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# Multiple routes to asexuality in an aphid species

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Cyclical parthenogens, including aphids, are important models for studying the evolution of sex. However, little is known about transitions to asexuality in aphids, although the mode of origin of asexual lineages has important consequences for their level of genetic diversity, ecological adaptability and the outcome of competition with their sexual relatives. Thus, we surveyed nuclear, mitochondrial and biological data obtained on cyclical and obligate parthenogens of the bird cherry–oat aphid, *Rhopalosiphum padi* (L.), to investigate the frequency of transitions from sexuality to permanent asexuality. Many instances of asexual lineages retaining the ability to produce males are known in aphids, so particular attention was paid to the existence of occasional matings between females from sexual lineages and males produced by asexual lineages, which have the potential to produce new asexual lineages. Phylogenetic inference based on microsatellite and mitochondrial data indicates at least three independent origins of asexuality in *R. padi*, yielding the strongest evidence to date for multiple origins of asexuality in an aphid. Moreover, several lines of evidence demonstrate that transitions to asexuality result from two mechanisms: a complete spontaneous loss of sex and repeated gene flow from essentially asexual lineages into sexual ones.

**Keywords:** cyclical parthenogenesis; evolution of sex; reproductive system; asexuality; microsatellite; cytochrome *b*

## 1. INTRODUCTION

Sexual reproduction is the predominant reproductive mode in eukaryotic organisms (Bell 1982). This is an evolutionary paradox, because asexual organisms transmit their genetic information twice as efficiently as do sexual organisms (Maynard Smith 1971; Williams 1975). Given this major 'cost of sex', the coexistence of sexual and asexual reproductive modes in the same organism is especially puzzling and suggests a short-term compensatory advantage to sexual reproduction (Williams 1975). Many balancing advantages of sexuality have been theorized to explain the maintenance of sexual reproduction (Kondrashov 1993). These theories are not mutually exclusive and pluralistic views have been defended (West *et al.* 1999) but, as yet, no theoretical consensus has been reached. With respect to this wealth of hypotheses, there is a relative lack of biological data on conspecific sexual and asexual organisms. Thus, progress in understanding the evolution of sex is likely to be made by accumulating more evidence from diverse biological models (Barton & Charlesworth 1998; West *et al.* 1999).

In this regard, organisms, such as aphids, that reproduce by cyclical parthenogenesis are especially useful because they undergo transitions to obligate parthenogenesis, leading to coexisting sexual (cyclical parthenogenesis) and asexual (obligate parthenogenesis) lineages. However, it is unclear whether conspecific sexual and asexual lineages remain in complete reproductive isolation, especially when they are sympatric. In fact, there is growing evidence that asexual lineages can undergo rare sex with their sexual counterparts (Innes & Hebert 1988; Browne 1992; Belshaw *et al.* 1999). Occasional gene exchange with sexual lineages may be important for the persistence of otherwise asexual lineages. Indeed, theoretical models have suggested that this process could be efficient at slowing down the accumulation of deleterious mutations (Hurst & Peck 1996); it could also generate a substantial amount of 'frozen' genetic diversity in asexual lineages (Vrijenhoek 1978). Interestingly, many instances of asexual lineages retaining the ability to produce males are known in aphids (Simon *et al.* 1991), freshwater flatworms (Pongratz *et al.* 1998), gall-wasps (Plantard *et al.* 1998), non-marine ostracods (Martens 1998), shrimps (Browne 1992) and snails (Samadi *et al.* 1997). However, even when these males are functional, their actual participation in sexual reproduction in nature often remains to be demonstrated (Lynch 1984; Innes & Hebert 1988; Little & Hebert 1996; Martens 1998). None the less, there are numerous aphid species in which asexual lineages have retained the ability to produce a small proportion of males at the time of sexual reproduction. Males produced by asexual lineages are usually functional in laboratory crossing experiments and are assumed to establish gene flow between sexual and asexual lineages, although there is no direct evidence that this occurs in the field (Blackman 1972; Dedryver *et al.* 1998; Rispe *et al.* 1999; Simon *et al.* 1999).

Among aphids, the bird cherry–oat aphid, *Rhopalosiphum padi* (L.), has been the subject of extensive studies that have allowed the characterization of three major reproductive lineages: first, sexual lineages (cyclical parthenogenesis) that alternate sexual and asexual reproduction and complete their annual life cycle on two different hosts—several asexual generations on cereals are followed by a single annual sexual generation in autumn on bird cherry, *Prunus padus* L., with production of cold-resistant eggs; second, asexual (obligate parthenogenesis) lineages that live year-round on cereals (however, most obligate parthenogens of *R. padi* in France retain the ability to produce males in autumn (Simon *et al.* 1991)); and third, intermediate lineages that not only produce both sexual

