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# The genetics of obligate parthenogenesis in an aphid species and its consequences for the maintenance of alternative reproductive modes

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Although loss of sex is widespread among metazoans, the genetic mechanisms underlying the transition to asexuality are poorly understood. Aphids are good models to address this issue because they frequently show reproductive-mode variation at the species level, involving cyclical parthenogens (CP) that reproduce sexually once a year and obligate parthenogens (OP) that reproduce asexually all year round. Here, we explore the genetic basis of OP in the cereal aphid *Sitobion avenae* by crossing several genotypes with contrasting reproductive modes and then characterising the reproductive phenotypes of F1 and F2 offspring. The analysis of phenotypic variation in F1 and F2 progenies suggests that at least two autosomal loci control OP in *S. avenae*. First, the transition to asexuality seems to depend on a single recessive locus, because the offspring from self-crossed cyclical parthenogenetic genotypes contain either 0 or 25% OP. Second, as we observed OP in the F1 progenies from crosses between CP and OP, and some CP in the offspring from outcrossed OP, a dominant 'suppressor' gene may also be involved, being inactive when in a recessive homozygous state in CP; this is the most parsimonious explanation for these results. This oligogenic inheritance of OP in *S. avenae* appears to be an efficient genetic system to generate new OP genotypes continually. It also allows asexuality-inducing alleles to be protected locally during harsh winters when extreme frost kills most OP, and then to spread very quickly after winter.
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## INTRODUCTION

In organisms with environmental sex determination such as cyclical parthenogens (CP), sexual and asexual generations alternate in the life cycle and transitions to obligate parthenogenesis (OP) often occur (Lynch, 1984; Vrijenhoek, 1998). In such cases, the initial spread of OP in the sexual populations, as well as the eventual equilibrium between both reproductive modes, strongly depends on the different mechanisms involved in transitions from sexuality to asexuality, such as mutation, hybridisation, microbial infection and genetic contagion (Simon *et al.*, 2003).

Contagious asexuality refers to a process whereby parthenogenesis-inducing alleles are spread by rare males produced in parthenogenetic lineages and that convert sexual into asexual lineages (Simon *et al.*, 2003; Sandrock and Vorburger, 2011). The effects of the meiosis-suppressing loci are most often limited to the female genetic pathway and a variable quantity of males in addition to asexual females can be produced by OP clones. In such systems, if males from OP clones are functional and if CP and OP populations are sympatric, crosses between sexual females from CP clones and males produced by OP clones can produce offspring with either reproductive mode (Innes and Hebert, 1988). In less common cases, the female genetic pathway is only partially affected and some sexual females are also produced by OP clones (Blackman, 1971), which increases the chances of contagious asexuality by allowing bi-directional gene flow. Contagious asexuality has been reported in a wide range of arthropods (Delmotte *et al.*, 2001; Lattorff *et al.*, 2005; Paland *et al.*, 2005; Halkett *et al.*, 2008; Sandrock and Vorburger, 2011). When studied, inheritance of obligate asexuality has been found to be mono- or oligogenic in most cases. A dominant sex-limited meiosis suppressor at a single locus has been hypothesised in the case of OP in *Daphnia pulex* (Innes and Hebert, 1988). However, it was later established that meiosis suppression is in fact owing to a dominant epistatic interaction among the products of at least four unlinked loci in several genomic regions (Lynch *et al.*, 2008). Recently, it was shown that the genomes of OP clones contain a transposable element that is inserted in one of the candidate genes and is completely absent of the genome of CP clones (Eads *et al.*, 2012). In the rotifer *Brachyonus calyciflorus*, OP clones are homozygous for a recessive allele op, which renders them unable to respond to the chemical signals that usually induce sexual reproduction in this species (Scheuerl *et al.*, 2011), whereas heterozygotes and homozygotes for the wild type are CP (Stelzer *et al.*, 2010). In insects, all-female parthenogenesis (thelytoky) has been found to be inherited as a single-locus recessive trait in two hymenopterans, the Cape honeybee *Apis mellifera capensis* (Lattorff *et al.*, 2005), and a solitary aphid endoparasitoid wasp, *Lysiphlebus fabarum* (Sandrock and Vorburger, 2011).

