

Automictic Parthenogenesis in the Turnip Sawfly, *Athalia rosae ruficornis* Jakovlev (Tenthredinidae, Hymenoptera)

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The turnip sawfly, *Athalia rosae*, has a unique reproductive system as most of other hymenopteran insects. Unfertilized eggs as a rule develop to haploid males and fertilized eggs develop to diploid females (Suomalainen *et al.*, 1987). Mature eggs dissected from ovaries of *A. rosae*, moreover, can be activated easily when being soaked in distilled water (Naito, 1982), and they usually develop into haploid males. We have experienced in the course of our studies on eggs activation in *A. rosae* (Sawa and Oishi, 1989) that apparent diploid females appear in a sporadic manner from eggs artificially activated.

We have examined this spontaneous occurrence of diploid females from unfertilized eggs by using a marker mutation, yellow fatbody (*yfb*) (Sawa and Oishi, 1989), with which we can distinguish + and *yfb* haploid males and +/+ , +/*yfb*, and *yfb/yfb* diploid females at the pupal stage. Using this mutation, it is also possible to recognize + and *yfb* haploid mosaic males. Since sex determination in *A. rosae* is based on the single-locus multiple-allele system (homo- and hemizyosity lead to male development and heterozygosity to female development, Naito and Suzuki, 1985), sex can also serve as a marker.

Heterozygous +/*yfb* diploid females were aged for 7 or 10 days and mature eggs dissected from their ovaries were activated artificially. From eggs of females aged 7 days, two + ↔ *yfb* haploid mosaic males and one +/+ diploid female were obtained out of 181 pupae examined. Out of 241 pupae from eggs of 10-day-old females, 3 + ↔ *yfb* haploid mosaic males, 2 +/+ diploid females, 4 +/*yfb* diploid females, and 3 *yfb/yfb* diploid females were obtained. Judging from the segregation of the *yfb* marker mutation, spontaneous automictic parthenogenesis must have taken place and the rate apparently increased as the parental females aged.

If we can induce the automictic parthenogenesis at a higher rate, it would enable us to study the ability of meiotically produced female nuclei to participate in development. Thus we attempted to induce automictic parthenogenesis by artificial means using mature eggs dissected from ovaries of unmated 7-day-old females.

We examined the effect of temperature. Eggs were exposed to ice-cold *Drosophila* Ringer solution for 60 min, in which the eggs were never activated, and then to warm distilled water (35–37°C) for 60 min, in which they were activated. Treated eggs were placed on wet filter paper and incubated at 25°C. Giemsa-stained specimens were prepared from normally developing 2-day-old embryos for chromosome examination. Table 1 shows the results.

As the temperature increased, individuals with chromosome numbers $2n$, $n/2n$, $3n$, $4n$ and others (mostly aneuploids) appeared at higher frequencies ($n=8$, Naito, 1982), while the percentage of embryos normally developing on the second day decreased rather drastically. Considering both viability of treated eggs and the frequency of individuals with chromosome numbers $2n$ or over, we chose the 36°C treatment and examined the effect further.

The results of rearing experiments of eggs taken from 7-day-old +/*yfb* females and given cold and heat (36°C) treatment are shown in Table 2. We obtained haploid males, haploid mosaic males, diploid males, diploid females, triploid females and gynandromorphs. Ploidy was determined by size and progeny testing. Gynandromorphs were detected by examinations of the external morphology and of the internal reproductive organs. Most probably, these gynandromorphs were haploid male and diploid female mosaics.

Although tetraploid individuals might have been expected from the results in Table 1, we did not obtain any in the rearing experiments. In a separate experiment, eggs given the same cold-heat treatment were reared, some 3rd instar larvae were dissected and gonadal tissues were examined cytologically. No tetraploid individuals were detected.

Table 1 Effect of cold and heat shock on parthenogenetic reproduction in eggs from diploid *Athalia rosae* females: Results of chromosome examination on 2-day-old embryos.