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forms in autumn but also give rise to parthenogenetic females that persist on cereals. Sexually produced eggs are the only life stage capable of diapause and resistance to frost. A theoretical model has shown that this ecological advantage is sufficient to explain the maintenance of sexual lineages and the geographical distribution of sexual and asexual lineages (Rispe *et al.* 1998; Rispe & Pierre 1998). In addition, this model showed that sexual and asexual life-cycle strategies can coexist even in cold or intermediate climates prone to occasional severe frosts if asexually produced males participate in sexual reproduction. This process actually shelters asexuality genes and gives rise to new asexual lineages after a cold winter lethal to overwintering parthenogenetic generations. Besides the ecological advantage of male production by asexual lineages (a 'hedging-your-bets' overwintering strategy), asexuality genes would accrue benefits from this gene flow in terms of increased genetic diversity of asexual lineages and the production of recombined genomes rid of deleterious mutations. These assumptions are supported by two empirical pieces of evidence. First, extensive surveys of the geographical distribution of mitochondrial variation have shown that sexual and asexual lineages have specific haplotypes (II and I, respectively). However, a few asexual lineages possess haplotype II (Martinez-Torres *et al.* 1996, 1997; Simon *et al.* 1996, 1999). Since the mitochondrial genome is maternally inherited, this pattern has been interpreted as the result of gene flow mediated by males produced by asexual lineages of haplotype I. Second, a substantial proportion of sexually produced individuals in the field are of asexual lineages, presumably resulting from crosses between cyclical-parthenogen and obligate-parthenogen lineages, as suggested by Rispe *et al.* (1999).

In a recent study, we compared the genetic structure of French sexual and asexual populations of *R. padi* using both allozyme and microsatellite markers (Delmotte *et al.* 2001). This analysis highlighted the impacts of reproductive mode on the genetic architecture of each kind of population. However, the precise genetic relationships between sexual and asexual lineages were not examined. Thus, here we investigated, first, the number of independent origins of asexuality and, second, the presence of gene flow between life cycles, which are two important components of the coexistence of sexual and asexual populations. For this purpose, we combined nuclear, mitochondrial and biological data obtained on sexual and asexual lineages of *R. padi*. First, additional sexual and asexual lineages were typed at seven microsatellite loci and added to the microsatellite dataset of Delmotte *et al.* (2001). The additional lineages helped to extend the range of sampling sites and dates. Second, the mitochondrial DNA (mtDNA) haplotypes of all the lineages were characterized by restriction fragment length polymorphism, and the main mtDNA haplotypes were sequenced to obtain estimates of divergence within and between life-cycle types. Because molecular markers do not predict reproductive mode with total accuracy, the reproductive modes of a subset of sexual and asexual lineages were characterized biologically, by laboratory experiments. These experiments allowed us to test the hypothesis of gene flow between sexual and asexual lineages, by which some sexually produced lineages inherit asexuality genes and consequently become obligate parthenogens (Blackman 1972; Simon *et al.* 1999).

## 2. MATERIAL AND METHODS

### (a) *Sampling of sexual and asexual lineages*

Aphid sampling was done in the early spring, when sexual and asexual lineages of *R. padi* are clearly separated in space, being on *P. padus* and cereals, respectively. Asexual lineages were sampled on cereal plants separated by at least 1 m in order to reduce collection of aphids from the same clone. Sexual lineages were collected on *P. padus* just after they hatched from eggs, ensuring that they did not start parthenogenetic reproduction. It is worth noting that these lineages have not necessarily kept their full potential for sexual reproduction and may have inherited asexuality genes, according to the gene-flow hypothesis described in § 1. Therefore, lineages collected on *P. padus* will be referred to hereafter as sexually produced lineages. A total of 188 asexual lineages were sampled in metropolitan France in 1994 ($n = 8$), 1995 ($n = 145$) and 1999 ($n = 35$), together with an additional two in the subantarctic Kerguelen islands in 1998, for comparison. Similarly, 224 sexually produced lineages were sampled across France in 1992 ($n = 13$), 1994 ($n = 3$), 1995 ($n = 204$) and 1999 ($n = 4$). Most sexual and asexual lineages collected in 1995 and 1999 were those already analysed for microsatellite variation in Delmotte *et al.* (2001).

### (b) *Microsatellite analysis*

In all, 30 new lineages were typed at seven microsatellite loci (S16b, S17b, R6.3, R5.29, R5.10, R2.73, R1.35) as described in Simon *et al.* (2001), and added to the 384 lineages already typed by Delmotte *et al.* (2001).

