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Apomictic parthenogenesis in a parasitoid wasp *Meteorus pulchricornis*, uncommon in the haplodiploid order Hymenoptera

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Abstract

Although apomixis is the most common form of parthenogenesis in diplodiploid arthropods, it is uncommon in the haplodiploid insect order Hymenoptera. We found a new type of spontaneous apomixis in the Hymenoptera, completely lacking meiosis and the expulsion of polar bodies in egg maturation division, on the thelytokous strain of a parasitoid wasp *Meteorus pulchricornis* (Wesmael) (Braconidae, Euphorinae) on pest lepidopteran larvae *Spodoptera litura* (Fabricius) (Noctuidae). The absence of the meiotic process was consistent with a non-segregation pattern in the offspring of

heterozygous females, and no positive evidence was obtained for the induction of thelytoky by any bacterial symbionts. We discuss the conditions that enable the occurrence of such rare cases of apomictic thelytoky in the Hymenoptera, suggesting the significance of fixed heterosis caused by hybridization or polyploidization, symbiosis with bacterial agents, and occasional sex. Our finding will encourage further genetic studies on parasitoid wasps to use asexual lines more wisely for biological control.

Keywords: Apomixis, arrhenotoky, automixis, clone, occasional sex, thelytoky

Introduction

The Hymenoptera is the third largest insect order, comprising more than 115,000 described species of sawflies, predatory, parasitoid or gall-making wasps, bees and ants (Sharkey, 2007). Arrhenotokous haplodiploidy (arrhenotoky), in which haploid males arise from unfertilized eggs through parthenogenesis whereas diploid females arise from fertilized eggs, is inherent and common, but thelytokous reproduction (thelytoky), where diploid females arise from unfertilized eggs through parthenogenesis, is also widespread in the order (Quicke, 1997).

The cytogenetic mechanism of thelytokous parthenogenesis involves automixis and apomixis. The former (automixis) fulfills the normal chiasmatic meiosis followed by central fusion, terminal fusion or gamete duplication to restore diploidy, but the latter (apomixis) lacks such a process of chiasmatic meiosis (White, 1984; Suomalainen *et al.*, 1987; Rabeling & Kronauer, 2013). Under automixis, diploidy is restored by fusion or duplication of nuclei after meiosis, thus, the heterozygosity of females decreases more or less at each generation by chromosome recombination (Pearcy *et al.*, 2006). By contrast, under apomixis, asexual diploid daughters are true clones of the mother due to a lack of genetic recombination. Therefore, genetic effects and evolutionary consequences will differ among the modes of thelytoky.

Behavioural and physiological characteristics ought to be compared between conspecific thelytokous and arrhenotokous populations of parasitoids for biological control (e. g., Ardeh *et al.*, 2005; Ramirez-Romero *et al.*, 2012). In thelytoky, moreover, to

what extent genetic variation can be maintained is different between automixis and apomixis (Beukeboom & Zwaan 2007), and thus it is important to understand the modes of thelytoky for management of biocontrol agents.

According to Haccou & Schneider (2004), mutational load and, thus, expected viability of females depend on the reproductive mode. Assuming that the effects of deleterious mutations are multiplicative with a selection coefficient that depends on the ploidy of the individual, they showed that the expected viability of diploid females is much less in apomixis (without chromosome combination) than in automixis (with chromosome combination that makes recessive deleterious mutations homozygous), while it is the highest in arrhenotokous haplodiploidy having haploid males, in which recessive deleterious mutations will be constantly removed. Moreover, they predicted that in Hymenoptera, the evolutionary transition from haplodiploidy to apomixis would be less likely than to automixis.

In fact, automixis is common throughout the four main groups of Hymenoptera, i.e., Tenthredinoidea (sawflies), Aculeata (bees, ants), Ichneumonoidea (parasitoids), and Proctotrupomorpha (parasitoids, gall-makers) (e.g., Suomalainen *et al.*, 1987; Mateo Leach *et al.*, 2009; Tagami & Miura, 2007; Rabeling & Kronauer, 2013; see Sharkey et al. (2012) for the phylogeny of these taxa); especially in the Proctotrupomorpha, automictic gamete duplication induced by bacterial endosymbionts is prevalent (Stouthamer & Kazmer, 1994; Pannebakker *et al.* 2004). On the contrary, although apomixis is the most common form of parthenogenesis in diplodiploid arthropods (Suomalainen *et al.*, 1987), it is uncommon in the Hymenoptera (Van Wilgenburg *et al.*, 2006; Rabeling & Kronauer, 2013); a few cases are known only from Tenthredinoidea (sawflies) and Proctotrupomorpha (parasitoids, gall-makers) (Suomalainen *et al.*, 1987; Vavre at al., 2004; Naito & Inomata, 2006; Adachi-Hagimori at al., 2008). Such rarity of apomixis in the haplodiploid order Hymenoptera supports the above theoretical prediction by Haccou & Schneider (2004). Nevertheless, we found a case of spontaneous apomixis in a parasitoid wasp of Ichneumonoidea, seemingly contradicting their prediction.

