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Segregation of autosomes during spermatogenesis in the peach-potato aphid (*Myzus persicae*) (Sulzer) (Hemiptera: Aphididae)

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Summary

Most aphids are cyclic parthenogens, so are ideal models in studies of the mechanisms and consequences of sex and recombination. However, owing to a shortage of physical and genetic markers, there have been few studies of the most fundamental genetic processes in these organisms. For example, it is not known whether autosomal segregation during male spermatogenesis is in Mendelian proportions: we address that question here. The aphid *Myzus persicae* has a typical karyotype of 2n = 12 in females (XX), while males are XO (2n = 11). During male meiosis, only the spermatocytes with an X chromosome are viable. We hypothesized that assortment of autosomes might be non-random because chromosomal imprinting leading to elimination of the paternal autosomes is seen in the closely related coccoids. In other aphid models, we have observed segregation distortions at single microsatellite loci (Wilson, 2000). Such distortions may have nothing to do with 'selfish' behaviour, but may be caused by mutation accumulation causing fitness differentials. Thus single-locus distortions might be predicted to be more likely to be detected via the male lines of clones that have lost the ability to reproduce sexually (male-producing obligate parthenogenesis (androcyclic)). Using microsatellites we show that genetic imprinting or selfish autosome behaviour does not occur in male *M. persicae*. Generally, loci segregated in Mendelian proportions in both sexes of cyclically parthenogenetic (holocyclic) clones. However, in androcyclic clones, segregation distortions consistently involved the same two autosomes. This is consistent with linkage of markers to deleterious mutations associated with a loss of sexual reproduction.

1. Introduction

Aphids (Hemiptera: Sternorrhyncha: Aphidoidea) and scale insects (Hemiptera: Sternorrhyncha: Coccoidea) are sister groups (Carver *et al.*, 1991). Many coccoids exhibit paternal genome elimination (PGE), the inactivation or, in some cases, total or partial physical elimination of the paternal genome of all males (see reviews by Hughes-Schrader, 1948; Brown & Nelson-Rees, 1961; Nur, 1990). Haig (1993) proposed a model for the evolution of PGE. In this model meiotic drive by the X chromosome caused female-biased sex ratios and the maternal set of autosomes in males was postulated to become effectively X-linked so as to exploit X-drive. He alluded to the possibility that a similar model might explain other unusual chromosome systems such as those in aphids.

Like some primitive scales, aphids have XX–XO sex determination, and have achiasmate spermatogenesis (cf. Herrick & Seger, 1999). Furthermore, aphids have a peculiar form of spermatogenesis in which the two groups of autosomes conduct what looks like a tug-of-war for the single X chromosome during anaphase I. At this time, one set of autosomes is visibly more heterochromatic than the other. The autosomes that finally capture the X chromosome survive to contribute to the next generation: those that fail are eliminated since only X-bearing sperm develop. Hence, the sex ratio arising from developing

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eggs favours females absolutely. Hamilton (1967) noted that any X-linked gene that caused all sperm to be X-bearing would drive the population extinct because no males would be produced. This constraint does not apply to aphids since male determination is essentially a mitotic phenomenon occurring in the ovaries of parthenogenetic females, under control of hormones entrained by abiotic conditions (Hales & Mittler, 1987). It seemed feasible that aphid autosomes might have some feature that determined their inclusion in the viable sperm, whether that feature was genetic or epigenetic (for example as a result of genomic imprinting). Imprinting is the marking of a particular part of a genome so that it is expressed differently from other parts. Our general prediction is that the paternal genome would be excluded. Further, male genome dispensability, rather than female, seems to be common and widespread, for example during hybridogenesis and gynogenesis in fish (W. Atmar, personal communication).