Because clonal amplification of genotypes can affect data interpretation (Sunnucks *et al.* 1997), copies of some asexual multilocus genotypes were removed from the microsatellite dataset (there was no copy of genotypes among sexually produced lineages). We calculated observed and expected heterozygosity, and performed Hardy–Weinberg exact tests and Fisher exact tests for genic and genotypic differentiation between sexually produced and asexual genotypes using GENEPOP 3.1 (Raymond & Rousset 1995). To compare the heterozygosities of sexually produced and asexual genotypes, an analysis of variance (ANOVA) was conducted using SAS software (SAS 1988). In order to investigate the relationships between sexually produced and asexual genotypes, a matrix of pairwise allele-shared distances ($D_{AS}$; Chakraborty & Jin 1993) between all genotypes was calculated using the microsatellite data, and a neighbour-joining (Saitou & Nei 1987) tree was constructed. POPULATION v. 1.2.01 (http://www.cnrs-gif.fr/pge/bioinfo) was used to calculate $D_{AS}$ and to construct neighbour-joining trees. The bootstrap support of nodes (1000 replicates) was calculated for the microsatellite tree. Bootstrap values were all less than 50% (and consequently not reported on the tree) because of the very large number of operational taxon units. Therefore, to assign multilocus genotypes to the sexual or asexual groups of lineages, we used a recently developed assignment method that assesses the probability that a given genotype belongs to a population (Cornuet *et al.* 1999). This Bayesian method, which requires no assumption of Hardy–Weinberg or linkage equilibrium, was performed using GENECLASS v. 1.0.02 ('leave-one-out' procedure; 10 000 simulations).

Table 1. Number of individuals sampled ($n_i$), number of microsatellite multilocus genotypes ($n_g$), mean number of alleles, expected and observed heterozygosity ($H_e$ and $H_o$, respectively) and the $P$ value of the exact test for Hardy–Weinberg proportions of sexual ($n=224$) and asexual ($n=90$) genotypes of *Rhopalosiphum padi*. (hs, highly significant, i.e. $p < 0.0001$.)

| mtDNA | $n_i$ | $n_g$ | mean number of alleles | $H_e$ | $H_o$ | exact test for Hardy–Weinberg proportions |
|---|---|---|---|---|---|---|
| asexual | | | | | | |
| hI | 158 | 68 | 5.85 | 0.596 | 0.832 | hs |
| hII | 32 | 22 | 5.57 | 0.642 | 0.530 | hs |
| sexual | | | | | | |
| hII | 224 | 224 | 12 | 0.672 | 0.518 | hs |

### (c) *Mitochondrial DNA analyses*

The mtDNA haplotypes of all sexually produced and asexual genotypes discriminated with microsatellites were characterized following Simon *et al.* (1996). Briefly, a segment of the mitochondrial genome containing the ND1 gene and portions of the 16S and cytochrome *b* genes was amplified by polymerase chain reaction (PCR) and cut with restriction enzymes that discriminate between the two main haplotypes (hI and hII) encountered in *R. padi* (Simon *et al.* 1996; Martinez-Torres *et al.* 1997). The haplotype information was then reported on the microsatellite neighbour-joining tree for all multilocus genotypes.

In addition, the mtDNA PCR products of three sexually produced lineages (with hII) and five asexual lineages (with either hII or hI) of *R. padi*, as well as two outgroups (*Rhopalosiphum maidis* and *Rhopalosiphum insertum*), were sequenced directly with an ABI 310 following the PE Applied Biosystems (Boston, MA, USA) protocol. A 1199-bp fragment including most of the cytochrome *b* gene was sequenced for each of the 10 samples. Sequences were aligned manually, and the phylogenetic relationships between the haplotypes were assessed using two character-based methods, maximum parsimony and maximum likelihood, and a distance-based method, neighbour-joining. Maximum-parsimony and maximum-likelihood trees were reconstructed using PAUP v. 4. (Swofford 1999); a neighbour-joining tree based on Kimura two-parameters genetic distance was constructed using MEGA v. 2.0 (Kumar *et al.* 2000). The trees were rooted using sequences of the outgroups.

### (d) *Biological characterization of reproductive modes*

In order to check the reproductive modes of the aphid lineages, a subset of sexually produced ($n=15$) and asexual lineages ($n=11$) were tested for their ability to produce sexual forms. These lineages, which were part of a collection of clones kept alive in the laboratory, were chosen, following microsatellite analysis, as representative of the main genetic variants found among the sexual and asexual lineages. Aphid lineages were induced to produce sexuals using standard methods (Simon *et al.* 1991), by placing aphids in long-night (8 L:16 D) low-temperature (12 °C) conditions in programme-controlled cabinets. The response to sex-inducing conditions of between five and 20 replicates was analysed for each lineage. Lineages producing both sexes were classified as cyclic parthenogens, those producing parthenogenetic females most often, along with males, were classified as obligate parthenogens and those producing both sexes, along with parthenogenetic females, were classified as intermediates.

## 3. RESULTS

### (a) *Overall patterns of microsatellite diversity*

Allelic diversity at the seven microsatellite loci ranged from four to 27 alleles per locus, with 86 alleles identified across all loci. Allelic diversity was twice as high in sexually produced populations as in asexual populations, which only possessed a subset of the alleles present in sexually produced lineages (table 1). The total number of genotypes discriminated by microsatellite markers was high ($n=314$ over 414 individuals) due to their high polymorphism (Simon *et al.* 2001). Each sexually produced lineage possessed a unique multilocus genotype, while 47% of the asexual lineages were copies of the same genotypes. No genotype was shared between sexually produced and asexual lineages. Moreover, Fisher exact tests for genic and genotypic differentiation indicated a high and significant separation between sexually produced and asexual groups of genotypes (both $p < 10^{-5}$).

