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Lack of detectable genetic recombination on the X chromosome during the parthenogenetic production of female and male aphids

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Summary

We used polymorphic microsatellite markers to look for recombination during parthenogenetic oogenesis between the X chromosomes of aphids of the tribe Macrosiphini. We examined the X chromosome because it comprises $\sim 25\%$ of the genome and previous cytological observations of chromosome pairing and nucleolar organizer (NOR) heteromorphism suggest recombination, although the same is not true for autosomes. A total of 564 parthenogenetic females of *Myzus* clones with three distinct reproductive modes (cyclical parthenogenesis, obligate parthenogenesis and obligate parthenogenesis with male production) were genotyped at three informative X-linked loci. Also, parthenogenetically produced males from clones encompassing the full range of male-producing reproductive strategies were genotyped. These included 391 *Myzus persicae* males that were genotyped at three X-linked loci and 538 males from *Sitobion* clones that were genotyped at five informative X-linked loci. Our results show no departure from clonality in parthenogenetic generations of aphids of the tribe Macrosiphini: no recombinant genotypes were observed in parthenogenetically produced males or females.

1. Introduction

Genetic recombination is a phenomenon generally associated with meiosis in sexually reproducing organisms. However, recombination in mitotic cells has been reported for several species, ranging from placental mammals (Cornforth & Eberle, 2001; Svetlova *et al*., 2001) to insects (Stern, 1936; Bartsch *et al*., 1997) and yeast (Huang & Keil, 1995). For example, in the yeast *Saccharomyces cerevisiae*, the recombination hotspot *HOT1* initiates mitotic recombination when inserted into novel locations throughout the genome (Huang & Keil, 1995).

In aphids, development of the parthenogenetic egg is essentially mitotic, even though the maturation division is equivalent to the first division of meiosis in sexually reproducing animals, and results in the formation of a single polar body (Blackman, 1978). Genetic recombination during parthenogenesis (termed 'endomeiosis') in aphids was suggested by Cognetti (1961) but various authors have since published data invalidating this concept. Blackman (1979) and Tomiuk & Wöhrmann (1982) gave evidence that the phenomenon does not generally occur, on the basis of selection experiments and allozyme investigations with large sample sizes. However, low levels of recombination during parthenogenetic oogenesis might not have been detected by the experiments mentioned above, because these studies used few markers of unknown genome location and low variability. Yet several lines of evidence suggest that recombination occurs during parthenogenetic oogenesis, specifically of aphid X chromosomes. Most of these concern nucleolar organizer regions (NORs) or ribosomal DNA (rDNA) arrays. NORs contain the genes that code for rRNA and are located on the terminal ends of the X chromosomes in most aphids. Interestingly, intra- and

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Table 1. *List of clones and their reproductive modes. The 'Female' and 'Male' columns list the number of female and male progeny genotyped for each clone. The alleles of each locus are listed in their X-chromosome haplotype association for each clone determined by the genotyping of male progeny. Allele sizes are in base pairs. It was not possible to determine the X-chromosome haplotypes for clone 014 because it cannot produce males*

