

Microsatellite isolation, linkage group identification and determination of recombination frequency in the peach-potato aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

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Summary

Fifteen polymorphic microsatellite markers were used to establish linkage groups and relative rates of recombination in male and female *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) (peach-potato aphid). We cloned nine markers from *M. persicae* and for these we report primer sequences and levels of allelic diversity and heterozygosity in four Australian *M. persicae* populations. Of the remaining six loci, four loci, also cloned from *M. persicae*, were obtained from G. Malarky (Natural History Museum, London) and two loci from *Sitobion miscanthi* were used. Additionally, the primer sequences of locus M77, a locus monomorphic in *M. persicae* but polymorphic in the closely related *Myzus antirrhinii*, are presented. Eleven of the 15 polymorphic markers were autosomal and four were X-linked. A linkage analysis was performed on a European pedigree of aphids containing five families with between seven and 11 offspring each. There was no linkage between any loci in females. In males, several pairwise comparisons yielded no recombinant offspring. With the exception of locus M40, these observations were supported in a linkage analysis performed on larger families produced from Australian *M. persicae* crosses. Locus M40 showed segregation consistent with involvement in a translocation between autosomes 1 and 3 in European samples but not in the Australian samples. From the Australian crosses we report an absence of recombination in males but high recombination rates in females. One X chromosome and four autosomal linkage groups were identified and tentatively assigned to chromosomes. The relevance of achiasmate meiosis to the evolution of sex is discussed.

1. Introduction

Chiasmata play two important roles in meiosis. First, they allow for the proper segregation of chromosomes by holding bivalents together after synaptonemal complex degradation (Gethmann, 1988; Wolf, 1994). Second, chiasmata are necessary for genetic exchange between chromosomes (Wolf, 1994).

Achiasmate meiosis has evolved 25–30 times (Burt *et al.*, 1991). It is common in many species of invertebrates, but rare in higher plants and has not been reported in vertebrates. In vertebrates, however, moderate to extreme differences in recombination rates exist between the sexes. For example, the female

human autosomal map length is approximately $1\frac{1}{2}$ times that of males (Ott, 1999), and in some marsupials recombination rates are up to 5 times higher in males (Bennett *et al.*, 1986; van Oorschot *et al.*, 1992), while in the Western Australian seahorse (*Hippocampus angustus*) preliminary data suggest recombination rates are about 10 times higher in females (Jones *et al.*, 1998). Achiasmate meiosis is always confined to the heterogametic sex in dioecious species (usually the male) (White, 1973). Examples include a mollusc, some mites, certain scorpions, the higher Diptera, some Hemiptera, females of several species of copepods, and the heterogametic females of the orders Trichoptera and Lepidoptera (Ar-Rushdi, 1963; White, 1973; Gethmann, 1988).

Numerous hypotheses have been proposed to explain how normal segregation can occur in the

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absence of chiasmata (Wolf, 1994). For example, the prolonged maintenance of the synaptonemal complex until metaphase I in female Lepidoptera could have evolved to fulfil the role of the absent chiasmata (Rasmussen, 1977). Chiasma formation is known to occur along autosomes during oogenesis in the birch aphid *Euceraphis betulae* (Koch) (Blackman, 1976). However, no chiasmata have been observed in over 1000 cells in the first meiotic prophase in males of that species, and only one autosomal chiasma was observed in over 500 prophase cells of male *E. punctipennis* (Blackman, 1976). Genetic evidence of crossing over during oogenesis but not spermatogenesis in aphids was found recently in a concurrent study on the segregation of an amplified esterase marker gene and a *de novo* fusion between autosomes 2 and 3 in the aphid *Myzus persicae* (Spence & Blackman, 2000). Our study extends this observation using a larger set of markers.

The cosmopolitan peach-potato aphid, *M. persicae*, is an economically important insect pest (Blackman & Eastop, 1984). It is a vector of many plant viruses and has developed resistance to a wide range of insecticides (Devonshire *et al.*, 1998). Typically *M. persicae* has a karyotype of $2n = 12$. Like all aphids, *M. persicae* possesses holocentric chromosomes, which are able to fragment to form stable dissociation products. By this mechanism, *M. persicae* displays extensive intra-specific karyotypic variation without morphological change (Blackman, 1987). Rearrangements include both fragmentations and translocations (Blackman, 1971; Blackman & Takada, 1977; Blackman *et al.*, 1978; Blackman, 1980; Spence & Blackman, 2000). The Australian *M. persicae* clones, and most of the European clones used in this study, had either the normal $2n = 12$ karyotype or an autosomal translocation between autosomes 1 and 3 ($2n = 12$).

