time via $neoX_p$, producing an $X_pneoX^1_p$-$neoX^2neoY^2$; $neoY^1_p$ is supposed to disappear (brackets). After heterochromatinisation (*h*) and substitution of chiasmate association by a nucleolar one, an $X_pneoX^1_pneoX^2_p$-$neoY^2_p$ arises. 3, The alternative that *neoX* associates nucleolarly by its *X*-segment: autosomes (A) plus Xy_p would produce an X_pneoX_pneoY, $+y_p$ which supposedly disappears (brackets). The end result $X_pneoX_pneoY_p$, does not differ from that of alternative 1, but note that X_pneoX_pneoY serves as a base from where the *cribrosa* complex may arise by multiplication of chromosomes.

the arms, Smith and Virkki.[9]) In *Blaps*, there are ring bivalents, however, so this argument is not very strong. Second, the translocate in *neoX*, derived from X_p, should associate with the parachute.

These arguments are eliminated if it is assumed that the incorporation of autosomes takes place via y_p, under a simultaneous elimination of a centric fragment (fig. 2). The y_p, usually minute in size and more or less dispensa-

[9] Smith, S. G., and Virkki, N., loc. cit.

ble genetically, has not been taken seriously as a possible point of anchorage for translocations. Such instances indeed were unknown until Lanier[10] found one in *Pityogenes fossifrons* (Scolytidae), then de Vaio and Postiglioni[11] found another such instance in *Botanochara angulata* (Chrysomelidae). It was found more recently in the northern race of *Blaps judaeorum*.[12] Thus, a firm factual basis exists for this evolutionary mechanism operating polyphyletically in the Xy_p beetles. Further advantages of this hypothesis are: 1, Favorite autosomal candidates for the translocation are the typical

