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Annexe II

ON SEX-DETERMINATION AND KARYOTYPIC EVOLUTION IN TETRANYCHIDAE

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Summary

The chromosome complements of 44 species of spider mites (Tetranychidae) are examined using the aceto-orcein squash technique. The total number of the species examined in this family is now 57, a figure representing approximately 10 % of the species concerned. The chromosome numbers range from n=2 to n=7. The type number of the family is 3 (found in 44% of the species). It is argued that the ancestral number is n=2 (21% of the species).

In the more primitive subfamily of the Bryobiinae both thelytokous and arrhenotokous species occur, whereas the subfamily of the Tetranychinae exclusively exhibite arrhenotoky. The karyotype evolution is discussed in connection with arrhenotoky. It is stated that karyotype information is a useful tool for the taxonomist.

Résumé

Au cours de cette étude, le nombre de chromosomes de 44 espèces d'acariens phytophages de la famille des Tetranychidae a été déterminé par la méthode du "squash" à l'aceto-orceine. Le nombre total des représentants de cette famille étudiés à ce point de vue, est ainsi porté à 57, ce qui correspond à environ 10% des espèces actuellement connues. Le nombre de chromosomes varie de n=2 à n=7. Le nombre n=3 trouvé pour 44% des espèces est le nombre type de la famille, tandis que le nombre n=2 rencontré dans 21% des espèces peut être considéré comme le nombre chromosomique ancestral.

Les espèces sont thélytoques ou arrhénotoques dans la sous-famille des Bryobiinae qui est la plus primitive, tandis que la sous-famille des Tetranychinae ne comprend que des espèces arrhénotoques. Du fait de l'arrhénotoquie, l'évolution du caryotype peut être très particulière.

Il apparaît enfin que l'étude des caryotypes constitue un instrument de travail intéressant pour le taxonomiste.

Introduction

The diverse species of the family of spider mites (Tetranychidae Donnadieu) all feed on higher plants (Spermatophyta). They form colonies on foliage and some species are serious pests in cultivated crops. There are two subfamilies : Bryobiinae Berlese and the Tetranychinae Berlese. Of these two, the former is considered more primitive than the latter (PRITCHARD & BAKER, 1955)

For a long time the Tetranychidae attracted the attention mainly of taxonomists and the applied entomologists. But the outstanding ability of spider mites to develop insecticide resistance also evoked interest into their formal genetics. The progress in this field was recently recorded by BALLANTYNE (1969).

As for karyology, a start has been made with a study concerning chromosome numbers and sex determination in 13 spider mite species (HELLE & BOLLAND, 1967). This study made clear that arrhenotoky and thelytoky are the modes of reproduction in the Tetranychidae. This was demonstrated by cytological means as well as by rearing experiments. The number of chromosomes in the species investigated was found to be low ranging from n=2 to n=7. As for the length of the chromosomes of all 13 species values between 1 and 2 were measured. Constrictions, if present, could not be seen and it could not be decided wether the chromosomes are holokinetic or monocentric. However, because of the presence of V-shaped chromosomes during anaphase it was believed that a localised centromere is present in some species.

A somewhat exceptional number of chromosomes (n=7) was found in the arrhenotokous species Neotetranychus rubi Trägardh. This species is taxonomically related to species having n=3 and n=4. It was therefore suggested that N. rubi had originated as a result of allopolyploidy. (HELLE & BOLLAND, 1967).

During the last two years the chromosome numbers of more than 40 other spider mite species were determined. The results are given and discussed in the present paper. So far, the chromosome complements of 57 species are known, a figure representing nearly 10% of the total number of spider mite species described.

Methods and material

Karyotype determinations were performed on eggs containing young undifferentiated embryonic tissue, using the orcein-squash technique.

For this technique, an egg is placed on a microscope slide and a droplet of 1% sodium citrate is added. Then a cover slip is put carefully on the object. After this treatment with sodium citrate, which takes about one minute, a droplet of 3% aceto-orcein stain is brought under the cover slip draining off the sodium citrate droplet with a piece of filtered paper. After a 5 to 15 minutes staining period of the intact egg, the actual squashing is done. The objects are embedded directly in an Euparal medium.

