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紅果蠅孤雌生殖的遺傳研究

Genetic Study of Parthenogenesis in *Drosophila albomicans*

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本論文係張家禎君（R00632018）在國立臺灣大學昆蟲學系暨研究所完成之碩士學位論文，於民國一百零二年十二月二十五日承下列考試委員審查通過及口試及格，特此證明

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誌謝

在張慧羽老師實驗室的四年，將在這裡畫下一個句點，雖然不是完美，但至少我問心無愧。時間是有限的，也許我所享受的大學玩樂時光相對於短，但是，科學研究的世界是如此令人著迷、如此令人廢寢忘食，當然，這一切的努力相當值得！

從大二進實驗室開始，張慧羽老師帶我一步一步前進，帶我認識遺傳學和進化學，教導我如何思考問題、尋找答案、解決問題，更告訴我許多做人做事的方法、以及面對困難時應有的心態。張老師對我而言，不僅僅是研究旅途的啟蒙者，更是我永遠的楷模。衷心的希望，我將來可以成為像張老師一樣令人欽佩的、成功的領航者。跟丁照棣老師、曹順成老師以及方淑老師實驗室的 joint lab meeting 時常點出我的盲點，讓我試著用另一個角度來看實驗數據，也使我上台報告的功力大幅的增加。感謝丁老師和方老師的耐心指導，和她們一起寫文章的數個月，讓我知道做研究不是只有一個人的努力就夠，更是需要多人的合作。感謝易玲輝老師的指導，雖然只有短短的幾個月，我也沒有做出成果，但在這嘗試的過程中，我也有不少的收穫和經驗。還有，文喬和心彥的協助，大量繁雜的行政工作都是依靠她們一一幫我們處理；實驗室的佳豪學長，他是我在實驗室僅次於張老師的靠山，我所會的實驗技術幾乎都透過他習得，也很感謝他源源不絕的關心，要吃飯要早睡要運動；以及丁老師和方老師實驗室的成員們：Ani、景賀、家宣、詩凡、宇謙、映璇、叡涵、維中，很感謝有大家的幫助，除了技術上的指導、研究進況的分享，還有不時的關心和鼓勵，也希望大家在研究工作上有豐沛的收穫、持續進步。昆蟲系一路上陪伴著我的同學們：繹梅、敏敏、博凱、宗學、羿宏、寄綸，非常高興這五年多來有他們的陪伴，也祝福大家的碩士研究工作一切順利。感謝所有出現在我生命中的人們。

現在只不過是我研究生涯的逗點，休息一下，我正準備帶著這四年所學會的知識和能力，繼續向前。期許自我，像螞蟻一般腳踏實地，也像老鷹般在高空中翱翔，時代的巨輪不停地滾動著，盼望我將是推動巨輪的一份子，現在的我，取之社會，未來的我，將回饋社會。

中文摘要

紅果蠅孤雌生殖的後代數遠低於有性生殖，且會行減數分裂產生單倍細胞，之後以只產生同結合型個體的配子重複為主要恢復二倍體的機制，因而導致遺傳變異降低。在這兩個不利條件之下，推測是兼性模式使孤雌生殖得以在自然界維持，因為有孤雌能力的雌蟲可以產生大量的有性生殖後代，且這些後代仍有行孤雌生殖的能力，所以遺傳變異可以進入孤雌生殖系統；再加上交尾後雌蟲仍可進行孤雌生殖，有助於保留孤雌生殖等位基因。經基因定位的實驗顯示，能否行孤雌生殖由一個主要的顯性遺傳因子控制，位於第二號染色體左臂 B_1 D_5 附近或其間；而後代數則受到許多數量性狀基因座的影響。有孤雌生殖能力的日本紅果蠅與臺灣烏來族群的親緣關係最接近，我們發現實驗室的烏來品系有行孤雌生殖的能力，論文中討論高比例的第二號染色體左臂的 B_1 到 D_5 區段逆位的異結合型和孤雌生殖長期保留在有性生殖品系中的關係。

關鍵字：兼性孤雌生殖、配子重複、基因定位、逆位

Abstract

Low fecundity and a very high percentage of gamete duplication mechanism which only generates homozygotes are two disadvantages of parthenogenetic *Drosophila albomicans*. Facultative mode may rescue parthenogenesis from these drawbacks. Females capable of parthenogenesis reproduce lots of offspring through sexual reproduction and those F_1 females can also perform parthenogenesis. In addition both sexual reproduction and parthenogenesis are performed after mating. Therefore, parthenogenetic factors can be amplified and genetic variation can be reintroduced into this system. Genetic mapping revealed a major genetic element, may be located on 2L chromosomal arm, with dominant effect for parthenogenetic capability and several genes with additive effect for parthenogenetic fecundity. Parthenogenetic *D. albomicans* was discovered in Japan. The population was probably derived from Taiwan population for flies in Wulai were the phylogenetically closest. A sexual strain originated from Wulai, Taiwan in 1970 was found to be able to perform parthenogenesis. This strain carries high $In(2L)B_1D_5$ heterozygosity, which may be a reason why parthenogenesis can sustain after long-term sexual reproduction with limited individuals per generation.

Keywords: facultative parthenogenesis, gamete duplication, genetic mapping, inversion

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Introduction

Parthenogenesis, in which unfertilized eggs develop into fertile adults, has recurrently evolved from sexual ancestors in many multicellular organisms. Although parthenogenesis is presumed to be advantageous over sexual reproduction because the latter has a so-called "twofold cost of sex" (Maynard Smith 1978), sexual reproduction is the predominant strategy of reproduction (Phillips 1903, Suomalainen 1950, Mittwoch 1978, Bell 1982, Suomalainen et al. 1987). The fecundity of some parthenogenetic insects was found to be lower than that of their sexual counterparts (Stalker 1954, Carson et al. 1957, Carson 1967, Lamb and Willey 1979, Hong and Ando 1998). However, parthenogenesis may be adaptive in harsh, high latitude and/or altitude, isolated environments or newly invaded marginal habits, where population size are frequently too small for females to find mates (Suomalainen 1950; Cuellar 1977). Geckos (*Heteronotia*) and grasshoppers (*Warrambia*) being able to colonize in Australian deserts may be attributed to parthenogenesis (Kearney 2003).

Automictic parthenogenesis performs meiotic recombination and diploidy is restored by three main cytological mechanisms, including gamete duplication, central fusion, and terminal fusion (Suomalainen 1950). Gamete duplication has been observed in some animals, such as phasmid, coccids, mites, and most facultative parthenogenetic *Drosophia* (Suomalainen et al. 1987). It is the chromosome doubling after meiosis or fusion after an extra round of mitosis and therefore enforces homozygosity at all loci. Different combinations of homozygous genotype may exist if the founding female is not fully homozygous. Terminal fusion, which is found in acridids, coccids, mites, and *Drosophila*, is the fusion of two nuclei derived from the same meiosis I nucleus

(Suomalainen et al. 1987). The offspring is completely homozygous unless recombination occurs. Central fusion, which is found in psychid moth and the only one obligate parthenogenetic *D. mangabeirai*, is the fusion of two nuclei derived from different meiosis I nuclei (Suomalainen et al. 1987). The offspring retains the genetic heterozygosity of the mother in the absence of recombination. Among facultative parthenogenetic *Drosophila*, *D. parthenogenetica* adopt fusion as the major diploidization mechanism to produce offspring by parthenogenesis (Stalker 1954). In *D. mercatorum* and the three species of *D. ananassae*-complex including *D. ananassae*, *D. pallidosa*, and *D. pallidosa*-like parthenogenetic offspring were produced mainly via gamete duplication (Carson et al. 1969, Carson 1973, Matsuda and Tobari 2004). The characterization of diploidization mechanisms allow us to understand the evolutionary consequence of parthenogenesis in populations because different diploidization mechanisms have different impacts on the genetic variability.

Parthenogenesis in *Drosophila* is thought to be evolved through spontaneous mutation (Templeton 1983). Although *D. mercatorum* and *D. parthenogenetica* from natural populations was found to have extremely low hatchability of unfertilized eggs, which was only 10^{-5} to 10^{-6}, selection could effectively improve it (Carson 1967, Stalker 1954). It was suggested that parthenogenesis may be regulated by polygenes because productivities were increased with generations. Nevertheless, Fuyama (1986) suggested that successful parthenogens will be hard to evolve through selection if parthenogenesis is controlled by a polygenic system. Each of these two seemingly conflict points may be responsible for one of the two phenotypes of parthenogenesis if there are only a few essential genes necessary for the capability while many genes influence the fecundity. Among all parthenogenetic *Drosophila*, only *D. ananassae*-complex was studied by

genetic mapping and a major gene located on its 2L arm was discovered (Matsuda and Tobari 2004).

