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Strong biases in the transmission of sex chromosomes in the aphid *Rhopalosiphum padi*

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Summary

The typical life cycle of aphids involves several parthenogenetic generations followed by a single sexual one in autumn, i.e. cyclical parthenogenesis. Sexual females are genetically identical to their parthenogenetic mothers and carry two sex chromosomes (XX). Male production involves the elimination of one sex chromosome (to produce X0) that could give rise to genetic conflicts between X-chromosomes. In addition, deleterious recessive mutations could accumulate on sex chromosomes during the parthenogenetic phase and affect males differentially depending on the X-chromosome they inherit. Genetic conflicts and deleterious mutations thus may induce transmission bias that could be exaggerated in males. Here, the transmission of X-chromosomes has been studied in the laboratory in two cyclically parthenogenetic lineages of the bird cherry-oat aphid *Rhopalosiphum padi*. X-chromosome transmission was followed, using X-linked microsatellite loci, at male production in the two lineages and in their hybrids deriving from reciprocal crosses. Genetic analyses revealed non-Mendelian inheritance of X-chromosomes in both parental and hybrid lineages at different steps of male function. Putative mechanisms and evolutionary consequences of non-Mendelian transmission of X-chromosomes to males are discussed.

1. Introduction

Following classical Mendelian inheritance, offspring inherit one copy of each gene from each parent. However, non-Mendelian segregations are relatively common in natural populations of plants, fungi, insects and mammals, affecting either autosomes or sex chromosomes (Lyttle, 1991). Non-Mendelian segregations can have diverse underlying factors, including chromosome deficiency, cytoplasmic incompatibility (Barr, 1980), segregation distorters (Lyttle, 1993; Silver, 1993) and the preferential transmission of the chromosome of a given parental origin through imprinting (Crouse, 1960).

In aphids, the typical annual life cycle involves several parthenogenetic generations followed by a single sexual one in autumn. All individuals, whether parthenogenetic or sexual, carry two sets of autosomes, but the number of sex chromosomes varies between sexes: parthenogenetic and sexual females carry two X-chromosomes (XX), whereas males carry only one (X0). Sexual individuals are produced by parthenogenesis. Previous genetic studies on parthenogenetic aphids have shown that no recombination event occurs during parthenogenesis (Blackman, 1979; Tomiuk & Wöhrmann, 1982; Sunnucks *et al.*, 1996; Simon *et al.*, 1999; Wilson *et al.*, 1999; Haack *et al.*, 2000; Hales *et al.*, 2000). In particular, no evidence of recombination of X-chromosomes in aphids has been found during the parthenogenetic production of sexual females and males in four aphid species (Hales *et al.*, 2002). Sexual females are thus genetically identical to their parthenogenetic mothers while the production of males involves the elimination of one X-chromosome. Consequently, a given parthenogenetic female is theoretically able to produce two types of male according to the X-chromosome transmitted. This sex determination system could exaggerate genetic conflicts between X-chromosomes and promote the selection of mechanisms favouring

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the preferential elimination of one X-chromosome during male production. In addition, deleterious recessive mutations could accumulate on sex chromosomes during the parthenogenetic phase and affect males differentially depending on the X-chromosome they inherit. Both genetic conflicts and deleterious mutations may thus induce transmission bias that could be exacerbated in males. However, no bias has been detected in the two aphid species in which it has been looked for: *Sitobion* near *fragariae* (Wilson *et al*., 1997) and *Acyrthosiphon pisum* Harris (Caillaud *et al*., 2002).

In the present study the transmission of X-chromosomes in *Rhopalosiphum padi* L. was followed in the laboratory using two X-linked microsatellite loci, through an entire reproductive cycle including sexual and asexual phases. Reciprocal crosses were performed between two cyclically parthenogenetic lineages and individuals were analysed genetically at various stages in the life-cycle: the initial parthenogenetic females, the sexual females and males they produced, the parthenogenetic females hatched from the eggs, and the males produced by these hybrid females. We show that the proportions of X-chromosomes in males do not match Mendelian ratios, suggesting that the elimination of one X-chromosome during male production is not always random in aphids.

