Chromosomal Abnormalities in an Anholocyclic Biotype of *Myzus persicae* (Sulzer)

In temperate climates the aphid *Myzus persicae* (Sulzer) often has a variable life-cycle, with the ability to produce sexuales in the autumn and lay overwintering eggs (holocyclic life-cycle), or to persist as parthenogenetic, viviparous forms throughout the year (anholocyclic life-cycle). Mild winters encourage survival of anholocyclic aphids which are potentially more dangerous as plant virus vectors. This paper concerns the discovery of chromosome abnormalities in one particular anholocyclic form of *M. persicae* which appears to be a distinct biotype, recognizable by its dark green colour and other characteristic features. This biotype has been described by several authors; it is apparently MÜLLER's[1] 'rein grüne Apteren' and WALDHAUER's[2] 'Klongruppe D', and it is equated by BÖRNER[3] with *Aphis dianthi* Schrank.

Material and method. The aphids studied were from clones of *M. persicae* established from individual field-collected virginoparae. The dark green biotype was obtained from potatoes and sugar-beet in the field in southern England, and from *Dianthus* in a glasshouse. Other material for comparison came from various sources in England and France, and from a random sample of a single field population. Metaphase chromosomes were studied in embryos from apterous virginoparae using a rapid Feulgen squash technique[4].

Result and discussion. The normal female karyotype of *M. persicae* was found to be $2n = 12$ (Figure 1a). The same number was observed by British and Canadian workers[5,6], although COGNETTI[7] has reported $2n = 14$ for *M. persicae* in Italy. The *X*-chromosome was identified from male embryos, in which it is unpaired (Figure 1b). All specimens of the dark green biotype examined had embryos with a chromosome complement of either $2n = 13$ (Figure 1c), or $2n = 14$ (Figure 1d). The former appears to differ from the normal karyotype in having 1 unpaired element ('S') and an extra pair of short autosomes, while the latter appears to have 2 unpaired elements of significantly different lengths ('S_1' and 'S_2') and 2 extra pairs of short autosomes.

On the assumption that chromosomes of similar length are homologous, relative lengths of the paired and unpaired chromosomes in the abnormal karyotypes are compared with the normal karyotype in Table I. The ratio of the summated lengths of *X*-chromosomes to autosomes remains similar in all forms, indicating a change which involves the autosomes only. The simplest hypothesis to account for the abnormal karyotypes is that a break has occurred in 1 of the autosome pair A3

