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ORIGINAL PAPER

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Effect of genome-plastome interaction on meiosis and pollen development in *Oenothera* species and hybrids

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Abstract *Oenothera villaricae* Dietrich and *Oe. picensis* Dietrich, complete translocation heterozygotes, are fully interfertile, giving rise to six discrete classes of true-breeding hybrids from a reciprocal cross. Associated with each parent and hybrid is a characteristic abortive non-staining pollen fraction easily distinguished from fully developed pollen under the light microscope. Pollen abortion has been associated with translocation rings in other angiosperm species, and may characterize such systems. The abortive pollen fraction is significantly different between reciprocal *Oenothera* hybrids, however ($P < 0.001$), indicative of partial cytoplasmic control. Pollen abortion is most severe in the F_1 hybrid generation, and ameliorates with successive generations of hybrid self-fertilization. Three-way analysis of variance shows significant effects on pollen stainability (a measure of the non-abortive fraction) for nucleus, cytoplasm and selfed hybrid generation, individually or in combinations. This result suggests a combined nucleocytoplasmic basis for the pollen abortion. Correlated with the observation of increased pollen abortion in *Oenothera* hybrids are meiotic findings of broken chromosome rings (chains, univalents), asymmetric anaphase chromosome distributions and trinucleate tetrads. To test the hypothesis that such anomalous meiotic events play a role in the mechanism of pollen abortion, meiotic disjunction frequency was determined for each parent, F_1 and F_9 selfed hybrid accessions. Three-way analysis of variance shows levels of significance comparable to those noted for pollen stainability ($P < 0.001$) for effects of nucleus, cytoplasm and selfed hybrid generation on disjunction frequency. A high degree of correlation ($r^2 = 0.984$) is noted between disjunction frequency and pollen stainability. We conclude that the abortive pollen grains are indeed the products of non-disjunctive meiotic events, which themselves are consequences of hybrid nucleocytoplasmic incompatibility.

Key words Translocation rings · Nondisjunction · Microsporogenesis · *Oenothera*

Introduction

The hollow, infertile pollen fraction in otherwise fertile *Oenothera* species has received comparatively little attention in research (Harte 1993). While the existence of two separate size classes of pollen, each bearing a separate Renner complex (seven-chromosome binary genomic subset) has been documented by random amplified polymorphic DNAs (Gambier and Mulcahy 1996), the basis for the shrunken, hollow, inviable grains has yet to be determined. The observation of 25–35% pollen abortion in translocation ring-forming *Oenothera* species, in contrast to less than 1% in bivalent-forming structural homozygotes (i.e., *Oe. hookeri* Johansen), piqued our interest in a possible genetic cause for this wasted reproductive potential. Published research on pollen abortion associated with translocation heterozygosity in rye (Thompson 1956; Lawrence 1958; Rees 1961) and upland cotton (Gwyn and Stelly 1989) has shown that pollen abortion is a heritable character associated with the translocation state and subject to selection.

In *Oenothera*, pollen abortion is a well-known consequence of hybridization, especially between translocation heterozygotes. Classical studies by Renner (1919) and Stubbe (1959, 1960) noted abortion of hybrid pollen carrying a given Renner complex combined with proplastids of an incompatible type. Renner (1937) also studied pollen abortion and recovery in the present species and hybrids, but without statistical techniques; details of his analysis are presented in the Discussion section.

Unlike the fully developed pollen, the abortive grains are shrunken and do not stain with aniline blue in lactophenol. The difference is easily appreciable on light microscopy with the $\times 20$ objective. We therefore undertook a comprehensive census of stainable versus nonstainable pollen in the complete translocation heterozygotes *Oe. villaricae* and *Oe. picensis* ssp. *picensis* as well as in

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their hybrids, which are known to form partial translocation rings of various sizes (Haustein 1952).

Normal synaptonemal complexes and chiasmata are prerequisites for correct distribution of homologues to opposite poles at metaphase I (Maguire 1995). To follow up our earlier qualitative finding of chains, univalents and trinucleate tetrads in the present species and hybrids (Chapman and Mulcahy 1997), meiotic disjunction frequency was quantified.

Materials and methods

Plant material

Material of *Oe. villaricae*, *Oe. picensis* ssp. *picensis* and reciprocal hybrids selfed more than nine generations was raised to maturity in a University of Massachusetts greenhouse. F₁ hybrids were generated as follows. Due to the primarily cleistogamous habit of *Oe. villaricae* and *Oe. picensis* (anthers dehiscence 24–48 h before opening of the corolla), emasculations were made in the 2-cm bud stage. Multiple crosses were made in both directions. Hybrids are morphologically distinguishable when seedlings are 2–3 cm high, at which time they may be transplanted. F₁ hybrids were raised to maturity and allowed to self-pollinate. F₂ seed was collected, germinated and raised to maturity in a similar fashion.

