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Importance of Karyology in aphid taxonomy

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Abstract. Cytological parameters are nowadays being increasingly and intelligently used in solving the taxonomic problems of dubious species. Aphids', in certain cases atleast, taxonomy is not stabilised at the species as well as at the subgeneric level. It is because of the usual occurrence of race or biotype in natural population. Occurrence of such taxonomic categories are possibly due to chromosomal rearrangements or otherwise that resulted from the association with a host plant, as evidenced in a few species, viz, *Myzus persicae*, *Brachycaudus helichrysi*, *Tuberolachnus salignus* etc. Several ecogeophysical factors are operative in establishing chromosomally distinguished races which demand the attention of taxonomists for further taxonomic evaluation of the concerned species. Attempt is therefore made to discuss the rationale of using the cytological informations in the identification of infraspecific categories of aphids.

Keywords. Aphid; biotype; karyology.

Aphids among the homopterans are possibly of special interest to the cytologists because of extensive polymorphism as well as complicated mode of life cycle (cyclical parthenogenesis) within the group itself. Furthermore, confusion over the taxonomic identity of the species of economic importance has arisen because several perfectly good biological species are very similar in their gross morphology. Such puzzling problems attracted the aphidologists to verify them cytotaxonomically. Their study includes the cytogenetical aspects of aphids that help the taxonomists to separate the closely related species of dubious taxonomic status by supplementing them with the evidences from karyotypical differences. Valuable bulk of data is available from the extensive studies of chromosomal variation of different aphids of higher taxonomic categories that may provide a guideline in clarifying the natural relationship of species as well as their probable way of phylogeny.

The range of karyotypic variation among aphids is broad. Sometimes a single genus may include several karyotypes. On the contrary, distantly related species are also with identical karyotypes. The conventional way of comparing identical species, in respect of morphological features could be well supplemented by certain cytological parameters, viz diploid number, structure, karyometrical analysis, chromosome formula (CF) (Khuda-Bukhsh and Pal 1984; Das *et al* 1985). Therefore, in the field of aphid taxonomy cytological studies can be used as a convenient tool in which somatic chromosomes in prophase or metaphase, by squashing the young embryos, can be obtained without difficulty which enables to study the basic feature of karyotypes, viz diploid number (2n), actual length of chromosomes (μm), relative length ($R^{1}\%$), total complement length (TCL), CF etc.

Homopteran chromosomes are holocentric which is also shared by Heteroptera, Mallophaga, Anopleura, Dermaptera, probably Lepidoptera, some scorpions and some centipedes. Due to lack of primary point of reference, aphid chromosomes appear featureless, rod or dot shaped depending on the size, excepting the occasional

occurrence of conspicuous secondary constriction in conventional preparations. But by employing recently developed advanced methods (C and G-banding) now it is possible to include specific pattern of bands along the length of a chromosome and accordingly it can be identified from its counterparts.

Sexual morphs, which generally occur in nature at the advent of adverse conditions like cold climate or can be produced artificially in glass-house from the cultures of virginoparae in late spring by inducing short photoperiod are indispensible for studying the meiotic stages, which is less touched by aphid cytologists in comparison to somatic chromosomes. For this practical reason present observations are made from the study on the somatic chromosomes.

Clonal variability of an aphid species in respect of life cycle, food specialization and morphology even in restricted ecological niche, often presents complicated situation to taxonomists. This may be evidenced from the following studies.

Myzus persicae (Sulzer) collected from two different host plants, *Nicotiana tabacum* (Solanaceae) and *Rhaphanus sativus* (Cruciferae) at different seasons and different localities of north east India which biologically might vary (Eastop 1973) has been found to vary karyotypically (tables 1 and 2). Sixty eight per cent of 79 totally examined plates were with $2n = 12$ which is also supported by the earlier reports (Blackman and Takada 1977; Kulkarni and Kacker 1981; Khuda-Bukhsh and Pal 1986; Kuznetsova 1969; Sun and Robinson 1966). Other aneuploids with $2n = 8$ and 13 may be due to dissociation, fusion or translocation being robertsonian fusion as the main operative force. In some cases occurrence of clear structural heterozygosity was noticed in one of the X-chromosomes of the diploid complement.

