Chromosoma (Berl.) 20, 155—172 (1966)

KARYOTYPE STABILITY AND DNA VARIABILITY IN THE ACRIDIDAE

B. JOHN and G.M. HEWITT
Department of Genetics, University of Birmingham

Received July 30, 1966

Abstract. The *Acrididae* are frequently quoted as one of the classic examples of karyotypic stability. Within the family, the *Cryptosacci*, for instance, are characterised by a majority of species having 23 chromosome arms in the male. The members are then related by Robertsonian sequences in which the basic karyotype is believed to consist of 23 acrocentric elements. Thus the 17-chromosome complement of male truxalines is argued to have been derived from the basic type by three successive centric fusions. Such an origin is at variance with the fact that the rod-shaped chromosomes in eight of the nine species utilised in this study turn out in fact to be telocentric. The scheme is also at variance with the finding that significant differences in DNA content exist both between species within the same chromosome group and between member species of the 17 and 23 groups. The concept of karyotypic stability is thus called to question and the relationship of karyotypes within the family must be considerably more complex than has formerly been supposed (Summary see p. 170).

Introduction

There are several techniques available for assessing the relationship of the karyotypes in related species. The most obvious and direct is a straight comparison of the dimensions and morphology of their respective mitotic complements. While this allows for the detection of many major structural alterations, chromosome phenotype is not usually sufficiently sensitive a criterion for gauging any but relatively gross changes. Indeed it may fail to reveal even these as can sometimes be seen from the behaviour of the chromosomes of two forms when they are brought together in a hybrid (JOHN and LEWIS, 1965).

One method which offers the possibility of increased resolution is the measurement of the relative quantity of DNA in equivalent nuclei of two, or more, related species. The potential inherent in this method is apparent in the pioneer studies of HUGHES-SCHRADER (1953, 1958) and WAHRMAN and O'BRIEN (1956). More recently SUNDERLAND and MCLEISH (1961) have devised and applied a much improved procedure to the study of plant nuclei while KEYL (1965) has employed a highly refined technique which has allowed him to compare the DNA content

of particular chromosomes or chromosome segments rather than simply entire nuclei.

A number of points of interest emerge from these several studies:

(i) In related species with markedly different chromosome numbers the same amount of DNA may be present (e.g., *Liturgousa* species, HUGHES-SCHRADER, 1953).

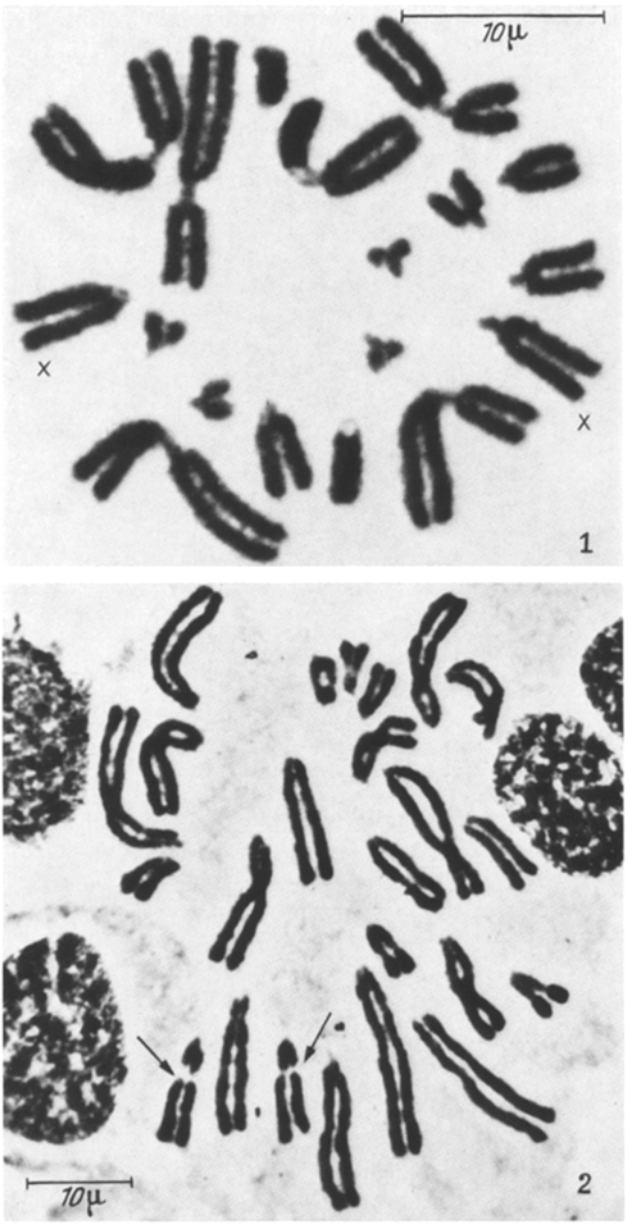
(ii) Appreciably different amounts of DNA may be found in species belonging to the same family and with the same number of chromosomes (e.g., *Bufo* species, ULLERICH, 1966, and see SUNDERLAND and MCLEISH, 1961).

(iii) Small, but significant, differences may be present in species with the same number of chromosome arms but showing Robertsonian relationships. Such differences may depend, in part at least, on variation in chromosome diameter (e.g., *Ameles* species, WAHRMAN and O'BRIEN, 1956).

(iv) 2:1 ratios in chromosome number but with a 1:1 ratio of DNA values and 1:1 ratios in chromosome number with accompanying 2:1 ratios in DNA values have been found between species in the same genus (e.g., *Thyanta* species, SCHRADER and HUGHES-SCHRADER, 1956; *Banasa* species, SCHRADER and HUGHES-SCHRADER, 1958; *Bucholzia* and *Enchytraeus* species, CHRISTENSEN, 1966). Such ratios have been taken as indicative of differential polynemy and differential polyteny respectively.

Finally (v) geometrical differences in the DNA content of the same cytological locus, as defined in terms of a specific polytene band, have been found between different sub-species of the same species (e.g., *Chironomus thummi* subspecies, KEYL, 1965).