The ichneumonoid wasp *Meteorus pulchricornis* (Wesmael) (Hymenoptera, Braconidae, Euphorinae) is a koinobiont parasitoid of lepidopteran larvae (Huddleston,

1980; Maeto, 1989; Berry & Walker, 2004; Stigenberg & Ronquist, 2011), having both arrhenotokous (biparental) and thelytokous (uniparental) strains (Fuester *et al.,* 1993), which are occasionally sympatric in East Asia (our unpublished data). The thelytokous strains show a marked degree of genetic variations among them (Abe *et al.*, 2013) and are promising biocontrol agents against noctuid and lymantrid pests (e.g., Marsh, 1979; Takashino et al., 1998; Liu & Li, 2006), but little is known of their genetic system. Similar intraspecific variation of reproductive modes, arrhenotoky and thelytoky, is also known in another congeneric species of potential use for biological control of tortricid pests (Bürgi & Mills, 2013), and thus it is imperative to understand the mechanism of thelytoky in this group of wasps.

Here, we describe the cytological mechanism of thelytoky, its genetic consequences, and involvement with bacterial symbionts in the thelytokous strains of *M. pulchricornis*, with discussion on the conditions that enable rare cases of apomictic thelytoky in the Hymenoptera.

Materials and methods

Insect strains and rearing

We used four thelytokous strains (KAGAWA_01_U, collected at Zentsuji, Kagawa Prefecture, Shikoku in 2001; KAGAWA_11_U, at Takamatsu, Kagawa Prefecture in 2011; and OSAKA_11#1_U and OSAKA_11#2_U, at Takatsuki, Osaka Prefecture, Honshu in 2011) and one arrhenotokous strain (KAGAWA_09_B, at Takamatsu in 2009) of *M. pulchricornis* from Japan. Each thelytokous strain was originated from a single female; and the arrhenotokous strain was originated from a single female, though its offspring were mated with males of the same population. The thelytokous strain KAGAWA_01_U has been maintained for more than 10 years (50 generations or more) in the laboratory without emergence of any adult male. All strains were cultured on larvae of *Spodoptera litura* (Fabricius) (Lepidoptera, Noctuidae) as host insects, and adult wasps were fed using honey solution. Wasps and host larvae were reared at 20–25 ^{o}C under a 16/8-h light-dark cycle.

Cytological technique

To clarify the chromosome complement of this species, ovaries were extracted from the pupae (on the 7th day after cocoon spinning) of the thelytokous strain KAGAWA_01_U in 1% sodium citrate solution and transferred onto a pre-cleaned slide on which the fixation and stain of chromosomes were made following the air-drying technique of Imai *et al.* (1977). Chromosomes of oogonia at the mitotic metaphase were examined under a microscope (Microphot FXA, Nikon) and photographed using a CCD colour camera (DS-5M-L1, Nikon).

To observe meiotic and mitotic stages in deposited eggs of the arrhenotokous (KAGAWA_09_B) and thelytokous (KAGAWA_01_U) strains, host larvae were dissected approximately every 5–10 min to extract the eggs until 3 h after oviposition, by which mitotic cell division started. Eggs were fixed on a microscopic slide by using the following fixatives in order: (1) distilled water:absolute ethanol:glacial acetic acid at 4:3:3, (2) absolute ethanol:glacial acetic acid at 1:1; and (3) glacial acetic acid. After drying, fixed eggs were stained using Vectashield Hard Set Mounting Medium with 4',6-diamidino-2-phenylindole (DAPI; Vector Laboratories) and covered using a cover glass. The following day, the slides were examined under a fluorescence microscope (BX50, Olympus), and the selected images were photographed using a CCD camera (DP70, Olympus).

Microsatellite markers and genotyping

For the development of microsatellite markers, genomic DNA was extracted from adult wasps of the thelytokous strain KAGAWA_01_U using the Qiaquick DNeasy tissue kit (Qiagen). Microsatellite loci were enriched and isolated by the combined procedures of Fischer & Bachmann (1998) and Hale *et al.* (2001), as precisely reported by Hamaguchi *et al.* (2007, 2011). Three sets of microsatellite primers (table 1) were established to study allele segregation.

DNA was extracted from each initial female and her progeny (in two or three generations) of the four thelytokous strains, and all individuals were genotyped using these three microsatellite markers. For DNA extraction, a middle leg removed from an

adult wasp or half a mature larva was incubated using 20 µl of 50 mM NaOH in a 0.2-ml tube at 95 ºC for 15 min. The solution, mixed using 20 µl of 0.2 mM Tris HCl (pH 8.0), was used as a template. PCR amplifications and fluorescent labeling of the amplified products were according to the method of Schuelke (2000) by using AmpliTaq Gold 360 Master Mix (Applied Biosystems) in 10 µl volumes. The PCR products were electrophoresed on an automated sequencer (ABI PRISM 310 Genetic Analyser) and analysed using GeneScan software (Applied Biosystems).