Parthenogenetic *D. albomicans* was found in Kiikatsuura, Japan, which was the first reported parthenogenesis in *D. albomicans* (Ohsako and Fuyama 1995). Strong natural selection in favor of parthenogenesis may be operated for an adaptation to a colder climate and that is why *D. albomicans* could invade from sub-tropical area, i.e., probably from Wulai, Taiwan to the temperate climate zone (Ohsako et al. 1994). The altitude of Wulai ranges from 200 to 500 meters above sea level which is the boundary of sub-tropical and temperate climates. Therefore, *D. albomicans* in Wulai may be able to do parthenogenesis. High $In(2L)B_1D_5$ heterozygosity was observed in winter due to small population size and inbreeding depression caused by high recessive deleterious mutation frequencies for both arrangements in Wulai populations (Chang et al. 1987; Yang et al. 2002) and high heterozygosity was also observed in the Japan population (Ohsako et al. 1994). The balancing selection may also maintain other alleles, such as parthenogenetic allele(s), accompanying with $In(2L)B_1D_5$ polymorphism.

In this study, we (1) checked parthenogenetic capability for a parthenogenetic strain and several sexual strains, (2) compared the reproductive patterns and mating propensity between a parthenogenetic strain and sexual strain, (3) revealed the co-existence of different diploidization mechanisms and their proportions, (4) mapped the important genetic elements for parthenogenesis, and (5) discussed factors for the maintenance of parthenogenesis in *D. albomicans*.

Materials and methods

Fly strains

The parthenogenetic *Drosophila albomicans* strain was a courtesy from Dr. Fuyama. It was established from a virgin female of an isofemale strain (KKU119), which was originated from Kiikatsuura, Japan in 1990, and maintained by parthenogenesis ever since (Ohsako and Fuyama 1995). All strains used in the study are shown on Table 1. Flies were maintained on standard cornmeal medium at 23℃ under 12:12 h LD cycle. Flies used for crosses were sexed within 8 hours after eclosion.

Table 1. List of strains, their collection year, collection site, and purpose in the study

Strains	Collection year	Collection site	Purpose in the study*
#55.1	1970	Taroko, Hwalien, Taiwan	1, 2, 3, 4, 5, 6
#56.1	1970	Wulai, Taipei, Taiwan	1, 2
#163.5-IA	1978	Okinawa, Japan	1, 2, 5
#193.1	1970	Wulai, Taipei, Taiwan	1,2
#281.4	1997	Xishungbanna, Yunnan, China	2
#296.6	2001	Lanyu, Taitung, Taiwan	2
#300.8	1992	Wulai, Taipei, Taiwan	2
KKU119[p]	1990	Kiikatsuura, Japan	1, 2, 3, 4, 5, 6
KKU202	1991	Kiikatsuura, Japan	1, 2
KKU204	1991	Kiikatsuura, Japan	1, 2
KKU210	1991	Kiikatsuura, Japan	1, 2

[p]Parthenogenetic strain
*1: Parthenogenetic capability; 2: Chromosomal inversion analysis; 3: Reproductive pattern; 4: Mating propensity; 5: Diploidization mechanism; 6: Genetic mapping

Parthenogenetic capability

The parthenogenetic capability was measured by the percentage of virgin females

produced offspring parthenogenetically in 4 weeks. Single virgin females were transferred twice a week until 4 weeks. Besides several strains listed in Table 1, F_1 females sexually produced by KKU119 X #55.1 and by KKU119 X #163.5-IA were checked.

Chromosomal inversion analysis

Third instar larvae were dissected, and their salivary gland polytene chromosomes were stained with lacto-aceto-orcein according to Lin and Chang (1986). An inbred line, #163.5-IA, is an *In*(*2L*)B_1D_5 homozygous strain arbitrarily assigned as I/I in our lab as inversion arrangement identification tool following the same criterion as in our previous paper (Chang and Lin 1995).

Reproductive patterns of parthenogenetic females

Each 4-day-old virgin female from KKU119 was put into vials and transferred to new vials every 3 days for 4 weeks. The total number of adult offspring parthenogenetically produced by each female was counted as fecundity of parthenogenesis. To study sexual reproduction performed by KKU119, we set single pairs of KKU119 X #55.1 when females were 4 days old or 11 days old. Single pairs were transferred to new vials twice a week for 4 weeks. The total offspring number produced by one pair indicated their fecundities. We excluded those pairs only producing female offspring or without offspring because they may lack successful mating. The comparison of fecundities between groups or weeks was done by Mann-Whitney U test. The sex ratio was calculated for each pair and each week. We excluded the pairs with less than 10 offspring in each week, in order to minimize the bias caused by small sample size. The sex ratio of offspring was calculated by the

number of males over total, and a ratio significantly lower than that of #55.1 X #55.1 was regarded as female biased due to males from the same strain by Chi-square test. The female offspring were genotyped to verify reproductive mode and estimated the proportion of offspring produced through sexual reproduction or parthenogenesis. For comparison, the fecundity of the sexual strain (i.e., #55.1) was measured with the same procedure as sexual reproduction of KKU119. In addition, hatchability of virgin KKU119 females was calculated by number of hatched larvae from 100 eggs, and that of #55.1 X #55.1 were also examined as comparison.

Mating propensity

The mating propensity of virgin female from KKU119 was indicated by percentage of copulation with that of #55.1 virgin females as control. Single females at 4 or 11 days old were put in vials, and then each was given a 4-day-old #55.1 male. The percentage of copulation pairs was counted twice (i.e., the first in 30 minutes and the second in 2 hours). The statistical analysis was adapted by Fisher's exact test.

Molecular markers

14 PCR (polymerase chain reaction)-RFLP (restriction fragment length polymorphism) markers were named after the PCR locus plus the restriction enzyme (e.g., a1350+*Hpa*II). An additional PCR-sequencing marker, a6 sequencing, was located on the centromeric 2R arm. The a6 sequencing marker was only conducted on the #55.1 cross because the a6 sequences were identical between #163.5-IA and KKU119. The marker information was listed on Table 3. Single-fly genomic DNA was extracted using the Puregene Cell and Tissue DNA Isolation Kit (Gentra Systems, USA) following the manufacture's protocol.

Diploidization mechanism

Diploidization mechanisms were determined by genotyping F_2 parthenogenetically produced by F_1 females and they were sexually produced by crossing the parthenogenetic strain and sexual strains. From the predicted genotypes shown on Table 2, recombinant F_2 provided a better resolution than non-recombinant ones for distinguishing the 3 possible diploidization mechanisms, so that recombination rates are crucial. Two molecular markers on the large neo-X chromosome arm, one near the centromere and the other near the telomere, were used as major indicators because their genetic distance was over 50 cM (Chang et al. 2008). The alleles from the parthenogenetic strain and the sexual sexual strains were assigned as "*P*" and "*S*" types, respectively. Since the expected recombination rate between these two markers is 50%, from homozygous recombinants we could confirm gamete duplication (GD) and make estimations. GD is actually the only category produced by one mechanism for sure. Among the other 8 possible genotypes, 2 (*PS,PP* and *PS,SS*) can be assigned to central fusion (CF) with recombination, and 2 (*PP,PS* and *SS,PS*) can be assigned to terminal fusion (TF) with recombination. The doubly heterozygous genotype (*PS,PS*) could be either CF (with or without recombination), or TF (with recombination between the proximal marker and the centromere). Therefore, additional three markers on the 2nd chromosome were used when both the neo-X chromosome arm markers were heterozygous. Molecular genotyping was also applied on the F_1 females for confirming that they were indeed sexually produced heterozygotes.

Table 2. All possible genotypes on a chromosome after F_1 parthenogenesis predicted by three diploidization mechanisms

Mechanisms	Genotypes[a]		
	With recombination -I[b]	With recombination-II[c]	Without recombination
Gamete duplication	*PP*,*SS*[d] *SS*,*PP*	*PP*,*PP* *SS*,*SS*	*PP*,*PP* *SS*,*SS*
Central fusion	*PS*,*PS* *PS*,*PP* *PS*,*SS*	*PP*,*PP* *PS*,*PS* *SS*,*SS*	*PS*,*PS*
Terminal fusion	*PP*,*PS* *SS*,*PS*	*PS*,*PS*	*PP*,*PP* *SS*,*SS*

[a] The genotypes of two markers on one chromosome are separated by a comma, and the one near centromere is left to the comma.
[b] r-I indicates a crossing over between two markers.
[c] r-II indicates a crossing over between the proximal marker and the centromere
[d] *S*: the allele from sexual strains; *P*: the allele from the parthenogenetic strain.