The paternal genome in scales appears to be imprinted after entering the egg but before fusing with the female pronucleus, the imprinting depending on the position of a given haploid genome in the egg cytoplasm (Chandra & Brown, 1975). While this process does not occur in the most primitive scales, we hypothesized that, because of the close relationship between scales and aphids, the preconditions existed for a similar development of paternal genome imprinting in aphids. Hales (1989) described an extreme case of irregular X-autosome chromosomal behaviour in an aphid. In *Schoutedenia ralumensis* (formerly *lutea*), there are two pairs of X chromosomes (X1X1–X2X2), and in females, two autosomes are physically attached, one to one X1, and the other to one X2 (X1AX1–X2AX2). Males receive both the Xs with attached autosomes, and the unattached Xs are lost at male determination of the oocyte. Pairing behaviour during male meiotic prophase indicates that these autosomes are homologues. One of the X chromosomes loses its autosomal material at late anaphase. It is not clear whether it is always X1, always X2, or random with respect to the X chromosome. Equally, it is not clear whether one member of the autosomal pair rather than the other is lost, or whether this is random. Haig (personal communication, 1991) suggested that upon fertilization the paternal X-attached A would detach and then attach itself to the maternal X that had previously lacked an attached A. In the absence of genetic markers for *Schoutedenia*, we hypothesized that in more 'normal' aphids, an association of one or more autosomes with the X might occur ('selfish autosome' behaviour). If this were so, allele frequencies, genetic correlations and evolution would be profoundly affected.

Selfish autosome behaviour in males could involve (i) homologous autosome pairs, with one member of the pair inherited from the father at the expense of the other, or (ii) associations between non-homologous autosomes. Using microsatellite markers we investigated whether there was either type of selfish autosome behaviour during spermatogenesis in *Myzus persicae*. Since spermatogenesis is achiasmate, segregation distortions were sought by screening autosomal loci in eggs fertilized by fathers bearing alleles different from those of the mother. Imprinting could be detected by the same means. If distortions were found, the mechanism would then need investigation. We considered also the possibility that the particular X chromosome present in a male (a random draw of the two in his mother) might influence the segregation of autosomes in spermatogenesis, i.e. that one X chromosome might be a more successful driving X than the other.

2. Materials and methods

(i) *Aphid breeding*

Maintenance of parthenogenetic cultures, induction of sexuals and egg collection is described elsewhere (Hales *et al*., 1989; Sloane *et al*., 2001). Briefly, in each replicate cage, single male aphids were mated with three to five females of a clone. Between two and 11 replicate cages were set up for each cross between two selected clones (Table 1). The number of replicate cages was subject to the availability of sexual forms. Clones used in each cross were chosen so that for a maximum number of loci, the alleles passed on to the progeny by the male parent could be distinguished from those of the female parent. Clones of two reproductive strategies, cyclic parthenogenesis (holocyclic) and male-producing obligate parthenogenesis (androcyclic), and two karyotypes, autosomal 1,3 translocated ($2n = 12$) and karyotypically normal ($2n = 12$), were used in crosses. Crosses consisted of combinations between seven holocyclic and three androcyclic clones (Table 1).

The allelic diversity of Australian *M. persicae* capable of sex is sufficiently low that clones cannot be found to generate crosses that are maximally informative at all loci (Wilson, 2000). Loci genotyped were those for which the male parent was heterozygous but the female parent was (i) homozygous with no alleles in common with the male, (ii) heterozygous with no alleles in common with the male, (iii) heterozygous with one allele in common with the male, or (iv) homozygous with the male heterozygous for the female's allele.

(ii) *Microsatellite genotyping*

The collection of eggs, DNA extraction from eggs and polymerase chain reaction (PCR) conditions for

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microsatellite markers are described in Sloane *et al.* (2001). To avoid bias results, DNA was extracted only from fertile eggs. Fertile eggs are black and glossy. Fertile eggs are easy to distinguish from clear, green or shrivelled brown non-viable eggs, which were tested and fail to amplify in PCRs. DNA was extracted from adults by salting-out (Sunnucks & Hales, 1996). The adults of a cross were genotyped alongside the eggs to ensure that contamination had not occurred during rearing of the sexual forms, and to allow easy comparison of allele sizes between parents and offspring. We have previously identified an X chromosome and four autosomal linkage groups: (i) myz3^{X}-myz25^{X}-M27^{X}-M86^{X}-s17b^{X}, (ii) myz2^{A}-myz9^{A}-S16b^{A}, (iii) M35^{A}-M63^{A}-M107^{A}, (iv) M37^{A}-M49^{A}-M55^{A}-M62^{A} and (v) M40^{A} (^{A} = autosomal locus, ^{X} = X linked locus) (Sloane *et al.*, 2001).