Pollen specimens

Four separate flowers were harvested from each of five randomly selected individuals of parent species, F₁, F₂ and F₉₊ selfed hybrid accessions. Pollen from each flower was transferred to a slide and stained with 0.001% aniline blue in lactophenol (equal parts water, lactic acid, glycerine and phenol) for 20 slides total per accession. At least 200 grains per slide were counted; for secure control, slides were pre-coded, taped and mixed before counting so that the genotype was hidden from the experimenter. Counted slides were stored until all data were in, then tapes were removed for sample identification and data analysis.

Meiotic specimens

From 10 to 50 meiotic buds approximately 1.5 mm in length were harvested at random from 10–20 individuals of parent species, F₁ and F₉₊ hybrid accessions. Buds were fixed in ethanol and acetic acid (3:1) and stained with acetocarmine after the method of Cleland (1972). Chromosome squashes were made to screen for meiotic figures; all slides with metaphase, anaphase or telophase figures were sealed with nail polish, precoded, taped and mixed before analysis. Slides were screened with the ×20 objective and individual cells scored with the ×100 oil objective. Five slides per accession were counted, with 22–50 meiotic events scored per slide. Only unambiguous instances were scored as disjunction or nondisjunction, defined as follows.

Disjunction

Meiotic events scored as disjunction were metaphase figures with unbroken rings and without evidence of univalents or chromosome fragments; anaphase figures with seven chromosomes approaching each pole; telophase figures with four symmetric groups of chromosomes; and tetrads with four symmetric nuclei.

Nondisjunction

Meiotic events scored as nondisjunction were metaphase figures with chains, univalents and/or chromosome fragments; anaphase

Fig. 1A–D Disjunctive versus nondisjunctive meiotic configurations in primary microsporocytes. Bars 10 μ m. **A** Disjunctive meiosis in hybrid L.V. showing intact ring of 14 chromosomes; **B** nondisjunctive meiosis in hybrid L.V. showing broken ring (chain) configuration. **C, D** Trinucleate tetrad figures showing aggregation of chromosomes into three offspring nuclei, and possible lost chromosomes (arrows)

or telophase figures with an asymmetric chromosome distribution; and tri- or binucleate tetrads.

Some examples of disjunctive versus nondisjunctive meiosis are presented in Fig. 1. Figure 1A and B shows light micro-

graphs at $\times 600$ magnification of *Oenothera* meiotic figures prepared with the crystal violet staining technique of Cleland (1972). For clarity, the confocal optical sectioning technique of Chapman and Mulcahy (1997) was used to generate Fig. 1C and D; in the acetocarmine squashes used to score disjunction frequency, only rarely are all chromosomes located in the same focal plane. Following scoring of all coded slides, tapes were removed for sample identification and data analysis.

Data analysis

Using the Jandel Scientific computer graphics package, bar graphs were generated for pollen stainability and meiotic disjunction frequency, as was a correlative line graph for comparison of the two data sets. Using the SYSTAT computer statistical package, three-way ANOVA tables were generated for pollen stainability and meiotic disjunction frequency.

Results

Mean pollen stainability values for parents and hybrids are presented in Fig. 2. Pollen census ANOVA (Table 1)

Fig. 2 Pollen stainability histogram for *Oe. villaricae* (line 1) and *Oe. picensis* (line 3) and hybrid generations F_1 , F_2 and F_{9+} . Hybrids 8, 10 and 11 are of the *Oe. villaricae* maternal lineage; hybrids 9, 12 and 14 of the *Oe. picensis* maternal lineage. Black bars parent species, white and grey bars hybrid species. Line identification numbers (1–14) are those given by Schwemmler (1938)

Table 1 Effects of hybrid genome, cytotype and generation on pollen stainability: analysis of variance. Dependent variable is pollen stainability (arcsine-square root transformed); $r^2=0.936$

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Genome	16641.530	3	5547.177	452.586	<0.001
Cytotype	20405.762	1	20405.762	1664.874	<0.001
Generation	421.977	2	210.989	17.214	<0.001
Genome $\times$ cytotype	28530.660	3	9510.220	775.924	<0.001
Cytotype $\times$ generation	45.852	2	22.926	1.870	0.156
Genomex $\times$ generation	565.564	6	94.261	7.691	<0.001
Error	4326.593	353	12.257		

shows highly significant effects ($P<0.001$) for genome $\times$ cytotype and genome $\times$ generation two-level interactions, as well as highly significant individual effects ($P<0.001$) for (nuclear) genome, cytotype and generation effects. No significant effect was detected for cytotype $\times$ generation. The dependent variable was frequency of stainable pollen (arcsine-square root transformed). The r^2 value of 0.936 indicates that the effects listed above account for most of the total variation in the sample. Combined-variable F -ratio values show that the greatest contribution to ANOVA came from genome $\times$ cytotype (775.9), which contributed more variation to ANOVA than the other combined variables (17.2, 1.9 respectively), or (interestingly) the single variable of genome (452.6). Comparison of single-variable F -ratio values shows that the greatest contribution of variation to ANOVA came from cytotype, followed by genome and generation.

