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CHROMOSOMAL POLYMORPHISM AND INTERRELATIONSHIPS IN *PISSODES* WEEVILS: ADDITIONAL CYTOGENETIC EVIDENCE OF SYNONYMY[1]

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Chromosome comparison and crossability tests compel relegating 6 of 10 Hopkins' *Pissodes* taxa to synonymy, namely, *utahensis* with *similis* Hopk., *nigrae* and *alascensis* with *rotundatus* Lec., *fraseri* and *piperi* with *dubius* Rand., and *curriei* with *affinis* Rand. Two, *dubius* and *rotundatus*, and possibly a third, *affinis*, of the four valid species revealed meiotic markers of semi-incompatibility not previously encountered; presumably, as in the *strobi* complex, the fourth, *similis*, is merely devoid of the necessary chromosomal diagnostic.

Introduction

My interest in the chromosome cytology of *Pissodes* weevils was aroused in 1955 by the discovery of numerical-morphological polymorphism in *P. approximatus* Hopkins (Smith, 1956). It has since been sustained by five major developments: (1) The SE-NW karyocline evidenced by natural populations of the species (Manna and Smith, 1959; Smith, 1970); (2) the interspecific polymorphism of the genus as a whole (Manna and Smith, 1959); (3) the joint autosomal/sex-chromosomal dichotomy — *XC/XC* : *XC/Ycc* — visible in *P. terminalis* Hopping (Manna and Smith, 1959; Smith, 1962; Smith and Takenouchi, 1962, 1969) and also in *P. fiskei* Hopkins (Smith and MacDonald, 1972); (4) the causally related semi-incompatibility systems (SIS) operative in the immediately foregoing species and again in two others here described in detail for the first time, as well as those exposed by crossing in *P. strobi* Peck, *P. nemorensis* Germar, and *P. approximatus* (Smith and Takenouchi, 1969); (5) the need to test the utility of karyotype comparison (Manna and Smith, 1959; Smith and MacDonald, 1972) and cytogenetic analysis (Smith and Takenouchi, 1969; Smith, 1970) as means of assessing the validity of Hopkins' (1911) so-called species and species complexes in a genus that is of considerable economic importance.

During the last 17 years, we at Sault Ste. Marie have investigated in greater or lesser detail the cytology of 21 of the 30 North American taxa that Hopkins (1911) recognized as distinct species and, in addition, *P. terminalis*, later described by Hopping (1920). As a result, 10 of these have been reduced to synonymy (Smith and Sugden, 1969; Smith and MacDonald, 1972). To date only 14 of the taxa have been fully documented, leaving 17, of which 8 are to be considered herein. Also, I now find that Manna and Smith's (1959) cytological descriptions of *P. dubius* Randall and *P. affinis* Randall both require amendment.

Material and Methods

The 10 weevil taxa investigated here are listed together with host and locality data in Table I. The *P. fraseri* Hopkins was generously donated by Dr. R. J. Kowal, U.S. Department of Agriculture, Forestry Service; the remainder were collected by institute staff or by field personnel of regional Canadian Forestry

[1]Contribution No. 230. Insect Pathology Research Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario. Manuscript received July 19, 1972.

Can. J. Genet. Cytol. 15: 83-100. 1973.

Service laboratories. The rearing and cytological techniques used are those detailed by Smith and Takenouchi (1969). It should be noted that the utility of karyotype analysis is somewhat impaired by colchicine pretreatment. The drug has a tendency to act differentially on the two arms of a chromosome so that arm ratios at fixation may, between individuals, depend in some degree on length of exposure and, within individuals, the stage of division at which it took effect. In the text and Table II, the female parent of a hybrid combination is consistently placed before the male parent. Because oocyte divisions are restricted to eggs, only oogonial complements were compared in the female sex. Photomicrographs, taken by J. A. MacDonald, are reproduced at a magnification of ca × 1575 unless otherwise stated.

TABLE I

Derivation of the *Pissodes* taxa investigated cytologically

Taxa	Number of ♀♀	Number of ♂♂	Host	Origin
similis Hopk.	0	2	Balsam fir "witch's broom"	Central Ontario
utahensis Hopk.	0	2	Alpine fir "witch's broom"	Interior, B.C.
affinis Rand.	7	4	Red pine bole	Central Ontario
curriei Hopk.	3	6	*Pinus* spp. boles	Interior, B.C.
dubius Rand.	1	22	Balsam fir bole	Central Ontario
fraseri Hopk.	2	7	Fraser fir bole	North Carolina
piperi Hopk.	3	7	Alpine fir bole	Interior, B.C.
rotundatus Lec.	7	33	Black spruce bole	Central Ontario
nigrae Hopk.	2	1	Black spruce bole	Nova Scotia
alascensis Hopk.	6	15	Englemann and white spruce boles	Interior, B.C.

TABLE II

Numbers of successful/unsuccessful testmatings within and between taxa

♀ ♂	*ut*	*af*	*cu*	*af.cu*	*cu.af*	*du*	*fr*	*pi*	*fr.du*	*ni*	*ro*	*al*
si	0/1											
af		2/0	8/1									
cu		3/2	2/2									
af.cu			1/0	0/2								
cu.af					0/1							
du							3/0	3/3				
fr						3/1	1/0	1/3				
pi						0/3	0/1	1/0				
fr.du						1/0			0/3			
ni												0/1
ro											0/5	0/13
al										0/1	0/8	0/2

Observations

P. similis Hopkins. The two specimens of this taxon, one which has not previously been studied cytologically, were both males. The colchicine pretreated individual provided gonial metaphases having 30 chromosomes. These consisted of two long metacentrics (*A*), two shorter near-metacentrics which, judging from first meiotic metaphases, are the *X* and *Y* sex chromosomes, two long submetacentrics (*B*), and 24 acrocentrics or subtelocentrics of graded length (*v.* Fig. 2). First meiotic metaphases (Fig. 1) usually had two bichiasmate (ring-shaped) bivalents, one equal-armed (*A*) and one unequal-armed (*B*), along with 12 unichiasmate (rod-shaped) bivalents, and an achiasmate *XY* pair smaller only than