In aphids, cyclical parthenogenesis is the ancestral reproductive mode and alternation of sexuality and asexuality is triggered primarily

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by photoperiod changes (Lees, 1959, 1960). Several species of economic importance have been shown to include lineages exhibiting different degrees of investment in sexual reproduction when reared in short-day conditions. Of these species, *Myzus persicae* Sulzer (Blackman, 1971; Vorburger *et al.*, 2003), *Rhopalosiphum padi* L. (Simon *et al.*, 1991; Delmotte *et al.*, 2002; Halkett *et al.*, 2008) and *Sitobion avenae* F. (Dedryver *et al.*, 1998, 2001; Simon *et al.*, 1999; Helden and Dixon, 2002) have been the most intensively studied. These aphids generally show reproductive variation ranging from CP genotypes, characterised by full commitment to the production of the sexual forms, to OP genotypes, producing essentially or exclusively parthenogens all year round, with intermediate strategies between these two extremes (for example, Blackman (1971); Dedryver *et al.* (2001); Halkett *et al.* (2008)). Given the considerable methodological constraints due to (1) their single annual event of sexuality, (2) the difficulty of obtaining F2 and F3 because of inbreeding depression and (3) the existence in aphids of an 'interval timer' that delays the possibility of sexual induction for several months after egg hatching (Lees, 1960), very few experiments have been conducted on the genetics of OP in aphids. Blackman (1972) is the only study to have focused on the inheritance of reproductive modes in *Myzus persicae,* although his interpretation of results was based on a very small number of F1 and F2 offspring. By crossing *M. persicae* genotypes with distinct reproductive phenotypes, the Blackman (1972) study concluded that (1) cyclical parthenogenesis is dominant, and OP, recessive and (2) that the mixed strategy (intermediate phenotype producing sexual and asexual forms in similar quantities) could be a modification of cyclical parthenogenesis because of an epistatic recessive suppressor. However, the phenotypes of the F2 progenies did not conform well to the second hypothesis.

The cereal aphid *S. avenae* feeds exclusively on Poaceae. It is thus easier to handle in terms of sexual morph production and mating than other species that shift from summer hosts to winter hosts to carry out sexual reproduction. In cyclical parthenogenetic genotypes of *S. avenae*, production of males and sexual females occurs in autumn and is induced by decreasing day length and temperature (Hand and Wratten, 1985). Mating and egg-laying take place on Poaceae in late November. Eggs overwinter in diapause, hatch spontaneously after 65–90 days of exposure to cold (Dedryver *et al.*, 1998) and initiate clonal lines that reproduce parthenogenetically during spring and summer.

More than 600 *S. avenae* clones originating from different regions of France have been characterised for their life-cycle (Simon *et al.*, 1999; Dedryver *et al.*, 2001) and their genotype (Dedryver *et al.*, 2008). At least in northern France, cyclical and obligate parthenogenetic populations are sympatric, and can exchange genes, as shown experimentally by crossing experiments (Dedryver *et al.*, 1998). Although evidence is only indirect, the fact that obligate parthenogens can be found early in the season, even after a very severe frost ($-10/-12\,^{\circ}C$) that normally would have killed all individuals that had overwintered parthenogenetically, suggests contagious asexuality in wild populations of *S. avenae* (Dedryver *et al.*, 2001). In this study, we investigated the genetic basis of OP in *S. avenae* by crossing several genotypes with distinct reproductive modes, characterising the reproductive phenotypes of their F1 and F2 offspring and testing genetic models for the inheritance of OP in *S. avenae*.

## MATERIALS AND METHODS

### Origin and reproductive phenotypes of parental clones

Seven *S. avenae* clones were used for the crossing experiments (Table 1). When induced for sexual morph production under the right stimuli (see below), clones CPRoum, CPR2, CP14 and CP5 produce only males and sexual females, and are therefore classified as CP genotypes. In contrast, clones OP1, OP21 and OP26 exposed to the same conditions mainly produce parthenogenetic females (>90%), and are therefore classified as OP genotypes.

As their collection in the field (see Table 1), these clones have been reared in controlled conditions at $20 \pm 1\,^{\circ}C$, L16:D8, as described in Dedryver *et al.* (1998). Some clones have been used in previous experiments and have not shown any change with time either in their reproductive phenotype (since 1990) or in their genotype, as assessed with five microsatellite loci (since 1999).

### Sex induction and crossing experiments

Tables 2 and 3 show the various successful crosses we performed and their timing. For each cross, sexual morph production was induced by exposing each parental clone to a short-day regime of L11:D13 at a temperature of $13 \pm 1\,^{\circ}C$ in an environmental chamber with a light intensity of 20 000 lx. This light/temperature regime corresponds to mean outdoor conditions of the first week of October in northern France. It was chosen as the most appropriate for the production of mating females and (to a lesser extent) males, based on prior studies on *S. avenae* (Hand and Wratten, 1985; Dedryver *et al.*, 1998).