Clearly, a DNA comparison is capable of reflecting evolutionary changes that may pass undetected from a simple comparison of chromosome morphology. We have therefore used such a method to compare the karyotypes of nine species of short horned grasshoppers (*Arrididae*), a family of orthopterans in which the number of chromosome arms is remarkably constant. There are two main sections within the family, one (the *Chasmosacci*) with 19 and the other (the *Cryptosacci*) with 23 chromosome arms in the male karyotype. About ninety-five per cent of the species belonging to the *Cryptosacci* have a male complement consisting of 23 rod-shaped elements. What is generally believed to be centric fusion has, however, led to a reduction in chromosome number through the production of metacentric members. In the subfamily *Truxalinae* this has resulted in a male complement with 17 members incorporating six metacentrics but retaining twenty-three chromosome arms (compare Figs. 1 and 2).



Figs. 1 and 2. Diploid chromosome complements in female C-metaphases of *Myrmeleotettix maculatus* (Fig. 1, ca. × 2,700, $2n = 18$) and *Schistocerca gregaria* (Fig. 2, ca. 1,350, $2n = 24$). Fig. 1 is an ovariole wall mitosis while Fig. 2 is a neuroblast mitosis from a 5-day old embryo. Secondary constrictions (arrows) are present in the M_8-members of the locust complement. Note that although the X-chromosome of *M. maculatus* also has a secondary constriction in male cells (JOHN and HEWITT, 1965) these constrictions are not developed in ovariole wall mitoses

There is then an extraordinary apparent stability of basic karyotype within acridids and it is with the nature of this stability that we shall be concerned in this paper.

Materials and Technique

Two main experiments were carried out. Both involved a comparison of the DNA content of 1C-spermatid nuclei using a Barr and Stroud integrating microdensitometer and the technique developed by MCLEISH and SUNDERLAND (1961). Spermatid nuclei which had just begun their morphological transition into spermatozoa where chosen for measurement because they offer a stage which is readily identified and one in which the chromosome material is uniformly distributed in

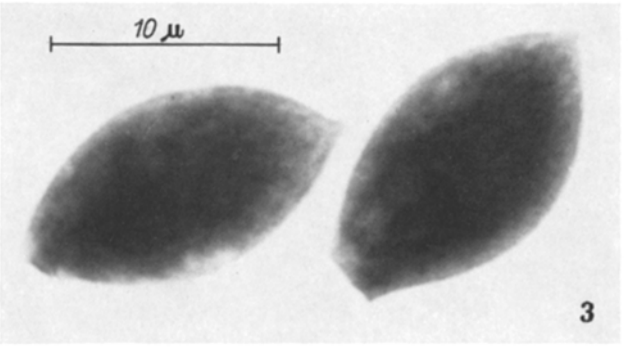


Fig. 3. 1C-spermatid nuclei from *Chorthippus parallelus* of the type used for DNA measurement (ca. × 2,700)

the nucleus (Fig. 3). Two kinds of spermatid are, of course, present in meiotic preparations; some contain and some do not contain the single X-chromosome which characterises the male. No attempt has been made to distinguish between them and each sample of spermatids has been taken at random.

The first comparison was made on four truxaline species — *Chorthippus brunneus, Myrmeleotettix maculatus, Chorthippus parallelus and Omocestus viridulus* — all of which share a complement of 17♂ (XO) and 18 ♀ (XX). The second comparison involves five species of locusts — *Locusta migratoria, Humbe tenuicornis, Schistocerca gregaria* and what have been provisionally named *Schistocerca cancellata* (ex. Argentina) and *Schistocerca paranensis* (ex. Nigaragua) — all of which have a complement with 23 ♂ (XO) and 24 ♀ (XX). In this second experiment, material of *Chorthippus brunneus* was also used to provide a common standard for comparison with the first.

In all cases the testes were removed from males of suitable age by vivisection under insect saline. They were then cleaned of the investing fat body and fixed for two hours in 4% neutral formalin. The fixed material was washed thoroughly in water for twenty-four hours, hydrolysed in N HCl at 60°C and transferred to Feulgen reagent (pH 3.3) for two hours. Finally the Feulgenised material was disrupted mechanically in glycerol on size 0 cover glasses, covered with melinex squares and lightly squashed between filter paper. The squash preparations were scanned under the integrating microdensitometer and the total amount of stain per nucleus was measured at a wavelength of 565 mµ for each of 10 nuclei from each of 5 individuals

in each species. Individuals were measured at random to obviate any possibility of progressive change in readings.

In both experiments the fixations, hydrolyses and staining were carried out at the same time and with the same batch of reagents and the same equipment. Squashing and DNA measurements were likewise synchronised for all individuals in any one experiment.

As McLeish and Sunderland (*loc. cit.*) point out, different species seem to require different periods of hydrolysis to achieve maximum staining. Six day old embryos of *Schistocerca gregaria* and testis follicles from young adults of the same species were therefore used in a preliminary run to determine the optimal time for hydrolysis. The rationale behind this pilot experiment is based on the assumption

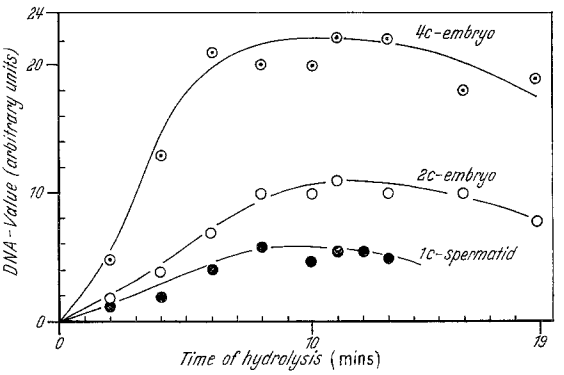


Fig. 4. Relationship between period of hydrolysis and intensity of Feulgen stain as determined by DNA value (arbitrary units) in 1C, 2C and 4C cell types of *Schistocerca gregaria*

that different tissues in the same species are more likely to require a different hydrolysis time than the same tissue from closely related species. The result of the experiment is shown in Fig. 4 where each point on the graph represents the mean absorption of 30 nuclei. There is a good correspondence between the 1C, 2C and 4C values, all of which plateau after about 8 mins hydrolysis. We have, therefore, employed a 10 min hydrolysis period throughout this study.

Observations

1. The Nature of the Rod-Shaped Chromosomes of Acridids

There have been two conflicting views concerning the nature of the rod-shaped chromosomes in the Acrididae. According to McClung (1914) and Makino and Momma (1950) some, at least, of these chromosomes are genuinely telocentric. Darlington (1936), White (1914) and Coleman (1943) on the other hand have all argued that these rods are acrocentric but that faulty fixation and unfavourable staining frequently obscures their real structure. This opinion was based on two lines of evidence:

(i) Small heads are frequently visible at metaphase both in spermatogonial and at second meiotic division.