2. Materials and methods

(i) *The biological model: the bird cherry-oat aphid* Rhopalosiphum padi

As in many aphid species, cyclically parthenogenetic lineages of *Rhopalosiphum padi* alternate several parthenogenetic generations and a single sexual generation within the annual life-cycle (Simon *et al*., 1991). In Europe, sexual reproduction occurs from the end of summer to the beginning of autumn on the winter-host *Prunus padus* L., while parthenogenetic reproduction occurs on many species of Poaceae in spring and summer (Dixon & Glen, 1971). The production of sexuals is triggered by decreasing temperature and day length (Hille Ris Lambers, 1966; Lees, 1966). By late summer, cyclically parthenogenetic lineages produce gynoparae (i.e. winged parthenogenetic females specialized in the production of sexual females) and males on summer-hosts, both of which then fly to the winter-host. There, gynoparae produce sexual females that mate and lay diapausing cold-resistant eggs. Eggs hatch by the end of February to beginning of March, each one initiating a new clonal lineage.

(ii) *Sexual inductions and reciprocal crosses*

In order to discriminate the X-chromosomes transmitted to their offspring, two cyclically parthenogenetic lineages differing in their genotypes at several microsatellite loci (named A and B) were selected. Lineage A was collected in Alsace (eastern France) during April 1992 and lineage B in Nord-Pas-de-Calais (northern France) during April 1993, both from *P. padus*. Aphids were maintained as parthenogenetic lineages under controlled conditions (18 °C and 16L : 8D) on wheat cv. Arminda.

To induce the production of sexual forms, individual aphids were placed in a program-controlled chamber in short-day conditions and low temperatures using a standard protocol (Simon *et al*., 1991). Briefly, after a period of about 15 days at 15 °C and 16L : 8D, a fourth instar larva (future winged female, generation G_0) was isolated and placed at 12 °C and 10L : 14D. After developing into a winged female, the individual was allowed to produce offspring from which the first-born larva (generation G_1) was isolated and grown in the same conditions. When developed into a wingless female, the aphid produced first gynoparae then males (generation G_2).

Males were collected at adult stages and split into two groups: one to investigate X-chromosome bias through genotyping and the other to produce hybrid lineages. When bias was detected in adult males, further analyses were done on embryos and young larvae to investigate at what stage(s) transmission bias was expressed. Embryos were obtained from females of generation G_1 dissected just after reaching adult moult. Gynoparae produced by each lineage were pooled on a winter-host *Prunus padus* enclosed in a muslin cage, where they gave birth to sexual females (generation G_3). Males from the alternative lineage were then introduced into the cage and allowed to mate with sexual females. Resulting eggs hatched as parthenogenetic females after a diapausing period of about 4 months on *P. padus* at a constant temperature of 5 °C. Five weeks later, each F_1 hybrid was isolated and placed on wheat cv. Arminda at 18 °C and 16L : 8D to maintain the hybrid individuals as clonal lineages. Up to 35 hybrid lineages from each of the two crosses were kept individually and reared for subsequent analyses.

X-chromosome microsatellite genotypes of hybrid lineages were then determined. Among all the lineages carrying the same X-genotype, one was randomly chosen for each different hybrid genotype and placed in sex-inducing conditions to produce males whose genotypes were determined.