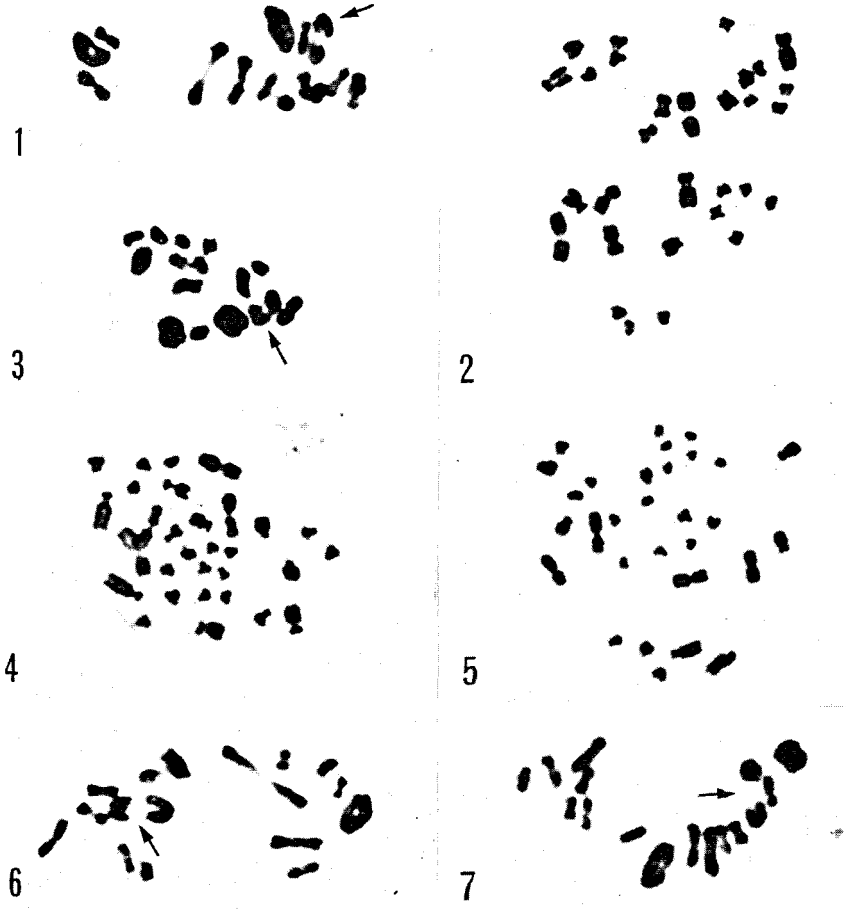
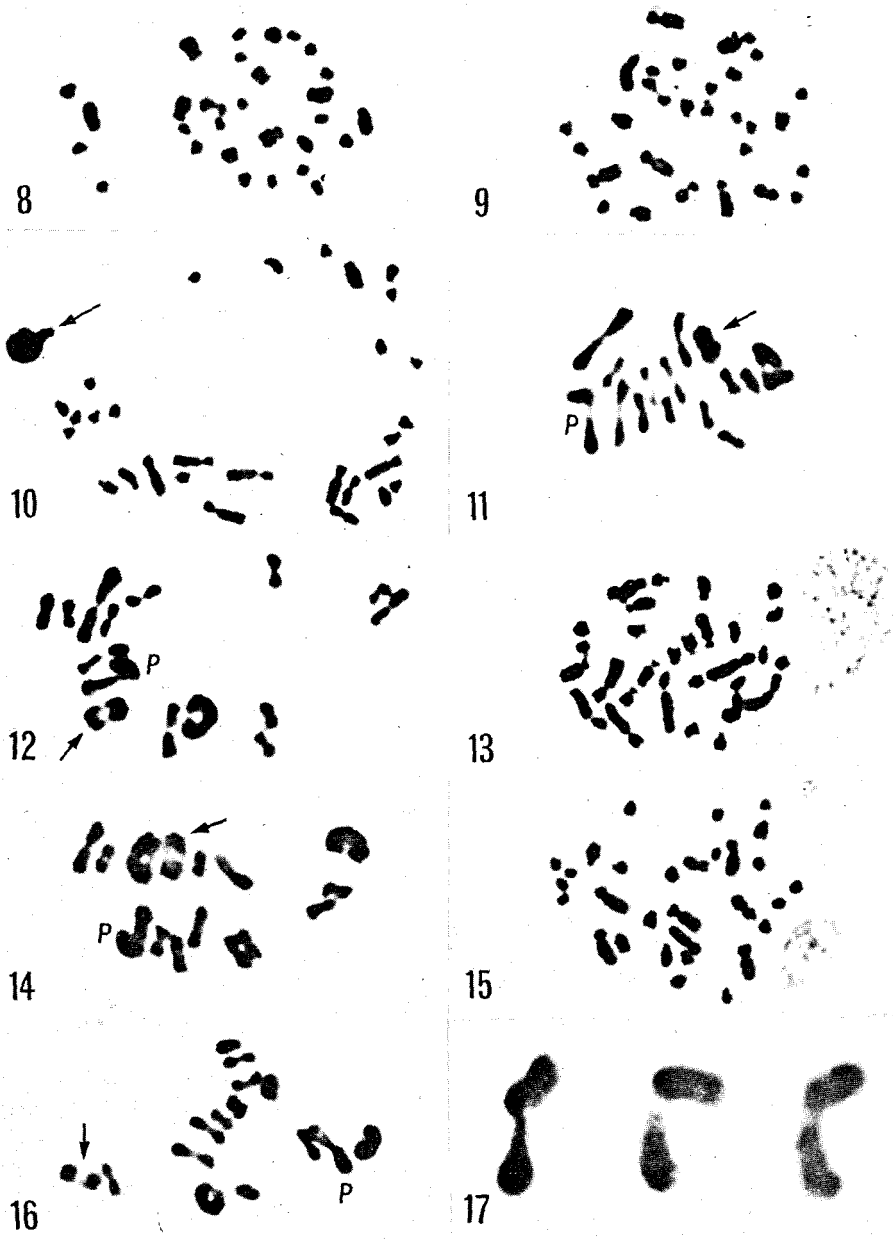


Fig. 1. MI in *similis* showing *A* ring, *B* ring, large *XY* (arrow) + 12 rods. Fig. 2. Spermatogonial metaphase in *utahensis* showing 2 *A*, 2 *B*, *X* and *Y* (smaller metacentrics) + 24. Fig. 3. MI in *utahensis* (cf. Fig. 1). Figs. 4, 5. Oogonial metaphases in *affinis* and *curriei*, respectively, showing 2 metacentric *A*, 2 large *X*, 2 subacrocentric *B* + 24 chromosomes. Figs. 6, 7. MI in *affinis* and *curriei* showing *A* ring, *B* rod and ring, respectively, *XY* (arrow) + 12 II.

the *A* and *B* rings and held together solely by the nucleolus. The untreated male had several first metaphases of the above type and a single spermatogonial or somatic anaphase showing 2 *A*, 2 *B*, and 24 shorter autosomes along with largish *X* and *Y* chromosomes; one of the longest acrocentric chromosomes in each group closely resembled the longer of the *c* chromosomes seen in the *strobi* complex (Smith, 1970). When rod-shaped, the *B* bivalent may be associated through either the long or the short arms.



P. utahensis Hopkins. This western taxon, phenotypically similar to *similis,* is also new to cytology. One of the two males examined was colchicined and contained gonial metaphases, the other only first meiotic metaphases. Both individuals appear to be chromosomally identical (Figs. 2, 3) to *similis.*

P. affinis Randall. Manna and Smith (1959) reported that this species gave gonial counts of $2n = 30$ and included six long chromosomes, two of which were the sex chromosomes. Of the specimens studied here only females were fed colchicine, and as a result they alone contained metaphases showing 30 chromosomes, which likewise included 6 long chromosomes (Fig. 4). In their Fig. 86, Manna and Smith code-lettered the large equal-armed ring bivalent *"A"* and the large unequal-armed one *"B".* It is now abundantly clear that their *B* chromosomes, if such they are, differ from those of *approximatus, nemorensis,* and *schwarzi* Hopkins (Smith, 1970) in having much shorter short arms, with an arm ratio of about 1:4 as against 1:2.5, and consequently have a lower mean number of ring bivalents per first metaphase. The *XY* pair is large (Fig. 6), larger even than that of *similis-utahensis.*

P. curriei Hopkins. This western taxon, which is morphologically indistinguishable from eastern *affinis,* has not previously been investigated cytologically. Nine individuals, three colchicined females (Fig. 5) and six untreated males (Fig. 7), were examined. They proved to be chromosomally inseparable from *affinis.*

P. dubius Randall. Compared with most other species, neither *dubius* nor its two closely similar allies (Smith and MacDonald, 1972) squashes well, so that satisfactorily spread first metaphases are rarely obtained. Furthermore, the extra pressure required frequently ruptures accompanying gonial metaphases, with consequent displacement or loss of chromosomes.

