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ORIGINAL ARTICLE

Direct tests of haplodiploid inheritance in *Thrips tabaci* (Thysanoptera: Thripidae) using parent-offspring SSR-GBS

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Abstract. Haplodiploidy is a sex determination system in which males are haploid and females are diploid. In the typical haplodiploid system, females inherit half of their genome from each parent, whereas males inherit a haploid genome exclusively from their mother. However, several exceptions to this pattern have recently been reported in some insects and spider mites, underscoring the need for rigorous genetic analyses of inheritance patterns in other haplodiploid species. Thrips (order Thysanoptera) are a major clade that exhibits haplodiploidy. Here, we investigated inheritance in the onion thrips, *Thrips tabaci*, by comparing parent-offspring genotypes using an amplicon-based microsatellite genotyping-by-sequencing method (Short Sequence Repeats-Genotyping by Sequencing, SSR-GBS). We successfully genotyped eight loci across eight families from the sexual lineage and additionally analyzed five families from the thelytokous lineage, including both diploid and triploid strains. In the sexual lineage, segregation conformed to arrhenotoky: F_1 females inherited one allele from each parent, and F_1 males carried a single maternal allele. In the thelytokous lineage, offspring inherited only alleles present in the mother. Compared with fragment-length genotyping, SSR-GBS increased the mean number of alleles detected per locus by 28% and reduced the rate of size homoplasy (alleles of the same size but different sequences), yielding clearer separation of the sexual and thelytokous lineages. These findings advance our understanding of the evolution of reproductive systems in this major haplodiploid clade and demonstrate the utility of SSR-GBS for high-resolution SSR genotyping.

INTRODUCTION

Haplodiploidy is a sex determination system in which males are haploid, carrying a single set of chromosomes, whereas females are diploid, carrying two sets of chromosomes (Normark, 2003). Within the Arthropoda, haplodiploidy has evolved independently at least 15 times (Normark, 2003). Under typical haplodiploidy, diploid females inherit half of their genome from each parent, whereas haploid males inherit their entire genome from their mother; this pattern is known as arrhenotoky. However, an increasing number of organisms with reproductive systems that deviate from this typical pattern have been reported. For example, in some mites and scale insects, paternal genome elimination (PGE) occurs, in which males inherit their genome from both parents but transmit only maternally derived genes to their offspring (Herbette & Ross, 2023). Haploid females have been reported in the false spider mite *Brevipalpus phoenicis* (Weeks et al., 2001). In several ant species, queens are produced by thelytokous parthenogenesis, males by androgenesis, and workers by sexual reproduction between the two clonal lineages (e.g. the little fire ant *Wasmannia auropunctata*; Fournier et al., 2005). In the yellow crazy ant *Anoplolepis gracilipes*, males are chimeras of paternal and maternal haploid cells (Darras et al., 2023). In the Varroa mite *Varroa destructor*, females have been reported to produce functionally diploid sons via parthenogenesis (Eliash et al., 2024). Thus, even in taxa where haplodiploidy is common, inheritance systems should be determined directly for each species using genetic markers (Normark, 2003; Ross et al., 2010; Rabeling & Kronauer, 2013; Vorburger, 2014; van der Kooi et al., 2017).

Within the Thysanoptera, haplodiploidy is the predominant system of sex determination and is thought to have evolved only once (Normark, 2003; De La Filia et al., 2015; Woldemelak, 2021). Sex allocation in some thysanopteran species can be influenced by female physiological condition and microbial symbionts (Katlav et al., 2020, 2021, 2022). However, in Thysanoptera, the mechanisms of sex determination and their molecular basis remain poorly characterized compared with those of Hymenoptera, scale insects, and mites. Cytogenetic data on chromosome numbers are available for fewer than two dozen thysanopteran species, and in all of them, males are haploid and females are diploid (Prussard-Radulesco, 1930; Bournier,

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1956; Risler & Kempter, 1961; Raizada, 1988; Brito et al., 2010; Jacobson et al., 2013b). Moreover, although several thelytokous thysanopteran species are polyploid, the inheritance patterns of genetic markers in these species remain unknown (Jacobson et al., 2013b; Nguyen et al., 2015).

The onion thrips, *Thrips tabaci*, is a globally distributed agricultural pest that damages a wide range of crops, including onions, leeks, cabbages, cotton, and tobacco (Gill et al., 2015; Varela & Fail, 2022). In addition to causing stippling and streaking through sap sucking, high densities can inhibit plant growth and cause plant death (Gill et al., 2015). *Thrips tabaci* is also an efficient vector of Tomato spotted wilt virus (TSWV, *Orthotospovirus tomatomaculae*) and Iris yellow spot virus (IYSV, *Orthotospovirus iridimaculaflavi*) (Gill et al., 2015). Resistance to neonicotinoids and pyrethroids has been reported in *T. tabaci* from multiple regions, complicating chemical control (Jouraku et al., 2019, 2024; Adesanya et al., 2020).

