Cytotaxonomy of ticks

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Summary

The material studied consisted of laboratory-bred specimens of 2 species of Prostriata and 8 species of Metastriata. *Ixodes ricinus* and *I. hexagonus* (Prostriata) have XX:XY sex-determining mechanisms with diploid complements of 28 and 26 acrocentric chromosomes respectively. All the species of Metastriata studied have XX:XO sex-determining mechanisms with diploid complements of 21 and 22 chromosomes in males and females respectively. *Haemaphysalis leporis palustris* is the only species with metacentric chromosomes. *Hyalomma* spp., *Dermacentor andersoni* and *Haemaphysalis leporis palustris* each have X-chromosomes larger than any of the autosomes, whereas *Rhipicephalus sanguineus* and *R. secundus* do not have conspicuously large X-chromosomes.

In all the species studied early male meiotic prophase is characterized by a period of growth similar to that of oocytes at first meiotic division. This nuclear growth of the primary spermatocyte is thought to have evolved together with the production of a large-sized spermatozoon.

The structure of the testes and their accessory glands in *Ixodes* differs significantly from that of the corresponding organs in Metastriata. *Ixodes* also differs from the other genera studied as regards the splitting of the integument during ecdysis. It is suggested that *Ixodes* should be given the status of a 'family'.

Introduction

The classification of ticks has been based on chitinous structures. The present study of the nuclear cytology of 10 species involving 5 genera was undertaken in the hope that it might assist in problems of identification. Papers published hitherto on the nuclear cytology of ticks contain inadequate, and in most cases inaccurate, information.

Nordenskiöld, (1909, 1920), although correctly stating the diploid chromosome number of *Ixodes ricinus* (Linneus) to be 28, dealt mainly with the developing spermatid. Bonnet (1907), as well as Tuzet and Millot (1937), undoubtedly underestimated the chromosome numbers of *Hyalomma aegyptium* (Neumann). In addition Tuzet and Millot must have made an error of identification for the ticks they studied were taken off cattle, and *H. aegyptium* is not found on mammalia. Dutt (1954), and Kahn and Feldman-Muhsam (1958) erroneously ascribed a median centromere to the X-chromosomes of *Hyalomma aegyptium* (Neumann). In addition Tuzet and Millot must have made an error of identification for the ticks they studied were taken off cattle, and *H. aegyptium* is not found on mammalia. Dutt (1954), and Kahn and Feldman-Muhsam (1958) erroneously ascribed a median centromere to the X-chromosomes of *Hyalomma* spp. The accounts given by Stella (1938) and Dutt (1954) on the cytology of *Rhipicephalus sanguineus* (Latreille) are in disagreement with my own data. Oppermann (1935), working on *Argas columbarum* (Shaw) (Argasidae are not included in this study), surveys the
entire course of spermatogenesis from spermatogonial divisions to the end of spermiohistogenesis. As a result of contradictory statements and inadequate drawings, his observations on the organization of bivalents are unconvincing, and the compound XY chromosome claimed for the male of *Argas columbarum* must be viewed with reserve.

**Material**

In the course of this study the following species were examined:

_Prostriata_

*Ixodes ricinus* (Linnaeus)  
*Ixodes hexagonus* (Leach)

_Metastriata_

*Haemaphysalis leporis palustris* (Packard)  
*Dermacentor andersonii* (Stiles)  
*Hyalomma marginatum* (Koch)  
*Hyalomma rufipes* (Koch)  
*Hyalomma excavatum* (Koch)  
*Hyalomma dromedarii* (Koch)  
*Rhipicephalus sanguineus* (Latreille)  
*Rhipicephalus secundus* (Feldman-Muhsam)

*Ixodes ricinus* was supplied and identified by Dr. Allan Campbell from the Moredun Institute (Gilmerton, Edinburgh). The specimens were originally collected in Scotland. *I. hexagonus* was collected off a hedgehog, caught in St. Andrews by Miss Elizabeth T. Callan, and identified on the basis of material received through Dr. A. Campbell.

*Haemaphysalis leporis palustris* and *Dermacentor andersonii* were sent by Dr. Glen M. Kohls, Director of the National Microbiological Institute (Rocky Mountain Laboratory, Hamilton, Montana).

