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R. M. Gambier · D. L. Mulcahy

The association between pollen size and Renner complex in *Oenothera villaricae* and *O. picensis* ssp. *picensis* and their hybrids: evidence for preanthesis pollen competition

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Abstract In both *Oenothera villaricae* Dietrich and *O. picensis* ssp. *picensis*, chromosomes are transmitted as two Renner complexes. Reciprocal combinations of the Renner complexes produce eight different F_1 hybrids, but only seven are viable. Each species, and each F_1 hybrid, produces three sizes of pollen, approximately 50% small sterile grains, 15% medium-sized viable grains and 35% large viable grains. Medium- and large-sized grains were separated manually and subjected to random amplified polymorphic DNAs (RAPDs) analysis. A pattern of RAPD amplifications was obtained which indicates that, for each species and F_1 hybrid, one specific Renner complex characterizes the medium- and another the large-sized viable pollen. The results indicate that pollen size is determined in part by the pollen genotype and in part by the genotype of the other pollen grains developing within the same anther.

Key words *O. villaricae* · *O. picensis* ssp. *picensis* · Renner Complexes · RAPDs · Pollen dimorphism

Introduction

In many species of *Oenothera* (2n = 14), haploid chromosome sets are transmitted from one generation to another in the form of Renner complexes. These are the products of multiple reciprocal translocations and result in rings of chromosomes at metaphase I of meiosis (Cleland 1972; Harte 1994). Species of *Oenothera* which carry Renner complexes exhibit an extreme reduction in both crossing-over and the random assortment of chromosomes. In fact, only two gametic genotypes are produced by a plant, and these are identical to the gametes that joined to make that plant now producing gametes. The adaptive significance of Renner complexes is demonstrated when such plants self pollinate. Instead of the 50% reduction in heterozygosity that is expected with each generation of selfing, all seeds of Renner complex species are as heterozygous as their parents. The restrictions on recombination which result from the reciprocal translocations are only the first part of this adaptive system. Even with these, selfing should normally result in one-half of the progeny being homozygous for one Renner complex or the other.

However, differing between species, there are three separate mechanisms for preventing the formation of homozygotes, despite selfing. In *O. muricata*, for example, one Renner complex is transmitted only through the egg and the other only through the pollen. Selfing combines the two dissimilar complexes. In *O. lamarckiana*, both complexes are transmitted through both sexes, and random fertilization does occur. However, the homozygotes are eliminated by sporophytically acting recessive lethals. The third case, much less well-known, is found in *O. villaricae* (formerly *O. berteriana*, see Schwemmle 1968) and *O. picensis* ssp *picensis* (formerly *O. odorata* see Schwemmle 1968). There, heterozygosity is maintained by the phenomenon of selective fertilization (Schwemmle 1968). In that system, both Renner complexes are transmitted through both sexes. However, fertilization is indeed selective: pollen tubes carrying either Renner complex are preferentially directed to embryo sacs containing the alternate Renner complex. This, again, reconstructs the heterozygous genotype.

O. villaricae and *O. picensis*, the subjects of the present study, collectively contain four different Renner complexes. Schwemmle referred to the Renner complexes of *O. villaricae* as "B" and "L" and those of *O. picensis* as "V" and "I". Like all species of *Oenothera*, subsect. *Munzia*, these two species are interfertile (Dietrich 1977; Stubbe and Raven 1979). By making reciprocal crosses

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R. M. Gambier
Department of Biology, Suffolk Community College, Seldon, N.Y., USA

D. L. Mulcahy (✉)
Department of Biology, University of Massachusetts, Amherst, Mass., USA

between the two parental species, it is thus possible to generate eight new combinations of Renner complexes and cytoplasm, (one combination is inviable). These combinations, and the line designations assigned by Schwemmle, 1 and 3 for the parents, etc., are shown in Fig. 1.