The life cycle of *M. persicae* encompasses apomictic parthenogenetic and sexual generations, with obligate alternation between unrelated host plants (Blackman, 1974). In temperate climates, the decreasing photoperiod and lower temperatures of autumn induce parthenogenetic females to produce male and sexual female offspring. After mating, sexual females lay eggs on peach trees. These eggs undergo physiological arrest over winter (diapause) before hatching as parthenogenetic females in spring (Blackman, 1974). However, even in temperate climates, certain genotypes have lost the ability to reproduce sexually, and reproduce by parthenogenesis throughout the year. Others are able to produce males but no sexual females (Blackman *et al.*, 1996).

In this study we utilized microsatellite markers to compare rates of recombination during spermatogenesis and oogenesis in *M. persicae* by genotyping the offspring of interclonal crosses.

2. Materials and methods

(i) Isolation and amplification of microsatellite markers

Microsatellites were cloned and screened largely following Taylor *et al.* (1994) and Sunnucks *et al.* (1996). In addition to the loci cloned by us, four loci (myz2, myz3, myz9, myz25) cloned by Gavin Malarky (Natural History Museum, London, UK) and two loci (S16b and S17b) isolated from *Sitobion miscanthi* by Wilson and Sunnucks (unpublished) have been used in this study.

(ii) Assignment of microsatellite markers to autosomes and the X chromosome

When a parthenogenetic female produces male offspring, one X chromosome is eliminated (Orlando, 1974; Blackman & Hales, 1986). On this basis X-linked microsatellites were identified following Wilson *et al.* (1997). In brief, loci are determined to be X-linked when a female genotype is heterozygous while males of the same clone are hemizygous. Conversely, loci that are heterozygous in both females and males of the same clone are considered to be autosomal. Chromosomal information is incorporated into the locus name of each microsatellite with ^A = autosomal, ^X = X-linked, and no superscript = currently unknown (Table 1).

(iii) Aphid breeding

M. persicae clones were collected from the primary host, *Prunus persica*, from one site in Bathurst, NSW, Australia, and from three sites in Tasmania, Australia. A collection was also made in Canberra, ACT, Australia, from vegetables (potatoes and broccoli). Collectors sent twigs or leaves bearing aphids to Macquarie University, Sydney, NSW. From these collections, approximately 60 clonal lineages were established.

Clones were maintained in synchronous culture on individual cabbage seedlings (var. Early Jersey Wakefield) at 20 °C at a regime of 16L:8D (hours of light:hours of dark). Under these conditions the aphids reproduce by continuous parthenogenesis. Clones were genotyped at all microsatellite loci. Pairs of clones with the greatest number of informative loci were selected for sexual crosses. Adult parthenogenetic females were transferred to a regime of 8L:16D at 15 °C to induce the sexual phase (Hales *et al.*, 1989).

Single male aphids were placed on a cabbage seedling with three to five females of a clone and allowed to mate. Depending on the number of sexual individuals that were available, between two and ten replicates of each cross were established. Males were

