THE ROYAL SOCIETY

Received 31 October 2003
Accepted 22 December 2003
Published online 31 March 2004

Occasional sex in an 'asexual' polyploid hermaphrodite

Thomas G. D'Souza[1*], Martin Storhas[1], Hinrich Schulenburg[1], Leo W. Beukeboom[2] and Nicolaas K. Michiels[1]

[1]*Institute for Animal Evolution and Ecology, Westphalian Wilhelms-University, Huefferstrasse 1, D-48149 Muenster, Germany*
[2]*Evolutionary Genetics Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, NL-9750 AA Haren, The Netherlands*

Asexual populations are usually considered evolutionary dead-ends because they lack the mechanisms to generate and maintain sufficient genetic diversity. Yet, some asexual forms are remarkably widespread and genetically diverse. This raises the question whether asexual systems are always truly clonal or whether they have cryptic forms of sexuality that enhance their viability. In the planarian flatworm *Schmidtea polychroa* parthenogens are functional hermaphrodites (as are their sexual conspecifics), copulate and exchange sperm. Sperm is required for initiation of embryogenesis but usually does not contribute genetically to the offspring (sperm-dependent parthenogenesis). Using karyology and genotyping of parents and offspring, we show that in a purely parthenogenetic population an estimated 12% of all offspring are the result of partial genetic exchange. Several processes of chromosome addition and loss are involved. Some of these result in an alternation between a common triploid and a rare tetraploid state. We conclude that genetic recombination does not necessarily require segregation and fusion within the same generation, as is the case in most sexual species. These occasional sexual processes help to explain the geographical dominance of parthenogens in our study species.

Keywords: parthenogenesis; recombination; evolution of sex; microsatellite; *Schmidtea polychroa*; Platyhelminthes

1. INTRODUCTION

The sex paradox, or the question of how sexual reproduction can be maintained in oogamous organisms in the face of its twofold cost, has largely been resolved—at least theoretically—by invoking the Red Queen hypothesis (Hamilton 1980; Hamilton *et al.* 1990; Lively 1996), the deterministic mutation hypothesis (Kondrashov 1988), stochastic models like Muller's ratchet (Barton & Charlesworth 1998) and sexual selection (Agrawal 2001; Siller 2001). Particularly when considering a combination of these mechanisms (pluralistic approach; Michiels *et al.* 1999; West *et al.* 1999), the benefits of sex appear to outweigh the costs under a wide array of conditions (Rice 2002). However, if sexuality is indeed advantageous, what maintains asexuality? A limited amount of sex or occasional sex may be sufficient to compensate for the long-term costs of clonality (Green & Noakes 1995; Hurst & Peck 1996; Beukeboom & Vrijenhoek 1998). A precondition for the occurrence of occasional sex is, however, that parthenogenetic eggs from one individual fuse with sperm from another, either by accident or because development depends on syngamy. Paternal chromosomes can either displace maternal chromosomes, leaving the ploidy level unchanged (Hedges *et al.* 1992; Spolsky *et al.* 1992) or paternal introgression can increase ploidy (Christensen *et al.* 1978; Tomiuk & Loeschke 1992; Goddard & Schultz 1993; Saura *et al.* 1993; Turgeon & Hebert 1994; Dufresne & Hebert 1994; Stenberg *et al.* 2000). Some parthenogens can indeed outcross with closely related sexuals (Normark 1996; Belshaw *et al.* 1999; Schneider *et al.* 2002). Irregular or partial fertilization leading to the incorporation of paternal genes is well known from sperm-dependent parthenogens (pseudogamy or gynogenesis; Schartl *et al.* 1995; Beukeboom *et al.* 1996*a*; Beukeboom & Vrijenhoek 1998). One restriction, however, is that all of them rely on temporal and spatial coexistence with sexual conspecifics or male sperm suppliers, strongly limiting the conditions under which occasional sex can take place.

This is different in hermaphroditic, sperm-dependent parthenogens where the male function of parthenogenetic conspecifics can provide sperm to trigger egg development (Beukeboom & Vrijenhoek 1998). In this study, we investigate the presence of occasional sex in a parthenogenetic population of a planarian flatworm. *Schmidtea polychroa* is a simultaneous hermaphrodite incapable of self-fertilization in which obligate outcrossing, diploid sexuals and polyploid parthenogens occur. Individuals of both reproductive modes produce cocoons that contain up to 10 eggs embedded in yolk. Parthenogens are sperm-dependent, usually triploid, sometimes tetraploid, and produce haploid sperm. This is achieved by elimination of one chromosome set (two in tetraploids) during spermatogonia differentiation. The resulting diploid spermatocytes undergo normal meiosis (Benazzi Lentati 1970). Crosses between sexuals and parthenogenetic individuals revealed that sperm from parthenogens is fertile (Benazzi Lentati 1970; Storhas *et al.* 2000). Since parthenogens are hermaphroditic and produce sperm, parthenogenetic populations are independent of sexual sperm donors. This explains why they can occur in purely parthenogenetic populations, as is the case in Central and Western Europe

* Author for correspondence (dsouzat@uni-muenster.de).

(Beukeboom *et al.* 1996*b*; Michiels & Kuhl 2003; Pongratz *et al.* 2003).