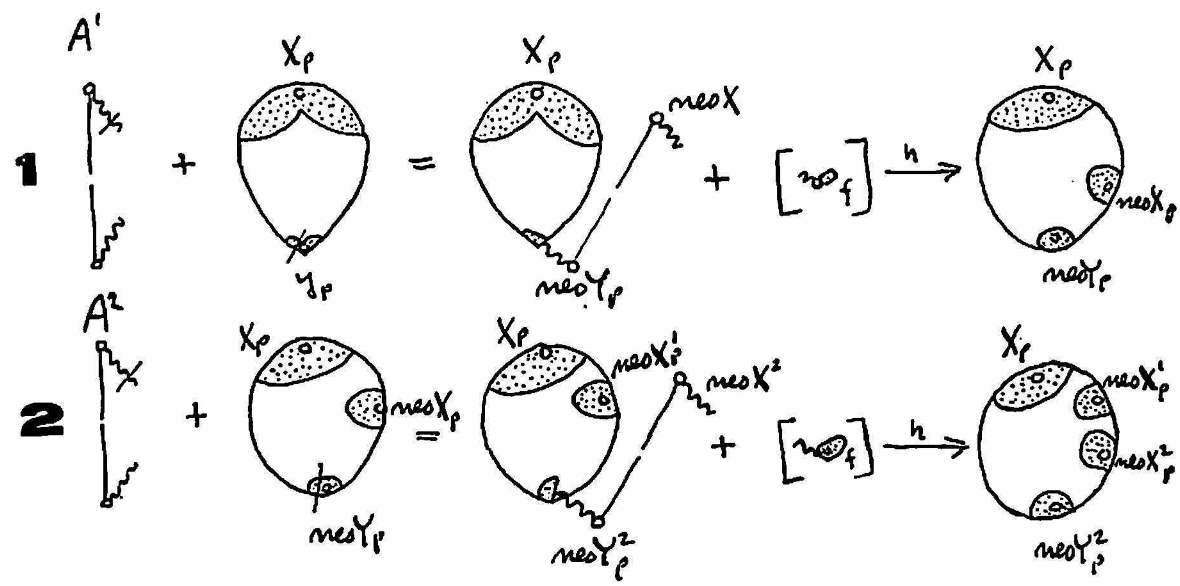

FIG. 2.—The present hypothesis. 1, First autosomal incorporation through a *Pit*-translocation: Preferably diphasic autosomes (A^1; wavy line: heterochromatic arms) plus Xy_p produce an $X_p neoX neoY_p$, plus a centric fragment, *f*, which usually disappears (brackets). Heterochromatinisation (*h*) and substitution of the chiasmate association by nucleolar one produces an $X_p neoX_p neoY_p$. 2, Second *Pit*-translocation, onto $neoY_p$, produces an $X_p neoX^1_p neoX^2 neoY^2_p$, plus a centric fragment, *f*, which supposedly disappears (brackets). The final result is numerically the same as in Lewis and John hypothesis: $X_p neoX^1_p neoX^2_p neoY^2_p$.

coleopteran diphasics as a piece lost from the heterochromatic arm is obviously genetically dispensable, also the initial association requires only one chiasma, in the euchromatic arm; 2, probable important genes of Y (y_p as

10 Lanier, G. N., State University College of Forestry, Syracuse, N. Y. Personal communication, 1971.

11 de Vaio, E. S., and Postiglioni, A., Número alto y cromosomas sexuales múltiples en *Botanochara angulata* (Germ.) (Coleoptera, Chrysomelidae, Cassidinae). A manuscript, Montevideo, 1973.

12 Wahrman, J., Nezer, R., and Freund, O., The "sex nucleolus" in beetles with multiple sex chromosomes, Exhibition at the 4th Intern. Chromos. Conf. Jerusalem, 1972.—Nezer, N., The Hebrew University, Jerusalem, Israel. Personal communication, 1973.

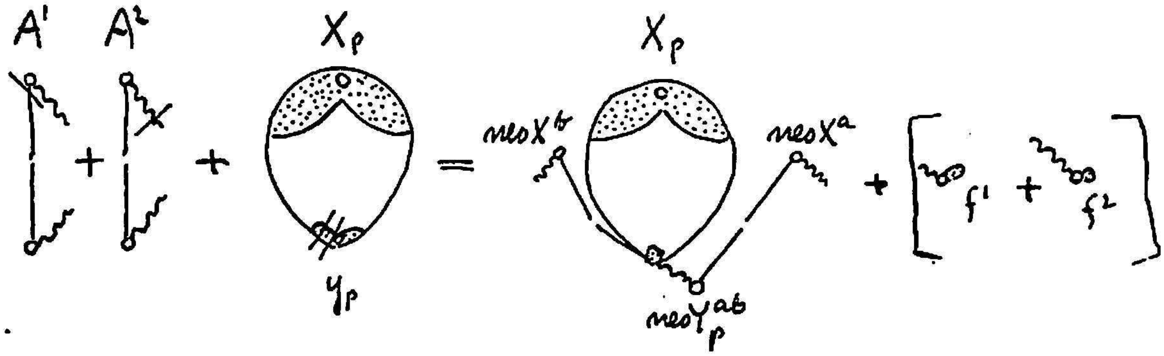

FIG. 3.—Simultaneous incorporation of two autosomes on the same y_p. The result is an $X_p neoX^a neoX^b neoY_p^{ab}$, as found in *B. sulcata*. Two centric fragments, f^1 and f^2, are formed and allegedly lost (brackets).

