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# Pushing the limits of whole genome amplification: Successful sequencing of RADseq libraries from single micro-hymenoptera (Chalcidoidea, *Trichogramma*)

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A major obstacle to high-throughput genotyping of micro-hymenoptera is their small size. As species are difficult to discriminate and because complexes may exist, the sequencing of a pool of specimens is hazardous. Thus, one should be able to sequence pangenomic markers (e.g. RADtags) from a single specimen. To date, whole genome amplification (WGA) prior to library construction is still a necessity as only ca 10ng of DNA can be obtained from single specimens. However this amount of DNA is not compatible with manufacturer's requirements for commercialised kits. Here we tested the accuracy of the GenomiPhi kit V2 on *Trichogramma* wasps by comparing RAD libraries obtained from the WGA of single specimens (generation F0 and F1, ca 1 ng input DNA for the WGA) and a biological amplification of genomic material (the pool of the progeny of the F1 generation). Globally, we found that ca 99% of the examined loci (up to 48,189; 109 bp each) were compatible with the mode of reproduction of the studied model (haplodiploidy) or a Mendelian inheritance of alleles. The remaining 1% (ca 0.01% of the analysed nucleotides) could represent WGA bias or other experimental / analytical bias. This study shows that the multiple displacement amplification method on which the GenomiPhi kit relies, could also be of great help for the high-throughput genotyping of micro-hymenoptera used for biological control or other organisms from which only a very low amount of DNA can be extracted such as human disease vectors (e.g. sand flies, fleas, ticks etc.).

1 **Pushing the limits of whole genome amplification: successful sequencing of RADseq**  
2 **libraries from single micro-hymenoptera (Chalcidoidea, *Trichogramma*)**

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11

12 **Abstract**

13 A major obstacle to high-throughput genotyping of micro-hymenoptera is their small size. As  
14 species are difficult to discriminate and because complexes may exist, the sequencing of a pool  
15 of specimens is hazardous. Thus, one should be able to sequence pangenomic markers (e.g.  
16 RADtags) from a single specimen. To date, whole genome amplification (WGA) prior to library  
17 construction is still a necessity as only *ca* 10ng of DNA can be obtained from single specimens.  
18 However this amount of DNA is not compatible with manufacturer's requirements for  
19 commercialised kits. Here we tested the accuracy of the GenomiPhi kit V2 on *Trichogramma*  
20 wasps by comparing RAD libraries obtained from the WGA of single specimens (generation F0  
21 and F1, *ca* 1 ng input DNA for the WGA) and a biological amplification of genomic material  
22 (the pool of the progeny of the F1 generation). Globally, we found that *ca* 99% of the examined  
23 loci (up to 48,189; 109 bp each) were compatible with the mode of reproduction of the studied

24 model (haplodiploidy) or a Mendelian inheritance of alleles. The remaining 1% (ca 0.01% of the  
25 analysed nucleotides) could represent WGA bias or other experimental / analytical bias. This  
26 study shows that the multiple displacement amplification method on which the GenomiPhi kit  
27 relies, could also be of great help for the high-throughput genotyping of micro-hymenoptera used  
28 for biological control or other organisms from which only a very low amount of DNA can be  
29 extracted such as human disease vectors (e.g. sand flies, fleas, ticks etc.).

30

31 **Running Head :** RADseq from low DNA amount through WGA

32 **Keywords :** DNA quantity, GenomiPhi, high-throughput genotyping, microarthropods, outside  
33 manufacturer recommendations, RAD.

34

## 35 INTRODUCTION

36 Parasitoid wasps (especially Chalcidoidea; Heraty et al. 2013) are increasingly used as biocontrol  
37 agents of many crop pests to reduce pesticide use (Austin et al. 2000). Among them, minute  
38 wasps of the genus *Trichogramma* (210 species worldwide, 40 in Europe), which develop within  
39 the eggs of ca. 200 species of moths damaging crops (e.g. corn, grapes, apple, pines; Consoli et  
40 al. 2010) are the most commercialized worldwide.