From tables 1 and 2 it is apparent that the populations collected from two different host plants do not significantly vary karyometrically but seasonal variation casts a significant change in karyotype. In both the clones chromosome formula stands $n = 2M + 2S + 2S^v$ but in response to seasonal variation, in clone-A one additional type of chromosome (i.e. long type; L) appears and accordingly the chromosome formula comes as $n = 1L + 1M + 2S + 2S^v$. In clone-B though CF remains the same but marked distinction could be observed between the second and fourth pairs of chromosomes.

Another clone collected from *R. sativus* during winter and summer from the plains

Table 1. Karyometrics of the haploid set of chromosomes of *M. persicae* from *N. tabacum*.

Locality	Season	Actual length ($\mu m \pm SE$)	Relative length ($R^{1}{}_{0}$)	TCL	CF
		5·97 ± 0·08	29·70		
		(6·30 ± 0·09)	(31·03)		2M + 2S + 2S^v
		5·77 ± 0·07	28·71		
	May	(5·80 ± 0·09)	(28·57)		
Darjeeling		(3·56 ± 0·11)	17·71	20·10	
(c. 70000 ft.)		(3·48 ± 0·07)	(17·14)	(20·30)	
	December	2·56 ± 0·09	12·74		
		(2·50 ± 0·07)	(12·32)		(1L + 1M + 2S + 2S^v)
		1·23 ± 0·08	6·12		
		(1·32 ± 0·08)	(6·50)		
		1·01 ± 0·05	5·02		
		(0·90 ± 0·08)	(4·43)		

Clone-A; Populations 1 and 2 (in parentheses).

Table 2. Karyometrics of the haploid set of chromosomes of *M. persicae* from *R. sativus*.

Locality	Season	Actual length (μm ± SE)	Relative length (R^1%)	TCL	CF
Darjeeling (*c* 7000 ft.)	May	6·09 ± 1·51	29·91		2M + 2S + 2S^v.
		(4·82 ± 0·09)	(25·06)		
		5·91 ± 1·03	29·03		
		(4·05 ± 1·20)	(21·81)		
	December	3·56 ± 0·08	17·49		
		(3·55 ± 1·38)	(19·12)	20·36	
		2·62 ± 1·21	12·82	(18·57)	
		(3·47 ± 0·07)	(18·69)		(2M + 2S + 2S^v)
		1·21 ± 0·05	5·94		
		(1·48 ± 0·08)	(7·97)		
		0·98 ± 0·08	4·81		
		(1·20 ± 1·02)	(5·28)		

Clone-B; Populations 1 and 2 (in parentheses).

Table 3. Karyometrics of the haploid set of chromosomes of *M. persicae* from *R. sativus* showing n = 4.

Season	Actual length (μm ± SE)	Relative length (R^1%)	TCL	CF
Summer	1·47 ± 0·33	35·08		
	(5·84 ± 0·17)	(37·77)		
	1·10 ± 0·32	26·25	4·19	
	(4·41 ± 0·19)	(28·53)		1L + 2M + 1S in both the cases
Winter	0·91 ± 0·30	21·72	(15·46)	
	(3·21 ± 0·13)	(20·76)		
	0·71 ± 0·13	16·95		
	(2·00 ± 0·13)	(12·94)		

Clone-C; Populations 1 and 2 (in parentheses).

of West Bengal (around Calcutta) exhibited 2n = 8 (table 3) which might be due to fusion of 1st and 6th and 3rd and 5th pairs of chromosomes of the normal karyotypes (tables 1 and 2).

Here the chromosome formula stands as n = 1L + 2M + 1S, very much akin to *Aphis* group, for which the preserved material were reexamined and the identification remained valid. However, in this clone seasonal variation does not hold any good distinctive criterion. Though the actual length of the chromosome seems to be longer in size yet it may be considered due to relative condensation of chromosomes during cell cycle.

Therefore, at least in case of *M. persicae*, it is established that in response to host plant, season or geographical distribution, several chromosomal races may be in nature which might have reflected on the morphology and/or biology of the species concerned. Such instances could be encountered with *Rhopalosiphum maidis* (Chattopadhyay *et al* 1982).