						Microsatellite loci				
Species	Clone	Lifecycle	Female	Male	X	*myz3*	*myz25*	*M27*	*M86*	*S17b*
Myzus persicae	003	andro	186	123	Xa	121	119	–	123	168
					Xb	111	123	–	097	168
	025	holo		22	Xa		119		137	
					Xb		121		131	
	034	holo	189	188	Xa	123	119	266	123	168
					Xb	125	121	266	135	168
	067	holo		20	Xa		123		135	168
					Xb		119		137	168
	068	holo		38	Xa		119		097	
					Xb		121		131	
Myzus antirrhinii	014	anholo	189		Xa					
					Xb					
						Sm11	*S10*	*S17b*	*S49*	*S43ii*
Sitobion avenae	Sa2	andro		12	Xa		094			122
					Xb		088			096
	Sa22	andro		10	Xa		094	202	096	
					Xb		088	216	138	
	Sa25	andro		6	Xa		094		122	
					Xb		088		096	
	Sa26	andro		44	Xa		088	204	116	
					Xb		094	208	096	
	Sa30	andro		8	Xa			210	130	
					Xb			218	124	
	Sa35	andro		8	Xa		094		122	
					Xb		088		096	
	BB22	andro		7	Xa	149		210	096	
					Xb	144		220	120	
	Sa5	holo		9	Xa		088		124	
					Xb		094		122	
	Sa23	holo		22	Xa		094	206	096	
					Xb		088	204	116	
	Sa48	holo		33	Xa	144		218	132	
					Xb	149		216	122	
	BB7	holo		13	Xa		094		124	
					Xb		088		122	
	Chav3	holo		22	Xa	148	088	218	134	
					Xb	144	118	200	096	
	Lec14	holo		4	Xa			204	096	
					Xb			218	136	
	V'dA16	holo		7	Xa	148	088	208	096	
					Xb	149	096	218	130	
	V'dA25	holo		33	Xa	148			096	
					Xb	144			116	
	Sa39	inter		3	Xa		088	216	134	
					Xb	118		198	096	
	Sa43	inter		4	Xa	094			122	
					Xb	088			096	
	Sa50	inter		10	Xa	148	094	218		
					Xb	144	088	216		
	BB54	inter		16	Xa	149	092	206	102	
					Xb	144	118	200	096	
	Herou3	inter		20	Xa		088	218		
					Xb		094	216		
Sitobion miscanthi	Sm195	holo		97	Xa		096		130	227
					Xb		120		112	229

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Table 1 (*Cont*.)

Species	Clone	Lifecycle	Female	Male	X	Microsatellite loci				
						Sm11	*S10*	*S17b*	*S49*	*S43ii*
Sitobion near *fragariae*	Snf17	holo		150	Xa	156	092	232	173	
					Xb	160	102	267	163	

Abbreviations: andro, androcyclic; holo, holocyclic; anholo, anholocyclic; inter, intermediate strategy in which the clone overwinters both by reproducing sexually and by continuous parthenogenetic reproduction.

interchromosomal exchanges also occur between rDNA arrays during mitosis in *Daphnia pulex*, a freshwater cladoceran that possesses, as do many aphid species, clones capable of either cyclic or obligate parthenogenesis (Crease & Lynch, 1991). The first indications that recombination of aphid X chromosomes might occur were the observations of Orlando (1974) and Blackman & Hales (1986) of an end-to-end association between the X chromosomes in developing parthenogenetic oocytes of *Megoura viciae* and *Amphorophora tuberculata*, respectively. Blackman & Hales (1986) suggested that this association might indicate terminalized chiasmata, raising the possibility of genetic exchange (Hales *et al*., 1997). Further, during male production, the X chromosomes remain together throughout prophase, linked by a large nucleolus-like body. At metaphase, this results in an XX bivalent (Blackman & Hales, 1986). This bivalent undergoes a 'mini-meiosis', again suggesting the possibility of exchange of genetic material (Blackman & Spence, 1996).

The second line of evidence is heteromorphism of X-linked NORs (Blackman & Spence, 1996; Mandrioli *et al*., 1999*a*, *b*). Mandrioli *et al*. proposed that the heteromorphism (observed both within clones and within individuals) was a consequence of unequal crossing over (recombination) (Mandrioli *et al*., 1999*b*), although it could result from sister-chromatid exchange (Blackman, 1979; Blackman & Spence, 1996). Third, structures interpreted as argentophilic bridges have been observed connecting the X chromosomes in mitotic metaphase of somatic cells (Mandrioli *et al*., 1999*a*, *b*). This somatic pairing of sex chromosomes via nucleolar material could presage a mechanism permitting recombination during the maturation division of the egg. Fourth, Mandrioli *et al*. (1999*a*) argued that some sequences within rDNA intergenic spacers of aphids show high sequence similarity with the consensus core region of human hypervariable minisatellites and microbial sequences, which are known hotspots of recombination in these species. However, these are very short sequences (10 bp) and it is possible that the similarity is due to chance.