Genetic mapping

Recombinant flies were produced through the following crossing scheme: KKU119 flies were crossed with #55.1 males, and their F_1 virgin females were collected and allowed to reproduce F_2 parthenogenetically. After that, each F_2 was placed in a vial and transferred to a new vial twice a week for consecutive four weeks. The fecundity (i.e., the number of F_3) was counted for each F_2. The F_2 was then genotyped, and checked for parthenogenetic capability (i.e., whether she produced offspring parthenogenetically in 4 week) as the phenotype. About 100 mainly homozygous recombinant virgin females were checked. The association between genotypes and phenotypes was revealed by Fisher's exact test. Linkage disequilibrium D' of those highly associated markers was calculated. When the linkage disequilibrium was high, we checked the recombinant individuals in order to reveal which marker is closer to the gene for parthenogenetic capability.

Table 3. Marker list with primer name, location, primer sequence, PCR annealing temperature, and restriction enzyme

Primer name	Location	Primer sequence	Annealing temperature (℃)	Restriction enzyme	Reference
a6	2R	F: GCTGTCACAGTCAATTTTAACAGG R: GATGCTTCTATGATGAACCCCAGT	56	NA*	This study
a28	2L	F: GGGGCACACTGATTTATTAAACAA R: TATTTTACGCCACAACTTGCAGCAC	57	*Alu*I	Chang et al. 2008
a52	neo-X	F: TATTCATCGCATTCCACAT R: GGCTTCCTCAATCAACTG	53	*Hae*III	Chang et al. 2008
a386	neo-X	F: GTTACGATTACGAAGAGTGC R: CTGCCGTGCTTATGTGAT	51	*Xmn*I	Chang et al. 2008
a708+	2R	F: GAAAAGGGCGAACAGATAGA R: AACAGGAACATAGAAATCAC	55	*Xmn*I	Chang, 2011
a1185	neo-X	F: ATTCTGTCGTTCGTTTTGA R: GATTTCGGCTTACATTATTG	49	*Sty*I	Chang et al. 2008
a1350new	neo-X	F: TACGACCCCGTCAAAGGCTGTG R: GGCTTGTATGCGATTCTGC	52	*Hpa*II	Chang, 2011
a1953	neo-X	F: GCCAACAGCGAGCCTTCT R: GCGACCCAAGCACGAATC	56	*Dde*I	Chang et al. 2008
A185D	2R	F: CAAACGCTCTGGAATAATGG	55	*Rsa*I	Chang, 2011

		R: CTCGGGAGTGTGGGGC			
c29	neo-X	F: CTGGGCAAAGAGTGTAGG R: CAGAAGGAGGGCGAAAA	57	*Rsa*I	Chang et al. 2008
c3242	neo-X	F: TTGAAGCGCAGTTTATGCAC R: TACAACCACGACCTGGACAA	62	*Ssp*I	Chang, 2011
c4081	2L	F: GCCCTGGAACAAAGTGAAAA R: AGTCTGCTGCGTATGGTCAA	52	*Hae*III	This study
c5237	2L	F: TATATGTTCCTCCTGATTGG R: AAGTTTAAACGCGAACTTTT	55	*Pst*I	Chang, 2011
c5665	2L	F: TGAAACATTATTACCGCCTG R: GATGACGACGACGATTCC	60	*Hae*III	This study
c7198	2L	F: GTGGGAAGCACGTTACAT R: CCATGAACAGTCTGAAGTTT	57	*Mse*I	Chang, 2011

*NA: not applicable

Results

Parthenogenetic capability

Among sexual strains, #163.5-IA (n = 16) and #55.1 (n = 72) were not found the performance of parthenogenesis, but #56.1, collected from Wulai, Taiwan was discovered to be able to perform parthenogenesis (i.e., at least 2.9% among 69 virgins). KKU202 (n = 133) did not perform parthenogenesis but KKU204 (i.e., at least 10% among 108 virgins) and KKU210 (i.e., 1% among 92 virgins) performed parthenogenesis. The F_1 females produced by crosses KKU119 X #55.1 and KKU119 X #163.5-IA can still perform parthenogenesis, but their parthenogenetic capability decreased to 75.3% and 69.0% for KKU119 X #55.1 and KKU119 X #163.5-IA, respectively (Table 4) while all (100%) KKU119 females can do parthenogenesis. The F_1 parthenogenetic capability did not show statistically significant ($\chi^2 = 1.28$, $p > 0.05$) difference between two cross sets.

Table 4. The parthenogenetic capability of sexually produced F_1 females and virgins from parthenogenetic strain and sexual strains

	Virgin (n)	Parthenogenetic capability (%)[a]
Parthenogenetic strain	KKU119 (34)	100.0
Sexual strains	#55.1 (72)	0.0
	#56.1 (69)	2.9
	#163.5-IA (16)	0.0
	#193.1 (130)	0.0
	KKU202 (133)	0.0
	KKU204 (108)	10.2
	KKU210 (92)	1.1
Crosses	F_1 from KKU119♀ X #55.1♂ (146)	75.3
	F_1 from KKU119♀ X #163.5-IA♂ (113)	69.0

[a] The parthenogenetic capability was measured by the percentage of those producing offspring among virgin females lived at least one month.

Although the parthenogenetic capability among sexually derived F_1 females was high, the average progeny number was only 1.4 ± 1.4 for those F_1 from KKU119X#55.1 and only 1.1 ± 1.1 for those from KKU119X#163.5-IA per female in a month; this is much lower than that produced by KKU119 females (Mann-Whitney *U* test, $p < 0.001$).

Chromosomal inversion *In*(*2L*)B_1D_5 analysis

All thirteen larvae from KKU119 and 10 from #55.1were homozygotes. Forty three offspring from 10 crosses between them were all *In*(*2L*)B_1D_5 heterozygotes. Determined by crossing with the #163.5-IA which is a *2L*(B_1D_5) I/I strain, KKU119 is N/N, and #55.1 is I/I. Other eight sexual strains were also checked for their *In*(*2L*)B_1D_5 arrangements (Table 5). Twelve larvae of #56.1 showed 75% heterozygous

Table 5. The *2L*(B_1D_5) in 9 sexual strains and a parthenogenetic strain

	Stocks		after crosses with #163.5-IA (no. of pair)		Status of strain
Strains	Heterozygote	Homozygote	N/I	I/I	
#55.1	0	10	0	3 (3)	I/I
#56.1	9	3	NA*	NA	polymorphism
#193.1	0	7	5 (1)	0	N/N
#281.4	8	1	NA	NA	polymorphism
#296.6	0	7	3 (2)	0	N/N
#300.8	5	0	NA	NA	I/N
KKU119	0	13	13 (3)	0	N/N
KKU202	0	11	0	4 (2)	I/I
KKU204	7	4	NA	NA	polymorphism
KKU210	7	3	NA	NA	polymorphism

*Not done

In(*2L*)B_1D_5. The strains, #193.1 and #296.6, were 100% homozygous *In*(*2L*)B_1D_5 with 7 larvae for each strain and through crossing with #163.5-IA they are determined to be

N/N. #281.4 showed 89% (n = 9) heterozygousity of *In*(*2L*)B_1D_5 and #300.62 showed 100% heterozygosity (n = 5). The heterozygosity of *In*(*2L*)B_1D_5 are 0% (n = 11), 58% (n = 11), and 63% (n = 10) for KKU202, KKU204, and KKU210, respectively. Four offspring of crossing between KKU202 and #163.5-IA showed all homozygous, indicating that KKU202 is I/I homozygous.

Reproductive patterns of parthenogenetic females

Parthenogenetic fecundity was significantly lower than that in sexual reproduction either by KKU119 or by #55.1 (Mann-Whitney *U* test, $p < 0.001$) and the fecundity of sexual reproduction by KKU119 was also significant lower than that of #55.1 (Mann-Whitney *U* test, $p < 0.001$). KKU119 parthenogenetically reproduced 43.0 ± 14.4 (n = 34) in 4 weeks, while 4-day-old KKU119 and #55.1 reproduced 182.9 ± 106.0 (n = 41) and 319.8 ± 100.3 (n = 18) after mating, respectively; 11-day-old from KKU119 and #55.1 reproduced 140.7 ± 77.9 (n = 21) and 358.6 ± 17 5.9 (n = 31), respectively. The comparison was shown in Figure 1. In addition, the hatchability was 23% (23/100) in virgin KKU119 which was lower than that of the sexual strain #55.1 X #55.1 (88/100 = 88%).