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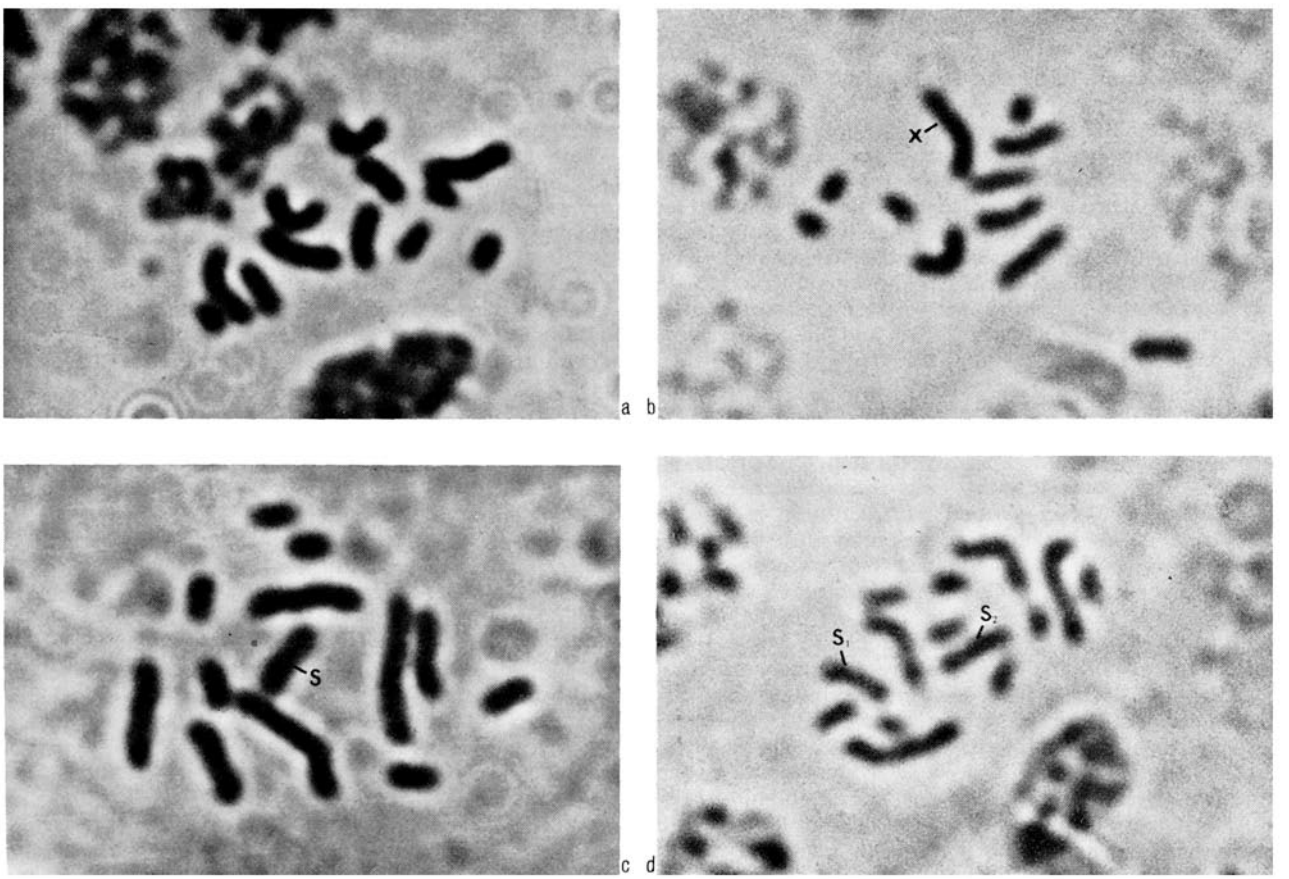

Fig. 1. a) Normal female karyotype of *M. persicae*, $2n = 12$. b) Male karyotype with unpaired *X*-chromosome. c) Karyotype of dark green, anholocyclic form, $2n = 13$. d) Karyotype of dark green, anholocyclic form, $2n = 14$.

of the normal karyotype to give the $2n = 13$ karyotype, and in 1 of both the A2 and A3 pairs to give the $2n = 14$ karyotype (Figure 2). Relative lengths of chromosomes in the abnormal karyotypes can be arranged to fit this hypothesis statistically (Table II).

This is apparently the first record of naturally occurring chromosome abnormalities in an aphid species. A similar chromosome break, but in both members of a pair, has been postulated as a mechanism of speciation in the genus *Anuraphis*, to account for anomalous chromosome numbers in 2 species[8]. Chromosome fragmentation per se is not generally accepted as a method by which a stable new configuration can arise[9], and perhaps some more complex structural rearrangement is involved. However, there has been of little critical work on species with holocentric chromosomes, in which fragmented chromosomes can still behave normally during somatic mitosis. This has been demonstrated in coccids, where chromosome fragments induced by irradiation of somatic cells have been maintained through many mitotic cycles[10]. Spontaneous fragmentation of chromosomes in coccids has also been described, and in at least one case (*Selenaspidus incisus*) fragments were transmitted from one parthenogenetic generation to the next[11]. The abnormal karyotypes in *M. persicae*, whether or not they are due to simple fragmentation, are reproduced faithfully not only

Table I. Relative chromosome lengths in *Myzus persicae*, assuming chromosomes of similar length to be homologous