41 It is acknowledged that successful and safe biological control depends on accurate genetic and  
42 phenotypic characterization of the strains released. Furthermore, host preferences and the  
43 potential of strains to hybridize with each other or with native species should be carefully studied.  
44 This is critical to avoid non-target effects such as gene introgression with indigenous species  
45 (Van Driesche & Hoddle 2016). However, probably because most species of chalcids are minute  
46 wasps (less than a few millimetres long) and are difficult to identify to species by non-specialists,  
47 strains are often released without in depth characterization.

48 RADseq, the sequencing of hundreds of thousands of DNA fragments flanking restriction sites  
49 (Miller et al. 2007) has been successfully used for population genetics or phylogeography  
50 (Emerson et al. 2010), to infer relationships between closely (Jones et al. 2013; Nadeau et al.  
51 2013; Wagner et al. 2013) or more distantly (Cruaud et al. 2014; Hipp et al. 2014) related species,  
52 to detect hybridization processes (Eaton & Ree 2013; Hohenlohe et al. 2011), to identify markers  
53 under selection and detect genes that are candidates for phenotype evolution (Hohenlohe et al.  
54 2010), or to better understand the genomic architecture of reproductive isolation (Gagnaire et al.  
55 2013). Thus, sequencing RAD markers appear relevant for in depth characterisation of  
56 *Trichogramma* strains used in biocontrol.

57 A major obstacle to RAD sequencing of oophagous parasitoids is their small size. Ideally, one  
58 should be able to sequence RAD markers from a single specimen. Indeed, species complexes  
59 may exist that are difficult to identify based on morphology only (Al Khatib et al. 2014; Kenyon  
60 et al. 2015; Mottern & Heraty 2014), which makes sequencing of a pool of specimens risky.  
61 However, to date, the DNA amount obtained from single specimens is not sufficient enough to  
62 build a RADseq library. Usually, for minute specimens, *ca* 10 ng are obtained while *ca* 150ng  
63 input DNA are required to build a RADseq library. Performing whole genome amplification  
64 (WGA) prior to library construction is thus a necessity. So far a few studies have formally  
65 examined the accuracy of WGA methods, mostly on human DNA and either a few loci (Hosono  
66 et al. 2003; Lovmar et al. 2003; Sun et al. 2005) or a higher number of SNPs and loci but always  
67 with 10ng of more input DNA (Abulencia et al. 2006; Barker et al. 2004; Blair et al. 2015;  
68 ElSharawy et al. 2012; Paez et al. 2004; Pinard et al. 2006). All studies have concluded that the  
69 multiple displacement amplification method (MDA; Dean et al. 2002; Lasken 2009), which  
70 relies on isothermal DNA amplification using a high-fidelity polymerase (bacteriophage phi29;  
71 Paez et al. 2004) and random hexamer primers to decrease amplification bias and increase  
72 product size, is among the most accurate.

73 So far, only one study has quantified sequence bias that might result from WGA prior to double-  
74 digest RAD sequencing (ddRADseq; Peterson et al. 2012); a variant of RADseq that uses two  
75 restriction enzymes to cut DNA instead of one enzyme and a DNA shearing system. In their  
76 study, Blair et al. (2015) use the Qiagen REPLI-g Mini Kit and 100 ng of input DNA (as  
77 requested by the kit) extracted from liver samples of specimens of the grey mouse lemur  
78 (*Microcebus murinus*). They conclude that the kit does not introduce bias for i) SNP calling as  
79 compared to what is obtained from native DNA of the same samples or ii) genome coverage as

80 compared to the published genome of *M. murinus*. Here we test the accuracy of the  
81 GE Healthcare Life Sciences™ illustra™ GenomiPhi V2 for the WGA of single *Trichogramma*  
82 wasps prior to RADseq library construction. As for the REPLI-g Mini Kit, WGA is performed  
83 using the MDA. However, the GenomiPhi kit requires 10 times less DNA (1 µl of input DNA at  
84 10ng/ µl) but still more than what can be extracted from single *Trichogramma* wasps. As a  
85 consequence, we had to push the limits of the kit, increasing the risk of inconsistent or not  
86 representative amplification of the genome. To test the accuracy of the GenomiPhi kit in these  
87 challenging conditions we compared RADtags obtained from the WGA of single individuals and  
88 RADtags obtained from the pool of their progeny (Fig 1). Thus, we compared RAD libraries  
89 obtained from a technical / artificial amplification (WGA) and a biological / natural  
90 amplification (pool of specimens).