Mated KKU119 females reproduced offspring not only by sexual reproduction but also by parthenogenesis. Sex ratio (male/total) was calculated for each pairs of KKU119 X #55.1 and #55.1 X #55.1 for each week. Since the males were from same strain, #55.1, we did the comparison of sex ratio, KKU119 X #55.1 produced female biased offspring in week 1, week 3 and week 4 (Figure 2). By genotyping the female offspring, we confirmed the parthenogenetically produced individuals existed and found that estimated frequency of

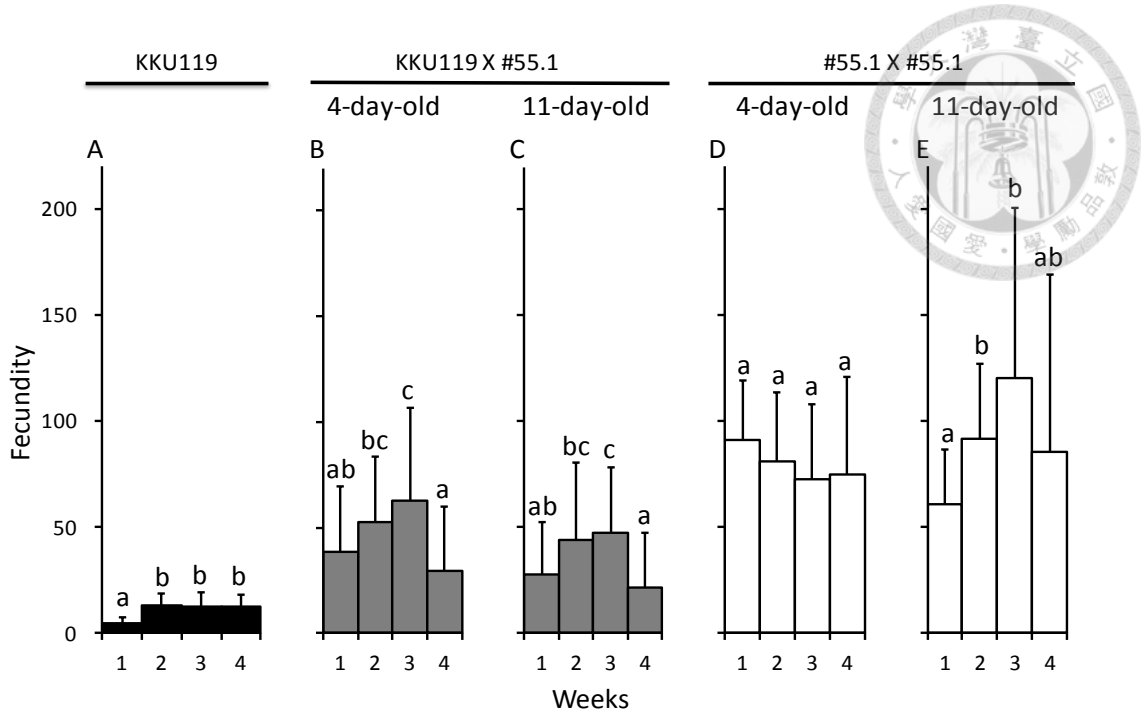


Figure 1. Pattern of fecundity of parthenogenesis and sexual reproduction by KKU119 and sexual reproduction by #55.1. (A) The parthenogenetic fecundity of individual virgin females from 3-4 days of age. (B) and (C) The fecundity of KKU119 X #55.1 with females crossed at 4- and 11-day old, respectively. (D) and (E) The fecundity of #55.1 X #55.1 with females crossed at 4- and 11-day old, respectively. X-axis denotes weeks after mating. Y-axis denotes fecundity. Error bars are SD. [a-c] different letters mean significant difference (Mann-Whitney *U* test, $p < 0.05$).

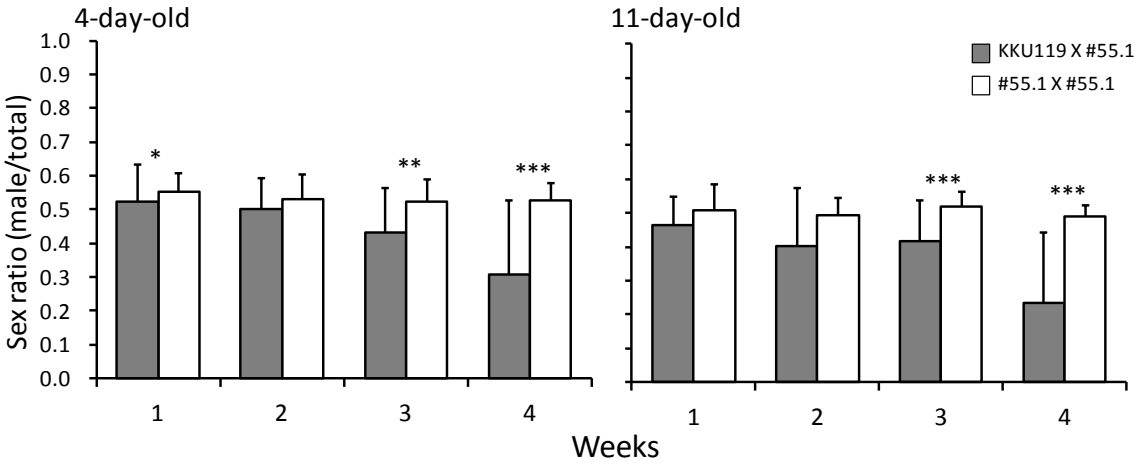


Figure 2. Comparison of the F_1 sex ratio between KKU119 X #55.1 and #55.1 X #55.1. Sex ratios were calculated for each pair with females crossed at 4- and 11-y old. X-axis denotes weeks after mating. Y-axis denotes sex ratio (male/ total). Error bars are SD. Mann-Whitney U test: [*] $p < 0.05$, [**] $p < 0.01$, [***] $p < 0.001$.

parthenogenetically produced females and the proportion of female offspring is positively correlated (R = 0.58, $p < 0.001$, Figure 3). It was revealed that these females still carry out parthenogenesis after mating.

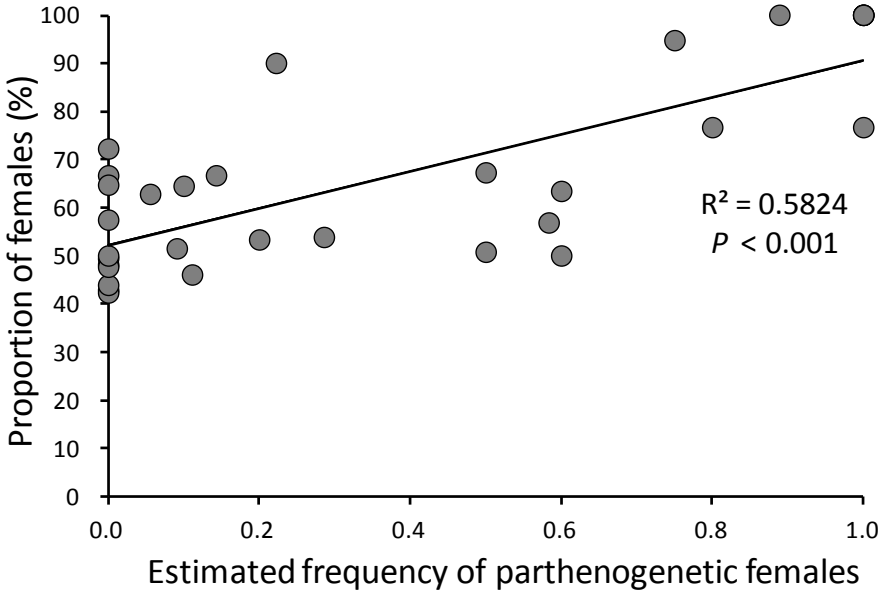


Figure 3. Positive correlation between the estimated frequency of parthenogenetically produced female and proportion of female among offspring produced by mated KKU119 females. The proportion of females was calculated as number of female against the total in one week. The frequency of parthenogenetically produced females was estimated as the number of parthenogenetically produced females among total number of genotyped females.