*Hyalomma rufipes* was identified and forwarded by Dr. T. Theiler, Director of Veterinary Services, Department of Agriculture (Onderstepoort, Union of South Africa). *H. dromedarii* was obtained from and identified by Dr. H. Hoogstraal, Head of the Department of Medical Zoology, U.S.A. Medical Research Unit No. 3 (Cairo, Egypt). *H. marginatum* was taken from a stock reared in the Department of Parasitology, Hebrew University of Jerusalem. Dr. B. Feldman-Muhsam identified the F₁ generation. *H. excavatum* was collected off a camel and forwarded by Mr. Z. Rosenwachs from the dairy of Beth-Govrin, Israel. The material was identified by myself on the basis of the chitinous structure of the female genital aperture.

*Rhipicephalus sanguineus* was supplied and identified by Dr. H. Hoogstraal. *R. secundus* females were collected off cattle and forwarded by Mr. Z. Rosenwachs. The F₁ generation reared in the laboratory were identified by myself.

With a few exceptions, this study is based on the offspring of a single female for each species. Apart from *H. leporis palustris*, which was reared on rabbits,
male rats were used as hosts. The ticks were secured on hosts by the pillbox/bandage method.

Engorged larvae and nymphs were required for the study of Ixodes, engorged nymphs and feeding imagos for all the other genera. Most of the ticks examined had, therefore, to be bred through at least one life-cycle. In Ixodes the development of sperm, and fertilization, occur prior to the imago's last blood meal. In all the Metastratiata which I have studied meiosis in males, and fertilization, do not take place unless the final blood meal is provided. This difference between Prostriata and Metastratiata was first described by Balashov (1956). However oviposition in both Prostriata and Metastratiata is conditioned by the feeding of females.

Cytological methods

Preparation of organs

Gonads, salivary glands, and central nervous systems were dissected out in insect Ringer's solution made up according to Ephrussi and Beadle's (1936) formula. Immediately after dissection such organs were squashed in a freshly filtered solution of 1% synthetic orcein (G. T. Gurr) in 45% acetic acid. On occasion, to prevent too great spreading of chromosomes, the organs were hardened by prefixation in 3 parts absolute alcohol: 1 part glacial acetic acid.

Preparation of eggs

For the purpose of investigating female meiosis in Hyalomma excavatum, ovaries at all stages of development in the adult feeding female and eggs from 0 to 8 h after oviposition, were fixed in bulk in acetic alcohol for \( \frac{1}{2} \) to 1 h. The Feulgen technique was subsequently employed.

The chromosomes of ticks

Ixodes ricinus (fig. 1, A to H)

Mitosis. Mitosis was studied in preparations of somatic tissues from replete larvae. The central nervous system and salivary glands are of convenient size for this purpose and can be easily manipulated under a binocular dissecting microscope.

Fig. 1, A, illustrates a mitotic metaphase in a salivary gland cell of a male larva. Fig. 1, B, illustrates a mitotic metaphase in a central nervous tissue cell of a female larva. Nordensköld's count of 28 chromosomes for the male diploid complement was confirmed, and was found to be valid also for the female. Sex is determined by an XX:XY mechanism, the male being heterogametic. Y is the smallest chromosome of the complement and is indicated in fig. 1, A. X is one of the medium-sized chromosomes and, therefore, not detectable at mitosis.

One pair of chromosomes in the mitotic complements of both males and females have subterminal constrictions. These constrictions are not centromeres, all the centromeres being terminal (evidence from 2nd meiotic
metaphase). Throughout mitosis none of the chromosomes are heterochromatic.

**Male meiosis.** Amongst 10 engorged male nymphs kept at 27°C, R.H. 100%, the shortest and longest pre-moultng periods recorded were 28 and 53 days respectively. It is, therefore, not surprising to find that, when taken within a few days after moultng, specimens differ as regards phase of spermatogenesis. Moreover, it was found that the two testes of one and the same individual may mature at different rates. One testis may contain young spermatids, whilst the other may contain primary spermatocytes only. The spermatogonia are arranged in cysts, each enclosed in a thin epithelial sheath. Even from squash preparations it is possible to observe that within each cyst all the divisions occur in synchrony.