For all experiments described hereafter, mass production of sexual morphs was obtained by placing two fourth-instar larvae of each clone (parental generation) on each of 80 wheat seedlings grown individually in plastic tubes and covered with cellophane bag (in the photoperiod/temperature regime described above). After 2 or 3 generations (6–8 weeks and 2 sub-cultures), fourth-instar larvae of sexual females were collected on plants and reared on other wheat seedlings in groups of 10 per seedling. Male larvae were removed as soon as they were born to avoid selfing, and reared separately. Crosses were performed by allowing 50 young sexual females to mate with 10–15 males of the same clone (selfing) or a different clone (outcrossing) on 25–30 vernalised wheat seedlings (cv. Orvantis, resistant to powdery mildew) grown in a 10-cm diameter plastic pot covered with a large cellophane bag ($20 \times 30$ cm). For each cross, there were 2–5 repetitions, depending on male availability. Ten days after egg laying started, seedling pots were transferred to a growth chamber with relative humidity kept at 80% to avoid egg desiccation, and held at $+5\,^{\circ}C$, L8:D16, until egg hatching 65–90 days later (Dedryver *et al.*, 1998). Egg hatching rates were most often around 60%. After the beginning of egg

**Table 1 Geographic origin, collection year, reproductive phenotype and genotypes of the parental clones at eight microsatellite loci**

| | *Geographic origin* | *Date of collection* | *Reproductive phenotype* | *Sm17* | *S4Σ* | *Sm10* | *S5L* | *Sm12* | *S16b* | *Sm11* | *S17b* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| CPRoum | Timisoara (Romania) | 1998 | CP[a] | 180 181 | 158 166 | 167 169 | 226 226 | 144 146 | 173 233 | 114 118 | 206 213 |
| CPR2 | Rennes (W. France) | 1987 | CP[b] | 180 182 | 158 166 | 154 169 | 222 226 | 122 141 | 182 207 | 114 118 | 200 212 |
| CP14 | Lecelles (N. France) | 1993 | CP[b] | 180 180 | 158 158 | 167 167 | 226 226 | 122 135 | 173 207 | 114 114 | 200 213 |
| CP5 | Rennes (W. France) | 1990 | CP[b] | 180 185 | 158 166 | 167 167 | 222 222 | 122 158 | 207 207 | 114 114 | 213 213 |
| OP1 | Rennes (W. France) | 1978 | OP[b] | 180 180 | 152 164 | 154 167 | 222 226 | 135 144 | 188 203 | 114 119 | 206 213 |
| OP21 | Rennes (W. France) | 1990 | OP[b] | 180 180 | 152 176 | 167 187 | NA | 122 144 | NA | 114 114 | NA |
| OP26 | Rennes (W. France) | 1990 | OP[b] | 180 185 | 158 176 | 167 167 | NA | 122 146 | NA | 114 114 | NA |

Abbreviations: CP, cyclical parthenogenetic; N., northern; NA, not assessed at this locus; OP, obligate parthenogenetic; W., western.
[a]This study.
[b]Dedryver *et al.*, 1998.

**Table 2 F1 crosses performed in this study: numbers of offspring and numbers of obligate parthenogenetic (OP) clones**

| | *Female × male* | *Year* | *Number of clones* | *OP clones* | $\chi^2_{66\%OP}$ (P) | $\chi^2_{50\%OP}$ (P) | $\chi^2_{33\%OP}$ (P) | $\chi^2_{25\%OP}$ (P) |
|---|---|---|---|---|---|---|---|---|
| *F1* | | | | | | | | |
| Selfing CP | CPRoum × CPRoum | 2002 | 39 | 0 | | | | |
| | CPR2 × CPR2 | 2002 | 31 | 0 | | | | |
| | CP14 × CP14 | 2002 | 45 | 11 | | | | 0.08 *(0.950)* |
| | CP5 × CP5 | 2002 | 65 | 0 | | | | |
| Outcrossing CP | CPR2 × CP14 | 2005 | 34 | 0 | | | | |
| | CPR2 × CPRoum | 2005 | 26 | 0 | | | | |
| | CPR2 × CP5 | 2005 | 11 | 0 | | | | |
| Outcrossing OP | OP1 × OP26 | 2006 | 44 | 29 | 0.011 (0.915) | | | |
| | OP1 × OP21 | 2006 | 22 | 14 | 0.09 (0.763) | | | |
| Outcrossing CP × OP | CPRoum × OP1 | 2006 | 60 | 24 | | 2.4 (0.12) | 1.2 (0.273) | |
| | CP14 × OP1 | 2006 | 63 | 16 | | | 1.79 (0.181) | 0.005 (0.942) |
| | CP5 × OP1 | 2006 | 29 | 10 | | 4.19 (0.04) | 0.017 (0.896) | |
| | CPR2 × OP1 | 2006 | 52 | 18 | | 4.9 (0.03) | 0.038 (0.845) | |
| | CP5 × OP26 | 2006 | 39 | 11 | | 7.4 (0.006) | 0.461 (0.497) | |
| | CPR2 × OP26 | 2006 | 41 | 9 | | 12.8 (0.0003 | 2.42 (0.122) | 0.203 (0.652) |
| Outcrossing OP × CP | OP1 × CP5 | 2006 | 9 | 3 | | | NA | |
| | OP1 × CPR2 | 2006 | 16 | 8 | | 0 (1) | | |

Abbreviations: CP, cyclical parthenogenetic; NA, not assessed; OP, obligate parthenogenetic.
$\chi^2$ tests carried out on the numbers of OP clones: $\chi^2$ values are given and the associated probability is shown in parentheses; threshold value of $\chi^2 = 3.84$ for d.f. $= 1$ and $\alpha = 0.05$.