Based on mitochondrial *cytochrome c oxidase subunit I* (*COI*) sequences, *T. tabaci* can be broadly divided into the tobacco-associated T lineage and two leek-associated lineages (L1 and L2) (Brunner et al., 2004). In the T and L1 lineages, males emerge from unfertilized eggs, whereas in the L2 lineage, females emerge from unfertilized eggs (thelytoky) and exhibit ploidy variation ranging from diploid to tetraploid (Jacobson et al., 2013a, b; Varela & Fail, 2022). Deuterotoky (parthenogenetic reproduction of both sons and daughters) has been reported in the United States and Hungary (Nault et al., 2006; Woldemelak, 2020). Despite this diversity in reproductive modes, most previous work has focused on phylogenetics. Although males are known to be haploid and females are diploid, direct tests of inheritance using parent-offspring comparisons have not been conducted (Jacobson et al., 2013a, b). In this study, we investigated the inheritance patterns of *T. tabaci* by comparing parent-offspring genotypes.

Microsatellite markers (Short Sequence Repeats; SSRs) have been widely used to infer inheritance patterns (Selkoe & Toonen, 2006; Guichoux et al., 2011). However, conventional capillary-electrophoresis-based SSR genotyping is vulnerable to size homoplasy (alleles that share the same fragment length but differ in sequence), which can reduce the resolution of phylogenetic and population genetic analyses (Estoup et al., 2002; Šarhanová et al., 2018). To address this problem, a short sequence repeats genotyping-by-sequencing (SSR-GBS) method has been developed (Vartia et al., 2016). In SSR-GBS, SSR loci are amplified by PCR, and the amplicons are sequenced on a next-generation sequencing platform, allowing direct readout not only of fragment length but also of SNPs and indels in the flanking regions. This discriminates a subset of size-homoplastic alleles and enables high-throughput analysis of many individuals across multiple loci (Vartia et al., 2016; Neophytou et al., 2018; Šarhanová et al., 2018).

We developed SSR-GBS protocol for *T. tabaci* and compared parent-offspring genotypes to elucidate inheritance patterns in the sexual and the thelytokous (diploid or triploid) lineages. To evaluate the utility of SSR-GBS, we compared datasets generated by different genotyping methods to test whether sequence information improves resolution.

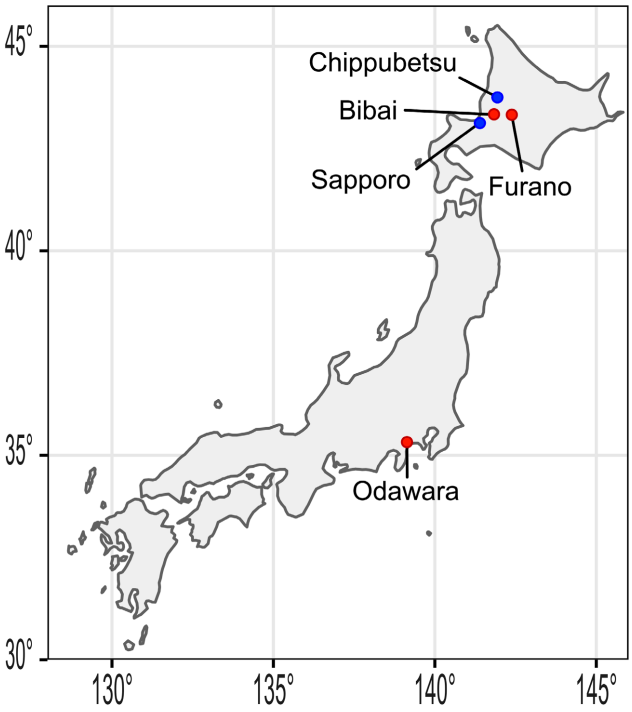

Fig. 1. Map of the sampling locations in Japan. Blue dots indicate sites where thelytokous lineages were collected (Sapporo and Chippubetsu) and red dots indicate sites where sexual lineages were collected (Bibai, Furano, and Odawara).

MATERIAL AND METHODS

Insects

The thrips used in this study were laboratory-reared individuals of *T. tabaci* derived from females collected in five onion fields in Sapporo (43°07′24″N, 141°23′44″E), Chippubetsu (43°45′05″N, 141°56′16″E), Bibai (43°20′12″N, 141°49′59″E), Furano (43°19′32″N, 142°23′01″E), and Odawara (35°19′20″N, 139°08′10″E) in Japan (Fig. 1). To determine the reproductive mode, we established multiple isofemale lines per field, each originating from a single female collected at that field. We verified the reproductive mode of each isofemale line by checking the sex of offspring produced by virgin females from unfertilized eggs: lines producing only males were classified as a sexual lineage, whereas those producing only females were classified as a thelytokous lineage. To avoid inbreeding depression, isofemale lines of the sexual lineage were pooled by field to form a single stock population. For the thelytokous lineages, a single isofemale line was used as the stock population for each region. All populations were maintained at 23°C. The ploidy of the isofemale lines of thelytokous lineages was determined by flow cytometry. We measured the genome size of single females using CyFlow Ploidy Analyzer and CyStain UV Ploidy kit (Sysmex, Japan). Adult males were used as a size standard because males of *T. tabaci* are haploid (Jacobson et al., 2013a).