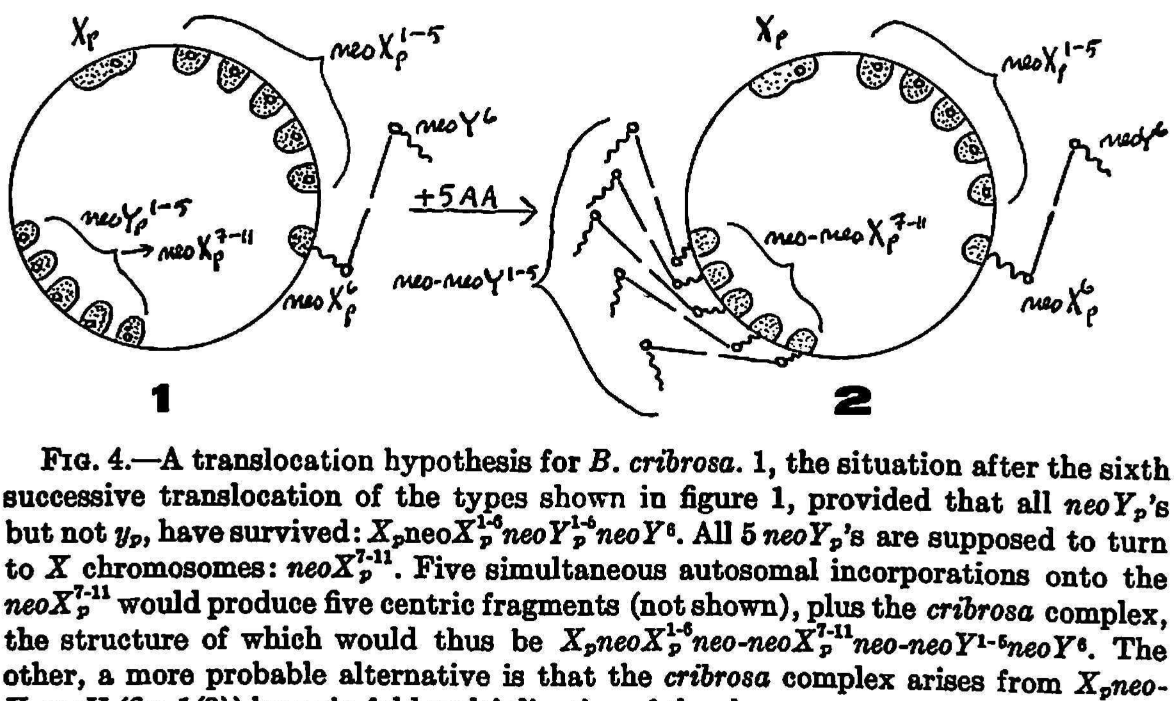

FIG. 4.—A translocation hypothesis for *B. cribrosa*. 1, the situation after the sixth successive translocation of the types shown in figure 1, provided that all $neoY_p$'s but not y_p, have survived: $X_p neoX_p^{1\text{-}6} neoY_p^{1\text{-}6} neoY^6$. All 5 $neoY_p$'s are supposed to turn to X chromosomes: $neoX_p^{7\text{-}11}$. Five simultaneous autosomal incorporations onto the $neoX_p^{7\text{-}11}$ would produce five centric fragments (not shown), plus the *cribrosa* complex, the structure of which would thus be $X_p neoX_p^{1\text{-}6} neo\text{-}neoX_p^{7\text{-}11} neo\text{-}neoY^{1\text{-}5} neoY^6$. The other, a more probable alternative is that the *cribrosa* complex arises from $X_p neo$-$X_p neoY$ (fig. 1(3)) by a six-fold multiplication of the chromosomes.

well as derivative $neoY_p$'s) have a chance to survive as it is not necessary for this chromosome to vanish completely; and 3, through progressive heterochromatinisation and subsequent repetition of the same type of translocation, this model meets the same requirement as did the Lewis and John model in which each autosomal incorporation increases the number of X chromosomes by one without altering the number of Y's.

Smith and Virkki[13] call this incorporation a *Pit*-translocation (*Pit* for *Pityogenes*). Most sex multivalents of *Blaps* seem to result from one to three *Pit*-translocations (fig. 5).

[13] Smith, S. G., and Virkki, N., loc. cit.