Eggs were genotyped at all informative autosomal loci. Male parents were also genotyped with informative X-linked microsatellite markers to determine which X chromosome haplotype he inherited from his mother. The X chromosome each male possessed was arbitrarily designated as Xa or Xb. We could then see whether there was a positive correlation between the autosomal alleles and the particular X chromosome inherited by the offspring from the father.

(iii) *Statistical analysis*

(a) *Single locus analysis*. To examine selfish inheritance of autosomes during spermatogenesis, only heterozygous loci in males could be informative, say AB, but female parents could be (i) CC, (ii) CD, (iii) AC, (iv) BC, (v) AA or (vi) BB (each letter pair represents the two alleles at the same single microsatellite locus). First, for each replicate cage in each cross, the two alleles inherited by the offspring from the male parent were counted at each locus. Heterogeneity χ^2 tests (Zar, 1974) were performed on each cross to test against the null hypothesis that each autosome homologue segregates into spermatocytes in one-to-one Mendelian ratios. In the absence of heterogeneity, the values in each replicate were then pooled for each cross, and χ^2 tests with Bonferroni correction (Sokal & Rohlf, 1995) were performed on these pooled values. If segregation of alleles is conforming to Mendelian expectations, the null hypothesis is that male alleles A and B will be found equally represented in the offspring of each replicate cage, as will the female alleles. Second, since recombination does not occur in males, but it does in females (Sloane *et al.*, 2001), the segregation of loci in females was compared in an identical way to that described for males. This approach allowed us to distinguish between whole chromosome and single-locus effects. We note that this difference in recombination would be confounded by any sex differences in selfish segregation.

(b) *Autosome allele + X haplotype associations in males*. Heterogeneity χ^2 tests were also used to see whether either allele at each autosomal locus preferentially segregated with the male parent's Xa or Xb chromosome. In the absence of heterogeneity, at each locus, the autosome allele – X haplotype associations in each cross were pooled across replicates, and for each autosomal locus χ^2 tests were performed to see whether the following four categories occurred in equal proportions: allele 1–Xa, allele 1–Xb, allele 2–Xa and allele 2–Xb.

(iii) *Pairwise comparisons analysis*

Linkage groups have been established by analysis of autosomal segregation during male spermatogenesis. Since there is no recombination in males, loci in different linkage groups must lie on different chromosomes (Sloane *et al.*, 2001). For the males, pairwise comparisons were performed on all autosomal locus pairs known to lie on separate chromosomes. Significant allelic correlations among loci would indicate non-random segregation between pairs of non-homologous autosomes. Because egg numbers per replicate were small, offspring genotypes were pooled across replicates in each cross. Chi-square tests were performed on the ratio of genotypes.

(iv) *Multi-locus analysis*

Multi-locus analysis was performed on seven crosses involving holocyclic male clones and five crosses involving androcyclic male clones. In each cross, counts were made of each multi-locus paternal genotype. Here we are exploring multi-locus associations between the paternal autosomal loci and thus maternal alleles were not incorporated into the analysis. Each possible multi-locus genotype therefore consisted of one paternal allele at each locus. Since there is no recombination in males, only one locus from each linkage group was used (i.e. each locus essentially represents one autosome). Observed counts for each genotype were performed using the 'Find Matching Samples' option in Microsatellite Toolkit for MS Excel 97 (Stephen Park). Chi-square tests with Monte Carlo simulation (10000 simulations) (SPSS for Windows) were then performed to determine whether any multi-locus genotypes were over- or under-represented in the offspring of each cross.

3. Results

(i) *Single-locus analysis*

Selfish autosome behaviour consistent with paternal imprinting did not occur. In every cross, the male

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Table 1. *Crosses established to examine selfish autosome behaviour during male spermatogenesis and possible segregation distortions with respect to life history characteristics. Segregation of linkage groups and individual loci in male and female clones respectively are also shown*