Crossing experiments

Virgin females were obtained by haphazardly collecting larvae from the stock populations and rearing them individually to adulthood. A virgin female of the sexual lineage was paired with a male, whereas a virgin female of thelytokous lineage was kept alone. Each female was kept for one week at 23°C in a 1.5-mL

microtube (Watson, Japan) with a 5 × 5 mm piece of cabbage leaf that served as both food and an oviposition substrate. Crossing combinations are shown in Table 2. The leaf was replaced every 2 or 3 days. After oviposition, the leaf piece was transferred to a Petri dish containing germinated fava beans, and the offspring were reared to the adult (F_1) stage. Parents and F_1 adults were stored in 100% ethanol. Families in which either parent died before preservation or from which no F_1 adults emerged were excluded from the subsequent analysis. We analyzed eight sexual, two diploid thelytokous, and three triploid thelytokous families. In families with 12 or fewer adults, all individuals were analyzed; in those with more than 12 adults, only a subset was analyzed (Table 2).

Library preparation and Illumina paired-end sequencing

Genomic DNA was extracted from single adult thrips using the DNeasy Blood & Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA libraries were prepared with a two-step PCR protocol. From the SSR primer sets developed previously (Jacobson et al., 2013a; Kobayashi & Hasegawa, 2013), we selected 11 sets whose successful amplification from the extracted genomic DNA was confirmed by agarose-gel electrophoresis. In the first PCR, SSR-containing loci were simultaneously amplified from genomic DNA with the QIAGEN Multiplex PCR Master Mix. Forward and reverse primers were extended at their 5′ ends with the tag sequences CAATGCATCAATGCGTG and TGCTCTTCCGATCTGAC, respectively (Supplementary Table S1). The primer mix contained all 11 primer pairs at 10 µM each. PCR reactions were carried out in 10 µL total volume containing 5 µL Multiplex PCR Master Mix, 0.2 µL primer mix, 2 µL genomic DNA, and 2.8 µL nuclease-free water. The thermocycling conditions were 30 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 30 s, followed by a final extension at 72°C for 1 min. In the second PCR, individual-specific indices, the Illumina P5 and P7 adapter sequences, and sequencing-primer binding sites required for the NovaSeq 6000 platform were added to libraries (Table S1). Reactions were carried out in 10 µL total volume containing 6 µL PrimeSTAR GXL Premix (Takara Bio, Japan), 0.5 µL each of the forward and reverse indexing primers (10 µM), 3 µL of the first PCR product, and 2 µL water. Cycling parameters were 20 cycles of 98°C for 30 s, 54°C for 1 min, and 68°C for 30 s, with a final extension at 68°C for 1 min. Finally, the second PCR products were pooled, and 300 µL was purified with SPRIselect beads (Beckman Coulter, USA) following the manufacturer's protocol. The library was submitted for 2 × 250-bp paired-end sequencing on an Illumina NovaSeq 6000 SP flow cell at Pre-made Library Sequencing Service of Novogene, Japan.

Sequence analysis and SSR-GBS genotyping

Raw Illumina reads were demultiplexed to the individual level using "process_shortreads" in Stacks v. 2.64 (Rochette et al., 2019) with default settings; reads that could not be assigned to any barcode were discarded. SSR primers and poly-G tails were removed using Cutadapt v. 4.6 (Martin, 2011). Poly-G sequences at both ends of the reads were trimmed first, followed by SSR primer trimming with a maximum error rate of 0.05. Reads lacking the expected primer sequence were discarded. Paired-end reads were merged using PEAR v. 0.9.6 (Zhang et al., 2014) with default settings. Merged reads were imported into R v. 4.5.1 (R Core Team, 2025) using Biostrings for locus-wise allele calling, which was conducted under a semi-manual framework with automated scripts and manual inspection. For each locus, alleles with a read proportion below 1 in 50,000 of the total reads at that locus were excluded. For each individual at each locus, a table of unique sequences with their read counts was generated. Allele calling was initially automated by selecting up to three of the most abundant sequences: the top sequence was always assigned as the first allele, while the second and third most abundant sequences were called as alleles 2 and 3 if they accounted for at least 33% and 16.6% of the read count of the top sequence, respectively. Histograms of reads and sequence length were also generated for each individual × locus to help manual inspection. Using these histograms along with the sequence frequency tables, automatically called alleles were systematically evaluated and manually curated (see Fig. S1). Genotypes derived from sequence identity were referred to as the "sequence-identity dataset", while those based solely on fragment length were defined as the "fragment-

Table 1. SSR markers used in this study. The "Chr" and "Position" indicate the chromosome number and position in the reference genome (GCA_040581495.1) to which the marker's primer sequences were mapped, as identified by BLAST. The loci in which nonspecific PCR products were detected were excluded from the analysis.