### (b) *Microsatellite diversity patterns among mtDNA haplotypes*

Out of 90 asexual multilocus genotypes (190 lineages sampled), 68 had mtDNA haplotype I (158 lineages) and 22 had mtDNA haplotype II (32 lineages). Most hII asexual genotypes were collected in western and northern France, with only three being collected in southern France. In contrast, all sexually produced genotypes had haplotype II.

The microsatellite data were analysed by separating asexual genotypes into haplotypes I and II. Sexually produced lineages (all with hII) and hII asexual lineages showed significant heterozygote deficit, while hI asexual lineages showed a strong excess of heterozygotes (table 1). The distribution of observed heterozygosity together with the analysis of relatedness between genotypes based on pairwise genetic distance ($D_{AS}$) clearly separate sexually produced lineages and hI asexual lineages; by contrast, hII asexual lineages were close to sexually produced lineages (figure 1). The observed heterozygosities of sexually produced genotypes and hII asexual genotypes were similar (0.530 and 0.518, respectively; ANOVA $p=0.6$), while hI asexual lineages had a significantly higher observed heterozygosity (0.832; ANOVA $p < 0.001$; figure 1*a*). Similarly, the mean number of shared alleles (m. ± s.e.) among sexually produced genotypes was similar to that of hII asexual genotypes (8.41 ± 0.011 and

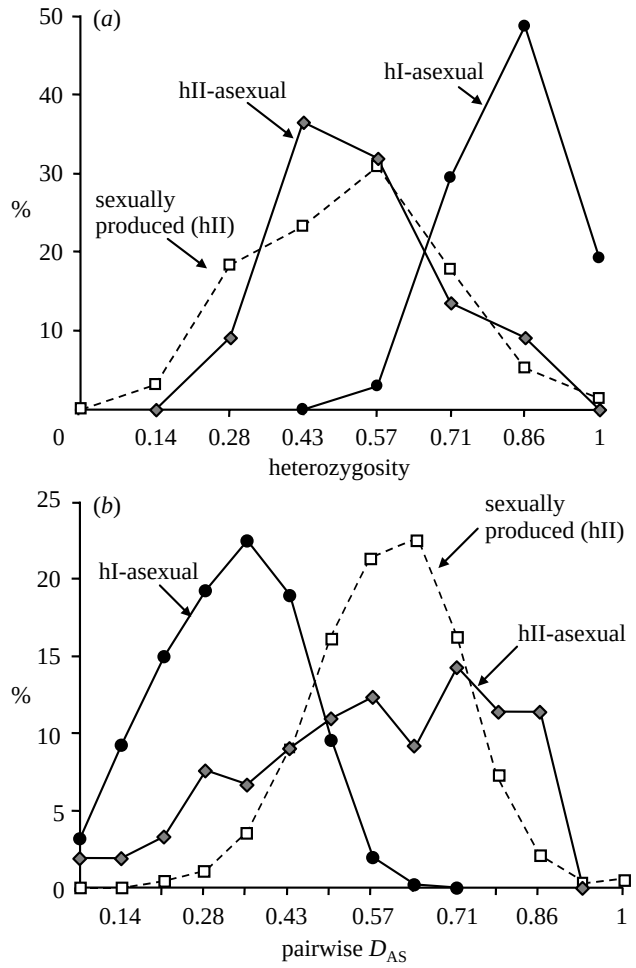


Figure 1. (*a*) Observed heterozygosity and (*b*) pairwise genetic distance ($D_{AS}$) in sexually produced ($n=224$), hII asexual ($n=22$) and hI asexual ($n=68$) genotypes of *Rhopalosiphum padi* based on seven microsatellite loci.

8.01 ± 0.20, respectively; $p > 0.4$) but significantly higher than among hI asexual genotypes ($4.63 \pm 0.033$, $p < 0.001$; figure 1*b*). Within asexual genotypes, the standard error was much higher among hII asexual genotypes than among hI asexual genotypes. This indicates that the hII asexual genotypes do not constitute a homogenous group: some of them resemble hI asexual genotypes and others resemble sexually produced genotypes.