**Table 3 F2 crosses performed in this study**

| *F1* | *Year* | *Putative F1 genotypes* | *F2 family* | *Total F2 genotypes* | *OP genotypes* |
|---|---|---|---|---|---|
| (CPR2 × CP14)[a] | 2009 | | | | |
| | | *Aa* | | | |
| Clone 2 | | | Segregating | 15 | 5 |
| Clone 14 | | | Segregating | 6 | 2 |
| Clone 18 | | | Segregating | 11 | 4 |
| Clone 26 | | | Segregating | 17 | 9 |
| | | *AA* | | | |
| Clone 27 | | | Fixed | 20 | 1 |
| Clone 23 | | | Fixed | 26 | 0 |
| Clone 29 | | | Fixed | 8 | 0 |
| (CP5 × CP5)[a] | 2007 | | | | |
| | | *AA* | | | |
| Clone24 | | | Fixed | 32 | 0 |
| Clone28 | | | Fixed | 41 | 0 |
| Clone42 | | | Fixed | 28 | 1 |
| (CPR2 × CP5)[b] | 2007 | | | 24 | 0 |
| (CPRoum × OP1)[b] | 2007 | | | 60 | 0 |
| (CP14 × OP1)[b] | 2007 | | | 60 | 2 |
| (CP5 × Sa1)[b] | 2007 | | | 36 | 0 |

Abbreviations: CP, cyclical parthenogenetic; OP, obligate parthenogenetic.
[a]F2 families from selfing F1 CP clones CPR2 × CP14 and CP5 × CP5 (*A*: major gene).
[b]F2 individuals from crosses among some F1 CP clones. Total numbers of genotypes and numbers of obligate parthenogenetic genotypes in the F2 progenies.

hatching, the number of parthenogenetic females newly born from eggs (fundatrices) were counted every 2 or 3 days. Each fundatrix was carefully removed with a fine brush, and reared on a wheat seedling (cv. Orvantis) at 12 ± 1 °C, L16:D8.

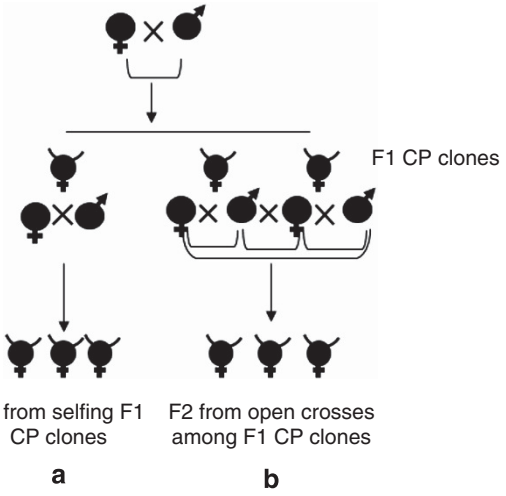


**Figure 1** Crossing design used to generate F2 offspring. F2 were produced (**a**) by selfing F1 CP clones or (**b**) by allowing sexual morphs of different CP clones from the same F1 progeny to mate freely, to reduce inbreeding depression. (♂: male; ♀: sexual female; ☿: parthenogenetic female).

To reduce the effects of inbreeding depression in crosses between CP F1 clones from several initial crosses, sexual forms produced by several clones from the same parental cross were allowed to mate freely (Figure 1b). However, selfings of CP F1 clones were also done (Figure 1a) to assess the heterozygosity of clone CP14 for the putative major gene *A*: each F1 sexual clone of the progenies from CPR2 × CP14, and CP5 × CP5 was selfed and 10 selfings were successful (Table 3).

## Genotyping the F1 progenies

Four complete progenies of CPRoum and CP14 selfings, and of CPRoum and CP14 crosses with OP1 were genotyped (207 clones) to check whether parental

alleles were in Mendelian proportions, and if there was no preferential mortality of some genotypes. In aphids, sexual and parthenogenetic females carry two copies of autosomes and X chromosomes (XX type). Males also have a diploid set of autosomes but are haploid for the X chromosome (X0 type). As transmission bias of sexual chromosomes (because of non-random elimination of either X chromosomes in males) has been documented in several aphid species, including *S. avenae*, genotyping was carried out using autosomal and X-linked genetic markers (Frantz *et al.*, 2005; Wilson and Sunnucks, 2006).

DNA from individual aphids was extracted by using the 'salting-out' protocol described by Sunnucks and Hales (1996). DNA was then resuspended in 20 µl of water. DNA extractions were checked and roughly quantified on a 1% agarose gel electrophoresis and dilutions were made correspondingly in order to reach a DNA concentration of about 10 ng µl$^{-1}$.