Marker	Primer (5'–3')	Size range	Chr	Position	Remarks	Reference
TMS62	F: TCCCTCGCTGCTTTCTGTCT R: GAGAGAGACGCGGAGGTGTCC	126–188	Not found	Not found		Kobayashi & Hasegawa 2013
TMS63	F: ATCTTCGCAGAGAAAGGAC R: AGACTTCTGCGGAAAAGGC	127–153	3	15642412–15642569	non-specific PCR products detected	Kobayashi & Hasegawa 2013
Ttab6	F: CACGCAAAACACTCTCTCCA R: AGTGGCGTCTGTGTTGAGAA	127–183	11	8061122–8061581	non-specific PCR products detected	Jacobson et al., 2013a
Ttab20	F: ACCGGAAGCTTTCAAATCG R: AATAAACCGTCGCGGAGACT	54–82	18	146140–146705		Jacobson et al., 2013a
Ttab24	F: GTAGAGCAGCACCGATAGGG R: CAGCCAGGACAACAGAGTGA	244–256	5	6333402–6333917		Jacobson et al., 2013a
Ttab27	F: AAGGTCAGGCATTGCGTTAT R: TACAAAGCGAGGACTCAGCA	261–287	18	7663144–7663560		Jacobson et al., 2013a
Ttab33	F: TCGTGGCATGACTCAAACG R: CCTCGGAACAAGGAGCCAG	96–164	11	8687264–8687536		Jacobson et al., 2013a
Ttab34	F: TTTGCTGTCCCTCGAAGCG R: CGATTCCATGTTTGTCTAAGAGTCC	82–106	12	5009513–5009787	non-specific PCR products detected	Jacobson et al., 2013a
Ttab47	F: TTCCTCGCGTGCCCTATG R: GTCGTGTAGCTGGAAGTGC	165–205	3	13918174–13918515		Jacobson et al., 2013a
Ttab48	F: TCGAACGGCTGGTGTGAAG R: GCGACCATTCGCGGTTC	141–157	14	9109567–9109888		Jacobson et al., 2013a
Ttab49	F: CGGACATGCGACATTCACC R: CGGAATTCGGAGCGAGCC	230–252	16	2149981–2150336		Jacobson et al., 2013a

Table 2. Overview of the original populations of individuals used in crossing experiments. Ploidy level was estimated from the genome size of females from the stock population measured by flow cytometry. *N* is the number of F_1 adults which were genotyped.

Family	*N*	Type	Ploidy	Mother	Father
F1	13	sexual	Not measured	Odawara	Furano
F2	2	sexual	Not measured	Odawara	Furano
F3	18	sexual	Not measured	Odawara	Bibai
F4	20	sexual	Not measured	Furano	Odawara
F5	20	sexual	Not measured	Furano	Odawara
F6	1	sexual	Not measured	Bibai	Furano
F7	12	sexual	Not measured	Bibai	Furano
F8	24	sexual	Not measured	Bibai	Furano
F9	12	thelytoky	2	Sapporo	
F10	6	thelytoky	2	Sapporo	
F11	4	thelytoky	3	Sapporo	
F12	12	thelytoky	3	Sapporo	
F13	12	thelytoky	3	Chippubetsu	

length dataset", which corresponds to a conventional microsatellite-style dataset and was used for comparison.

For each SSR marker, its position on the reference genome (GCA_040581495.1; Gao et al., 2024) was obtained using BLASTn under the default settings. The primer sequences in GenBank (AB627091-AB627098, JX402997-JX403007) were used as queries. From the results, the hit with the lowest E-value is reported in Table 1.

Statistics

Data analyses were performed separately for the sequence-identity and fragment-length datasets. Genotyping outputs were analyzed in R v. 4.4.2 (R Core Team, 2025). For parents for which genotypes were obtained, pairwise genetic distances were computed as Lynch distances using the polysat package v. 1.7 (Clark & Jasieniuk, 2011). Based on allele sharing Lynch distance is suitable for polyploid genotype data (Clark & Jasieniuk, 2011). Finally, principal coordinates analysis (PCoA) was performed with the ape package v. 5.8 (Paradis & Schliep, 2019).

