No slave to sex
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Fully asexual lineages cannot purge accumulating mutations from their genome through recombination. In ancient asexuals that have persisted without sex for millions of years, this should lead to high allelic divergences (the ‘Meselson effect’) as has been shown for bdelloid rotifers. Homogenizing mechanisms can counter this effect, resulting in low genetic diversity within and between individuals. Here, we show that the ancient asexual ostracod species Darwinula stevensoni has very low nucleotide sequence divergence in three nuclear regions. Differences in genetic diversity between embryos and adults furthermore indicate that up to half of the observed genetic changes in adults can be caused by somatic mutations. Likelihood permutation tests confirm the presence of gene conversion in the multi-copy internal transcribed spacer sequence, but reject rare or cryptic forms of sex as a general explanation for the low genetic diversity in D. stevensoni. Other special mechanisms (such as highly efficient DNA repair) might have been selected for in this ancient asexual to overcome the mutational load and Muller’s ratchet. In this case, our data support these hypotheses on the prevalence of sex, even if the two extant ancient asexual groups (bdelloids and darwinulids) seem to follow opposite evolutionary strategies.

Keywords: ancient asexuals; Meselson effect; mutational load; gene conversion; DNA repair; asexual reproduction

1. INTRODUCTION
Several hypotheses predict that asexual lineages are doomed to early extinction (Maynard Smith 1978, 1998; Butlin 2002). Nevertheless, a few groups have apparently survived without sex for many millions of years. These ancient asexuals (Judson & Normark 1996), so-called because they defy ruling evolutionary theory, are assumed to carry genetic evidence of their long-term asexuality. The latter has been demonstrated for the bdelloid rotifers (aexual for ca. 30 Myr), which show high allelic divergence at the DNA sequence level (Mark Welch & Meselson 2000). This is known as the ‘Meselson effect’: through non-homologous accumulation of mutations, allelic divergences in individuals should be larger in long-term asexuals than in sexuals. Although this effect is accepted as a genetic test for the status of ancient asexuality, its powers are asymmetrical. Presence of the effect indicates long-term amixis but its absence does not necessarily imply sex (Butlin 2000). Low allelic divergences can also be caused by homogenizing mechanisms such as gene conversion (Butlin 2000) between alleles or highly efficient DNA repair (Schön & Martens 1998). Comparative research on ancient asexuals constitutes an alternative approach to test some of the more than 20 hypotheses related to the ‘queen of problems in evolutionary biology’ (i.e. the prevalence of sex in the living world; Bell 1982), especially those referring to the irreversible accumulation of (deleterious) mutations (Kondrashov 1988) and to the stochastic loss of least-corrupted genotypes (Muller’s ratchet; Muller 1964). If special mechanisms are developed to counter the deleterious effects in the absence of sex, the validity of the hypotheses is supported.

Here, we test for the Meselson effect in a representative of the ostracod family Darwinulidae, the second putative ancient asexual animal group beside bdelloid rotifers. The fossil record shows that the ostracod family Darwinulidae has reproduced fully asexually for 200 Myr (Martens et al. 2003) and neither sexual nor mixed extant populations or close sexual relatives are known (Martens 1998). The type species Darwinula stevensoni has been fully asexual for 20–25 Myr (Straub 1952), which makes it the longest living, extant asexual species known. It is common, cosmopolitan and ubiquitous (Rossetti & Martens 1996, 1998), with a general purpose genotype (Van Doninck et al. 2002) and has, for an animal less than 1 mm in size, an unusually long lifespan of up to 4 years in subarctic populations (Ranta 1979).

Morphological (Rossetti & Martens 1996, 1998) and allozyme (Rossi et al. 1998) studies show a low variability in D. stevensoni. Estimates of genetic variability at the DNA level have so far been published from the nuclear internal transcribed spacer (ITS) 1 region and the mitochondrial cytochrome oxidase subunit I (COI) gene, showing no (Schön et al. 1998) or little divergence in ITS1. Neither of these studies constitute a test for the Meselson effect, either because direct sequencing was used (Schön et al. 1998) or because the sample scheme was too restricted (Gandolfi et al. 2002) to draw conclusions about allelic divergence of a multiple copy region.