Table 1. Details of *Myzus persicae* microsatellites cloned in this study

Locus	Motif	Size range (bp)	Primer sequence (5'–3')	PCR prog.	Na (n)	B	S	NT	CAN	GenBank accession no.
M35 ^A	(AT) ₉ ~(AC) ₂₂ impure	178–198	F: GGCAATAAAGATTAGCGATG R: TGTGTGTATAGATAGGATTTGTG	PMS1	6 (45)	0.533 {0.580} (15)	0.714 {0.571} (7)	0.500 {0.570} (10)	0.857 {0.755} (7)	AF233240
M37 ^A	(AC) ₁₆	155–157	F: GTGTGAGTAAGTCGTATTG R: TTGTATTATGTACCTGTGC	PMS1	2 (44)	0.467 {0.500} (15)	0.571 {0.408} (7)	0.500 {0.445} (10)	0.571 {0.408} (7)	AF233241
M40 ^A	(AC) ₁₇	123–135	F: ACACGCATACAAGAATAGGG R: AGAGGAGGCAGAGGTGAAAC	PMS1	4 (44)	0.667 {0.567} (15)	0.500 {0.625} (6)	0.667 {0.562} (9)	0.857 {0.724} (7)	AF233242
M49 ^A	(AC) ₃₁	130–199	F: CCCATACATACCTCCAAGAC R: AGAGAGAAAATAGGTTTCGTG	PMS2	16 (45)	0.867 {0.856} (15)	0.857 {0.837} (7)	1.000 {0.858} (9)	0.857 {0.796} (7)	AF233243
M55 ^A	(CA) ₁₆ CT(CA) ₈	119–129	F: TTAATCAATAACTGCTCAC R: GAAGTAGGCAGACACG	PMS1da	4 (33)	0.750 {0.681} (12)	0.000 {0.375} (4)	0.600 {0.560} (5)	0.500 {0.403} (6)	AF233244
M62 ^A	(CA) ₆ ~(CA) ₁₂	127–143	F: CGCTGGGGACGAAAAACCTG R: AACAAAAACCGAAAAACCCG	PMS1a	5 (34)	0.200 {0.620} (10)	0.000 {0.375} (4)	0.429 {0.663} (7)	0.571 {0.459} (7)	AF233245
M63 ^A	(AC) ₂₉	163–207	F: GCGGTTTTCTTTGTATTTTCG R: GATTATGGTGCTCGGTGG	PMS2	7 (45)	0.733 {0.662} (15)	0.857 {0.653} (7)	0.667 {0.623} (9)	0.857 {0.735} (7)	AF233246
M77	(TA) ₇	138	F: AACTGCAATCGTGTATATAC R: TTATATTGTATGGCGGCGG	PMS1	1 (46)	0.000 {0.000} (15)	0.000 {0.000} (7)	0.000 {0.000} (10)	0.000 {0.000} (7)	AF233247
M86 ^X	(CA) ₂₃	97–141	F: TCCACTAAGACCTCAAACAC R: ATTTATTATGTCGTTCCGCC	PMS1	9 (44)	0.533 {0.651} (15)	1.000 {0.736} (6)	0.556 {0.747} (9)	0.714 {0.612} (7)	AF233248
M107 ^A	(AT) ₁₁	133–145	F: TAAAAAACACACAATACACA R: GACACCAATGAATGACC	PMS1	6 (44)	0.600 {0.598} (15)	0.667 {0.444} (6)	0.556 {0.512} (9)	0.857 {0.582} (7)	AF233239

Locus name^A = autosomal locus, Locus name^X = X-linked locus.

PCR program PMS1: $v = 62$, $w = 61$, $x = 59$, $y = 57$ and $z = 55$; PMS2: $v = 55$, $w = 53$, $x = 51$, $y = 49$ and $z = 47$. For cycling details of PCR programs see text.

d, plus 1 × BSA and 0.01% DMSO in PCR; a, 2 × normal primer concentration.

Na, number of alleles in the four populations of *M. persicae* listed, (n) = number of individuals.

All populations are in Australia, ACT (Australian Capital Territory), New South Wales (NSW), Tasmania (TAS): B, Bathurst, NSW; S, Sorell, TAS; NT, New Town, TAS; CAN, Watson, ACT. No brackets, observed heterozygosity; curly brackets, expected heterozygosity; parentheses, number of individuals.

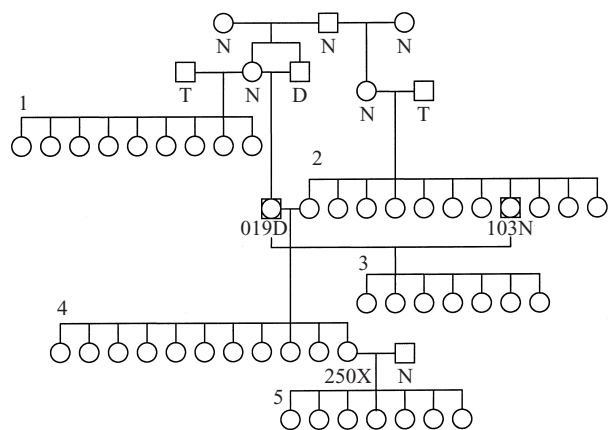


Fig. 1. Pedigree of European *Myzus persicae* clones showing the five families on which linkage analysis was performed. All families were phase-known for the female linkage analyses. For the males, families 3 and 4 were phase-known, while families 1, 2 and 5 were phase-unknown. Note that for family 3 the male parent was clone 103N and the female parent 019D, while for family 4 the male parent was clone 019D. T, autosomal 1,3 translocation; N, normal karyotype ($2n = 12$); and D, dissociated chromosome 2. Clone 250X has a *de novo* fusion of chromosomes 2 and 3.

left with the females for 1 week before being removed from cages. Females were left for a further week to lay eggs before being removed. Eggs were then allowed to develop for a further 2 weeks prior to collection for immediate or future genetic analysis.