Chromosome	Total chromosome length (%)					
	Normal karyotype	S.E.	Dark green biotype (a)	S.E.	Dark green biotype (b)	S.E.
X	14.15	±0.34	14.45	±0.13	13.37	±0.22
X	14.15		14.45		13.37	
A1	11.08	±0.26	10.30	±0.10	11.12	±0.20
A1	11.08		10.30		11.12	
A2	8.70	±0.09	8.66	±0.08	5.21	±0.09
A2	8.70		8.66		5.21	
A3	7.95	±0.20	4.74	±0.06	4.57	±0.10
A3	7.95		4.74		4.57	
A4	4.21	±0.07	4.18	±0.05	3.88	±0.07
A4	4.21		4.18		3.88	
A5	3.85	±0.09	3.68	±0.05	3.49	±0.08
A5	3.85		3.68		3.49	
S_1	–		8.00	±0.28	9.26	±0.22
S_2	–		–		7.69	±0.25
Ratio *XX*/AA	0.395		0.407		0.364	

Table II. Relative chromosome lengths in *Myzus persicae*, arranged in accordance with the hypothesis that fragmentation of A2 and A3 autosomes gives the abnormal karyotypes of the dark green form

Chromosome	Total chromosome length (%)				
	Normal karyotype	Dark green biotype (a)	S.E.	Dark green biotype (b)	S.E.
X	14.15	14.45	±0.13	13.37	±0.22
X	14.15	14.45		13.37	
A1	11.08	10.30	±0.10	11.12	±0.20
A1	11.08	10.30		11.12	
A2	8.70	8.66	±0.08	9.26	±0.22
A2	8.70	8.66		(5.38 + 3.62) = 9.00	±0.16
A3	7.95	8.00	±0.28	7.69	±0.25
A3	7.95	(4.88 + 3.49) = 8.37	±0.12	(5.04 + 3.35) = 8.39	±0.17
A4	4.21	4.44	±0.06	4.57	±0.10
A4	4.21	4.44		4.57	
A5	3.85	3.97	±0.06	3.88	±0.07
A5	3.85	3.97		3.88	

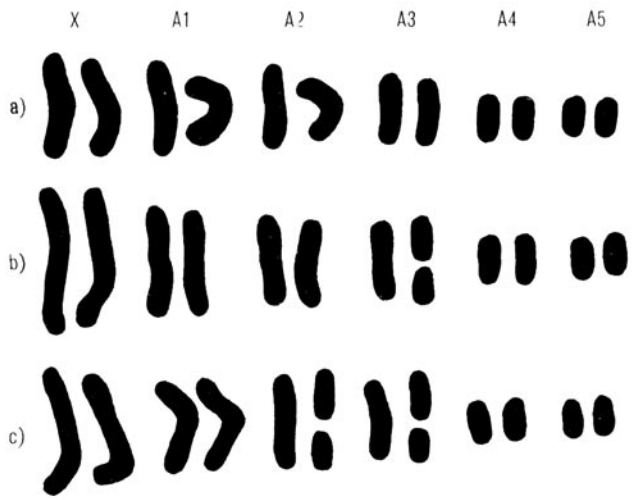

Fig. 2. a) Normal female karyotype of *M. persicae*, pairing assumed homologs. b) Chromosomes of $2n = 13$ karyotype, arranged according to the hypothesis that it has arisen from the normal form by chromosome breakage. c) Chromosomes of $2n = 14$ karyotype, arranged according to the same hypothesis.

Table III. Association of karyotype with a morphometric character in apterous virginoparae of *Myzus persicae* from various clones reared at 20 °C

Origin of clone	Colour	Karyotype of embryos	Number of aphids measured	Antennal seg. VI Processus terminalis / Base
Silwood Park (holocyclic)	normal	$2n = 12$	70	3.86 ± 0.02
Silwood Park (anholocyclic)	normal	$2n = 12$	80	3.83 ± 0.02
Bangor	dark green	$2n = 12$	19	3.99 ± 0.05
Trumpington (anholocyclic)	dark green	$2n = 13$	20	4.77 ± 0.07
Silwood Park (anholocyclic)	dark green	$2n = 13$	20	4.55 ± 0.05
Silwood Park (anholocyclic)	dark green	$2n = 14$	18	4.57 ± 0.08

through successive mitoses but through successive generations of parthenogenetic individuals. The unpaired elements may, however, constitute a barrier to gametogenesis, and this could be the direct cause of the anholocyclic character of the biotype.

