Novel microsatellite markers suggest the mechanism of parthenogenesis in *Extatosoma tiaratum* is automixis with terminal fusion

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Abstract

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Parthenogenetic reproduction is taxonomically widespread and occurs through various cytological mechanisms, which have different impact on the genetic variation of the offspring. *Extatosoma tiaratum* is a facultatively parthenogenetic Australian insect (Phasmatodea), in which females oviposit continuously throughout their adult lifespan irrespective of mating. Fertilized eggs produce sons and daughters through sexual reproduction and unfertilized eggs produce female offspring via parthenogenesis. Here, we developed novel microsatellite markers for *E. tiaratum* and characterised them by genotyping individuals from a natural population. We then used the microsatellite markers to infer the cytological mechanism of parthenogenesis in this species. We found evidence suggesting parthenogenesis in *E. tiaratum* occurs through automixis with terminal fusion, resulting in substantial loss of microsatellite heterozygosity in the offspring. Loss of microsatellite heterozygosity may be associated with loss of heterozygosity in fitness related loci. The mechanism of parthenogenetic reproduction can therefore affect fitness outcomes and needs to be considered when comparing costs and benefits of sex versus parthenogenesis.

**Key words.** Apomixis; Automixis; *Extatosoma tiaratum*; heterozygosity; microsatellite markers; parthenogenesis

**Introduction**

Thelytokous parthenogenesis, the production of females from unfertilised eggs, is widespread among insects, including Lepidoptera, Orthoptera, Hemiptera, Coleoptera and Hymenoptera (Simon *et al.*, 2003). Parthenogenetic species are often derived from close sexual ancestors and can be obligate, cyclic or facultative parthenogens (Simon *et al.*, 2003). Thelytokous parthenogenetic females are
often referred to as an evolutionary paradox because, all else being equal, they can reproduce twice as
good as sexual females by producing female only offspring; yet they are often out-competed by their
sexual counterparts (Williams, 1975; Smith & Maynard-Smith, 1978; Bell, 1982). Facultatively
parthenogenetic species, which can reproduce parthenogenetically when unmated, provide an
opportunity to compare directly the consequences of sex and parthenogenesis. The cytological
mechanism of parthenogenesis is an important component of this comparison, because each
mechanism has a different outcome on the individual-level heterozygosity, which has important
implications for individual fitness and genetic diversity at the population-level.

The two most common cytological mechanisms of parthenogenesis are apomixis and
automixis, each resulting in different levels of heterozygosity (Suomalainen et al., 1987). Apomictic
parthenogenesis occurs through mitosis and the resulting offspring are identical clones of their mother
(Suomalainen et al., 1987). This mechanism involves no recombination and thus the ratio of
heterozygote to homozygote loci in the offspring is identical to that of their mother (Pearcy et al.,
2006). In contrast, meiosis takes place in automictic parthenogenesis and diploidy is restored through
one of the following mechanisms: gamete duplication, central fusion (fusion of non-sister nuclei),
terminal fusion (fusion of the resulting sister nuclei), or random fusion (Suomalainen et al., 1987;
Pearcy et al., 2006). Automixis with central fusion has similar outcomes to apomixis as
heterozygosity is restored in most loci, except for those loci far from the centromere, which have high
recombination rates. In automixis with gamete duplication, heterozygosity is entirely lost, resulting in
homozygosity at all loci (Suomalainen et al., 1987; Pearcy et al., 2006). In terminal fusion, loci close
to the centromere may be heterozygous as a result of crossing over between the chiasma and telomere
(Rabeling & Kronauer, 2013). Changes in heterozygosity levels can directly affect fitness (see
Chapman et al., 2009), so it is important to determine the mechanism of parthenogenesis when
comparing it with sexual reproduction.

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Polymorphic nuclear genetic markers such as microsatellite loci, can be used to determine the cytological mechanism of parthenogenesis by estimating the rate of transition (R) of heterozygous loci in mothers to homozygous loci in their offspring for each locus (Pearcy et al., 2006). If parthenogenesis is derived through apomixis, no heterozygous loci are expected to change to the homozygous state and $R = 0$. In contrast, with gamete duplication R is always expected to be one (complete homozygosity), while with automixis the expected value of R varies depending on the position of each locus relative to the centromere. For loci closer to the centromere (with lower recombination rates), a higher transition rate to homozygosity is expected (see Pearcy et al., 2006).