Mating propensity

In 30 minutes after setting up single pairs, not even one KKU119 female (n = 20) mated while among # 55.1 females about 63.2% 4-day-old females (n = 19) and 30% 11-day-old ones (n = 20) did. In 2 hours, mating propensity of 4-day-old and 11-day-old parthenogentic females increased to 35% and 10%, respectively, but they were still lower than those of sexual females (4 day-old: 84.2%; 11-day-old: 75%). Using #55.1 as control, all four comparisons (i.e., two age groups x two observation

periods) showed significantly lower mating propensity for KKU119 females (Fisher's exact test, $p < 0.05$, Table 6). In addition, all 4-day-old KKU119 females (n = 39) reproduced male offspring in 4 weeks and 32% of 11-day-old KKU119 females (n = 31) did not reproduce any male offspring

Table 6. Mating propensity of virgins from KKU119 and #55.1

			Mating propensity			
			30 min		2 h	
Age group (days old)	Strain	Sample size	%	*p*-value*	%	*p*-value
4	KKU119	20	0	< 0.001	35.0	< 0.01
	#55.1	19	63.2		84.2	
11	KKU119	20	0	< 0.05	10.0	< 0.001
	#55.1	20	30.0		75.0	

*Fisher's exact test

Diploidization mechanisms

Based on the prediction shown in Table 2, the diploidization mechanisms determined from the genotyping result of 200 F_2 is shown in Table 7. Since there was no difference between the two sets ($\chi^2 = 0.33$, $p > 0.05$), the data were pooled together. Among these F_2, 90 of them were determined to be gamete duplication (GD), 5 terminal fusion (TF) cases and one central fusion (CF) with markers on neo- X chromosome arm. Two individuals were heterozygous for both markers (*PS, PS*) on the neo-X chromosome arm but genetic markers on the 2nd chromosome showed that one of them with all three markers heterozygous and the other with both available markers homozygous. The former one has a high possibility to be CF because all five markers on both 2nd and neo-X chromosome arm were heterozygous, while the latter

Table 7. F_2 genotypes and the inferred diploidization mechanisms

Genotype[a]	Mechanism[b]	No. of F_2		
		#55.1 cross	#163.5-IA cross	total
PS,*PS*	CF[c]	1	0	1
PS,*PS*	TF[c]	0	1	1
PS,*SS*	CF	0	1	1
PP,*PS*	TF	1	3	4
SS,*PS*	TF	1	0	1
PP,*PP*	TF, GD, or CF	34	41	75
SS,*SS*	TF, GD, or CF	12	15	27
SS,*PP*	GD	29	21	50
PP,*SS*	GD	19	21	40
Total		97	103	200

[a] The genotypes of two markers on the neo-X chromosome arm are separated by a comma: the proximal a1350+*Hpa*II, the distal c29+*Rsa*I. S: the allele from sexual strains (i.e., #55.1 and #163.5-IA); P: the allele from the KKU119.

[b] Three diploidization mechanisms for short, TF: terminal fusion; CF: central fusion; GD: gamete duplication.

[c] These two individuals were further determined by the 2nd chromosome markers (see text).

one is still undistinguishable for it could be either CF with a crossing over between the proximal marker and the centromere on the 2nd chromosome or TF with a crossing over between the proximal marker and the centromere on the neo-X chromosome arm. To sum up, 6 of these F_2 progeny were determined to be TF, 2 CF and 90 GD. One hundred and three out of 200 (i.e., over half of the data) cannot be determined, and therefore, we adopted two approaches to estimate the frequencies.

The first estimation method was based on the confirmed data of fusion. With the assumption of 50% recombinants between two markers on the neo-X chromosome arm, the TF proportion was estimated to be 6% (i.e., 6*2/200) and CF to be 1% (i.e., 2/200 although uncertain whether it was CF or TF. The GD thus was estimated as 93.5%. The other method is based on the confirmed GD frequencies. GD frequencies

were 90% (i.e., 90*2/200). The two methods did not show statistically significant difference, the average GD frequency was 92%.

Multiple diploidization mechanisms can occur in single females. There were 19 F_1 females in #55.1 cross and 23 F_1 females in #163.5-IA cross produced more than one offspring. Among them, 10 F_1 in #55.1 cross and 5 F_1 in #163.5-IA cross provided clear evidence for having both GD and TF mechanisms in one individual (Table 8).

Table 8. Females produced parthenogenetic offspring by multiple diploidization mechanisms

Cross set	F_2 number	Gamete duplication	Fusion*	Uncertain
KKU119 X #55.1	2	1	1	
	4	1	1	2
	4	2	1	1
KKU119 X #163.5-IA	2	1	1	
	3	1	1	1

*Fusion: terminal fusion or central fusion

Genetic mapping

Among F_2, 53.3% (n = 105) performed parthenogenesis and produced 16.7 ±16.2 offspring in 4 weeks. The fecundity formed a continuous distribution (Figure 4). Since *PS* frequency was too low (i.e., in average 1.5% among all markers), the data was excluded from the analysis for simplicity. Firstly, the association between genotypes and parthenogenetic capability (i.e., whether or not able to perform parthenogenesis) was done for each 14 PCR-RFLP marker. Six (a28, a708+, c4081, c5665, c5237, and c7198) of the 7 markers on the 2nd chromosome showed statistically significant association (Fisher's exact test, $p < 0.01$ for c5237 and $p < 0.001$ for other 5 markers) with parthenogenetic capability (Figure 5) but none of 7 markers on the neo-X chromosome arm showed significant association (Fisher's exact test, $p > 0.05$). High

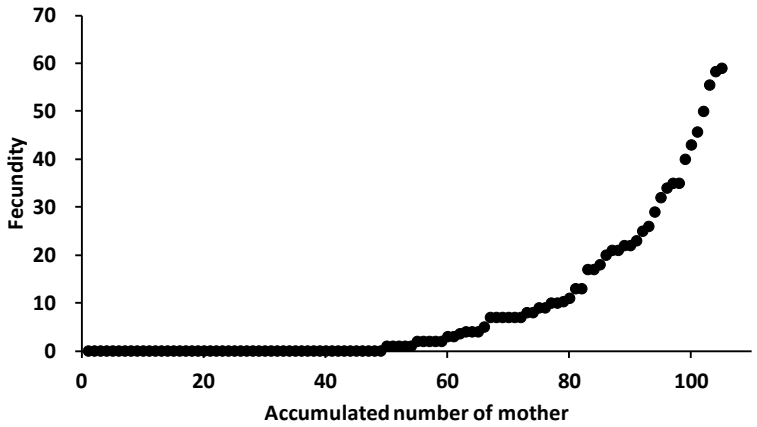


Figure 4. Fecundity of F_2 through parthenogenesis. Each dot represents an F_2 female and orders with fecundity in 4 weeks. Sample size includes individuals *PP*, *PS*, and *SS*.

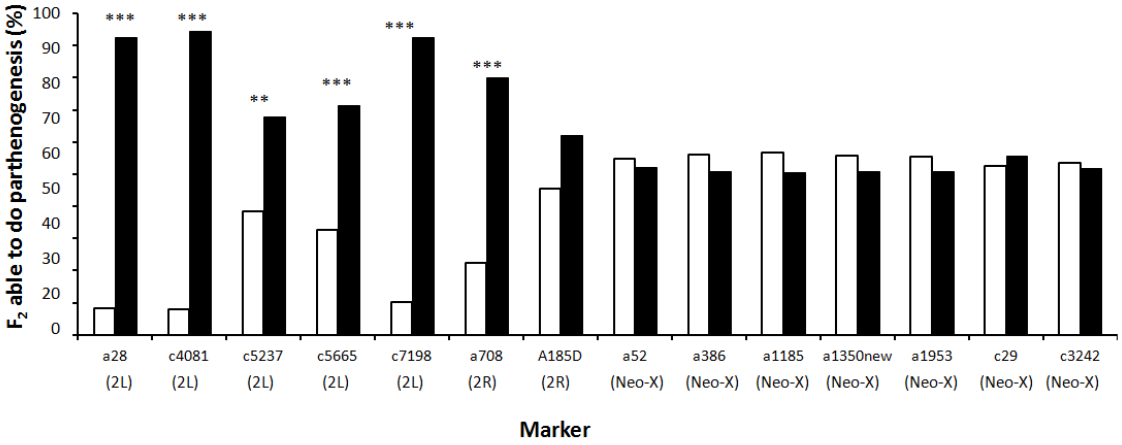


Figure 5. The proportion of F_2 individuals able to perform parthenogenesis with *PP* (black) or *SS* (white) genotypes for each marker. Sample size is 100 to 105 Marker with significant association with genotypes and F_3 offspring through Fisher's exact test, according to Fisher's exact test $^{**}p < 0.01$, $^{***}p < 0.001$. Locations of markers are shown in parentheses.

linkage disequilibrium values appeared among all 6 markers strongly associated with parthenogenetic capability (Table 9). Recombinant individuals were further checked with a28,which has known location near centromere revealed by *in situ* hybridization (Table 3), and c5237, c5665, and a708+ were excluded for they were significantly different from a28 which was always associated with parthenogenetic capability in

Table 9. Linkage disequilibrium for markers shown to be highly correlated with parthenogenesis

	a708+	c4081	c5237	c5665	c7198
a28	0.93	1.00	0.59	0.71	1.00
a708+		0.92	0.42	0.68	0.91
c4081			0.63	0.74	1.00
c5237				0.54	0.61
c5665					0.69

those pair-wise comparison (Fisher's exact test, $p < 0.01$, Table 10). The amount of recombinants was too small to discriminate the contribution of a28, c7198 and c4081. Secondly, the fecundity was significantly correlated with the number of *PP* for all the other markers (ANOVA test, $p < 0.01$). The three capability related markers (i.e., a28, c7198, c4081) were excluded for their lack of resolution power. Through "jackknife", the insignificance was not shown for excluding any one of those markers.