The rate of transition to homozygosity of a heterozygous locus during parthenogenesis is expected to be 0 in apomixis and automixis after premeiotic doubling, 0–1/3 in automixis with central fusion, 1/3–1 with terminal fusion, and 1 with gamete duplication (Pearcy, et al., 2006, 2011). A 95% confidence interval (CI) of the observed rate of transition for each microsatellite locus was estimated by the adjusted Wald method (Agresti, & Coull, 1998).

Antibiotic treatment

The effects of antibiotic treatments on the production of male offspring were determined using five female wasps of the thelytokous strain KAGAWA_01_U. The wasps and their female offspring were provided a cotton ball containing honey solution with 5% tetracycline hydrochloride for two days before oviposition. The number of wasps that emerged and their gender were recorded for five generations. The mortality of wasps increased markedly when they were fed a honey solution with over 5% tetracycline hydrochloride in a preliminary experiment, thus, we fixed the concentration of tetracycline hydrochloride for this experiment.

Diagnostic PCR for checking bacterial infection

To check bacterial infection in the thelytokous strain KAGAWA_01_U, e.g. *Wolbachia*, *Spiroplasma*, *Richettsia*, and *Cardinium*, PCR amplifications were conducted using the bacterial general primers for the 16S rRNA gene, which amplify this gene across all known bacteria (Weisburg *et al.,* 1991).

DNA was extracted from individual specimens (n = 12) by crushing them by using a

clean plastic rod in 30 µl of 5% Chelex, and incubating with 2 µl of 0.5 mg/ ml proteinase K at 56 ºC for 12 h. Homogenates were boiled at 99.9 ºC for 3 min to inactivate the proteinase K, and then, they were used as templates for PCR. PCR amplifications were conducted under the following conditions: 16.5 µl of AmpliTaq Gold PCR Master Mix (PE Applied Biosystems, Tokyo, Japan), 1.3 µl forward and reverse primers (10 pmol/µl) and 13.9 µl of sterile water, to obtain a total PCR reaction volume of 33 µl. All reactions were performed using an ABI thermal cycler (PE Applied Biosystems PCR System 9700) with the following program: initial denaturing at 96 ºC for 10 min; 40 cycles at 95 ºC for 1 min; annealing at 55 ºC for 1 min and 72 ºC for 1 min 30 s; and final extension of 72 ºC for 1 min 30 s. Primers 27f (5'-GAG AGT TTG ATC CTG GCT CAG-3') and 1495r (5'-CTACGGCTA CCT TGT TAC GA-3') were used (Weisburg et al., 1991). The PCR included a negative control (sterile water instead of DNA) and a positive control (*Wolbachia* of *Ephestia kueniella*) to detect contamination or a mistake in the PCR method. The PCR products were resolved on a 1.5% agarose gel, stained using ethidium bromide and visualised under a UV transilluminator. To confirm that DNA was properly extracted, the mitochondrial cytochrome oxidase-I (COI) gene primers, COI 1 (5'-CTT TAT CAA CAT TTA TTT TGA TTT TTT-3') and COI 2 (5'-TAC TCC AAT AAA TAT TAT AAT AAA TTG-3') (an annealing step at 53 ºC), were used to amplify mitochondrial DNA as a positive control to check the template DNA quality (Hoshizaki & Shimada, 1995).

Results

Cytological observations of arrhenotokous and thelytokous strains

We counted 20 chromosomes at the mitotic metaphase in the pupal oogonia of the thelytokous strain (fig. 1), composed of the two longest (indicated using black arrows), two second longest (with white arrows) and 16 other chromosomes in eight cases. This showed that the thelytokous strain is diploid with a haploid set of 10 chromosomes.

In the arrhenotokous strain, 10 bivalent chromosomes were observed in the first metaphase of meiosis just after oviposition (fig. 2a). Within 1 h of oviposition, a reduction division of chromosomes (fig. 2b) and subsequent second phase of meiosis proceeded resulting in four sets of chromosomes (fig. 2c), presumably pronucleus and

polar bodies. Mitotic cell division was observed to start about 2 h after oviposition.

In the thelytokous strain, we counted 20 univalent chromosomes in deposited eggs just after oviposition (fig. 3a). This complement of 20 chromosomes remained unchanged for at least 1 h (fig. 3b, c). We examined more than 100 cases but never observed nuclear division and chromosomal complements in the haploid number (n = 10), nor polar bodies, until mitotic cell division started approximately 1.5 h after oviposition (fig. 3d, e). These observations did not show meiosis in the egg maturation stages after oviposition in the thelytokous strain.