Conversely, loci located far away from the centromere have lower transition rates due to recombination. Accordingly, the range of the expected rates of transition to homozygosity ($r$) is between 0 and 1/3 for central fusion, 1/3 for random fusion and between 1/3 and 1 for terminal fusion (Pearcy et al., 2006).

Here we investigate the mechanism of parthenogenetic reproduction in a facultatively parthenogenetic insect, *Extatosoma tiaratum* (Phasmatodea). *Extatosoma tiaratum* is native to the rainforests of tropical and subtropical Queensland and northern New South Wales, Australia (Gurney, 1947). Females commence ovipositing irrespective of mating (Alavi et al., 2016; Schneider & Elgar, 2010), and continue to oviposit throughout their adult phase, producing viable sons and daughters via fertilised eggs, and only daughters from unfertilised eggs by parthenogenesis (Carlberg, 1983). We isolated 18 nuclear microsatellite markers for *E. tiaratum* and investigated their patterns of bi-allelic inheritance in progeny from sexual matings, as well as inheritance patterns in parthenogenetically produced progeny, to determine the cytological mechanism of parthenogenesis. In arthropods, bacterial endosymbiotic infection (most commonly by *Wolbachia*) may be responsible for parthenogenetic reproduction (resulting in gamete duplication, Stouthamer et al., 1999). However, we
did not screen for \textit{Wolbachia} infection in this study, as this bacteria is unlikely to be responsible for parthenogenesis in the Phasmatodea order and has never been associated with parthenogenesis in facultatively parthenogenetic species (Perez-ruiz \textit{et al.}, 2015).

\textbf{Materials and methods}

\textit{Laboratory stock population}

A stock population of \textit{E. tiaratum} was derived from eggs and juveniles obtained from existing cultures maintained by the Melbourne Zoological gardens and the Melbourne Museum (both in Victoria, Australia), however the exact origin of these populations was unknown. All insects were housed in cylindrical containers (height: 23 cm; diameter: 25 cm), enclosed by a fine mesh. The cages were kept in climate-controlled conditions (24–26°C; 50% humidity; 12:12 h Light:Dark cycle). Males and females were housed separately. All eggs were incubated under climate-controlled conditions in plastic containers (37 × 36 × 30cm) containing moist sand (sprayed with water weekly). First instar nymphs were collected after hatching and stored in 100% Ethanol in -20 °C for later DNA extraction.

\textit{Field collection}

We collected \textit{E. tiaratum} from a natural population at Crystal Cascades, north Queensland, Australia (16°57′42″S 145°40′46″E) in December 2014 over three nights. The insects were collected manually.

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from the lower accessible branches of trees along a 2 km trail. The insects (N = 6 females, 5 males) were all at the juvenile stages (1st–5th instar) at the time of collection. *Extatosoma tiaratum* is nocturnal and inconspicuous (see Bian *et al.*, 2016), making field collections challenging. Nevertheless, as the laboratory stock was of unknown origin, we used wild, outbred insects to characterize our microsatellite markers. Following collection, the insects were moved to the laboratory and housed individually under similar conditions to the laboratory population before DNA was extracted at the adult stage.

### Next-generation sequencing and de novo genome assembly

DNA was extracted from limb tissue (legs) of a single first instar individual (1–7 days old) from the stock population, using a QIAGEN DNA Easy kit (Qiagen), following the manufacturers recommendations. The 454 next generation sequencing platform (Schuster, 2008) was used to identify microsatellite loci for *E. tiaratum*. Approximately 10 µg genomic DNA was extracted from limb tissue as above. The DNA was then nebulized, ligated with 454 sequencing primers and tagged with a unique oligo sequence allowing sequences to be separated from pooled species DNA sequences using post-run bioinformatic tools. The sample was then analysed using high throughput DNA sequencing on 1/16 of a 70 x 75 mm PicoTiterPlate using the Roche GS FLX (454) system (Margulies *et al.*, 2005) at the Australian Genome Research Facility (AGRF, Brisbane, Australia).