Male	Female	No. of replicates	No. of eggs (average per replicate)	Loci genotyped	Segregation of linkage groups in males		Segregation of loci in females	
Holocyclic	*Holocyclic*							
1. 042 (N)*	031 (N)	9	39 (4)	myz2, M49	myz2	✓	myz2	✓
					M49	✓	M49	✓
2. 042 (N)*	015 (N)	10	46 (5)	M40, M49, M63, myz2	M40	✓	M40	✓
					M49	✓	M49	✓
					M63	✓	myz2	✓
					myz2	✓		
3. 033 (N)*	015 (N)	11	128 (12)	M35, M49-M55, myz2-myz9	M35	✓	M35	×
					M49-M55	✓	($^{M35}\chi^2 = 4{\cdot}9$, $P < 0{\cdot}05$)	
					myz2-myz9	✓	M49	✓
							M55	✓
							myz2	✓
							myz9	✓
4. 033 (N)*	031 (N)	2	29 (15)	M49-M55, myz2-myz9	M49-M55	✓	M49	✓
					myz2-myz9	✓	myz2	✓
							myz9	✓
5. 020 (T)*	033 (N)	5	35 (7)	M40, M37-M49, M63-M107, myz2-myz9	M40	✓	M49	✓
					M37-M49	✓	myz2	✓
					M63-M107	✓	myz9	✓
					myz2-myz9	✓		
6. 020 (T)*	031 (N)	10	101 (10)	M40, M49, M63-M107, myz9	M40	✓	M49	✓
					M49	✓	M63	×
					M63-M107	✓	($^{M63}\chi^2 = 7{\cdot}2$, $P < 0{\cdot}01$)	
							M107	✓
							myz9	✓
7. 015 (N)*	033 (N)	5	48 (10)	M40, M35-M107, M49-M55, myz2-myz9-S16b	M40	✓	M35	✓
					M35-M107	✓	M49	✓
					M49-M55	✓	M55	✓
					myz2-myz9-S16b	✓	myz2	✓
							myz9	✓
Androcyclic	*Holocyclic*							
8. 003 (T)*	031 (N)	7	50 (7)	M40, M35-M63, M49, myz2-myz9	M40	✓	M35	✓
					M35-M63	✓	M63	✓
					M49	–	M49	✓
					myz2-myz9	×	myz2	–
					($^{myz2}\chi^2 = 3{\cdot}1$; $^{myz9}\chi^2 = 5{\cdot}5$, $P < 0{\cdot}05$)		myz9	✓

9. 003 (T)*	015 (N)	3	23 (7)	M35-M63, M37-M49-M55, myz2-myz9, M40	M35-M63	✓	M40	✓
					M37-M49-M55	✓	M35	✓
					myz2-myz9	✓	M55	✓
							myz2	× ($^{myz2}\chi^2 = 4{\cdot}5,\ P < 0{\cdot}05$)
							myz9	✓
10. 003 (T)	055 (N)	5	93 (19)	M35-M63, myz2-myz9	M35-M63	✓	M63	✓
					myz2-myz9	✓	myz2	✓
11. 003 (T)	066 (N)	6	37 (6)	M35-M63, myz2-myz9	M35-M63	✓	M35	✓
					myz2-myz9	✓	M63	✓
							myz9	✓
12. 010 (T)*	015 (N)	6	45 (8)	M40, M35-M63, M37-M55, myz2-myz9	M40	✓	M40	✓
					M35-M63	×	M35	✓
					($^{M35}\chi^2 = 7{\cdot}4$; $^{M63}\chi^2 = 4{\cdot}0$) ($P < 0{\cdot}05$)		M55	✓
					M37-M55	✓	myz2	✓
					myz2-myz9	×	myz9	✓
					($^{myz2}\chi^2 = 3{\cdot}5$; $^{myz9}\chi^2 = 3{\cdot}1$) ($P > 0{\cdot}05$)			
13. 010 (T)*	055 (N)	9	102 (11)	M40, M35-M63, M37-M55 myz2-myz9	M40	✓	M40	✓
					M35-M63	×	M63	✓
					($^{M35}\chi^2 = 4{\cdot}2$; $^{M63}\chi^2 = 3{\cdot}6$) ($P < 0{\cdot}05$)		myz9	✓
					M37-M55	✓		
					myz2-myz9	×		
					($^{myz2}\chi^2 = 3{\cdot}5$; $^{myz9}\chi^2 = 5{\cdot}5$) ($P < 0{\cdot}05$)			
14. 004 (T)*	015 (N)	6	45 (8)	M40, M35-M107, M49-M55, myz2-myz9	M35-M107	✓	M40	×
					M49-M55	✓	($^{M40}\chi^2 = 9{\cdot}0,\ P < 0{\cdot}01$)	
					myz2-myz9	✓	M35	✓
							M107	✓
							M49	✓
							M55	✓
							myz2	× ($^{myz2}\chi^2 = 4{\cdot}9,\ P < 0{\cdot}05$)
							myz9	✓
15. 004 (T)	055 (N)	4	65 (16)	M35, myz2-myz9	M35	×	myz2	✓
					($^{M35}\chi^2 = 4{\cdot}6,\ P < 0{\cdot}05$)			
					myz9	✓		
16. 004 (T)	066 (N)	4	54 (14)	M35, myz2-myz9	M35	✓	M35	✓
					myz2-myz9	✓	myz9	✓

