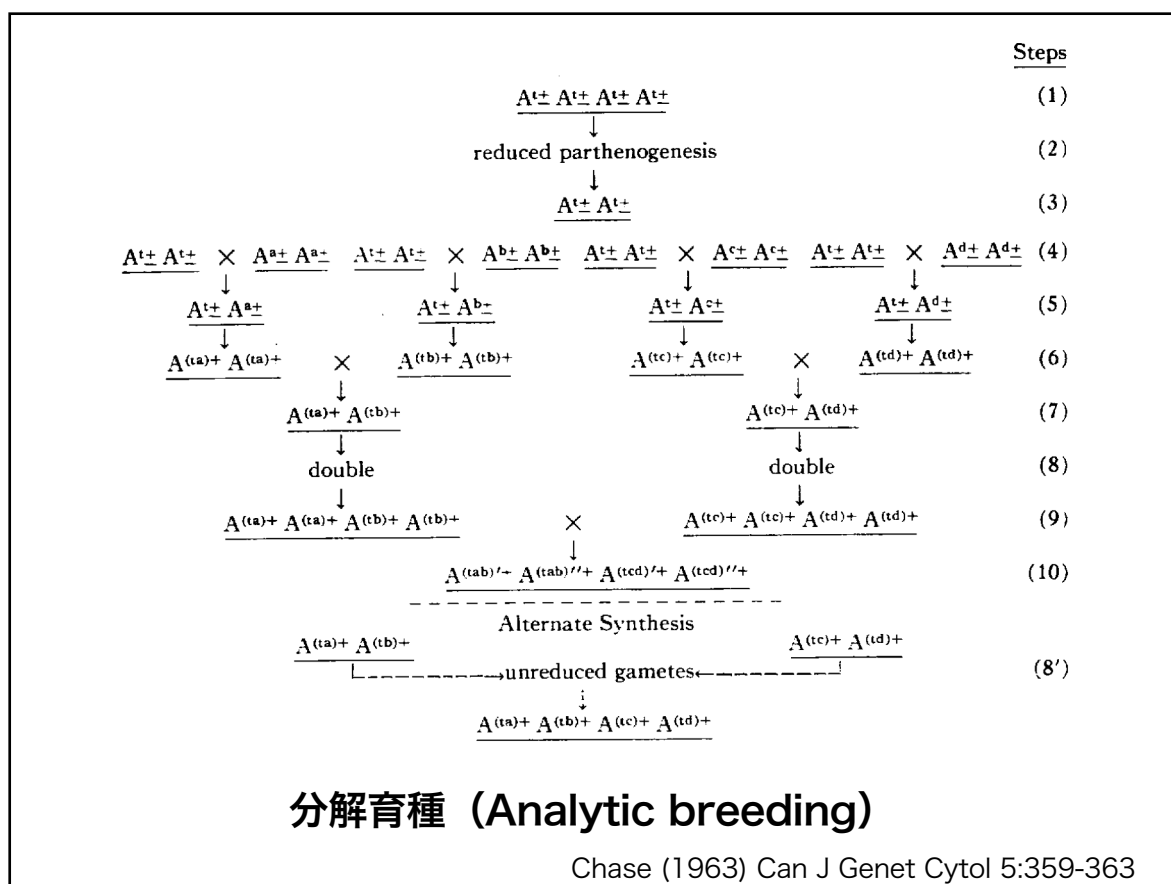


倍数性操作（単数体化と倍数体化）

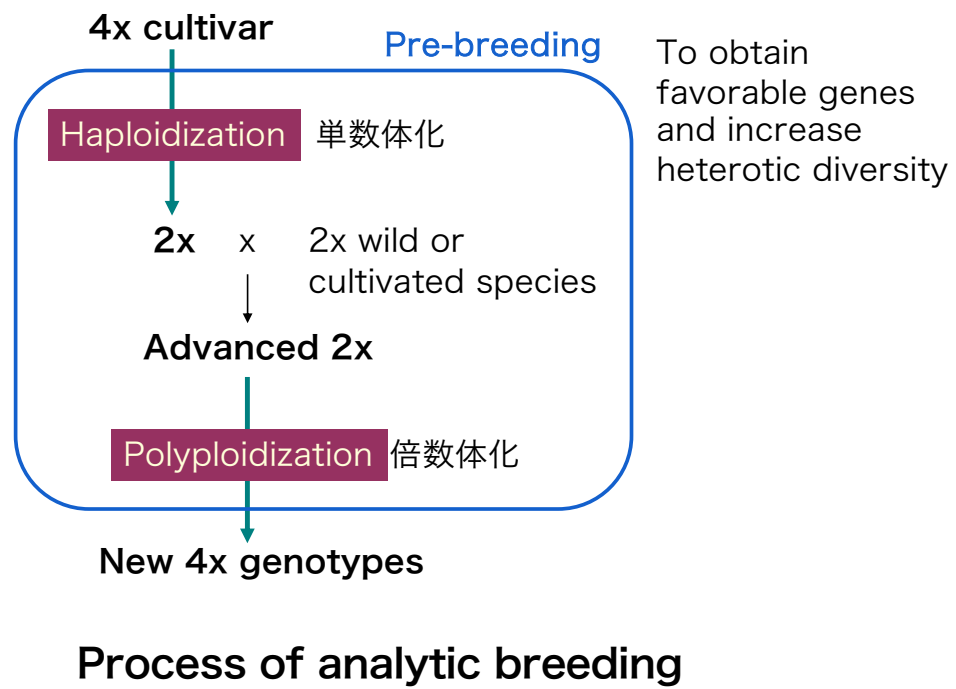


Ploidy manipulation
(haploidization and
polyploidization)