Preferably, a species was cultivated in the laboratory. This presented no difficulties with polyophageous species accepting bean (Phaseolus vulgaris L.) as sustenance. A detached leafculture (cf HELLE, 1965) was then used which made it possible to examine the progeny of mated as well as that of unmated females. The following species were kept in culture on bean : Eutetranychus banksi, Eu. orientalis, Eu. eliei, Oligonychus bessardi, O. sylvestris, Eotetranychus imerinae, Eo. paracybelus, Eo. ranomafanae, Tetranychus atlanticus, T. ludeni, T. neocaledonicus and T. tumidus. Munger cells (of GUTIERREZ, 1967) were used for those species which could not be reared on beans. For this method we depended on the availibility of the hostplant in the immediate surroundings of the laboratory. In this way the greater part of the species were studies. For some species we had to make do with examples of eggs gathered in the field. The species, localities and hostplants are presented in Table I.

Results

It was possible to determine the number of chromosomes in all species under investigation. Often, several dozens of metaphase-plates were found in the embryonic tissue of one egg. Therefore, also the species where the sample of eggs studies was small, the number of chromosomes of the particular species was determinable.

The chromosome numbers of the various species are given in Table I ; photomicrographs of the karyotypes are presented in Plates I and II.

TABLE I

Chromosome numbers in different spider mite species. Egg number in parentheses.

N = The Netherlands M = Madagascar

Species	Location	Hostplant	Number of chromosomes in randomly taken eggs	in eggs obtained from unfertilized females
Subfamily Bryobiinae Berlese				
Bryobia rubrioculus (Scheuten)	Amsterdam (N.)	Malus sp.	8 (43)	
Bryobia praetiosa Koch	Amsterdam (N.)	Gramineae	8 (14)	
Porcupinychus insularis (Gut.)	Ihosy (M.)	Sida rhombifolia L.	8(10) 4(7)	4(2)
Petrobia harti (Ewing)	Tananarive (M.)	Oxalis corniculata L.	4(23) 2(49)	2(14)
Petrobia latens (Müller)	Amsterdam (N.)	Gramineae	8(14)	
Subfamily Tetranychinae Berlese				
Eonychus curtisetosus Gut.	Betioky (M.)	Grewia lavanalensis H. Bn.	4(3) 2(1)	
Eonychus grewiae Gut.	Maevatanana (M.)	Grewia flavicans H. Bn.	4(1) 2(6)	
Eurytetranychus madagascariensis Gut.	Tuléar (M.)	Nerium oleander L.	6(4) 3(3)	
Eutetranychus grandidieri Gut.	Ihosy (M.)	Phragmites mauritianus Kunth	4(5) 2(6)	2(9)
Eutetranychus eliei Gut. and Helle	Tuléar (M.)	Plumeria alba L.	8(4) 4(3)	
Eutetranychus ranjatoi Gut.	Befandriana-Sud (M.)	Rinorea greveana H. Bn.	6(7) 3(1)	
Eutetranychus orientalis Klein	Israël	Citrus spec.	6(5) 3(4)	3(3)
Eutetranychus banksi (McGregor)	Florida U.S.A.	Citrus spec.	6(16) 3(12)	3(4)
Oligonychus sylvestris Gut.	Tananarive (M.)	Sida rhombifolia L.	4(3) 2(3)	2(7)
Oligonychus andrei Gut.	Ihosy (M.)	Grewia lavanalensis H. Bn.	4(3) 2(3)	

Oligonychus randriamasii Gut.	Ampanihy (M.)	Croton sp.	4(9)	2(4)	
Oligonychus gossypii (Zacher)	Majunga (M.)	Grangeria sp.	4(5)	2(5)	
Oligonychus bessardi Gut.	Tananarive (M.)	Oxalis corniculata L.	8(4)	4(3)	4(14)
Oligonychus virens Gut.	Ankazobe (M.)	Melinis minutiflora P. B.	8(2)	4(1)	
Oligonychus monsarrati Gut.	Tananarive (M.)	Panicum maximum Jacq.	8(2)	4(4)	
Oligonychus pratensis (Banks)	Tuléar (M.)	Dactyloctenium capitatum A. Camus	8(3)		
Oligonychus chazeaui Gut.	Majunga (M.)	Hyphaene shatan Boj.	8(6)	4(1)	
Oligonychus coffeae (Nietner)	Tuléar (M.)	Vitis vinifera L.	6(6)	3(3)	
Oligonychus quercinus Hirst	Amsterdam (N.)	Quercus robur L.	6(6)	3(7)	
Anatetranychus tephrosiae (Gut.)	Tuléar (M.)	Mundulea pungens Vigu.	6(9)	3(4)	
Eotetranychus befandrianae Gut.	Ampanihy (M.)	Croton sp.	4(3)	2(2)	
Eotetranychus sakalavensis Gut.	Befandriana-Sud (M.)	Phyllanthus sp.	4(7)	2(4)	
Eotetranychus tulearensis Gut.	Befandriana-Sud (M.)	Bauhinia sp.	4(13)	2(6)	
Eotetranychus ranomafanae Gut.	Ranomafana (M.)	Rosa sp.	10(8)	5(10)	
Eotetranychus rinoreae Gut.	Befandriana-Sud (M.)	Rinorea greveana H. Bn.	6(3)	3(2)	
Eotetranychus friedmanni Gut.	Tananarive (M.)	Solanum auriculatum Ait.	6(4)	3(3)	
Eotetranychus imerinae Gut.	Tananarive (M.)	Erythrina macrophylla D. C.	6(11)	3(5)	3(14)
Eotetranychus paracybelus Gut.	Ivato (M.)	Tephrosia vogelii Hook.	6(5)	3(3)	3(6)
Eotetranychus roedereri Gut.	Ankazobe (M.)	Cephalostachyum sp.	6(2)	3(2)	
Eotetranychus grandis Gut.	Majunga (M.)	Hippocratea sp.	6(6)	3(2)	