### Microsatellite isolation and characterization

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Unique sequence contigs possessing microsatellite motifs were identified using the software GDD2 (Meglécz et al., 2010). Primer 3 (Rozen & Skaletsky, 2000) was used to design optimal primer sets for each unique contig where possible. A selection of 48 contigs including di-, tri-, and tetra-nucleotide repeats, were used for subsequent analysis. Loci were initially screened for polymorphism using template DNA from eight individuals from the laboratory population. Loci were pooled into ten groups of four, labelled with unique fluorophores (FAM, NED, VIC, PET) and co-amplified by multiplex PCR using a Qiagen multiplex kit (Qiagen) and an Eppendorf Mastercycler S gradient PCR machine (see Blacket et al., 2012). Genotyping was subsequently performed using an Applied Biosystems 3730 capillary analyzer (AGRF) and product lengths were scored manually and assessed for polymorphisms using GeneMapper version 4.0 (Applied Biosystems).

Polymorphic loci were selected and pooled into three groups for multiplexing based on observed locus specific allele size ranges, and checked using DNA obtained from wing and leg tissue of 11 field collected individuals from Crystal Cascades, north Queensland. Reaction matrices for all PCR amplification consisted of 5 µL Qiagen multiplex mix, 4 µL of primer mix (0.2 µmol/L of each primer) and 2 µL of template DNA. PCR conditions consisted of an initial 15 minutes denaturing step at 94 °C, followed by 40 cycles of 94 °C for 30 seconds, 59 °C for 1:30 minutes, and 72 °C for 1:00 minutes, with a final extension step of 60 °C for 30 minutes.

Microsatellite profiles of the field specimens (supplementary Table 1) were examined using GeneMapper version 4.0 and alleles scored manually. The Excel Microsatellite Toolkit was used to estimate expected \( H_e \) and observed \( H_o \) heterozygosities and number of alleles \( N_A \), while examination of conformation to Hardy–Weinberg equilibrium (HWE), the inbreeding coefficient \( F_{IS} \) and linkage disequilibrium estimates between all pairs of loci were conducted using GENEPOP.
Significance was adjusted for multiple comparisons using the sequential Bonferroni procedure where necessary (Rice, 1989). Finally, all loci were assessed using MICRO-CHECKER to check for null alleles (van Oosterhout et al., 2004). Null alleles are created by failure in PCR amplification and can be caused by mutations in primer binding regions or because PCR conditions are not ideal (Selkoe et al., 2006). The frequency of null alleles was calculated using the Brookfield method (Brookfield, 1996). In order to calculate genotyping error rate, we genotyped 33 individuals drawn randomly from the laboratory population in duplicate and determined the number of mismatches (Hoffman & Amos, 2005).

Patterns of inheritance

To investigate inheritance patterns of the microsatellite loci, we collected sexually produced eggs from three families derived from the laboratory stock. Each male-female pair was housed in a separate cage in climate-controlled conditions (as above), until copulation took place (determined by the presence of a spermatophore – a protein capsule containing spermatozoa that is transferred from the male to the female). Eggs were collected a week after copulation and were incubated as above. A subset of early instar nymphs ($N=45$) from three distinct families was used to determine patterns of microsatellite allele inheritance. DNA was extracted and microsatellite markers were amplified as above. PCR products were sent to AGRF for genotyping. Microsatellite profiles were then scored manually using GeneMapper version 4.0. We estimated the probability of conformance to Mendelian expectations using $G$-tests ($\alpha = 0.05$). Significance values were adjusted for multiple comparisons using the sequential Bonferroni correction method where necessary (Rice, 1989).
Mechanism of parthenogenesis

Five virgin females from the stock population were isolated and housed in individual cages as above. These virgin females had no contact with males from the early juvenile phase of their lifecycle thus ensuring that eggs produced were created parthenogenetically. Females commenced laying eggs approximately 30 days after the final juvenile moult. Eggs were collected weekly and incubated as above. First instar nymphs ($N = 169$ from five virgin mothers) were collected upon hatching. DNA was extracted and microsatellite markers were amplified as above. PCR products were genotyped at AGRF and microsatellite profiles were scored as above.

Results

Next-generation sequencing and de novo genome assembly

A total of 133048 sequence reads (average length = 435.3 ± 0.33 bp) were obtained from the 454 Next Generation Sequencing (NGS) run. Previous studies indicate that these figures are not excessive as they are commonly achieved by NGS analyses using only 1/16th of a 70 × 75 mm picoTitre Plate (Miller et al., 2012, 2013). Nonetheless these data represent approximately 4.4 % of the ~1.3 Gb stick insect genome (Soria-Carrasco et al., 2014).