1



2



3

单数体化
Haplodization

4

A Haploid Plant of the Potato Variety Katahdin

A PLANT with a somatic chromosome number of 24 was found among an F_1 population of a cross between the tetraploid *Solanum tuberosum* variety Katahdin and the cultivated South American diploid species *Solanum rybinii*. This plant resembles the female parent Katahdin and lacks any of the distinctive characters of the male parent *S. rybinii*. It is therefore presumed to be a haploid plant of Katahdin which arose through parthenogenesis.

First parthenogenetic haploid

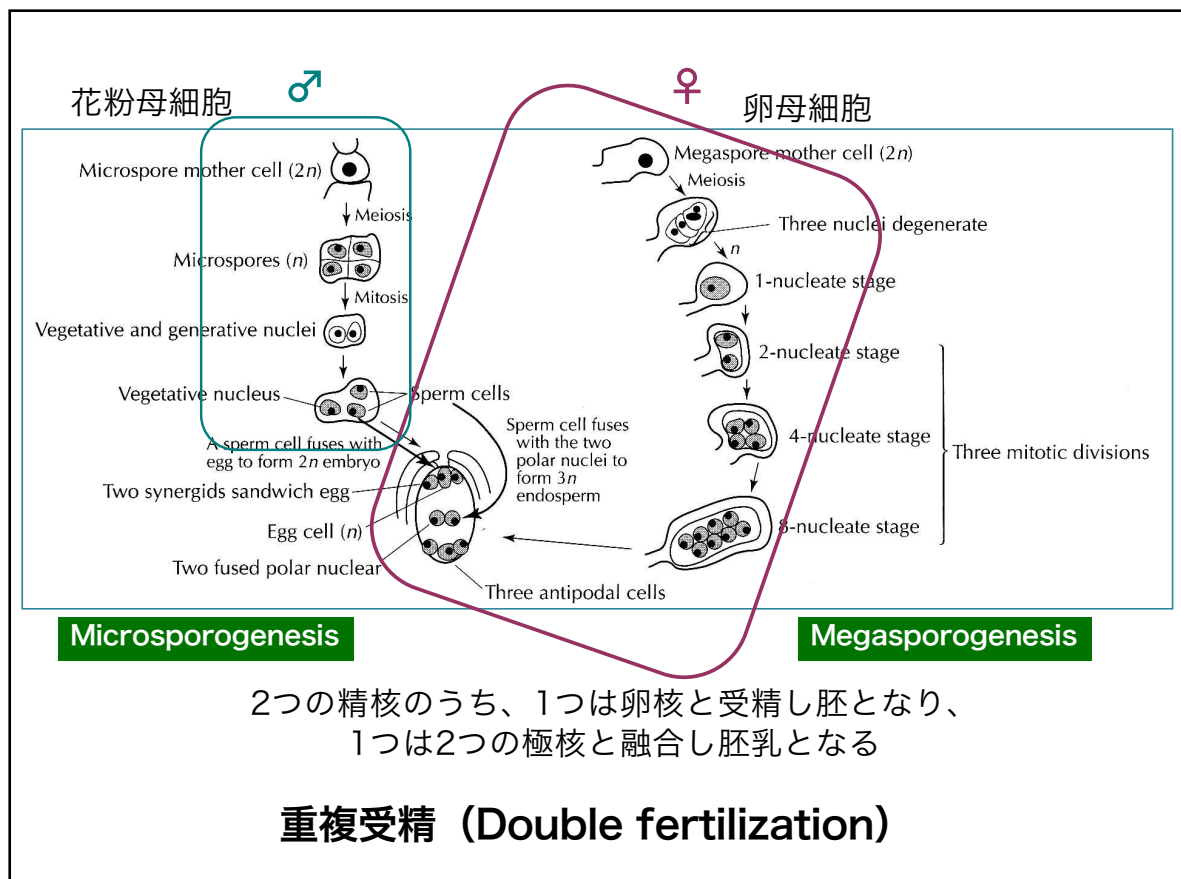
単為生殖による単数体作出



Fig. 1. Plants of haploid (right) and triploid F_1 hybrid

Hougas & Peloquin (1957)
Nature 180:1209-1210

5



6

Ploidy levels in ovules of the cross
cv. Merrimack x *Solanum phureja*

4x x 2x

	胚乳 (Endosperm)		胚 (Embryo)		
正常発育	6x	99%	4x	39%	➡ 2n卵
崩壊	5x	1%	3x	2%	➡ 単数体
			2x	8%	
			None	52%	