Allele segregation in thelytokous strains

All 3 microsatellite loci examined were heterozygous in the four thelytokous strains examined, except for MP5230 in the strain OSAKA_11#2_U, and no male or homozygous female progeny were produced from heterozygous females (table 2). The upper 95% CI of the rate of transition to homozygosity of every heterozygous locus was lower than 0.05 on pooled data of all thelytokous strains (table 3), indicating that the cytogenetic mechanism of this thelytokous diploidisation is apomictic or less probably automixis with central fusion.

Effect of antibiotic treatment on a thelytokous strain

We found no males in the 99 wasps produced by females treated using tetracycline hydrochloride for up to five generations (see the electronic supplementary material, fig. S1), indicating that the thelytoky of this species is not caused by the infection of any bacterial endosymbionts.

Bacterial infection in a thelytokous strain

Diagnostic PCR using the bacterial general primers showed no bacterial infection in the thelytokous wasps. This is consistent with the results of the antibiotic treatment described above and supports its indication that no bacterial endosymbionts cause thelytoky in this species.

Discussion

Our results reveal that the thelytoky of *M. pulchricornis* is apomixis without meiosis, thus, making no genetic recombination as a result to maintain heterozygosity in subsequent generations. While no observation of the transition to homozygosity of heterozygous loci in the thelytokous strains cannot exclude automixis with central fusion (Pearcy *et al.,* 2006; Rey *et al.,* 2011), the diploid number of univalent chromosomes in the metaphase just after oviposition refutes it. Although automixis after premeiotic doubling may show the same number of bivalent chromosomes in the metaphase, the fact that no nuclear division and polar bodies were observed within about 1 h after oviposition, in which meiosis occurred in the arrhenotokous strain, indicate the absence of meiosis in the thelytokous strain.

In the Hymenoptera (Proctotrupomorpha), a case of apomictic thelytoky is known to be induced by endosymbiotic bacteria *Rickettsia* (Hagimori *et al.,* 2006; Adachi-Hagimori *et al.*, 2008); this is in addition to the many cases of automictic thelytoky (gamete duplication) induced by *Wolbachia* (Stouthamer & Kazmer, 1994; Tagami & Miura, 2007). Recently, *Wolbachia*–induced thelytoky was observed in a braconid parasitoid as its first case in Ichneumonoidea (Kremer *et al.*, 2009), although the cytogenetic mechanism is unknown. No positive evidence, however, was given for the induction of thelytoky by any bacterial symbionts in *M. pulchricornis*, indicating that the apomictic thelytoky of this species is spontaneous, i.e., genetically determined by the wasp itself. Nevertheless, the possible cause of thelytokous reproduction by horizontal gene transfer from a bacterial symbiont once existed to the nucleus of wasp cannot be excluded (Dunning Hotopp, 2011).

Although automictic thelytoky is widely distributed in the Hymenoptera, apomictic thelytoky is known for only two species of Tenthredinoidea and three species of Proctotrupomorpha. *Pachyprotasis youngiae* (Tenthredinidae) is the only known triploid species in the Hymenoptera (Naito & Inomata, 2006), and *Strongylogaster* (= *Thrinax*) *macula* (Tenthredinidae) shows achiasmatic meiosis with the expulsion of a single polar body, though with the occasional emergence of haploid males (Peacock & Sanderson, 1939). Recently, a similar apomictic cloning mechanism of achiasmatic

meiosis with the expulsion of a single polar body has been found in two parasitoid wasps of Proctotrupomorpha, which likely originated from interspecific hybridization in *Trichogramma cacoeciae* (Trichogrammatidae) (Vavre at al., 2004) or was obviously induced by the endosymbiotic bacteria *Rickettsia* in *Neochrysocharis formosa* (Eulophidae) (Adachi-Hagimori at al., 2008). The female-producing eggs of the unisexual spring generation in a gall-maker *Neuroterus quercusbaccarum* (= *N. baccarum*) (Cynipidae) of Proctotrupomorpha, which undergoes seasonal cyclic parthenogenesis, are also regarded to be produced through apomixis (Doncaster, 1916; Dodds, 1939; Suomalainen *et al.,* 1987). Nevertheless, the spontaneous and noncyclic apomixis of diploid thelytoky, without expulsion of polar bodies, in the present species *M. pulchricornis* is new to the Hymenoptera.