Generally, there is no paternal genetic contribution to offspring, because the paternal chromosome set is expelled from the zygote. Hence, purely parthenogenetic populations are essentially clonal. Surprisingly, we found that such a purely parthenogenetic population showed more diverse triploid microsatellite genotypes at localities where tetraploid parthenogens were also present (L. Gerace and N. K. Michiels, unpublished data). Tetraploids can originate from triploid oocytes that fail to expel the paternal chromosome set after syngamy. However, a mechanism explaining the origin of triploid offspring from tetraploids with the generation of new genotypes is unknown. Since parthenogenetic *S. polychroa* populations are often genetically diverse (Storhas 2001), we ask here whether reciprocal transitions between triploid and tetraploid parthenogens may represent a mechanism for occasional sex. We collected a sample of triploid and tetraploid adult *S. polychroa* and compared karyotypes and microsatellite genotypes of all adults ($n = 184$) and offspring of all 23 tetraploid adults and 23 randomly chosen triploids. We thus provide evidence of genome gain ($3x \rightarrow 4x$) and genome loss ($4x \rightarrow 3x$), which confirm a two-step cycle from triploidy to tetraploidy and back.

2. MATERIAL AND METHODS

(a) *Collection and culture*

In June 2000, we collected 200 adult *S. polychroa* from a purely parthenogenetic population of *S. polychroa* in the Ammersee, a 46 km^2 lake in Bavaria, Germany (Beukeboom *et al.* 1996*b*; Pongratz *et al.* 2003). Individuals were kept in isolation in 200 ml vials at room temperature and a natural light cycle for three weeks. They were fed minced beef liver every third day and cleaned within 12 h after feeding. Because flatworms store sperm and collection took place during the reproductive season, they produced fertile cocoons (see § 1 for description of genetic system). Cocoons were collected daily ($n = 1297$) and stored singly. Offspring hatching within three weeks ($n = 4165$; 3.91 ± 1.9 per cocoon) were fed twice and allowed to grow for one week before further analysis. We deliberately used field-collected animals with unknown mating partners rather than individuals from known crosses, to obtain an estimate of occasional sex under natural conditions.

(b) *Karyology*

Karyotypes were determined using colchicine to arrest the cell cycle at metaphase (Redi *et al.* 1982). Chromosomes were counted using phase contrast microscopy (Beukeboom *et al.* 1996*b*). Karyology of adults revealed 169 triploid ($3x = 12$), 25 tetraploid ($4x = 16$) and four mosaic (triploid–tetraploid) individuals. One hundred and fifty-seven triploids and 23 tetraploids produced cocoons. For a comparison between $3x$ and $4x$ parents, we karyotyped all offspring of the 23 tetraploids as well as those of 23 randomly chosen triploids. Among 802 analysed offspring we identified triploids and tetraploids, but also rare cases of mosaic triploid–tetraploid ($n = 3$) and pentaploid ($n = 2$) karyotypes. These five cases were excluded from further analyses.

(c) *Microsatellite analyses*

Genomic DNA was isolated from juvenile worms with the Nucleon Bacc DNA Extraction kit, as outlined (Pongratz *et al.* 2001). DNA isolation from adults was performed using a CTAB-based protocol as described (Schulenburg *et al.* 2001). Two trinucleotide microsatellite loci, *SpATT12* and *SpATT20*, were subsequently amplified using PCR and sized on an ABI 310 Genetic Analyser (Applied Biosystems). For detailed protocols, see Pongratz *et al.* (2001). Unusual results (e.g. presence of supernumerary alleles) were checked in a second PCR analysis. To assess whether inefficient primer binding accounts for unusual cases, those observed for *SpATT12* were tested with a new primer set (*SpATT12U1*: 5′-CGGTTAGATTTTGCT GGATGA; *SpATT12L2*: 5′-GGAATGGAACGGATATT TTAGG) and the following PCR profile: 2 min at 95 °C, 35 cycles of 20 s at 95 °C, 1 min at 50 °C, 1 min at 72 °C, and a final period of 5 min at 72 °C.

(d) *Duplication of* SpATT12

We consistently found *SpATT12* alleles of intermediate size (26–34 repeat units) and large size (46–55 repeat units) together. To check for possible duplication of *SpATT12* we sequenced alleles of both size classes. PCR products were purified using Microcon-50 Microconcentrators (Millipore Ltd), cloned into *E. coli* DH5α using pGEM-T Vector System (Promega Ltd), plasmids purified with Wizard Minipreps DNA Purification System (Promega Ltd), cycle sequenced using ABI Prism BigDye Terminator Cycle Sequencing Kit (Applied Biosystems Ltd) and analysed on an ABI 310 Genetic Analyser (Applied Biosystems Ltd). DNA sequences were obtained for both strands. The flanking regions of the long fragments differed from the previously published sequence for *SpATT12* (EMBL AF201314) and were submitted (EMBL AJ516026). These results indicate that the two size classes represent a duplicated locus. As a consequence, up to six alleles could be amplified in triploids and eight in tetraploids using the original primer pair. Each linkage group of alleles with intermediate and large size was considered as one allele for further analysis. The presence of the duplicated locus does not affect our conclusions.