The karyotype and life history characteristics of each clone are shown (N, karyotypically normal 2n = 12; T, autosomal 1,3 translocation 2n = 12). The number of replicates and eggs produced in each cross, and the informative loci used to genotype eggs are shown. * Crosses used in multi-locus analysis. Heterogeneity χ^2 tests were performed on each locus in each cross for informative loci in male and female clones. (–) represents loci that showed heterogeneity across replicate cages. For crosses in which replicates were not heterogeneous a χ^2 test was performed on pooled replicates. In males, because there is no recombination, loci on the same linkage group show identical segregation patterns, unless some samples failed to amplify in PCRs. In females, however, there is substantial recombination, so loci were considered separately. ✓, pooled replicates segregated in Mendelian proportions; ×, pooled replicates showed significant deviation from Mendelian proportions.

Table 2. *The association between autosomal linkage groups and the X chromosome haplotype in males during spermatogenesis*

Male	Linkage group	Autosome allele – X haplotype association
1. 042 (N) (H)	M49	✓
	myz2	✓
2. 042 (N) (H)	M40	✓
	M49	✓
	M63	✓
	myz2	✓
3. 033 (N) (H)	M35	✓
	M49-M55	$\times$ ($^{M49}\chi^2 = 3{\cdot}8$, $^{M55}\chi^2 = 4{\cdot}3$) ($P < 0{\cdot}05$)
	myz2-myz9	✓
4. 033 (N) (H)	M49-M55	✓
	myz2-myz9	✓
5. 020 (T) (H)	M37-M49	✓
	M63-M107	✓
	myz2-myz9	✓
6. 020 (T) (H)	M40	✓
	M49	✓
	M63-M107	✓
7. 015 (N) (H)	M40	$\times$ ($^{M40}\chi^2 = 5{\cdot}1$) ($P < 0{\cdot}05$)
	M35-M107	✓
	M49-M55	✓
	myz2-myz9-S16b	✓
8. 003 (T) (A)	M40	✓
	M49	✓
	M35-M63	✓
	myz2-myz9	$\times$ ($^{myz2}\chi^2 = 8{\cdot}6$, $^{myz9}\chi^2 = 6{\cdot}2$) ($P < 0{\cdot}05$)
12. 010 (T) (A)	M40	✓
	M35-M63	✓
	M37-M55	✓
	myz2-myz9	✓
13. 010 (A) (A)	M40	✓
	M35-M63	✓
	M37-M55	✓
	myz2-myz9	✓
14. 004 (A) (A)	M35-M107	✓
	M49-M55	✓
	myz2-myz9	✓

✓, the autosome alleles at each locus segregated in Mendelian proportions with each of the two possible X chromosome haplotypes during spermatogenesis; $\times$, there was a significant deviation ($P < 0{\cdot}05$) from the expected proportions of each autosome allele – X chromosome haplotype association; H, holocyclic; A, androcyclic.

parent passed on each of his alleles at every autosomal locus to some offspring. Significant heterogeneity among replicates of the same cross was observed only once for inheritance via the paternal line: for locus M49 in a cross involving the androcyclic clone 003 (Table 1). Apart from this exception, for each locus in each cross, it was considered valid to perform χ^2 tests on the pooled replicate values, to investigate deviation from expected Mendelian proportions.