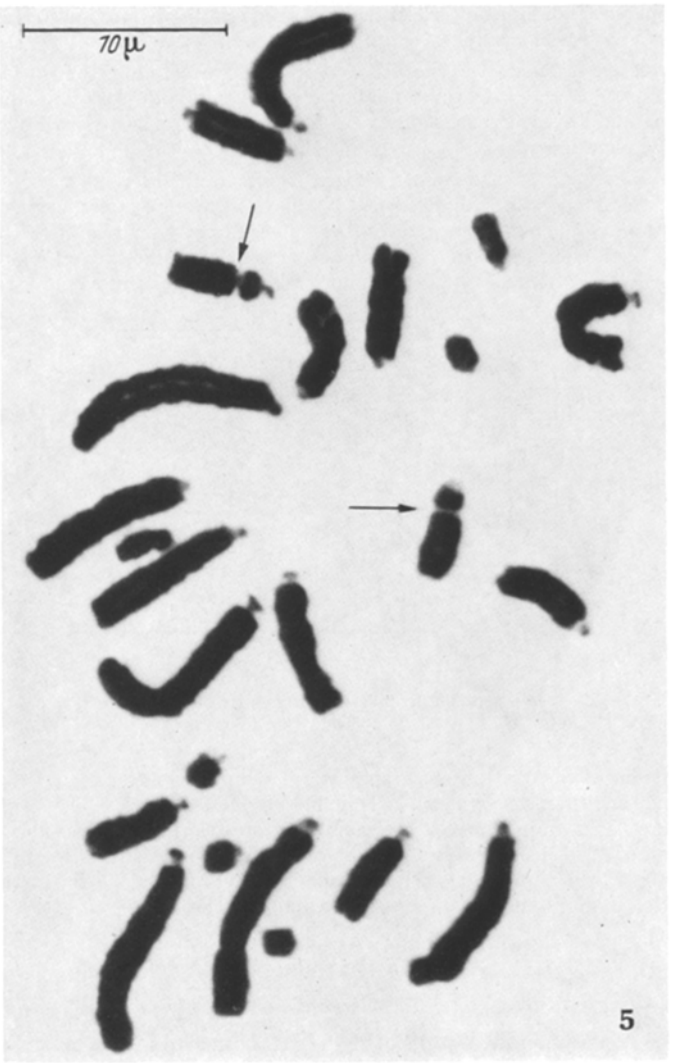


Fig. 5. Metaphase chromosome complement of a neuroblast mitosis from a female embryo of *Locusta migratoria* (orcein, ca. 2,700, $2n = 24$) to demonstrate the acrocentric nature of the chromosomes in this species. Note the secondary constrictions (arrows) in the M_8 members

(ii) So-called ditactic bivalents are sometimes produced in which association is maintained exclusively by what is interpreted to be the short arms.

The latter point is particularly convincing and in cognizance of it we have in our earlier studies assumed that the rod-shaped chromosomes which characterise the species utilised in this study were all acrocentric.

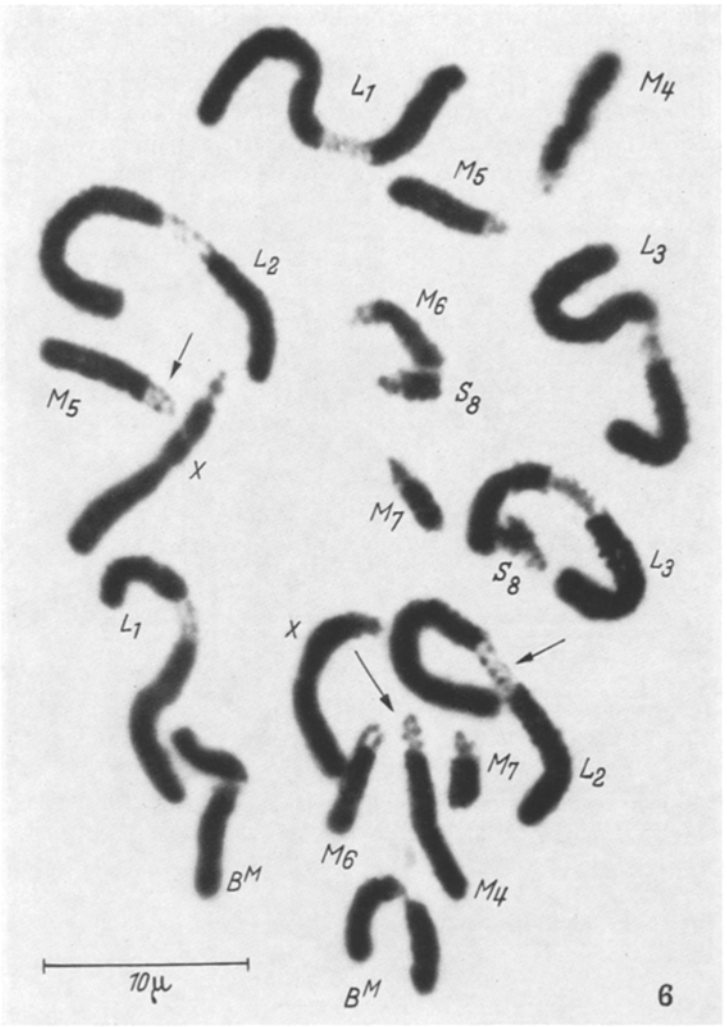
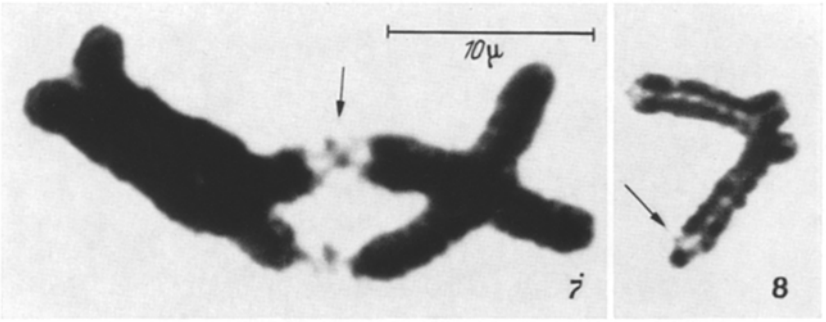