Microsatellite isolation and characterization

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A total of 3257 unique sequence contigs possessing microsatellite motifs were identified by GDD2 analysis of which 2361 contigs had optimal priming sites adjacent to microsatellite motifs. Forty-eight contigs were selected for subsequent analysis, 33 of which contained di-nucleotide repeats, 9 containing tri-nucleotide repeats, and 6 containing a tetra-nucleotide repeat. The initial screening analysis found 18 loci to be polymorphic, 11 were monomorphic and 19 failed to amplify. The 18 polymorphic loci were then pooled into three groups for multiplexing based on observed locus specific allele size ranges (Table 1). The genotyping error rate was 0.02 ± 0.005 (mean ± SE) for the 18 isolated microsatellite markers, with values > 0.03 for loci ET17, ET27 and ET39 (0.07, 0.05 and 0.06 respectively, Table 1). These three loci were thus excluded from further analyses. The majority of the loci were characterized by moderate to high levels of genetic variation with an average of 4.7 ± 0.58 alleles per locus while microsatellite heterozygosity estimates ranged between 0 and 1 (mean ± SE = 0.5 ± 0.07) among loci in the field collected specimens (Table 2). Locus ET29 was monomorphic in all field-collected samples (Supplementary Table S1). Linkage disequilibrium analysis confirmed marker independence, indicating no evidence of significant linkage between loci, while MICRO-CHECKER analysis suggested that there may be null alleles present at loci ET8, ET20, ET24 and ET40 (Table 2). All loci conformed to Hardy–Weinberg expectations according to Fisher’s exact tests and FIS estimates did not show any evidence for heterozygote excess in the Crystal Cascades population (Table 2).

Patterns of allele inheritance

The genotypes of 45 progeny from three families derived from the stock population showed conformity with Mendelian biallelic segregation patterns at all 15 microsatellite loci (Supplementary
Family A had 31 distinct alleles in total with two polymorphic (heterozygous) loci in the mother’s and eight in the father’s genome. Family B had a total of 32 alleles with eight polymorphic loci in the mother’s and six in the father’s genome. Family C had 29 distinct loci in total with two polymorphic loci in the mother’s and seven in the father’s genome. ET26 was the only monomorphic locus in all three families.

Mechanism of parthenogenesis

The proportion of homozygous offspring produced parthenogenetically by heterozygous mothers ranged between 0.44 – 1, depending on the locus (Table 3). Polymorphism was rare in offspring at most loci, despite at least one heterozygous mother for each locus (except loci ET2, ET20, ET29 and ET40 which were homozygous in all five mothers). Therefore most loci had homozygosity transition rates (R) close to 1, with progeny from heterozygous mothers becoming mostly homozygous. The only exception is locus ET6 with 62 heterozygous offspring out of a total of 111 (R = 0.44). The observed R value for this locus is significantly different from the expected rates under apomixis or automixis with gamete duplication, central fusion and random fusion (Table 4). None of the loci had R values significantly different to those expected under automixis with terminal fusion. Therefore, the most likely mechanism of parthenogenesis in *E. tiaratum* is automixis with terminal fusion.