Manna and Smith (1959) examined four *dubius* specimens, all males, of which two contained only a few substandard spermatogonial divisions. They reported a count of 30 chromosomes, of which two so-called metacentric pairs formed relatively large ring or rod bivalents. I have since examined preparations made from a colchicined female (Figs. 8, 9) and male (Fig. 10) and from 21 untreated males. Six of the longest chromosomes in oogonial metaphases have arm ratios of 2+:1, and the two somewhat shorter near-metacentrics are, judging from male meiosis, the *X* chromosomes. Two of the longest autosomes look like typical *B* submetacentrics, but since no large metacentric autosomes are seen in the complement, *A* chromosomes do not in fact occur in the species (Figs. 8-10). In my males the two remaining pairs of submetacentrics have been code-lettered *D* and *P* for the following reasons. In sideview first metaphases (Figs. 11, 12) the former,

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Figs. 8, 9. Oogonial metaphases in *dubius* showing 6 long submetacentrics + 24 shorter chromosomes (2 short ones missing from Fig. 9; note the reduced differentiation between arms of submetacentrics in Fig. 8. Fig. 10 (× 1750). Spermatogonial metaphase in *dubius* (1 shorter chromosome, arrow, partially obscured); note particularly the absence of *A* metacentrics and the close similarity of the 6 long chromosomes. Figs. 11, 12. MI in *dubius* (*XY* arrowed). In Fig. 11 the large bivalents are unichiasmate, L-L, S-S, and L-S (= *P*). In Fig. 12, 1 bivalent is bichiasmate and 2 are unichiasmate, L-L, and L-S (*P*). Fig. 13. Oogonial metaphase in *fraseri*; note the 6 long submetacentrics. Fig. 14. MI in *fraseri* (cf. Figs. 11 and 12). Fig. 15. Oogonial metaphase in *piperi* (cf. Figs. 8, 9, 13). Fig. 16. (× 1750). MI in *piperi* (cf. Figs. 11, 12, 14). Fig. 17 (× 4250). *P* bivalents in *pi, du* × *fr* and *fr* × *du.*

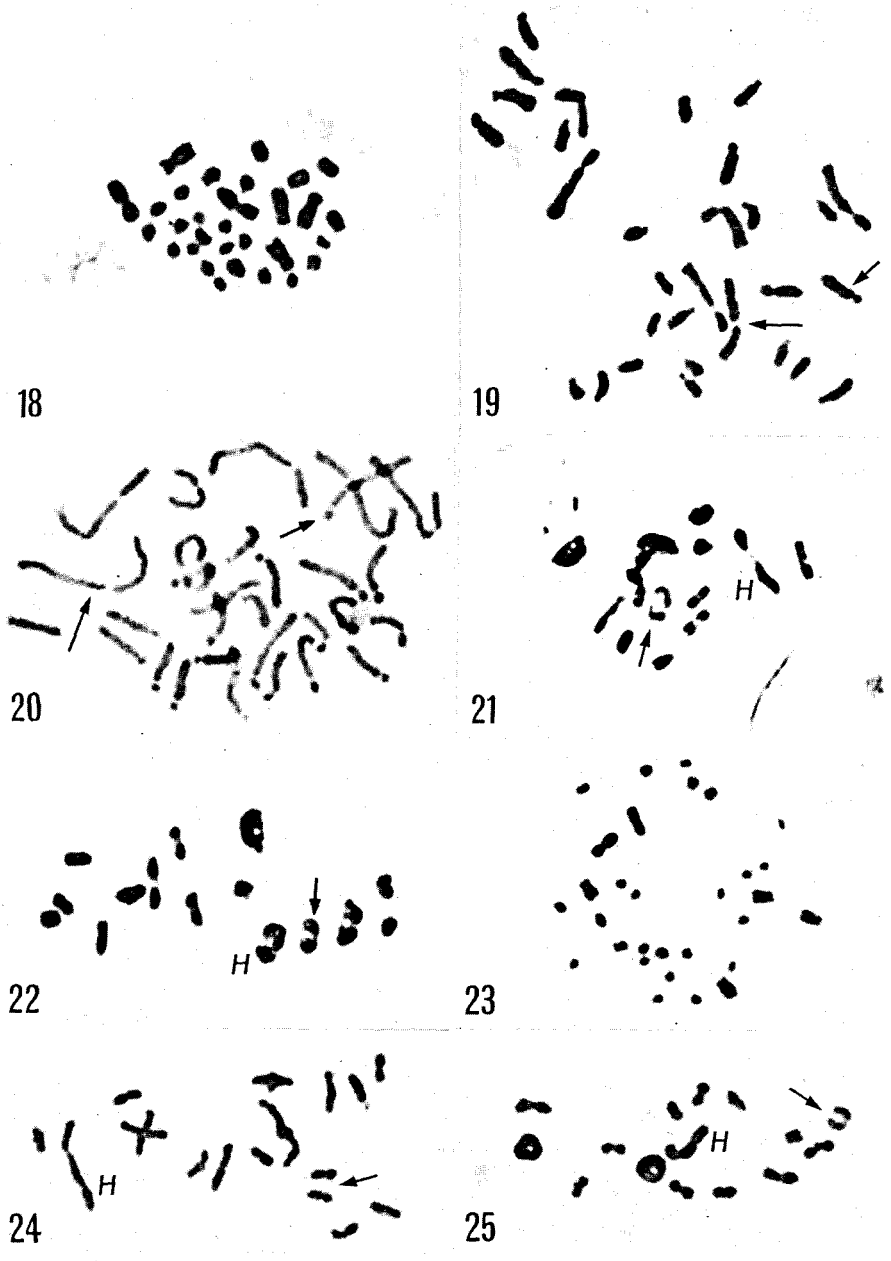

Fig. 18. Oogonial metaphase in *rotundatus* showing 2 *H* metacentrics and 4 meta-acrocentrics. Figs. 19, 20. Spermatogonial metaphases in *rotundatus* showing 1 long unmatched metacentric (long arrow) and 1 unmatched acrocentric (short arrow). Figs. 21, 22. MI in *rotundatus*; in Fig. 21 the *H* II is a rod, in Fig. 22, a ring. Fig. 23. Oogonial metaphase in *nigrae* (cf. Fig. 18). Figs. 24, 25. MI in *nigrae* (cf. Fig. 21).

even more so than the *B* bivalent, is unequal-armed, appearing either as a bichiasmate *D*-shaped ring or more commonly as a unichiasmate rod. The *P* chromosomes at first metaphase appear in the form of a unichiasmate L-shaped bivalent, the long arm of one component being joined to the short arm of the other, thereby indicating that it is heterozygous for a pericentric inversion (Smith, 1970). A re-examination of Manna and Smith's preparations established beyond doubt that they also contain this heteromorphic *P* bivalent. As stated earlier, because of difficulties in studying first meiotic metaphases in eggs nothing is known of how the *P* chromosomes associate in female meiosis.