Genetic studies of continuously parthenogenetic aphids from the field in general conform with the proposition that recombination during parthenogenesis does not occur (Sunnucks *et al*., 1996; Simon *et al*., 1999; Wilson *et al*., 1999; Hales *et al*., 2000; Haack *et al*., 2000). Additionally, previous laboratory studies have shown stability of the intergenic spacer of rDNA within parthenogenetic clones of aphids, despite variability in the field (Shufran *et al*., 1991; Black, 1993; Fenton *et al*., 1998).

Despite this, the cytological observations are sufficiently tantalizing to justify a specific investigation of X-chromosome recombination during parthenogenesis. Furthermore, cryptic recombination could potentially provide a means of generating genetic variation and could be a contributing factor to the persistence of obligate parthenogens (Mark Welch & Meselson, 2000; Simon *et al*., 2002). Recombination in asexuals would influence the application of most theories on the evolution of sex. For example, the Red Queen hypothesis does not require sexual recombination *per se*, only genotypic diversity (Simon *et al*., 2002). The tenet that asexual organisms can generate much less genetic variation than their sexual counterparts is fundamental to most theories of the evolution and maintenance of sex. In fact, most theories assume that asexual organisms are truly clonal (Lynch, 1984; Kondrashov, 1993). In order to evaluate the alternate groups of models for the evolution of sex (e.g. mutational models versus environmental models; see Kondrashov, 1993), it is necessary to measure parameters in well-characterized model species (Birky, 1999; West *et al*., 1999). Although the examples of NOR heteromorphism might be a consequence of rare or past sexual stages (Hales *et al*., 1997), they are circumstantial evidence of some form of parthenogenetic recombination in aphids and warrant further investigation.

In previous work, we have described development of microsatellite markers for aphids in the genera *Myzus* and *Sitobion*, and identified microsatellite linkage groups for *Myzus* and X-linkage for both genera (Sunnucks *et al*., 1996; Wilson *et al*., 1997; Simon *et al*., 1999; Wilson, 2000; Sloane *et al*., 2001). In this work, we have used these markers to look for recombination between the X chromosomes, during parthenogenetic oogenesis of both female and male-producing eggs, in different aphid species of the tribe

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Macrosiphini. We examined the X chromosome because it comprises ~25% of the genome (unpublished data) and because previous research, as outlined above, indicates that recombination might occur between germ-line aphid X chromosomes during the development of the parthenogenetic egg.

2. Materials and methods

(i) *Choice of aphid material*

Cyclical parthenogenesis (holocycly; the alternation of several parthenogenetic generations with one sexual generation) is the dominant mode of reproduction in aphids. However, some species exhibit a wide range of reproductive strategies besides cyclical parthenogenesis. These include obligate parthenogenesis (anholocycly), obligate parthenogenesis with male production (androcycly) and an intermediate strategy in which the clone over-winters both by reproducing sexually and by maintaining continuous parthenogenetic reproduction (Blackman, 1971; Dedryver *et al*., 1998). Because all aphid reproductive strategies involve a parthenogenetic phase, it is possible to maintain clones representative of all reproductive strategies in continuously parthenogenetic laboratory cultures. Here, we tried to examine aphids displaying the full range of reproductive strategies. Five species from two genera of the tribe Macrosiphini were used: *Myzus persicae*, *Myzus antirrhinii*, *Sitobion avenae*, *Sitobion miscanthi* and *Sitobion* near *fragariae*.

(ii) *Aphid breeding*

Three *Myzus* clones (*M. persicae* clones 003 and 034 and *M. antirrhinii* clone 014) were used to look for recombination between the X chromosomes during the parthenogenetic production of females. Each of these clones uses a different reproductive strategy (Table 1). In addition, 27 clones, representing the three reproductive strategies that involve male production, were used to look for recombination between X chromosomes during the parthenogenetic production of males. These include five *M. persicae* clones, 20 *S. avenae* clones and one clone of each of *S. miscanthi* and *S.* near *fragariae* (Table 1).

(*a*) *X-chromosome recombination in parthenogenetic production of females.* *Myzus* clones were maintained in synchronous culture on individual cabbage seedlings (var. Early Jersey Wakefield) at 20 °C at a photoperiodic regime of 16L:8D (hours of light: hours of dark). Under these conditions, the aphids reproduce by continuous parthenogenesis. Once they were adult, female offspring of successive generations of each clonal lineage were collected. 186 female offspring of clone 003, 189 of clone 014, and 189 of clone 034 were collected for genotyping at all available, informative X-linked loci (Table 1).

