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Genetic analyses of several *Drosophila ananassae*-complex species show a low-frequency major gene for parthenogenesis that maps to chromosome 2

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Summary

Parthenogenetic strains of several species have been found in the genus *Drosophila*. The mode of diploidization in the eggs of females has been found to be post-meiotic nuclear fusion. The genetic basis for this parthenogenesis is not understood but is believed to be under the control of a complex polygenic system. We found parthenogenetic females in an isofemale strain (LAE345) of *D. pallidosa*-like collected in 1981 at Lae, Papua New Guinea, and established a parthenogenetically reproducing strain. Parthenogenetic strains of *D. ananassae* and *D. pallidosa* collected at Taputimu, American Samoa had also been established by Futch (1972). *D. ananassae*, *D. pallidosa* and *D. pallidosa*-like are very closely related species belonging to the *ananassae* complex of the *ananassae* species subgroup of the *melanogaster* species group. Using these three species, we found that more than 80% of females from parthenogenetic strains produced progeny parthenogenetically and that inter-specific hybrid females also produced impaternate progeny. In the present report, we demonstrate that the mode of parthenogenesis of *D. ananassae* appears to be the post-meiotic nuclear doubling of a single meiotic product, and that a major gene responsible for the parthenogenesis maps to the left arm of the second chromosome of *D. ananassae*. We also suggest that the genetic basis for parthenogenesis capacity may be identical among the three closely related species. We discuss the function of the gene required for parthenogenesis and its significance for the evolutionary process.

1. Introduction

Parthenogenesis in animals is a well-known phenomenon. The first attempt to demonstrate the genetic basis of parthenogenesis in *Drosophila* was carried out by Stalker (1951, 1954). He tested the response to selection for parthenogenetic capacity in *D. parthenogenetica* and found that the rate of parthenogenesis had risen about 20-fold (from 0·08% to 1·55%) by the seventeenth unisexual generation. Such a large response to selection has also been observed in *D. mercatorum* (Carson, 1967). He alternated unisexual generations with bisexual ones and obtained a 60-fold increase in parthenogenetic rate. Templeton *et al.* (1976*b*) reported a 1000-fold increase over a single unisexual generation of *D. mercatorum* using highly heterozygous females from natural populations as the source of genetic variability. In his review of parthenogenesis in the genus *Drosophila*, Templeton (1983) concluded that 'the genetic basis for parthenogenesis in *Drosophila* is not simple – there is no gene for parthenogenesis. Rather, the experimental evidence indicates parthenogenesis is a complex polygenic trait which can be subdivided into many components affecting very diverse attributes of the fly.'

The mechanism of parthenogenesis in diploid females is known to be automictic via a variety of post-meiotic nuclear fusion events that produce diploid progeny. Several models of the mechanism of parthenogenesis in *Drosophila* have been proposed (Templeton, 1983). As models of post-meiotic nuclear fusion, three types have been proposed on the basis of the behaviour of genetic markers: central fusion (Murdy & Carson, 1959; Fuyama, 1986), terminal fusion (Stalker, 1954; Futch, 1979) and post-meiotic nuclear doubling of a single meiotic product (Carson, 1973; Carson *et al.*, 1969; Futch, 1973*a*, 1979; Templeton *et al.*, 1976*a*).

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Table 1. *List of parthenogenetic strains used in the experiments*

Species	Strains	Locality	References
D. ananassae	*y-Im* *y-Br*	Taputimu, Tutulia, American Samoa	Futch (1972)
D. pallidosa	*pal-Im* *pal-Br*	Taputimu, Tutulia, American Samoa	Futch (1972)
D. pallidosa-like	*pal*-l-*Im* *pal*-l-*Br*	Lae, Papua New Guinea	Matsuda & Tobari (1999)

Table 2. *Cytological sites of the loci and map distances on the X and second chromosomes of* D. ananassae

	X chromosome					Second chromosome									
Loci:	*Om(1A)*	*y*		*Om(1D)*	*Bx*	*Om(2A)*	*Sb*	*Om(2G)*	*Om(2H)*	*Om(2C)*	*Arc*	*Om(2I)*	*Pr*	*Om(2F)*	
Cytological site	1A		5D	15B	16B	34C	—	38B	41B	44C	—	49C	—	55A	
Map distance		25·7		46·1	8·0		3·2	3·2	7·6	2·4	0·4	8·0	24·5	20·1	

Data from Tobari (1993*b*), Matsubayashi *et al*. (1992).