### (c) *Mitochondrial DNA sequence data*

Sequences of the mtDNA fragment (1199 bp) revealed 83 polymorphic sites, of which 29 were phylogenetically informative. In the *R. padi* sequences we found 24 variable sites including 19 informative sites, allowing the discrimination of seven haplotypes over eight sequenced samples. Phylogenetic analysis based on genetic distance (neighbour-joining) revealed a monophyletic origin for each of the haplotype groups I and II (node supported by a bootstrap value of 93) and a strong sequence divergence between haplotype groups; this result was also found with maximum parsimony and maximum likelihood (data not shown). Indeed, the mean (m. ± s.e.) genetic distance variation (Kimura two-parameters) within haplotype groups was never more than $9 \times 10^{-4}$ ($5 \times 10^{-4}$); in contrast it was 0.013 ($\pm 3 \times 10^{-3}$) between the two

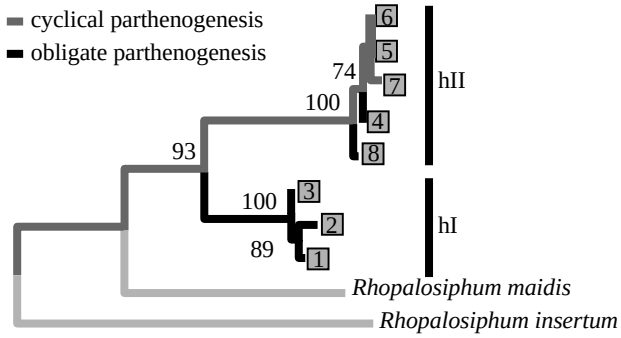


Figure 2. Rooted neighbour-joining tree based on Kimura two-parameter distances of 10 mitochondrial haplotypes (1199 bp comprising cytochrome *b*). The numbers at the nodes are bootstrap percentages (1000 replicates). Changes in reproductive mode are mapped along the neighbour-joining tree, assuming that sexual reproduction is the ancestral reproductive mode and that reversion to sexuality is impossible.

groups, equivalent to 43% of the genetic distance between *R. padi* and *R. maidis*. The five asexual lineages of *R. padi* were distributed among the two well-supported clades: the first clade (hI) was entirely asexual; the second clade (hII) comprised asexual and sexually produced lineages. Under the assumption that asexual lineages cannot revert to sexuality, at least three independent losses of sex are needed to account for the pattern of mtDNA variation among sexual and asexual lineages (figure 2).

### (d) *Combined microsatellite, mitochondrial and biological data*

The mtDNA haplotypes (I or II) of all genotypes were superimposed on the microsatellite neighbour-joining tree (figure 3). The microsatellite tree separated the genotypes into two main groups, sexual and asexual, confirming the results on genic and genotypic differentiation between reproductive modes. The separation between these groups was somewhat arbitrary, but the 'sexual group' comprised the large majority of sexually produced lineages, and the 'asexual group' comprised the large majority of asexual lineages (figure 3).

The 'sexual group' comprised most of the sexually produced genotypes (90.3%, $n=185$), all of which had mtDNA haplotype II. This sexual group also included 11 asexual genotypes, which all also had haplotype II. These asexual genotypes were clearly distinct from the genotypes of the 'asexual group', a result confirmed by assignment tests ($p < 0.01$). In this sexual group, the sexually produced lineages that were tested biologically were all cyclical parthenogens, while the asexual lineages that were tested biologically (including the Kerguelen genotype) were all obligate parthenogens (table 2).

The 'asexual group' comprised most of the asexual genotypes (80%, $n=79$), with either haplotype I ($n=68$) or haplotype II ($n=11$). All hI asexual genotypes clustered in this group. However, this asexual group also included 19 sexually produced genotypes (14 of them being monophyletic). This grouping of sexually produced genotypes in the asexual group was well supported since all genotypes but one were significantly assigned to this

group ($p < 0.01$). In this asexual group, the lineages that were tested biologically (comprising asexual lineages and sexually produced lineages) were either obligate parthenogens or intermediates (table 2).

Combining all the available information reveals that asexual genotypes have a minimum of three independent origins, as supported by both nuclear and mitochondrial trees (figures 2 and 3). First, one origin of asexuality led to the monophyletic clade of hI asexual genotypes. These genotypes comprise most asexual genotypes (75.6%, $n = 68$), and have presumably diverged through clonal evolution, as suggested by their short branch lengths on the microsatellite neighbour-joining tree. A second origin of asexuality led to hII asexual genotypes that clustered into the asexual group of the microsatellite tree. These genotypes comprised 12.2% ($n = 11$) of asexual genotypes. A third origin of asexuality (with apparently multiple occurrences, according to the microsatellite tree) led to hII asexual genotypes clustered into the 'sexual group'. These genotypes represented 12.2% ($n = 11$) of asexual genotypes.

## 4. DISCUSSION

To our knowledge, this study represents the first detailed investigation of the origin, evolution and diversity of asexual lineages in an aphid. Moreover, it is also the first time that a combination of powerful markers has been used to study the routes to asexuality at an intraspecific level. The key findings are: first, that there are strong genetic differences between sexual and asexual lineages of *R. padi* in France; second, that there is evidence for multiple origins of asexual lineages; and third, that there is evidence that transitions to asexuality result from at least two mechanisms: a spontaneous loss of sex and repeated gene flow from essentially asexual lineages into sexual ones.