(*b*) *X-chromosome recombination in parthenogenetic production of males.* To induce the parthenogenetic production of males in *M. persicae*, adult females of clones 003, 034, 025, 067 and 068 were transferred from short night conditions (above) to long nights (8L:16D or 10L:14D) at 15 °C (Hales *et al*., 1989). Males of *S. avenae* were induced according to a procedure described in Dedryver *et al*. (1998), and *S. miscanthi* and *S.* near *fragariae* males were induced by transferring females from short nights to long nights (8L:16D at 15 °C). The numbers of males collected from each clone are detailed in Table 1.

Aphids show XX/XO (female/male) sex determination and, typically, a pool of sons from a given parthenogenetic mother contains 50% sons with each of the mother's X chromosomes (Wilson *et al*., 1997; Wilson, 2000). Thus, in the absence of recombination, two genotypic classes of sons are expected from each mother, whereas, in the case of recombination, there would be more than two.

(iii) *DNA extraction and microsatellite genotyping*

Whole aphids were crushed in 50 μl of 5% Chelex® 100 resin (Bio-Rad) (w/v in 10 mM Tris pH 8, 0·1 mM EDTA, pH 8) and then boiled for 10 min. Samples were then pulse centrifuged. Supernatant (1 μl) was used as template in standard isotopic polymerase chain reactions (PCRs), as described in Sloane *et al*. (2001) for the *Myzus* clones and in Wilson (2000) for the *Sitobion* clones. All individuals of each clone were genotyped at each heterozygous X-linked locus. We currently have five X-linked markers in both *M. persicae* (*myz3*, *myz25*, *M27*, *M86* and *S17b*) (Sloane *et al*., 2001) and the genus *Sitobion* (*Sm11*, *S10*, *S17b*, *S49* and *S43ii*) (Wilson, 2000). However, not all markers were useful for detecting recombination in all clones, and *M27* was not used for technical reasons. Informative heterozygous loci for each clone are listed in Table 1.

(iv) *Test for linkage between X-linked markers*

Two-point linkage analysis was performed on Australian crosses and a pedigree of European *M. persicae* (Sloane *et al*., 2001) using the program LINKMFEX (Version 1.5) (R. G. Danzmann). Analysis was performed on the maternal line only, because there can be no X-chromosome recombination in males (only one X chromosome). For each pairwise comparison, the LOD (likelihood of the odds calculated by the method of maximum likelihood) scores were calculated for individual families, as well as the ratio of recombinant and non-recombinant offspring genotypes summed across each family. The sample size, number of

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Table 2. *Two point linkage analysis performed on X-linked microsatellite markers in female Myzus persicae*

	M86	*myz25*	*myz3*	*S17b*
M86	–			
myz25	$N = 31$ (**38**) $\theta = 0{\cdot}45$ (**0·45**) $Z = 0{\cdot}06$ (**0·09**)	–		
myz3	$N = 73$ $\theta = 0{\cdot}01$ $Z = 19{\cdot}7$	$N = 30$ $\theta = 0{\cdot}47$ $Z = 0{\cdot}03$	–	
S17b	$N =$ **27** $\theta =$ **0·33** $Z =$ **0·66**	$N =$ **27** $\theta =$ **0·44** $Z =$ **0·07**	– – –	–

Linkage analysis was performed on Australian crosses and a European pedigree (bold) of *Myzus persicae* (Sloane *et al.*, 2001). N is the number of offspring summed across each family, θ is the recombination value giving the maximum LOD score and Z is the maximum LOD score. '–' represents an uninformative comparison (one locus homozygous). For *M86–myz3*, only one X-chromosome recombinant offspring genotype was observed.

families and maximum LOD score for each pairwise comparison are shown in Table 2.