Table 10. Number of recombinant F_2 categorized into genotypes and whether they produced F_3 offspring parthenogenetically

Paired of markers with recombination	Genotypes	Number of F_2		*p*-value*
		without F_3	with F_3	
a28, c5237	*SS*, *PP*	15	1	< 0.001
	PP, *SS*	2	17	
a28, c5665	*SS*, *PP*	15	0	<0.001
	PP, *SS*	3	12	
a28, a708+	*SS*, *PP*	8	0	< 0.01
	PP, *SS*	1	7	

*Fisher's exact test

After 9 generations of sexual reproduction between KKU119 and #55.1

KKU119 females performing sexual reproduction with #55.1 males for 9 generations, then 10^{th} generation (F_{10}) virgins were collected to perform parthenogenesis. 14 vials among 23 vials of F_{10} (n = 254) reproduced F_{11} parthenogentically. F_{11} reproduced parthenogenetically were checked their

parthenogenetic capability and the genotypes (Table 11). Among 14 females, 4 of them performed parthenogenesis with low fecundity (i.e., one offspring for 3 mothers and 3 offspring for one mother). The genotypes of a28, a7198, and c4081 were *SS* for all tested F_{11}, except the one with 3 offspring carrying *PP* for c7198. The marker c29 and a1350new did not show any association with parthenogenesis.

Table 11. Parthenogenetic capability and genotypes after 9 generation of sexual reproduction between KKU119 and #55.1

Parthenogenetic capability and fecundity of F_{11} females	no. of F_{11}	a28	a1350new	c29	c4081	c7198
3 offspring	1	*SS*	*PP*	*PP*	*SS*	*PP*
1 offspring	1	*SS*	*SS*	*PP*	*SS*	*SS*
	2	*SS*	*PP*	*PP*	*SS*	*SS*
without offspring	2	*SS*	*SS*	*PP*	*SS*	*SS*
	5	*SS*	*SS*	*PP*	*SS*	*SS*
		SS	*SS*	*SS*	*SS*	*SS*
	2	*SS*	*PP*	*SS*	*SS*	*SS*
	1	*SS*	*PP*	*PP*	*SS*	*SS*

Discussion

Disadvantages of parthenogenesis

Low fecundity and loss of genetic variation are two disadvantages of parthenogenesis in *Drosophila albomicans* and most parthenogenetic *Drosophila* species also have these two disadvantages (Stalker 1954, Carson 1967, Futch 1972, Templeton 1983). According to the cytological studies in *D. mercatorum,* lack of paternal basal body of centrosome and a high failure rate of *de novo* centrosome formation during embrogenesis (Eisman and Kaufman 2007) may be the reason for low fecundity. High frequency of gamete duplication performed by most parthenogenetic *Drosophila* leads to the loss of genetic variation, such *as D. albomicans, D. ananassae*-complex and *D. mercatorum* (This study, Carson et al. 1969, Carson 1973, Matsuda and Tobari 2004). In other words, most parthenogenetic *Drosophila* may suffer loss of genetic heterozygosity.

Sustention of parthenogenesis in natural populations

Facultative mode of parthenogenesis contributes to the sustention of parthenogenesis in *D. albomicans*. Although parthenogenetic fecundity is low, the amount of offspring produced by sexual reproduction is significantly higher; therefore, alleles for parthenogenesis would be amplified through the contribution of sexual reproduction. Moreover, the genes could express in those heterozygous F_1 female offspring. Since sexual reproduction amplified parthenogenesis alleles are functional, in natural populations, alleles can be propagated through sexual reproduction. All virgins (n = 39) in KKU119

females mated with males when they were young (4 days old), thus genetic variation will be reintroduced by sexual reproduction. Moreover, the mated females performing parthenogenesis is also helpful for the sustention because parthenogenetically produced females can again generate about 43 daughters while sexually produced F_1 females can only give birth to about 1 daughter parthenogenetically. Additionally, predominant gamete duplication is advantageous for parthenogenesis under facultative condition for it can eliminate alleles deleterious for parthenogenesis and, through recombination, incorporate beneficial ones. To answer why parthenogenesis is sustained in natural populations, our study suggests that facultative mode of parthenogenesis is the main contribution through amplification of alleles for parthenogenesis and reintroduced genetic variation.

Parthenogenesis is adaptive for females to colonize in the isolated area or the border of distribution with the small population size in which it is difficult to find mates. This geographic parthenogenesis has been documented in various organisms (Cuellar 1977, Bell 1982, Suomalainen et al. 1987). In *Drosophila*, there is a supportive evident for parthenogenesis being beneficial for colonization. *Drosophila mangabeira* is the only one in this genus that reproduces absolutely by parthenogenesis, and they may use very specialized breeding sites in Central and South America (Carson et al. 1957). Facultative parthenogenesis is performed in most *Drosophila* species. Even though, there is also an example that facultative parthenogenesis facilitates population dispersal, which is *D. albomicans*. The isofemale for establishing the parthenogenetic *D. albomicans* strain was indeed collected at the distribution border of the species (Ohsako and Fuyama 1995),

and females there may have difficulty to meet males after cold winters. Parthenogenesis may rescue the populations from extinction. In addition, females tend to produce offspring by parthenogenesis after mating if they were old, about one third of old females from the parthenogenetic strain did not reproduce any male offspring even males available. The time of meeting males might be an indicator of population density. This phenomenon might be advantageous for the population, because parthenogenetic fecundity of females produced by parthenogenesis is much better than that of those produced sexually.

Unlikely to evolve to obligate parthenogenesis eventually

The only species *D. mangabeirai* performing obligate parthenogenesis was reported, and they operate central fusion as the major diploidization mechenisms (Murdy and Carson 1959). There were about 50% of its unfertilized eggs could develop to adults while no more than 8% was found in facultative parthenogenetic species (Carson et al. 1957, Markow 2013). It may suggest that central fusion is a more suitable mechanism for obligate parthenogenesis.

In a natural population with facultative parthenogenesis, the spontaneously successful development of unfertilized eggs is rare (Templeton 1983). However it provides the possibility to select for parthenogenetic strains (Kramer and Templeton 2001). In our long-term culture of parthenogenesis, there is a common evolutionary consequence in parthenogenetic lineages, which is increased the hatch rate and fecundity under selection effectively (Stalker 1954,

Carson 1967).The mating propensity also reduced obviously, which might be resulted from 20-year parthenogenesis without males and therefore without natural selection acting on the maintenance of mating behavior. This is also observed in *D. mercatorum* (Carson et al. 1982). The parthenogenetic strains may be similar to a situation of no males in natural populations. Due to no selection for the maintenance of mating propensity, potentially, those females lose the mating ability and only can perform parthenogenesis eventually, which is so-called obligate parthenogenesis. However, in nature, it is likely that there are other environmental factors leading to the loss of males. There is little genetic variation as the resource for selection to adapt the changed environments because of gamete duplication. Therefore, it is unlikely to that a parthenogenetic strain can evolve to a successful obligate parthenogenetic strain from under changed environments not to mention one with central fusion, which is more suitable for obligate parthenogenesis. In natural populations, because of abundant genetic variation, it is not impossible to evolve to obligate parthenogenesis.