91

## 92 **MATERIALS AND METHODS**

93

### 94 **Sampling and experimental design**

95 Males and females (F0) were taken from the strain collection hosted by the Biological Resource  
96 Centre "Egg Parasitoid Collection" (EP-Coll, Sophia-Antipolis, France) (Marchand et al. 2017).  
97 All specimens belong to the species *Trichogramma brassicae* Bezdenko, 1968. Ten crossing  
98 experiments were attempted (Fig. 1). Ten pairs (F0) of male (haploid) / female (diploid) were  
99 sorted out from the rest of the specimens. Each pair was then reared in individual glass tubes and  
100 left free to mate. Droplets of honey were provided as food and eggs of *Ephestia kuehniella*  
101 (Pyralidae) were used as hosts. Females and males were killed in ethanol 70% before emergence  
102 of the F1 generation. Emerging females of the F1 generation were kept separated from males (no

103 mating) and reared in new glass tubes (1 female per tube). As for F0, droplets of honey were  
104 provided as food and eggs of *Ephestia kuehniella* were used as hosts. Females F1 were killed  
105 before the emergence of the F2 generation (males only, arrhenotokous parthenogenesis). For  
106 each cross, all males F2 were pooled prior to DNA extraction.

107

#### 108 **DNA extraction and whole genome amplification**

109 DNA extraction was performed with the Qiagen DNeasy 96 Blood & Tissue Kit, following  
110 manufacturer protocol with the following modifications to increase DNA yield: two successive  
111 elutions (50  $\mu$ L each) were performed with heated buffer AE (55°C) and an incubation step of 15  
112 minutes followed by plate centrifugation (6000 rpm for 2 minutes).

113 DNA was quantified with a Qubit® 2.0 Fluorometer (Invitrogen). To fit as much as possible the  
114 recommendations of the GenomiPhi protocol (1  $\mu$ l DNA input at 10 ng/ $\mu$ l), ethanol precipitation  
115 of DNA was performed prior to WGA. 1/10 volume of sodium acetate 3M pH 5.2 was added to  
116 the extract. Then, 2 volumes of cooled absolute ethanol were added to the mix. The mix was  
117 incubated at -20°C overnight. The mix was then centrifuged (30 min, 13 000 rpm, 4°C) and the  
118 pellet was washed with 500  $\mu$ l of cooled ethanol 70%. After another centrifugation (15 min,  
119 13 000 rpm, 4°C), the pellet was dried at room temperature and resuspended in 4  $\mu$ l of sterile  
120 molecular biology ultrapure water, as a total resuspension of the pellet would not have been  
121 obtained in a smaller volume. Concentrate DNA was quantified with a Qubit® 2.0 Fluorometer  
122 (Invitrogen). DNA extracts were then subjected to Whole Genome Amplification using the  
123 GenomiPhi™ V2 DNA Amplification kit (GE Healthcare) with 1  $\mu$ l of concentrate DNA used as  
124 input. The resulting DNA was quantified with a Qubit® 2.0 Fluorometer (Invitrogen).