Treatment	Parental genotype	No. of eggs activated (%)	No. of normal embryos (%)	No. of embryos examined (%)	No. of embryos with chromosome number:					
					<i>n</i>	<i>2n</i>	<i>n/2n</i> mosaic	<i>3n</i>	<i>4n</i>	Others
None	<i>+/+</i>	382 (100)	356 (93.2)	114	114	0	0	0	0	0
	<i>yfb/yfb</i>	399 (100)	332 (83.2)	102	100	1	0	0	0	1
35°C	<i>+/+</i>	524 (100)	263 (50.2)	116	82	19	2	9	0	4
	<i>yfb/yfb</i>	523 (100)	220 (36.1)	106	72	12	0	16	3	3
36°C	<i>+/+</i>	438 (100)	173 (39.5)	98	17	34	5	16	20	6
	<i>yfb/yfb</i>	727 (100)	131 (18.0)	102	31	35	5	16	12	3
37°C	<i>+/+</i>	650 (100)	44 (6.3)	42	1	14	0	3	12	12
	<i>yfb/yfb</i>	643 (100)	30 (4.7)	30	1	7	0	2	12	8

Table 2 Effect of cold and heat shock on parthenogenetic reproduction in eggs from diploid *Athalia rosae* females: Results of rearing experiments.

Treatment	Parental genotype	No. of eggs activated (%)	No. of larvae hatched (%)	No. of pupated and examined (%)	♂ ^a					♀ ^a					Gyn-andro-morph	Others	
					<i>+ yfb</i>	<i>+/yfb</i>	<i>+/+</i>	<i>+/yfb</i>	<i>yfb/yfb</i>	<i>+/+</i>	<i>+/yfb</i>	<i>yfb/yfb</i>	<i>+/+/yfb</i> and/or <i>+/yfb/yfb</i>				
36°C	<i>+/yfb</i>	2045 (100)	307 (15.0)	186 (9.1)	38	28	3	0	5	2	10	43	7	27	0	5	18

^a Ploidy was determined on two grounds, size and progeny testing. It is difficult to determine whether a triploid female is *+/+/+*, *+/+/yfb* or *+/yfb/yfb*, especially when they appear sporadically as in the present experiments and not all at once, hence "and/or" heading.

These results suggest that most, if not all, tetraploid individuals die before the 3rd instar larval stage and probably none survive to adulthood.

Next, we examined the fate of eggs from triploid females by giving the same cold-heat treatment. Triploid females were obtained by crossing diploid females and diploid males. When mature eggs from triploid females were activated artificially, most of them developed abnormally and cytological examinations of 2-day-old embryos indicated that they were aneuploids with chromosome numbers ranging from $n+1$ to $2n-1$. Small number of normal embryos did develop, however, and some developed still farther. Apparently only in rare cases, chromosome disjunction in triploids occur so as to result in the production of euploids.

The frequency of normally developing 2-day-old embryos greatly increased in eggs from triploid females given the same cold-heat treatment. Out of 548 eggs treated, 131 were normally developing on the second day. Of these 129 were successfully examined cytologically: 33 had the eu-triploid chromosome number, 34 were either $3n+1$ or $3n-1$, 9 were eu-hexaploids, 3 were aneuploids close to $6n$, and 50 were more extensive aneuploids.

Table 3 shows the results of rearing experiments. Many larvae died during early larval stages. Five 3rd instar larvae were sacrificed for cytological examination and all had ovaries and all proved to be triploids. The remaining larvae were allowed to develop further, and 12 out of 13 adults eclosed were shown to be triploid females upon progeny testing.

Table 3 Effect of cold and heat shock on parthenogenetic reproduction in eggs from triploid *Athalia rosae* females: Results of rearing experiments.

Treatment	Parental genotype	No. of eggs activated (%)	No. of larvae hatched (%)	No. pupated and examined (%)	♂		♀	
					+ / + / +, + / yfb / yfb and/or + / + / yfb	yfb / yfb / yfb	+ / + / +, + / yfb / yfb and/or + / + / yfb	yfb / yfb / yfb
36°C	+ / + / yfb	258 (100)	68 (26.4)	13 (5.0)	1 ? ^a	0	12	0

^a Not confirmed.

The present results, taken together, demonstrate that recombination took place not only between the centromere and the *yfb* locus but also between the centromere and the sex determination locus. Appearance of individuals such as haploid-haploid mosaic males and haploid male-diploid female mosaics (gynandromorphs) shown in Tables 1 and 2 clearly indicates that more than one nucleus of the four meiotically produced nuclei can participate in development independently with or without fusion among themselves. There remains another possibility, however, that at least in some cases the first meiotic division failed and formed a restitution nucleus, which then proceeded to second division and one of the daughter nuclei initiated development alone or following fusion with the sister nucleus. Some of the diploids and tetraploids from treated eggs of diploid females, and triploids and hexaploids from those of triploid females could have arisen this way.

References

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