期待されるのは

単為生殖 (parthenogenesis) による単数体とは

2つの精核が2つの極核と受精し、卵細胞は受精なしに発達した

Wangenheim et al. (1960) Zeitschrift für Vererbungslehre 91:391-399

7

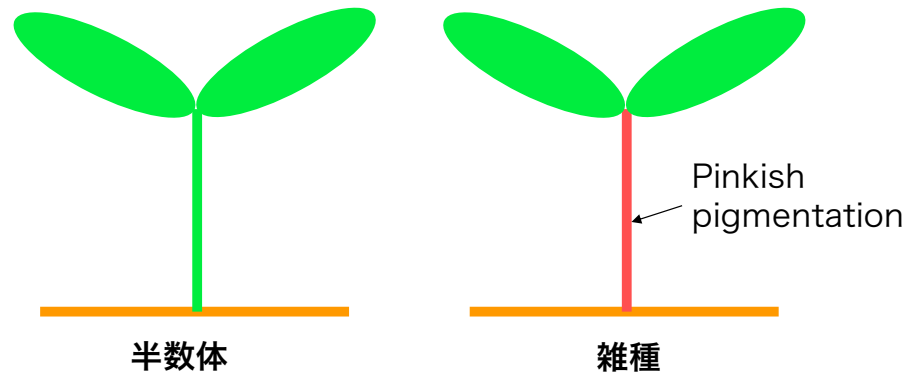
Elite *Solanum phureja* pollinators
単数体誘発系統 (haploid inducer)

単数体誘発系統	選抜マーカー	備考
lvp35	Embryo spot	Hermesen and Verdenius (1973) Euphytica 22: 244-269
lvp48	Embryo spot	
1.22	Hypocotyl color	High frequency of haploids, developed by Peloquin
No. 460	Embryo spot	=lvp35
No. 461	Embryo spot	=lvp48
No. 253	Hypocotyl color	Very high frequency of haploids 入倉 (1975) 北海道農業試験場報告第112号

8

単数体の選抜マーカー

胚軸マーカー (Hypocotyl color marker) (1.22, No. 253)



欠点

有色品種の単数体は着色するので、雑種との識別がつかない
Haploids from pigmented cultivars also show pinkish hypocotyl.

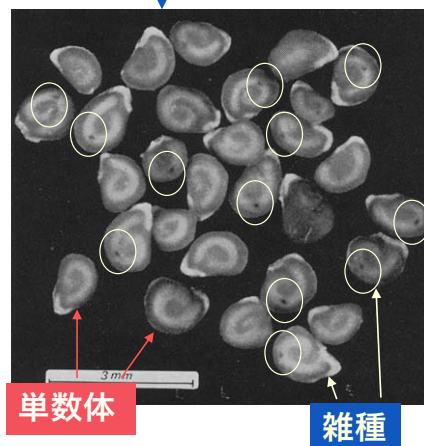
9

単数体の選抜マーカー

胚斑点マーカー (Embryo spot marker) (lvp35, lvp48)

4倍体品種

単数体誘発系統の花粉



単数体誘発系統の持つ単一顕性遺伝子の働きにより、雑種胚は色素を発現し青紫色の斑点として現れる

Hermesen and Verdenius (1973)
Euphytica 22: 244-269

10



PP
self

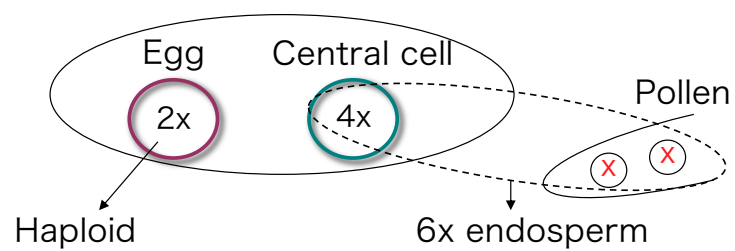
Pp
self

胚斑点マーカの遺伝的分離

11

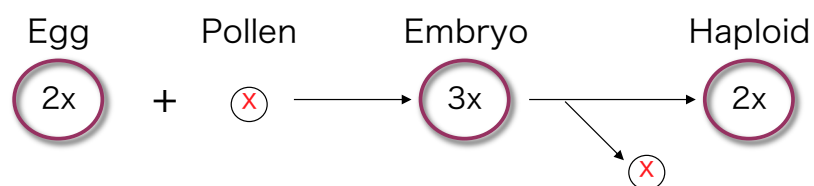
単数体誘発機構

(1) 単為生殖



Wangenheim et al. (1960) Zeitschrift für Vererbungslehre 91:391-399

(2) *S. phureja* 染色体の選択的離脱



Clulow et al. (1991) TAG 82:545-551

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4倍体品種から単数体誘発系統を用いて得られる 単数体作出効率

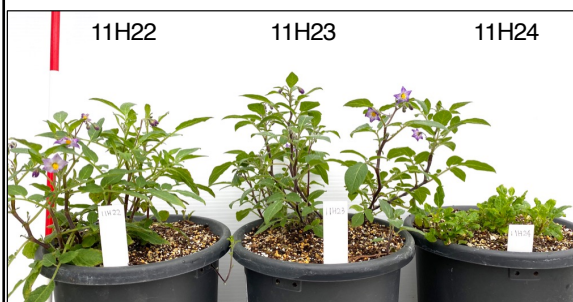
単数体/ 授粉花数	単数体/ 100果実	引用文献
28/6041	?	Hougas et al. (1958)
1/36	5.0	Kawakami and Matsubayashi (1960)
222/49004	1.3	Hougas et al. (1964)
?	53.7	Hermesen (1969)
174/757	33.2	Cipar and Lawrence (1972)
10674/86583	33.8	入倉 (1975)
6/2055	0.7	Ruhde and Hanneman (1979)
662/14898	10.4	Kotch and Peloquin (1987)