We have estimated nucleotide sequence divergence of D. stevensoni within and between individuals from natural populations over a wide geographical range including South Africa, southern France, Italy, Belgium and Ireland. Three nuclear regions have been PCR-amplified, cloned and sequenced from individual ostracods: intron and exon regions of the single copy gene hsp82, the interspersed regions ITS1/ITS2 and a calmodulin intron (CaD8s). Comparisons are made with sequence data of hsp82 from Cyprideis torosa, a fully sexual ostracod species.
Table 1. Mean genetic distance of *Cyprideis torosa* and *Darwinula stevensoni*, estimated as HKY 85 (Hasegawa et al. 1985) within and between populations.

(CI, confidence interval; B, Belgium; F, France; EU, between different European populations; EU–AFR, between European and South African populations; I, between two Italian populations. Belgian individuals are either adult females (A) or embryos (E). All other individuals are adult females. 95% CI in the *C. torosa* dataset is especially high owing to the presence of a single, very different clone in individual Ct1; the variable positions of this clone are mainly located in the intron region of *hsp82*.)

<table>
<thead>
<tr>
<th>genomic region</th>
<th>species</th>
<th>comparison</th>
<th>number of comparisons</th>
<th>mean HKY 85</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>hsp82</em></td>
<td><em>C. torosa</em></td>
<td>B, A</td>
<td>13</td>
<td>0.0497</td>
<td>0.0294</td>
</tr>
<tr>
<td><em>hsp82</em></td>
<td><em>D. stevensoni</em></td>
<td>B, A</td>
<td>24</td>
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<td>0.0046</td>
</tr>
<tr>
<td><em>hsp82</em></td>
<td><em>D. stevensoni</em></td>
<td>B, E</td>
<td>14</td>
<td>0.0056</td>
<td>0.0007</td>
</tr>
<tr>
<td><em>hsp82</em></td>
<td><em>D. stevensoni</em></td>
<td>F</td>
<td>26</td>
<td>0.0073</td>
<td>0.0004</td>
</tr>
<tr>
<td><em>hsp82</em></td>
<td><em>D. stevensoni</em></td>
<td>EU</td>
<td>66</td>
<td>0.0110</td>
<td>0.0002</td>
</tr>
<tr>
<td><em>hsp82</em></td>
<td><em>D. stevensoni</em></td>
<td>EU–AFR</td>
<td>71</td>
<td>0.0110</td>
<td>0.0004</td>
</tr>
<tr>
<td><em>ITS</em></td>
<td><em>D. stevensoni</em></td>
<td>I</td>
<td>12</td>
<td>0.0019</td>
<td>0.0006</td>
</tr>
<tr>
<td><em>CadDs</em></td>
<td><em>D. stevensoni</em></td>
<td>EU</td>
<td>16</td>
<td>0.0034</td>
<td>0.0006</td>
</tr>
<tr>
<td><em>CadDs</em></td>
<td><em>D. stevensoni</em></td>
<td>EU–AFR</td>
<td>24</td>
<td>0.0051</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

2. MATERIAL AND METHODS

(a) Ostracods

Specimens of the ancient asexual ostracod species *D. stevensoni* were collected from the following lakes, rivers and springs: Hollandsersgatreek (Belgium), Lake Sibaya (Republic of South Africa), Lough Ballyquirke (Ireland), ‘Cava’ and ‘Canale’ of Mantova, (Italy) and Clue de la Fou (France). Adult females of the sexual ostracod species *C. torosa* were sampled from Hollandsersgatreek (Belgium). All ostracods were stored in 95% ethanol or frozen at −20 °C before DNA extraction.

(b) PCR amplification, cloning and sequencing

DNA was extracted from individual ostracods with a modified Chelex method (Schön et al. 1998); individual embryos were manually removed from the brooding pouch of living, adult females before DNA extraction. Eight hundred and fifty base pairs of *hsp82* containing intron and exon regions were amplified with primers hsp8.X (5′-ACGTCTTCAAGARTGRTCYTCCCAATCRTCRTTNGT) and hsp1.2 (5′-TGCTCTAGAGCAGACARTTGGGTGNTNGTTT) and the following PCR protocol in a Progene DNA amplificator (Technne): 5 min at 95 °C, 10 × 50 s at 95 °C, 50 s at 50 °C, 2 min at 72 °C, 35 × 50 s at 95 °C, 50 s at 55 °C, 2 min at 72 °C; 10 min at 72 °C. Amplification occurred in 25 μl volumes with 0.5 U Taq DNA Polymerase (Amersham Pharmacia Biotech) and the provided buffer, 1.5 mM MgCl₂, 200 μM dNTPs (Promega), 10 pmol of each primer and 10 pg DNA. The 890 base pair (bp) stretch of ITS was amplified (with primers ITS1 and ITS4) as given (White et al. 1990) and 411 bp of CadDs as in Schön (2001). Cleaning and cloning of PCR products as well as DNA preparation from positive clones and sequencing occurred as previously described (Schön 2001).