An year round cytological survey on a macrosiphine aphid, *Brachycaudus helichrysi* also speaks in favour of the existence of several chromosomal races in natural

populations. *B. helichrysi* collected from *Artemisia vulgaris* (Compositae) showed 2n = 12. Six homomorphic pairs with n = 2M + 2S + 2S^{v}, 4·65 μm and 1·00 μm being the length of the chromosome from longest to shortest. But in the sample collected from *Brassica napus* (Cruciferae), the karyology varied to a great extent (table 4).

Here an additional type (long type; L) of chromosome appears indicating 2n = 10 with chromosome formula as n = 1L + 1M + 2S + 1S^{v}. Another distinctive criteria between the two populations is that the maximum and minimum size difference exists between the 2nd and 3rd pair and 5th and 6th pair of chromosomes in the population from *A. vulgaris* which measures 5·32 and 3·06 R^{1}%, respectively in contrast to 8·90 and 4·55 R^{1}% size difference between the 2nd and 3rd and 4th and 5th pair of chromosomes respectively, in the sample collected from *B. napus*. We encountered similar incidence while working with a lachnine species, *Tuberolachnus salignus*. Populations established at distantly related host plants markedly varied karyotypically. Populations collected from *Pinus cassia* (Pinaceae) exhibited 2n = 14, (i) n = 1M + 5S + 1S^{v} and in some instances (ii) 6S + 1S^{v}. However, the M-type chromosome of the former can be omitted since it remains in the boundary line (R^{1}% – 20·14). So the CF is n = 6S + 1S^{v} (table 5). But the populations collected from *Salix babylonica* (Salicaceae) presented a different picture with 2n = 22; n = 4S + 7S^{v} which may arise as a result of fragmentation of the chromosomes (table 6). Thus it seems that host plant variation affects the karyotype of the species.

Sometimes a special problem arises in determining the exact identity of an aphid when one unfortunately deals with a mixed population of two or more species cohabiting the same host plant. To exemplify the situation reference is made to rose infesting aphids *Macrosiphum rosae* (Linn) and *Sitobion rosaeiformis* (Das). Very often they are found in close, in one and the same colony and live colours of the two species overlap to a great extent from greenish to pinkish. In this connection it may be pointed out that *M. rosae* has world wide distribution although entirely absent in eastern Asia, Japan in particular (Eastop 1966; Blackman and Eastop 1984). However, Eastop (1966) opines that *M. rosae* has been replaced in far eastern countries by several species including *Sitobion ibarae* (Mats.) and *S. rosaeiformis*. Simultaneous find of both *M. rosae* and *S. rosaeiformis* in India does not substantiate Eastop's contention. But from our cytological studies these two confusing species

Table 4. Karyometrics of the haploid set of chromosomes of *B. helichrysi*.

Host plants	Actual length (μm ± SE)	Relative length (R^{1}%)	Diploid No (2n)	TCL	CF
A. vulgaris	4·65 ± 0·07	28·40	12	16·37	n = 2M + 2S + 2S^{v}
	(3·75 ± 1·90)	(32·75)			
	3·87 ± 0·07	23·64			
	(3·12 ± 1·23)	(27·24)	(10)	(11·45)	(n = 1L + 1M + 2S + 1S^{v})
	3·00 ± 1·03	18·32			
	(2·10 ± 1·00)	(18·34)			
B. napus	2·35 ± 1·10	14·35			
	(1·50 ± 0·93)	(13·10)			
	1·50 ± 0·09	9·16			
	(0·98 ± 1·03)	(8·55)			
	1·00 ± 0·49	6·00			
	(——)	(——)			

Data in parentheses are of samples from *B. napus*.

Table 5. Karyometrics of the populations a and b of *T. salignus*.