Locus duplication alone could not explain that in some offspring ($n = 5$ and 18 for *SpATT12* and *SpATT20*, respectively) allele number exceeded ploidy level. The occurrence of supernumerary alleles can be explained by amplification of maternal yolk cells (Pongratz *et al.* 2001) or genetic mosaicism. The latter is a rare event that results from the incomplete constriction of polar bodies during female meiosis or incomplete exclusion of paternal chromosomes after fertilization during early embryogenesis (Benazzi Lentati 1970).

(e) *Mutations versus recombination*

Differences between parents and offspring in microsatellite alleles were assumed not to be due to mutation, when: (i) alleles differed by more than four unit changes; (ii) alleles at both loci had changed simultaneously; (iii) the ploidy level had increased or decreased; or (iv) new supernumerary alleles in offspring that exceeded the number of chromosomes appeared. Analysis was limited to individuals with known karyotype and genotype. For parent–offspring comparisons, only families with at least three analysed offspring were considered.

3. RESULTS

(a) *Chromosome gain and loss*

Twelve per cent of the offspring produced by tetraploid adults (total $n = 259$) were triploid and 5.1% of the offspring of triploid adults (total $n = 448$) were tetraploid. A

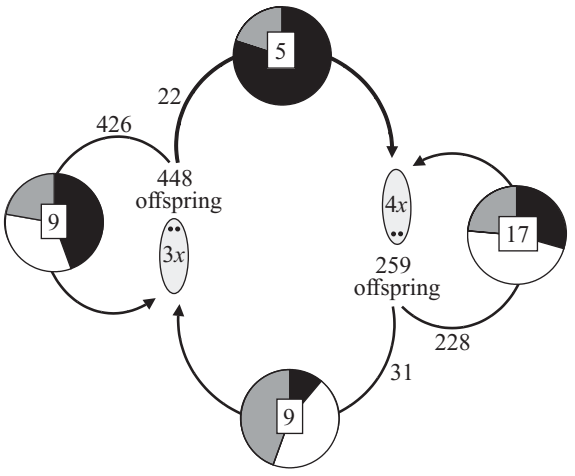

Figure 1. Chromosome gain and loss in *Schmidtea* parthenogens. Schematic overview of chromosome and marker changes in offspring produced by 23 triploid and 23 tetraploid parthenogenetic *S. polychroa* (represented by worm cartoons). Arrows mark transitions from adults to offspring with number of offspring next to each arrow. Pie diagrams show composition of offspring that had a two-locus genotype that differed from their mother and the type of difference (gain, black areas; loss, white areas; gain plus loss, grey areas).

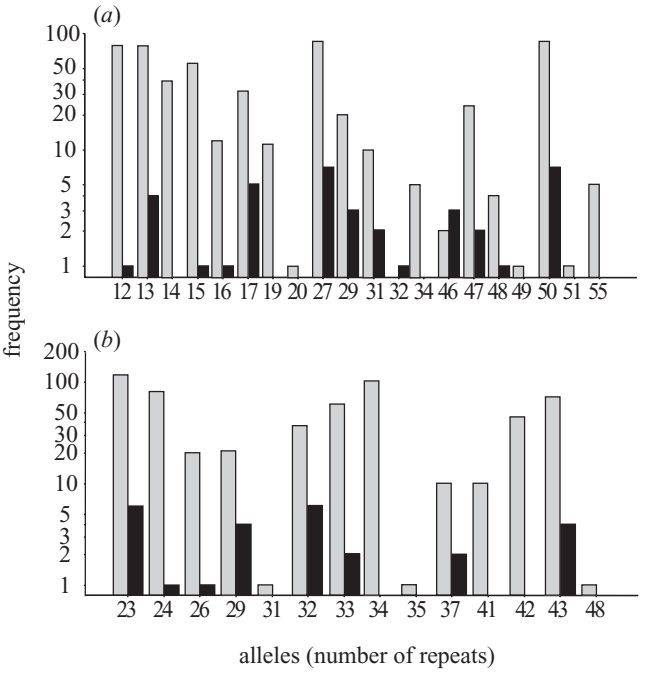

Figure 2. Allele distribution in triploid and tetraploid *Schmidtea*. Frequencies of microsatellite alleles in adults (grey bars) and newly gained alleles in offspring (black bars) for two loci (*a*) *SpATT12* and (*b*) *SpATT20*. Allele labels indicate repeat number.

comparison of the proportion of karyotypically aberrant offspring between triploid and tetraploid parents revealed a trend for more aberrant progeny in tetraploids (Mann–Whitney $U = 152.5$, $n_{3x} = 22$, $n_{4x} = 20$, exact $p = 0.075$). Aberrant offspring were found in 11 out of 22 and 12 out of 20 analysed triploid and tetraploid families, respectively ($\chi^2 = 0.423$, d.f. = 1, $p = 0.551$). The number of offspring was significantly higher in triploid (29.11 ± 17.95) than in tetraploid parents (16.67 ± 14.29) (Mann–Whitney $U = 1057.0$, $n_{3n} = 156$, $n_{4n} = 23$, exact $p < 0.001$).

(b) *Microsatellite marker gain and loss*

In microsatellites most mutations lead to length changes of one repeat unit, but changes of up to four units are possible (DiRienzo *et al.* 1994; Brohede *et al.* 2002). 7.5% ($n = 53$) of all genotyped offspring (total $n = 707$) differed from the maternal genotype at one or both investigated loci. Out of these, 15.1% ($n = 8$) showed only a single repeat unit difference. When including changes in a single allele of up to four repeat units, up to 24.5% ($n = 13$) of the divergent offspring could have originated by mutation. For a conservative analysis of paternal inheritance, we assigned all these cases to mutation and ignored them for further analysis.