(c) Sequence analyses

Processed sequences were viewed with Chromas 1.45, aligned with CLUSTAL W (Thompson et al. 1994) and checked manually. Variable sites were identified by eye and by using the SEQUENCE computer package (B. Schüren). Genetic and taxonomic identity of all sequences was confirmed by BLAST (Altschul et al. 1997). A total of 129 cloned sequences were analysed in both directions.

Genetic distances were estimated with PAUP v. 4.05 (Swofford 1998), using the HKY85 (Hasegawa et al. 1985) method under the assumption of equal rates for variable sites. For estimates at different levels (individuals, within and between populations), values of the lower levels were excluded. For example, for comparisons within populations, no intra-individual estimates were used. Cloning with subsequent sequencing can generate technical artefacts owing to Taq errors or mutations in the bacterial host. Obvious errors (point mutations and indels restricted to single clones) were excluded before further analysis; original and edited data matrices can be obtained from the first author on request. A subset of the edited sequences has been submitted to GenBank, submission numbers AJ534952-AJ534968.

(d) Statistical analyses

Student’s t-tests and tests for correlations were conducted with the program STATISTICA. If different populations were tested against each other, geographically closest populations were chosen (e.g. *hsp82* from the South of France was tested against ITS from Italy). Details of the statistical analyses can be obtained from the first author on request. Permutation likelihood tests (McVean et al. 2002) were conducted with ‘no frequency cut-off of rare alleles’, Watterson theta estimate (θ) of population mutation rates, τ = 20 and 10 000 permutations. Owing to the low number of segregating sites (0–6), tests were not applicable to individual *hsp82* sequences of *D. stevensoni* from South Africa and individual ITS and CadDs sequences. Highest composite log likelihoods were obtained with 4Nf,τ = 10 and 10 000 grid points for *D. stevensoni* (D.s) and 4Nf,τ = 200 and 100 grid points for *C. torosa*. Values of p had similar significance levels for all datasets but ITS, if 4Nf,τ = 20 or 50 (D.s) were used. pτ, a and pτ, a are calculated by methods similar to published permutation-based tests (McVean et al. 2002) and describe the correlation between recombination and physical distance. pD,τ and pA,τ for gene conversion (GC) are new parameters of likelihood permutation tests (McVean et al. 2002).

3. RESULTS

We found that genetic divergence of *hsp82* is significantly higher for the sexual species *C. torosa* than for the ancient asexual *D. stevensoni*, both within individuals (mean HKY 85 (Hasegawa et al. 1985) distances of 0.090 and 0.0089, respectively; not shown in table 1) and between individuals from the same population (table 1; t-test: p < 0.001). In *D. stevensoni*, mean genetic diversity of
**Figure 1.** Mean genetic distances (HKY 85; Hasegawa et al. 1985) of \(hsp82\), ITS and CadDs within individual *Darwinula stevensoni* genomes. Error bars indicate 95% CIs of means. Numbers below individual codes indicate numbers of sequenced clones from each PCR product. Unless specifically mentioned, all individuals are adult females. \(hsp82\): RSA, Republic of South Africa; F1,2, two specimens from France; IRL, Ireland; B1A, B2A, two specimens from Belgium; B3E, B4E, two embryos from Belgium; I1, 2, two specimens from Italy; B, Belgium.

\(hsp82\) is low at all levels of comparison: within individuals (figure 1), within and between populations (table 1). Mean genetic divergence of both ITS and CadDs in *D. stevensoni* (maximum of 0.002 and 0.0036, respectively) is significantly smaller than that of \(hsp82\) (table 1; figure 1; \(t\)-test: \(p < 0.0001\)) at the individual and population level.

Also, Watterson theta estimates (see table 2; 0.0028–0.00614) indicate low mutation rates of *D. stevensoni* in all examined genomic regions or low effective population size.