Gambier (1994) found that each parental species and also each of the new F_1 combinations produces three distinct sizes of pollen. The large- and medium-sized grains are viable, but the third, small form is shriveled and clearly inviable. This was not the first report of pollen polymorphism in *Oenothera*. For example, pollen grains are dimorphic in *O. muricata* (Cleland 1972) and, presumably, the larger pollen carries only the Renner complex which is transmitted through the pollen. (It is of course also possible that in *O. muricata* there is no relationship between Renner complex and pollen size and only some of the larger grains result in fertilization). We decided to determine if in *O. villaricae* and *O. picensis* ssp. *picensis* and their hybrids there is an association between the two Renner complexes and the two sizes of viable pollen. If such an association exists, it would be possible to identify, by inspection, the genotypes of pollen from segregating plants, thus allowing a particularly convenient study of several topics in reproductive biology.

If the two morphs of viable pollen from each plant do carry specific Renner complexes, then large- and medium-sized pollen on each plant should produce RAPD fingerprints that differ from each other. Furthermore, since there are only four Renner complexes in all, there should be only four random amplified polymorphic DNA (RAPD) fingerprints found among the 18 possible pollen types (large- and medium-sized pollen grains from each of nine lines). The distribution of RAPD fingerprints should correspond to the distribution of Renner complexes among the nine possible diploid genotypes; that is, the 2 pollen types from line 13 should equal those from line 14. Furthermore, line 13 should contain one fingerprint (and only one) found in line 1 plus one from line 3, etc. We therefore analyzed RAPDs from each of the two sizes of viable pollen produced by each of the nine genotypes.

Materials and methods

Seeds of *Oenothera villaricae* (which contain Renner complexes B and L) and *O. picensis* ssp *picensis* (Renner complexes V and I) and all seven possible F_1 hybrids, nine genotypes in all, including reciprocal crosses (see Fig. 1), were generously provided by B. Schwemmle.

Plants of each line represented in Fig. 1 were grown in screened greenhouses and pollen from each was placed into a humid atmosphere for 30 min before being manually separated under a dissecting microscope. Two sizes of viable pollen were distinguishable, but an effort was made to select the largest of the large viable grains and the smallest of the medium viable grains to minimize the frequency of misclassification. DNA was extracted from samples of 100 grains by