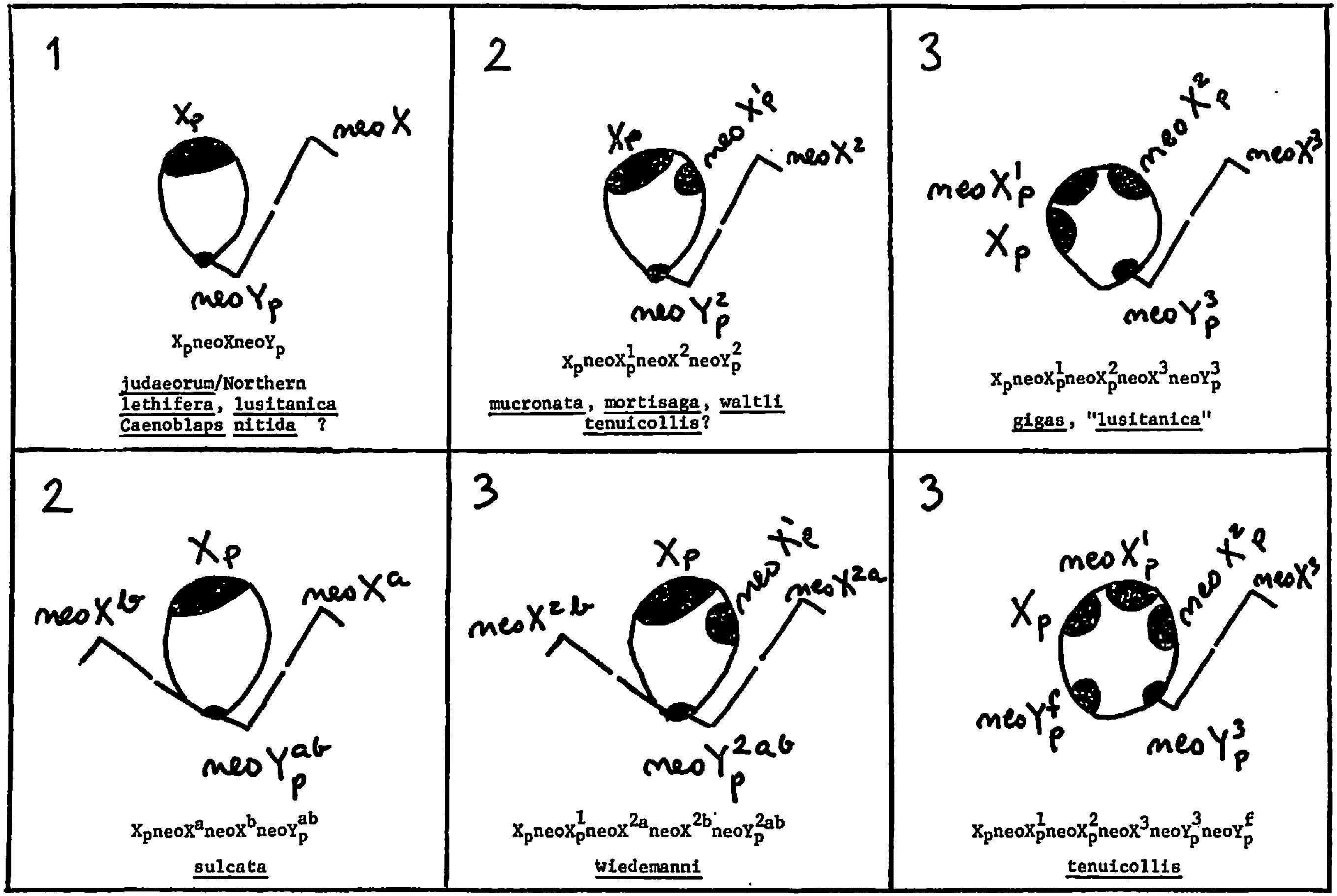

FIG. 5.—The six simplest parachute modifications of *Blaps*, all supposedly resulted from *Pit*-translocations. The number in the left upper corner gives the number of autosomes incorporated.

Complications of the schema are:

1. *B. sulcata* and *wiedemanni* (figs. 3 and 5).—In these, there has been a practically simultaneous *Pit*-translocation on the same Y chromosome. In *wiedemanni*, this has occurred after one simple *Pit*-translocation and subsequent heterochromatinisation of the neo-chromosomes.