Name of the host plant	Actual length ($\mu m \pm SE$)	Relative length ($R^1\%$)	TCL	CF
	4·10 ± 0·33	20·14		
	(2·90 ± 0·09)	(18·29)		
	3·50 ± 0·29	17·19		
	(2·75 ± 0·23)	(17·35)		
	3·30 ± 0·25	16·21		$n = 1M + 5S + 1S^v$
P. cassia	(2·50 ± 0·21)	(15·77)	20·35	
	2·80 ± 0·31	13·75		
	(2·35 ± 0·18)	(14·82)	(15·85)	
	2·55 ± 0·29	12·53		$(n = 6S + 1S^v)$
	(2·00 ± 0·13)	(12·61)		
	2·15 ± 0·27	10·56		
	(1·80 ± 0·17)	(11·35)		
	1·95 ± 0·30	9·58		
	(1·55 ± 0·18)	(9·77)		

Data in parentheses are of samples showing $n = 6S + 1S^v$.

Table 6. Karyometrics of the haploid set of chromosomes of *T. salignus* collected from *S. babylonica* showing n = 11.

Chromosome pair	Actual length ($\mu m \pm SE$)	Relative length ($R^1\%$)	TCL	CF
I	5·00 ± 0·12	14·68		
II	4·25 ± 0·08	12·48		
III	4·00 ± 1·02	11·77		
IV	3·50 ± 0·08	10·27		
V	3·10 ± 1·23	9·10		
VI	3·00 ± 0·07	8·81	34·05	$n = 4S + 7S^v$
VII	3·00 ± 0·08	8·81		
VIII	2·50 ± 0·09	7·34		
IX	2·25 ± 0·08	6·60		
X	1·90 ± 0·07	5·58		
XI	1·55 ± 0·09	4·55		

could be readily identified as the diploid number and the relative length varies widely between them (table 7).

Cytological characterisation at the higher taxonomic categories of aphids is also noteworthy from our study. For example the genus *Aphis* of the tribe Aphidini is cytologically distinguished as $2n = 8$ with $n = 1L + 2M + 1S$ from that of the genus *Myzus* of the tribe Macrosiphini with $2n = 12$, $n = 2M + 2S + 2S^v$. Similarly cinarine aphids feeding on Cupressaceae, have $n = 6$ as opposed to $n = 5$ for other *Cinara* feeding on Pinaceae (Blackman 1980; Das *et al* 1985).

It is apparent that karyotypic variation within the species seems to occur frequently in aphids, which have been recently reviewed elsewhere (Blackman 1980). Differences in diploid number (other than polyploidy) may be the resultants of dissociation or fusions involving the elements of normal set, or due to the presence of additional supernumerary or B-chromosomes. Exchange of chromosomal segments of unequal length results detectable structural heterozygosity in karyotype.

Table 7. Karyometries of the haploid set of chromosomes of *S. rosaeiformis* and *M. rosae* (in parenthesis).

Chromosome pair	Actual length (μm ± SE)	Relative length (R'%)	TCL	CF
I	3·58 ± 0·23	21·21		
	(2·49 ± 0·09)	(26·80)		
II	2·42 ± 0·22	14·34		
	(2·16 ± 0·08)	(23·38)		
III	2·15 ± 0·19	12·74		n = 1M + 3S + 5S^{v}
	(1·99 ± 0·08)	(21·38)	16·88	
IV	1·85 ± 0·18	10·96		
	(1·47 ± 0·07)	(15·80)	(9·30)	
V	1·60 ± 0·23	9·48		(n = 3M + 2S)
	(1·19 ± 0·07)	(12·72)		
VI	1·45 ± 0·19	8·59		
	(——)	(——)		
VII	1·38 ± 0·16	8·18		
	(——)	(——)		
VIII	1·25 ± 0·08	7·41		
	(——)	(——)		
IX	1·20 ± 0·07	7·11		
	(——)	(——)		

Chromosomal rearrangements are associated particularly with anholocyclic aphids, *M. persicae* and its asexual offshoots provide most studied example of this phenomenon (Blackman 1985).