Apomixis definitely has the advantage of maintaining favorable gene combinations in a fixed manner, with no recombination in meiosis. Although apomictic thelytoky is the most common type of parthenogenesis found in arthropods (Suomalainen *et al.,* 1987), it is rare within the haplodiploid order Hymenoptera, while automictic thelytoky is rather frequent, as already pointed out by Mateo Leach *et al.* (2009) and by Rabeling & Kronauer (2013). In haplodiploidy, deleterious mutations can be effectively eliminated from haploid males, and thus, the estimated expected viability is high for diploid females. The expected viability of females is the highest in haplodiploidy (arrhenotoky), low in automictic thelytoky, and definitely the least in apomictic thelytoky as well as in diplodiploidy (amphimixis) (Haccou & Schneider, 2004). Therefore, the transition from haplodiploidy to apomictic thelytoky will be more difficult than that to automictic thelytoky. Then, what are the conditions that cause rare cases of apomictic thelytoky in the Hymenoptera?

It is most likely that fixed heterosis, caused by triploidization in *P. youngiae* (Naito & Inomata, 2006) or by hybridization in *T. cacoeciae* (Valve *et al.*, 2004), has overwhelmed the fast accumulation of deleterious mutations in diploidy. On the other hand, apomictic thelytoky of *N. formosa* is caused and maintained by the infection of bacterial symbionts (Adachi-Hagimori, *et al.*, 2008), where horizontal transmission is involved. In those cases, males are seldom or never known, or if present, they are

infertile (Adachi-Hagimori, *et al.*, 2008).

The cyclic parthenogenesis of *N. quercusbaccarum* involves a generation of haploid males (Doncaster, 1916; Dodds, 1939), thus, deleterious mutations can be eliminated from the males. In addition, in the case of probably spontaneous apomixis of *S. macula*, occasionally appearing haploid males indicate facultative thelytoky or deuterotoky (Peacock & Sanderson, 1939), in which unmated females produce both diploid females and haploid males. Such occasional sexual reproduction (D'Souza & Michiels 2010) may cover the cost of apomictic parthenogenesis.

In the present species *M. pulchricornis*, apomixis is unlikely to be caused by polyploidization, specific hybridization, or bacterial symbiosis. Thelytokous strains usually do not produce males for many generations in the laboratory, but both arrhenotokous and thelytokous strains are sometimes sympatric in the fields (our unpublished data). Thus, it could be suggested that apomictic thelytoky of this species is primarily facultative in combination with occasional arrhenotoky, that is nothing but deuterotoky in nature, and deleterious mutations would be regularly eliminated from occasional haploid males. The examined strains of obligate thelytoky may be dead ends recently derived from a spectrum of deuterotokous strains. Further investigation of nuclear gene flow among sympatric strains of so-called arrhenotoky and thelytoky will test this suggestion.

Our finding suggests the possibility of operational shifting between sexual and asexual reproduction in parasitoid wasps, and thus, it will encourage further genetic studies to use asexual lines of wasps more wisely for biological control.

Supplementary Material

The supplementary material for this article can be found at http://www.journals.cambridge.org/ber

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Table 1. Primer sequences used for genotyping three microsatellite loci.

Locus	MP5185	MP5230	MP5291
Forward primer	5'-CGCAATAACA CAAACGCACG-3'	5'-CCTAATGTTCC ATCGTTGATG-3'	5'-GAAGCTTGGA GATATCGCAC-3'
Reverse primer	5'-GATGGAAGGA CGCTCGAGG-3'	5'-GTGGTAACCGT GCAAGTGG-3'	5'-GAATTACCCA TGGCAACGTC-3'
Annealing temp. (℃)	55	55	55
Repeat motif	(GT)10	(GT)10	(GT)10
Size (bp)	183	173	225
Accession no.	AB808651	AB808652	AB808653

Table 2. Genotype of the initial females and their parthenogenetically produced progeny in the four thelytokous strains for three microsatellite loci. Numbers in parenthesis indicate the number of progeny with that genotype.

Locus	Strain	Genotype of initial female (bp)	Genotype of progeny (bp)		
			Generation 1	Generation 2	Generation 3
MP5185	KAGAWA_01_U	188/192	188/192 (9)	188/192 (27)	-
	KAGAWA_11_U	188/192	188/192 (2)	188/192 (47)	-
	OSAKA_11#1_U	188/190	188/190 (2)	188/190 (2)	188/190 (18)
	OSAKA_11#2_U	190/192	190/192 (4)	190/192 (46)	-
MP5230	KAGAWA_01_U	189/191	189/191 (9)	189/191 (27)	-
	KAGAWA_11_U	189/191	189/191 (2)	189/191 (47)	-
	OSAKA_11#1_U	187/193	187/193 (2)	187/193 (2)	187/193 (18)
	OSAKA_11#2_U	191/191	191/191 (4)	191/191 (46)	-
MP5291	KAGAWA_01_U	244/250	244/250 (9)	244/250 (27)	-
	KAGAWA_11_U	248/250	248/250 (2)	248/250 (47)	-
	OSAKA_11#1_U	242/244	242/244 (2)	242/244 (2)	242/244 (18)
	OSAKA_11#2_U	246/250	246/250 (4)	246/250 (46)	-

Table 3. Observed rates of transition to homozygosity of heterozygous loci during parthenogenesis. No, number of progeny; Nt, number of transitions to homozygosity; R, observed rate of transition; CI, confidence interval.