The segregation of autosome homologues via the paternal line was in Mendelian proportions for all seven crosses involving holocyclic male clones (Table 1). However, in the pooled results for androcyclic males, significant, or near significant distortions occurred in all three male clones (003, 004 and 010) ($P < 0{\cdot}05$) although these deviations were insignificant after Bonferroni correction. Each of these clones also had the A1,3 karyotype. Four loci from two separate linkage groups (M35-M63 and myz2-myz9) were consistently involved. M35 and M63 are thought to lie on autosome 1 (Sloane *et al.*, 2001), one of the chromosomes involved in the translocation. M35, M63, myz2 and myz9 showed segregation distortions in male 010 in both crosses in which he was involved. M35 showed distortions in male 004 in one of the two crosses in which he was involved, while myz2 and myz9 showed segregation distortions in male 003, in one of the four crosses in which he was involved. For M35 and M63 the same alleles (size 186–207) were always favoured over the other two (196–187). However, there was no pattern for myz2 and myz9. It was expected that if distortions occurred via the paternal line, then loci in the same linkage group would show the same abnormal segregation patterns

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(i.e. they are not independent events). This pattern did occur; however, because some samples failed to amplify, the results were not always significant for each locus in the linkage group.

For the holocyclic females, significant heterogeneity among replicates occurred in only one cross for locus myz2 (Table 1). The inheritance of pooled autosome alleles via the maternal line was also in Mendelian proportions, with the exception of four loci: M35, M63, myz2 and M40 (Table 1). M35, M63 and myz2 were involved in distortions via paternal androcyclic lines. The M40 distortion occurred in only one cross, in one female clone. For females, significant results were patchily distributed over families in no clear pattern. When they did occur, it was difficult to determine whether the same alleles were preferentially inherited. The different female clones used in each cross meant that they did not share the same alleles, which made comparison difficult.

Table 3. *Pairwise comparisons showing significant segregation distortions*

Male	Locus pair	Chi-square value
4. 033 (H) (N)	myz2-M55	3·2 ($P > 0·05$)*
	myz9-M55	4·3 ($P < 0·05$)
5. 020 (H) (T)	M49-M63	3·5 ($P > 0·05$)*
	M49-M107	3·9 ($P < 0·05$)
	M37-M63	2·9 ($P > 0·05$)*
	M37-M107	3·9 ($P < 0·05$)
7. 015 (H) (N)	M35-M40	4·1 ($P < 0·05$)
	M40-M107	4·8 ($P < 0·05$)

*Loci in the same linkage group. e.g. myz2 and myz9, should show identical distortions with loci in other linkage groups, e.g. M55. The reason for a significant result for myz9-M55, but not for myz2-M55, is because some samples failed to amplify in myz2 PCRs. This reduction in sample size was sufficient to make the myz2-M55 segregation distortion insignificant. This was also the case for M49-M63 and M37-M63.

(ii) *Autosome allele + X haplotype associations in males*

Only three cases of significant non-random association between autosomal linkage groups and the male X chromosome were observed. Two of these occurred in normal 2n = 12 holocyclic males and the other in an A1,3 translocated androcyclic male (Table 2). These distortions showed no trend across loci or families, with different loci and different clones involved in each case. Therefore, the X chromosome plays little if any active role in capturing a haploid autosome set during male spermatogenesis.

(iii) *Analysis of association between autosomal linkage groups*

Of the 82 pairwise comparisons performed in holocyclic males, between loci known to lie on separate chromosomes, only eight comparisons from three separate crosses showed segregation distortions ($P < 0·05$) (Table 3). The distortions were not reproduced for the same locus or clone in independent crosses. These deviations were insignifcant after Bonferroni correction. Sixty-six pairwise comparisons were performed in androcyclic males and no distortions were observed.

(iv) *Multi-locus analysis*

In only one cross, involving the androcyclic male clone 010, was there a significant over-representation of some multi-locus genotypes ($P < 0·05$). However, these genotypes did not share the same alleles across the multiple loci. In the remaining four crosses involving androcyclic male clones, and the seven crosses involving holocyclic male clones, there was no over- or under-representation of multi-locus genotypes inherited via the paternal line.

4. Discussion

(i) *No evidence of genomic imprinting in* M. persicae

Spermatogenesis in aphids provides an apparently ideal arena for preferential removal of the paternal genome. We now have conclusive evidence that no 'abnormal' segregation occurs in aphids because we examined segregation at the level of single loci, locus pairs and multiple loci, using microsatellite markers distributed on four of the five autosome pairs of *M. persicae* (Sloane *et al.*, 2001). We found no evidence of extreme selfish autosome behaviour, in which a homologue(s) of paternal origin was selectively eliminated during male meiosis, since all paternal alleles were represented in at least some progeny. We also found no evidence of partial selfishness, in which some non-homologous autosomes were preferentially inherited together via the paternal line more often than expected by chance. None of the loci pairs known to lie on different chromosomes showed consistent significant associations in either the pairwise or the multi-locus analyses. The passage of autosomes through *M. persicae* spermatogenesis essentially occurs according to Mendelian expectations. Thus, despite the unorthodox nature of spermatogenesis (achiasmate, and the loss of a haploid set of autosomes each sexual generation), the end result is 'normal' male meiosis.