Mean meiotic disjunction frequency values for parents and hybrids are presented in Fig. 3. Disjunction frequency ANOVA (Table 2) shows a statistical profile similar to Table 1; i.e., highly significant combined effects of genome $\times$ cytotype and genome $\times$ generation ($P<0.001$ and $P<0.005$, respectively), and highly significant individual effects ($P<0.001$) for genome, cytotype

Fig. 3 Meiotic disjunction frequency histogram for *Oe. villaricae* and *Oe. picensis* (black bars) and hybrid generations F_1 and F_{9+} (light grey and dark grey bars). Note maternal lineages as in Fig. 2

Table 2 Effects of hybrid genome, cytotype and generation on meiotic disjunction frequency: analysis of variance. Dependent variable is meiotic disjunction frequency (arcsine-square root transformed); $r^2=0.978$

Source	Sum-of-squares	df	Mean-square	F-ratio	P
Genome	3805.412	3	1268.471	187.689	<0.001
Cytotype	4927.952	1	4927.952	729.164	<0.001
Generation	80.866	1	80.866	11.965	<0.001
Genome $\times$ cytotype	7892.844	3	2630.948	389.288	<0.001
Generation	1.221	1	1.221	0.181	0.672
Genome $\times$ generation	98.013	3	32.671	4.834	<0.004
Error	419.018	62	6.758

Fig. 4 Regression plot of mean meiotic disjunction frequency versus mean pollen stainability for parents and hybrids. Bars (X,Y) = standard error in pollen abortion and meiotic disjunction frequency, respectively. $r^2=0.984$

and generation. The largest contribution to variation in the meiotic census as measured by F -ratio is attributable to cytotype (729.164), followed by genome $\times$ cytotype combined effect (389.288). The regression plot of meiotic disjunction frequency versus pollen stainability (Fig. 4) shows a linear relationship with high correlation ($r^2=0.983$) between these variables.

Discussion

The basis for stable, nonmutational pollen abortion in *Oenothera* translocation heterozygotes has received sporadic attention from researchers since the classic work of Renner (1937) on the present species and hybrids, in which he attributed the abortive fraction to meiotic nondisjunction due to incomplete homology between allo-specific chromosome complexes. To explain the observed recovery of pollen fertility with hybrid selfing, Renner hypothesized selection for improved homology in the pairing regions. Through limited meiotic crossover exchange in these regions, the chromosomes of complex A might acquire pairing elements of complex B, thus acquiring sequence-level identity and improved homology

at meiosis in selfed progeny. Alternatively, Linder (1967) attempted, but was unable, to attribute the shrunken, hollow grains to the action of a genetic lethal. We here present quantitative evidence for the mechanism of pollen abortion. The shrunken grains are the products of nondisjunctional meiosis, but the basis for nondisjunction itself is more complex. Certain Renner complex pairings, i.e., that of hybrids 11 and 12 (L.V complexes; see Fig. 2), exhibit parental-level disjunction frequencies regardless of maternal lineage; hybrids 8 vs 9 and 13 vs 14 (B.I and B.V pairings), by contrast, display marked reciprocal differences in disjunction frequency and pollen stainability. Taken together, these results suggest that neither the hybrid nucleus nor the cytoplasm is wholly responsible for nondisjunction; rather, an interaction between the two systems appears to influence chromosomal behavior beginning in pachytene. The effects of this interaction culminate in the viability or nonviability of pollen.

A weaker, yet still highly significant, effect on disjunction frequency is exerted by selfed hybrid generation. In a cytoplasmic background incompatible with the hybrid nucleus (i.e., *picensis* maternal lineage lines 9 and 14), some degree of pollen fertility is recovered with nine or more generations of self-fertilization. One logical explanation for this phenomenon is given by Renner's model (1937) of intracellular selection for crossover types homozygous for the pairing regions. Alternatively, selection for increased density of recombination nodules in these regions could give rise to increased chiasma probability and improved disjunction frequency. Irrespective of the specific mechanism of selection, new species could arise from the reciprocal cross *Oe. villaricae* $\times$ *Oe. picensis* only within the constraints of hybrid nucleocytoplasmic compatibility. In cases of cytoplasmic hybridization intolerance, the nascent species either fails outright due to embryonic lethality, or the genome and plastome coevolve.

That the cytoplasmic genes evolve in response to the nucleus is beyond dispute; macroevolutionary changes concomitant with the transition from symbiont to organelle have resulted in transfer of most plastid and mitochondrial genes to the nucleus (Gillham 1994). The converse process, however, i.e., evolution of the nucleus in response to the organelles, is quite rare. In *Oenothera*, the only known example of nucleus-to-organelle gene transfer is that of a nuclear gene fragment encoding the 18S cytoplasmic rRNA, which has been integrated into the mitochondrial genome (Schuster and Brennicke

1988). Nuclear microevolution of the present sort, i.e., change in selfed hybrid meiotic disjunction frequency against a cytoplasmic background intolerant to hybridization, has never before been quantified. The present statistical results are at best circumstantial evidence in support of Renner's model. Further studies are needed to determine the mechanism for asynapsis and to document selfed hybrid pairing-region exchange at the DNA sequence level.

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