Schizotetranychus australis Gut.	Tuléar (M.)	Mundulea pungens Vigu.	12(9)	6(3)	
Tetranychus roseus Gut.	Majunga (M.)	Medemia nobilis Gall.	8(15)	4(10)	
Tetranychus viennensis Zacher	Goes (N.)	Prunus avium L.	6(17)	3(3)	
Tetranychus panici Gut.	Ankazobe (M.)	Panicum uvulatum Stapf.	8(3)	4(2)	
Tetranychus ludeni Zacher	Tananarive (M.)	Thunbergia alata Boj.	6(10)	3(4)	3(8)
Tetranychus kaliphorae Gut.	Ankazobe (M.)	Kaliphora madagascariensis Hook	6(3)	3(1)	
Tetranychus atlanticus McGregor	Bosnia (Yugoslavia)	Humulus lupulus L.	6(12)	3(11)	3(8)
Tetranychus neocaledonicus André	Louisiana (U.S.A.)		6(29)	3(2)	3(15)
Tetranychus neocaledonicus André	Ihosy (M.)	Gossypium hirsutum L.	6(1)	3(1)	3(9)
Tetranychus tumidus Banks	Louisiana (U.S.A.)	" "	12(36)	6(20)	6(192)

With the exception of Oligonychus pratensis, in all bisexual species two classes of eggs were found, apparently having the haploid and diploid numbers. In those species in which eggs were studies obtained from virgin females the haploid number occured. Also was found that eggs deposited by unmated females , resulted in males only. These two facts reflect the haplo-diploid sex determination of the bisexual species.

As for O. pratensis, only 3 eggs were examined and the 8 chromosomes found presumably represent the diploid number. The sex ratio in O. pratensis is such that the females outnumber the males by far.

In the populations of the species Bryobia praetiosa, B. rubrioculus and Petrobia latens no males were observed. For these particular species only one class of eggs was found with a number of 8 chromosomes, presumably representing the diploid number. Tetranychus tumidus is a species which exhibits the for the genus rather unusual numbers of 6 and 12 chromosomes. An interesting point about the populations of this species under study is noteworthy. It was observed that in the particular population sex aberrants occurred which had a male appearance but which were extremely large in size. Closer examination of these giant males learned that they were actually intersexes, however, with male characteristics predominating. The frequency of these giant males also occurred in progenie of unfertilized females. Since it is up till now impossible to determine the chromosome number in adult spider mites, one can only speculate about their cytogenetic basis. The 192 examined eggs obtained from unmated females of T. tumidus only showed the normal haploid number of 6. In table II a review is given of the sizes of various Tetranychus species, measured on 10 living adults of each species, to give an impression of the deviating size of T. tumidus.

A survey of the variation in chromosome numbers within the various genera examined (including those mentioned in HELLE & BOLLAND, 1967) is presented in Table III.

Discussion

Three subjects for discussion come to the fore : i.e. sex-determination, karyotype-evolution and the usefullness of karyotype information for taxonomic purposes.

We can be short about sex-determination, as the results presented here do not open new wiew points. All bisexual species clearly exhibit an arrhenotokous parthenogenesis. This has been demonstrated with an arbitrarily chosen number of species and generations, by cytological and by rearing means both. The fact that virgin females do produce eggs, facilitates the evidence for arrhenotoky. It should be mentioned, however, that most species of which unmated females were isolated had a lower egg-production than most species of which females had. This phenomenon was demonstrated before with T. neocaledonicus by GUTIERREZ (1967).