(iv) DNA extraction and microsatellite amplification

Individual eggs were crushed in 30 μ l of 5% Chelex 100 resin (Bio-Rad) (w/v in 10 mM Tris pH 8, 0.1 mM EDTA pH 8), then incubated at 65 $^{\circ}$ C for 15 min, and boiled for a further 6 min. Samples were then pulse centrifuged. Extraction blanks (Chelex but no egg) were always included. DNA was extracted from adult aphids using a salting-out protocol as described in Sunnucks & Hales (1996). All DNA extractions were stored at -20° C until samples were genotyped.

Immediately prior to genotyping, Chelex extracts were thawed, vortexed, and centrifuged at 14000 rpm for 3 min. With the exceptions detailed in Table 1, loci were screened using standard acrylamide gel isotopic microsatellite PCR in 10 μ l volumes, containing 0.5 U *Taq* polymerase (Promega), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2 mM $MgCl_2$, 200 μ M of dGTP, dCTP, dTTP, 20 μ M of dATP, 10 pmol of each primer, ~ 50 ng of DNA and 0.05 μ l of [$\alpha^{33}P$]dATP at 1000 Ci/mmol. PCR touchdown programs were as follows: initial denaturation 94 $^{\circ}$ C 2 min, followed by one cycle of each: *v*, *w*, *x*, and *y* $^{\circ}$ C 30 s, 72 $^{\circ}$ C 45 s, 94 $^{\circ}$ C 15 s, then 30 cycles annealing at temperature *z* $^{\circ}$ C; final extension 72 $^{\circ}$ C for 2 min (see Table 1 legend for annealing temperature details).

Reference DNA can be obtained from the authors where confirmation of allelic length identity is necessary. This has proven helpful for making data sets completely comparable among studies from different laboratories (Terradot *et al.*, 1999; Hales *et al.*, 2000).

(v) Linkage analysis in European multi-generational pedigree of *M. persicae*

Aphids used in this linkage analysis originated from clones of *M. persicae* collected from Germany, the United Kingdom, France and Italy. Crosses between pairs of these clones were established by R. Blackman's research group to study the inheritance of amplified esterase genes responsible for insecticide resistance (Blackman *et al.*, 1996). These crosses were established without any knowledge of the microsatellite genotypes of the clones.

The pedigree comprised multiple generations of related families, but was divided into five subfamilies for linkage analysis, since chromosomal rearrangements could affect recombination. Fig. 1 shows the karyotype and phase status (whether allelic haplotypes are known or unknown) of the parents of each family, and the number of offspring in each family. Note that we use the term *recombination* when referring to sister chromatid exchange and chiasmata formation, and *independent assortment* as the random association of the autosomes in the absence of recombination.

The recombination fraction giving the maximum LOD (likelihood of the odds calculated by the method of maximum likelihood) score was calculated for each informative pairwise comparison in both sexes, by summing the number of recombinant and non-recombinant genotypes, and substituting the values into the following equations:

$$(a) Z = \log_{10}[2^{n+r} \cdot \theta^r \cdot (1-\theta)^n] \text{ (phase-known families)}$$

$$(b) Z = \log_{10}2^{n+r-1}[\theta^r \cdot (1-\theta)^n + \theta^n \cdot (1-\theta)^r] \text{ (phase-unknown families) (Ott, 1991)}$$

where *n* is the number of non-recombinant genotypes, *r* is the number of recombinant genotypes, θ is the recombination fraction and *Z* is the LOD score.

A positive LOD score implies that linkage may exist, while negative LOD scores indicate an absence of linkage (Ott, 1991). A LOD score of 3 is taken as the critical value for significant evidence of linkage (Morton, 1955). If linkage does exist, the score will continue to increase above the critical value as more family data are added (Ott, 1991).