### (a) *Genetic characteristics of sexual and asexual lineages*

Asexual lineages of *R. padi* possess a subset of the microsatellite allelic diversity found in their sexually produced counterparts. This is most parsimoniously interpreted as a consequence of asexual aphid lineages being derived from sexual ones (Moran 1992). This seems to be generally true for asexual eukaryotes (Williams 1975; Bell 1982). There are profound differences in the level of heterozygosity not only between sexually produced and asexual lineages but also within asexual lineages (table 1 and figure 1). In large sexually reproducing panmictic populations, Hardy–Weinberg equilibrium is expected. However, sexually produced lineages in *R. padi* show heterozygote deficit, which probably results from effects such as substructure within a sample including several years and sites, inbreeding or selection (Delmotte *et al.* 2001). By contrast, hI asexual genotypes show a heterozygote excess, which could result from an increase in heterozygosity with the age of asexual lineages, as predicted by population genetic theory (Birky 1996; Judson & Normark 1996). According to this prediction, hI asexual lineages in *R. padi* would have undergone long-term asexuality, while hII asexual genotypes, which are much less heterozygous, would have a more recent origin. The long-term persistence of hI asexual lineages is also supported by the mtDNA phylogenetic analysis, which suggests that hI and hII have evolved separately for about 400 000 years (general cytochrome *b* molecular clock of 2.5% divergence per million years applied to the present sequence data; Martinez-Torres *et al.* 1996). Accordingly, we found that the putatively oldest asexual lineages (hI) are also more diversified than hII asexual lineages (measuring the number of multilocus genotypes), which is expected if new asexual lineages arise by mutations within extant asexual lineages. The stepwise mutation process acting on the hI asexual group is illustrated by the distribution of their relatedness, which is narrow in comparison with that of the polyphyletic hII asexual lineages (figure 1*b*). The existence of long-term asexuality is often controversial and difficult to demonstrate (Judson & Normark 1996): it could be demonstrated in *R. padi* by studying phylogenetic relationships within and between alleles of asexual lineages, as recently done on putatively ancient asexual aphids and rotifers (Normark 1999; Welch & Meselson 2000).

### (b) *Multiple origins of asexual lineages*

Asexuality is widely distributed among plants and animals, and has evolved many times (Bell 1982). However, there are great differences between taxa in their propensity to evolve asexual derivatives. Birds and mammals do not produce natural clones, while some invertebrates show a high incidence of asexual taxa emergence at the family, genus or species levels (Vrijenhoek 1998). Asexual lineages may even arise repeatedly at the intraspecific level, as has been reported in daphnia and ostracods (Crease *et al.* 1989; Hebert *et al.* 1989; Martens 1998). This study is, to our knowledge, the first to demonstrate the repeated emergence of asexual lineages in aphids. Actually, our microsatellite and mtDNA phylogenies reveal at least three independent origins for asexuality in *R. padi*. This number of transitions to asexuality is the most conservative estimate. We were cautious about inferring greater numbers of independent origins of asexual lineages because of low bootstrap values for branches of the microsatellite neighbour-joining tree. A better estimate would require sequencing of nuclear markers and more mtDNA haplotypes in sexual and asexual genotypes.

The polyphyletic origin of asexual lineages is a feature that has important consequences for their levels of genetic diversity and ecological adaptability, and the outcome of competition with their sexual relatives (Crease *et al.* 1989; Butlin *et al.* 1999). Thus, the mode of origin of asexuality has to be taken into account when testing theories on the evolution of sex, since high diversity in asexual lineages can decrease the short-term advantage of sexuality under the tangled bank or the red queen hypotheses (Case & Taper 1986; Howard & Lively 1994, 1998).

### (c) *Mechanisms of loss of sexuality*

It is now clear that no single mechanism is responsible for the transition to asexuality in animals. Different processes, such as infectious agents, sperm-dependent parthenogenesis, interspecific hybridization and sex-limited meiosis suppression, can lead to the generation of asexual lineages (Innes & Hebert 1988; Stouthammer *et al.*