Matsuda & Tobari (1999) isolated parthenogenetic females from an isofemale strain (LAE345) of *D. pallidosa*-like collected in Lae, Papua New Guinea, and established a parthenogenetically reproducing strain. *D. pallidosa*-like, which only inhabits Papua New Guinea, is recognized as a new species closely related to *D. pallidosa*, belonging to the *ananassae* complex of the *ananassae* species subgroup in the *melanogaster* species group (Tobari, 1993*a*; Tomimura *et al*., 1993). Among these three species, *D. ananassae*, *D. pallidosa* and *D. pallidosa*-like, inter-specific hybrids can be obtained under laboratory conditions, although there is some ethological isolation. Parthenogenetic strains of *D. ananassae* and *D. pallidosa* collected in Taputimu, American Samoa, were established by Futch (1972) and he has maintained them. Dr Futch kindly donated these parthenogenetic stocks of *D. ananassae* and *D. pallidosa*. In the present experiment using these three closely related species, we found that the mode of parthenogenesis appears to be post-meiotic nuclear doubling of a single meiotic product and that a major gene mapping to the second chromosome is essential for parthenogenesis.

2. Materials and methods

(i) *Stocks*

Table 1 lists the parthenogenetic stocks used in the present experiment. The '*Im*' stocks are impaternate female strains that have been maintained without males for many generations. We prepared a 'Bridge' bisexual stock (-*Br*) for each species by crossing *Im* females with males from the parental stocks, from which the *Im* stocks were derived. The F_1 males were backcrossed to the *Im* females, and males (B_1) arising from the crosses were backcrossed to the *Im* females. Males (B_2) arising from these crosses were also backcrossed to the *Im* females in turn. These backcross cycles were repeated. After five or six backcross generations, the parthenogenetic capacity of virgin females from the *Br* stocks was comparable to those of females from the *Im* stocks. The following mutant stocks of *D. ananassae* were used: *Om(1A)15* (Optic morphology), *y* (yellow), *Om(1D)9* and *Bx* (Beadex) on the X chromosome; *Sb* (Stubble bristles), *Arc* (Arc wings), *Om(2I)90*, *Pr* (Prickly bristles), *Om(2F)27a* and e^D (Ebony dominant) linked with a balancer NG2 on the second chromosome; and *Cu* (Curled wings) on the third chromosome. The genetic symbols follow the description by Moriwaki & Tobari (1975, 1993). Cytological locations of some of these loci are found in Table 2 (Matsubayashi *et al*., 1992). The species names follow the description by Tobari (1993*a*).

(ii) *Crossing experiments*

Inter-specific hybrids among *D. ananassae*, *D. pallidosa* and *D. pallidosa*-like can be obtained in the laboratory, although pre-mating isolation among these three species has been recognized (Futch, 1973*b*; Tobari, 1993*a*; Tomimura *et al*., 1993; Doi *et al*., 2001; Yamada *et al*., 2002). Both sexes of F_1 hybrids are fertile. Virgin females younger than 7 days old from the *Im* stock were used to cross with approximately 7-day-old males from the *Br* stocks, because older females rarely mate. Experiments were carried out at 25 °C using a cornmeal, yeast, glucose and agar medium.