Discussion

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We isolated 18 microsatellite markers for *E. tiaratum*, three of which had high genotyping error rates possibly caused by stutter patterns (loci ET 17, ET27 and ET39, Table 1). The remaining 15 microsatellite markers were independent and conform to Mendelian expectations. Our preliminary analysis suggests that the population in Crystal Cascades is moderately genetically diverse (average 4.7 ± 0.58 alleles per locus) with only one monomorphic locus (ET29, Supplementary Table1). This level of microsatellite polymorphism is greater than that found in two sexually reproducing populations of another phasmid, *Bacillus rossius*, (with one and three monomorphic loci out of five, Andersen et al., 2005). The presence of multiple polymorphic loci in the genome of individuals from the Crystal Cascades population suggests that all the individuals were produced sexually, given the mechanism of parthenogenesis (automixis with terminal fusion) will lead to high individual homozygosity. The population was in HWE, with $F_{IS}$ values indicating no evidence of heterozygosity excess or deficit (Table 2). However, larger samples sizes are required to confirm these results, as there was a suggestion that four loci (ET8, ET20, ET24 and ET40) contained null alleles. Microsatellite analysis using 15 microsatellite markers suggests that the most likely mechanism of parthenogenesis in *E. tiaratum* is automixis with terminal fusion. Most heterozygous loci in mothers (except ET6) were homozygous in their parthenogenetically produced offspring, thereby excluding either apomixis, automixis with central fusion, or random fusion mechanisms of parthenogenesis (Table 3, Table 4). Loci ET6 had high transition rates to heterozygosity, which distinguishes the mechanism from gamete duplication. Further cytogenetic analyses are required to confirm this mechanism. The lower rate of transition to homozygosity in locus ET6 suggests that this locus might be located further away from the centromere and therefore have higher recombination rates (given that the mechanism is automixis with terminal fusion). Although endosymbiotic infection is unlikely to be responsible for the occurrence of parthenogenetic reproduction in Phasmatodea (Perez-ruiz et al., 2015), we are unable to completely rule out this possibility. Nonetheless, our data
indicate that parthenogenesis leads to severe loss of microsatellite heterozygosity in *E. tiaratum*. Loss of microsatellite heterozygosity may be associated with loss of heterozygosity at fitness related loci and therefore could be an important component of determining reproductive fitness (Vali et al., 2008). Loss of heterozygosity can result in inbreeding depression when homozygosity is associated with increased genetic load due to the accumulation of recessive deleterious alleles (partial dominance hypothesis: Charlesworth & Charlesworth, 1999; Roff, 2002).

Studies in various organisms report correlations between loss of heterozygosity and reduced fitness (Chapman et al., 2009). Such fitness costs may explain why most obligate or cyclic parthenogenetic species reproduce by apomixis (e.g. Johnson & leefe, 1999; Mark Welch & Meselson, 2000; Delmotte, et al., 2001; Vavre et al., 2004; Tsutsui et al., 2014) while automixis is mainly observed among facultative or geographic (only in certain populations) parthenogens (e.g. Matsuura et al., 2004; Pearcy et al., 2006; Sekiné & Tojo, 2009; Kellner & Heinze, 2011). Apomictic populations may persist over a greater number of generations while avoiding inbreeding depression, whereas automictic populations would quickly become inbred (Schwander & Crespi, 2009). The specimens collected from Crystal Cascades showed high heterozygosity for most loci in all individuals, suggesting they were produced sexually. While this is a small sample size from a single population, this observation is consistent with the prediction that automictic parthenogenesis occurs sporadically, for example in cases of mating failure (Stalker, 1956; Schwander et al., 2010). It is noticeable that other factors apart from inbreeding depression may be responsible for reduced fitness in parthenogenetic eggs, for example developmental constraints associated with an absence of paternal factors (see Engelstaedter, 2008).
In the stick insect order Phasmatodea, obligately parthenogenetic species such as *Bacillus whitei*, *Sipyloidea sipylus*, *Carausius morous*, and obligately parthenogenetic species of *Timema* genus reproduce by apomixis (Pijnacker, 1966, 1967; Marescalchi et al., 1991; Schwander & Crespi, 2009). In contrast, automixis has been reported in the facultatively parthenogenetic *Bacillus rossius* and *B. atticus* (Pijnacker, 1969; Marescalchi et al., 1993). This observation is consistent with the above argument that apomictic lineages are generally more viable as they are more successful at maintaining genetic diversity. The transition from sexual reproduction to automixis does not involve drastic cytological changes and occurs at low frequencies in many normally sexually reproducing species (Schwander et al., 2010). This frequency can increase once there is a selective advantage to automixis, for example through mating failure (Schwander et al., 2010). The adaptive significance of automictic parthenogenesis is not fully understood, but potential fitness costs associated with a loss of heterozygosity at fitness related loci, may be evident at a diverse range of life history traits.