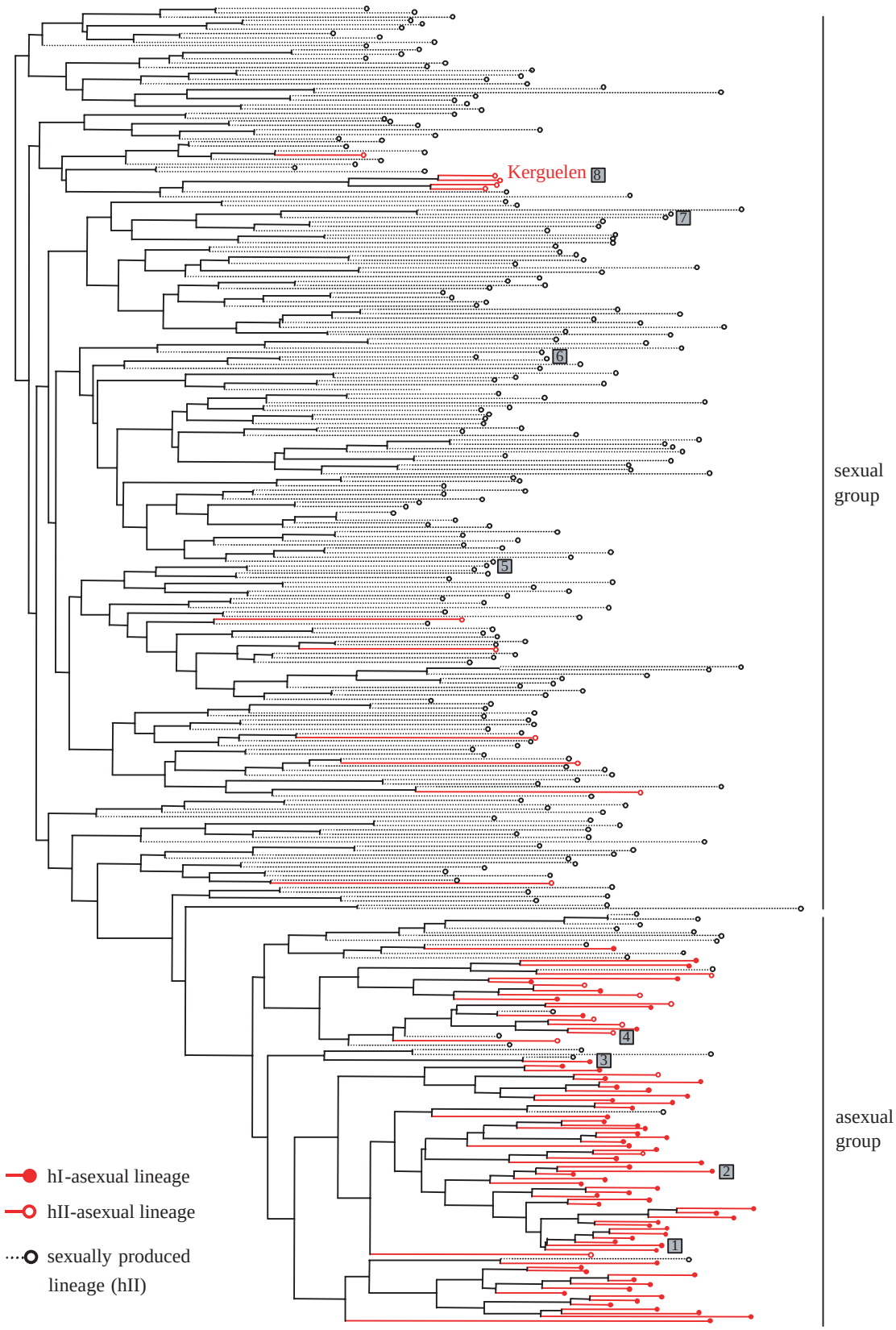


Figure 3. Neighbour-joining tree based on allele shared distance ($D_{AS}$) calculated with seven microsatellite loci for 90 asexual (solid lines) and 224 sexually produced (dashed lines) multilocus genotypes of *Rhopalosiphum padi*, collected on cereals and *Prunus padus*, respectively. Mitochondrial variation, scored as two different haplotypes (hI and hII), was also assessed for all multilocus genotypes and is reported on the tree. Framed numbers in grey boxes refer to lineages for which the haplotype was sequenced (see figure 2).

Table 2. Biological characterization of the actual reproductive mode of a subset of sexually produced (collected on *Prunus padus*) and asexual (collected on cereals) lineages of *Rhopalosiphum padi*.

(Sexual and asexual groups are based on the microsatellite neighbour-joining tree. CP, cyclical parthenogens; OP, obligate parthenogens producing males.)

| | sexual group | asexual group |
|---|---|---|
| sexually produced lineages | CP, hII ($n=11$) | OP, hII ($n=1$)<br>intermediate, hII ($n=3$) |
| asexual lineages | OP, hII ($n=5$) | OP, hI ($n=4$)<br>OP, hII ($n=1$)<br>intermediate, hII ($n=1$) |

1993; Vrijenhoek 1998). Among the different modes of origin of asexual lineages in *R. padi*, *Wolbachia*-induced parthenogenesis is very unlikely since this bacterium has never been detected in aphids (West *et al.* 1998). Also, there is no evidence for sperm-dependent asexuality in aphids (Blackman 1987; Beukeboom & Vrijenhoek 1998). Three remaining mechanisms could constitute routes to asexuality in *R. padi*: mutation(s) at the gene(s) controlling the production of sexual forms, hybridization and recurrent gene flow via males produced by asexual lineages, which transmit asexuality genes (Simon *et al.* 1999).