There is a strong effect of age class on genetic variability of \(hsp82\): means in embryos reach only half of those in adults (table 1; figure 1; \(t\)-test: \(p < 0.0001\)) at the individual and at the population levels. Genetic divergences of \(hsp82\) from the same geographical region are of the same order of magnitude for individuals and populations (\(t\)-test: \(p > 0.37\)). Throughout Europe, however, we observe an effect of latitude on genetic variability for \(hsp82\); both individuals and populations show significantly higher, mean genetic divergence of \(hsp82\) at northern latitudes than in southern locations (\(t\)-test: \(p < 0.0001\)). The pattern is not as clear for the genetic variability of the CadDs region for which fewer individuals were analysed.

New and more powerful likelihood permutation tests (McVean et al. 2002) based on the Fisher–Wright population model determine whether recombination or mutations have generated the observed pattern of nucleotide sequence divergence (which is small but present) in *D. stevensoni*. Multiple mutations are herewith regarded as the null hypothesis, whereas significant deviations of the estimated parameters indicate recombination. Sequence data of the sexual ostracod species *C. torosa* were also analysed with likelihood permutation tests to verify the validity of the tests.

As expected, highly significant \(p\) values were obtained for \(hsp82\) in the population of this sexual ostracod for all three permutation parameters (see table 2). The outcome is similar, if the single, highly variable sequence of one of the two individuals is excluded (not shown). In \(hsp82\) of *D. stevensoni*, only one significant value (\(p_{\text{null}}\)) is found when sequences of all screened populations are pooled (table 2). No evidence for recombination appears if sequence data from each population of *D. stevensoni* are analysed separately.

We have also tested for cryptic (intra-individual) forms of sex by repeating the analyses with individual \(hsp82\) datasets from *D. stevensoni* (table 3). In this case, the test only picks up a single signature of \(p_{\text{null}}\) in one specimen from the Belgian population for which the maximal genetic divergence of \(hsp82\) was observed. The result is not confirmed by \(p_{\text{max}}\), which is considered to be the most powerful parameter (McVean et al. 2002). Permutation likelihood tests indicate some recombination in the ITS region (significant \(p_{\text{max}}\); see table 2); this result is congruent with the signal for gene conversion. No recombination is found in the CadDs region (table 2).

Likelihood permutation tests can indeed be modified to test for gene conversion instead of recombination with genetic change (G. McVean, personal communication). If the modified tests are used, significant deviations are only found in \(hsp82\) from *C. torosa* and ITS from *D. stevensoni* (tables 2 and 3).

**4. DISCUSSION**

Compared with the sexual ostracod species *C. torosa*, *D. stevensoni* shows significantly lower levels of genetic diversity within and between individuals. Mean genetic diversity in *D. stevensoni* is very low for all three examined genomic regions and for all levels of comparison: within and between individuals and populations. The low estimates for comparisons between populations are especially puzzling. Such low genetic divergences are opposite to the
prediction of the Meselson effect (Mark Welch & Meselson 2000) and confirm the exceptionally low genetic variability in Darwinula stevensoni at the population level obtained by direct sequencing (Scho¨n 2002). All parameters describe the correlation between recombination and physical distance. B, Belgium; A, adult; E, embryo; F, France; I, Italy. Bold p values are significant at the 0.01 level.)

Table 3. Results of the likelihood permutation test (McVean et al. 2002) for recombination or GC (last column) in hsp82 of individual Darwinula stevensoni.