RESULTS

The Illumina NovaSeq run generated 10,023,042 raw reads, of which 4,050,146 passed quality control and were used for genotyping. On average, 2,012 reads were obtained per locus per individual. In total, we analyzed 13 families (sexual: 8; diploid thelytokous: 2; triploid thelytokous: 3) and genotyped 177 individuals (21 parents and 156 F_1 offspring). Three individuals were excluded from the analyses because they had fewer than 100 reads at all loci (Tables 4 and 5). In families 3 and 4 (both sexual), we observed many offspring carrying alleles at TMS63, Ttab6, and Ttab34 that were absent from their parents. Because these anomalies occurred only at specific loci and the number of alleles detected exceeded those expected given the ploidy level, they were attributed to PCR errors. These loci were therefore excluded from subsequent analyses. The proportions of individuals requiring manual curation of automatic genotypes were 0% (0 individuals) at TMS62, 2.3% (4 individuals) at Ttab20, 25.0% (44 individuals) at Ttab24, 5.6% (10 individuals) at Ttab27, 11.9% (21 individuals) at Ttab33, 16.4% (29 individuals) at Ttab47, 2.8% (5 individuals) at Ttab48, and 1.1% (2 individuals) at Ttab49.

Table 3. The total number of alleles (N_a) and observed heterozygosity of the sexual females (H_o) for two datasets: based on fragment length and sequence identity. The homoplasy rate is calculated as the number of fragment-length alleles containing hidden variation (SNPs or indels) divided by the total number of fragment-length alleles, as defined in Šarhanová et al. (2018). Total reads represent the number of reads used for genotyping, and mean reads are calculated by dividing total reads by the total number of individuals.

Locus	Ttotal reads	Mean reads	Fragment length		Sequence identity		Homoplasy rate
			N_a	H_o	N_a	H_o	
TMS62	969248	5296.437	6	0.776	11	0.789	0.500
Ttab20	290698	1588.514	5	0.539	6	0.539	0.200
Ttab24	332884	1829.033	4	0.592	9	0.592	1.00
Ttab27	651412	3559.601	10	0.776	12	0.776	0.100
Ttab33	193558	1051.929	11	0.921	15	0.921	0.364
Ttab47	302292	1651.869	10	0.833	13	0.833	0.300
Ttab48	244719	1329.995	8	0.800	10	0.813	0.250
Ttab49	648558	3544.033	9	0.816	9	0.816	0
Overall	454171	2481.426	7.875	0.757	10.625	0.760	0.339

In the parental genotype dataset, size homoplasy was detected at TMS62, Ttab24, Ttab27, Ttab33, Ttab47, Ttab48, and Ttab49, and in every case it was observed both between and within lineages. In the sequence-identity dataset, the total number of alleles increased by an average of 28% per locus relative to the fragment-length dataset (Table 3). Fig. 2 shows the clustering of parental samples based on principal coordinates analysis (PCoA). PCoA based on the fragment-length dataset did not separate triploid thelytok-

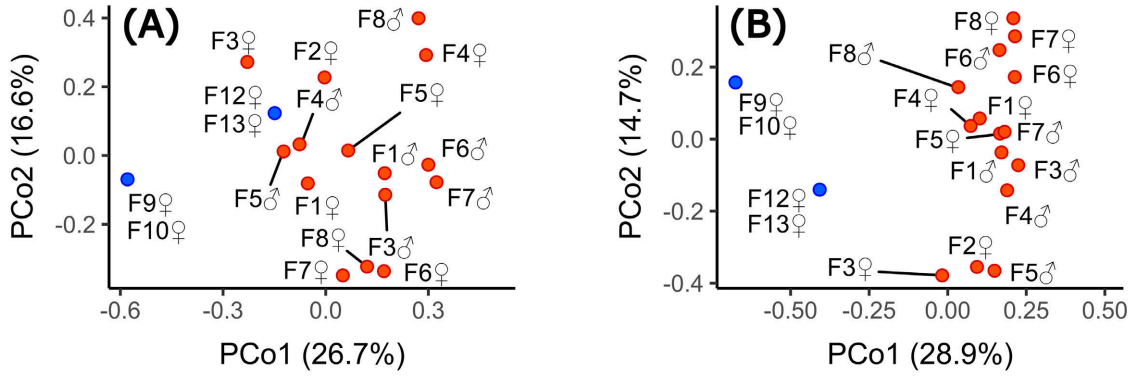

Fig. 2. Principal coordinate analysis (PCoA) using two datasets of 19 parents (♀: mother, ♂: father) based on Lynch distance (red: sexual lineage, blue: thelytokous lineage). (A) fragment-length dataset, (B) sequence-identity dataset. In the sequence-identity dataset, two lineages were clearly separated.

Table 4. Genotype of parents and F_1 individuals (sexual lineages). The numbers of each allele indicate the fragment length (bp) of the alleles excluding the primer regions, and the letters indicate sequence identity.