P. fraseri Hopkins. Representatives of this taxon, obtained from the Great Smokey Mountains in North Carolina, and the one immediately below, which originated from British Columbia, were not available to Manna and Smith (1959). I have examined cytological preparations of two colchicined *fraseri* females and seven untreated males that consequently lacked usable gonial metaphases. As in *dubius,* oogonial complements consisted of 2 *B,* 2 *D,* 2 *P,* and 22 acrocentric autosomes of graded size as well as 2 *X* chromosomes (Fig. 13). Male first meiotic metaphases (Fig. 14) were indistinguishable from those of *dubius.*

P. piperi Hopkins. Three colchicine pretreated females (Figs. 15) and seven untreated males (Fig. 16) of *piperi* were examined and found to have karyotypes corresponding to those of the foregoing two taxa.

P. rotundatus Leconte. This taxon, like the morphologically identical more eastern, maritime, *nigrae* and the more western, montane, *alascensis* (Smith and MacDonald, 1972) was not represented in Manna and Smith's (1959) investigation. Seven colchicined females, and one colchicined, one Na-citrate pretreated, and 31 untreated males of *rotundatus* were studied by me.

Although 5 of the 30 chromosomes seen in spermatogonial metaphases are relatively large, only the longest is near-metacentric; the other 4 can be matched in pairs and have arm ratios of ca 2:1 and ca 3:1 (Fig. 20). Of the 25 remaining chromosomes, 2 are metacentric and appear to be the sex chromosomes; the others are acrocentrics of graded size, but the longest, like the near-metacentric, lacks a morphological equivalent (Figs. 19, 20). In females, on the other hand, all the long gonial chromosomes, including the near-metacentrics, can be matched in pairs (Fig. 18).

First spermatocyte metaphases (Figs. 21, 22) include a large heteromorphic (*H*) bivalent formed by a metacentric united terminally to an acrocentric chromosome; it obviously represents the spermatogonial near-metacentric and one of the longer acrocentrics, seeming the longest. Often at late diakinesis (Fig. 42) and exceptionally at metaphase (Fig. 22) the *H* bivalent takes the form of an extremely asymmetrical ring that owes its lopsided appearance to the second arm of the metacentric being joined to the short arm of the acrocentric. Since this particular bivalent occurs along with a nucleolus-conjoined pair, it obviously cannot be the sex-determining bivalent. Moreover, in this species it is especially clear under phase-optics that the *X* and *Y* are held together at diakinesis and prometaphase (cf. Figs. 28 and 29) solely by the nucleolus; they most commonly appear as two somewhat curved rods often lying well apart and separated by an unusually large mass of nucleolar material. At metaphase, the other four long chromosomes, the *B*'s and *D*'s, form either rings or rods with the components most often joined at the ends of the long arms, rarely the short arms.

P. nigrae Hopkins. Only three specimens of this taxon were examined cytologically, two females and one male, all of which had been fed colchicine. As in *rotundatus,* spermatogonial metaphases gave counts of $2n = 30$ and included the odd near-metacentric. Oogonial complements, (Fig. 23), however, have this chromosome in duplicate. Prometaphases (Fig. 24) and first metaphases (Fig. 25) included the *H* bivalent.

P. alascensis Hopkins. Six females and 15 males of *alascensis* were available and most were colchicined. They proved to be chromosomally indistinguishable (Figs. 26-29) from *rotundatus* and *nigrae.*

Results of Crossability Tests

P. similis-utahensis. These rarely encountered taxa were only once on hand simultaneously: a newly emerged *similis* female and an old *utahensis* male. Following pairing, copulation was observed, but the female died without laying eggs.

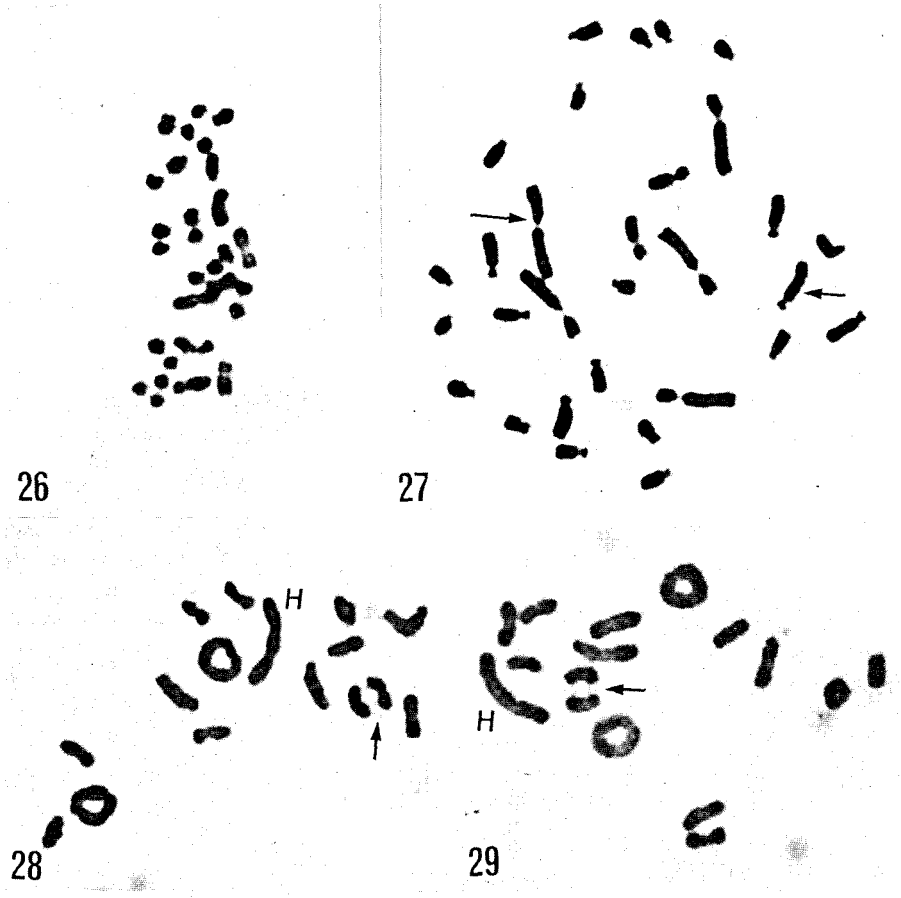

Fig. 26. Oogonial metaphase in *alascensis* (cf. Figs. 18 and 23). Fig. 27 (× 1750). Spermatogonial metaphase in *alascensis* (cf. Figs. 19, 20). Figs. 28, 29. Prometaphase I in *alascensis* (cf. Figs. 21, 24, 25).

P. rotundatus-nigrae-alascensis. One small shipment of *nigrae* was received from Nova Scotia but at a time when only one male and one young virgin female *alascensis* were available. The appropriate pairings were made (Table II), but because both females died prematurely, the negative results are inconclusive. At various times, however, concurrent supplies of local *rotundatus* and western *alascensis* were sufficient to permit a total of 21 intertaxon and 7 intrataxon tests to be made (Table II). Even though copulation was observed in both reciprocal combinations, none produced larvae. In view of their identical exo- and endo-phenotypes, this complete inability to demonstrate the obvious close affinity of the two taxa by means of consanguinity tests points to deficiencies in the procedure employed. Of these, the most obvious is that of failing to subject individuals to a period of cold-rest, a practice that, because of high ensuing mortality, had been discontinued earlier in the general investigation. Notably, the only culture obtained was established by using a field-collected *alascensis* female.