(iii) *Genotypic analyses*

Genotypes of A and B and of F_1 hybrid lineages and of the males produced by these lineages were determined using microsatellites. DNA of individual aphids was extracted using the 'salting-out' method (Sunnucks & Hales, 1996) and resuspended in 20 μl

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of TE Buffer. Two X-linked microsatellite loci (S17b and R3.171) respectively cloned and developed from *Sitobion miscanthi* Takahashi (Wilson *et al*., 2004) and *R. padi* (Simon *et al*., 2001) were used. Microsatellite polymerase chain reactions (PCR) were carried out following Simon *et al*. (2001). After PCR cycles each reaction was analysed by automated capillary electrophoresis and the actual size of microsatellite alleles was determined using manual electrophoresis in polyacrylamide gel as described in Delmotte *et al*. (2001). X-linked microsatellite loci allowed the distinction between X-chromosomes carried by parthenogenetic females of parental lineages. The four X-chromosomes were named X_1 (alleles 161 at locus S17b and 226 at locus R3.171) and X_2 (161–246) for parental lineage A (females are X_1X_2), and X_3 (169–209) and X_4 (165–209) for parental lineage B (females are X_3X_4). Since both parental lineages were homozygous at one locus or the other, recombination events that could have occurred during oogenesis remained undetectable. Therefore, maternally-inherited X-chromosomes were denoted according to their observed microsatellite genotype. For instance, the offspring produced by the cross between male of parental lineage A carrying X_2 and female of parental lineage B (X_3X_4) will be labelled $X_2X_{3\text{-like}}$ when it carries the alleles characteristic of X_3 and $X_2X_{4\text{-like}}$ when it carries the alleles characteristic of X_4.

(iv) *Statistical analyses*

For each parental and F_1 lineage, the likelihood of an even ratio of the two X-chromosomes in males given the observations was checked through the calculation of the probability of the observed frequencies under the hypothesis of equal probabilities of transmission of both X-chromosomes. Calculations were achieved with the binomial function of Microsoft Excel 97.

3. Results

(i) *Production of hybrid lineages*

Hatching success strongly depended on the direction of the cross, ranging from about 18% only in cross ♂A ∗ ♀B (35 parthenogenetic females hatched from 200 eggs) to more than 96% in cross ♂B ∗ ♀A (241 from 250). A strong difference in egg mortality rate was thus observed between the two reciprocal crosses.

All the 35 hybrid lineages from the ♂A ∗ ♀B cross and 34 randomly chosen lineages from ♂B ∗ ♀A were retained and reared for subsequent analyses.

(ii) *Genotypes of offspring*

The frequencies of the eight possible X-chromosome microsatellite genotypes in the hybrid lineages are presented in Table 1. Three genotypes were missing ($X_1X_{4\text{-like}}$ in cross ♂A ∗ ♀B, and $X_4X_{1\text{-like}}$ and $X_4X_{2\text{-like}}$ in ♂B ∗ ♀A), and one genotype was at very low frequency (only three occurrences of $X_1X_{3\text{-like}}$ in ♂A ∗ ♀B).

Table 1. *Genotypic frequencies in hybrid lineages obtained from the crosses between parental lineages A (carrying X_1 and X_2) and B (carrying X_3 and X_4) according to their sex chromosomes genotypes*

(a) Cross ♂A ∗ ♀B

	♂A	$\underline{X_1}$ (161–226)	$\underline{X_2}$ (161–246)	Total	
♀B					
$X_{3\text{-like}}$ (169–209)		3	15	**18**	$P=1.00$
$X_{4\text{-like}}$ (165–209)		0	17	**17**	
Total		**3**	**32**	35	
			$P<0.001$		

(b) Cross ♂B ∗ ♀A

	♂B	$\underline{X_3}$ (169–209)	$\underline{X_4}$ (165–209)	Total	
♀A					
$X_{1\text{-like}}$ (161–226)		16	0	**16**	$P=0.86$
$X_{2\text{-like}}$ (161–246)		18	0	**18**	
Total		**34**	**0**	34	
			$P<0.001$		

Alleles (given as number of base pairs) at the two X-linked loci are given in parentheses (S17b and R3.171). The X-chromosome transmitted by the male is underlined.

One of the X-chromosomes was then preferentially (X_2 in ♂A ∗ ♀B) or exclusively (X_3 in ♂B ∗ ♀A) transmitted by males in each cross direction. On the contrary, no significant departure from Mendelian proportions (1 : 1) was observed in X-chromosomes transmitted by females.