Four males were dissected immediately after moultng. In their testes no stages later than pre-diplotene nuclei could be found, and in pre-diplotene nuclei the chromosomes stain poorly. Fig. 1, c, illustrates the 14 bivalents of an early diplotene nucleus, obtained from a tick 36 h after moultng. During the first meiotic division 13 pairs of autosomes and the XY pair of sex chromosomes always form 14 bivalents; no univalents were observed. The XY pair forms only 1 chiasma. In fig. 1, c, X and Y are associated by a chiasma that is not quite terminal. In most diplotenes, however, X and Y are terminally associated. Amongst the autosomes 1- and 2-chiasma bivalents are found. The mean chiasma frequency per cell was calculated at $14.76 \pm 0.16$. Fig. 1, e, illustrates full first meiotic metaphase from a tick 24 h after moultng; most associations within bivalents appear to be terminal.

First meiotic anaphase is followed by an interphase when the nucleus contains a network of entangled chromatids. At second meiotic prophase (fig. 1, f, X-type) the chromatids are long and show a spiral structure. They are attached to one another at their centromeres, but otherwise widely separated. Fig. 1, g to h illustrate full second meiotic metaphases of the X-containing and Y-containing types (the first meiotic division being always reducational for the sex-chromosomes). The chromosomes are more contracted and about the size of mitotic chromosomes. At full metaphase secondary constrictions are often visible; these constrictions do not appear to correspond with those found in mitosis.

**Ixodes hexagonus** (fig. 1, j; fig. 2, a to e)

**Mitosis.** Somatic divisions were studied in larval nervous tissue. The diploid number is 26 for male and female (fig. 1, j). Centromeres are all terminal. The sex chromosomes of *I. hexagonus*, unlike those of *I. ricinus*, are indistinguishable at this stage. Also in contrast to *I. ricinus* there are only 2 pairs of large chromosomes in the complement of *I. hexagonus*.

**Male meiosis.** Shortly after leaving the host, the nymphs' testes are well developed, with some of the cysts already containing maturing spermatids. A specific feature of meiotic timing in *I. ricinus* appears to be the absence of diplotene prior to ecdysis. As in *I. ricinus*, the 2 testes of a single individual
Fig. 2. All of *Ixodes hexagonus*. A and B, male diplotene. C, male diakinesis. D, male 1st metaphase. E, male 2nd prophase.
and the cysts within 1 testis differ greatly as to the stage of maturation which they have reached. It was, therefore, possible to follow meiosis from early diplotene (fig. 2, A, B) to late second meiotic prophase (fig. 2, E) in nymphs before and after ecdysis.

The most interesting feature of meiosis in the male of *I. hexagonus* is presented by the behaviour of one of its large bivalents. At diplotene a delay in condensation of segments of this bivalent causes a sixfold difference in length (fig. 2, A) between it and the other large bivalent. In these relatively uncondensed segments the chromosome strands are often below the limit of resolution (they are shown in the drawings as broken lines). The interpretation of the structure of this bivalent is supported (a) by the symmetrical distribution of condensed segments in associated homologues; (b) by their occurrence in all diplotenes; (c) by the persistence of an unevenly condensed bivalent during diakinesis (fig. 2, c). By full first meiotic metaphase, this constriction has disappeared (fig. 2, D). Some of the diplotene configurations of this particular bivalent (fig. 2, B) could only be explained on the assumption that homologues are associated by 2 chiasmata, and this assumption is confirmed by the occurrence of 2 large ring bivalents during first meiotic metaphase (fig. 2, D). This chromosome pair may represent the sex bivalent of *I. hexagonus*. I suggest that the original X- and Y-chromosomes may have become fused to a pair of autosomes, this suggestion being supported by comparison of chromosome numbers and sizes between *I. hexagonus* and *I. ricinus*. Diplotene nuclei of *I. hexagonus* may, like those of *I. ricinus*, contain up to 3 2-chiasma bivalents.

*Haemaphysalis leporis palustris* (fig. 3, A to H)

*Mitosis.* The mitotic complements of *H. l. palustris* were studied in oogonial and spermatogonial divisions of engorged nymphs. The male diploid number is 21 (fig. 3, A). The female diploid number is 22 (fig. 3, B). Sex is therefore determined by an XX:XO mechanism, the male sex being heterogametic. X is the biggest chromosome of the complement. In 2 male nymphs some gonadal cells contained 22 chromosomes. The extra chromosome was not the X.