Thelytokous species are found in the Bryobiinae and not in the Tetranychinae. It is interesting to point out here that several times a number of Tetranychus species in the laboratory switched over to thelytokous reproduction. In all cases known, such thelytokous strains could only be maintained for a limited number of generations.

It needs no argument that arrhenotoky is the ancestral type of reproduction of the Tetranychidae. The few data about other families of the Prostigmata s.s. (Suborder Trombidiformes), to which belong the Tetranychidae, indicate that arrhenotoky is the predominant type of reproduction.

So far, the chromosome complements of 57 species of Tetranychids are known. After this important extension of karyotype information, the first thing noticeable is the numerical variation in chromosome numbers in most genera, like Eutetranychus, Oligonychus, Eotetranychus, Schizotetranychus and Tetranychus. It is clear that the former suggestion that a reduction in number of phylogenetic relationship between genera would exist in the subfamily of the Tetranychinae, is too simplistic. (HELLE & BOLLAND, 1967).

In the following discussion on the karyotypic evolution, it must be postulated that our knowledge is inadequate on two points. Notwithstanding the great number of metaphase-plates of the various species examined, we are still uncertain about the condition of the centromere. This implies that in case of diffusecentricity, processes like fragmentation in the increase in chromosomes may be involved. The second hindering gap in our knowledge is the relatively low number of examined species of the subfamily of the Bryobiinae.

As can be seen in Table III, n=3 is the type number of the family. It occurs in 44% of the species investigated. The numbers n=2 and n=4 represent 21% and 26% of the species concerned.

In the random sample of 7 species of the Bryobiinae, the number n=3 is not found, all species of this primitive subfamily exhibit haploid complements with either 2 or 4 chromosomes. This may be an indication that the ancestral chromosome number of the family is not 3 but probably 2.

The number n=2 is found in the phylogenetically important genus Eonychus. Owing to the number of anal setae and the absence of tenent hairs on the empodium, this genus, endemic for Madagascar, belongs to the Tetranychinae. Eonychus, however, shows an abundance of primitive characteristics, like the form of the empodium, the peritremata, the aedeagus and the reticulation of the propodosoma, indicating the close relationship with the Bryobiinae. Undoubtedly, Eonychus is the least specialized genus of the Tetranychinae in the genera involved. Apart from these morphological criteria, Eonychus can also be considered as unspecialised because of biological reasons, since Eonychus, like the Bryobiinae, has no ability to produce silk.

The production of silk-structures is an important evolutionistic step for the Tetranychinae. It offers protection against rain and many predator mites. This type of specialization can be followed step by step in the genus Eotetranychus The species E. befandrianae, E. sakalavensis and E. tulearensis belong to the less specialized group within this genus. They only produce a few threads to cover an egg mass. In other species within the genus Eotetranychus the production of silk-structures is further developed (GUTIERREZ, HELLE & BOLLAND, 1970). There are also indications in Eotetranychus that the species with n=2 are less specialized.

Based on the current data, we are inclined to presume that n=2 is the primary chromosome number. Unfortunately, there is little information about chromosome numbers in other families of the Prostigmata s. s. The only data existing concern the family Harpyrhynchidae, two species of which were examined by OLIVER & NELSON (1967). Both species have n=2 and 2n=4 and are arrhenotokous.

With respet to changes in chromosome numbers, it is tempting to hypothesize that polyploidy contributed to speciation in spider mites. The case of Neotetranychus rubi Trägård with n=7(whereas related species show n=3 and n=4) has already been mentioned before (HELLE & BOLLAND, 1967). The present investigations offer new material for this supposition. We want to draw the attention to the genus Schizotetranychus, of which the species S. schizopus shows a haploid complement with n=3, whereas S. australis has n=6. We further mention Tetranychus tumidus with n=6, which belongs to the more advanced species of

Tetranychus, each with n=3. The big size of T. tumidus, as compared to related species (see Table II) together with the occurrence of sex-aberrants, are phenomena that can be connected with a recent speciation of T. tumidus by doubling of the chromosome complement. One could continue this view by conceiving the species with n=5 (like for instance Eotetranychus ranomafanae) as allopolyploids. And to return to the Bryobiinae, the situation in Petrobia can also be seen in the same light.

Polyploidy as a factor for speciation in bisexual animals is rather unpalatable for many cytologists (cf. WHITE, 1961 ; SUOMALAINEN, 1958). However, it is relevant to consider the peculiarities of arrhenotoky, since the evolutionary possibilities resulting from arrhenotoky seems different from those animals with diploid sexes.