(vi) Linkage analysis of Australian *Myzus persicae*

The karyotypes of the parents, the loci examined, and the number of eggs collected from each cross involving Australian *M. persicae* clones are shown in Table 2. The grandparent genotypes were unknown in all

Table 2. The karyotype of each parent (*T* = autosomal 1,3 translocation $2n = 12$; *N* = normal $2n = 12$) used in Australian *Myzus persicae* crosses, the number of replicates and eggs collected per cross, and the loci used to genotype eggs. Loci analysed: pairwise comparisons giving significant LOD scores with no recombination in males are shown in parentheses. LOD scores: male LOD scores are given, superscripts refer to loci pairs listed in previous column; maximum female LOD scores for the same loci pairs are given in parentheses. *One recombinant offspring genotype

Male	Female	Replicates	No. of eggs	Loci analysed	LOD scores
020 (T)	031 (N)	10	106	(M63–M107) ¹ , M40, M49, myz9	$z^1 = 22$ (0.6)
033 (N)	031 (N)	2	30	(myz2–myz9) ¹ , (M49–M55) ²	$z^1 = 7$ (0.1), $z^2 = 6$
003 (T)	031 (N)	7	50	(M35–M63) ¹ , (myz2–myz9) ² , M40, M49	$z^1 = 13$ (0.1), $z^2 = 12$ (0.5)
033 (N)	015 (N)	11	135	(M49–M55) ¹ , (myz2–myz9) ² , M35	$z^1 = 36$ (2.2), $z^2 = 37$ (0.5)
042 (N)	015 (N)	10	50	myz2, M49, M40, M63	–
015 (N)	033 (N)	5	54	(M35–M107) ¹ , (M49–M55) ² , (myz2–myz9) ³ , (myz2–S16b) ⁴ , (myz9–S16b) ⁵ , M40	$z^1 = 14$, $z^2 = 14$ (0.2), $z^3 = 14$ (0.2), $z^4 = 14$, $z^5 = 14$
020 (T)	033 (N)	5	41	(M49–M37) ¹ , (M63–M107) ² , (myz2–myz9) ³ , M40	$z^1 = 11$, $z^2 = 9$, $z^3 = 7$
042 (N)	031 (N)	9	44	myz2, M49	–
031 (N)	040 (N)	10	55	(M35–M63) ¹ , (M35–M107) ² , (M63–M107) ³ , (M37–M49) ⁴ , (myz2–myz9) ⁵	$z^1 = 9$, $z^2 = 9$, $z^3 = 9$, $z^4 = 9$, $z^5 = 9$
047 (N)	066 (N)	3	55	M37, M40	–
031 (N)	054 (N)	9	52	M63, S16b	–
003 (T)	015 (N)	3	23	(M35–M63) ¹ , (M37–M49) ² , (M37–M55) ³ , (M49–M55) ⁴ , (myz2–myz9) ⁵ , M40	$z^1 = 5$, $z^2 = 5$, $z^3 = 5$, $z^4 = 4$, $z^5 =$ 6 (0.1)
004 (T)	015 (N)	6	45	(M49–M55) ¹ , (M35–M107) [*] , (myz2–myz9) ² , M40	$z^1 = 11$ (0.1), $z^* = 9$ (0.2), $z^2 = 12$ (0.1)
010 (T)	015 (N)	7	45	(M35–M63) ¹ , (M37–M55) ² , (myz2–myz9) ³ , M40	$z^1 = 11$, $z^2 = 11$, $z^3 = 11$ (0.9)
010 (T)	055 (N)	9	102	(M35–M63) ¹ , (M37–M55) ² , (myz2–myz9) ³ , M40	$z^1 = 28$, $z^2 = 27$, $z^3 = 27$

families so it was not possible to determine which two alleles were received as a haplotype from each parent (phase-unknown). Therefore the allelic haplotypes were assigned, and the number of recombinants and non-recombinants were substituted into the phase-unknown equation above. We chose to use this approach rather than examine phase-known families. Such analysis would require hatching eggs from a cross, raising several generations of parthenogenetic aphids, followed by inducing sexuals and crossing these sexuals to produce third-generation eggs. The entire process would take at least 6 months.

Pairwise comparisons and LOD score calculations were performed as in the European linkage analysis. For pairwise comparisons not showing complete association in males, the G-test for goodness of fit with Bonferroni correction for multiple comparisons (Sokal & Rohlf, 1995) was performed to determine whether there was a significant deviation from a one-to-one ratio of recombinants versus non-recombinants. It was assumed that if there were no male recombination, then loci on the same chromosome would show complete association, whereas those on different chromosomes would assort independently. Significant but not total association would imply non-random assortment of autosomes.

3. Results

(i) Details of microsatellite loci isolation

Approximately 9000 recombinant colonies were screened. Sequences were obtained from 16 dinucleotide microsatellite-containing plasmids. Primers were designed for 11 regions (the remainder were unsuitable because of characteristics of the flanking regions) and microsatellite loci were successfully amplified from 10 of these (Table 1).