Hougas et al. (1958) J Hered 43:103-106; Kawakami, K. & M. Matsubayashi (1960) Jpn J Breed 10:10-18; Hougas et al. (1964) Crop Sci 4:593-595; Hermesen (1969) Euphytica 18:183-189; Cipar & Lawrence (1972) Am Potato J 49:117-119; 入倉 (1975) 北海道農業試験場報告第112号; Ruhde & Hanneman (1979) Am Potato J 56:587-589; Kotch & Peloquin (1987) Am Potato J 64:137-141

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薬培養による普通品種からの単数体作出効率

Haploids/ anthers	Haploids/ 100 anthers	References
1/?	?	Irikura (1975)
34/1770	1.92	Mix (1983)



Dr. Yukio Irikura is the first man who developed a monohaploid ($2n=x=12$) from *Solanum verrucosum* using anther culture (Irikura and Sakaguchi 1972).

Irikura (1975) Potato Res 18:133-140
Mix (1983) Potato Res 26:63-67
Irikura & Sakaguchi (1972) Potato Res 15:170-173

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単為生殖と薬培養の比較

	単為生殖	薬培養
(1) Genetic composition	Female (卵細胞)	Male (花粉)
(2) Applicability	<i>S. acaule</i> (4x)	<i>S. acaule</i> (4x)
	<i>S. demissum</i> (6x)	<i>S. demissum</i> (6x)
	<i>S. tuberosum</i> (4x)	<i>S. tuberosum</i> (4x)
	<i>S. verrucosum</i> (2x)	<i>S. verrucosum</i> (2x)
		<i>S. bulbocastanum</i> (2x)
		<i>S. chacoense</i> (2x)
		<i>S. fendleri</i> (4x)
		<i>S. hjertingii</i> (4x)
		<i>S. phureja</i> (2x)
		<i>S. polytrichon</i> (4x)
		<i>S. stenotomum</i> (2x)
		<i>S. stoloniferum</i> (4x)
(3) Efficiency	Very high in <i>S. tuberosum</i>	?

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倍数体化 Polyploidization

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倍数体作出法 Polyploidization method

(1) Asexual polyploidization

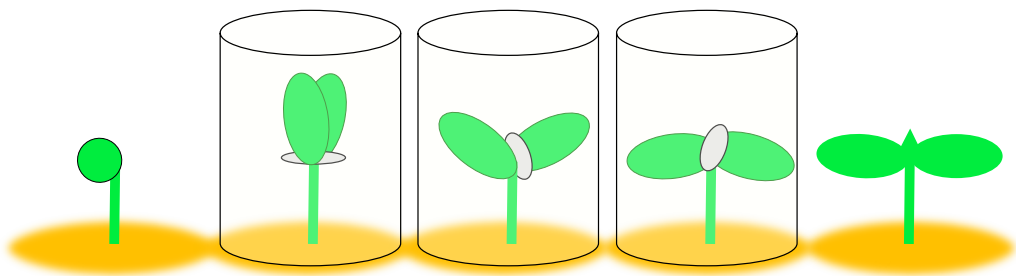
- (a) Somatic chromosome doubling by colchicine treatment
- (b) Chromosome doubling through *in vitro* culture

(2) Sexual polyploidization

- (a) Unilateral sexual polyploidization ($4x \times 2x$)
- (b) Bilateral sexual polyploidization ($2x \times 2x$)

17

実生植物のコルヒチン処理法



双葉が展開する直前から直後にかけて、脱脂綿か毛糸を成長点に当て、0.15%コルヒチン水溶液で膨潤させ3日間処理する。この間蒸発を防ぎ、毎日コルヒチン溶液を継ぎ足す。

問題点

コルヒチン水溶液が蒸発し高濃度になると薬害を起こしやすいキメラ植物（正常な枝と倍加した枝が混じる）になる場合が多い

18

組織培養による染色体倍加法



Euphytica 30 (1981) 239-246

CHROMOSOME DOUBLING THROUGH ADVENTITIOUS SHOOT FORMATION ON IN VITRO CULTIVATED LEAF EXPLANTS FROM DIPLOID INTERSPECIFIC POTATO HYBRIDS

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²Foundation ITAL, Wageningen, the Netherlands.

Received 15 August 1980

425 of 450 plantlets produced via *in vitro* culture from 51 F₁ hybrids of *Solanum etuberosum* x *S. pinnatisectum* were phenotypically chromosome-doubled.