125

#### 126 **RADseq library construction**



127 Library construction followed Baird et al. (2008) and Etter et al. (2011) with modifications  
128 detailed in Cruaud et al. (2014). The *PstI* enzyme was chosen as cutter. The number of expected  
129 cut sites was estimated with an *in silico* digestion of the genome of *T. pretiosum* (assembly  
130 Tpre\_1.0, 196Mb) using a custom script. The experiment to test the accuracy of WGA for  
131 RADseq of micro-hymenoptera is part of a larger project that aims at resolving the phylogenetic  
132 relationships of European *Trichogramma* wasps. Thus more samples (N=40) than what was used  
133 to answer our technical question were included in the library. About 250ng of input DNA was  
134 used for each sample. The quantity of P1 adapters (100nM) to be added to saturate restriction  
135 sites (result=3uL) as well as the optimal time for DNA sonication on a Covaris S220  
136 ultrasonicator to obtain fragments of 300 – 600 bp (results = duty cycle 10%, intensity 5,  
137 cycles/burst 200, duration 70s) that are both specific to the studied group were evaluated in a  
138 preliminary experiment. After tagging with barcoded P1 adapters and prior to sonication,  
139 samples were pooled eight by eight. Five pools were thus obtained, and each pool was sheared  
140 and then tagged with a different barcoded P2 adapter. 2\*125nt paired-end sequencing of the  
141 library was performed at MGX-Montpellier GenomiX on one lane of an Illumina HiSeq 2500  
142 flow cell.

143

#### 144 **Data analysis**

145 Cleaning of raw data was performed with the wrapper RADIS (Cruaud et al. 2016) that relies on  
146 Stacks (Catchen et al. 2013; Catchen et al. 2011) for demultiplexing of data and removing PCR  
147 duplicates. Data analysis was performed with Stacks v1.46. Individual loci were built using  
148 *ustacks* [m=3 ; M=1 ; N=2 ; with the removal (r) and deleveraging (d) algorithms enabled].  
149 Catalogs of loci were built with *cstacks* (n=2) for each of the four crosses. Females F1 and their

150 progeny were analysed first (4 data sets), other catalogs grouping all specimens involved in the  
151 cross were built (4 other data sets). *sstacks* was used to map individual loci to the catalog.  
152 *rxstacks* was then used to correct genotype and haplotype calls : i) Loci for which at least 50% of  
153 the samples (when a pair composed of one female F1 and a pool of males F2 was analysed) or  
154 25% of the samples (when F0, F1 and F2 were analysed together) had a confounded match to the  
155 catalog were removed; ii) excess haplotypes were pruned; iii) SNPs were recalled after removal  
156 of possible sequencing errors using the bounded SNP model (--bound\_high 0.1), and iv) loci  
157 with an average log likelihood less than -10.0 were discarded. After this filtering step, *cstacks*  
158 and *sstacks* were rerun. The program *populations* was then used to compare the RADtags  
159 obtained with or without WGA (parsing of the *haplotypes.tsv* and *populations.log* files). Loci  
160 were kept only if i) they had a minimum stack depth of 10 and ii) all samples had a sequence.  
161 Analyses were performed on the Genotoul Cluster (INRA, Toulouse).

162

## 163 RESULTS

164 On the ten attempted crosses, only three led to enough F2 males ( $N > 100$ ) to get a sufficient  
165 amount of DNA for RADseq library construction without WGA. Consequently RADseq libraries  
166 were constructed only on these crosses. DNA extraction of one third of the tested specimens  
167 provided an amount of DNA that stand below the detection limit of the Qubit (Table 1). WGA  
168 was not attempted on these specimens. For other specimens, the average amount of DNA  
169 obtained with the Qiagen kit was 10.4 ng (min = 6.2 – max = 13.9) (Table1). After DNA re-  
170 concentration, the average DNA quantity used as input for the WGA was *ca* 1.0 ng (0.17 – 2.9).  
171 In average, 947.5 ng of DNA was obtained with the WGA (226 - 2393).