All loci, with the exception of M77, were polymorphic in the *Myzus persicae* samples examined (Table 1). Allelic diversity ranged from two to 16 alleles per locus. Observed and expected heterozygosities are reported from samples obtained from four populations of *M. persicae* collected in spring from *Prunus persica*, the primary host on which sexual reproduction and over-wintering takes place. In general, high levels of heterozygosity were observed in all populations at all loci (Table 1).

Although M77 is monomorphic in all *M. persicae* assayed, it is heterozygous in *M. antirrhinii* (Terradot *et al.*, 1999; Hales *et al.*, 2000). M77 can be used as a simple diagnostic marker for distinguishing *M. antirrhinii* from *M. persicae* since morphological separation of the two species is often difficult (see Hales *et al.*, 2000). All other *Myzus* loci cloned by us, M35, M37, M40, M49, M55, M62, M63, M86 and M107, have been used in this study.

(ii) Linkage analysis in European multi-generational pedigree of *M. persicae*

(a) Female linkage analysis

Some loci were uninformative in certain families, with one or both parents being homozygous at the loci concerned. Examination of females in each family showed no linkage among autosomal or among X-linked loci, with all LOD scores being less than 3. When LOD scores were calculated from recombinants and non-recombinants summed across all five families, only the pairwise comparison between *myz2^A* and *S16b^A* showed evidence of linkage ($Z = 3.2$, when $\theta = 0.15$). *Myz9^A*–*S16b^A* and *M49^A*–*M62^A* produced the next highest LOD scores of 1.3 ($\theta = 0.27$) and 1.9 ($\theta = 0.22$) respectively.

(b) Male linkage analysis

Pairwise comparisons that showed no recombination in normal ($2n = 12$), A1,3 translocated or dissociated chromosome 2 male parents were *M49^A*–*M62^A*, *myz2^A*–*myz9^A*, *myz2^A*–*S16b^A*, *myz9^A*–*S16b^A*, *M55^A*–*M62^A* and *M49^A*–*M55^A*. The pairwise comparisons of *M37^A*–*M62^A*, *M63^A*–*M107^A*, *M35^A*–*M63^A*, *M37^A*–*M49^A* and *M37^A*–*M55^A* produced no recombinants in normal or A1,3 translocated males. The dissociated chromosome 2 male parent in family 4 was uninformative for these comparisons. For *M35^A*–*M107^A* no recombinants occurred with the normal male parent in family 3 (the only informative family) (see Table 3 for LOD scores). In contrast, all the pairs mentioned above did show recombination via the maternal line.

Independent assortment of loci *M35^A*–*M40^A*, *M40^A*–*M63^A* and *M40^A*–*M107^A* occurred in families with a normal father (families 3 and 5 for *M40^A*–*M63^A* and *M40^A*–*M107^A*; family 3 for *M35^A*–*M40^A*) or one with a dissociated chromosome 2 (family 4 for *M40^A*–*M63^A*). In contrast, however, non-independent assortment of these loci, via the paternal line in families with a translocated male parent, was identified (family 1 for *M35^A*–*M40^A*; families 1 and 2 for *M40^A*–*M63^A*; family 2 for *M40^A*–*M107^A*). It seems likely that the *M37*–*M49*–*M55*–*M62* group is A2 but a dissociation will not be detectable by independent assortment.

LOD scores for all other pairwise comparisons in males were less than 3.

(iii) Linkage analysis of Australian *Myzus persicae*

The linkages observed in the European analysis were for the most part reflected in the Australian *M. persicae* crosses. Five loci pairs showed no recombination in either normal or translocated males:

Table 3. The pairwise comparisons showing no male recombinant genotypes in the European linkage analysis