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Tetraploidization through 4x x 2x crosses

Ploidy levels of progeny

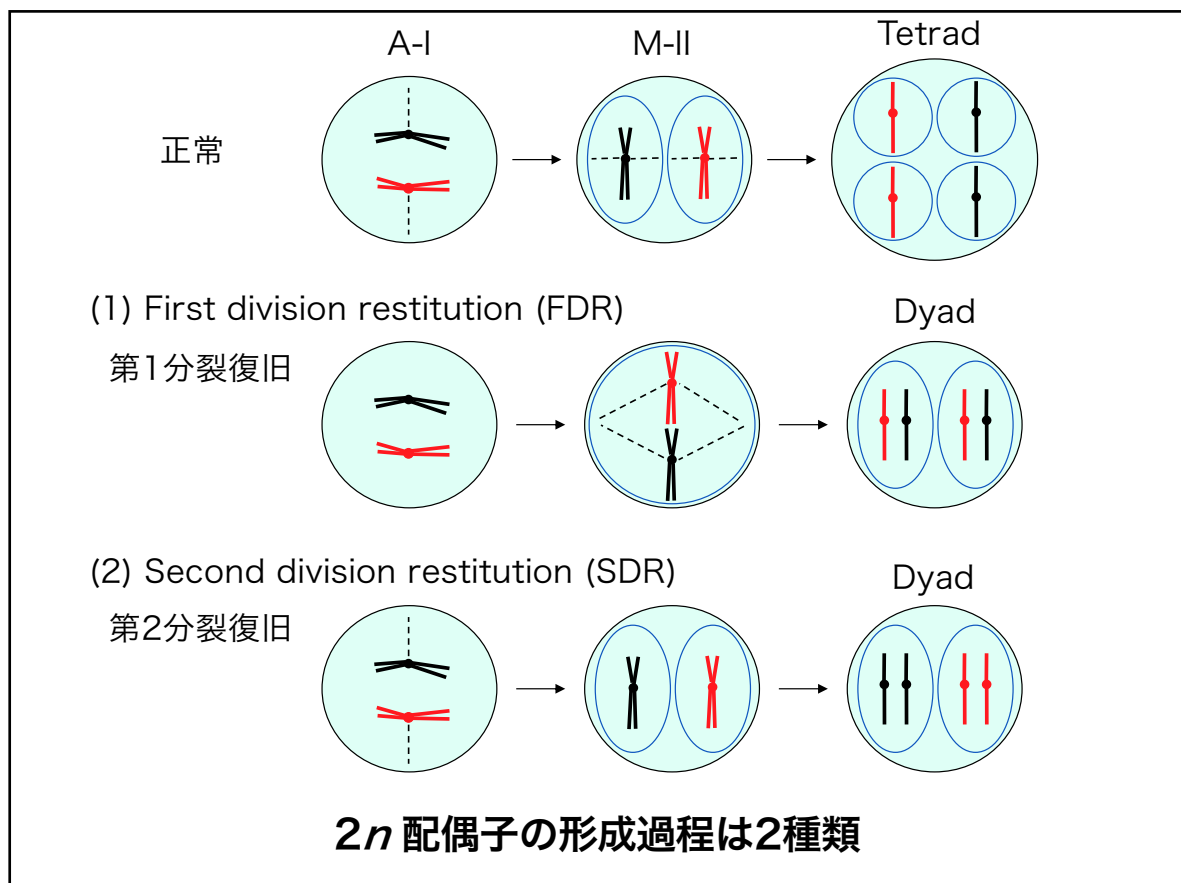
	2x	3x	4x
4x x 2x	0.6%	7.6%	91.9%
2x x 4x	3.3%	1.9%	94.8%