One major genetic element for parthenogenesis

There may be one major genetic element contributing to parthenogenetic capability with dominant effect and several quantitative loci for parthenogenetic fecundity with addictive effect. After 370 generations of parthenogenesis, essential genes exist in every individual and all females are able to do so. After crossing with a sexual strain, F_2 were mainly homozygous recombinants due to meiotic recombination and high frequency of gamete duplication performed by heterozygous F_1. About half F_2 were able to perform partheneogenesis and the fraction of females capable of parthenogenesis decreased 50%

(i.e., from almost 100% in KKU119 to nearly half in F_2). It is consistent with the expectation that, for each marker, 50% F_2 carrying homozygous alleles from KKU119 and the other 50% carrying homozygous alleles from #55.1. It suggests that there may be a major gene for parthenogenesis. However, due to different chromosomal inversion arrangements carried in KKU119 and #55.1, we could not tell apart whether there is one gene or a cluster of genes inside the inversion loop. Moreover, the sexual strain, #55.1 does not have parthenogetic capability, so the ability in F_1 heterozygous females is only caused by alleles from the parthenogenetic strain. High proportion (75%) of heterozygous F_1 could perform parthenogenesis. The percentage of F_1 capable for parthenogenesis may be underestimated because, based on a Poisson distribution, with mean of 1.4 the portion with less than 1 cannot be observed, and the probability of less than 1 is 0.247. Therefore, it suggests that the major element contributes to parthenogenesis by dominant effect.

In addition, there are several quantitative loci for parthenogenetic fecundity. The fecundity of F_2 showed a continuous distribution as expectation of a quantitative trait (Figure 4), which may suggests many loci with additive effect contributing to parthenogenetic fecundity. It is supported by the significant correlation between the fecundity and the number of *PP*. Since almost all F_2 performing parthenogenesis carried *PP* for a28, c7198, and c4081, we did not know the contribution of this region for their fecundity. After the genotypes of these three markers were excluded from the analysis, the correlation was also significant ($p < 0.01$). It is suggests that the fecundity is regulated by other loci. Though jackknifing, the significant correlation is always shown no matter which marker was deleted from the re-sampling; it may correspond to quantitative loci. However, we could not map these minor genes because of limited

sample size.

Further, we determine the location of those markers strongly associated with parthenogenetic capability in order to locate the gene for parthengenetic capability. There is an inversion loop on the 2L arm in the region between B_1 and D_5, two markers, a28 and a708+, with known locations revealed by *in situ* hybridization (Chang et al. 2008, Chang 2011), and recombination rates among 7 markers on the 2nd chromosome (Figure 6). The genetic distances were roughly estimated by recombination rate, but

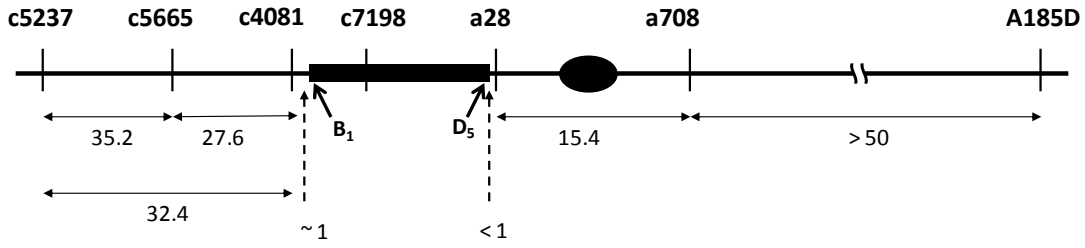


Figure 6. The genetic distances are shown by percentage of recombination rate. The recombination rate statistically indistinguishable from 50% was marked as > 50 cM. Black horizontal line shows 2nd chromosome, and black oval dot represents a centromere. The black bar indicates *In*(*2L*)B_1D_5 region and D_5 is near the centromere. Dashed arrows point the distance between c4081 to breakpoint B_1 and that between a28 and breakpoint D_5 (See text).

were all underestimated due to the large spacing (i.e., > 10%). The marker a28 had *in situ* location showing that it is very near to D_5 at the basal region of 2L (Chang et al. 2008). Since there was only 1 recombinant individual between c4081 and a28, c4081 may be either located near a28 or near B_1 which is a breakpoint of the inversion The marker a708+ is at the basal region of 2R arm based on *in situ* hybridization (Chang 2011). The recombination rate between a708+ and a28 was 15.4% and the recombination rate between a708+ to c4081 was 16.2% indicating that c4081 is more likely to be located near B_1 breakpoint of the inversion (Figure 6); the recombination

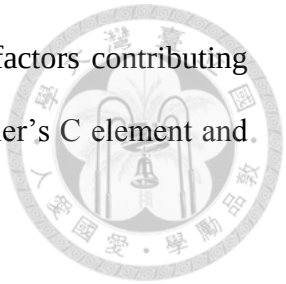
rate 17.3% between a708+ and c7198 indicates that c7198 should be next to c4081 and relatively closer to the distal end of 2L. However, conflicting to the previous reasoned determination, 2 recombinants with a crossing over between closely linked a28+c4081 and c7198 (i.e., *PP*, *PP*, *SS* and *SS*, *SS*, *PP* in order of a28, c4081, and c7198) indicate the possible location of c7198 at either side of the former two markers with a distance of 1.9 cM; one recombinant with a crossing over between closely linked c7198+a28 and c4081 (i.e., *PP*, *PP*, *SS* in order of c7198, a28, c4081) indicates that c7198 should be next to a28 and closer to the centromere. This conflict could be solved by supposing c7198 located between c4081 and a28. Therefore, the marker c7198 is probably located in the B_1D_5 inversion loop. 2 recombinant individuals were generated by double crossing over between c7198 and both of the other two markers (*PP*, *SS*, *PP* and *SS*, *PP*, *SS* in the order of c4081, c7198, a28), and one single crossing over between c4081 and c7198 suggests that the crossing may occur outside the breakpoint B_1 with the estimated genetic distance about 1 cM. Since no crossing over was found outside the breakpoint D_5, the distance between a28 and the breakpoint D_5 is estimated to be less than 1 cM (Figure 6).

Suppose there is a parthenogenetic capability gene, and the gene must be from the parthenogenetic stain for the other strain didn't produce any parthenogenetic offspring (Table 4). We assumed that F_2 with this gene were able to do parthenogenesis and those without the gene could not perform parthenogenesis. We estimated the recombination rates between the supposed parthenogenetic capability gene and those highly associated markers, which were 7.7%, 8.7%, and 6.7 % for a28, c7198, and c4081, respectively. These three markers are related to a heterozygous inversion loop, and the parthenogenetic capability gene may be located near or inside the loop. The marker

c7198 inside the loop revealed a 1 % double crossing over rate. However, we could not exclude the possibility of the parthenogenetic capability gene inside the inversion loop for we do not know the actual location of c7198. If outside the loop, the capability gene may be located near to the B_1 breakpoint because its 21% recombination rate with a708+ was higher than the 16.2% recombination rate between a708+ and c4081. In addition, it looks contradictory that the sum of distance between c5237 and c5665 and distance between c5665 and c4081 is much larger than distance between c5237 and c4081 (i.e., 0.352 + 0.276 >> 0.324). This is because of the high proportion of double crossing over (i.e., 0.14 for *PP*, *SS*, *PP* plus *SS*, *PP*, *SS* in the order of c5237, c5665, c4081). The rate of single crossing over is 0.352 for between c5237 and c5665 and 0.276 for between c5665 and c4081, and the double crossing over rate is too high. Besides, both the two map distances are also underestimated. A185D may be located at the distal end of 2R arm because its distance with all other 6 markers exceeded 50 cM.

The locations of parthenogenesis gene in *Drosophila* may be different among species. A major gene for parthenogenesis was discovered in *D. ananassae*-complex species and Matsuda and Tobari (2004) mapped it to their 2L arm and that for *D. albomicans* is also located in its 2L arm. However the 2L of *D. ananassae* is Muller's element E while that of *D. albomicans* is Muller's element B. These two lineages may adopt different genes to achieve parthenogenesis. It is still possible that the same gene responsible for parthenogenesis in both species, for the two elements are two arms of the 2nd autosome in *D. albomicans*, and the gene may be relocated by a pericentric inversion. The parthenogenesis gene mapped in *D. ananassae* might involve the post meiotic nuclear doubling after meiosis (Matsuda and Tobari 2004). Fuyama (1986) indicated that *D. melanogaster* can perform gynogenetic reproduction (i.e., females can

reproduce by mating with sterile males) and there were recessive factors contributing for parthenogenesis located between *Tft* locus and *nw* locus on Muller's C element and *Gl* and *Sb* locus on Muller's E element.