Fig. 6. C-mitosis from an ovariole wall cell of an individual of *Myrmeleotettix maculatus* with two B_m chromosomes ($2n = 16 + XX + 2B^m$). Note that the rod-chromosomes are clearly telocentric (arrows) and have a centric organisation equivalent to one half of that present in the metacentric elements (orcein, ca. × 2,700)

Makino and Momma (*loc. cit.*), on the other hand, have argued that the supposed short arms seen by Darlington, White and Coleman in fact represent fused centromeric chromomeres. As is so often the case in science the truth may well lie between these two viewpoints. Thus in *Locusta migratoria* the chromosomes appear to be genuinely acrocentric. The short arms are particularly well seen in the mitoses of the giant neuroblast cells of developing embryos (Fig. 5). Moreover at early ana-

phase in neuroblast mitoses it is possible to see that both long and short arms flex on each side of the centromeric contriction. In *Humbe tenuicornis* and the three "species" of *Schistocerca*, on the other hand, no short arms can be found even in neuroblasts (see, for example, Fig. 2).

In the 17-chromosome species, colchicine treatment reveals that the centromere of the metacentric members has a duplicate organisation



Figs. 7 and 8. A comparison of centric organisation in metacentric (Fig. 7) and telocentric (Fig. 8) diakinesis bivalents of *Chorthippus brunneus*. Ca. × 2,700

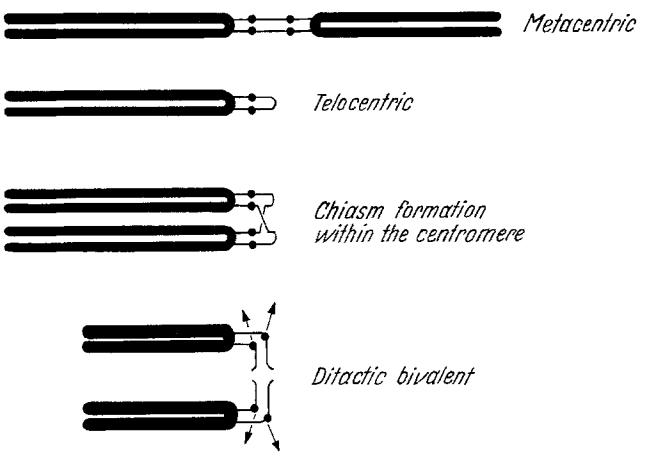


Fig. 9. The relationship between the meta and telocentric elements found in truxaline grasshoppers (compare with Fig. 6) and a possible mode of origin for ditactic bivalents in telocentrics

identical to that described by Lima-de-Faria (1956) in plant chromosomes. The rod-shaped chromosomes, on the other hand, are terminated by a structure which is equivalent to one-half of the centric structure found in the metacentrics (Fig. 6). Clearly they are genuinely telocentric. The same demonstration can be made in meiotic bivalents (Figs. 7 and 8).

The one problem that remains is that of interpreting the nature of the ditactic bivalents which unquestionably occur on rare occasions in the telocentrics of both *Chorthippus* and *Schistocerca*. Since the centromere

has a compound organisation and unquestionably duplicates itself in company with the rest of the chromosome there is no reason why a chiasma may not occur within its limits. If we assume that the association of homologues in a ditactic bivalent is chiasmate then their production in telocentrics can be accommodated in terms of chiasma formation within the limits of the centromere (Fig. 9). The only sensible alternative would be to assume that the association is non-chiasmate.

2. The Relationship between Karyotype and DNA Content

a) The 17-chromosome types. Superficially all four karyotypes appear very similar in morphology (Fig. 10) and it is tempting to assume that this implies homology in the sense that we are dealing with the same chromosomes throughout all four species. Certainly the relative lengths of the members of all four complements are extremely suggestive of homology and in all four it is the third largest of the medium telocentrics (M_6) that is heteropycnotic at first meiotic prophase. There are, however, differences in the organisation of the X-chromosome. In two of the species the X has a secondary constriction which is responsible for organising a small nucleolus at male meiosis. In *Chorthippus parallelus* this constriction is proximal to the centromere while in *Myrmeleotettix maculatus* it is near median. There are also differences in heterochromatic content for in *M. maculatus* the centric regions of all the autosomes other than the M_6-pair are enclosed in small but well marked heteropycnotic blocks at zygotene-pachytene. In the three other species there is no heterochromatic material in the centric zones.

The similarity in karyotype is paralleled in three of the four species by a marked similarity in DNA content (Fig. 11). In the case of *Ch. brunneus*, however, there is a significant difference in DNA value when compared with the other species (Table 1).

Table 1. *Analysis of variance of the DNA values for the 17-chromosome species shown in Fig. 11*

Item	dF	SS	MS	VR	P
		a) All 4 species			
1. Between species	3	410.66	136.88	42.372	$\ll 0.001$***
2. Between individuals	16	51.69	3.231	3.895	< 0.001***
3. Within individuals	180	149.30	0.829	—	—
4. Totals	199	611.65			
		b) Omitting the *Ch. brunneus* values			
1. Between species	2	18.62	9.314	2.666	0.1—0.2 Not sig.
2. Between individuals	12	41.92	3.493	3.715	< 0.001***
3. Within individuals	135	126.95	0.940	—	—
4. Totals	149	187.49			