2. *B. tenuicollis* (fig. 5).—Wahrman et al.[14] left open the question whether this is a case of $X^1X^2X^3Y$ or $X^1X^2X^3X^4Y^1Y^2$, because they could not decide whether or not a heteromorphic bivalent always found close to the sex chromosomes really belongs to them. It seems conceivable that this "bivalent" is a recently incorporated autosomal pair now taking the position of $neoY^3_p/neoX^3$. The second Y ($neoY^f_p$; f for fragment) could be a surviving centric fragment. These fragments obviously are capable of parachute association by virtue of their Y-segment and they should survive if they happen to contain important genes. But they have disappeared in most cases.

3. *B. cribrosa.*—This is a puzzle. Before becoming aware of the recent findings of the Israeli team of Wahrman, Nezer and Freund, the author was seemingly successful in leading this complex from six repeated *Pit*-translocations, in which all centric fragments but the first (where y_p participated) survived as nucleolus-associated $neoY^f_p$'s, and these $neoY^f_p$'s served as bases for five more practically simultaneous *Pit*-type translocations. Unfortunately, this interpretation was not correct. The Israeli team[15] has shown convincingly by analysing the heterochromasy and arm relationships of the chromosomes involved, that all X chromosomes are attached to the nucleolus. The metacentrics sticking out from it are the $neoY$'s. They tentatively suggest this complex arose from multiplication by six of the condition they found in the northern race of *Blaps judaeorum*. But this condition almost certainly is a $X_pneoXneoY_p$, the multiplication of which would lead to the erroneous structure proposed by the author.

There is an escape from this seeming dead-end if one assumes that *cribrosa* belongs to a different lineage of development, based on a translocation similar to the one proposed by Lewis and John, with the difference that the association to the parachute is by virtue of the X_p-segment carried by the $neoX$ rather than by a second chiasma of $neoY$. In other words, the original formula would be X_pneoX_pneoY (fig. 1 (3)). An X_p-segment containing sex determinators immediately forces the autosome in question to an X status. Competent sex multivalents of this type are not known but some abortive ones have been found.[16]

[14] Wahrman, J., Nezer, R., and Freund, O., Multiple sex chromosome mechanisms with "segregation bodies", Chromosomes Today 4 (in press).

[15] Nezer, R., The Hebrew University, Jerusalem, Israel. Personal communication, 1973.

[16] Smith, S. G., and Virkki, N., loc. cit.

There now are two ways:

1. If one goes along with the Israeli workers who propose the partial multiplication of the sexual part of the karyotype, one gets the *cribrosa* structure as $6X_p$ $6(neoX_p)$ $6(neoY)$.

2. One also can speculate the repetition of the original translocation (repeatedly with X_p, or with $neoX_p$'s) with successive heterochromatinisation and nucleolar association of the neo-chromosomes. After the sixth autosomal incorporation, the parachute would appear as in figure 4(1). It thus may be argued that the nucleolus-associated fully heterochromatinised $neoY$'s, devoid of sex determinators or any other important specific genes, have turned to X chromosomes simply because the colossally boostered parachute blister[17] does not permit them to separate from the X's but drags them behind still in anaphase I. This of course requires a change in the orientation pattern of the multivalent; the orientation of the device is supposed to derive from a somatogrammic order of the chromosomes at the beginning of the blister synthesis rather than from a prometaphasic collaboration of all centromeres involved.[17] Assuming that five of the fully heterochromatic $neoX_p$'s (perhaps only the ex-$neoY$'s) simultaneously incorporate five additional autosomal pairs, it would result again in the *cribrosa* multivalent. The formula for this would be $X_p neoX_p^{1\text{-}6}$ $neo\text{-}neoX_p^{1\text{-}5}$ $neoY^{1\text{-}6}$ (fig. 4(2)). The great difference between these alternatives is in the number of autosomes incorporated: 1 pair in the former, 11 pairs in the latter. Autosomal changes should thus give the key to the solution of the problem.