3. Results and discussion

(i) *No evidence of X-chromosome recombination during parthenogenesis*

At each of the X-linked loci, the 564 parthenogenetic females had heterozygous genotypes identical to those of their mothers – their X chromosomes were unaffected by recombination. Similarly, the 929 parthenogenetically produced males showed no signs of recombination – they always inherited one of two possible X-chromosome haplotypes (Table 1). Thus, it appears that recombination during the parthenogenetic production of both female and male aphids is not a source of genetic variation. If such recombination does occur, it is very rare.

This raises questions about the behaviour of X chromosomes during parthenogenetic oogenesis (Orlando, 1974; Blackman & Hales, 1986). Blackman & Spence (1996) reported that rDNA can be concentrated on only one X chromosome in obligately parthenogenetic aphids and proposed that the terminalized chiasma suggested by Blackman & Hales (1986) and common to the parthenogenetic development of both male and female oocytes provides a possible mechanism. That is, the concentration of rDNA on one X chromosome is the result of unequal crossing over between the X chromosomes. However, we find no evidence of X chromosome recombination. The observations of Blackman & Spence (1996) and those presented in this paper are not mutually exclusive. Ribosomal DNA is located in a telomeric or subtelomeric position on the X chromosomes of most aphids (Blackman & Spence, 1996). Although we have some data on linkage between the X-linked microsatellite loci used in this research (Table 2), their exact positions on the X chromosome are unknown. It is likely that they are not located in the telomeric or subtelomeric region of the X chromosomes.

We are convinced by the evidence that aphids can show recombination and non-equal exchange either between homologues or between sister chromatids within rDNA arrays during parthenogenetic reproduction (Mandrioli *et al.*, 1999*b*; Blackman & Spence, 1996). However our results indicate that this might be a peculiarity of that important functional region rather than a general process over the whole X chromosome. As outlined in the introduction, cytological studies suggest that X chromosomes are much more likely to be affected by any recombination during parthenogenetic oogenesis than are autosomes. There are no reported indications of recombination in aphid autosomes during parthenogenetic oogenesis, apart from those of Cognetti (1961 and subsequent papers). Cognetti's work was based on sectioned material, which can be highly misleading when considering chromosome distribution and behaviour. Overall, our data suggest that recombination on the X chromosome is, at most, rare and localized in aphids during parthenogenetic oogenesis, regardless of reproductive strategy. Other mechanisms might generate genetic variation in the absence of true meiosis. For example, unequal sister-chromatid exchange or replication slippage will give novel DNA complements on individual chromosomes (Blackman, 1979) but these processes cannot be addressed by the methods used here.

(ii) *Is the lack of recombination a result of closely linked markers?*

We were able to investigate the linkage of the *M. persicae* markers using the pedigreed material described in Sloane *et al.* (2001). All locus pairs, with the exception of *myz3* and *M86*, are freely segregating in females (Sloane *et al.*, 2001). Additionally, all X-linked *Sitobion* markers appear to be freely segregating (Wilson, 2000). Thus, there is little likelihood that the absence of recombination during parthenogenesis in aphids is caused by close linkage of the markers we used.

(iii) *Conclusion*

Thus, we reaffirm the conclusions of Blackman (1979) and Tomiuk & Wöhrmann (1982). Apomixis in aphids

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produces daughters that are genotypically identical to their mothers and sisters. Sons are also truly clonal except for the random inheritance of one of two maternal X chromosomes (Wilson *et al*., 1997; Wilson, 2000). Clonal identity, however, is known to be altered by mutation and chromosomal rearrangements, and might also be affected by rare unequal sister-chromatid exchanges and possible chiasmata in the telomeric rDNA arrays. This accords with findings for other parthenogenetic groups, such as *Daphnia* (Crease & Lynch, 1991), ostracods (Schon *et al*., 1998) and rotifers (Arkhipova & Meselson, 2000; Mark Welch & Meselson, 2000), all of which show life-cycle characteristics similar to those of aphids. In the Class Bdelloidea (Phylum Rotifera), sexual reproduction has never been found. Sequence divergence analysis shows that this metazoan taxon has evolved entirely asexually over the past several million years (Mark Welch & Meselson, 2000).

Our work further validates aphids as a good model for investigating the evolution of sex: their life cycle incorporates amphimixis (sexual reproduction) with much female recombination (Sloane *et al*., 2001) and apomictic reproduction with no recombination (present work).

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