172 *In silico* digestion of the genome of *T. pretiosum* revealed 59,433 *PstI* cut sites (i.e. 118,866  
173 tags). An average of 2\*3,757,867 reads (109 bp) was obtained for the different samples after  
174 quality filtering, demultiplexing and removal of PCR clones (Table 1). Two females F1  
175 (TRIC00027\_1103 and TRIC00027\_3103) were represented by much less reads than other  
176 samples (595,204 and 1,991,305 respectively). The number of tags recovered by *ustacks* and  
177 *cstacks* varied but was comparable among the samples and in line with the predictions made on  
178 the genome of *T. pretiosum* when these two females were excluded from calculation (average  
179 number of *ustacks* tags = 132,787; average number of *cstacks* tags = 128,293, Table1).

180 The comparison of the loci obtained after filtering steps with *rxstacks* and *populations* revealed  
181 that, in average, 97.6% of the loci were homozygous and identical for females F1 and the pool of  
182 males F2 (min=96.8% - max=98.2%, Table 2). In average, 0.7% (0.3%-1.3%) of the loci were  
183 heterozygous and identical in both samples. Thus there was a *ca* 98.3% (97.4%-99.0%) exact  
184 match between the loci of the females F1 included in the library (and whose DNA was amplified  
185 with WGA) and the whole progeny of the F1 generation (pool of males whose DNA was not  
186 amplified).

187 Between 1.0 and 2.6% of the loci were not identical between analysed pairs (Table 2). A careful  
188 inspection of the haplotypes revealed that *ca* 60% of these differences could be explained by the  
189 experimental setup, *i.e.* the sequencing of a single female of the F1 generation versus the  
190 sequencing of the whole progeny of the F1 generation (pool of males heterozygous, female F1  
191 homozygous with an allele present in the pool of males; pool of males with three alleles, female  
192 F1 homozygous with an allele present in the pool of males; pool of males with three alleles,  
193 female F1 heterozygous with two alleles present in the pool of males). About 40% of the  
194 observed SNPs were not compatible with either the experimental setup (pool of males and

195 female F1 homozygous but with different alleles; female F1 heterozygous, pool of males  
196 homozygous with an allele present in the female F1; female F1 heterozygous, pool of males  
197 homozygous with an allele not present in the female F1; pool of males heterozygous, female F1  
198 homozygous with an allele not present in the pool of males; females F1 and pool of males  
199 heterozygous with only one of the two alleles in common. Globally, 99.3% (98.9 - 99.6%) of the  
200 shared loci were either identical or displayed differences that could be explained by the  
201 experimental setup. The first cross was used to check in details the overall coherence of the  
202 haplotypes from the parental generation to the whole progeny of the F1 generation (Table 3).  
203 98.8% of the 32,913 loci shared by the four samples displayed haplotypes consistent with  
204 experimental the setup and a Mendelian inheritance of alleles (97.3% being homozygous and  
205 identical between samples). SNPs observed in 385 loci (which represent 1.2% of the loci and  
206 0.01% of the analysed nucleotides) were not compatible with the mode of reproduction of the  
207 studied model (haplodiploidy) or a Mendelian inheritance of alleles. Considering the haplotype  
208 observed in the pool of males F2 as a reference, questionable SNPs could be categorized into  
209 five categories as listed in Table 4. In *ca* 90% of the situations, SNPs found either in the male F0  
210 (21%), the female F0 (32.5%) or the female F1 (36.6%) were incompatible with haplodiploidy or  
211 with a Mendelian inheritance of alleles. 96 cases (*ca* 25.0%) represented situations where one  
212 allele was missing for the female F0 or the female F1 to fit with a Mendelian inheritance of  
213 alleles (possible cases of allele drop-out).

214

## 215 **DISCUSSION**

216 Here we compare RAD libraries obtained from a technical / artificial amplification of DNA  
217 (WGA of single specimen of micro-hymenoptera, F0 and F1 generations) and a biological /