Genotyping of *S. avenae* individuals was achieved through analyses at eight aphid microsatellite loci polymorphic in *S. avenae*. The loci Sm10, Sm11, Sm12 and Sm17 are described in Sunnucks *et al.* (1996) and were isolated from *Sitobion miscanthi*, a species closely related to *S. avenae* (Sunnucks and Hales, 1996). S16b and S17b are described in Wilson *et al.* (2004), S4Σ was cloned from *S. avenae* by Simon *et al.* (1999), as was S5L (Simon *et al.*, unpublished). Sm11 and S17b are X-linked (Wilson *et al.*, 1997), the other markers are autosomal.

Amplification reactions were carried out as in Dedryver *et al.* (2008) in a S1000 programmable thermal controller (2008, Bio-Rad Laboratories, Hercules, CA, USA). For loci Sm10, S5L, S16b and S17b, annealing took place at 56 °C, for Sm17 at 53 °C, at 64 °C for Sa4Σ, at 52 °C for SM11 and 55 °C for Sm12. A 1.5% agarose gel electrophoresis was used to check for good amplification and the concentration of the PCR products. These were diluted with water before electrophoresis.

One microlitre of diluted PCR products was added to 5 µl of high-dye formamide containing 1% of 500 LIZ DNA ladder (Applied Biosystems, by Life Technologies Corporation, Carlsbad, CA, USA) and electrophoresis was performed in the capillary sequencer ABI 3130 Xl (Applied Biosystems). Allele calls were automatically assigned by GeneMapper (version 3.7, Applied Biosystems, by Life Technologies Corporation) and visually checked.

### Phenotyping the F1 and F2 progenies

All F1 and F2 progenies from the different crosses were reared for more than a year before exposure to sex-inducing conditions to allow complete disappearance of the interval timer known to inhibit the production of sexual forms in aphids (Dedryver *et al.*, 2012). For each clone, two fourth-instar alatiform larvae from the continuous parthenogenetic lines reared at 20 °C, L16:D8 were kept on a wheat seedling covered with a Cellophane bag (as described above) and allowed to reproduce at 13 °C, L11:D13. Three replicates (batches) per clone were done. For each replicate, a sub-sample of 50 larvae was transferred every 2 weeks to a new seedling to avoid overcrowding. After 6 weeks, numbers of sexual females, males and parthenogenetic females on each seedling were recorded. In most cases, few males were produced by either CP or OP clones, because our experimental conditions were optimal for sexual female production, but not for male production (Hand and Wratten, 1985). Consequently, male production was not taken into consideration in further analyses. A grand total of 1335 aphid clones were analysed for their reproductive phenotype. The strong differences between clones producing mostly sexual females and clones producing almost exclusively parthenogenetic females allowed characterising CP and OP clones, respectively.

The numbers of parthenogenetic females produced by OP clones were not precisely assessed, but ranged from 40 to 60 per seedling batch. Production of parthenogenetic females by CP clones appeared to be negligible (0–5 per batch).

A two-way ANOVA implemented in S-Plus 6.2 (Insightful Corporation, Seattle, WA, USA) was performed to assess the effects of reproductive mode (CP vs OP) and of the cross (CPRoum × OP1, CPR2 × OP1 and CP14 × OP1) on the numbers of sexual females produced by F1 progenies of the three crosses between CP and OP clones. Conformity to expected proportions of clones with different reproductive modes in the F1 progenies was further assessed by $\chi^2$ tests.

## RESULTS

### Characterisation of the different reproductive modes

Figure 2 shows the typical bimodal distribution of abundance classes of sexual females produced per batch for the cumulated progenies of the three crosses between CP clones (CPRoum, CPR2, CP14) and the OP clone OP1, compared with the distribution of the progeny produced by the parents. On the basis of the numbers of sexual females produced, CP clones could be distinguished from OP clones in the F1 progenies ($F_{1,\ 391} = 1545$, $P < 0.001$) and the effect of the cross (CPRoum × OP1, CPR2 × OP1 and CP14 × OP1) on the number of sexual females produced was also significant ($F_{2,\ 391} = 7$; $P < 0.001$). CP clones produced a mean of 43–45 sexual females per batch, whereas OP clones produced a mean of <3 sexual females per batch.

### Microsatellite analysis of F1 clones

For the six autosomal loci, allele frequencies conformed to Mendelian proportions in the four F1 families genotyped (Table 4). Mendelian proportions were also observed for the two X-linked loci in the offspring of both selfings, indicating no transmission bias involving the sexual chromosome in males of CPRoum and CP14. Conversely, F1 progenies from crosses involving OP1 males were not in Mendelian proportions for either of the two X-linked loci, presumably because of the lack of transmission of one of the two X chromosomes in OP1.