Loci pairs	Family					All families
	1	2	3	4	5	
myz2–myz9	2.4 (0.02)	3.0 (0.02)	2.1 (0.03)	3.3 (0.02)	* (0.03)	11.4 (0.1)
myz2–S16b	1.2 (0.4)	* (1.0)	1.2 (–0.07)	3.0 (*)	* (2.1)	6.0 (3.2)
myz9–S16b	1.2 (1.2)	* (1.0)	1.2 (–0.3)	* (*)	3.0 (0.03)	6.0 (1.2)
M63–M107	* (*)	3.0 (0.5)	2.1 (*)	* (0.02)	1.8 (*)	7.5 (0.4)
M37–M55	2.4 (*)	* (0.02)	2.1 (*)	* (0.5)	* (*)	4.8 (0.2)
M49–M55	2.4 (0.02)	* (0.5)	2.1 (0.1)	3.3 (0.02)	* (*)	8.1 (0.05)
M49–M62	2.4 (1.3)	2.7 (*)	2.1 (–0.3)	3.0 (1.0)	* (*)	11.1 (1.9)
M37–M62	2.4 (*)	2.7 (*)	2.1 (*)	* (0.2)	* (*)	7.8 (0.2)
M55–M62	2.4 (0.02)	* (*)	2.1 (0.03)	3.0 (0.5)	* (*)	8.1 (0.2)
M37–M55	2.4 (*)	* (0.02)	2.1 (*)	* (0.5)	* (*)	4.8 (0.2)
M35–M63	2.4 (0.02)	* (1.0)	2.1 (*)	* (0.02)	* (0.3)	4.8 (0.1)
M35–M107	* (*)	* (0.02)	2.1 (*)	* (0.2)	* (*)	2.1 (0.04)
M37–M49	2.4 (*)	3.0 (0.02)	2.1 (*)	* (0.02)	* (0.3)	8.1 (0.0)

The maximum male LOD scores ($\theta = 0$) for each family, as well as the cumulative LOD scores across all families are given. Numbers in parentheses are the maximum female LOD scores for the same loci pairs.

* Uninformative cross.

M35^A–M63^A, M37^A–M49^A, M49^A–M55^A, M63^A–M107^A and myz2^A–myz9^A. No recombinant offspring were observed for myz2^A–S16b^A or myz9^A–S16b^A in normal males, or for M37^A–M55^A in translocated males (Table 2). It was not possible to compare segregation patterns between normal and translocated males involving S16b^A, or M37^A–M55^A and M55^A–M63^A. Of the approximately 1900 pairwise comparisons performed between loci on the same chromosomes, only one recombinant progeny genotype occurred via the male line, for M35^A–M107^A involving a translocated male parent. Apart from this single event, M35^A–M107^A showed no recombination in normal or translocated males. There was no significant evidence of linkage in any pairwise comparisons in females, with all LOD scores being less than 3.

Apart from those loci that are physically linked, after Bonferroni correction, no loci pairs showed significant deviations from independent inheritance via the male line.

4. Discussion

(i) Linkage groups based on no recombination in males versus substantial recombination in females

The failure to detect linkage groups in European female *M. persicae* was probably a consequence of (i) small sample size and/or (ii) low marker density. When data from females alone were used it was not possible to distinguish between loci located far apart on the same chromosomes, and loci assorting independently on different chromosomes. Failure to detect female linkages in the Australian crosses, despite substantially larger sample sizes in comparison

with the European material, suggests that a low marker density prevented the detection of the linkage groups in females.

Pairs of loci showing no recombinant offspring via the male line must lie on the same chromosome. Pairs of loci that produced a mixture of recombinant and non-recombinant offspring via the male line must be on different chromosomes. Based on linkage analyses of Australian *M. persicae*, the autosomal linkage groups are: (i) myz2^A–myz9^A–S16b^A, (ii) M37^A–M49^A–M55^A–M62^A (iii) M35^A–M63^A–M107^A and (iv) M40^A. Since normal *M. persicae* have five pairs of autosomes, our current suite of markers are distributed on all but one of the chromosome pairs. Myz3^X–myz25^X–M86^X–S17b^X are X-linked, as indicated by hemizyosity in males (Wilson *et al.*, 1997).

In the European linkage analysis, M40^A assorted independently from M35^A–M63^A–M107^A via the paternal line when the male parent was karyotypically normal or had a dissociated chromosome 2, but not when the male parent had the A1,3 translocation. This pattern of segregation is consistent with M40^A being located on the translocated section of autosome 3 and M35^A–M63^A–M107^A being on autosome 1, or vice versa, though it is unlikely that the fragment would have three markers. We conclude that M35^A–M63^A–M107^A are on autosome 1 and M40 on autosome 3 in European *M. persicae*.

It is important to note that the association seen in European aphids between M40^A and the translocation, did not occur in the Australian crosses. Locus M40^A did not show linkage via the paternal line with any locus in Australian crosses regardless of the paternal karyotype. These data suggest that the A1,3 translocation in Australian *M. persicae* is not the same as

that in European aphids, even though it too is associated with insecticide resistance. The translocation in Australia must represent either an event independent from that in Europe, or the same event with subsequent modification involving the movement of locus M40 to an autosome with no other marker.