218 natural amplification (pool of the progeny of the F1 generation). We push the limits of the kit  
219 used for the WGA (GenomiPhi) by using ca 90% less DNA (ca. 1.0ng) than the required amount  
220 specified on the manufacturer's protocol (10ng). Globally, we show that 99% of the examined  
221 loci (up to 48,189; 109 bp each) were compatible with haplodiploidy and either identical among  
222 specimens or compatible with a Mendelian inheritance of alleles. These results are consistent  
223 with observations by Blair et al. (2015) who used the Qiagen REPLI-g Mini Kit and 100 ng of  
224 input DNA and showed that SNP calling between ddRAD libraries from native and amplified  
225 DNA presented a > 98% match (up to 11,309 loci examined). They are also in agreement with  
226 older studies that attempted to quantify bias induced by multiple displacement amplification  
227 method (MDA) on which the GenomiPhi kit relies (> 99% match; Barker et al. 2004; ElSharawy  
228 et al. 2012; Paez et al. 2004), though with more input DNA (10ng).

229 To the exception of two samples, for which the construction of the library seems to have failed  
230 (much less reads were obtained), comparable numbers of tags were obtained. This indicates that  
231 the coverage of the genome is the same regardless if native or amplified DNA is used as  
232 suggested by previous studies on the potential bias induced by MDA (Abulencia et al. 2006;  
233 Blair et al. 2015; Paez et al. 2004). Studies have suggested that WGA may induce allele dropout  
234 especially when the starting amount of DNA is low (<1ng) (Handyside et al. 2004; Lovmar et al.  
235 2003; Lovmar & Syvänen 2006; Sun et al. 2005). ElSharawy et al. (2012) and Blair et al. (2015)  
236 concluded that MDA had no significant effect on levels of homozygosity. Here about 1% of the  
237 loci retained by our analytical pipeline (ie ca 0.01% of the examined nucleotides) presented  
238 problematic SNPs that were not compatible with the biology of *Trichogramma* wasps or a  
239 Mendelian inheritance of alleles. 0.3% of the SNPs were possible cases of allele drop-out (one  
240 allele was missing for the female F0 or the female F1 to fit with a Mendelian inheritance of

241 alleles). A larger sampling would be required to examine these few problematic SNPs in more  
242 details. Here, a correlation may exist between the number of problematic SNPs and the quantity  
243 of input DNA used for the WGA [less bias in haploid male F0 (1.5 ng; 81 problematic SNPs;  
244 0.002% of the examined nucleotides) as compared to female F0 (0.39 ng; 125; 0.003%) and  
245 female F1 (0.35 ng; 141; 0.004%)] but no definite conclusion can be drawn. It is noteworthy that  
246 if these problematic SNPs can indeed result from bias caused by WGA, other explanations are  
247 possible (competition between fragments for ligation of P1 or adapters, mutation during  
248 enrichment PCR, sequencing error). Indeed, although they are less frequent, bias are also  
249 observed in the pool of males F2 (29 problematic SNPs; 0.0002% of the examined nucleotides).  
250 Regarding the possible improvements of our protocol. Extraction failed for a third of our  
251 specimens (especially single males that are much smaller than females). Here we used the  
252 Qiagen kit 96-well-plate format in order to facilitate the processing of many specimens at a time.  
253 However, especially for precious specimens, DNA yield could be increased with the spin-column  
254 format, as higher centrifuge speed could be used. Furthermore, for projects that aim to target a  
255 high number of specimens, re-concentration on SPRI beads may be used instead of using ethanol  
256 precipitation of DNA. Indeed, such methods are compatible with robotic sample preparation.  
257 However, while DNA yield could be better, working with very low amount of buffer to  
258 resuspend DNA could be troublesome.

259

## 260 CONCLUSION

261 In this study we pushed the limits of the GenomiPhi kit V2 and successfully built RADseq  
262 libraries from single micro-wasps (*Trichogramma*). Globally, we found that *ca* 99% of the  
263 examined loci (up to 48,189; 109 bp each) were compatible with the mode of reproduction of the

264 studied model (haplodiploidy) and/or a Mendelian inheritance of alleles. The remaining 1% (ca  
265 0.01% of the analysed nucleotides) could represent WGA bias or other experimental / analytical  
266 bias. It is noteworthy that the GenomiPhi kit V2 (and the new GenomiPhi kit V3) are affordable  
267 and easy to use by the vast majority of laboratories, which is an important point to consider given  
268 the increasing demand for the genomic characterisation of parasitoids used in biocontrol  
269 programs or other disease-transmitting micro-arthropods (e.g. sand flies, fleas, ticks etc.).