If one plausibly assumes that simplicity reflects a phylogenetically more primitive stage, the *Blaps* species with low chromosome numbers are rendered more primitive than those with high chromosome numbers. They also have more simple sex chromosome systems. The initial karyotype probably has been a primitive $9^{II} + Xy_p$ of *Pleocoma* type[18]: all autosomes metacentric, with euchromatic arms that form one chiasma each. It is just this kind of karyotype that has allegedly given rise to the adephagan karyotypes $18^{II} + Xy$ through a complete series of centric fissions.[19] Similarly, in *Blaps*, a trend of centric fissions seems to operate increasing the autosomal number to 18 pairs. Additional rearrangements may follow, especially pericentric inversions or accretion of heterochromatic second arms, that tend to mask Robertsonian relationships in Coleoptera.[20] Peri-

[17] Nezer, R., loc. cit.

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

[19] Smith, S. G., The cytology of *Sitophilus* (*Calandra*) *oryzae* (L.), *S. granarius* (L.), and some other Rhynchophora (Coleoptera), loc. cit.

[20] Smith, S. G., and Virkki, N., loc. cit.

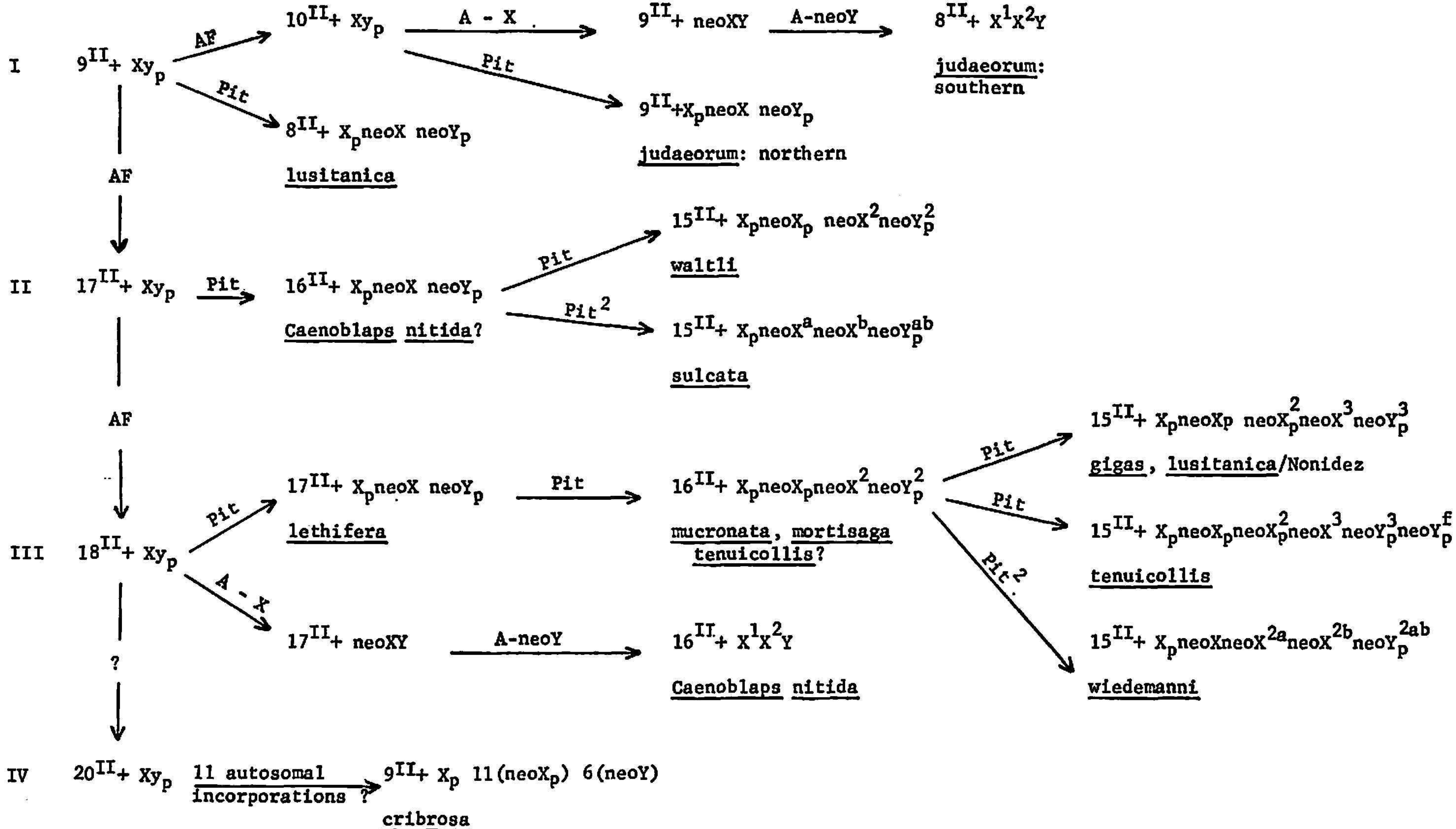

FIG. 6.—Probable interrelationships of the *Blaps* and *Caenoblaps* karyotypes. AF = autosomal centric fission(s); A–X = autosome-to-X fusion; A–*neoY* = autosome-to-*neoY* fusion; *Pit* = Pityogenes-type translocation: autosome-to-y_p; Pit^2 =second *Pit*-translocation to the same y_p or *neo*Y_p.

centric inversions open the gate for further centric fissions; thus the increase of autosomal number can exceed 18 pairs without need for speculating on polyploidy as Lewis and John[21] did.

Figure 6 suggests a general plan for evolution of the presently known *Blaps* karyotypes. It is not to be taken as a phylogenetic tree, it merely suggests the most probable evolutionary stages that can explain the contemporary karyotypes.