Table 3. *Parthenogenetic capacity of the* Im *stocks of* D. ananassae (y-Im), D. pallidosa (pal-Im) *and* pallidosa-*like* (pal-l-Im), *and of* F_1 *hybrid females*

Strains	No. of virgin females tested	No. of virgin females producing progeny	% of virgin females producing progeny	Parthenogenetic capacity[a] (range)
y-Im	43	35	81·4	8·1 (1–28)
pal-Im	26	24	92·3	8·8 (1–23)
pal-l-Im	61	59	96·7	12·6 (1–28)
F_1 (*y-Im*/*pal-Br*)	13	13	100·0	13·7 (2–19)
F_1 (*y-Im*/*pal*-l-*Br*)	59	57	96·6	7·9 (1–30)

[a] Average number of progeny per female per 3 weeks.

Table 4. *Parthenogenetic capacity of* F_1 *and* F_2 *females obtained by crossing of* y-Im *females to* Sb *males of* D. ananassae

Genotypes	No. of virgin females tested	No. of virgin females producing progeny	% of virgin females producing progeny
F_1 [*y*/+ ; +/*Sb*]	60	0	0
F_2 [*Sb*/+]	20	2	10·0
F_2 [+/+]	8	6	75·0

(iii) *Parthenogenetic capacity*

Virgin females were kept singly in a vial and transferred to fresh vials every week for 3 weeks. The number of adult flies produced by each virgin female was counted and used as a measure of her parthenogenetic capacity.

3. Results

(i) *A gene responsible for parthenogenesis is located on the second chromosome*

We examined the parthenogenetic capacity of virgin females from each of the three *Im* stocks and inter-specific hybrid females. The hybrid females were obtained from the crosses between *y-Im* females of *D. ananassae* and males from the *Br* stocks of other species. Table 3 shows that more than 80% of females produced offspring parthenogenetically and that each female yielded about 10 female progeny; the range was from 1 to 30. Egg to adult viability of the *Im* strain of *D. ananassae* was about 4·4–9·4% (10 flies/229 eggs per female to 33 flies/351 eggs per female for 25 days). There are no significant differences in parthenogenetic capacity of females between the parental species and inter-specific hybrids. These data suggest that the genetic basis for the parthenogenetic capacity could be identical among these species.

To examine the mode of inheritance of the parthenogenetic trait in *D. ananassae*, F_1 females (*y*/+ ; +/*Sb*) obtained from crosses between *y-Im* females and Sb/e^D males were tested for parthenogenetic capacity. None of the F_1 females expressed parthenogenetic capacity (Table 4). But 8 out of 28 F_2 females, 2 of which were *Sb*/+ and 6 of which were wild types (+/+), did show parthenogenetic capacity. This indicates that a chromosomal recessive gene or genes are responsible for parthenogenesis. Based on the mitotic chromosome configurations of neurocytes of ganglia of the third instar larvae, the parthenogenetic females and all F_1 progeny were shown to be diploid.

Table 4 shows that the percentage of F_2 [+/+] females producing progeny is much higher than that of F_2 [*Sb*/+] females. This indicates that second chromosomes have a major effect on parthenogenesis. To check the X chromosome effect on parthenogenesis, we performed crossing experiments as follows: F_1 females from crosses between *y-Im* females and wild-type males of non-parthenogenetic strains were backcrossed to males from the *y-Br* strain. There was no difference in the frequencies of impaternate females between the two genotypes of B_1 virgin females (60% (9/15) in *y*/*y* and 53% (10/19) in *y*/+). To examine a possible effect of the third chromosome, F_1 females obtained from the crosses between *y-Im* females and *Cu* males were backcrossed to males from the *y-Br* strain. There was no difference in the percentage of impaternate females between the two B_1 genotypes (42% (55/130) in *Cu*/+ and 45% (41/91) in +/+. To test for a possible fourth chromosome effect, we crossed males of non-parthenogenetic strain carrying *spa* on the fourth chromosome to females from the *y-Im* stock. In the F_2 generation, 2 [*spa*] and 8 [*spa*/+] virgin females among 60 females were shown to have parthenogenetic capacity. The data presented above show that the second chromosome has a major role in the regulation of parthenogenesis and that the X, third and fourth chromosomes do not have appreciable effects on parthenogenesis.