The centromeres of the sex chromosome and 7 pairs of autosomes are terminal, but 3 autosome pairs are metacentric, amongst them the largest autosome. The largest autosome frequently shows 2 constrictions at mitotic metaphase (marked by arrows in fig. 3, A). One of these constrictions, probably the more terminal one, is the site of a nucleolar organizer, as will be described later.

*Male meiosis.* In males of all the Metastriata studied the mechanism of meiotic prophase is identical in outline. The description of early prophase up to diplotene is illustrated from *H. l. palustris* and *H. marginatum*.

To describe early meiotic prophase in male Ixodidae one cannot apply, offhand, orthodox terminology such as leptotene, zygotene, and pachytene. The occurrence of bivalents is the first indication that meiosis is under way.
Fig. 3, c and d illustrate such bivalents in a male of *H. l. palustris* after ecdysis and before feeding as an adult. Stages from diplotene onwards do not occur in the adult male Metastriata prior to feeding, therefore fig. 3, c and d illustrate a pre-diplotene stage, most likely pachytene. In fig. 3, c it is possible to recognize the chromosome configurations as pairs of homologues. Gradually, however, it becomes impossible to distinguish chromosomes within a bivalent, and the sex univalent from autosomal bivalents.

Meiotic phases from diplotene onwards were studied in material obtained from feeding males. The overall appearance, degree of fuzziness and stainability of *H. l. palustris* early diplotene chromosomes is similar to that of comparable stages illustrated for other species. One testis, removed from a tick which had fed for 5 days, gave an unusual view of diakinesis (fig. 3, e). The peculiarity consists in the largest autosome bivalent being invariably associated with a bulky nucleolus. The nucleolus is always attached subterminally to both chromosomes of the bivalent. I think the nucleolus breaks down before the onset of prometaphase. At diakinesis chiasma positions are often difficult to make out owing to the degree of contraction of the chromosomes, and frequently the sex univalent cannot be identified with assurance. At 1st prometaphase (fig. 3, f) the nucleolus is absent and the bivalents are compact. One of the autosome bivalents (marked by an arrow) is characterized by a subterminal constriction, which may represent the site of the nucleolar organizer. At 1st anaphase the sex univalent passes undivided to one pole, giving rise to 2 types of spermatocytes.

Fig. 3, g, h represent early and late 2nd meiotic prophases, with and without an X-chromosome. The largest autosome is easily identified as metacentric. Owing to their smaller size the 2 other metacentric autosomes are not so easily identifiable: they are indicated by arrows in the drawings.

**Hyalomma spp.** (fig. 4, a to h; fig. 5, a to c)

Since *Hyalomma dromedarii*, *H. marginatum*, *H. excavatum*, and *H. rufipes* are not identifiable on the basis of their nuclear cytology, they will be described together.

**Mitosis.** All *Hyalomma* spp. studied have diploid chromosome numbers of 21 in males and 22 in females (fig. 4, a, b). Sex is determined by an XX:XY mechanism, the males being heterogametic. The acrocentric X is the largest chromosome of the complement and easily distinguished from the autosomes, all of which are also acrocentric. The autosomes are similar in size and cannot be distinguished from one another with certainty.

**Male meiosis.** As the adult male starts feeding, the bivalents become less and less stainable and the spermatocyte nucleus increases enormously in volume. By the time the spermatocyte nucleus has reached about half its final size it becomes impossible (by the aceto-orcein squash technique) to discern chromosome structure. With the reappearance of stainable bivalent structures, the nucleus has reached its maximal size (fig. 4, c). Each nucleus of this size always contains 1, and only 1, heterochromatic chromosome.
I think this object is probably the sex univalent. A nucleolus can be seen in association with an autosomal bivalent. The invisibility of the chromosomes in younger spermatocyte nuclei may perhaps indicate that they are in a phase comparable to the 'lampbrush' chromosomes of oocytes. In male ticks a typical diplotene follows.

At diplotene 10 1-chiasma bivalents and a sex univalent were normally found in *H. marginatum*, *H. excavatum* and *H. dromedarii* (fig. 4, D), but in *H. rufipes* (fig. 4, E) many diplotene nuclei contained one 2-chiasma bivalent. Since terminal chiasmata are common the sex univalent often cannot be distinguished at diplotene.