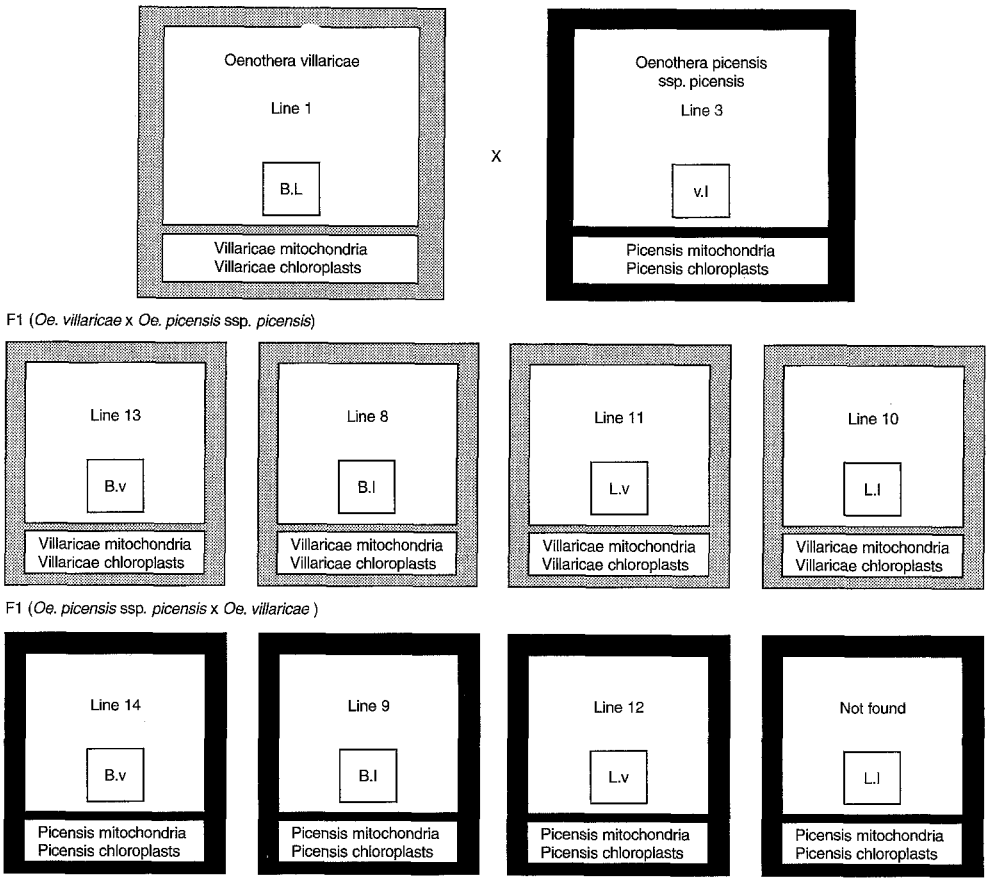

Fig. 1 Renner complexes found in *O. villaricae* and *O. picensis* ssp. *picensis* and their hybrids (Schwemmle 1968)

the method of Jhingan (1992), with the modifications of José X. Chaparro (School of Forestry, University of North Carolina, Raleigh). The extraction buffer consisted of 10 m*M* EDTA pH 8.0; 100 m*M* TRIS-HCl pH 7.5; 700 m*M* NaCl and 625 m*M* potassium ethyl xanthogenate (Fluka Chemicals). The extraction procedure was as follows:

1) Crush 100 pollen grains in 5 µl of PEX buffer, centrifuge and crush again.
2) Vortex, centrifuge and crush once again; add 2 µl of PEX buffer.
3) Vortex, centrifuge and then incubate at 65 °C for 5–10 min.
4) Pellet debris by spinning 5 min at 11,000 rpm on the microfuge and transfer 5 µl of supernatant to a new tube.
5) Add an equal volume of cold isopropanol to the supernatant, mix gently and pellet DNA by spinning 5 min at 11,000 rpm on the microfuge.
6) Wash pellet in 70% ethanol, dry and resuspend in 2.5 µl ddH_2O.

Polymerase chain reaction (PCR) conditions

The resulting 2.5 µl of DNA solution was mixed with 10.15 µl of reaction mixture. The reaction mixture was as follows: 1.25 µl 10 × buffer (100 m*M* TRIS-HCl pH 8.3, 500 m*M* KCl, 15 m*M* $MgCl_2$, 0.1% Difco gelatin); 1.0 µl dNTPs (2.5 m*M* of each dNTP); 0.55 µl 10 m*M* $MgCl_2$, 0.55 µl 10 m*M* primer; 0.1 µl *Taq* (DNA polymerase), 6.75 µl H_2O. Several primers from Operon Kit A were used. The PCR conditions were 94 °C for 1 min, followed by 40 cycles of 94 °C for 1 min, 34 °C for 2 min and 74 °C for 2 min. Some reactions were run on a Barnstead/Thermolyne thermocycler (Model DB 66935, Dubuque, Iowa) and others on a Perkin/Elmer thermocycler (Model 480, Norwalk, Conn.).

Electrophoresis

After PCR, the samples were placed in a 2% agarose gel (1% FMC NuSieve, 1% Sigma Low EEO agarose, 1 × TBE buffer) that had been poured at least 2 before use. The samples were run for 4 h at 155 V. Staining of the gel was done with 0.01% ethidium bromide in water.