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Table 5. *Parthenogenetic capacity of B_1 females resulting from crosses of F_1 females obtained by crossing* y-Im *females to males from each of the marker stocks,* (*A*) Pr Om(2F)27a, (*B*) Om(2I)90, (*C*) Sb Arc/e^D, *to males from the* y-Br *stock*

Genotypes of B_1 females	No. of virgin females tested	No. of virgin females producing progeny	% of virgin females producing progeny
(A)			
Pr Om(2F)/++	10	8	80·0
++/++	10	6	60·0
(B)			
[*Om(2I)*/+]	32	12	37·5
[+/+]	16	12	75·0
(C)			
[*Sb Arc*/++]	183	10	5·5
[*Sb* +/++]	138	39	28·3
[+ *Arc*/++]	120	17	14·2
[++/++]	160	96	60·0

Table 6. *Genotypes and numbers of impaternate progeny arising from virgin B_1 females obtained from crosses of F_1* y ++/+ Om(1D)9 Bx *females to* y-Br *males of* D. ananassae

Genotypes of impaternate progeny of B_1 virgin females	No. of progeny
y++/*y*++	73
Om(1D)9 Bx/*Om(1D)9 Bx*	55
y Om(1D)9 Bx/*y Om(1D)9 Bx*	22
++/++	55
y+ *Bx*/*y*+ *Bx*	1
Om(1D)9 +/*Om(1D)9* +	14
y Om(1D)9 +/*y Om(1D)9* +	13
+ *Bx*/+ *Bx*	4
Om(1D)9 +/++	1
Om(1D)9 Bx/++	1
Om(1D)9 Bx/*Om(1D)9* +	1
Total	240

% females heterozygous for non-recombinant X (*Om(1D)9 Bx*): 0·42% (2/240).
% females heterozygous for recombinant X (*Om(1D)9* +): 0·42% (2/240).

Table 7. *Genotypes and number of progeny of hybrid B_1 virgin females obtained from crosses of* (*A*) F_1 ++ (pal-Im)/Om(1D)9 Bx *females* × ++(pal-Br) *males and* (*B*) F_1++(pal-l-Im)/Om(1D)9 Bx *females* × ++(pal-l-Br) *males*

Genotypes of progeny of B_1 virgin females	No. of progeny (A)	No. of progeny (B)
++/++	94	21
Om(1D)9 Bx/*Om(1D)9 Bx*	49	9
+ *Bx*/+ *Bx*	9	0
Om(1D)9 +/*Om(1D)9* +	21	2
Om(1D)9 +/++	3	0
+ *Bx*/++	0	0
Total	176	32

(ii) *The gene responsible for parthenogenesis maps to the left arm of the second chromosome*

To determine the site responsible for parthenogenesis on the second chromosome, we used three dominant marker stocks: *Pr Om(2F)27a*, *Om(2I)90* and *Sb Arc*. F_1 females from the crosses between females from the *y-Im* stock and males from each of the marker stocks were crossed to males from the *y-Br* stock. B_1 females produced by these crosses were used to test parthenogenetic capacity in the genetic intervals defined by the dominant visible markers. Table 5A and B shows that the region essential for parthenogenesis is located on the left side of the *Om(2I)90* locus of the second chromosome. The genetic map distance of this putative locus from *Pr* or *Om(2I)90* is about 40% [1/2(6/10+2/10)] or about 30% [1/2(4/16+12/32)], respectively. For more precise mapping of the locus determining parthenogenesis, we used *Sb Arc* markers located on the left arm of the second chromosome. F_1 ++ (*Im*)/*Sb Arc* females were crossed to males from the *y-Br* stock, and from B_1 females produced by this crossing, *Sb Arc*/++, *Sb* +/++, + *Arc*/++ and ++/++, were selected to check parthenogenetic ability. Table 5C shows that over 60% of the ++/++ females show parthenogenetic ability, but that only 5·5% of *Sb Arc*/++ females do so. The percentages of virgin recombinant females (*Sb* +/++ and + *Arc*/++) producing progeny are intermediate between those of non-recombinant females. These data indicate that the gene(s) responsible for parthenogenesis maps between the *Sb* and *Arc* loci of *D. ananassae*. We named the gene responsible for parthenogenesis, which is located between *Sb* and *Arc*, *parth*