In *M. persicae* polymorphism is very common in natural populations which are the products of autosomal dissociation giving 2n = 13 karyotype or autosome 1,3-translocation, resulting in 2n = 12 with a marked structural heterozygosity. Takada *et al* (1978) could rear a triploid clone (2n = 18) of *M. persicae* in the laboratory which originated by the fertilization of an unreduced diploid oocyte nucleus of autosome 1,3-translocation variety, by a normal haploid gamete. Morphologically it differed from the diploid form having a broader, more globose shape and thicker and shorter appendages and biologically it is less fecund. Such triploid clone if exists in nature and accordingly collected, might get a status of a new species unless the knowledge of its origin and cytology is known. In the field of agriculture, horticulture, forestry, misidentification of aphids may seriously affect the control strategies. Here we can cite the example of cytological separation of *Rubus* feeding *Amphorophora* species which were morphologically identified as one and the same species (Blackman *et al* 1977). Karyological investigation in taxonomic studies therefore seems logical.

In this connection it may be pointed out that DNA determination can be applied for further karyotype analysis since the amount of DNA/genome is usually constant and characteristic for each species, yet may differ considerably between species. If the karyotype is variable with large chromosome number, comparison of DNA content may provide clues to the nature of differences. If there are differences in chromosome number between the karyotype of different species, the DNA determination will show whether higher numbers are likely to be due to polyploidy. For example, Kuznetsova (1975) compared DNA content of *Anuraphis subterranea* (n = 13) with that of *A. farfarae* (n = 6). The larger n-value of *A. subterranea* was clearly not due to

polyploidy, as the DNA value for this species was less than that for *A. farfarae*. However, such a study has not yet been attempted by Indian cytologists and the same is true for gel electrophoretic studies as well.

References

Blackman R L 1980 Chromosome numbers in the Aphididae and their taxonomic significance; *Syst. Entomol.* **5** 7–25

Blackman R L 1985 Aphid cytology and genetics; *Proc. Int. Aph. Symp.*, Jabolana 1981, Polaska Acudemia Nauk, Warszawa

Blackman R L and Eastop V F 1984 *Aphids on the world's crops: An identification and information guide* (John Wiley and Sons) p 466

Blackman R L, Eastop V F and Hills M 1977 Morphological and cytological separation of *Amphorophora* Buckton (Homoptera: Aphididae) feeding on European raspberry and blackberry (*Rubus* spp.); *Bull. Entomol. Res.* **67** 285–296

Blackman R L and Takada H 1977 The inheritance of natural chromosomal polymorphisms in the aphid *Myzus persicae* (Sulzer); *Genetica* **47** 9–15

Chattopadhyay D, Das P L and Raychaudhuri D 1982 Karyotype variation of *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae); *Entomon* **7** 441–446

Das P L, Chattopadhyay D and Raychaudhuri D 1985 Comparative karyological studies of two cinarine aphids (Homoptera: Aphididae) from Kashmir valley; *Entomon* **10** 317–322

Eastop V F 1966 A taxonomic study of Australian Aphidoidea (Homoptera); *Aust. J. Zool.* **14** 399–592

Eastop V F 1973 Biotypes of Aphids; *Bull. Entomol. Soc. N.Z.* **2** 40–51

Khuda-Bukhsh A R and Pal N B 1984 Karyomorphology of five species of aphids; *Environ. Ecol.* **2** 329–332

Khuda-Bukhsh A R and Pal N B 1986 Cytogenetical studies on aphids (Homoptera: Aphididae) from India II. Karyomorphology of five species of *Myzus*; *Entomon* **11** 135–140

Kulkarni P P and Kacker R K 1981 A simple technique for preparation of somatic chromosomes of aphids; *Bull. Zool. Surv. India* **3** 277–278

Kuznetsova V G 1969 On the chromosome number in *Myzodes persicae* (Homoptera: Aphididae); *Cytologia* **14** 399–592

Kuznetsova V G 1975 Investigation of aphids with different karyotypes by DNA cytophotometry; *Dokl. Akad. Nauk SSSR* **224** 457–459

Sun R Y and Robinson A G 1966 Chromosome studies on 50 species of aphids; *Can. J. Zool.* **44** 649–653

Takada H, Blackman R L and Miyazaki M 1978 Cytological, morphological and biological studies on a laboratory reared triploid clone of *Myzus persicae* (Sulzer); *Kontyu (Tokyo)* **46** 557–573