Locus	Strain	No	Nt	R (95% CI)
MP5185/MP5291	KAGAWA_01_U	36	0	0.00 (0.00–0.12)
MP5185/MP5291	KAGAWA_11_U	49	0	0.00 (0.00–0.09)
MP5185/MP5291	OSAKA_11#1_U	22	0	0.00 (0.00–0.18)
MP5185/MP5291	OSAKA_11#2_U	50	0	0.00 (0.00–0.09)
	Pooled	157	0	0.00 (0.00–0.03)
MP5230	KAGAWA_01_U	36	0	0.00 (0.00–0.12)
MP5230	KAGAWA_11_U	49	0	0.00 (0.00–0.09)
MP5230	OSAKA_11#1_U	22	0	0.00 (0.00–0.18)
	Pooled	107	0	0.00 (0.00–0.04)

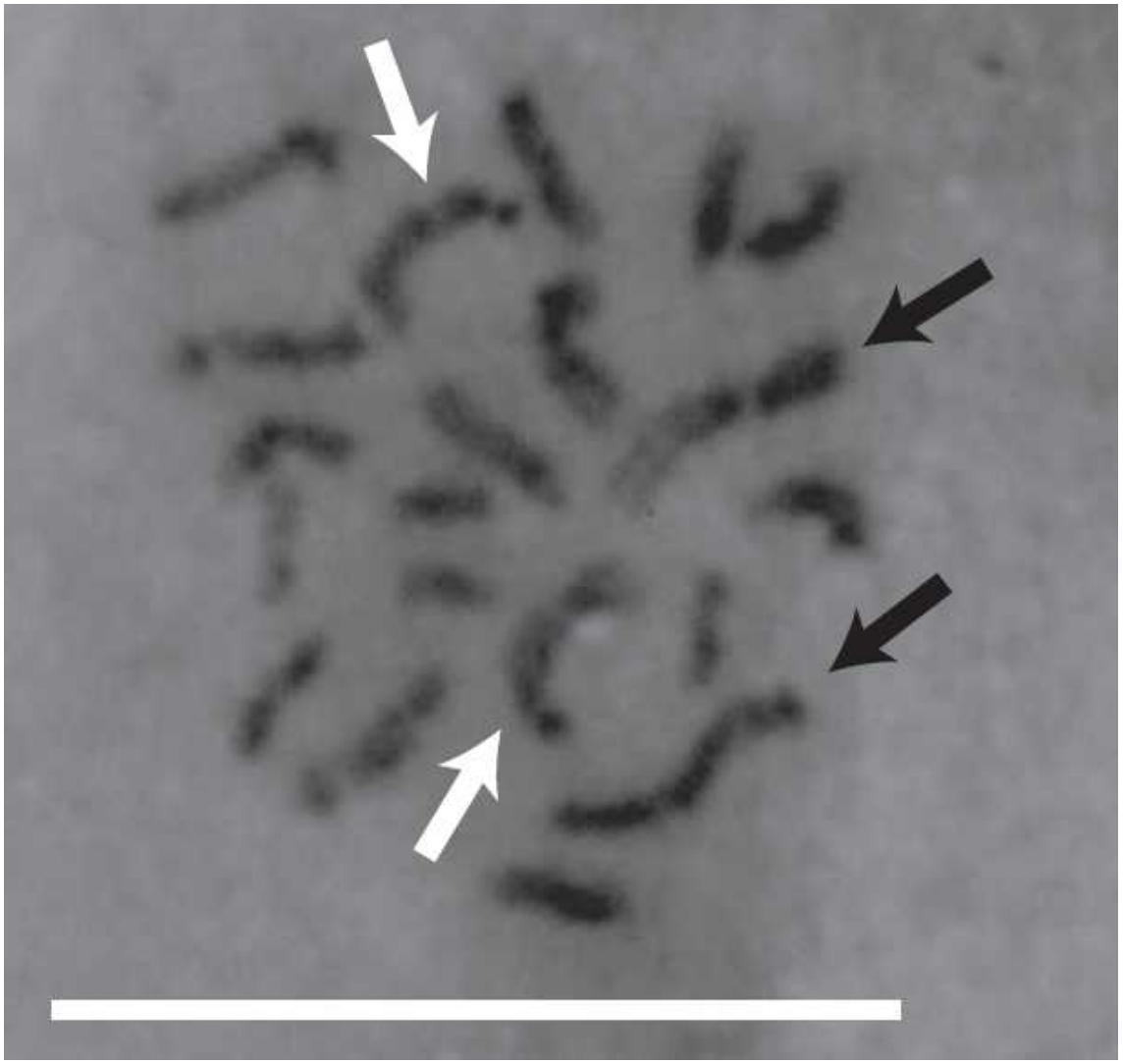

Fig. 1. Metaphase of mitosis in a pupal ogonium of the thelytokous strain. The pairs of two longest and two second longest chromosomes are indicated using black arrows and white arrows, respectively. Scale bar represents 10 μm.