P. affinis-curriei. Eleven of the 14 reciprocal tests of *affinis* against *curriei* were successful (Table II), a result that compares favourably with the four out of six intra-taxon pairings. Females had the expected 30 gonial chromosomes (Figs. 30, 32), and meiosis in F_1 males (Figs. 31, 33) was completely regular — no multiple associations were observed and, despite one of the longer rod bivalents appearing unequal (Fig. 33, *U*), both divisions proceeded entirely normally. Although there are thus no obvious meiotic impediments to fertility, at least on the male side, all three attempted F_2 matings were nevertheless unsuccessful (Table II). Again I attribute this failure to the parents being paired as soon as they emerged. The lone *af. cu* × *cu* backcross, made after a long period of cold-rest, provided a few progeny, but no satisfactory preparation was obtained.

P. dubius-fraseri-piperi. Out of the 21 crossability tests attempted within this group, 10 were successful (Table II). Six of seven reciprocal *du-fr* pairs yielded progeny. Three of six *du* × *pi* pairings gave progeny, but all three reciprocals failed. Similarly, although one of four *fr* × *pi* tests was successful, the lone reciprocal failed. Meiosis in all F_1 males (Figs. 34, 35, 37) examined appeared normal and entirely indistinguishable from their parental types, i.e., all

TABLE III

Numbers of unsuccessful crossability tests against more remotely related species*

♀ ♂	*af*	*cu*	*fr*	*pi*	*ro*	*al*	*st*	*ne*	*sw*	*ob*
af							1			
cu							1	1		
fr										1
ro									2	
al									1	
ne	1	1								
sw	1				1	3				
ap					2					
fa				1						
ob			3							

*st = *strobi*, *ne* = *nemorensis*, *sw* = *schwarzi*, *fa* = *fasciatus*, *ob* = *obscurus* Roelofs.

hybrid males carried a heteromorphic *P* bivalent (Fig. 17) and all hybrid females had a balanced gonial complement (Fig. 36). One *fr. du* × *du* mating provided adult progeny (Fig. 38), but three attempted F_2 matings failed.

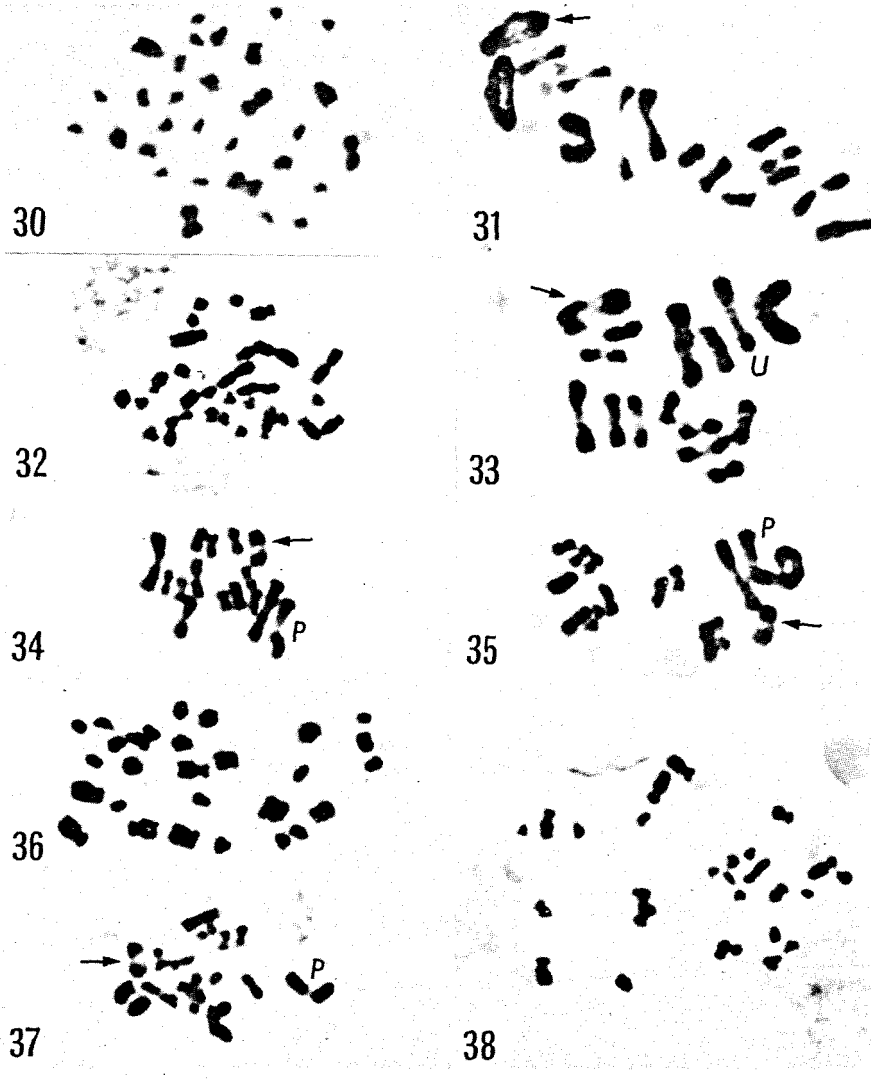

Fig. 30. Oogonial metaphase in *affinis* × *curriei* showing 2 *A*, 2 *B*, 2 *X* + 24. Fig. 31. MI in *affinis* × *curriei* showing *A* ring, *B* rod with pairing between short arms, large *XY* (arrow) and 12 rod bivalents. Fig. 32. Oogonial metaphase in *curriei* × *affinis* (cf. Fig. 30). Fig. 33. MI in *curriei* × *affinis*; note the unequal bivalent (*U*) and the nucleolar material associated with the *XY* (arrow). Figs. 34, 35. MI in *dubius* × *fraseri* showing the pericentrically inverted bivalent (*P*). Fig. 36. Oogonial metaphase in *fraseri* × *dubius* showing 6 submetacentrics. Fig. 37. MI in *fraseri* × *dubius* (cf. Figs. 34 and 35). Fig. 38. Oogonial metaphase in *fraseri* × *dubius* ♀ × *dubius* ♂ backcross derivative showing 6 long submetacentrics (incomplete plate).

Additional Interspecific Testcrosses

A limited number of tests of the taxa here documented were carried out using more distantly related species that happened to be on hand concurrently. As summarized in Table III, none of these pairings was successful.

Discussion

From their comparison of the karyotypes of 12 *Pissodes* species (or 9 according to Smith and Sugden, 1969), Manna and Smith (1959) concluded that "they all have the same major arms irrespective of chromosome number," i.e., they exhibit a monophyletic Robertsonian relationship. These authors concede that this is an oversimplification: interspecific *XY* size differences and autosomal isomery, etc., are evidence of other structural changes having been involved. Indeed, even visibly identical meiotic complements may in fact conceal the existence of structural dissimilarities between taxa (Smith, 1970). This has been most convincingly demonstrated by their meiotic consequences in hybrids between sibling species of *Eyprepocnemis plorans* Charp. (John and Lewis, 1965). These grasshopper hybrids contained univalents, asymmetrical bivalents, multiple associations, and, rarely, bridges and fragments. Even so, the validity of Manna and Smiths generalization has been in part established by Smith and Takenouchi (1969) and Smith (1970) through examination of hybrids between nine loosely allied *Pissodes* taxa. The former authors were unable to determine the chromosome homologies of four of the remaining five species that Manna and Smith studied because whenever they were paired all but the fifth, *P. notatus* Fabricius, which became available later, failed to outcross. Significantly, *notatus* is most closely related to the species complex with which it was hybridized.*

Based on the host associations established by Smith and Sugden (1969), and/or the morphometric comparisons made by Smith and MacDonald (1972), and especially in view of the chromosomal equivalence and/or pairing homologies demonstrated herein, I feel entirely confident in synonymizing *utahensis* with *similis; nigrae* and *alascensis* with *rotundatus; fraseri* and *piperi* with *dubius;* and *curriei* with *affinis,* even though corroboration through hybridization has not been obtained for all combinations. Suffice it to recall that where intraspecific, intertaxon hybrids were obtained, they were entirely devoid of the meiotic irregularities characterizing the *Eyprepocnemis* hybrids or, more pertinently, of the high pairing failure exhibited by the *notatus* interspecific hybrids. The only possible anomaly encountered was the unequal rod bivalent that occurred in males obtained by crossing *affinis* and *curriei.* It will be considered later.