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Table 8. *Recombination frequencies in the X chromosome of the impaternate B_1 females heterozygous for* y++/+ Om(1D) Bx *of* D. ananassae

Regions	Standard map distance	% recombination frequency in parthenogenetic females		
		ananassae (*ST*/*ST*)	*pallidosa* (*ST*/*In*(*XL*)*A*[a])	*pallidosa*-like (*ST*/*In*(*XL*)*A* + *In*(*XR*)*A*[b])
Om(1A)–y	25·7	29·4	—	—
y–Om(1D)	46·1	39·2	—	—
Om(1D)–Bx	9·8	14·2	13·1	6·3
No. of progeny		240	176	32

[a] *In(XL)A*, 4A;10D.
[b] *In(XR)A*, 16C;19D.
(Tobari *et al*., 1993; Tobari, 1993*b*).

(parthenogenesis). This is the first report of mapping of a major gene responsible for parthenogenesis in *Drosophila*.

(iii) *The mode of the parthenogenesis is post-meiotic nuclear doubling of a single meiotic product*

We carried out an experiment to determine the mechanism of parthenogenesis in *D. ananassae* and its relatives. We made crosses between females from *y-Im* and *Om(1D)9 Bx* males. *Om(1D)9* and *Bx* are semi-dominant alleles, enabling us to distinguish heterozygotes and homozygotes. F_1 females were backcrossed to males of *y-Br*. B_1 [+ *Om(1D)9 Bx*/*y* ++] virgin females were selected to test parthenogenetic capacity. Table 6 shows that most of the parthenogenetically produced progeny are homozygous for each locus, *y*, *Om(1D)9* and *Bx*. The frequency of females heterozygous for non-recombinant *Om(1D)9 Bx* was 0·42% and of females heterozygous for recombinant *Om(1D)9* was 0·42%. These results demonstrate that diploidy is predominantly restored by post-meiotic nuclear doubling of a single meiotic product.

Similar crossing experiments were carried out using parthenogenetic stocks of *D. pallidosa* and *D. pallidosa*-like and *Om(1D)9 Bx* of *D. ananassae*. These results show that most progeny were homozygous: 98·3% in *pallidosa* and 100% in *pallidosa*-like (Table 7). The mechanism of parthenogenesis is thus shown to be the same among the three species.

Table 8 shows that there are no significant difference in the frequency of meiotic recombination in the X chromosome between parthenogenetic females and bisexual wild-type females in *D. ananassae*, *D. pallidosa* and *D. pallidosa*-like hybrids. This suggests that the MI stage of meiosis in parthenogenetic females must be normal.

4. Discussion

Most of the progeny produced parthenogenetically were homozygous for the mutant loci on the X chromosome used in the experiment, and there was no significant difference in the frequency of meiotic recombination between parthenogenetic females and bisexual wild-type females. In addition, the cytological observations reveal that there are no distinguishable differences in meiosis II between parthenogenetic females and bisexual wild-type females (data not shown). Therefore, we can conclude that post-meiotic nuclear doubling of a single meiotic product is the predominant mechanism of parthenogenesis of *D. ananassae* and its relatives.