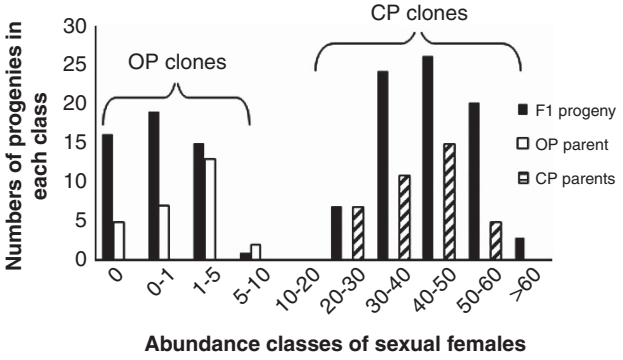


**Figure 2** Bimodal frequency curve of abundance classes of sexual females in the progenies of three crosses between cyclical and obligate parthenogenetic clones of the aphid *S. avenae*. (black bars), compared with the monomodal frequency curves of abundance classes of sexual females in the progeny of the OP (white bars) and CP (hatched bars) parents.

**Table 4 Numbers of F1 clones genotyped from four crosses and $\chi^2$ values for conformity to Mendelian proportions at eight microsatellite loci**

| *F1* | N *clones* | *Sm17* | *S4Σ* | *Sm10* | *S5l* | *Sm12* | *S16b* | *Sm11* | *S17b* |
|---|---|---|---|---|---|---|---|---|---|
| CPRoum × CPRoum | 39 | 0.52 | 0.19 | 1.99 | 0.04 | 0.29 | 2.34 | 0.95 | 0.11 |
| CP14 × CP14 | 45 | NA | NA | NA | NA | 0.37 | 0.89 | NA | 0.06 |
| CPRoum × OP1 | 60 | 0.43 | 0.12 | 0.48 | 0.57 | 0.38 | 1.7 | 11.04 | 4.42 |
| | | | | | | | | *0.01* | *0.05* |
| CP14 × OP1 | 63 | NA | 1.02 | 1.02 | 1.02 | 0.71 | 0.68 | 28.5 | 13.5 |
| | | | | | | | | *0.01* | *0.01* |

Abbreviations: N: clone number; NA: not assessed due to homozygosity at this locus.
P values shown in italics; Sm11 and S17b are X-linked while other loci are autosomal.

### Reproductive phenotypes in the offspring obtained by crossing CP clones

Three of four selfings and the three outcrosses produced 100% CP clones in F1 (Table 2). In F2 families from selfing three F1 (CP5 × CP5) clones, 100 clones out of 101 were CP (Table 3). Similarly, F2 progenies from crosses among several CPR2 × CP5 F1 clones gave 100% CP clones (Table 3).

Conversely, selfing CP14 gave 75.6% CP and 24.4% OP F1 clones. These percentages are not significantly different from a 3:1 ratio (Table 2). Selfing F1 clones from the CPR2 × CP14 cross produced two kinds of F2 families in similar numbers: three fixed families with around 100% CP clones, and four families segregating for CP/OP (Table 3).

### Reproductive phenotypes in the offspring of OP × OP and CP × OP crosses

Outcrossing OP clones produced heterogeneous F1 progenies with a majority (around 66%) of OP clones (Table 2). Outcrossing CP and OP clones also gave heterogeneous F1 progenies, with percentages of OP clones varying between 22 and 50% (usually around 33%, Table 2). F2 progenies from crossing some CP F1 clones gave 0–3% OP clones (Table 3).

### Testing genetic models for the inheritance of OP in *S. avenae*

Selfing and outcrossing CP clones showed that cyclical parthenogenesis is a dominant character over OP and suggest that these reproductive phenotypes are controlled by a major gene (*A*) with two alleles, A and a (Table 5). Under this genetic model, genotypes of CPRoum, CP5 and CPR2 would be AA (CP-A), CP14 Aa (CP-B) and F1 OP clones aa. Arguments in favour of this hypothesis include (1) that F2 progenies from CP5 and CPR2 parents are homogenous (100% sexual clones) and (2) that two kinds of F2 families (fixed and segregating) were obtained after selfing F1 clones from a CP-A × CP-B cross (Table 3).

Results from the OP clone outcrosses, however, are not compatible with a monogenic model, and more generally with the previous hypothesis that OP clones should be aa (Table 5). For example, a minority of CP clones were observed in F1, when only OP clones should be produced if parents were aa. Likewise, results from crosses between CP and OP clones showed segregation in F1, whereas no OP clones should be produced under the monogenic hypothesis.

Let us consider a model involving two independent genes (*A*) and (*S*), a dominant suppressor of (*A*), inactive in the recessive state. In this case, genotypes of CP clones would be AAss (CP-A) or Aass (CP-B), and OP clones, AASs (Table 5). This hypothesis predicts the production of (1) 75% OP clones in OP × OP crosses (or 66%, which fits with the observed proportions, if we assume that SS F1 clones are unviable), and (2) 50% CP clones in CP × OP crosses, which differs from observations (the proportion was closer to 33%). It also conforms to the 100% sexual clones observed in the F2 from CP-A × OP crosses (in this case, all F2 clones would be AAss). However, in F2 progenies from CP-B × OP crosses, some OP should be produced (3% were observed).