On the basis of chromosome length we suggest that the linkage group M37^A–M49^A–M55^A–M62^A is most likely to be on autosome 2 and the remaining group (myz2^A–myz9^A–S16b^A) on autosome 4 or 5. Australian *M. persicae* would have M40 on the remaining autosome and no markers on autosome 3, while European counterparts have no markers on one of the small autosomes.

(ii) Evolution of achiasmate meiosis

We can not unequivocally say the single recombinant genotype produced via the male line in the Australian crosses was the result of a real recombination event. It is possible that a single repeat unit mutation at locus M107 in the father produced the ‘false’ recombinant genotype. However, if it was a real recombination event, this would support the cytological evidence of rare chiasma formation during spermatogenesis in *E. punctipennis* (Blackman, 1976), and would imply that males have not completely lost the machinery for recombination. *Myzus persicae* might be viewed as being at the very extreme end of the spectrum of quantitative variation in rates of recombination.

There is a common association between achiasmate meiosis and species possessing holocentric chromosomes. Examples include aphids (Blackman, 1987), scorpions, the Lepidoptera and Trichoptera (White, 1973). Perhaps the absence of localized centromeric function has been important in affecting the mechanical role of chiasmata in insect species with holocentric chromosomes.

The evolution of sex differences in recombination has been reviewed elsewhere (Trivers, 1988; Charlesworth, 1996), so will not be discussed in depth here. In essence it has been argued that the evolution of the Y chromosome has been associated with a quantitative decline in the rate of recombination in the heterogametic sex. Views on the selective forces causing degradation of the proto-Y chromosome vary (for differences of opinion see Haldane, 1922; Huxley, 1928; and Trivers, 1988). Whilst the Y-degradation argument is plausible in mammals in which the Y chromosome has a dominant male-determining effect, it is not applicable to all animal groups, e.g. insects (Blackman, 1985). Using data on chiasma frequency from a range of taxa, Burt *et al.* (1991) found that quantitative sex differences in recombination rates are not related to sex or gamety, and concluded that achiasmate meiosis did not evolve from a gradual

decline in recombination in the heterogametic sex. These conclusions were based on karyological observations, and could be affected by sex-specific (or gamety-specific) differences in position of chiasmata, and of undetected chiasmata. A re-analysis of the data using map lengths based on marker loci would be most interesting. Nonetheless, we feel the conclusions reached by Burt *et al.* (1991) have merit when insect sex determination is considered.

XX/XO sex determination is seen in most insect orders. Does this represent an extreme evolutionary end-point of the degradation process, in which the Y chromosome was completely lost? This reasoning does not seem plausible, in that it implies that selection to reduce recombination leading to evolution of the proto-Y ultimately removed genes determining maleness. In insects ancestral sex determination was more likely XX/XO. In fact in cyclically parthenogenetic aphids a Y chromosome could never persist since only females survive over the summer months (Blackman, 1985). The appearance of a Y chromosome in insect orders which commonly contain species with XO males (e.g. Hemiptera, Odonata, Orthoptera) is often the result of a *de novo* centric fusion of an X chromosome and an autosome. In this case the Y chromosome has no male-determining function and sex is determined by genic balance (Blackman, 1985). Inhibition of recombination in male aphids must be dependent on a dosage effect governed by the number of X chromosomes.

(iii) Adaptive significance of the absence of male recombination in aphids

The lack of autosomal recombination and XO hemizygous system in male aphids could be viewed as a mild form of haplodiploidy. This could act as a mechanism to eliminate genetic defects from the population, since lethal mutations cannot be ‘shuffled’ and deleterious sex-linked mutations are exposed on the single male X chromosome. Indeed the accumulation of deleterious mutations under functional parthenogenesis was implicated in the non-representation of one X chromosome in male *Sitobion miscanthi* aphids, and similarly in reduced male fertility in *S. near fragariae* (Wilson, 2000). Recombination in females may provide the new potentially favourable gene combinations. Alternatively, the absence of recombination in males may also provide a means to maintain favourable gene combinations.

We have confirmed that male *M. persicae* have essentially no recombination, while females have substantial levels. Males are hemizygous for the X chromosomes, and thus *M. persicae* represents a particularly useful class of organism, one in which the

achiasmata, heterogametic sex is hemizygous. Quantitative investigations of female recombination rates, including comparison of X and autosomal rates, could make a substantial contribution to understanding sex/gamety differences. The basic genetic findings and X chromosome and autosomal linkage groups we have established here provide a platform from which to start.

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