Establishment of genome mutations in zygogenetic bisexual animals meets with serious difficulties. These difficulties result from the fact that for propagation the mutant depends on a sexual partner of the wild-type. It may be that the mutation is expressed in some phenotypical change with causes an instantaneous reproductive isolation of the mutant. In case of polyploidy, for instance, an increase in size can result in physical non-correspondance of the genitalia, or in lack of mutual attraction. But even if the mutant is full compatible with normal, lots of hindrances can prevent establishment. If sex is balanced by a chromosomal mechanism, the upset in balance may cause sterility amongst the descendants (cf MULLER, 1925).

In animals with an arrhenotokous reproduction the chance of establishment of genome mutations seems more favourable, for this reproductive type offers an escape to the mutation, as the mutant female can mate with her parthenogenetically produced sons. This would be an opportunistic way in which instantaneous differentiation can occur resembling the possibilities of monoecious organisms. Arrhenotoky can be considered in this respect as a kind of "monoecy in time". However, it is difficult to appraise the evolutionistic value of this possibility inherent to arrhenotoky. Especially with respect to polyploidy, the question arises whether tetraploid females can produce diploid sons.

The genetic basis of the haplo-diploid sex-determination, however, is still obscure. In a study on the occurrence of giant males and intersexes in an inbred line of Tetranychus urticae Koch it was shown that the giant males in this inbred line were diploid and produced visible spermatids (HELLE, unpublished). We mention these facts to illustrate that diploidy is not per se, male-determining.

TABLE II

Range in size of different adults of Tetranychus-species.

	female		male	
	length	width	length	width
T. urticae	490-515	290-303	310-325	165-195
T. pacificus	490-530	280-295	325-340	165-180
T. atlanticus	500-530	290-310	295-325	170-185
T. neocaledonicus	490-530	300-325	295-340	170-185
T. tumidus	610-630	390-415	375-400	210-230
" (uniparental giant males)			520-570	270-310

TABLE III

Chromosome complements in different genera of the Tetranychidae

Genus	Species examined	Number of species with haploid complement					
		2	3	4	5	6	7
Bryobia	3			3			
Tetranycopsis	1	1					
Porcupinychus	1			1			
Petrobia	2	1		1			
Subfamily Bryobiinae	7	2		5			
Eonychus	2	2					
Eurytetranychus	2		1		1		
Eutetranychus	5	1	3	1			
Oligonychus	12	4	3	5			
Panonychus	1		1				
Anatetranychus	1		1				
Neotetranychus	1						1
Eotetranychus	12	3	6	2	1		
Schizotetranychus	2		1			1	
Tetranychus	12		9	2		1	
Subfamily Tetranychinae	50	10	25	10	2	2	1
Family Tetranychidae	57	12	25	15	2	2	1

Our sceptical attitude towards polyploidy is due to our conviction that a more detailed study is needed for the cases mentioned. More normal processes, like dissociations or fragmentations can result in similar figures.

Remains an evaluation of karyotypic information for taxonomic purposes. It is beyond doubt that karyotypic information is a useful tool for the acarologist. In a heterogeneous genus like Oligonychus the chromosome numbers are indicative for the division of a number of sub-units. An integration of the karyotypic information, with morphological and biological disciplines, will be presented in a separate paper (GUTIERREZ, HELLE & BOLLAND, 1970).

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PLATE I. Photomicrographs of mitotic stages in egg squashes of different spider mite species. Magnification 1900X.

1. B. rubrioculus, 2n=8
2. B. praetiosa, 2n=8
3. P. harti, 2n=4
4. P. harti, n=2
5. E. grewiae, 2n=4
6. E. grandidieri, n=2
7. E. orientalis, 2n=6
8. E. orientalis, n=3
9. O. sylvestris, n=2
10. O. randramasii, n=2
12. O. bessardi, 2n=8
13. O. bessardi, n=4
14. O. monsarrati, 2n=8
15. O. monsarrati, n=4

Plate II. Photomicrographs of mitotic stages in egg squashes of different spider mite species. Magnification 1900X.

16. O. coffeae, 2n=6
17. A. tephrosiae, n=3
18. E. befandrianae, n=4
19. E. ranomafanae, 2n=10
20. E. ranomafanae, n=5
21. E. imerinae, 2n=6
22. E. paracybelus, 2n=6
23. E. roederi, 2n=6
24. S. australis, 2n=12
25. T. kaliphorae, 2n=6
26. T. atlanticus, 2n=6
27. T. atlanticus, n=3
28. T. neocaledonicus, 2n=6
29. T. tumidus, 2n=12
30. T. tumidus, n=6