Family	Locus	Parental genotype		F_1 genotype	
		Male	Female	Male	Female
1	TMS62	134a	126a/130a		130a/134a×7, 126a/134a×6
1	Ttab20	66	66/74a		66/74a×8, 66/66×5
1	Ttab24	244a	247a/247a		244a/247a×13
1	Ttab27	275	261/275		261/275×7, 275/275×6
1	Ttab33	132	124b/132		124b/132×10, 132/132×3
1	Ttab47	194	170a/170a		170a/194×13
1	Ttab48	151	143/155		151/155×9, 151/143×4
1	Ttab49	230	233/236		230/236×7, 230/233×6
2	TMS62	Not determined	126a/126a		126a/134c×2
2	Ttab20	Not determined	66/66		66/66×2
2	Ttab24	Not determined	244a/253a		244a/244a×1, 244a/253a×1
2	Ttab27	Not determined	266/266		266/276×2
2	Ttab33	Not determined	120a/124a		120a/124a×2
2	Ttab47	Not determined	170a/192		170a/170a×1, 170a/192×1
2	Ttab48	Not determined	151/151		151/151×2
2	Ttab49	Not determined	224/236		224/236×1, 236/236×1
3	TMS62	130c	126a/126b	126a×2, 126b×1	126a/130c×8, 130c/126b×7
3	Ttab20	66	66/66	66×3	66/66×15
3	Ttab24	244a	247a/253b	247a×2, 253b×1	244a /253b×8, 244a/247a×7
3	Ttab27	263a	263a/266	266×2, 263a×1	263a/266×8, 263a/263a×7
3	Ttab33	116a	120a/120a	120a×3	116a/120a×15
3	Ttab47	192	183/183	183×3	192/192**×10, 183/192×5
3	Ttab48	151	153a/153b	153b×2, 153a×1	151/153a×8, 151/153b×7
3	Ttab49	230	224/236	236×3	230/236×12, 224/230×3
4	TMS62	134a	126a/126a	126a×9	126a/134a×11
4	Ttab20	74a	54/82	54×7, 82×2	54/74a×6, 74a/82×5
4	Ttab24	244a	244a/244a	244a×9	244a/244a×11
4	Ttab27	263a	287/287	287×9	263a/287×11
4	Ttab33	120a	115/116b	115×5, 116b×4	116b/120a×9, 115/120×2
4	Ttab47	170a	170a/196	170a×6, 196×3	170a/196×7, 170a/170a×4
4	Ttab48	151	143/151	143/151*×4, 143×3, 151×2	151/151×10, 143/151×1
4	Ttab49	230	230/236	230×6, 236×3	230/230×6, 230/236×5
5	TMS62	130c	130c/130c	130c×9	130c/130c×11
5	Ttab20	66	66/74a	66×5, 74a×4	66/66×8, 66/74a×3
5	Ttab24	253a	244a/244a	244a×9	244a/253a×11
5	Ttab27	263a	261/261	261×9	261/263a×11
5	Ttab33	120a	96/113a	113a×6, 96×3	113a/120a×7, 96/120a×4
5	Ttab47	170a	170a/192	192×5, 170a×4	170a/192×7, 170a/170a×4
5	Ttab48	151	147/149b	149b×7, 147×2	147/151×7, 149b/151×4
5	Ttab49	230	230/243	243×5, 230×4	230/243×8, 230/230×3
6	TMS62	130a	130c/130c		130a/130a**×1
6	Ttab20	66	66/66		66/66×1
6	Ttab24	244a	244a/253a		244a/253a×1
6	Ttab27	278	275/275		275/278×1
6	Ttab33	116a	116a/116a		116a/116a×1
6	Ttab47	196	165b/192		192/196×1
6	Ttab48	147	143/157		147/157×1
6	Ttab49	230	230/233		230/233×1
7	TMS62	184	130a/134a	134a×8, 130a×4	
7	Ttab20	66	66/74a	66×8, 74a×4	
7	Ttab24	244a	244a/253b	244a×7, 253b×5	
7	Ttab27	269	266/275	266×8, 275×4	
7	Ttab33	116a	116a/128	116a×9, 128×3	
7	Ttab47	192b	173/165b	173×9, 165b×3	
7	Ttab48	151	143/143	143×12	
7	Ttab49	230	230/233	233×7, 230×5	
8	TMS62	126a	130a/134a	130a×7, 134a×2	126a/130a×9, 126a/134a×6
8	Ttab20	54	66/74a	66×5, 74a×4	54/66×11, 54/74a×4
8	Ttab24	244a	244a/244a	244a×9	244a/244a×15
8	Ttab27	287	266/275	275×6, 266×3	275/287×8, 266/287×7
8	Ttab33	113a	116a/128	128×6, 116a×3	113a/116a×8, 113a128×7
8	Ttab47	196	165b/173	165b×5, 173×4	165b/196×10, 173/196×5
8	Ttab48	147	143/143	143×9	143/147×15
8	Ttab49	230	230/233	233×5, 230×4	230/233×11, 230/230×4

*Two alleles detected in a haploid male genotype. **Expected allele not detected.