(iii) *Male production by parental lineages A and B*

The frequencies of the two expected male types produced by parental lineages A and B are showed in Table 2. For lineage A, both types of males (X_1 and X_2) were found at the adult stage. By contrast, for lineage B only X_3 males were found at the adult stage. However, both types of males (X_3 and X_4) were present at embryonic and early larval stages.

(iv) *Male production by hybrid lineages*

In the following, hybrid lineages are indicated by the pair of X-chromosomes inherited, with the paternally inherited X-chromosome underlined and placed in first position. In total, five different

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Table 2. *X-chromosomes frequencies in males produced by parental lineages at various developmental stages*

	Parental lineage A		Parental lineage B	
Stage	X_1 (161–226)	X_2 (161–246)	X_3 (169–209)	X_4 (165–209)
Male embryos	NG	NG	5	3
Male larvae	NG	NG	8	11
Adult males	16	15	40	0

NG, not genotyped.

genotypes were obtained: three from cross ♂A * ♀B ($\underline{X_1}X_{3\text{-like}}$, $\underline{X_2}X_{3\text{-like}}$ and $\underline{X_2}X_{4\text{-like}}$) and two from cross ♂B * ♀A ($\underline{X_3}X_{1\text{-like}}$ and $\underline{X_3}X_{2\text{-like}}$). In three lineages ($\underline{X_2}X_{3\text{-like}}$, $\underline{X_2}X_{4\text{-like}}$ and $\underline{X_3}X_{1\text{-like}}$) the proportions of the two types of males significantly departed from Mendelian expectations at adult stage (Table 3). For the two lineages that did not significantly differ from Mendelian proportions in the first 20 males ($\underline{X_1}X_{3\text{-like}}$ and $\underline{X_3}X_{2\text{-like}}$), an additional batch of 12 males was analysed to increase the statistical power of the experiment, but there was still no significant bias.

4. Discussion

Contrary to earlier studies on aphids (Wilson *et al.*, 1997; Caillaud *et al.*, 2002) – with the exception of unpublished evidence of transmission bias in sex chromosomes in *Sitobion* (A. Wilson, PhD thesis, Macquarie University, Australia, 2000) – this work revealed non-Mendelian inheritance of X-chromosomes in males in two field-collected cyclically parthenogenetic lineages of the aphid *R. padi* and in their hybrids produced in the laboratory. In lineage A, the two types of males reached adult stage in equal proportions. This suggests that the transmission bias observed in the progeny resulted from either a low value in sexual and/or sperm competition of males carrying X_1 or a reduced viability of their offspring. In lineage B, bias was detected at an earlier stage and was due to strong larval mortality of males carrying X_4. Biases were also observed at male production in three out of five hybrid lineages and might have been even more pronounced had we followed the success at gamete transmission of males produced by hybrid lineages (Fig. 1).

Several mechanisms are often invoked to account for transmission biases, including chromosome deficiency caused by deleterious mutations, cytoplasmic incompatibility, meiotic drive and imprinting.

The succession of numerous generations of diploid parthenogenetic females during the asexual phase is likely to promote the accumulation of deleterious mutations in aphid lineages (Wilson *et al.*, 2003). Deleterious mutations certainly could have time to accumulate in lineages A and B of *R. padi* that have been maintained parthenogenetically for 9 years (*c.* 320 generations) and 8 years (*c.* 290) respectively. The haploid X-chromosome in males is particularly exposed to the deleterious effects of recessive mutations that otherwise remain silent in diploid females (Hales *et al.*, 1997). The high larval mortality of males carrying X_4 observed in this study could be due to such a mutation accumulation on this chromosome. Deleterious mutations accumulated on X_4 could have been passed on to $X_{4\text{-like}}$ offspring and through recombination at oogenesis to $X_{3\text{-like}}$ and account for the low proportion of males carrying these two X-chromosomes in some hybrid lineages. Evidence for deleterious effects of mutation accumulation in parthenogenetic lineages of aphids are lacking. Mutation accumulation experiments in the grape phylloxera *Daktulosphaira vitifoliae* showed significant decline in fitness after *c.* 20 clonal generations, but this strongly depended on both phylloxera genotypes and life history traits that were measured (Downie, 2003). The production of aborted and viable male embryos in equal proportions by a clone of the vetch aphid *Megoura viciae* led Crema (1981) to propose that recessive lethal factors were present in one of the two X-chromosomes. Since the clone of *M. viciae* has been reared in the laboratory for many years in permanent parthenogenesis, mutations could have accumulated independently on the X-chromosomes, producing detrimental effects at male production in the same way as observed in *R. padi*.