Our current work shows that there is no selective elimination of paternal autosomes in male *M. persicae*, so Haig's model (Haig, 1993) does not seem to apply

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to aphids. Further, a model of paternal genome loss would predict the loss of the paternal X chromosome at male determination. However, we have already shown that X chromosome loss at male determination in aphids is random (Wilson *et al.*, 1997), unless offspring ratios are disrupted by mutation accumulation (Wilson, 2000). It seems reasonable to assume that Mendelian segregation, rather than the imprinting seen in coccoids, was the ancestral state and has been conserved in aphids.

(ii) *The effect of life cycle history on autosome segregation*

Non-Mendelian autosome segregation could occur in *M. persicae* because of (i) male–female incompatibility and (ii) mutations associated with life cycle characteristics. If segregation distortions were to occur, there was an *a priori* expectation they would more likely be seen in androcyclic male clones, rather than holocyclic male clones. Androcyclic clones are essentially asexual clones that are capable of producing males (but no sexual females). In the absence of sexual reproduction, recessive and partially recessive deleterious and lethal mutations will not be purged from the genome as efficiently as in a life cycle that incorporates a sexual generation each year (Lynch *et al.*, 1993). If such mutations were linked to our microsatellite loci, segregation distortions could be observed in the offspring of an androcyclic male mated with a female of a different clonal lineage. This may account for the distortions involving M35, M63, myz2 and myz9 in the androcyclic males, especially clone 010. However, each of these clones also has the A1,3 karyotype. This makes it difficult to distinguish between the effects of the translocation and distortions associated with deleterious mutations. Mutation accumulation is a more plausible argument. If the translocation were the cause of the segregation distortions, one would predict consistent distortions across independent crosses involving clones with the translocation. This, however, was not the case. Further, myz2 and myz9 are not on the chromosomes involved in the translocation (Sloane *et al.*, 2001). Although the distortions were not significant after Bonferroni correction, it should be noted that the Bonferroni method is a harsh test that may fail to detect important patterns. Corrections for multiple comparisons depends on the inference to be made (Chapman *et al.*, 1999; Curach & Sunnucks, 1999). Repeatable patterns from independent observations could indicate important phenomena that would be dismissed if stringent Bonferroni corrections were applied. The facts that the same loci were involved repeatedly, and that androcyclic lines were disproportionately affected as predicted, suggest that the distortion phenomena have a biological basis, and are not chance. It is then somewhat unexpected that female holocyclic lines should also show distortions at the same loci. The fact that the same loci are involved in females is consistent with deleterious alleles being involved, as it may suggest that harmful variation can exist closely linked to the markers concerned.

The *t* haplotypes in mice (e.g. review by Schimenti, 2000) and *Drosophila* SR (sex ratio) drive chromosomes (e.g. review Carvalho *et al.*, 1999) are classic examples of selfish chromosome behaviour. Even in these well-studied examples of segregation distortion, the nature of the loci involved in distorted segregation ratios, the exact molecular mechanisms involved in the process and the effect on species evolution are still speculative. Several studies on linkage maps also report segregation distortions involving selectively neutral microsatellite loci (Kocher *et al.*, 1998; Sakamoto *et al.*, 2000) as seen in this study, although these studies are not able to propose clear reasons for the distortions or present evolutionary outcomes. The concentration of our observations in androcyclic lines is consistent with deleterious recessive alleles (above) being the cause of segregation distortions.

(iii) *Conclusion*

Microsatellite markers have provided a means to demonstrate for the first time that aphid autosomes generally segregate in Mendelian proportions. This has put to rest the hypothesis of selfish autosomes, proposed by us on the basis of the apparent cytogenetic 'fight' between haploid autosome sets for the X chromosome, and because of the extreme case of genomic imprinting in the closely related coccoids. This study adds to the growing body of data on basic aphid genetic processes that will aid future evolutionary and applied aspects of aphid research.

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