Acknowledgments

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Conflicts

The authors have no conflict of interest to declare.
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**Table 1** Primer sequences, repeat motifs, size ranges, genotyping error rates and Genebank Accession number for 18 microsatellite markers isolated from *Extatosoma tiaratum*.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences (5’–3’)</th>
<th>Repeat motif</th>
<th>Size (bp)</th>
<th>Error rate</th>
<th>Genebank Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiplex 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET2</td>
<td>AGAGCAACTCAGCTGACGAA</td>
<td>ACT</td>
<td>90</td>
<td>0</td>
<td>KP938274</td>
</tr>
<tr>
<td></td>
<td>TCTCATCTCTTTGATCATTTGTTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET14</td>
<td>AGATTACCACGTGGCAAGCAT</td>
<td>AG</td>
<td>188</td>
<td>0.01</td>
<td>KP938277</td>
</tr>
<tr>
<td></td>
<td>GTGACGAGTCTCTTGCAGTGTTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET16</td>
<td>ATGGTCGACGAGCAGTACAAT</td>
<td>AC</td>
<td>103</td>
<td>0</td>
<td>KP938275</td>
</tr>
<tr>
<td></td>
<td>CTGCTTCTGCAAAGAGTGAGG</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ET26</td>
<td>CATGCTACAACCTTTGGCTC</td>
<td>AGGT</td>
<td>404</td>
<td>0</td>
<td>KP938279</td>
</tr>
<tr>
<td></td>
<td>TACTGATCCACCATTGAGGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET30</td>
<td>TTGAAAGTGGCAGACCTG</td>
<td>AC</td>
<td>150</td>
<td>0.01</td>
<td>KP938276</td>
</tr>
<tr>
<td></td>
<td>TAAACGGTGCCATTTGGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET40</td>
<td>TCATACGATTCAGAACCAGA</td>
<td>AG</td>
<td>229</td>
<td>0.01</td>
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</tr>
<tr>
<td></td>
<td>CAGGCAATGAGCGATAGAGAA</td>
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<td></td>
</tr>
<tr>
<td>Multiplex 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET6</td>
<td>GTACTGAGACCTCTACGAACTAATGT</td>
<td>AG</td>
<td>292</td>
<td>0</td>
<td>KP938284</td>
</tr>
<tr>
<td></td>
<td>TGACATTGCTGGTCAACAGG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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ET11  TCTTCTACGCCCCACGACAT   AG  149  0.03  KP938282
       AGAGGGTTGATTCGCTGTGA
ET15  AGATACCAGTGCAAGCAT   AC  319  0  KP938285
       CAGTACACGAGTGATGGTCG
ET24  TCCGGAAGATAGCATTACATTTAAACA  AG  90  0  KP938280
       GCGGGAAAATTTTCGAGTAG
ET27  TCCCTACAGCTCTGACTATCCG  AAT  131  0.05  KP938281
       TGATCACAGTCAAAGTTGGACAATA
ET29  GCAAGCGACTTAAGCTTCCC  AC  202  0  KP938283
       TCACCTCGAGACCAGCAAAT
ET8   CTGCAAGTAGCCAACCTTCTCTG  AAT  121  0.03  KP938288
       ATGCTGTTGCTTGGAATGCT
ET17  TATCTCAGGAGAACGAGCG   AC  130  0.07  KP938289
       AGAGACGTGTCAGTCAGCC
ET20  TCCCTATGACATCACCACCC   AG  214  0.02  KP938291
       CAGACAAACGAAACGGATGG
ET35  CACCTCCTCTCCCTCTCACA  AC  108  0  KP938287
       AACATGGCTTGTGATGCATTT
ET39  GCCTCAGTGTGGGAAGTTGT   AG  98  0.06  KP938286
       CTGACAGGTACTGGCTGGCT
ET42  ACTTTCACCTGTCGGCTGCTG  AAT  181  0  KP938290
       GAATTTCGGAGTGGCTTCAA

Multiplex 3

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Table 2  Characterization of 15 microsatellite markers estimated from 11 individuals from the Crystal Cascades, North Queensland, Australia