(i) *First mechanism*

Recurrent mutations at the gene(s) controlling sexuality could generate all the genetically distinct asexual lineages. First, one ancient mutation would have given birth to an asexual genotype (the ancestor of all hI asexual genotypes, from which the hI asexual genotypes were derived by mutation). A second (group of) mutation(s) would have given birth to more recent hII asexual genotypes. An example of recent loss of sex by spontaneous mutation is provided by asexual lineages from the Kerguelen islands. *R. padi* has been introduced to these subantarctic islands, presumably through French settlements, during the last two centuries (Remaudière & Etienne 1988). In this area, cyclical parthenogenesis is counterselected because the climate is mild enough to allow permanent parthenogenesis, and the host on which sexual reproduction takes place is absent. Accordingly, populations of *R. padi* in the Kerguelen islands consist of only two genotypes (indicating a rather small number of introduction events; M. Hulle, unpublished data) that are both strictly obligate parthenogens and are closely related to French sexual lineages. The most likely explanation is that *R. padi* was originally introduced as sexual lineages, which have spontaneously lost sexual reproduction under these specific environmental conditions.

(ii) *Second mechanism*

Hybridization, which is a very common route to asexuality in both animals and plants (Lynch 1984), is another possible origin of asexual lineages in *R. padi*. It has been shown that aphid hybrids have a diminished ability to produce sexual forms, except for rare males (Rakauskas 1999). Hybridization could account for the high heterozygosity in hI asexual *R. padi*, and would explain the high genetic divergence between the two mitochondrial haplotypes. However, this hypothesis does not explain the presence of hII asexual genotypes with low heterozygosity grouped with the majority of the sexually produced lineages. Furthermore, although interspecific hybridization is not rare in aphids (e.g. Müller 1985; Guldemond 1990; Sunnucks *et al.* 1997; Rakauskas 1999), it has not been reported to date in *R. padi* (Simon *et al.* 1999).

(iii) *Third mechanism*

The third mechanism supposes that some lineages lost the ability to produce sexual females by mutation (for example hI asexual lineages or, more recently, some hII asexual lineages). Occasional matings between females from sexual lineages (which are all hII) and males from asexual lineages would produce asexual genotypes having the maternal haplotype (Martinez-Torres *et al.* 1997; Simon *et al.* 1999). Two lines of evidence support the existence of such gene flow. First, we expect that these matings would give rise to hII asexual lineages positioned in the microsatellite neighbour-joining tree either in the sexual or in the asexual group, depending on the inherited set of genes. Accordingly, hII asexual genotypes have a large distribution of relatedness (figure 1*b*) and are distributed all over the microsatellite tree (figure 3), suggesting that some of them have captured the genetic diversity of sexual lineages. Second, such gene flow should result in the regular emergence of asexual lineages among sexually produced lineages, as already found by Rispe *et al.* (1999). Again, here, several sexually produced lineages were shown to be obligate parthenogens or intermediates (table 2) and clustered into the 'asexual group'. These lineages would have inherited asexuality genes from male-producing parthenogens or intermediates during sexual reproduction. Gene flow mediated by male-producing obligate parthenogens also occurs in daphnia (Hebert 1981; Innes & Hebert 1988). Thus, in both aphids and daphnia, asexuality genes could spread in a contagious fashion and rapidly convert cyclical parthenogens into obligate parthenogens. However, little is known about the nature of the genes involved in the loss of sex, which may differ between the two taxa (meiosis-suppressor genes in daphnia (Hebert 1981) and periodicity genes or genes regulating hormonal expression in aphids (Simon *et al.* 1999)). Thus, studying differential gene expression in cyclical and obligate parthenogens submitted to sex-inducing conditions would be extremely valuable for identifying the genes involved in the loss of the ability to produce sexual forms as well as for understanding the complexity of the mechanisms implicated in the switch from parthenogenesis to sexual reproduction in aphids.

### (d) *Frequency of transitions*

Several mechanisms are responsible for the multiple losses of sexuality in *R. padi*. Spontaneous mutations are indicated by the evolution of asexual lineages in the Kerguelen islands. Such mutations possibly also explain the existence of obligate parthenogens with predominantly sexual-like genotypes, although these could also

result from gene flow between life cycles. Male-mediated gene flow between sexual and asexual lineages is strongly indicated by the presence of obligate parthenogens possessing typically asexual genotypes in sexually produced lineages. However, the frequency of such transitions to asexuality must be low in order to explain the strong differentiation between sexual and asexual lineages, unless there exist strong selection pressures that eliminate most of the newly generated asexual lineages. In aphids, winter cold is a primary factor that selects against asexual lineages. In addition, the viability of newly asexual lineages resulting from gene flow between two well-differentiated gene pools (sexual and essentially hI asexual lineages) could be reduced, as has been reported for $F_1$ hybrid aphids (Müller 1985; Guldemond 1990; Via *et al*. 2000).

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