The fact that biases in X-chromosome transmission could result from mutation accumulation during clonal reproduction has two types of implication. First, we expect the occurrence of biases to increase with the duration of asexual reproduction. This could be tested either by using lineages maintained in the laboratory in permanent parthenogenesis during various time spans or by comparing populations with an obligate sexual phase each year with those that have lost sex in the life-cycle. Second, deleterious mutations acquired during a parthenogenetic regime should spread among populations via crosses, as seemed to occur for our hybrid lineages of *R. padi*. Many aphid species encompass both cyclically parthenogenetic (sexual) lineages and essentially asexual ones still capable of producing some sexual forms, creating a potential for gene exchanges between these two (Dedryver *et al.*, 1998; Simon *et al.*, 2002; Vorburger *et al.*, 2003; Halkett *et al.*, 2005). Essentially asexual lineages are thus more prone to accumulate deleterious mutations that could be passed on through the sexual

Table 3. *X-chromosomes frequencies in males produced by the five hybrid lineages*

Lineage	Sample size	Male genotype	Parental origin	Observed	*P*
$\underline{X_1}X_{3\text{-like}}$	32	$\underline{X_1}$ (161–226)	♂	17	0.86
		$\overline{X_{3\text{-like}}}$ (169–209)	♀	15	
$\underline{X_2}X_{3\text{-like}}$	20	$\underline{X_2}$ (161–246)	♂	18	<0.001
		$\overline{X_{3\text{-like}}}$ (169–209)	♀	2	
$\underline{X_2}X_{4\text{-like}}$	20	$\underline{X_2}$ (161–246)	♂	18	<0.001
		$\overline{X_{4\text{-like}}}$ (165–209)	♀	2	
$\underline{X_3}X_{1\text{-like}}$	20	$\underline{X_3}$ (169–209)	♂	17	0.003
		$\overline{X_{1\text{-like}}}$ (161–226)	♀	3	
$\underline{X_3}X_{2\text{-like}}$	33	$\underline{X_3}$ (169–209)	♂	14	0.49
		$\overline{X_{2\text{-like}}}$ (161–246)	♀	19	