Second meiotic divisions precisely resemble those already described for other species; they clearly show that all *Hyalomma* chromosomes (fig. 4, G, H) possess terminal centromeres.

**Female meiosis.** Stella (1938) stated that maturation, with the expulsion of both polar bodies, occurs inside the female in the ovary. In none of my material did I obtain meiotic divisions from ovarian squash preparations. Only in the case of *H. excavatum* did I follow this question systematically. Ovarian tissues, and eggs before and after oviposition, were prepared for this purpose. Meiotic divisions were found only in eggs fixed 0 to 2 h after oviposition. The earliest stainable chromosomes from layed eggs were at 1st meiotic pre-metaphase 'stretch' stages (fig. 5, B). Apart from the pre-metaphase 'stretch', female meiosis is characterized by the X-bivalent forming 2 chiasmata in most cells (fig. 5, B, C).

*Dermacentor andersoni* (fig. 5, D, E)

*D. andersoni* is not easily distinguishable from the *Hyalomma* spp. on the basis of nuclear cytology, but there is one minor difference. The X-chromosome of *D. andersoni* is larger than the autosomes, but less so than the X of *Hyalomma*.

*Rhipicephalus* spp. (fig. 5, F to K)

The diploid chromosome numbers of *R. secundus* (fig. 5, F to H) and of *R. sanguineus* (fig. 5, J, K) are 21 and 22 for males and females respectively. *Rhipicephalus* chromosomes differ little in size from one another and the X-chromosome is not identifiable at mitosis. Contrary to Dutt's observations, in my material the sex chromosomes are not the largest chromosomes of the complement, neither are they nor any other chromosomes metacentric. With the exception of the sex chromosome's relative size the description of *Hyalomma* and *Dermacentor* is valid also for the 2 species of *Rhipicephalus*.

**Incidental observations on the anatomy of tick testes and their accessory glands**

During the course of my cytological studies I found that the testes and accessory glands of *Ixodes* differ significantly from those of the other 4 genera. I can confirm Douglas's (1943) observations on *Dermacentor andersoni*, and have found his description also valid for all the Metastriata that I have studied.
Fig. 5. A, male diakinesis of *Hyalomma dromedarii*. B, female pre-metaphase 'stretch' stage, and C, female 1st metaphase of *H. excavatum*. D, oogonial metaphase, and E, spermatogonial metaphase of *Dermacentor andersoni*. F, spermatogonial metaphase, G, oogonial metaphase, and H, male early diplotene of *Rhipicephalus secundus*. J, spermatogonial metaphase, and K, oogonial metaphase of *R. sanguineus*. 
The reproductive system of the Prostriata type genus *Ixodes* differs from the reproductive system of Metastriata in that the testes are continuous across the midline, as well as in the number and distribution of accessory gland lobes.

In regard to testes *Ixodes* falls between the Metastriata and the Argasidae (Robinson and Davidson, 1913–14). In the Argasidae the testis is a single median organ. The structure of accessory glands differs widely in all 3 groups.

**Moulting**

In the course of breeding ticks I found that the integuments of larval and nymphal stages of the Metastriata split posterior to the scutum and capitulum during ecdysis, whereas the integuments of larval and nymphal stages of Prostriata split between scutum and capitulum.

**Discussion**

The 4 *Hyalomma* species studied (and *H. aegyptium* as described and illustrated by Dutt), all conform to a single karyotype, with \(2n = 22\) or \(21\), all chromosomes acrocentric, and an \(XX:XO\) sex-determining mechanism. The karyotype of *Dermacentor andersoni* differs little from those of the *Hyalomma* species. It too, has \(2n = 22\) or \(21\), all chromosomes acrocentric and an \(XX:XO\) sex-determining mechanism, but its X-chromosome is more difficult to distinguish from the larger autosomes, being relatively smaller than the X-chromosome of *Hyalomma*. This minor difference does not provide a clear-cut distinction even between genera, let alone between species.