Most of the cases of parthenogenesis found in *Drosophila* species are facultative and their mechanisms are automictic (Carson, 1967; Templeton, 1983), so the mode of parthenogenesis in *D. ananassae* and its relatives is not exceptional. The gene we mapped on the second chromosome is a maternal gene that might mediate the post-meiotic nuclear doubling of a single meiotic product without insemination after completion of female meiosis (Doane, 1960). Three maternal genes – *gnu*, *plu* and *png* – of *D. melanogaster* have been reported to regulate entry into S-phase after fusion of two pronuclei derived from each parent and to promote early cleavage division (Shamnski & Orr-Weaver, 1991; Lee *et al*., 2001). The wild-type product of the *parth* gene mapped in the present experiment might have a role in repression of DNA replication prior to fertilization and also in the early cleavage divisions. The molecular identification of the product of the gene will facilitate our understanding of the mechanism of parthenogenesis. As in parthenogenetic strains of *D. mercatorum*, high mortality rates of eggs (90·6–95·6%) were observed in *D. ananassae* and its relatives. This high rate of mortality of impaternate progeny might be explained by an uncoupling of DNA replication and nuclear division and could result from an initiation of cleavage division of haploid eggs and/or an uncoupling of DNA replication and mitosis in the cleavage division.

During the course of these experiments, we found exceptional males in the *Im* parthenogenetic strains.

They were recovered with a frequency of 0·14–0·35%, and their karyotypes were diploid X0. Futch (1973*b*) also found sterile males in the *ananassae* parthenogenetic strain. These data cannot be explained by post-meiotic nuclear doubling of a single meiotic product. Carson (1967) reported that the rate of production of X0 males ranged from 0·27% to 0·72% in *D. mercatorun-Im* strains. He suggested that non-disjunction of the X chromosome and post-meiotic central fusion could explain the recovery of X0 males. Matsuda & Tobari (1994) found the primary non-disjunction rate to be 0·21% in crossing experiments using bisexual strains of *D. ananassae*. The X0 males that appeared in *Im* strains of *D. ananassae* may also have been due to X non-disjunction and post-meiotic central fusion. The observed non-recombinant heterozygotes (0·42%) and recombinant heterozygotes (0·42%) cannot be explained by post-meiotic nuclear doubling of a single meiotic product either. These heterozygotes, which appeared at low frequency, might derive from 'central fusion' of pronuclei and/or 'terminal fusion' of pronuclei, respectively. Similar results were also reported by Futch (1972, 1973*b*, 1979). In *D. mercatorum*, the occurrence of heterozygous impaternate progeny was explained as the result of the fusion of two of the four meiotic products (Carson *et al*., 1969; Templeton *et al*., 1976*a*). It is hard to explain why two or more mechanisms are concomitant in parthenogenesis. We need further experiments to elucidate the mechanism(s) of these exceptional cases.

Futch (1972) found that parthenogenetic females were rare in collections (1–10%). He made a systematic examination for parthenogenesis in many geographical stocks from Mexico, Hawaii, Palmyra Island, Marshall Islands, Fiji, Cook Islands and Papua New Guinea populations and found that only females of *D. pallidosa* from the Western Samoa, American Samoa and Tonga populations, and of *D. ananassae* from the Western Samoa and American Samoa populations, had parthenogenetic capacity. We also established many isofemale lines collected in the species area of *D. ananassae* and its close relatives: *D. pallidosa*, Taxon-K and *D. papuensis*-like of the *ananassae* complex (Matsuda & Tobari, 1999). Parthenogenetic females were found only in *D. pallidosa*-like inhabiting Lae, Papua New Guinea. The results of the above studies show that parthenogenetic capacity has been kept in the restricted island populations in low frequency. In the Samoan islands, populations of the two species, *D. ananassae* and *D. pallidosa*, have kept the *parth* gene. This may suggest the occurrence of some gene flow between them, as Futch (1972, 1973*a*) suspected. *D. pallidosa*-like collected from Lae, Papua New Guinea, are morphologically nearly indistinguishable from *D. pallidosa*, and carry the '*D. pallidosa* chromosome', but are ethologically isolated (Tomimura *et al*., 1993). This may indicate that the *parth* gene has been derived from their ancestral species and kept in their populations during the course of speciation.

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