<table>
<thead>
<tr>
<th>Locus</th>
<th>Na</th>
<th>(H_O/H_{E})</th>
<th>HWE</th>
<th>(F_{IS})</th>
<th>Frequency of null alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET2</td>
<td>2</td>
<td>0.36/0.31</td>
<td>1</td>
<td>-0.20</td>
<td>0.000</td>
</tr>
<tr>
<td>ET6</td>
<td>2</td>
<td>0.27/0.51</td>
<td>1</td>
<td>0.37</td>
<td>0.290</td>
</tr>
<tr>
<td>ET8</td>
<td>6</td>
<td>0.40/0.68</td>
<td>0.35</td>
<td>0.50</td>
<td>0.270</td>
</tr>
<tr>
<td>ET11</td>
<td>6</td>
<td>0.64/0.80</td>
<td>1</td>
<td>0.26</td>
<td>0.110</td>
</tr>
<tr>
<td>ET14</td>
<td>9</td>
<td>0.64/0.74</td>
<td>1</td>
<td>0.14</td>
<td>0.009</td>
</tr>
<tr>
<td>ET15</td>
<td>2</td>
<td>0.27/0.25</td>
<td>1</td>
<td>-0.12</td>
<td>0.000</td>
</tr>
<tr>
<td>ET16</td>
<td>5</td>
<td>0.90/0.69</td>
<td>1</td>
<td>-0.32</td>
<td>0.000</td>
</tr>
<tr>
<td>ET20</td>
<td>8</td>
<td>0.50/0.86</td>
<td>1</td>
<td>0.59</td>
<td>0.400</td>
</tr>
<tr>
<td>ET24</td>
<td>8</td>
<td>0.60/0.86</td>
<td>0.48</td>
<td>0.34</td>
<td>0.120</td>
</tr>
<tr>
<td>ET26</td>
<td>7</td>
<td>0.90/0.78</td>
<td>1</td>
<td>-0.13</td>
<td>0.120</td>
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<tr>
<td>ET29</td>
<td>1</td>
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<td>NA</td>
<td>0.000</td>
</tr>
<tr>
<td>ET30</td>
<td>2</td>
<td>0.18/0.17</td>
<td>0.74</td>
<td>0.00</td>
<td>0.000</td>
</tr>
<tr>
<td>ET35</td>
<td>3</td>
<td>0.45/0.58</td>
<td>1</td>
<td>0.19</td>
<td>0.070</td>
</tr>
<tr>
<td>ET40</td>
<td>6</td>
<td>0.54/0.84</td>
<td>0.38</td>
<td>0.32</td>
<td>0.140</td>
</tr>
<tr>
<td>ET42</td>
<td>4</td>
<td>0.70/0.73</td>
<td>1</td>
<td>0.32</td>
<td>0.220</td>
</tr>
</tbody>
</table>

Number of alleles (Na), observed (HO) and expected (HE) heterozygosities, Hardy-Weinberg equilibrium p-values (HWE) and inbreeding coefficient (FIS). P-values indicate the probability that HO differs from HE. Locus ET29 is monomorphic in this population, therefore HO, HE, HWE and FIS values are not available (NA).

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Table 3: Number of homozygous and heterozygous offspring produced parthenogenetically by heterozygous mothers and rates of transition to homozygosity (R)

<table>
<thead>
<tr>
<th>Informative loci</th>
<th>Hetero mother</th>
<th>Homo offspring</th>
<th>Hetero offspring</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET6</td>
<td>4</td>
<td>49</td>
<td>62</td>
<td>0.44</td>
</tr>
<tr>
<td>ET8</td>
<td>1</td>
<td>22</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>ET11</td>
<td>2</td>
<td>64</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>ET14</td>
<td>3</td>
<td>91</td>
<td>1</td>
<td>0.99</td>
</tr>
<tr>
<td>ET15</td>
<td>5</td>
<td>158</td>
<td>1</td>
<td>0.99</td>
</tr>
<tr>
<td>ET16</td>
<td>1</td>
<td>36</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>ET24</td>
<td>2</td>
<td>71</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>ET26</td>
<td>3</td>
<td>89</td>
<td>1</td>
<td>0.99</td>
</tr>
<tr>
<td>ET29</td>
<td>3</td>
<td>101</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>ET30</td>
<td>1</td>
<td>34</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>ET42</td>
<td>1</td>
<td>150</td>
<td>1</td>
<td>0.99</td>
</tr>
</tbody>
</table>

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Table 4 G-tests for observed and expected rates of transition to homozygosity (R) depending on the mechanism of parthenogenesis (P = 0.05). R is not significantly different from expected rates under automixis with terminal fusion for any loci.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Gamece duplication</th>
<th>Central fusion</th>
<th>Random fusion</th>
<th>Terminal fusion</th>
<th>Apomixy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r=1</td>
<td>r=0–1/3</td>
<td>r=1/3</td>
<td>r=1/3–1</td>
<td>r=0</td>
</tr>
<tr>
<td>ET6</td>
<td>****</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET8</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET11</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET14</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET15</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET16</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET24</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET26</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET29</td>
<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
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<td>1</td>
<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
<tr>
<td>ET42</td>
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<td>****</td>
<td>****</td>
<td>1</td>
<td>****</td>
</tr>
</tbody>
</table>

**** shows statistically significant differences with P <0.0001

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