Table 5. Genotype of parents and F_1 individuals (thelytokous lineages). The numbers of each allele indicate the fragment length (bp) of the alleles excluding the primer regions, and the letters indicate sequence identity.

Family	Locus	Parental genotype	F_1 genotype
9	TMS62	130b/134b	130b/134b×11
9	Ttab20	70/74b	70/74b×11
9	Ttab24	253c/256a	253c/256a×11
9	Ttab27	263b/282	263b/282×11
9	Ttab33	120b/164	120b/164×11
9	Ttab47	203/205	203/205×11
9	Ttab48	149a/149a	149a/149a×11
9	Ttab49	229/235	229/235×6
10	TMS62	130b/134b	130b/134b×6
10	Ttab20	70/74b	70/74b×6
10	Ttab24	253c/256a	253c/256a×6
10	Ttab27	263b/282	263b/282×6
10	Ttab33	120b/164	120b/164×6
10	Ttab47	203/205	203/205×6
10	Ttab48	149a/149a	149a/149a×6
10	Ttab49	229/235	229/235×6
11	TMS62	Not determined	130b/156/188×4
11	Ttab20	Not determined	66/70/74b×4
11	Ttab24	Not determined	244b/247b/256b×4
11	Ttab27	Not determined	263b/263c/279×4
11	Ttab33	Not determined	113b/118/122×4
11	Ttab47	Not determined	165a/170b/179×4
11	Ttab48	Not determined	141/151/157×4
11	Ttab49	Not determined	224/230/252×4
12	TMS62	130b/156/188	130b/156/188×12
12	Ttab20	66/70/74b	66/70/74b×12
12	Ttab24	244b/247b/256b	244b/247b/256b×12
12	Ttab27	263b/263c/279	263b/263c/279×12
12	Ttab33	113b/118/122	113b/118/122×12
12	Ttab47	165a/170b/179	165a/170b/179×12
12	Ttab48	141/151/157	141/151/157×11, 141/157*×1
12	Ttab49	224/230/252	224/230/252×12
13	TMS62	130b/156/188	130b/156/188×12
13	Ttab20	66/70/74b	66/70/74b×12
13	Ttab24	244b/247b/256b	244b/247b/256b×12
13	Ttab27	263b/263c/279	263b/263c/279×12
13	Ttab33	113b/118/122	113b/118/122×12
13	Ttab47	165a/170b/179	165a/170b/179×12
13	Ttab48	141/151/157	141/151/157×12
13	Ttab49	224/230/252	224/230/252×12

*Fully heterozygous to partially homozygous.

ous individuals from sexual individuals, whereas PCoA based on the sequence-identity dataset divided the samples into two clusters corresponding to the sexual (L1) and thelytokous (L2) lineages.

Tables 4 and 5 summarize the F_1 allele combinations and their frequencies for all families and across all loci analyzed. In the sexual lineage, males carried a single maternal allele, whereas females carried one maternal and one paternal allele. However, in families 3, 4, and 6 we detected 4 males with two alleles or 11 females lacking paternal alleles, yielding genotypes that deviated from expectations of haplodiploidy (see Discussion). With one exception, parents and offspring in the thelytokous lineage shared identical multilocus genotypes (fully heterozygous to partially homozygous; Table S13).

BLAST search showed that most SSR loci were located on different chromosomes. Only two loci, Ttab20 and Ttab27, were located on the same chromosome (Chr18; Table 1). Recombination can influence the inheritance pattern observed between these two loci. In Family 8, the recombination fraction between Ttab20 and Ttab27 was 12/24 = 0.5, which did not deviate from the expected independence (binomial test, $p = 1$).

DISCUSSION

We characterized inheritance at 8 SSR loci in the sexual and thelytokous lineages of *T. tabaci* and demonstrated the utility of a sequence informed SSR-GBS approach. The sexual lineage conformed to expectations under arrhenotokous haplodiploidy, whereas the thelytokous lineage was most consistent with thelytoky. Together, the number of detected alleles and the results of PCoA showed that SSR-GBS mitigated size homoplasy and increased discriminatory power, delivering higher resolution at lower operational costs than conventional genotyping based on fragment length. The two loci, Ttab20 and Ttab27, are located on the same chromosome, but no significant linkage disequilibrium between these loci was detected in our samples. This is consistent with the fact that they are separated by a relatively large physical distance (approximately 7 Mb; Table 1) and suggests that the marker set used in this study is minimally affected by recombination.