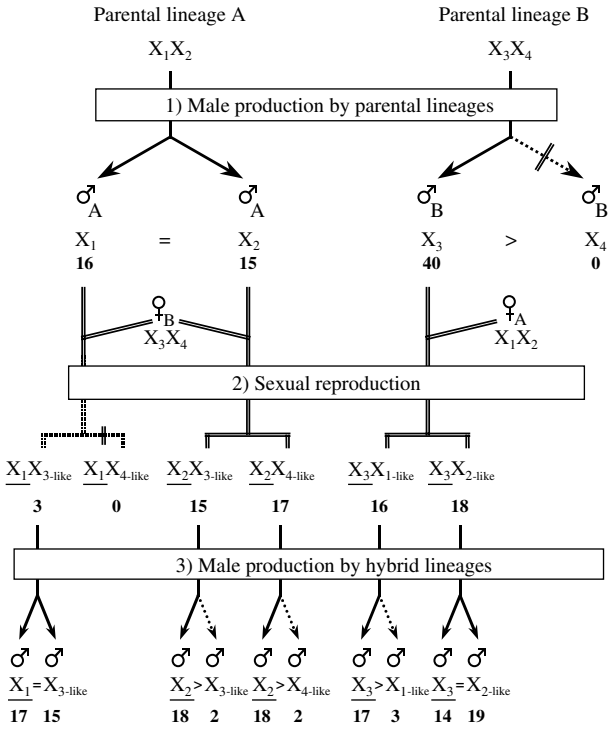

Fig. 1. Transmission of sex chromosomes observed during the whole cycle of reproduction for two cyclically parthenogenetic lineages of *Rhopalosiphum padi*. Dotted lines represent X-chromosomes transmitted at low frequency. Paternally inherited X-chromosomes are underlined.

reproduction with their sexual relatives. If such an accumulation actually occurs in the wild and is not mainly an artefact due to long rearing in the laboratory, this would have profound consequences on the fitness of the resulting offspring, especially on their male function, which is particularly exposed to the effects of deleterious mutations.

In addition to the direct observations of frequent biases in *R. padi*, we have found a large difference in hatching rates in the reciprocal crosses. Such an asymmetry in hybrid viability is often attributed to nuclear-cytoplasmic incompatibilities, which in arthropods usually involve micro-organisms (e.g. *Wolbachia*). Aphids are known to host various maternally inherited symbiotic bacteria (Moran & Telang, 1998; Russell *et al*., 2003), including *Wolbachia* (Gómez-Valero *et al*., 2004). One can envisage that lineages A and B of *R. padi* harboured different symbiotic strains that were responsible for the asymmetrical hatching rates in the crosses.

In all cases where X-chromosome transmission bias was observed in hybrid lineages, the paternal X-chromosome was always favoured. An alternative hypothesis accounting for bias in *R. padi* could thus involve the existence of an imprinting phenomenon. The concept of imprinting was first used to describe the selective elimination of paternal chromosomes in sciarids (Crouse, 1960). At present, it mainly refers to a mechanism of gene regulation consisting of differential expression of paternally and maternally derived genes (Constância *et al*., 1998; Spencer *et al*., 2004). Genomic imprinting has been mostly studied in mammals but examples of selective elimination and/or inactivation of a given parental chromosome have also been documented in insects and have been showed to be involved in sex determination in a few groups including dipterans (White, 1973; Stuart & Hatchett, 1988, 1991; Sánchez & Perondini, 1999) and coccids (White, 1973; Brown & Chandra, 1977), a sister group of aphids. Imprinting has never been reported in aphids, perhaps because it has never been investigated thoroughly.

In aphids, the haploid state of X-chromosome in males and the absence of recombination at male production mean that a paternal X-chromosome transmitted to sons experiences no recombination event (Blackman, 1987; Sloane *et al*., 2001). This peculiar process may lead to the differentiation of male-specialized X-chromosomes that could accumulate genes enhancing the male function. Favouring the transmission of such X-chromosomes to males as a whole linkage group could therefore represent an efficient mechanism of producing high-fitness males. This could favour the evolution of imprinting in

aphids, especially since they usually have large X-chromosomes (Blackman, 1985).

We have shown here that biases in transmission of X-chromosomes affected the two lineages of *R. padi* and some of their hybrids and suggested biases can differ in their origins and effects. Therefore, the phenomenon of biased inheritance of X-chromosomes could be more common in aphids than previously thought. Several important phenotypic traits (e.g. wing polymorphism in male of *A. pisum*: Caillaud *et al.*, 2002; Braendle *et al.*, 2005) are controlled by genes located on X-chromosome, which accounts for about a quarter to a third of the haploid aphid genome. Thus, biases in the transmission of X-chromosome would certainly have dramatic consequences on population genetics and evolutionary ecology of aphids. Further analyses are required to assess the frequency of these biases in natural populations of *R. padi* and other aphid species and to assess the relative importance of underlying factors.

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