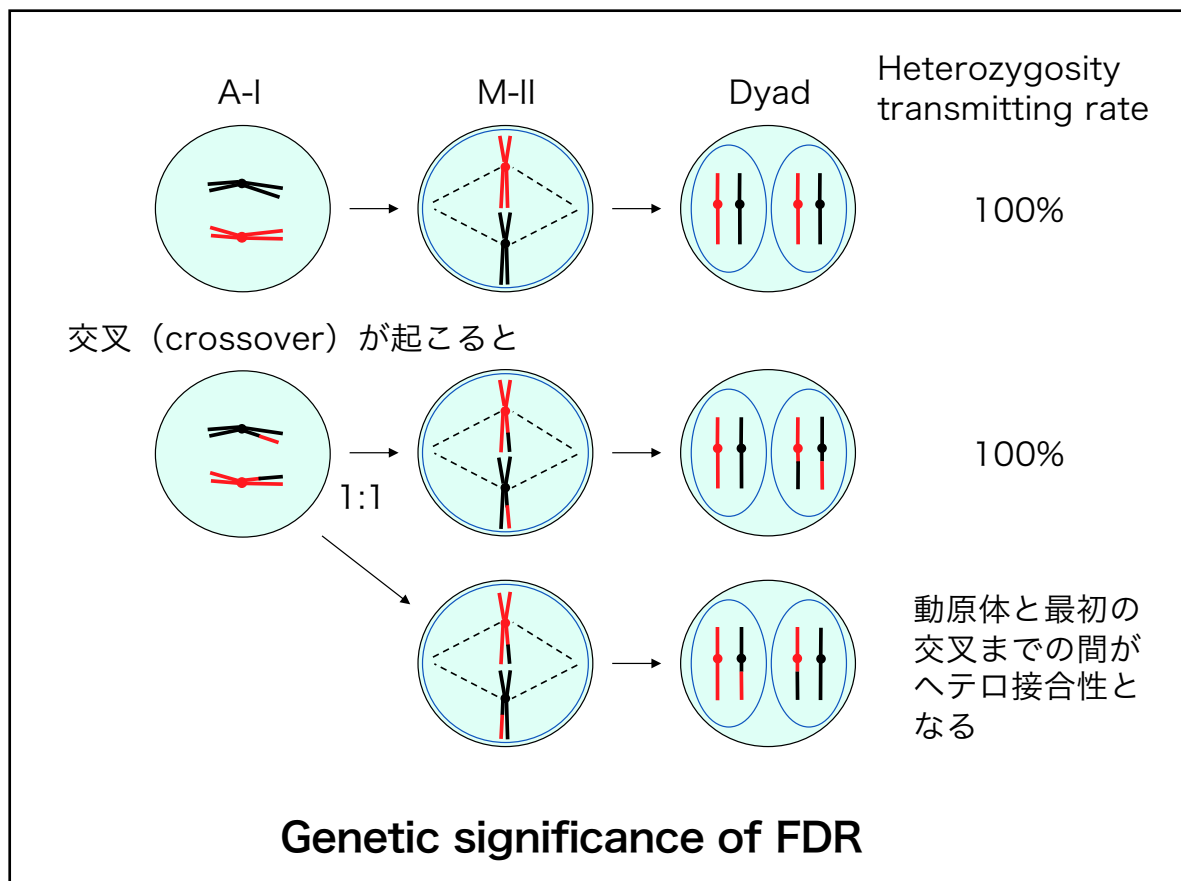
2n 配偶子による

Hanneman & Peloquin (1968)
Am Potato J 45:255-261

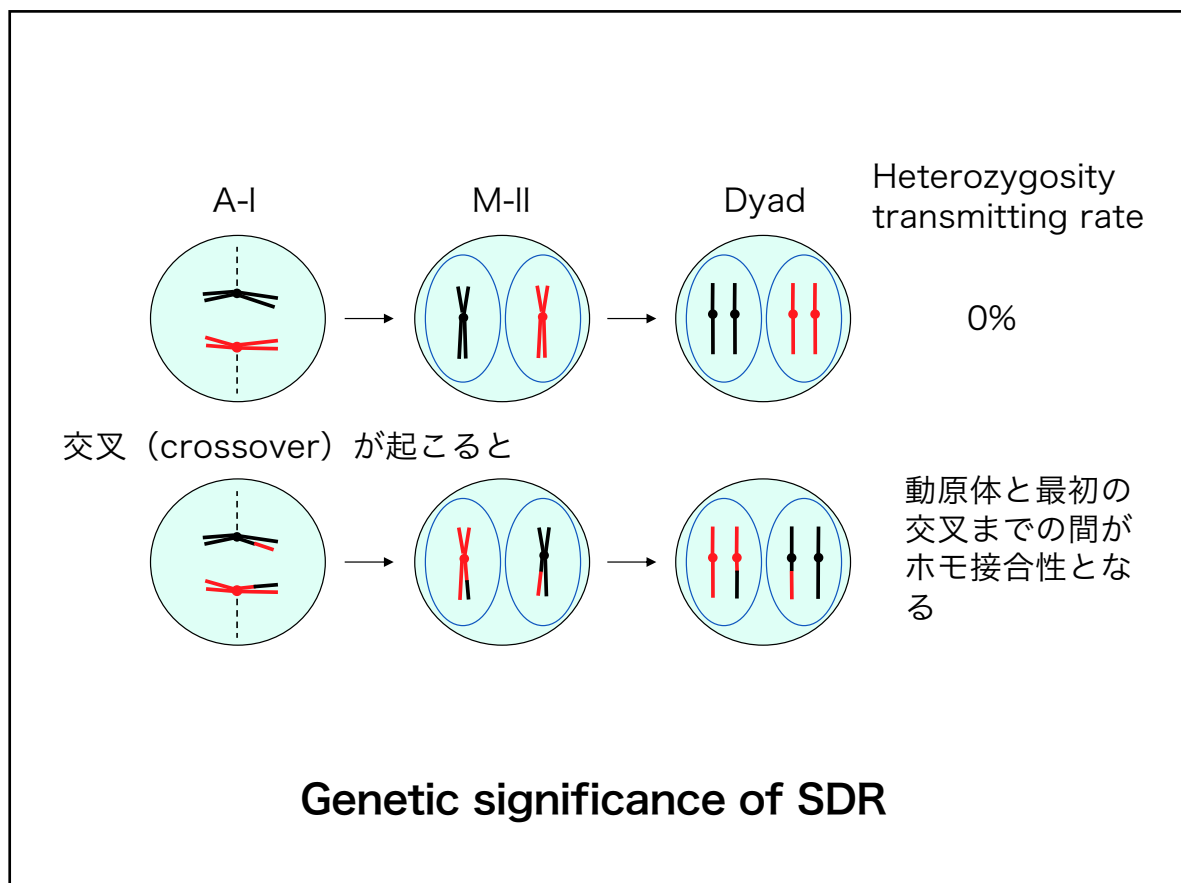
20



21



22



23

Average heterozygosity transmitting rate (%)

Cross combination	%
4x cultivar x 2x SDR	38
4x cultivar x 4x cultivar	63
4x cultivar x 2x FDR	80

A large fraction of heterotic effects and epistatic interactions are transmitted from parent to offspring with FDR.