Preservation of parthenogenesis through *In(2L)*B_1D_5 polymorphism

In three sexual strains originated from the same location of the parthenogenetic strain, only one of them could not perform parthenogenesis which carried *In*(*2L*)B_1D_5 homozygtes while the other two have high *In*(*2L*)B_1D_5 heterozygosity. Moreover, a 40-year long term laboratory cultured *D. albomicans* sexual strain, #56.1, originated from Wulai population, which has the closest phylogenetic relationship to Japan population (Ohsako et al. 1994), was able to do parthenogenesis under such strong influence of genetic drift and lack of selection for parthenogenesis. In contrast, another two sexual strains, #55.1, which originated from Hualien and #193.1, which originated from Wulai did not perform parthenogenesis and no heterozygous chromosomal inversion was observed. The high *In*(*2L*)B_1D_5 heterozygosity probably is a main factor for the sustention of parthenogenesis in the laboratory strain. Further studies on parthenogenesis natural populations should be done in order to understand the association of sustention of parthenogenesis and the inversion polymorphism.

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Appendix

Appendix 1. Genotypes and phenotypes of F_2 reproduced parthenogenetically by heterozygous F_1 and statistic analysis for each marker

	Fecundity	a28	a52	a386	a708+	a1185	a1350 new	a1953	A185D	c29	c3242	c4081	c5237	c5665	c7198
D5-3-1	0	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*
D2-3-10	0	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*
D5-2-1	0	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D3-2-3	0	*SS*	---	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*
D3-4-2	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*
D3-3-4	0	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*
D3-2-1	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*
D2-3-3	0	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*
D5-1-1	0	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*
D3-1-10	0	*PS**	*SS*	*PP*	*SS*	*PS**	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*
D2-3-13	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*
D1-2-4	0	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*
D5-1-3	0	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*
D5-3-7	0	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*
D4-1-6	0	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	---	*PP*	*SS*	*SS*	*SS*	*SS*
D1-2-5	0	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*
D5-4-3	0	*SS*	*SS*	*PP*	*SS*	*PS**	*SS*	*PS**	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*
D5-3-3	0	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*SS*
D5-4-2	0	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*
D2-3-15	0	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*

M2-4-4	0	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*
M18-2-1	0	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*
M10-17-1	0	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*
M5-16-2	0	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*SS*
M5-3-4	0	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PS**	*PP*	*PP*	*SS*	*SS*	*PS**
M1-5-1	0	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*
M18-3-1	0	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*
M5-2-2	0	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*
M5-2-1	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*
M4-4-1	0	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*
M1-5-3	0	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*
M1-22-1	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*SS*
M5-18-3	0	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*PS**	*SS*	*SS*	*PP*	*SS*
M7-4-2	0	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*
M7-4-3	0	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*
M5-7-2	0	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*
M5-7-1	0	*SS*	---	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*SS*
M7-12-3	0	*SS*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*
M7-12-1	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*
M7-10-3	0	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*SS*
M7-4-4	0	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*
M5-15-2	0	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*
M5-18-1	0	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*SS*
M9-7-3	0	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*
M10-21-1	0	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*
M24-3-4	0	*SS*	*PS**	*SS*	*SS*	*PS**	*SS*	*PP*	*PS**	*PS**	*SS*	*SS*	*PP*	*SS*	*SS*

M13-3-2	0	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*
M13-12-1	0	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*
M19-2-1	0	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*
D2-2-5	1	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*
D3-1-4	1	*SS*	*PP*	*PP*	*SS*	*PS*[*]	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*
D5-2-3	1	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*
D5-2-6R	1	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*
M1-10-2	1	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D4-1-7	2	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PS*[*]	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D5-2-5	2	*PP*	---	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
D2-3-17	2	*PP*	---	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*
M1-16-1	2	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*
M5-3-1	2	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*
D2-1-2	3	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D3-1-14	3	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D2-3-5	4	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*PS*[*]	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*
D2-3-1	4	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PS*[*]	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*
D3-1-3	4	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*
D5-2-2	4	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*
D1-2-1	5	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*
D2-2-3	7	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D1-2-2	7	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*
D3-3-1	7	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D4-1-3	7	*PP*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*
M5-3-3	7	*PP*	---	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*
M13-1-1	7	*PP*	*SS*	*SS*	*PP*	*PS*[*]	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*

D2-3-11	8	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*
M19-1-2	8	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
M28-1-1	9	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*
M29-3-3	9	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
M2-6-1	10	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*	*SS*	*PP*	*PS**	*PP*	*SS*	*SS*	*PP*
M9-7-1	10	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*
D1-2-3	10	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*
M5-6-1	11	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
M2-4-2	13	*PP*	*PP*	*SS*	*PP*	*PS**	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*	*PP*
M5-8-1	13	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*
M7-10-2	17	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*
M13-1-2	17	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*SS*	*PP*
M9-9-1	18	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*	*PP*
M24-3-3	20	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*SS*	*PP*	*PP*
M2-11-1	21	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*
M19-1-1	21	*PP*	*SS*	*SS*	*PP*	*SS*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*
D3-3-3	22	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
M19-5-1	22	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PS**	*PP*	*PP*	*PP*	*PP*
D3-1-15	23	*PP*	*PP*	*SS*	*PP*	*PS**	*SS*	*PP*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*
D4-4-1	25	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*SS*	*PS**	*SS*	*PP*	*PP*	*PP*	*PP*
M18-4-1	26	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*
D2-4-1	29	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
D4-1-5	32	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*SS*	*SS*	*SS*	*PP*	*PP*	*PP*	*PP*
M2-4-1	34	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*
D2-3-7	35	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*
D1-1-3	35	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PS**	*PP*	*SS*	*PP*	*SS*	*PP*	*PP*

M7-4-1	40	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*
M5-2-3	43	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*SS*	*SS*	*PP*
D2-2-7	46	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*
D2-3-9	50	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
D2-4-2	56	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*
D2-1-4	58	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*SS*	*SS*	*PP*	*PP*	*PP*	*SS*	*PP*	*PP*
M13-8-1	59	*PP*	*SS*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*	*SS*	*PP*
	PS	1	1	0	0	7	0	3	3	3	3	0	0	0	1
	PP	56	53	57	56	61	53	57	45	61	48	55	53	56	55
	SS	48	46	48	49	37	52	45	57	40	54	50	52	49	49
	(exp.) PP	52	50	53	53	49	53	51	51	51	51	53	53	53	52
	(exp.) SS	52	50	53	53	49	53	51	51	51	51	53	53	53	52
	***p*-value (Chi-square test)**	**0.43**	**0.48**	**0.38**	**0.49**	**0.02**	**0.92**	**0.23**	**0.23**	**0.04**	**0.55**	**0.63**	**0.92**	**0.49**	**0.56**
	PS/total (%)	1.0	1.0	0.0	0.0	6.7	0.0	2.9	2.9	2.9	2.9	0.0	0.0	0.0	1.0
	SS + w/o offspring	44	22	21	38	16	23	20	31	19	25	46	32	33	44
	SS + w/ offspring	4	24	27	11	21	29	25	26	21	29	4	20	16	5
	PP + w/o offspring	4	24	28	11	30	26	28	17	27	23	3	17	16	4
	PP + w/ offspring	52	29	29	45	31	27	29	28	34	25	52	36	40	51
	Sum	104	99	105	105	98	105	102	102	101	102	105	105	105	104
	***p*-value (Fisher's exact test)**	**$6.29E^{-20}$**	**0.84**	**0.70**	**$2.07E^{-09}$**	**0.68**	**0.70**	**0.69**	**0.11**	**0.84**	**1.00**	**$2.44E^{-21}$**	**0.003**	**$8.63E^{-05}$**	**$6.13E^{-19}$**

---Not available, **PS* were excluded from the analysis

Appendix 2. Recombination rate (%) of markers on neo-X chromosome and 2nd chromosome

Neo-X chromosome

	a52	a386	a1185	a1350new	a1953	c29
a386	27.3					
a1185	33.0	37.8				
a1350new	39.2	45.7	17.3			
a1953	26.8	32.4	44.8	52.0		
c29	46.9	42.6	51.6	46.5	30.6	
c3242	43.8	45.1	20.0	10.8	53.5	50.5

2nd chromosome

	a28	a708+	A185D	c4081	c5237	c5665
a708+	15.4					
A185D	44.6	56.9				
c4081	1.0	16.2	43.1			
c5237	33.7	39.0	45.1	32.4		
c5665	28.8	30.5	40.2	27.6	35.2	
c7198	1.9	17.3	45.5	2.9	32.7	29.8