A three-gene model, involving a recessive restorer (*b*), inhibiting the effects of (*S*), gives a better prediction of the observed percentage of CP clones produced in CP-A × OP crosses (37.5%), but is not compatible with the homogenous F2 observed for such crosses (Table 5).

**Table 5 Genetic models of the inheritance of obligate parthenogenesis in the aphid *Sitobion avenae*: observed and predicted % of OP clones in F1 and F2**

| *Type of cross* | *Observed OP%* | *Predicted OP%: one-gene model (postulated parental genotypes)* | *Predicted OP%: two-gene model (postulated parental genotypes)* | *Predicted OP%: three-gene model (postulated parental genotypes)* |
|---|---|---|---|---|
| CP-A × CP-A | | *AA* × *AA* | *AAss* × *AAss* | *AAssBb* × *AAssBb* |
| | F1: 0% | F1: 0% | F1 0% | F1 0% |
| | F2: 0% | F2: 0% | F2: 0% | F2: 0% |
| CP-B × CP-B | | *Aa* × *Aa* | *Aass* × *Aass* | *AassBb* × *AassBb* |
| | F1: 25% | F1: 25% | F1: 25% | F1: 25% |
| CP-A × CP-B | | *AA* × *Aa* | *Aass* × *Aass* | *AAssBb* × *AassBb* |
| | F1: 0% | F1: 0% | F1: 0% | F1: 0% |
| | F2: heterogeneous | F2: heterogeneous | F2: heterogeneous | F2: heterogeneous |
| OP × OP | | *aa* × *aa*[a] | *aass* × *aass*[a] | *aassBb* × *aassBb*[a] |
| | F1: 66% | F1: 100% | F1: 100% | F1: 100% |
| | | | *AASs* × *AASs*[b] | *AASsBb* × *AASsBb*[b] |
| | | | F1: 66% | F1: 50% |
| | | | (if SS lethal) | (if SS lethal) |
| | | | F1: 75% | F1: 56% |
| | | | (if SS not lethal) | (if SS not lethal) |
| CP-A × OP | | *AA* × *aa*[a] | *AAss* × *aass*[a] | *AAssBb* × *aassBb*[a] |
| | F1: 22–50% | F1: 0% | F1: 0% | F1: 0% |
| | F2: 0% | F2: heterogeneous | *AAss* × *AASs*[b] | *AAssBb* × *AASsBb*[b] |
| | | | F1: 50% | F1: 37.5% |
| | | | F2: 0% | F2: heterogeneous |
| CP-B × OP | | *Aa* × *aa*[a] | *Aass* × *aass*[a] | *AassBb* × *aassBb*[a] |
| | F1: 25% | F1: 50% | F1: 50% | F1: 50% |
| | F2: 3% | F2: heterogeneous | F2: heterogeneous | F2: heterogeneous |
| | | | *Aass* × *AASs*[b] | *AassBb* × *AASsBb*[b] |
| | | | F1: 50% | F1: 37.5% |
| | | | F2: heterogeneous | F2: heterogeneous |

Abbreviations: CP, cyclical parthenogenetic; OP, obligate parthenogenetic.
[a]If OP clones derive from CP2 selfing and are aa
[b]If OP clones result from (*A*) suppression by (*S*).
(for the three-gene model, several parental genotypes are possible and could give somewhat different results). [*A*] major sex gene, [*S*] sex suppressor, [*b*] sex restorer.

## DISCUSSION

### Range of reproductive phenotype variation in *S. avenae*

Earlier works on reproductive-mode variation in aphids have identified several phenotypes ranging from exclusive production of sexual forms (when exposed to sex-inducing stimuli under standardised conditions) to the sole production of parthenogens, with the inclusion of mixed strategies. In particular, in *M. persicae* and *R. padi*, respectively, Blackman (1972) and Halkett *et al.* (2008) report— in addition to purely cyclical and obligate parthenogens—the existence of 'intermediate' genotypes producing roughly equal numbers of sexual and parthenogenetic morphs. Intermediates have also been reported in field populations of *S. avenae* (Dedryver *et al.*, 2001), but the numbers of sexual females and males produced were much lower than for the two above species. Here, crossing cyclical and obligate parthenogens gave F1 progenies whose production of sexual females was clearly bimodal. Male production was very low in all cases, and could not be used to study the genetic determinism of the male function. Therefore, reproductive-mode variation displayed by the *S. avenae* genotypes used in this study fell into two well-differentiated categories: (1) cyclical parthenogens (CP), which produce large quantities of sexual females in response to short-day conditions, and (2) obligate parthenogens (OP), which produce few sexual females and a large quantity of asexual females when exposed to the same conditions. Phenotypic variation may be greater under a larger range

of genotypes and/or environmental conditions. However, as our aim was to explore the genetic basis of OP, we focused on *S. avenae* genotypes with contrasting reproductive phenotypes characterised in the same controlled environmental conditions.