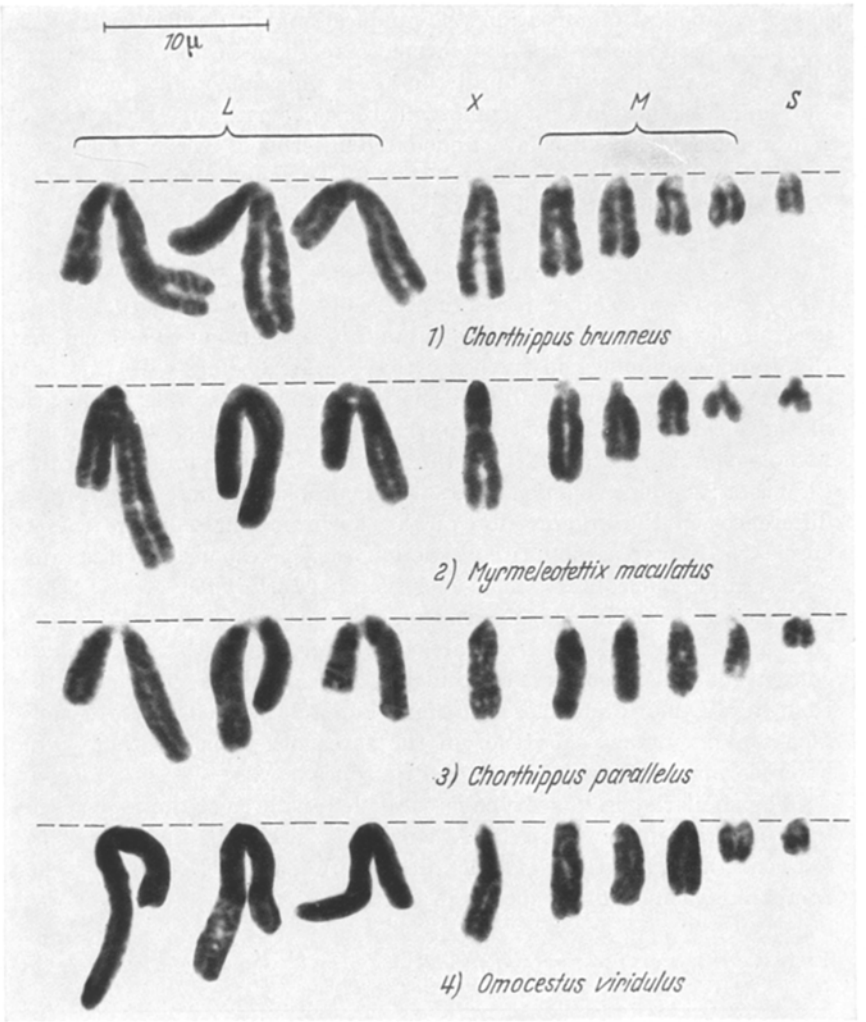


Fig. 10. A comparison of haploid karyotypes from spermatogonial mitoses of the grasshopper species utilised in this study. Ca. × 2,100

b) The 23-chromosome types. The extent of karyotypic similarity is again pronounced. In all five species there are four long, five medium and three short pairs with the X ranking amongst the longest members of the complement (Fig. 12). Nevertheless there are also clear differences in cytomorphology. Thus in the three "species" of *Schistocerca* the six S-chromosomes are relatively much shorter in *cancellata* and *paranensis* than they are in *gregaria*. Again in *Locusta migratoria*, as we have already seen, the chromosomes are all acrocentric.

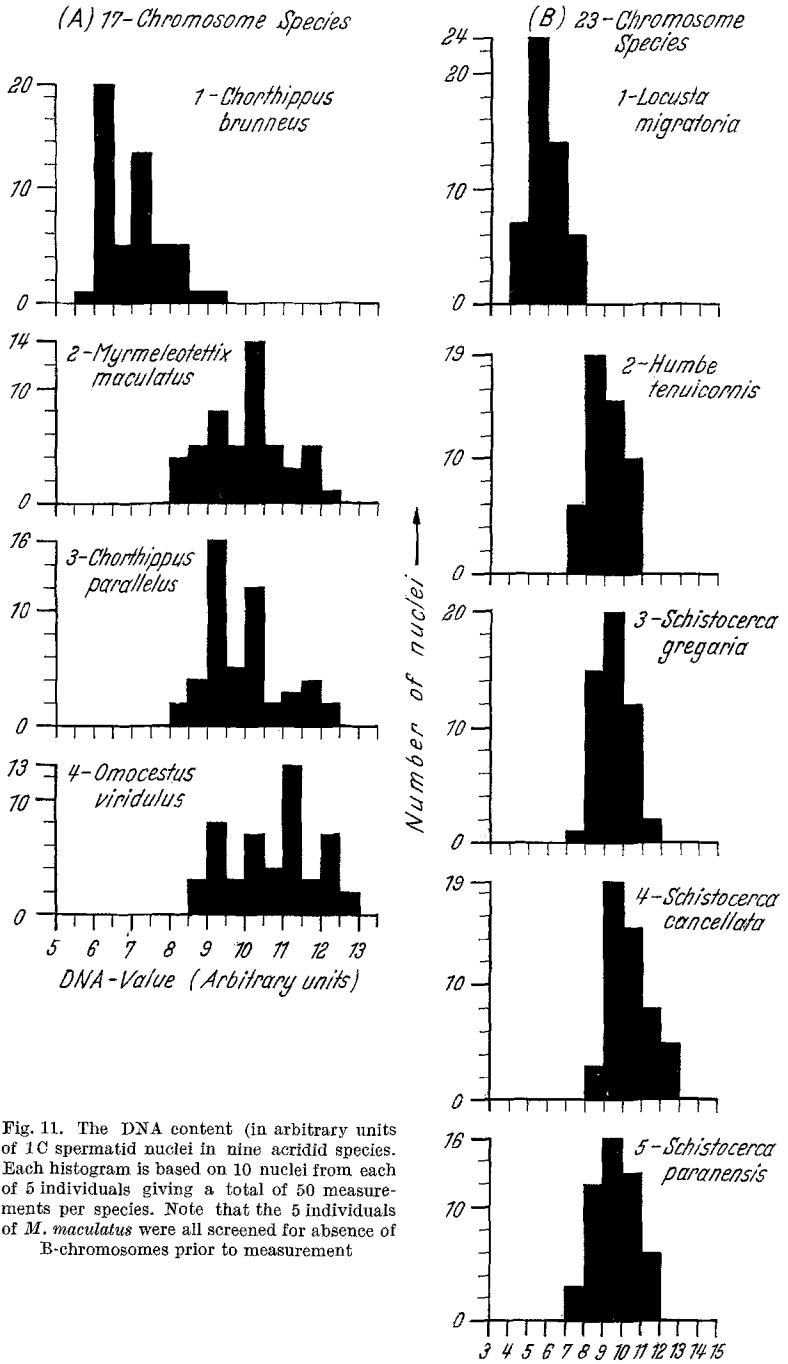


Fig. 11. The DNA content (in arbitrary units of 1C spermatid nuclei in nine acridid species. Each histogram is based on 10 nuclei from each of 5 individuals giving a total of 50 measurements per species. Note that the 5 individuals of *M. maculatus* were all screened for absence of B-chromosomes prior to measurement