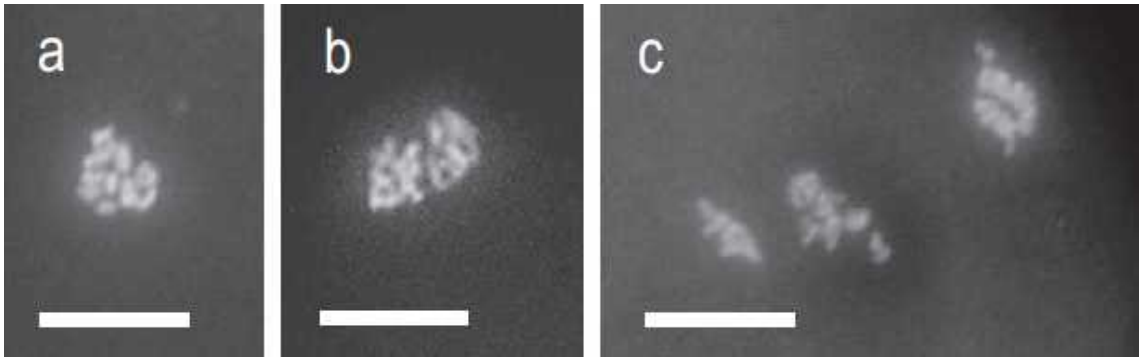

Fig. 2. Chromosomes in the deposited eggs of the arrhenotokous strain. (a) First metaphase of meiosis after oviposition, showing 10 bivalents, (b) first anaphase (20 min after oviposition), and (c) second meiosis (30 min after oviposition). Scale bar represents 10 μm.