The chromosome complements of *Rhipicephalus sanguineus* and *R. secundus* are similarly identical with \(2n = 22\) or \(21\), all chromosomes acrocentric; and an \(XX:XO\) sex-determining mechanism. The 2 *Rhipicephalus* species differ from *Hyalomma* and *Dermacentor* in that their X-chromosomes do not exceed in size the largest autosomes.

*Haemaphysalis leporis palustris*, also \(2n = 22\) or \(21\), \(XX:XO\), has 3 pairs of metacentric autosomes and its karyotype thus differs distinctly from *Hyalomma, Dermacentor* and *Rhipicephalus*. A study of more species within this genus might yield useful information.

Morphological features such as anal grooves, sexual dimorphism of mouth parts, the testes and their accessory glands, the splitting of integument during ecdysis, as well as the timing of spermatogenesis and mating, separate *Ixodes* (Prostriata) from the 4 other genera (Metastriata) described in this study. These morphological and biological differences are correlated with a major difference in karyotype. I propose that *Ixodes ricinus* and *I. hexagonus*, with diploid complements of 28 and 26 acrocentric chromosomes respectively and both having \(XX:XY\) sex-determining mechanisms, should be separated from Metastriata as a distinct 'family'. For a decision of this kind to be valid, however, information from a larger number of species is required.
Further research into the cytology of ticks should consider the possibility that *Haemaphysalis* (so far the only ixodid genus known to have metacentric chromosomes), may form a natural link between genera of Metastriata having diploid numbers of 22 or 21 acrocentric chromosomes, and *Ixodes*.

According to White (1954), the XO and $X_O X_2 X_3 O$ sex-determining mechanisms of spiders are presumed to be secondary derivatives of the $X_1 X_2 O$ system. It seems significant that 37 species described up to 1952 have terminal centromeres. Those which are XO in the male have 10 pairs of autosomes. In the majority of cases, species with an $X_1 X_2 O$ mechanism have 12 or more pairs of autosomes. White's hypothesis that XO mechanisms are secondary derivatives of the $X_1 X_2 O$ system would therefore have to account for the most drastic reshuffling, if not loss, of genetic material. There are at least 2 possibilities other than White's hypothesis to explain the occurrence of XO and $X_1 X_2 X_3 O$ mechanisms in spiders. Mites investigated by Schrader (1923), Patau (1936) and Cooper (1937, 39) have diploid complements of 6 chromosomes with male haploidy. Gamasidae (Sokoloff 1934) have diploid complements of 21 chromosomes or more. This situation, and the fact that spiders with $X_1 X_2 O$ and $X_1 X_2 X_3 O$ mechanisms have in most cases a larger number of autosomes than those with XO mechanisms, suggests that polyploidy may have played a role in the early evolution of arachnids.

An alternative to the above hypothesis would be the assumption that ticks and spiders with XO mechanisms and 10 pairs of autosomes may have evolved from a common stock, whilst ticks and spiders with XY and $X_1 X_2 O$ mechanisms respectively and 12 or 13 pairs of autosomes may have descended from another stock of ancestral arachnids.

Ixodoidea and Gamasidae form the suborder Parasitiformes. The most outstanding feature of the fully-grown Parasitiformes spermatozoon is its comparatively large size, of the order of 1 mm length for Ixodoidea and 0.13 mm for Gamasidae. Large spermatozoa are also found in Ostracods and Polyxenus (Myriapoda). Schmalz (1912), Sokoloff (1934), Nordenskiöld (1909, 1920), and Oppermann (1935) stressed the fact that primary spermatocytes of such animals go through a period of growth similar to oocytes at first meiotic division.

The description by Oppermann tallies with what I have here described for *H. l. palustris* and *H. marginatum*, and is also true for the other species which I have studied. Sokoloff's observation that chiasmata are completely terminalized by the onset of the second exaggerated growth stage of the spermatocyte must be viewed with reserve. The 2 growth stages described by Oppermann refer to nuclear growth while Sokoloff's second exaggerated growth stage undoubtedly refers to an increase in cytoplasmic material. Despite the difference between Prostriata and Metastriata as regards the commencement of the second growth period, the overall morphology of their bivalents throughout meiosis is the same. The exceptional nuclear growth of the primary spermatocyte of Parasitiformes, comparable to oocytes, may well be correlated with the evolution of an exceptionally large-sized spermatozoon.
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