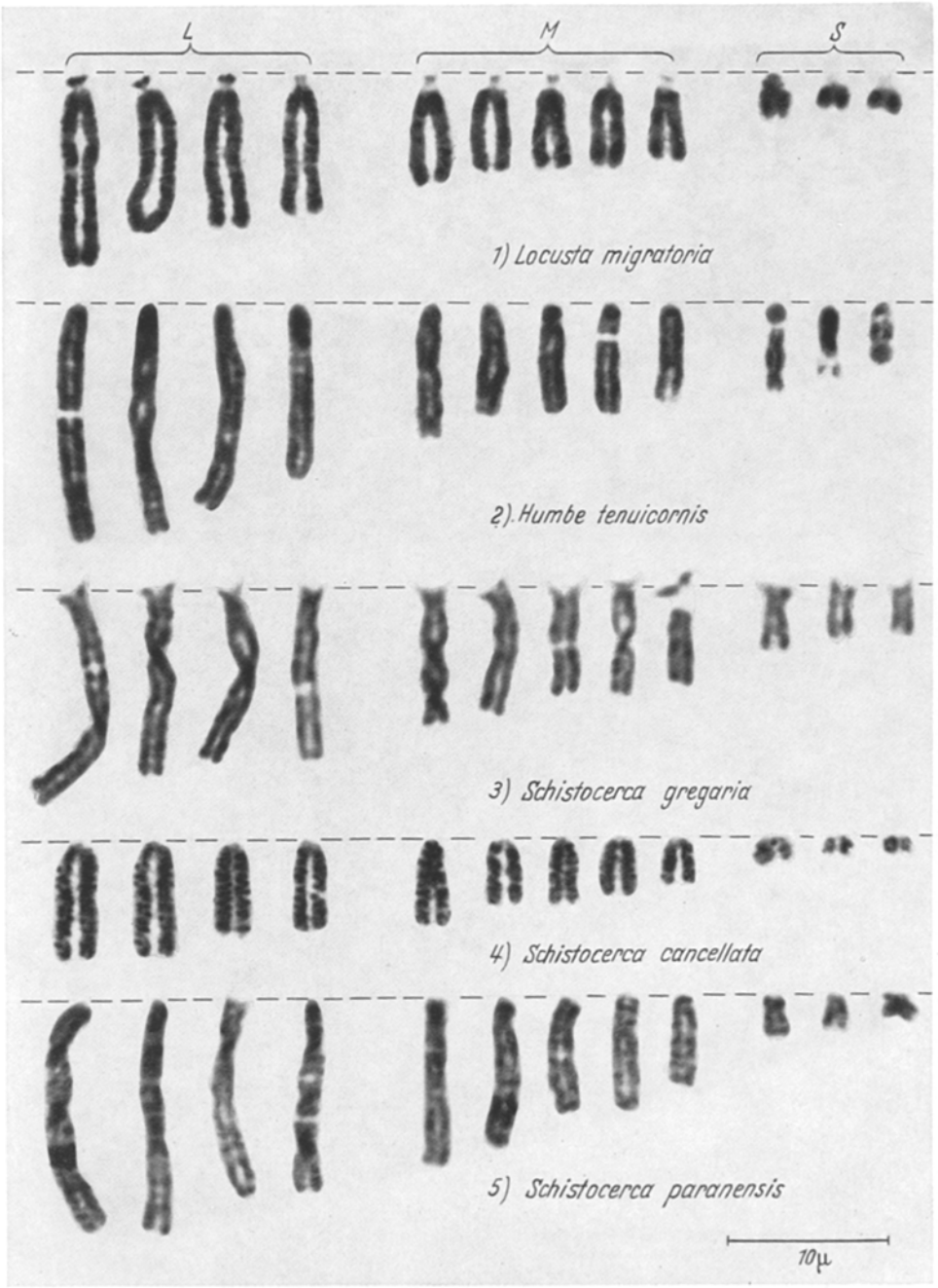


Fig. 12. A comparison of haploid karyotypes in the five locust species utilised in this study. With the exception of *S. cancellata* which is from an ordinary somatic cell the chromosomes are taken from neuroblast metaphases. Note that in all five species the X-chromosome belongs to the L-class. Ca. × 2,100

When we turn to the DNA data there is heterogeneity here too for there are significant differences between the five species (Tables 2). A large part of this difference unquestionably stems from *Locusta migratoria* which clearly has a distinctive and much reduced DNA content

Table 2. *Analysis of variance of the DNA values for the 23-chromosome species shown in Fig. 11*

Item	dF	SS	MS	VR	P
		a) All 5 species			
1. Between species	4	608.38	152.095	61.727	$\ll 0.001$***
2. Between individuals	20	49.28	2.464	2.923	< 0.001***
3. Within individuals	225	189.70	0.843	—	—
4. Totals	249	847.36			
		b) Omitting the *L. migratoria* values			
1. Between species	3	43.08	14.360	5.594	0.01—0.001**
2. Between individuals	16	41.08	2.567	—	—
3. Within individuals	180	159.92	—	—	—
4. Totals	199	244.08			
		c) Omitting both the *L. migratoria* and the *H. tenuicornis* values			
1. Between species	2	21.98	10.989	3.389	~ 0.05
2. Between individuals	12	38.92	3.243	—	—
3. Within individuals	135	118.09	—	—	—
4. Totals	149	178.99			

(Fig. 11). If the analysis of this interspecific variation is repeated with the omission of the *migratoria* data the values of the remaining four species are still significantly different. This difference, however, disappears if the *Humbe tenuicornis* values are also omitted.

Discussion

If the DNA values of the 17 and 23-chromosome species are converted to the same relative scale using the *Ch. brunneus* standard employed in the second experiment it is clear that, on average, the 23-chromosome forms have less DNA than the 17 (Table 3). It is true that the value for *Ch. brunneus* is lower than that shown by the locust species but the three other truxalines are significantly higher. This has two interesting implications:

(i) The apparent stability of the karyotype in acridids is obviously misleading since quite marked changes in chromosome organisation have evidently taken place during evolution. It is impossible as yet to decide whether the differences which we have demonstrated here

Table 3. *Individual mean 1C-DNA values for nine species of acridids. The data for the 17-chromosome species have been converted to the same relative scale as that of the 23-chromosome types. Each value given represents the mean of 10 spermatid nuclei*

Individual No.	23-chromosome types					17-chromosome types			
	Species					Species			
	L. m.	H. t.	S. g.	S. c.	S. p.	Ch. b.	M. m.	Ch. p.	O. v.
1	5.7	9.3	9.4	10.4	9.2	8.8	13.5	13.3	14.1
2	6.2	9.5	9.6	10.6	9.8	9.8	14.2	13.8	14.2
3	6.6	9.5	10.0	10.7	10.1	9.9	14.2	14.3	15.9
4	6.6	9.6	10.1	11.1	10.6	10.6	14.3	15.1	16.2
5	6.8	10.0	10.8	11.5	11.0	10.6	15.0	15.4	16.5
$\bar{x}$	6.4	9.6	10.0	11.1	10.1	10.0	14.2	14.4	15.4

arise directly from simple gain or loss or whether the duplication/deficiency differences have themselves arisen secondarily as a consequence of primary structural change. As we have recently shown in the case of the grasshopper *Eyprepocnemis plorans* (JOHN and LEWIS, 1965) apparent karyotypic stability is fully compatible with a marked structural reorganisation of the karyotype.