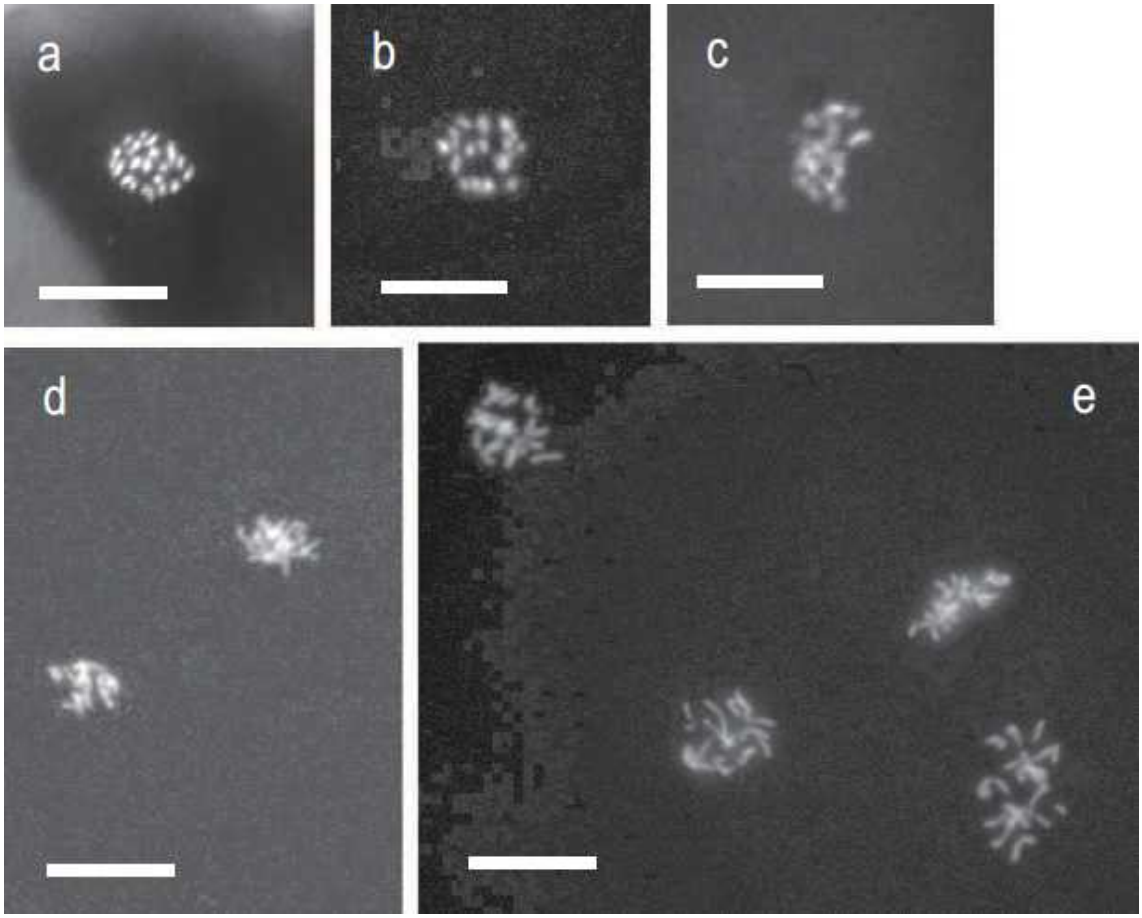

Fig. 3. Chromosomes in the deposited eggs of the thelytokous strain. (a) Metaphase just after oviposition, showing 20 univalents, (b) metaphase (45 min after oviposition), (c) metaphase (1 h and 25 min after aviposition), (d) first mitotic division (1 h and 50 min after oviposition), and (e) subsequent mitotic division (2 h and 45 min). Scale bar represents 10 µm.

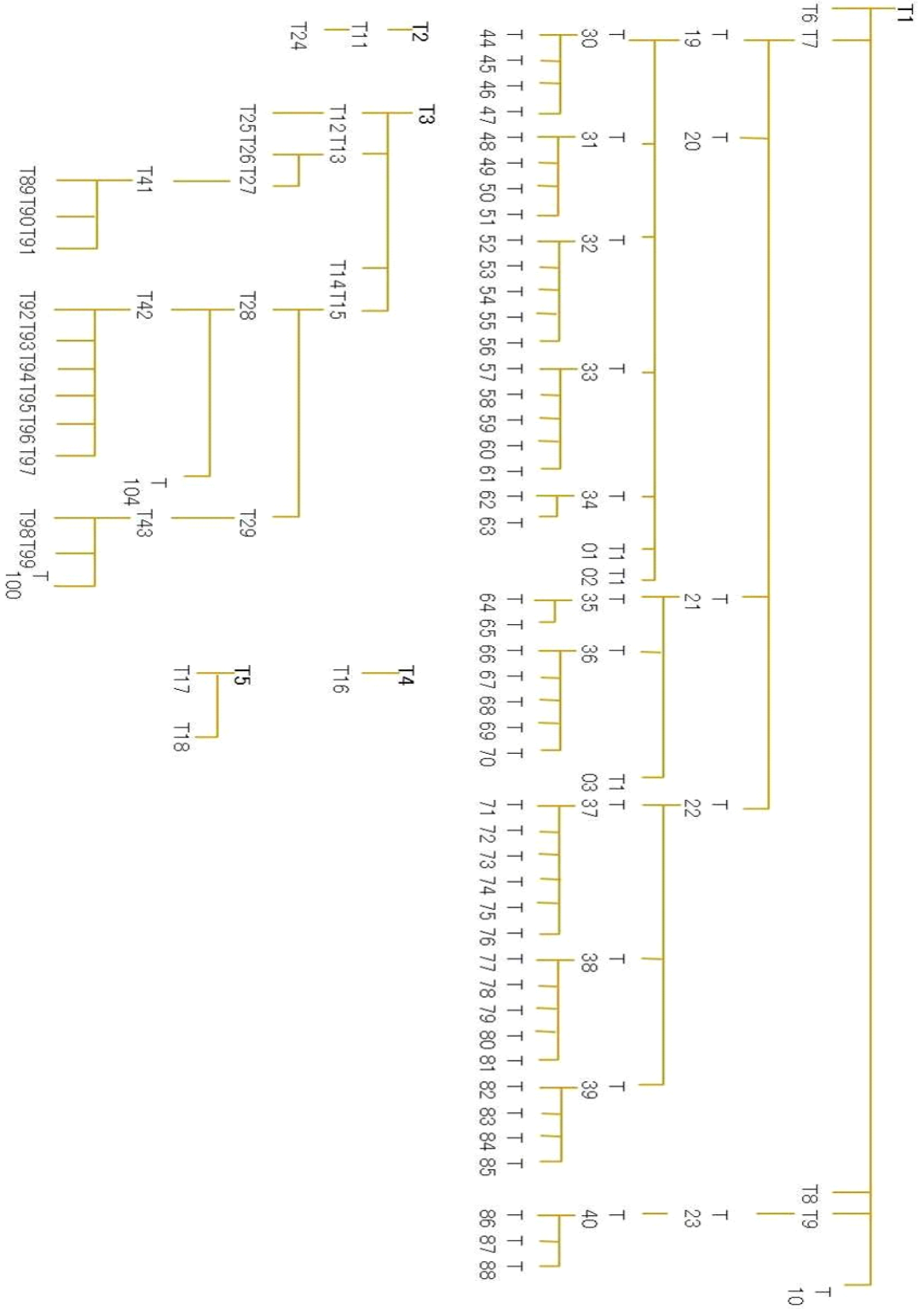

Fig. S1. Five family trees of all thelytokous females treated using tetracycline hydrochloride. Numbers are individual codes.