<table>
<thead>
<tr>
<th>dataset</th>
<th>species</th>
<th>number of individuals</th>
<th>number of clones</th>
<th>number of segregating sites</th>
<th>\theta_w</th>
<th>D</th>
<th>\rho_{Lk_{max}}</th>
<th>\rho_{p2, d}</th>
<th>\rho_{p3, d}</th>
<th>\rho_{Lk_{max}} GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsp82, B</td>
<td>C.t.</td>
<td>2</td>
<td>13</td>
<td>130</td>
<td>0.104</td>
<td>-1.395</td>
<td>0.006</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>hsp82, all</td>
<td>D.s.</td>
<td>8</td>
<td>80</td>
<td>25</td>
<td>0.006</td>
<td>0.365</td>
<td>0.7820</td>
<td>0.0051</td>
<td>0.1025</td>
<td>0.5110</td>
</tr>
<tr>
<td>hsp82, B, A</td>
<td>D.s.</td>
<td>2</td>
<td>24</td>
<td>19</td>
<td>0.006</td>
<td>1.162</td>
<td>0.4980</td>
<td>0.0218</td>
<td>0.0872</td>
<td>0.1940</td>
</tr>
<tr>
<td>hsp82, B, E</td>
<td>D.s.</td>
<td>2</td>
<td>14</td>
<td>10</td>
<td>0.003</td>
<td>1.808</td>
<td>0.7240</td>
<td>0.5888</td>
<td>0.4391</td>
<td>0.3690</td>
</tr>
<tr>
<td>hsp82, F</td>
<td>D.s.</td>
<td>2</td>
<td>21</td>
<td>13</td>
<td>0.004</td>
<td>0.707</td>
<td>0.920</td>
<td>0.3758</td>
<td>0.7807</td>
<td>0.6990</td>
</tr>
<tr>
<td>ITS, I</td>
<td>D.s.</td>
<td>2</td>
<td>12</td>
<td>6</td>
<td>0.002</td>
<td>-0.905</td>
<td>0.0</td>
<td>0.2022</td>
<td>0.2022</td>
<td>0.0</td>
</tr>
<tr>
<td>CadDs, all</td>
<td>D.s.</td>
<td>4</td>
<td>24</td>
<td>4</td>
<td>0.002</td>
<td>0.947</td>
<td>0.2370</td>
<td>0.3427</td>
<td>0.3427</td>
<td>0.0760</td>
</tr>
</tbody>
</table>

(provisional)
described for humans (Clark 1996). Lower levels of metabolic activity and/or fewer cell divisions in embryos compared with adults could also have contributed to the observed, significant difference in genetic diversity between the age classes.

The exceptionally low genetic variability in the single copy gene(s) at all levels, even the interpopulation comparisons, shows that there is no apparent Meselson effect in *D. stevensoni*. This becomes even more clear if maximal genetic diversities within individual ostracods and bdelloid rotifers are compared directly (figure 2). Owing to the asymmetry of the test (Butlin 2000, 2002), however, this does not imply that *D. stevensoni* is not an ancient asexual, as several mechanisms could counter the accumulation of mutations.

Haploidy could be an elegant way to avoid the accumulation of mutations for millions of years, because first, it makes all slightly deleterious mutations pseudodominant, thereby increasing the efficiency of purifying selection (Lynch et al. 1993). Second, haploidy reduces the number of gene loci that can mutate, especially in combination with a large population size (Haigh 1978; Lynch & Gemmell 1998). This genetic pattern cannot have been caused by a recent selective sweep, as darwinulids are poor dispersers (they are the only ostracods without dry-resistant eggs). Also, some genetic divergence was observed in a mitochondrial gene and no speed-up in molecular rates of mitochondrial evolution has been found (see Schön et al. 1998) for more details), not even within the family of Darwinulidae as a whole (Schön et al. 2003). European and African populations of *D. stevensoni* have been separated for an estimated 7.6–4.4 Myr (Schön et al. 1998) and haploid, nuclear sequences should have accumulated a significant amount of genetic change, but this is not the case.

The absence of the Meselson effect in *D. stevensoni* can also not be explained by rare syngamy nor by cryptic sex, as permutation likelihood tests identify recombination (with genetic exchange) as a possible explanation for one out of 21 instances in *hp82* only. Most likely, this single deviation is due to a technical artefact such as PCR jumping or PCR recombination (Paabo et al. 1990; Bradley & Hillis 1997). In the apparent absence of rare or cryptic sex, other homogenizing mechanisms need to be identified. These should also be more efficient than sex in the removal of genetic changes considering that genetic divergence within individuals of *D. stevensoni* is significantly lower than within the sexual ostracod *C. torosa* (figure 2) and genetic divergence is low between populations. Such potential mechanisms have been suggested.