KEYL (1965) has demonstrated that, in terms of both primary spermatocytes and salivary gland nuclei, *Chironomus thummi thummi* has about twenty seven per cent more DNA than *Ch. thummi piger*. This difference depends upon the fact that certain of the bands in the polytene chromosomes of *thummi thummi* contain 2,4,8 or 16 times the amount of DNA present in homologous bands of *thummi piger*. Here we have a distinctive and localised method for increasing DNA content which introduces a new dimension in our approach to chromosome organisation. ULLERICH (1966) has likewise concluded that the differences in DNA content which he found between three species of the genus *Bufo* may also be due to a localised duplication of the type suggested by KEYL.

(ii) The precise phylogenetic relationship of the 17 and 23-chromosome forms is still largely a matter for conjecture. It is generally believed that the 17 types are secondarily derived from the 23 by successive centric fusions but it is no more easy or reliable to read the direction of evolution from comparative karyology than it has been from comparative anatomy. Indeed the demonstration that both acro- and telocentric types occur within the 23-chromosome group must mean that our appreciation of the problems of karyotypic change in acridids has, to date, been too naive.

Ideas concerning the relationship between rod and v-shaped chromosomes have been — indeed in a measure still are — dominated by the belief that strictly telocentric chromosomes either do not exist (NAWASCHIN, 1916; LEWITSKY, 1931; MULLER, 1940) or if they exist are unstable

(RHOADES, 1940) or if stable that they are rare (WHITE, 1957, 1959). There is therefore a long and unfortunate history to the whole issue and one which still prejudices contemporary thinking on the subject.

The field of disbelief in telocentric chromosomes and their stability has certainly progressively receded and there is now unambiguous evidence in plants for the occurrence of breakage within the centromere (misdivision) leading to the production of stable telocentrics (DARLINGTON and LACOUR, 1950; MARKS, 1957). Of course, so long as these examples were confined to plants it could be argued that they had no necessary relevance for animals. Even when in 1956 LIMA-DE-FARIA did provide convincing evidence for the existence of terminal centromeres in *Mecostethus* (= *Stethophyma*) *grossus*, WHITE, one year later, could still write "LIMA-DE-FARIA has recently claimed that in the acrocentric chromosomes of the grasshopper *Mecostethus* what we interpret as the 'short arm' is in fact the centromere, but we cannot consider our interpretation as disproved by this photographs". That small heavily stained bodies can appear regularly at the centromere ends in telocentrics is clear from the observations of MARKS (1957) and these are no different from the bodies which WHITE has repeatedly used in support of the existence of short arms (see, for example, Fig. 15 in WHITE, 1965).

The tenacity with which WHITE has clung to his view stems in no small part from his belief in the applicability of the telomere concept to the problem of relating V and rod-shaped chromosomes. This problem was first clearly stated by MULLER (1940), the originator of the telomere concept, and we quote "The difficulty of the formation of two rods from one V arises from the fact that the one V has one centromere and two telomeres whereas the two rods considered together have two centromeres and four telomeres — no 'rods' being quite terminal in attachment". Yet, in fact, where stable telocentrics arise by misdivision each of the division products of the initially single centromere must be capable of functioning both as telomere and centromere.

Faced with the necessity of deriving two rods from one metacentric in the eumastacids *Moraba scurra* and *M. viatica* WHITE (1957, 1964) has, therefore, resorted to inventing a new process — dissociation — which is a form of translocation in which one new centromere and two new telomeres are supplied by a hypothetical donor chromosome which, as yet, has been given no material form. He rejects the possibility of simple fission by misdivision because it does not fit with his conception of centromere structure. An examination of the C-mitotic figures of *Moraba viatica* — one of the species in which dissociation has been claimed — gives every reason to believe that the morabines too may possess genuinely telocentric elements (see, for example, Fig. 8 in WHITE, CARSON and CHENEY, 1964).

It is clearly time to re-examine the whole issue of fusion and fission and in doing so to replace preconceptions about the nature and potentialities of centromere organisation with an objective evaluation of these properties. Whether two telocentrics can undergo simple fusion to produce a metacentric is not known. There is certainly evidence to show that stable telocentrics may show a non-random tendency towards temporary centric associations at metaphase (MARKS, 1957). There is even a suggestion that associations of this type, though reversible, can persist through several successive mitotic cycles (WOLF, 1960). Indeed if MATTHEY (1963) is correct in his interpretation of the polymorphism he claims in *Acomys* hybrids, telocentric fusions may be considerably more permanent than has formerly been believed.

MORRISON (1954) has described a spontaneous interchange heterozygote in *Triticum aestivum* which he believed was produced by the reunion of non-homologous telocentrics shortly after their origin. Such a reunion of the non-homologous products of broken centromeres immediately following misdivision constitutes a very distinctive type of fusion but breakage and reunion within the centromeres of two stable telocentrics would, of course, lead to the same end product. Thus KAYANO and NAKAMURA (1960) have found a fusion heterozygote in *Acrida lata*, a grasshopper which they describe as normally possessing 23 telocentric chromosomes.

Summary

1. The mean DNA content of 1C-spermatid nuclei of nine species of acridids has been compared using an integrating microdensitometer. Four of the species used share a diploid male complement of 17 consisting of 6 metacentric and 11 telocentric members. In the other five the equivalent complement includes 23 rod-shaped elements which are telocentric in four but acrocentric in one. There are thus 23 principal chromosome arms in all the species involved in the comparison.

2. The demonstration of stable telocentrics in acridid species runs contrary to the hypothesis that centric fusions between acrocentric elements have led to the production of numerically reduced karyotypes in the Cryptosacci.