There are clear phenotypic differences between aphids with normal and abnormal karyotypes. Colour is not a reliable character, as the intensity of pigmentation varies with age, food and temperature, and some dark green clones are cytologically normal. However at constant temperature a simple morphometric ratio, that of the lengths of the processus terminalis to the base of antennal segment VI (WALDHAUER[2]), is a reliable character for distinguishing aphids of abnormal karyotype. In both $2n = 13$ and $2n = 14$ forms this ratio is larger than in the normal form, with scarcely any overlap (Table III). No morphological differences were detected between aphids with $2n = 13$ and $2n = 14$ karyotypes. This may indicate that the $2n = 13$ form is a relatively old parthenogenetic line which has had time to evolve a characteristic phenotype, and that the $2n = 14$ form arose from it only relatively recently[12].

Résumé. Le karyotype normal de *Myzus persicae* (Sulzer) provenant de diverses sources fut de $2n = 12$. Une forme anholocyclique verte de cette espèce possède un karyotype de $2n = 13$ ou $2n = 14$, avec 1 ou 2 autosomes non appariés. Une comparaison des dimensions relatives des chromosomes des karyotypes normaux et anormaux suggère que ces derniers dérivent des chromosomes par fragmentation.

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Mutagenic Effect of UV-Light and X-Rays on *Streptomyces nigrifaciens* and Yield of the Antifungal Substance

The antibiotic production by micro-organisms can be enhanced by the production of suitable strains. KELNER[1], SAVAGE[2], DULANEY et al.[3], DULANEY[4], HOVARTH[5] induced mutants of *Streptomyces* species by irradiation giving higher yield of antibiotics. A strain of *Streptomyces nigrifaciens* Waks. isolated from soil was found antagonistic to *Colletotrichum capsici* and a few other fungi (GUPTA[6]). The present investigation reports the effect of UV-light and X-rays in *S. nigrifaciens* on the survival and induction of mutants in view of the production of the antifungal substance.

Single spore suspension of *S. nigrifaciens* was obtained following KELNER's method[7]. 4 ml of the suspension was distributed in sterilized bacterial tubes and exposed to UV-light and X-rays for 5, 10, 15 and 20 min. X-ray irradiation was done from Cu-anticathode Machellet tube at 30 K.V.-10 M.A. and for exposure to UV-light, the suspension was transferred to a small watch glass. The irradiated spores were immediately plated out in 5 replicates on Czapek's agar and incubated for 10 days at 28 °C (± 2 °C). Control plates in equal number were run simultaneously under similar conditions.

Several colonies showed morphological differences but after repeated sub-culturing, only 2 mutants remained stable which were tested further for the production of the antifungal substance on yeast extract-glucose-asparagine medium. The antifungal substance was assayed by the spore germination test of *Colletotrichum capsici* (GUPTA[6]).

The effect of UV-light and X-rays on the survival of *S. nigrifaciens* has been presented in Table I. It is evident that the percentage of spore survival decreased with the increase in exposure time. The results show that the spores of *S. nigrifaciens* are more resistant to UV-light than to X-rays.

The 2 mutants selected for the production of the antifungal substance were: 1. Asporogenous mutant: Light brown to yellow in colour, slimy and transparent, non-sporulating. 2. Pigmented mutant: Vegetative growth thick, colonies slightly raised with abundant grey aerial mycelium producing dark brown soluble pigment on Czapek's agar. The production of the antifungal substance by these two mutants is presented in Table II.

It is evident from Table II that the asporogenous mutant has lost its capacity to produce antifungal substance, whereas the pigmented one showed slight increase over the parent culture.

UV-light and X-rays have long been used for irradiation to obtain mutants. According to HOLLAENDER[8], UV-light is supposed to produced gene mutation while X-rays cause predominantly chromosomal aberrations and breaks. In the present investigation 2 stable mutants were obtained. The spores of *S. nigrifaciens* are more

Table I. Effect of UV-light and X-ray irradiation on the survival of spores of *S. nigrifaciens*

Exposure time (min)	UV-light No. of spores survived	Survival (%)	X-rays No. of spores survived	Survival (%)
5	166.00	71.24	81.60	34.37
10	120.00	51.84	25.60	10.78
15	78.00	33.47	14.80	6.23
20	47.20	20.25	5.80	2.40
Control	233.00		237.40	

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