In line with the concept current in 1959, Manna and Smith assumed that *Pissodes* sex chromosomes are chiasmate. They therefore considered that in those species in which the sex pair appeared at first metaphase as a lopsided ring — "with the long arm of one associating with the short arm of the other of the two J-shaped submetacentric X and Y chromosomes" — it must be heterozygous for a pericentric inversion. Their inability to see such a configuration in certain other species was rationalized as due to its smaller size or unfavourable orientation. John and Lewis (1960) have since demonstrated that the typical coleopteran large-*X*/small-*y* bivalent has the appearance of a parachute (Xy_p) because the chromosomes are

*The exception, *notatus,* crossed readily (30 +ve, 9 −ve), but extensive though highly variable failure of pairing in the markedly vestigial testes rendered all 22 F_1 males tested completely sterile (Smith, unpublished); the total failure experienced using six hybrid females as mothers demonstrates that they also suffered from similiar meiotic irregularities.

held together solely by the nucleolus, i.e., it is achiasmate. This has since been almost universally verified and quite certainly holds for all species of *Pissodes* studied to date* irrespective of the relative size of the chromosomes. The role played by the nucleolus is in fact plainly evident in several of Manna and Smith's photographs, particularly in their Figs. 88, 92, and 110, even though they were taken from preparations stained with Feulgen and light green. A re-examination of the original slides of *P. fasciatus* Le Conte, *P. radiatae* Hopkins, and *affinis* shows that the *X* and *Y* do not necessarily associate in reverse sequence, as they claimed. Although angle of vision undoubtedly introduces a certain element of subjectivity, it now seems that reverse pairing is no commoner than 'straight' pairing. With pairing non-particulate, this of course is expected; accordingly it is no longer necessary to postulate a pericentric inversion in the sex chromosomes of the male.

A chromosomal sex-determining mechanism can perpetuate only with one sex homogametic (typically *XX* ♀ in Coleoptera) and the other heterogametic (typically *XY* ♂). The same principle must therefore operate where, as in *dubius*, the male is invariably heterozygous for an autosomal pericentric inversion; in the female the corresponding pair of chromosomes must form a symmetrical bivalent, one that is homozygous for the uninverted sequence (Fig. 39). That these relatively inverted autosomes are permanently linked in inheritance with the sex chromosomes parallels the situation obtaining in SIS-carrying *terminalis* (Smith and Takenouchi, 1969) and *fiskei* (Smith and MacDonald, 1972), in both of which the two sexes have different numbers of autosomes, and also in *strobi, approximatus* and *nemorensis*, in which they are either homozygous (♀ ♀) or heterozygous (♂ ♂) for a pair of morphologically indistinguishable but genetically distinct autosomes. The joint sexual dichotomy in *terminalis* was formally interpreted by Smith and Takenouchi (1969) as due to translocation of an essential 'factor' (+) from an acrocentric autosome (*c*) to the *Y*, whereby c^- and Y^+, on the one hand, and *c* and *X*, on the other, were rendered complementary and inevitably sex-linked in heredity. Their linkage in $2n = 34$ *strobi* and in $2n = 30$ *approximatus* and *nemorensis* was demonstrated by the presence or absence of numerical differences between the sexes in derivatives of hybrids with $2n = 28$ *schwarzi* (both ♀ and ♂ *CC*) depending on the direction in which the cross was made. It is logical but by no means obligatory — because chiasma formation is excluded from the short arm — to conclude that the plus factor in *dubius* is sited within one of the relatively inverted segments of the *P* chromosomes and thereby permanently excluded from crossing over: the inverted segment clearly constitutes a genuine differential segment. A hypothetical model for the evolution of the SIS in *terminalis-strobi-approximatus-nemorensis* was proposed by Smith and Takenouchi (1969) and is here extended to include *dubius* and *rotundatus* (Fig. 39).

The situation in *rotundatus* is less straightforward. Here we are faced with an obvious quantitative difference between sexes: the male lacks virtually one complete arm that is present in duplicate in the female. Such an imbalance is by no means unique when Coleopteran sex-determining systems are considered, *cf.* the different sizes of the chromosomes in the typical Xy_p bivalent or of the chromo-

*Takenouchi (1958) gave the formula 18_{11}+ XY for *P. nitidus* Roelofs and illustrated (Fig. 54) the sex pair at meta-anaphase as a rod configuration. He has since informed me that these are purely transcriptional errors and actually refer to *Cryptorhynchidius insidiosus* (Roelofs), which, as he shows in his Fig. 56, is in fact an Xy_p species. The correct diploid formula for *nitidus* is 2*A* + 2*B* + 24 acrocentrics + *XX/XY*; the *A*s are metacentric, the *B*s submetacentric, and the sex chromosomes fairly large and nucleolar.

somes in derivative neo-*XY* systems (Smith, 1952, on *Tribolium;* Virkki, 1972, on *Omophoita cyanipennis* Fabr.) or again of the elements comprising the tertiary *XXXX/XXY* in *Chilocorus stigma* Say (Smith, 1959), and, at the extreme, of the *XX/XO* system common among other beetles. However, a comparable sex-limited inequality of autosomal material is, I believe, unknown in the order. Indeed, to find a parallel we have to turn to the classic *XX/XO* Orthopteroid *Gryllotalpa hexadactyla* L. in which the males of certain 'races' are *XA/Oa* and females *XA/XA* (Payne, 1912; White, 1951; Carmenzind and Nicklas, 1968). The normal size differences between sex chromosomes are attributed to erosion of that portion of the chromosome permanently confined to the male line (Smith, 1952, on *Tribolium*; John and Shaw, 1967). But the autosomal inequality in *rotundatus* is plainly not due to loss from the male but to gain by the female; beyond doubt this species has a female/male autosomal N.F. of 34/33, whereas all others, with the possible exception of inadequately known *fiskei*, have one of 32 in both sexes. This deviation could most readily be explained by the large *H* chromosome of *rotundatus* having achieved metacentry by a one-step intercalation of a long heterochromatic segment were it not that no supernumerary chromosomes have been observed in any of the 6,900 *Pissodes* individuals that have been examined at this institute. The obvious alternative is by gradual accretion. While heterochromatin in Coleoptera typically manifests heteropycnosis (e.g. in Chilocorine lady-bird beetles, Smith 1965a,b), we have never directly observed any substantial allocyclic segments in *Pissodes* autosomes, although circumstantial evidence exists for heterochromatic minor arms in certain *approximatus* hybrids (Smith, 1970). Be that as it may, I here offer for consideration a photograph Fig. 40) of a pachytene cell from a *rotundatus* female. If the two triple-armed complexes each represents three acrocentric bivalents with their minor arms fused