Three types of genetically divergent offspring were recognized: (i) offspring with new non-maternal alleles (marker gain) ($n = 14$); (ii) offspring with missing maternal alleles (marker loss) ($n = 15$); and (iii) offspring with new non-maternal and missing maternal alleles (marker gain plus loss) ($n = 11$) (figure 1). New non-maternal alleles in offspring are most probably of paternal origin. This is supported by the observation that nearly all new alleles (95% for *SpATT12* and 100% for *SpATT20*) are present in the adult population (figure 2). Although common alleles should more often be inherited paternally, they also have a higher likelihood of already being present in the mother individual. For this reason, a perfect correlation between allele frequencies in adults and offspring is not expected. We never found more than one new allele per locus per offspring, indicating that only a single paternal set is included at a time.

(c) *Integration of chromosome and marker changes*

As expected, genetic changes from parents to offspring coincided mainly with a change in ploidy ($3x \rightarrow 4x$ and $4x \rightarrow 3x$), but were also found in cases where ploidy did not change ($3x \rightarrow 3x$ and $4x \rightarrow 4x$) (figure 1). Most offspring with changed ploidy were genetically identical to their mother (77.3% for $3x \rightarrow 4x$; 71% for $4x \rightarrow 3x$) most probably because parents shared the same alleles and the resolution of the analysis was low. Genetic changes in offspring with increased ploidy ($3x \rightarrow 4x$; $n = 5$) involved the addition of alleles in four cases, and gain plus loss in the remaining case. In offspring with genetic changes and decreased ploidy ($4x \rightarrow 3x$; $n = 9$), 'loss' and 'gain plus loss' of alleles accounted for four cases each. Gain alone occurred once in the offspring of a mother with increased homozygosity, making loss less detectable. During the transition $3x \rightarrow 4x$, gain was particularly common, where the $4x \rightarrow 3x$ transition involved mainly loss, with or without gain. This difference was marginally non-significant (Fisher's exact test, $p = 0.051$). Among offspring that were produced without a change in ploidy, there was no difference between triploids and tetraploids in the relative frequency of marker gain, loss or gain plus loss (Fisher's exact test, $p = 0.867$). Among triploids ($3x \rightarrow 3x$) gain, loss or gain plus loss is observed in four, three and two cases. Among tetraploids ($4x \rightarrow 4x$) the values are 5, 8 and 4.

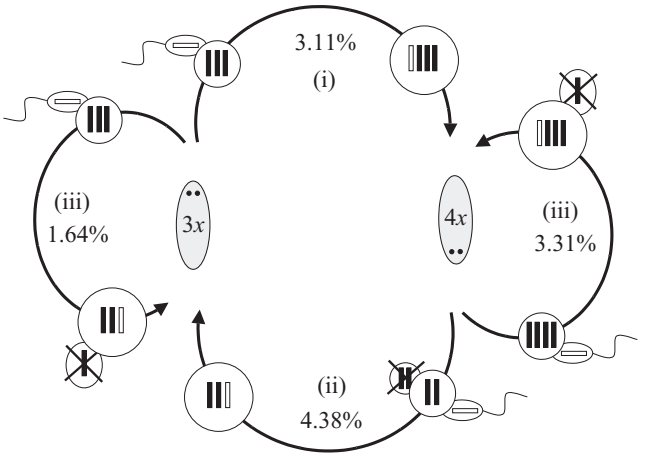

Figure 3. Mechanisms of occasional sex in parthenogenetic *Schmidtea*. Roman numbers refer to the mechanism described in § 4. Filled and open bars represent maternal and paternal chromosome sets, respectively. The number of bars reflects the level of ploidy in egg, sperm and zygote. Ploidy increase ($3x \rightarrow 4x$) is presumably caused by syngamy of triploid egg with haploid sperm, while in the cases with ploidy decrease ($4x \rightarrow 3x$) a reduction of the genome most probably precedes through meiosis. Within triploid and tetraploid lineages ($3x \rightarrow 3x$, $4x \rightarrow 4x$) displacement of the maternal chromosome set by paternal chromosomes is supposed to take place. The percentages indicate the importance of each process relative to the total number of offspring produced (see table 1).

(**d**) *Random mating model*

Occasional sex is invisible when added alleles are identical to alleles already present, or when an allele disappears that was present in two copies. Since both processes can lead to an underestimate of occasional sex, we estimated this invisible fraction in the observed data by conducting a simulation that creates new single-locus genotypes from the known maternal genotypes of the 23 triploid and 23 tetraploid focal individuals under random mating. Sperm alleles are randomly drawn from the total allele distribution for all genotyped individuals ($n = 194$). We simulated all four sexual transitions assuming the most likely mechanism (see § 4 and figure 3). In a first step, the maternal single-locus genotype was randomly reduced by 0, 1 or 2 alleles (0 for $3x \rightarrow 4x$ transition, 1 for $3x \rightarrow 3x$, 1 for $4x \rightarrow 4x$ and 2 for $4x \rightarrow 3x$). In a second step, we added one paternal allele. Each transition was simulated for 60 offspring per maternal genotype and repeated for both loci. *All* offspring generated in this way had undergone some form of sex. The fraction of offspring with a genotype identical to that of their mother allows the estimation of how many genetically divergent offspring were not recognized as such in the empirical data.