**Gene conversion** between alleles can effectively homogenize genomic regions (Rourke & East 1997; Butlin 2000, 2002). In *D. stevensoni*, the modified permutation likelihood tests for gene conversion (McVean et al. 2002) give significant departures for the multiple ITS region only (table 2). Homogenization of this region should act on both alleles and copies effectively causing concerted evolution. Most documented cases of gene conversion (15 out of 17; see electronic Appendix A available on The Royal Society’s Publications Web site) so far come from gene families (Charles et al. 1997; Rourke & East 1997; Wang et al. 1999; Lazzaro & Clark 2001) or multi-copy regions like rRNA clusters (Benevolenskaya et al. 1997) and ITS (Fuerst Aguilar et al. 1999). Data from well-studied organisms such as yeast, *Drosophila* and *Arabidopsis* suggest that gene conversion can occur anywhere in the genome (e.g. Bertrand et al. 1997; Abdullah & Borts 2001; Langley et al. 2001; Haubold et al. 2002). Thus, although gene conversion is known to happen in single copy genes through repair of double strand breaks (Osman & Subramani 1998; Johnson & Jasin 2000), no evidence of this has been found in *D. stevensoni*. No gene conversion has as yet been detected in bdelloid rotifers (see Butlin 2000; Mark Welch & Meselson 2000), but only single copy genes have thus far been analysed in this group.

Although screening of mother–daughter pedigrees in non-marine, asexual ostracods has so far only confirmed apomixis as the asexual reproductive mode (e.g. Chaplin 1994), the occurrence of auto- mixis in *D. stevensoni* cannot at the moment be ruled out as a potential explanation for the low genetic diversity. However, only one out of three types of auto- mixis does effectively increase homozygosity, when diplidy is restored by terminal fusion of two haploid products after the second meiotic division (Butlin et al. 1998; Maynard

Figure 2. Maximal genetic distance (HKY 85; Hasegawa et al. 1985) of *hp82* within individual genomes. For *Darwinula stevensoni* and *Cyprideis torosa*, individuals with maximal means of genetic divergence are shown. Error bars show 95% CIs of means. Distances for bdelloid rotifers were recalculated from GenBank sequences (Mark Welch & Meselson 2000) of which *Philodina roseola* showed maximal individual divergence.
This type of automixis still implies recombination (Butlin et al. 1998), which, because of the increase in homozygosity, will not be detectable within but only between individuals. The permutation likelihood tests applied here (see above) do not signal the presence of such recombination. Moreover, even automixis could not account for the exceptionally low genetic distances between populations of this species. Alternative mechanisms must therefore be sought to explain the overall homogeneity of the genome in D. stevensoni.

Highly efficient DNA repair (Schön & Martens 1998) is one possible mechanism. If chemical changes in DNA are actively repaired before they become manifested as mutations, a low genetic divergence within individuals will result. The higher genetic variability in a mitochondrial gene (COI) of D. stevensoni (Schön & Martens 1998; Schön et al. 1998) hints at such a mechanism as DNA repair in mitochondrial DNA being less efficient than in nuclear DNA (LeDoux et al. 1992). The presented results indicate that such efficient DNA repair would also operate in germ line cells.

Speculation aside, we have shown that D. stevensoni, which has survived for millions of years in the complete absence of sex, prevented the accumulation of mutations. If highly efficient DNA repair or other, special genetic mechanisms have been selected for to overcome the negative effects of the mutational load hypothesis and Muller’s ratchet, then our data support the validity of these hypotheses to explain the prevalence of sex in the animal and plant kingdoms (Maynard Smith 1978). Earlier data on the bdelloid, however, demonstrate that such accumulations can be handled without deleterious effects over long time-frames (Mark Welch & Meselson 2000), even if the molecular rates of evolution appear unaffected (Mark Welch & Meselson 2001). However, in the absence of special genetic mechanisms, the bdelloids have adopted alternative ecological strategies, such as large population size and very short life cycles of a few days only. Both extant ancient asexual animal groups have thus persisted without sex for millions of years, albeit with opposite evolutionary strategies. It shows that at least these groups are no slaves to sex (Dagg 2000).

We thank Roger K. Butlin from Leeds University, Giampaolo Rossetti from the University di Parma and Karine Van Doninck. The group of Matthew Meselson from Harvard University is thanked for their help with the amplification of Smith 1998). This type of automixis still implies recombination (Butlin et al. 1998), which, because of the increase in homozygosity, will not be detectable within but only between individuals. The permutation likelihood tests applied here (see above) do not signal the presence of such recombination. Moreover, even automixis could not account for the exceptionally low genetic distances between populations of this species. Alternative mechanisms must therefore be sought to explain the overall homogeneity of the genome in D. stevensoni.

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