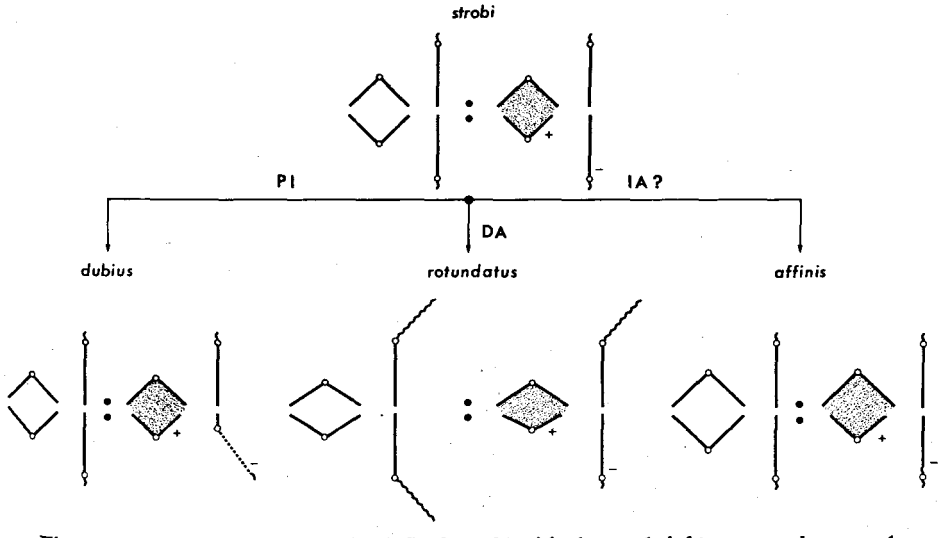

Fig. 39. Diagram comparing the SIS of *strobi* with those of *dubius, rotundatus,* and possibly *affinis*. Note that PI = pericentric inversion, DA = distal accretion, and IA = interstitial accretion (of heterochromatin); for further explanation see text.

into a chromocenter, the arms in question should consist of heterochromatin. The three long independent bivalents are then the *B, D,* and *H,* the last judging from relative gonial lengths being the longest, yet revealing no evidence of heteropycnosis. Whether chiasmate exchange could occur within the paired *H* arms is an open question. Primary oocyte metaphases are virtually impossible to obtain, but the pachytene genome strongly suggests that if the extra *H* arms do indeed consist of heterochromatin, they may, like the constitutive sex chromatin in mammals, become meiotically isopycnotic and hence free to form chiasmata.

For comparison, Fig. 41 is a photograph of a diplotene cell from a *rotundatus* male. Note here the presence of four large rings and a long rod, one among which is the *H* bivalent, and in particular the apparent totally euchromatic nature of all major arms, including those of the *H* pair. But although both arms of the *H* bivalent may be associated at diakinesis (Fig. 42) and even metaphase (Fig. 22), only one of the unions necessarily persists into metaphase. This ectopic pairing is the only direct evidence that one arm of the near-metacentric consists mostly, if not entirely, of heterochromatin. My colleague T. J. Ennis has restained these two preparations with quinacrine mustard and finds no intensely fluorescing arms in either; but then in *Chilocorus,* for example, not all segments of demonstrably late-replicating heterochromatin fluoresce intensely, nor do they stain differentially with Giemsa (Ennis, unpublished). Irrespective of the true nature of the second *H* arm, invariable sexual dichotomy of the species unquestionably marks the pair, both meiotically and mitotically, as being homoeologous to the c/c^- bivalent in the *strobi* complex and the P/P^- bivalent in *dubius* (Fig. 39).

The structural dissimilarities Smith (1970) demonstrated in *Pissodes* were interspecific rearrangements: a pericentric inversion in a *B* submetacentric and causally related reciprocal interchanges between twin *b/b* acrocentrics. In the intraspecific hybrid males studied herein, meiotic association and anaphase disjunction were invariably regular and hence indistinguishable from those in the parents. The only 'anomaly' encountered is the unequal rod bivalent observed in *curriei* × *affinis* hybrid males (Fig. 33, *U*). Whether the inequality results from a valid interparental structural difference — a quantitative difference between homologues in *affinis* relative to *curriei* — though likely, remains uncertain. The possibility is open that one allopatric taxon (Table I) carries an appreciable chromosome segment not present, or as well developed, in the other: a segment that (1) judging from the regularity of pairing in hybrids, is situated proximal to the proterminal chiasma-forming region (Smith, 1970) and (2) judging from phenotypic overlap (Smith and Sugden, 1969), consists of inert or quasi-inert heterochromatin. If so, then high-quality oogonial and spermatogonial F_1 metaphases, which mine are not, should equally reveal autosomal heterozygosity. There is also the possibility that the *U* bivalent is actually a meiotic manifestation that both taxa possess, as *rotundatus* does, an innate SIS; in other words, the *U* bivalent is in fact a genuine c/c^- bivalent

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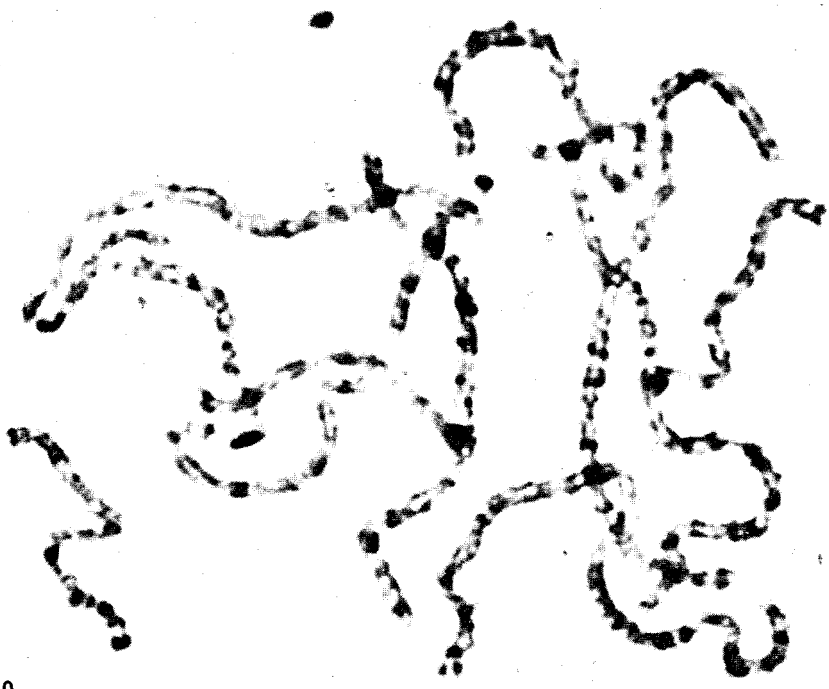
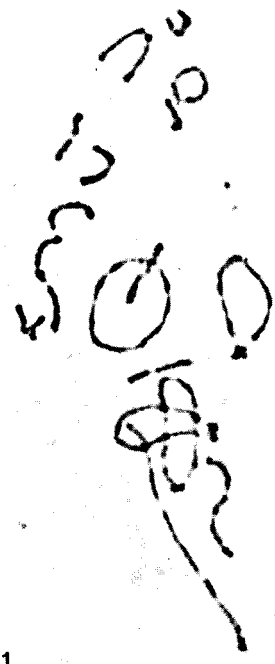
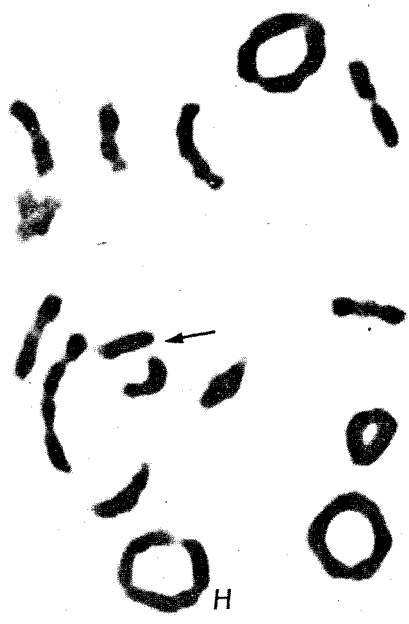
Fig. 40 (× 2300). Pachytene in *rotundatus* ♀ illustrating the particulate nature of all major-arm pairing. Fig. 41. (× 1425). Diplotene in *rotundatus* ♂; note that heteropycnosis is restricted to the terminial knobs. Fig. 42 (× 2330). Late diakinesis in *rotundatus* ♂ showing the heteromorphic bivalent (*H*) in the form of a ring. Under phase-contrast nucleolar material is visible between the *X* and *Y* (arrow).