FDR型 $2n$ 配偶子を使うとヘテロシスを伝達しやすい

24

2-2n MICROSPORES **4-n MICROSPORES**

2n pollen formation by parallel spindle (*ps*)

平行紡錘体によって作られた
2n花粉（全数性花粉）
潜在*ps*遺伝子による

2n pollen

Peloquin (1981) In: Plant Breedin II, Iowa State Univ Press. p117-149

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2-2n MICROSPORES **4-n MICROSPORES**

(1) First division restitution (FDR)

(2) Second division restitution (SDR)

Peloquin (1981) In: Plant Breedin II, Iowa State Univ Press. p117-149

***psps* により作られる2n花粉はFDRと同等である**

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集団中の ps 遺伝子頻度

Species	ps frequency	Ref.
<i>S. brevicaule</i>	0.46	1
<i>S. canasense</i>	0.31	2
<i>2x S. gourlayi</i>	0.26	2
<i>4x S. gourlayi</i>	0.80	1
<i>S. infundibuliforme</i>	0.30	2
<i>S. sparsipilum</i>	0.39	1
<i>S. tarjense</i>	0.20	2
<i>S. verrucosum</i>	0.12	2
<i>S. phureja</i>	0.47	1
<i>S. stenotomum</i>	0.42	1
<i>S. tuberosum</i> ssp. <i>andigena</i>	0.85	1
<i>S. tuberosum</i> ssp. <i>tuberosum</i>	0.69	3

- 1) Watanabe and Peloquin (1989) TAG 78:329-336
 2) Peloquin et al. (1989) Genome 31:1000-1004
 3) Iwanaga and Peloquin (1982) TAG 61:161-169

4倍種で ps 遺伝子頻度高いのは $2n$ 花粉を利用して4倍体となったことを意味する

27

$2n$ gameteはunreduced gamete（非還元配偶子）か？

通常 $2n$ gameteはunreduced gamete（非還元配偶子）と呼ばれる。

しかし、 ps 遺伝子（ $psps$ 潜性ホモ型）により形成された $2n$ pollen（ $2n$ 花粉）は減数分裂第1分裂で染色体数が半分に減数している（＝還元している）のでunreduced gamete（非還元配偶子）とは言えない。このため ps 遺伝子により形成される $2n$ pollen（ $2n$ 花粉）は全数性花粉と訳される。

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Number of *ssp. tuberosum* haploids producing $2n$ eggs, based on $2x \times 4x$ crossability

Parental tetraploid	No. of haploids			Haploids with $2n$ eggs (%)
	Screened	Producing fruits	Producing seed	
Atlantic	131	93	78	59.5
Chippewa	43	25	23	53.5
Merrimack	114	95	85	74.6
Redsen	5	2	0	-
W231	42	23	21	50.0
W760	46	31	29	63.0
Total	381	269	236	61.9