270

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275

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399  
400 **DATA ACCESSIBILITY**

401 Cleaned reads are available as a NCBI Sequence Read Archive (#SRP136713).

402

403 **AUTHOR CONTRIBUTIONS**

404 A.C. and J.-Y.R. designed the study, analysed the data and wrote the manuscript. G. Groussier  
405 reared *Trichogramma* strains. G. Genson and L.S. performed molecular work. All authors  
406 commented on the manuscript.

407

408 **TABLES AND FIGURES**

409

410 **Figure 1. Experimental setup**

411 Photo *Trichogramma brassicae* © J.-Y. Rasplus

412

413 **Table 1. Extraction, whole genome amplification and sequencing results**

414 \* reads obtained after demultiplexing and quality filtering with *process\_radtags*; \*\* reads  
415 obtained after removal of PCR clones (input reads for the *ustacks* step); \*\*\* one catalog was  
416 built for each cross

417

418 **Table 2. Pairwise comparison of loci obtained for females of the F1 generation and pools of**  
419 **males of the F2 generation.** Analysed loci have been first corrected by *rxstacks* for genotype

420 and haplotype calls and filtered with *populations*. Only loci that were present in the two samples  
421 with a stack depth of 10 were kept.

422

423 **Table 3. Comparison of loci obtained for the first crossing experiment.** Analysed loci have  
424 been first corrected by *rxstacks* for genotype and haplotype calls and filtered with *populations*.  
425 Only loci that were present in the four samples with a stack depth of 10 were kept.

426

427 **Table 4. Categories of SNPs not compatible with the mode of reproduction of the studied**  
428 **model (haplodiploidy) or a Mendelian inheritance of alleles and number of occurrences of**  
429 **each case.** The different situations are illustrated by examples taken from the analysis of the 385  
430 questionable SNPs.

431

432

**Table 1** (on next page)

Extraction, whole genome amplification and sequencing results

\* reads obtained after demultiplexing and quality filtering with *process\_radtags*; \*\* reads obtained after removal of PCR clones (input reads for the *ustacks* step); \*\*\* one catalog was built for each cross

1 **Table 1**

Cross #	Sample code	Description	qDNA (ng)	Input DNA for WGA (ng)	Output DNA from WGA (ng)	Input DNA for RAD library (ng)	Demultiplexed Reads* (forward only)	Cleaned reads** (forward only)	ustacks : Nb of loci	ustacks : Nb of loci***
1	TRIC00027_2101	Male F0, haploid, WGA	11.5	1.5	500	169.0	6,774,680	5,173,711	136,623	130,060
1	TRIC00027_2102	Female F0, diploid, WGA	10.6	0.39	1048	203.3	4,073,370	3,179,891	128,212	122,860
1	TRIC00027_2103	Female F1, diploid, WGA	6.20	0.35	2393	281.2	4,597,505	3,566,986	130,565	124,845
1	TRIC00027_2199	Pool of haploid males F2 (n=933), no WGA	735.4	N.A.	N.A.	269.0	4,818,385	3,745,752	127,709	125,047
2	TRIC00027_1101	Male F0, haploid, WGA	Too low	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2	TRIC00027_1102	Female F0, diploid, WGA	Too low	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
2	TRIC00027_1103	Female F1, diploid, WGA	9.4	0.17	1128	164.6	774,450	595,204	43,763	42,062
2	TRIC00027_1199	Pool of haploid males F2 (n=229), no WGA	359.6	N.A.	N.A.	270.6	5,878,301	4,437,984	127,380	125,302
3	TRIC00027_3101	Male F0, haploid, WGA	Too low	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
3	TRIC00027_3102	Female F0, diploid, WGA	13.9	2.9	390	247.7	7,006,980	5,365,499	147,718	140,690
3	TRIC00027_3103	Female F1, diploid, WGA	10.7	0.43	226	137.3	2,616,330	1,991,305	93,606	89,543
3	TRIC00027_3199	Pool of haploid males F2 (n=1415), no WGA	670.7	N.A.	N.A.	228.4	7,510,904	5,764,475	131,267	129,249