40



41



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in but another guise. Were this the case, the autosomal complements of *affinis* and *curriei* should then be balanced in the female and to some degree unbalanced in the male. Once the unequal bivalent has been observed in the hybrids, first metaphases in the parental-type males were again searched, but no such diagnostic pair could be invariably picked out.

None of the remaining nine species recognized by Hopkins (Table IV) has been studied chromosomally, but specimens of all except *californicus, barberi,* and *murrayanae* were seen in the Canadian National Collection. In the absence of cytological evidence to the contrary, but fully aware that Hopkins' key is far from being foolproof or for that matter free from error (Smith and Sugden, 1969; Smith and MacDonald, 1972; and herein), I am prepared to accept unreservedly only *P. costatus* Mannerheim as a biologically valid species. Hopkins' *burkei* presents special difficulties: (1) In his Plate IV, *burkei* is depicted as having an obvious anterior spot on the elytra, despite which he placed it in Subdivision B, one that accommodates only taxa having "Elytra usually without distinct anterior spots"; (2) He states "This species is quite distinct from *P. rotundatus* but is more closely allied to *P. piperi.*" Now both *burkei* and *piperi* are assigned to Series c7, one having "Elytral striae with punctures very irregular"; nevertheless a comparison of his illustrations (Plate IV) reveals that in elytral punctation *burkei* has a much closer similarity to *rotundatus* than to *piperi,* which according to the present cytogenetic analysis is conspecific with *dubius;* (3) Despite his having several if not many *burkei* from which to choose, the one Hopkins selected (Fig. 25) is close to 22% larger than the one of *rotundatus* (Fig. 24), yet he states that *burkei* varies in length "from 6 to 7.7 mm" and *rotundatus* "from 6 to 7.3 mm." Hopkins was, of course, over-impressed with the role that host tree might play in speciation (see below regarding *deodarae*); one cannot therefore but suspect that in allying *burkei* with *piperi* he was deeply influenced, as I am, by his own *burkei* specimens having been reared out from the thick bark of *Abies lasiocarpa* because it is the known host of *piperi* and especially since he was under the impression, subsequently proved in part correct, that *rotundatus* and it close allies are restricted to *Picea.* We have never found any species to include one subspecies that, under natural conditions, breeds in *Picea* and another that breeds in *Abies,* although almost all taxa we have reared can be forced to breed in *Pinus banksiana.* In this context, then, it appears

TABLE IV

Synonymy proposed for eight cytologically unknown *Pissodes* taxa*

Hopkins' species with host tree		Proposed synonymy with host tree	
Sp. 3 = *barberi*	?*Picea*	*Abies*	*similis* Hopk. = Sp. 1
Sp.11 = *deodarae*	*Cedrus*	*Pinus*	*nemorensis* Germ. = Sp. 10
Sp.12 = *californicus*	*Pinus*	*Pinus*	*schwarzi* Hopk. = Sp. 8
Sp.14 = *webbi* ?	*Pinus*		
Sp.20 = *puncticollis*	*Picea*	*Pinus* &/or *Picea*	*rotundatus* Lec. = Sp. 24
Sp.21 = *murrayanae*	*Pinus*		
Sp.22 = *coloradensis*	*Picea*		
Sp.25 = *burkei*	*Abies*	*Abies*	? = Sp. 25

*Allopatric taxa that are reduced to junior synonymy merit recognition as subspecies (Smith, in press).

that *burkei* may indeed be a species distinct from *rotundatus,* just as *barberi,* considered by Smith and Sugden (1969) a synonym of *Abies*-associated *similis-utahensis,* is most unlikely to be the *Picea*-breeder Hopkins surmised.

Hopkins' *P. deodarae* specimens were reared out of *Cedrus deodara* saplings growing in the State of Georgia on a plot near, I understand, a mill engaged in sawing pine logs. He noted, along with some highly questionable morphological differences from *nemorensis,* that *deodarae* is "more distinctly separated by its habit and host," and concisely documents his faith in the host-selection principle by commenting, "It is not impossible that this is an example of the origin of species through mutation and change of habit and host." It is the considered opinion of W. J. Brown, Coleopterist (retired), Entomological Research Institute, Ottawa, that Hopkins was undoubtedly dealing with *nemorensis,* a conclusion with which I concur wholeheartedly.

The cytological evidence detailed herein amply confirms the morphometric evidence (Smith and MacDonald, 1972) that *nigrae* and *alascensis* are mere subspecies of *rotundatus,* all three of which occur in *Picea* and/or *Pinus.* Likewise, Hopkins' sp. 20, *P. puncticollis,* and sp. 22, *P. coloradensis,* breed in *Picea,* and sp. 21, *P. murrayanae,* breeds in *Pinus.* Hopkins placed these three between sp. 19, *nigrae,* and sp. 23, *alascensis*; clearly, unless otherwise dictated, all five taxa are best treated as subspecies of *rotundatus.*

P. californicus was erected by Hopkins on the basis of only a single adult female "collected . . . at the same time and place [and from the same tree species] as those referred to *P. yosemite,* and were not recognized at the time as distinct" [from it]. Unambiguous cytogenetic reasons have already been given for synonymizing the latter with *schwarzi* (Smith and Takenouchi, 1969; Smith, 1970). As shown in Table IV, Hopkins' key lists *californicus* as sp. 12, and since this places it, along with *nemorensis* (sp. 10) and *deodarae* (sp. 11). between *schwarzi* (p . 8) and *yosemite* (sp. 13), it seems indisputable that *californicus* is yet another subspecies of *schwarzi.* Finally, by the same reasoning but with somewhat less confidence, in view of its restriction to Arizona and New Mexico, his sp. 14, *webbi,* can probably also be equated with *schwarzi.*

To recapitulate, in the absence of contrary cytogenetic evidence, it appears simple logic to relegate to synonymy seven of the eight Hopkins' taxa listed in Table IV. The eighth, *burkei,* alone stands a reasonable chance of proving to be a legitimate species; if not, and were it to become available for chromosome analysis, its assignment to either the *rotundatus* or the *dubius* complex should, in view of their highly individualistic SIS karyotypes present no problem.

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