In the sexual lineage, we observed the inheritance pattern expected under arrhenotokous haplodiploidy. In some individuals, genotypes inconsistent with haplodiploidy were observed; however, because these anomalies were confined to specific loci and can be explained by allelic dropout or PCR-induced false alleles, they most likely reflect technical artifacts rather than an unusual mode of inheritance (see Tables S2–S14). Based on these results alone, we cannot fully exclude PGE, in which paternally derived chromosomes are eliminated during early development, but our data do not provide positive evidence for such mechanisms.

In the thelytokous lineage, except for a single case, offspring shared the same genotype as their mothers (Table 5). Mechanisms of diploid thelytoky in insects are commonly classified into four models: apomixis (no meiosis; the maternal genotype is transmitted unchanged), central-fusion automixis (heterozygosity is relatively preserved), terminal-fusion automixis (most heterozygosity is lost), and gamete-duplication automixis (complete loss of heterozygosity) (Sperling & Glover, 2023). Our observations are consistent with central-fusion automixis or apomixis and are difficult to reconcile with terminal-fusion or gamete-duplication models. For triploid thelytoky, meiosis is generally impeded by an odd number of chromosome sets; accordingly, apomixis is often invoked, and our results likewise favor apomixis. Nevertheless, genotype data alone cannot strictly distinguish among the mechanisms of thelytoky (e.g. apomixis, central-fusion or terminal-fusion automixis). Cytological studies of the presence or absence of meiosis and the mode of ploidy restoration during early embryogenesis, together with whole-genome analyses, will be required to clarify how thelytoky is achieved in this species (Tsutsui et al., 2014; Lacy et al., 2024).

Although rare, hybridization between sexual and thelytokous lineages can occur in *T. tabaci* (Li et al., 2015). Hybridization between sexual and thelytokous lineages may facilitate the establishment of polyploid thelytokous lineages and further influence gene flow between them (Schneider et al., 2003; Crespo-López et al., 2007; Mishina et al., 2021). From an applied perspective, introgression of insecticide resistance alleles via such hybridization could pose challenges for pest management (Jouraku et al., 2024). Therefore, identifying the mechanisms of gamete formation under arrhenotoky and thelytoky in *T. tabaci* will provide insights not only into evolutionary questions but also into risk assessment of the evolution of insecticide resistance in this species.

In Thysanoptera, thelytoky has been reported in 20 species and deuterotoky in 2 species (Woldemelak, 2021), suggesting that diverse reproductive modes can evolve from arrhenotoky (van der Kooi & Schwander, 2014). Because unusual reproductive mechanisms have been reported in other arrhenotokous taxa, comparable diversity is likely to occur within Thysanoptera as well. Indeed, terminal-fusion automixis has been reported in *Heliothrips haemorrhoidalis*, strongly suggesting that multiple ploidy-restoration mechanisms exist in Thysanoptera (Pomeyrol, 1929; Prussard-Radulesco, 1930; Bournier, 1956; Nguyen et al., 2015). Future research that comprehensively elucidates inheritance patterns across Thysanoptera will provide insights into their evolution and contribute to broader questions about parthenogenesis, haplodiploidy, and sociality.

Given the diversity of reproductive modes in *T. tabaci*, this species can be a promising model for studying the evolution of reproductive strategies (Woldemelak, 2021). The inheritance patterns revealed here provide a foundation for investigating that diversity. We also show that SSR-GBS offers superior allele detection compared with conventional fragment length scoring. Because anthropogenic introductions are recurrent in pest thrips, population genetic analyses are essential for inferring invasion dynamics and tracking populations. For analyzing large numbers of individuals at high resolution and low cost, SSR-GBS is therefore a highly useful approach (Cao et al., 2019; Dedukh et al., 2022).

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Fig. S1. Histograms of read counts and lengths illustrating manual curation.

Table S1. Primer list used in the 1st and 2nd PCR.
Table S2. Genotypes of all individuals of family 1 (sexual).
Table S3. Genotypes of all individuals of family 2 (sexual).
Table S4. Genotypes of all individuals of family 3 (sexual).
Table S5. Genotypes of all individuals of family 4 (sexual).
Table S6. Genotypes of all individuals of family 5 (sexual).
Table S7. Genotypes of all individuals of family 6 (sexual).
Table S8. Genotypes of all individuals of family 7 (sexual).
Table S9. Genotypes of all individuals of family 8 (sexual).
Table S10. Genotypes of all individuals of family 9 (thelytokous).
Table S11. Genotypes of all individuals of family 10 (thelytokous).
Table S12. Genotypes of all individuals of family 11 (thelytokous).
Table S13. Genotypes of all individuals of family 12 (thelytokous).
Table S14. Genotypes of all individuals of family 13 (thelytokous).