Polyploid genotypes regularly contain fewer alleles than chromosomes, for example, an *ab* triploid, representing the indistinguishable genotypes *aab* or *abb*. To accommodate for such situations, we used allele likelihood rather than absolute frequency in all calculations (simulated and observed). For example, in an *abc* triploid, each allele accounted for one-third, whereas in an *ab* triploid *a* and *b* accounted for one-half each. We controlled for mutations in three ways: (i) no mutations, all changes of paternal origin; (ii) single repeat unit changes regarded as mutations; and (iii) up to four repeat unit changes regarded as mutations. As controlling for mutations did not affect the outcome, we show only the mutation-free results (table 1).

Out of all offspring generated through sexual processes, 76.9% and 85.6% (*SpATT12* and *SpATT20*) can be recognized as genetically different from the maternal genotype (table 1). Adjusting the observed frequencies accordingly, it is clear that the change is larger in the $3x \rightarrow 4x$ transition than in all other transitions. This must be attributed to the fact that this step involves only one sexual event (inclusion of paternal chromosomes) whereas all others involve two steps (loss and gain), increasing the likelihood of detectable genetic change. The percentage of offspring that resulted from sexual processes is *ca*. 12% for both loci. These values include all offspring with ploidy change and/or genetic change.

4. DISCUSSION

(**a**) *Mechanisms of occasional sex*

In combination with published accounts, our data suggest that genetic exchange in parthenogenetic *S. polychroa* is caused by several mechanisms (figure 3).

(i) *Syngamy*. Offspring with increased ploidy ($3x \rightarrow 4x$) and changed genotype can be explained by introgression of a single paternal chromosome set, implying an incomplete chromosome exclusion. This mechanism was previously shown for offspring from crosses between sexual and parthenogenetic *S. polychroa* (Benazzi Lentati 1970), as opposed to crosses between parthenogens, as in this study.

(ii) *Meiosis and syngamy*. Chromosome loss ($4x \rightarrow 3x$) involving genetic-marker addition requires reduction of the maternal complements as well as inclusion of paternal chromosomes. This may result from meiotic reduction of tetraploid oogonia to diploid eggs and/or irregular degeneration of the paternal chromosomes (Benazzi Lentati 1970). Again, this process was only known before from crosses between sexual and parthenogenetic biotypes. Four observed cases with genome loss only might represent offspring where gain remained undetected because of similarities between the parents.

(iii) *Syngamy and chromosome displacement*. Offspring that differ genetically from their mother do not always show a changed ploidy level ($3x \rightarrow 3x$ and $4x \rightarrow 4x$). Such cases cannot be explained by mechanisms (i) and (ii). Instead, stochastic chromosome exclusion needs to be invoked as maternal chromosomes must be expelled, while paternal chromosomes remain in the zygote. The data do not allow any distinction between displacement of whole or incomplete chromosome sets. In contrast to other planarians (Beukeboom *et al.* 1998), aneuploidy has never been observed in *S. polychroa*. Hence, regular numbers of chromosomes (either $3x$ or $4x$) are always restored, irrespective of the elimination mechanism. Further clarification of the chromosome displacement process requires the study of more polymorphic loci and controlled crosses.

Table 1. Estimates for the rate of occasional sex.
(Absolute and relative frequencies of genetically different offspring at each locus and transition mechanism relative to the total number of offspring per category (raw data in columns 1–5). Simulation results (detectable percentage) show the maximum level of detectable change, assuming 100% occurrence of the transition mechanism with the given maternal genotypes and paternal allele frequency (see § 3). This value is used to adjust the observed data (adjusted). The final column shows the frequencies of genetically different offspring relative to the total number of offspring ($n = 707$). For $3x \rightarrow 4x$ and $4x \rightarrow 3x$ the lower value considers the adjusted genetic changes only, whereas the upper value includes all observed offspring with ploidy change irrespective of any detected genetic changes.)

		empirical data			simulation-adjusted data			
			diverging			adjusted		
locus	transition	*n*	*n*	%	% detectable	*n*	%	% of total
SpATT12	$3x \rightarrow 3x$	426	9	2.1	77.4	11.6	2.7	1.64
SpATT12	$3x \rightarrow 4x$	22	5	22.7	70.3	7.1	32.3	1.00–3.11
SpATT12	$4x \rightarrow 3x$	31	6	19.3	88.6	6.8	21.8	0.96–4.38
SpATT12	$4x \rightarrow 4x$	228	16	7.0	74.9	21.4	9.4	3.03
SpATT12	total	707	36	5.1	76.9	46.9	6.6	6.6–12.12
SpATT20	$3x \rightarrow 3x$	426	6	1.4	86.1	7.0	1.6	0.99
SpATT20	$3x \rightarrow 4x$	22	4	18.2	58.7	6.8	31.0	0.96–3.11
SpATT20	$4x \rightarrow 3x$	31	7	22.6	97.7	7.2	23.1	1.02–4.38
SpATT20	$4x \rightarrow 4x$	228	20	8.8	85.7	23.4	10.2	3.31
SpATT20	total	707	37	5.2	85.6	44.4	6.3	6.3–11.79