2



**Table 2** (on next page)

Pairwise comparison of loci obtained for females of the F1 generation and pools of males of the F2 generation

Analysed loci have been first corrected by *rxstacks* for genotype and haplotype calls and filtered with *populations*. Only loci that were present in the two samples with a stack depth of 10 were kept.

1 **Table 2**

Pair of samples	Nb of shared loci	Percentage of identical loci (homozygous)	Percentage of identical loci (heterozygous)	Percentage of loci with differences possibly explained by the experimental setup	Percentage of loci with differences not explained by the experimental setup
Cross #1 Female F1 x pool of males F2	48,189	97.7	1.3	0.6	0.4
		Total percentage of identical loci 99.0		Total percentage of loci with differences 1.0	
Cross #2 Female F1 x pool of males F2	5,184	96.8	0.6	1.5	1.1
		Total percentage of identical loci 97.4		Total percentage of loci with differences 2.6	
Cross #3 Female F1 x pool of males F2	20,095	98.2	0.3	0.9	0.6
		Total percentage of identical loci 98.5		Total percentage of loci with differences 1.5	

2

**Table 3** (on next page)

Comparison of loci obtained for the first crossing experiment.

Analysed loci have been first corrected by *rxstacks* for genotype and haplotype calls and filtered with *populations*. Only loci that were present in the four samples with a stack depth of 10 were kept.

1 **Table 3**

<b>Studied samples</b>	<b>Nb of shared loci</b>	<b>Percentage of identical loci (homozygous)</b>	<b>Percentage of loci consistent with the experimental setup and Mendelian inheritance of alleles</b>	<b>Percentage of loci not consistent with the experimental setup and Mendelian inheritance of alleles</b>
Cross #1 - female F0 & male F0 - one female F1 - progeny of the F1 generation	32,913	97.3	98.8	1.2

2

**Table 4**(on next page)

Categories of SNPs not compatible with the mode of reproduction of the studied model (haplodiploidy) or a Mendelian inheritance of alleles and number of occurrences of each case.

The different situations are illustrated by examples taken from the analysis of the 385 questionable SNPs.

1 **Table 4**

Description	Male F0	Female F0	Female F1	Pool of males F2	Occurrences
Male F0 incompatible	<b>A/G</b>	G	G	G	81 (21.04 % of the problematic SNPs ; 0.002% of the analysed nt)
	<b>TG</b>	GA	GA	GA	
Female F0 incompatible	C	<b>C/T</b>	C	C	125 (32.47 % of the problematic SNPs ; 0.003% of the analysed nt)
	A	<b>A</b>	A/G	A/G	
	G	<b>A</b>	G	G	
Female F1 incompatible	C	C	<b>A/C</b>	C	141 (36.62 % of the problematic SNPs ; 0.004% of the analysed nt)
	T	C/T	<b>C</b>	C/T	
	GG	AG/GG	<b>GG/GT</b>	AG/GG	
	T	A	<b>A</b>	A/T	
Pool of males F2 incompatible	<b>T</b>	<b>T</b>	<b>T</b>	<b>C/T</b>	29 (7.53% ; of the problematic SNPs ; 0.0008% of the analysed nt)
	A	A/G	A/G	A	
	AA	AA	AA	<b>CC</b>	
	C	T	C/T	<b>C</b>	
Combination of the different situations	<b>C/G</b>	C/G	C/G	<b>C</b>	9 (2.34%; of the problematic SNPs ; 0.0002% of the analysed nt)

2

3

# Figure 1

Experimental setup

Photo *Trichogramma brassicae* □ J.-Y. Rasplus