3. Significant differences in DNA content exist between some of the species within both the 17 and the 23 chromosome groups as well as between the groups. In three of the species belonging to the 17 chromosome group the DNA content is higher than that of the 23 chromosome group This, coupled with the existence of both acro and telocentric elements, must mean that the apparent karyotypic stability generally claimed to exist within acridids is both misleading and spurious.

Acknowledgements. We are grateful to the Anti-Locust Research Centre for supplying the locusts used in this study.

References

CHRISTENSEN, B.: Cytophotometric studies on the DNA content in diploid and polyploid *Enchytraeidae* (*Oligochaeta*). Chromosoma (Berl.)**18**, 305—315 (1966).

COLEMAN, L.C.: Chromosome structure in the *Acrididae* with special reference to the X-chromosome. Genetics **28**, 2—8 (1943).

DARLINGTON, C.D.: Crossing-over and its mechanical relationships in *Chorthippus* and *Stauroderus*. J. Genet. **33**, 465—500 (1963).

—, and L.F. LA COUR: Hybridity selection in *Campanula*. Heredity **4**, 217—248 (1950).

HUGHES-SCHRADER, S.: The nuclear content of deoxyribonucleic acid and interspecific relationships in the mantid genus *Liturgousa*. Chromosoma (Berl.) **5**, 544—554 (1953); — The DNA content of the nucleus as a tool in the cytotaxonomic study of insects. Proc. Xth. Int. Congr. Entomol. 1956, **2**, 935—944 (1958).

JOHN, B., and G.M. HEWITT: The B-chromosome system of *Myrmeleotettix maculatus* 1. The mechanics. Chromosoma (Berl.) **16**, 548—578 (1965).

—, and K.R. LEWIS: Genetic speciation in the grasshopper *Eyprepocnemis plorans*. Chromosoma (Berl.) **16**, 308—344 (1965).

KAYANO, H., and K. NAKAMURA: Chiasma studies in structural hybrids V. Heterozygotes for a centric fusion and for a translocation in *Acrida lata*. Cytologia (Tokyo) **25**, 476—480 (1960).

KEYL, H.-G.: A demonstrable local and geometric increase in the chromosomal DNA of *Chironomus*. Experienta (Basel) **21**, 191 (1965).

LEWITSKY, G.A.: The morphology of the chromosomes. Bull. appl. Bot. **27**, 19—173 1931).

LIMA-DE-FARIA, A.: The role of the kintetochore in chromosome organisation. Hereditas (Lund) **42**, 85—160 (1956).

MAKINO, S., and E. MOMMA: Observations on the structure of grasshopper chromosomes subjected to a new acetocarmine treatment. J. Morph. **86**, 229—252 (1950).

MARKS, G.E.: The cytology of *Oxalis dispar* (BROWN). Chromosoma (Berl.) 8, 650—670 (1957); — Telocentric chromosomes. Amer. Naturalist **91**, 223—232 (1957).

MATTHEY, R.: Polymorphisme chromosomique intraspécifique et intraindividuel chez *Acomys minous* BATE (*Mammalia-Rodentia-Muridae*). Étude cytologiques des hybrides *Acomys minous* ♂ × *Acomys cahirinus* ♀. Le méchanisme des fusions centriques. Chromosoma (Berl.) **14**, 468—497 (1963).

MCCLUNG, C.E.: A comparative study of the chromosomes in orthopteran spermatogenesis. J. Morph. **25**, 651—749 (1914).

MCLEISH, J., and N. SUNDERLAND: Measurements of deoxyribosenucleic acid (DNA) in higher plants by Feulgen photometry and chemical methods. Exp. Cell Res. **24**, 527—540 (1961).

MORRISON, J.W.: Chromosome interchange by misdivision in *Triticum*. Canad. J. Bot. **32**, 281—284 (1954).

MULLER, H.J.: Bearings of the *'Drosophila'* work on systematics, p. 185—268 in: The new systematics. Oxford: Clarendon Press 1940.

NAWASCHIN, S.: Sur quelques charactères de l'organisation interne des chromosomes, p. 185—214. Réc. d'Art Sci. dédié à C. TIMIRIASEFF. Moscow 1916.

RHOADES, M.M.: Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics **25**, 483—520 (1940).

SCHRADER, F., and S. HUGHES-SCHRADER: Polyploidy and fragmentation in the chromosomal evolution of various species of *Thyanta* (*Hemiptera*). Chromosoma (Berl.) **7**, 469—496 (1956); — Chromatid autonomy in *Banasa* (*Hemiptera: Pentatomidae*). Chromosoma (Berl.) **9**, 193—215 (1958).

SUNDERLAND, N., and J. MCLEISH: Nucleic acid content and concentration in root cells of higher plants. Exp. Cell Res. **24**, 541—554 (1961).

ULLERICH, F.-H.: Karyotyp und DNS-Gehalt von *Bufo bufo*, *B. viridis*, *B. bufo* × *B. viridis* und *B. calamita* (*Amphibia, Anura*). Chromosoma (Berl.) **18**, 316—342 (1966).

WAHRMAN, J., and R. O'BRIEN: Nuclear content of DNA in chromosomal polymorphism in the genus *Ameles* (*Orthoptera: Mantoidea*). J. Morph. **99**, 259—270 (1956).

WHITE, M.J.D.: Cytogenetics of orthopterid insects. Advanc. Genet. **4**, 267—330 (1951); — Some general problems of chromosomal evolution and speciation in animals. Surv. Biol. Progr. **3**, 109—147 (1957); — Cytogenetics of the grasshopper *Moraba scurra* l. Meiosis of interracial and interpopulation hybrids. Aust. J. Zool. **5**, 285—304 (1957); — Chiasmatic and achiasmatic meiosis in african eumastacid grasshoppers. Chromosoma (Berl.) **16**, 271—307 (1965).

— H.L. CARSON, and J. CHENEY: Chromosomal races in the australian grasshopper *Moraba viatica* in a zone of geographic overlap. Evolution (Lawrence, Kansas) **18**, 417—429 (1964).

WOLF, B.E.: Zur Karyologie der Eireifung und Furchung bei *Cloeon dipterum* L. (BENGTSSON) (*Ephemerida, Baetididae*). Biol. Zbl. **79**, 153—198 (1960).

Dr. B. JOHN
Department of Genetics
University of Birmingham
Birmingham 15, England

Dr. G. M. HEWITT
School of Biological Sciences
University of East Anglia
Norwich, England