The first species cluster (I) evolved from the primitive $9^{II} + Xy_p$ stage directly by a *Pit*-translocation (*lusitanica*), or after one autosomal centric fission: the $10^{II} + Xy_p$ stage. From this, one *Pit*-translocation leads to *judaeorum*/northern, whereas the other lineage follows the more conventional way of *neoXY*-formation followed by a translocation between an autosome and *neoY*: *judaeorum*/southern. As is to be expected, this multiple is purely chiasmate, and nucleolus is not needed. Both races of *judaeorum* still have most of their autosomes as euchromatic, monophasic metacentrics.[22]

The cluster II developed from $17^{II} + Xy_p$ stage through two repeated *Pit*-translocations which were nearly simultaneous for *sulcata*. The position of *Caenoblaps nitida* is uncertain due to scanty information on the association mode of the multivalent.

Cluster III, the largest, starts from the fully fissioned karyotype $18^{II} + Xy_p$ suggesting a chiasmate alternative for the sex multiple of *Caenoblaps nitida*, a translocation history paralleling that of *judaeorum*/southern. In addition, there is a history of three successive *Pit*-translocations the last pair of which is double for *wiedemanni*, while *tenuicollis* appears in two sites due to the uncertainty of the karyotype structure.

Now arises this puzzle: If one insists on accommodating *cribrosa* in this general plan of fissions and translocations, the initial karyotype should have had 20 pairs of autosomes. As just mentioned, this number *can* be derived by fissions and additional rearrangements from 9 pairs, but one would not expect monophasic, euchromatic metacentrics among the autosomes thus arisen. As several of such autosomes still seem to occur in the *cribrosa* karyotype,[23] the partial polyploidy hypothesis of the Israeli workers gains in creditability.

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[21] Lewis, K. R., and B. John, loc. cit.

[22] Wahrman, J., R. Nezer, and Freund, O., The "sex nucleolus" in beetles with multiple sex chromosomes Exhibition at the 4th Intern. Chromos. Conf. Jerusalem, 1972.

[23] Nezer, R., loc. cit.