**Putative genetic control of OP in *S. avenae***

Our results on *S. avenae* cannot be fully compared with those of Blackman (1972), because we did not observe 'intermediate' clones in our F1 and F2 progenies. Nevertheless, we confirmed Blackman's results on the dominance of CP vs OP: we found that selfing one of our CP genotypes gave 25% F1 OP offspring and that crosses between this CP genotype and OP genotypes also gave heterogeneous progeny. However, Blackman's genetic model fails to account for the production of heterogeneous progeny in most of our *S. avenae* CP genotypes crossed with OP genotypes.

Suppressor genes are well known in plants where they are particularly involved in the expression of nuclear male-sterility (Li *et al.*, 2004). In insects, suppressors negatively modulate the 'Notch' pathways, regulating cellular differentiation in *Drosophila* (Nagel and Preiss, 2011) and are involved in the failure in male and female gonad development in the mosquito *Anopheles gambiae* (Magnusson *et al.*, 2011). Our hypothesis that reproductive mode in *S. avenae* is determined by the combined effect of one major gene (*A*) and one suppressor (*S*) is the simplest explanation for the heterogeneous F1 progeny observed in the OP × OP and CP × OP crosses, although observed proportions of OP clones were often significantly lower than those predicted according to our two-gene model. We have no evidence that this difference between observed and predicted proportions is linked to the transmission bias detected in X male chromosome of OP1, because (*A*) and (*S*) appear to be autosomal: outcrosses of CP clones with OP1 gave heterogeneous progenies whatever the crossing direction, although OP1 males apparently transmit only one sexual chromosome. Conversely, departure from expected proportions could be because of preferential mortality of one of the genotypes (for example, SS), despite the fact that autosomal alleles are in Mendelian proportions in the genotyped F1 progenies. Further, this would have no consequence at the genotyped microsatellite loci, because it would affect only the chromosomal region of the considered locus. It is also possible that the suppressor (*S*) is partially inefficient, perhaps because its expression depends upon environmental conditions. Such environmental dependence has been observed for several kinds of suppressors, particularly those controlling nuclear male-sterility in plants. For example, expression of male-sterility in wheat depends both on temperature and photoperiod (Guo *et al.*, 2006). In insects, several temperature-sensitive lethal genes have been described in *Drosophila melanogaster* (Dudick *et al.*, 1974; Neuburger *et al.*, 2006). The suppressor (*S*) may belong to a pool of environmentally sensitive genes responsible for the switch from sexual to asexual reproduction in aphids (Simon *et al.*, 2010). Further research is needed to compare the proportions of both aphid reproductive modes in progenies of CP × OP crosses obtained in different conditions of photoperiod and temperature. Finally, the involvement of a third gene restoring sexuality is an alternative explanation for some of the proportions observed in CP × OP F1, but it implies having heterogeneous F2 in such crosses, which does not fit with our data. Even if none of the tested genetic models fully explain the outcomes of all the crosses we performed on CP and OP *S. avenae* genotypes, it is likely that OP in this aphid is controlled by a limited set of loci. Further genetic analyses on a wider range of phenotypes are nevertheless needed to explore all the mechanisms underlying reproductive-mode variation in the species.

**Consequences for the maintenance of alternative modes of reproduction**

Reproductive mode variation in *S. avenae* may be maintained in field populations by (1) crosses between heterozygous CP genotypes and (2) crosses between CP and OP genotypes or between different OP genotypes (these latter crosses should be rare, however, due to the small number of mating females produced by OP clones). This inheritance mode of OP appears as an efficient genetic system for the continual production of new obligate parthenogenetic genotypes in the wild. It also allows asexuality-inducing alleles to be protected locally during harsh winters when extreme frost kills most obligate parthenogens, and to spread very quickly after winter. Moreover, repeated transitions to OP maintain a high level of genetic diversity in OP populations, allowing their persistence in the long term owing to higher evolvability (Simon *et al.*, 2003; Sandrock and Vorburger, 2011). Despite the high potential of this contagious mechanism to generate obligate parthenogens, its actual incidence in the wild is largely unknown (Simon *et al.*, 2003; Halkett *et al.*, 2008; Sandrock and Vorburger, 2011). To date, for *S. avenae*, we have only indirect evidence of its occurrence in field populations (Simon *et al.*, 1999; Dedryver *et al.*, 2001, 2008). Current efforts to identify regulatory networks of polymorphic genes involved in the control of reproductive-mode variation in aphids (Le Trionnaire *et al.*, 2007, 2009; Simon *et al.*, 2010) and other cyclical parthenogens (Simon *et al.*, 2011; Eads *et al.*, 2012) through quantitative trait loci and functional genomic approaches are promising avenues of future research to identify asexuality-induced alleles and quantify the actual importance of contagious obligate asexuality in the field.

## DATA ARCHIVING

This article does not report new empirical data or software.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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