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) reported a 1000-fold increase over a single unisexual generation of *D. mercatorum* (Carson, 1967). He alternated unisexual generations with bisexual ones and obtained a 60-fold increase in parthenogenetic rate. Templeton *et al.* (1976) 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 automic 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, 1973a, 1979; Templeton *et al*., 1976a).
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; 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 ethiological 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 impaternal 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 F₁ males were backcrossed to the Im females, and males (B₁) arising from the crosses were backcrossed to the Im females. Males (B₂) 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(2F)/90, Pr (Prickly bristles), Om(2F)/27a and e³b (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 (1993a).

(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, 1973b; Tobari, 1993a; Tomimura et al., 1993; Doi et al., 2001; Yamada et al., 2002). Both sexes of F₁ 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.
Parthenogenesis in Drosophila

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 F₁ hybrid females

| Strains       | No. of virgin females tested | No. of virgin females producing progeny | % of virgin females producing progeny | Parthenogenetic capacity* (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)                      |
| F₁ (y-Im/pal-Br) | 13                          | 13                                     | 100.0                                | 13.7 (2–19)                      |
| F₁ (y-Im/pal-l-Br) | 59                          | 57                                     | 96.6                                 | 7.9 (1–30)                       |

*Average number of progeny per female per 3 weeks.

Table 4. Parthenogenetic capacity of F₁ and F₂ 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 |
|-----------------|-------------------------------|----------------------------------------|--------------------------------------|
| F₁ [y/ + : +/Sb] | 60                            | 0                                      | 0                                    |
| F₂ [Sb/+]       | 20                            | 2                                      | 10.0                                 |
| F₂ [+/+ ]       | 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, F₁ females (y/ + ; +/Sb) obtained from crosses between y-Im females and Sb/e₀ males were tested for parthenogenetic capacity. None of the F₁ females expressed parthenogenetic capacity (Table 4). But 8 out of 28 F₂ females, 2 of which were Sb/+ and 6 of which were wild type (+/+), 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 F₁ progeny were shown to be diploid.

Table 4 shows that the percentage of F₂ [+/+] females producing progeny is much higher than that of F₂ [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: F₁ 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 imparthenate females between the two genotypes of B₁ virgin females (60% (9/15) in y/y and 53% (10/19) in y/ +). To examine a possible effect of the third chromosome, F₁ 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 imparthenate females between the two B₁ 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 F₂ 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.
Table 5. Parthenogenetic capacity of B₁ females resulting from crosses of F₁ 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/e³, to males from the y-Br stock

| Genotypes of B₁ 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                                 |

(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. F₁ 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. B₁ 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. F₁ ++ (Im)/Sb Arc females were crossed to males from the y-Br stock, and from B₁ 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
Table 8. Recombination frequencies in the X chromosome of the impaternate Bx females heterozygous for y +/+ Om(1D) Bx of D. ananassae

| Regions                  | Standard map distance | ananassae (ST/ST) | pallidosa (ST/In(XL)A) | pallidosa-like (ST/In(XL)A + In(XR)A) |
|--------------------------|-----------------------|-------------------|------------------------|---------------------------------------|
| 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                     |                                       |

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

(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-In and Om(1D)/9 Bx males. Om(1D)/9 and Bx are semidominant alleles, enabling us to distinguish heterozygotes and homozygotes. F1 females were backcrossed to males of y–Br. B1 [+ 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 (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 automic (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, plus and ping – 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 (Shamanski & 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 (1973b) 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. mercatorum-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 crosses 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, 1973a, 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., 1976a). 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, 1973a) 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 (Tomiura 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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