Results

Consistent differences between complexes were only found with primers OPA18 (5′AGGTGACCGT3′) and OPA20 (5′GTTGCGATCC3′). The best results were obtained using primer OPA18. We observed no differences in the products of the Barnstead/Thermolyne and the Perkin/Elmer thermocyclers. RAPD amplifications performed with primer OPA18 are given in Fig. 2. When the medium viable grains of Fig. 2 are considered, it is clear that the medium pollen of lines 3, 8, 9, and 10 all exhibit the same RAPD fingerprint. The only Renner complex found in all four of these lines is complex I. If the medium grains of these lines are indeed complex I, this would require that the large grains of lines 3 = Complex V, 8 = B, 9 = B and 10 = L. The fingerprints shown in Fig. 2 (large grains) are compatible with this conclusion in that there $3 \neq 8 = 9 \neq 10$. If the large grains of line 3 = V then, since among large grains $3 = 11 = 12 = 13 = 14$, these last four should also be V. This leads to the conclusion that the medium grains of 11 and 12 should be L, and those of 13 and 14 should be B. A confirmation of this conclusion is that the medium grains of line 11 do equal the medium grains of line 12,

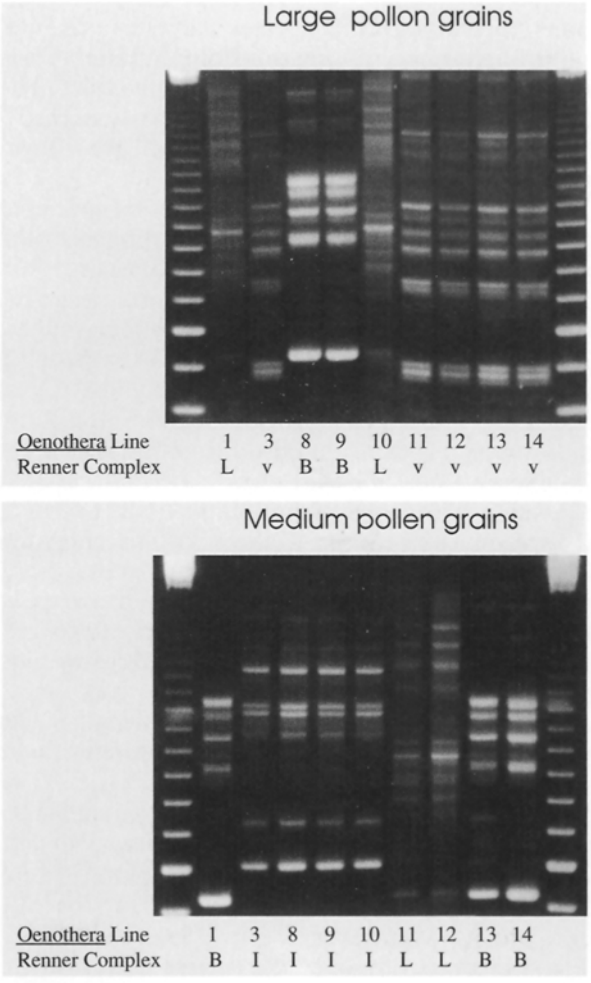

Fig. 2 RAPD markers and the Renner complexes for the large- and medium-sized pollen grains produced by *O. villaricae* and *O. picensis* ssp. *picensis* and their hybrids. Compare the distribution of the four patterns *B*, *L*, *V*, and *I* with the distribution of the same Renner complexes in Fig. 1. The *extreme right* and *left lanes* in each gel are a 100-bp ladder, with the particularly bright band being 800 bp

and medium 13 does equal medium 14. Continuing, it is seen that if large grains of line 10 = L, so too do the large grains of line 1, which indicates that medium grains of line 1 contain complex B. The identity of medium grains of line 1 is confirmed by the match between them and the already identified medium grains of lines 13 and 14, all of which contain complex B.