Werner & Peloquin (1991) Genome 34:975-982

2倍体に4倍体を交配し種子が取れたものは2倍体親の $2n$ eggが機能して4倍体の種子ができた、と仮定する

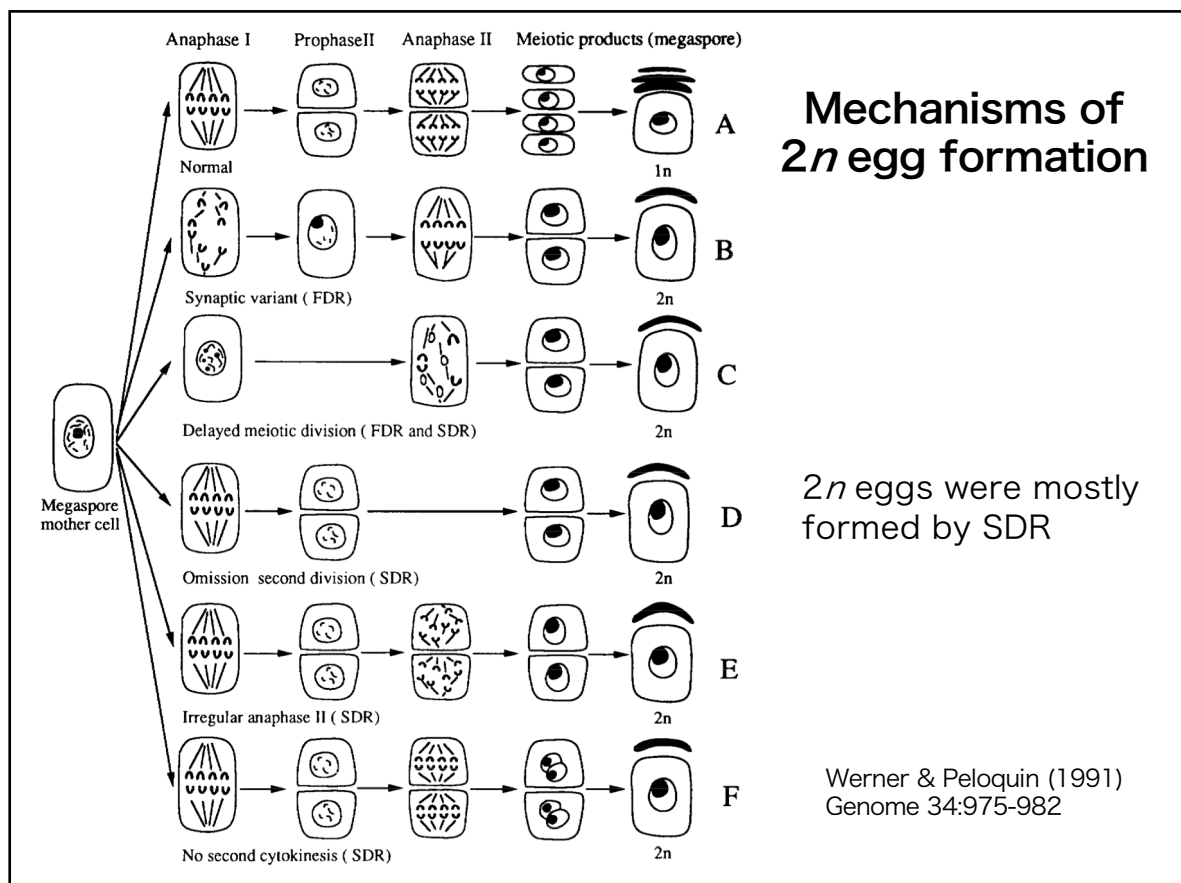
29

Number of diploid wild species producing $2n$ eggs, based on $2x \times 4x$ crossability

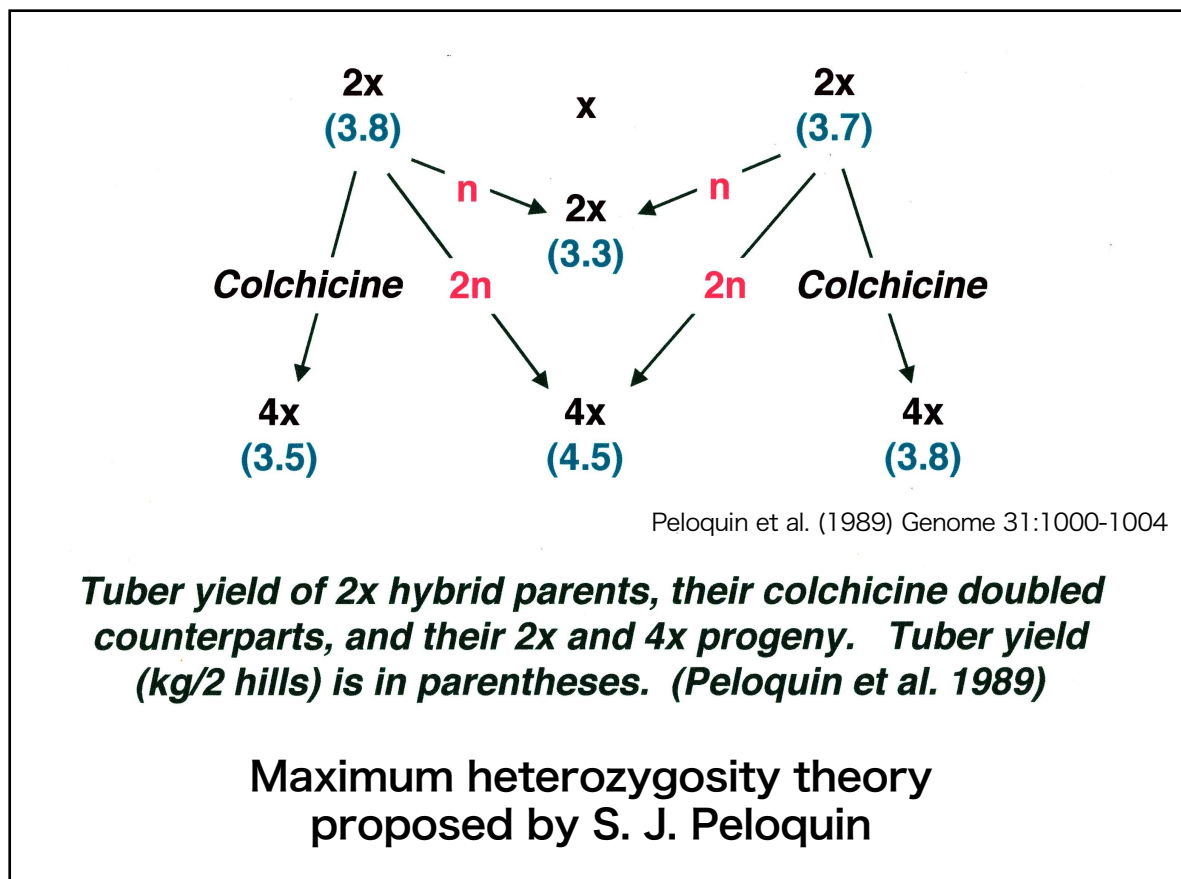
Diploid species	PI number	No. of plants			Plants with $2n$ eggs (%)
		Screened	Producing fruits	Producing seed	
<i>S. berthaultii</i>	218215	3	0	0	-
<i>S. brevicaule</i>	233961	24	23	17	70.8
<i>S. chacoense</i>	275139	24	14	2	8.3
<i>S. sparsipilum</i>	230502	16	12	2	12.5
	310933	33	28	5	15.1
<i>S. tarijense</i>	217457	15	6	2	13.3
	230466	3	3	0	-
	414148	9	6	2	22.2
Total		127	92	30	23.6

Werner & Peloquin (1991) Genome 34:975-982

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