(**b**) *Estimating occasional sex*

Our estimates for occasional sex underestimate the true level of genetic exchange for several reasons. First, our liberal definition of mutations resulted in presumed mutation rates of 3.1% and 1.4% for *SpATT12* and *SpATT20*. These are even higher than values from hypermutable loci (Brohede *et al.* 2002). This means that a number of sexual events may have been falsely rejected. Second, our sample contained only 10% tetraploids. Since the presence of tetraploids plays a key role in occasional sex, and parthenogenetic populations routinely have up to 20% tetraploids, with extremes up to 80%, sex may be more common in other parthenogenetic populations (Beukeboom *et al.* 1996*b*). Third, incorporated paternal alleles may have been identical to maternal alleles and hence gone undetected. Fourth, a lost allele may not have been picked up because a second identical copy may have been present on one of the other homologues. Although the simulation corrects for the effect of the latter two, it is still an underestimate because two-allele genotypes in triploids (e.g. *aab* and *abb*) and two- or three-allele genotypes in tetraploids (e.g. *aabc*, *abbc* and *abcc*) are not differentiated in the microsatellite analysis. This may explain why we found only 20–30% genetically recombined offspring in $3x \rightarrow 4x$ and $4x \rightarrow 3x$ transitions, where 100% was expected. Another explanation for this discrepancy is assortative mating between related individuals rather than random mating as in the simulation. Non-random mating is likely as sperm trading and variation in male functionality between clonal lineages may favour mating among parthenogens with a similar phenotype (Storhas 2001; Michiels & Kuhl 2003).

Until now, we have assumed that all observed changes are due to the sexual mechanisms proposed above. Alternative mechanisms, however, may not require genetic exchange. For instance, the $3x \rightarrow 4x$ transition could be due to chromosome endoduplication, whereas some of the $4x \rightarrow 3x$ transitions may follow from chromosome loss. Although we cannot exclude these mechanisms, they must only play a minor role, if any. There is neither evidence from the planarian literature that they exist, nor can they explain the genetic differences involving marker gain that we find between parents and offspring.

(**c**) *Sexual processes in parthenogens*

In all mechanisms leading to offspring that differ genetically from their mother, egg and haploid sperm have fused. Fusion of polyploid, parthenogenetic eggs with sperm is a known source of genetic variability (Tomiuk & Loeschke 1992; Hobaek *et al.* 1993; Stenberg *et al.* 2000). However, such additions usually lead to ever-increasing ploidy series. This may increase diversity within the higher ploidy levels, but not at the lower levels as the reciprocal mechanism is missing (Stenberg *et al.* 2000). Unique to *S. polychroa*, genetic exchange with ploidy reduction as well as genetic exchange within ploidy levels is possible.

(**d**) *Consequences for the paradox of sex debate*

Most models on the evolution of sex consider obligate sexual and obligate asexual organisms. The present study illustrates that a mixed mode of reproduction (occasional sex) is also possible, even in the absence of sexual conspecifics. Occasional sex should also benefit from combining advantages of both sex and asex (Wright 1939; Hastings 1992; Hurst & Peck 1996). As reviewed by Hurst & Peck (1996) most theories explaining the maintenance of sex also work with a limited amount of sexual reproduction or recombination. For example, the incorporation of beneficial mutations is accelerated in asexual populations if they show low recombination rates (Pamilo *et al.* 1987; Green & Noakes 1995) or low segregations rates (Hedrick & Whittam 1989; Green & Noakes 1995). Low rates of recombination can generate genotypes with low mutation load and prevent the decrease of population

fitness due to accumulation of deleterious mutations through Muller's ratchet (Pamilo *et al.* 1987). This is true for large population sizes (Bell 1988) or low mutation rates (Charlesworth *et al.* 1993). The deterministic mutation hypothesis also predicts an advantage for rare sex. Under low mutation rates and truncation selection, it is sufficient to prevent the accumulation of mutations beyond the level in sexuals (Kondrashov 1985; Hurst & Peck 1996).

It is currently unknown why occasional sex evolved in the case of *S. polychroa*. Benefits from combining sex and asex could lead to the origin of occasional sex. By contrast, it could be the consequence of different interests between male and female gametes. To maximize maternity, eggs are under selection to eliminate the male pronucleus from the zygote, while sperm is under selection to stay in the zygote leading to increased paternity. Moreover, by fusing with maternal pronuclei, sperm behave as parasites as an increase in ploidy leads to a reduction of fitness. To restore fitness the number of maternal chromosomes must be reduced, either via meiosis or chromosome elimination. This results in a conflict of interest between sperm and egg and may explain the different mechanisms of occasional sex in *S. polychroa*.

Finally, this study shows how important rare types (i.e. tetraploids) are. In terms of offspring number, tetraploids have only 57% of the fitness of a triploid, and are probably rare for this reason. As a consequence, we have either discarded or pooled them with triploids in previous studies (Weinzierl *et al.* 1998, 1999*a*,*b*). Yet, they play a key role concerning genetic exchange; tetraploids are involved in more than 80% of the sexual processes found in this study. Hence, the presence or absence of tetraploids, however rare, may be a good indicator for occasional sex in natural populations. Another important attribute of the *S. polychroa* system is that the sexual process presented here depends on a two-step cycle from triploidy to tetraploidy and back. Such a process takes at least two generations. Such long-term alternation of genome increase and decrease is not commonly considered when thinking about sex.