Discussion

Although there are several mechanisms for generating bimodality of pollen within an anther (Horner and Street 1978; Mulcahy et al. 1992), the results of the present study suggest that in these *Oenothera* species the size of any pollen grain is determined, in part, by the Renner complex that is contained within the individual pollen grain and in part also by the complex which is

contained in the other pollen type that shares the same anther. For example, in lines that contain Renner complex V, (lines 3, 11, 12, 13 or 14), the V complex always occupies the large pollen from each line (see Fig. 2). Complex I, in contrast, when present, is always found in the medium pollen. (See lines 3, 8, 9, and 10). Complexes L or B will each be in the medium pollen if either of them is segregating from complex V or in the large pollen if segregating with complex I. If L and B are together in a hybrid, as is the case in line 1, the L complex will occupy the larger pollen grains. These observations suggest that the tendency of Renner complexes to occupy the larger of the viable pollen type in any combination can be represented by following hierarchy: V > L > B > I.

The observed lack of recombination support reports that, according to vegetative characteristics, these different F_1 lines breed true (Schwemmle 1968; Chapman unpublished). These results, while useful, are, at least for the authors, somewhat surprising since hybrids between two randomly chosen Renner complex species of *Oenothera* only rarely assemble into a ring of 14 chromosomes without additional reciprocal translocations. The hybrids between *O. villaricae* and *O. picensis* ssp *picensis* are no exceptions to this statement. Five of these nine F_1 hybrids (lines 8, 9, 13, 14 and 10) do not form a ring of 14 chromosomes at meiosis. (See Haustein 1952, quoted in Kirk and Tilney-Bassett 1967; Chapman, in preparation). Since anything other than a ring of 14 chromosomes allows for the possibility of recombination, genetic segregation might be expected in the interspecific progeny. However, Harte (1994) states (p 39) that "A recombination of alleles by free segregation or crossing-over can occur [in interspecific hybrids of *Oenothera*] but seems to be the exception. Instead constancy or at least partial constancy of the hybrid is observed". It thus appears that despite the apparent possibility for meiotic recombination, the highly heterozygous individuals remain true breeding, and this requires some explanation.

It is possible that segregation does occur but is undetected. Schwemmle (1968) and Chapman (in preparation) worked with 12 vegetative characteristics and reported that each of the nine lines breeds true. One exception to this statement was seen in line 10, which segregates for both petal spot and pollen fertility. Perhaps this pair of markers is comparable to the *brevistylis* marker described by de Vries (1913; see Harte 1994, p 39), located at the ends of chromosome arms. With respect to the molecular markers, Fig. 2 shows at least 22 strong amplicons that are consistent for all samples of the four presumed Renner complexes. No strong amplicon is inconsistent with (that is, segregating from) the presumed Renner complexes. There are many additional consistencies among fainter amplicons but also inconsistencies. It has not been possible for us to determine whether inconsistencies among faint amplicons indicate technical artifacts or genetic segregation.

Considering the total number of markers, we should be able to detect segregation of chromosome arms if it is occurring. For example, without segregation, meiosis is characterized by a ring of 14 chromosomes, which separates into two groups, that is, two linkage groups of 7 chromosomes each. In the five lines in which segregation seems possible, there are three pairs of linkage groups. Line 10, for example, produces rings of 8 and 4 chromosomes each and also one bivalent. With 22 molecular markers, each chromosome should be marked by an average of 22/14 or 1.6 markers; the ring of 8 would then be marked by 8/14 of the 22 markers, or 12.6. The ring of 4 should carry approximately 6.3 markers, and the bivalent 3.2 markers. Figure 2 indicates no obvious differences between the meiotic products of the nonsegregating lines (1, 3, 11 and 12) and the possibly segregating lines (13, 14, 8, 9 and 10). This we interpret as evidence that viable pollen grains contain intact (nonsegregated) Renner complexes.

How can the reported meiotic segregation in five of the hybrid lines be reconciled with the absence of recombinants in the viable pollen? One possibility is that recombinant haplotypes are nonviable. Whatever the mechanism, the present study indicates that each of the two sizes of pollen produced in these 9 genotypes of *Oenothera* is characterized by a specific Renner complex. These pollen genotypes can be identified by inspection, a fact which should make these and related species particularly useful for investigating the relationship between pollen genotype and pollen performance.

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