The authors thank Iris Michiels, Oliver Adrian and Norbert Pongratz for help in collecting flatworms, Barbara Hasert and Gaby Niester for technical support in the laboratory, Ruzica Bruvo and Markus Haber for helpful discussions and Laurence Hurst, Joel Peck, Peter van Dijk, Koen Martens, Isa Schön, Tim Sharbel, Verena Brauer, Claus-Peter Stelzer and two anonymous referees for useful comments on earlier drafts of the manuscript.

REFERENCES

Agrawal, A. F. 2001 Sexual selection and the maintenance of sexual reproduction. *Nature* **411**, 692–695.

Barton, N. H. & Charlesworth, B. 1998 Why sex and recombination? *Science* **281**, 1986–1990.

Bell, G. 1988 Recombination and the immortality of the germline. *J. Evol. Biol.* **1**, 67–82.

Belshaw, R., Quicke, D. L. J., Volkl, W. & Godfray, H. C. J. 1999 Molecular markers indicate rare sex in a predominantly asexual parasitoid wasp. *Evolution* **53**, 1189–1199.

Benazzi Lentati, G. 1970 Gametogenesis and egg fertilization in planarians. *Int. Rev. Cytol.* **27**, 101–179.

Beukeboom, L. W. & Vrijenhoek, R. C. 1998 Evolutionary genetics and ecology of sperm-dependent parthenogenesis. *J. Evol. Biol.* **11**, 755–782.

Beukeboom, L. W., Seif, M., Mettenmeyer, T., Plowman, A. B. & Michiels, N. K. 1996*a* Paternal inheritance of B chromosomes in a parthenogenetic hermaphrodite. *Heredity* **77**, 646–654.

Beukeboom, L. W., Weinzierl, R. P., Reed, K. M. & Michiels, N. K. 1996*b* Distribution and origin of chromosomal races in the freshwater planarian *Dugesia polychroa* (Turbellaria: Tricladida). *Hereditas* **124**, 7–15.

Beukeboom, L. W., Sharbel, T. F. & Michiels, N. K. 1998 Reproductive modes, ploidy distribution, and supernumerary chromosome frequencies of the flatworm *Polycelis nigra* (Platyhelminthes: Tricladida). *Hydrobiologia* **383**, 277–285.

Brohede, J., Primmer, C. R., Moller, A. & Ellegren, H. 2002 Heterogeneity in the rate and pattern of germline mutation at individual microsatellite loci. *Nucleic Acids Res.* **30**, 1997–2003.

Charlesworth, D., Morgan, M. T. & Charlesworth, B. 1993 Mutation accumulation in finite outbreeding and inbreeding populations. *Genet. Res.* **61**, 39–56.

Christensen, B., Jelnes, J. & Berg, U. 1978 Long-term isozyme variation in parthenogenetic polyploid forms of *Lumbricus lineatus* (Enchytraeidae, Oligochaeta) in recently established environments. *Hereditas* **88**, 65–73.

DiRienzo, A., Peterson, A. C., Garza, J. C., Valdes, A. M., Slatkin, M. & Freimer, N. B. 1994 Mutational processes of simple-sequence repeat loci in human populations. *Proc. Natl Acad. Sci. USA* **91**, 3166–3170.

Dufresne, F. & Hebert, P. D. N. 1994 Hybridization and origins of polyploidy. *Proc. R. Soc. Lond.* B **258**, 141–146.

Goddard, K. A. & Schultz, R. J. 1993 Aclonal reproduction by polyploid members of the clonal hybrid species *Phoxinus-eos-neogaeus* (Cyprinidae). *Copeia* **3**, 650–660.

Green, R. F. & Noakes, D. L. G. 1995 Is a little bit of sex as good as a lot? *J. Theor. Biol.* **174**, 87–96.

Hamilton, W. D. 1980 Sex versus non-sex versus parasite. *Oikos* **35**, 282–290.

Hamilton, W. D., Axelrod, R. & Tanese, R. 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl Acad. Sci. USA* **87**, 3566–3573.

Hastings, I. 1992 Why is sex so frequent? *Trends Ecol. Evol.* **7**, 278–279.

Hedges, S. B., Bogart, J. B. & Maxson, L. R. 1992 Ancestry of unisexual salamanders. *Nature* **356**, 708–710.

Hedrick, P. W. & Whittam, T. S. 1989 Sex in diploids. *Nature* **342**, 231.

Hobaek, A., Weider, L. J. & Wolf, H. G. 1993 Ecological genetics of Norwegian *Daphnia*. III. Clonal richness in an Arctic apomictic complex. *Heredity* **71**, 323–330.

Hurst, L. D. & Peck, J. R. 1996 Recent advances in understanding of the evolution and maintenance of sex. *Trends Ecol. Evol.* **11**, 46–52.

Kondrashov, A. S. 1985 Deleterious mutations as an evolutionary factor. II. Facultative apomixis and selfing. *Genetics* **111**, 635–653.

Kondrashov, A. S. 1988 Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–440.

Lively, C. M. 1996 Host–parasite coevolution and sex—do interactions between biological enemies maintain genetic variation and cross-fertilization? *Bioscience* **46**, 107–114.

Michiels, N. K. & Kuhl, A. 2003 Altruistic sperm donation in a sperm-dependent parthenogenetic hermaphrodite is stabilized by reciprocal sperm exchange. *J. Zool.* **259**, 77–82.

Michiels, N. K., Beukeboom, L. W., Greeff, J. M. & Pemberton, A. J. 1999 Individual control over reproduction: an underestimated element in the maintenance of sex? *J. Evol. Biol.* **12**, 1036–1039.

Normark, B. B. 1996 Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. *Evolution* **50**, 734–745.

Pamilo, P., Nei, M. & Li, W.-H. 1987 Accumulation of mutations in sexual and asexual populations. *Genet. Res.* **49**, 135–146.

Pongratz, N., Gerace, L., Alganza, A. M., Beukeboom, L. W. & Michiels, N. K. 2001 Microsatellite development and inheritance in the planarian flatworm *Schmidtea polychroa*. *Belg. J. Zool.* **131**, 71–75.

Pongratz, N., Storhas, M., Carranza, S. & Michiels, N. K. 2003 Phylogeography of competing sexual and parthenogenetic forms of a freshwater flatworm: patterns and explanations. *BMC Evol. Biol.* **3**, 23.

Redi, C. A., Garagna, S. & Pellicciari, C. 1982 Chromosome preparation from planarian blastemas: a new procedure suitable for cytogenetic and cytochemical studies. *Stain Technol.* **57**, 190–192.

Rice, W. R. 2002 Experimental tests of the adaptive significance of sexual recombination. *Nature Rev. Genet.* **3**, 241–251.

Saura, A., Lokki, J. & Suomalainen, E. 1993 Origin of polyploidy in parthenogenetic weevils. *J. Theor. Biol.* **163**, 449–456.

Schartl, M., Nanda, I., Schlupp, I., Wilde, B., Epplen, J. T., Schmid, M. & Parzefall, J. 1995 Incorporation of subgenomic amounts of DNA as compensation for mutational load in a gynogenetic fish. *Nature* **373**, 68–71.

Schneider, M. V., Beukeboom, L. W., Driessen, G., Lapchin, L., Bernstein, C. & Van Alphen, J. J. M. 2002 Geographical distribution and genetic relatedness of sympatrical thelytokous and arrhenotokous populations of the parasitoid *Venturia canescens* (Hymenoptera). *J. Evol. Biol.* **15**, 191–200.

Schulenburg, J. H. G. v. d., Hancock, J. M., Pagnamenta, A., Sloggett, J. J., Majerus, M. E. N. & Hurst, G. D. D. 2001 Extreme length and length variation in the first ribosomal internal transcribed spacer of ladybird beetles (Coleoptera: Coccinellidae). *Mol. Biol. Evol.* **18**, 648–660.

Siller, S. 2001 Sexual selection and the maintenance of sex. *Nature* **411**, 689–692.

Spolsky, C. M., Phillips, C. A. & Uzzel, T. 1992 Antiquity of clonal salamander lineages revealed by mitochondrial DNA. *Nature* **356**, 706–708.

Stenberg, P., Terhivuo, J., Lokki, J. & Saura, A. 2000 Clone diversity in the polyploid weevil *Otiorhynchus scaber*. *Hereditas* **132**, 137–142.

Storhas, M. 2001 *Sex versus asex in a hermaphroditic flatworm*. Münster: Schüling.

Storhas, M., Weinzierl, R. P. & Michiels, N. K. 2000 Paternal sex in parthenogenetic planarians: a tool to investigate the accumulation of deleterious mutations. *J. Evol. Biol.* **13**, 1–8.

Tomiuk, J. & Loeschke, V. 1992 Evolution of parthenogenesis in the *Otiorhynchus scaber* complex. *Heredity* **68**, 391–397.

Turgeon, J. & Hebert, P. D. N. 1994 Evolutionary interactions between sexual and all-female taxa of *Cyprinotus* (Ostracoda, Cyprididae). *Evolution* **48**, 1855–1865.

Weinzierl, R. P., Berthold, K., Beukeboom, L. W. & Michiels, N. K. 1998 Reduced male allocation in the parthenogenetic hermaphrodite *Dugesia polychroa*. *Evolution* **52**, 109–115.

Weinzierl, R. P., Beukeboom, L. W., Gerace, L. & Michiels, N. K. 1999*a* Spatial and ecological overlap between coexisting sexual and parthenogenetic *Schmidtea polychroa* (Tricladida; Platyhelminthes). *Hydrobiologia* **392**, 179–185.

Weinzierl, R. P., Schmidt, P. & Michiels, N. K. 1999*b* High fecundity and low fertility in parthenogenetic planarians. *Invertebr. Biol.* **118**, 87–94.

West, S. A., Lively, C. M. & Read, A. F. 1999 A pluralist approach to sex and recombination. *J. Evol. Biol.* **12**, 1003–1012.

Wright, S. 1939 Statistical genetics in relation to evolution. In *Evolution: selected papers of Sewall Wright* (ed. W. B. Provine), pp. 282–341. University of Chicago Press.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.