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An Attempted Analysis of the Tetrads in the Complex Heterozygote Species of the Sub-genus *Oenothera*

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Summary. The statistical parameters of a pollen population of *Oenothera purpurata* in which the mendelian factor of pollen lethality segregates, showed that the pollen population analysed at anthesis in the *Oenothera* sub-genus quite faithfully conserves the segregation indicated in the tetrads. Following on this fact, the segregation "active grains, inactive grains, empty grains" was analysed in the pollen of the complex heterozygotes. The large standard deviation of the percentages of the three pollen classes and the value of the correlation between the percentages of grain classes taken two by two signify that the segregation of the three potential classes of microspore differs from one tetrad to another. By superimposing the correlation coefficient estimated between the percentage of the three grain classes of a bifactorial segregation of corn pollen on the correlation coefficient of the three classes of *Oenothera* pollen, it is possible to know with certainty the distribution of the potentially active, inactive and empty microspores in the tetrads. This distribution, which is different depending on whether the species is heterogamous or isogamous, allows us to confirm that the empty grains carry the recombined complexes which become lethal as a result of this recombination.

It has been demonstrated (Jean 1974) that the empty pollen grains of the complex heterozygote species of the subgenus *Oenothera* represent haploid individuals of recombined complexes. It would be interesting to know the segregation "active grains, inactive grains, empty grains" in the tetrads resulting from the meiosis of anther mother cells. In the pollen of the majority of angiosperm species, at the point of anthesis, the ties between the four pollen grains originating from one particular tetrad are destroyed by dissolution of the callosic wall of the mother-cell. This means that, after sampling, the grains are randomly dispersed in a microscopic preparation. The Oenotheras pollen, however, is a different case, the viscine filaments which are juxtaposed globules of sporopollenine (Jean 1971), binding together the grains of any tetrad. In this way the pollen population should conserve an oriented distribution of the grains in a microscopic preparation. This was proved when we found among the homozygous *Oenothera purpurata* a plant whose pollen showed the segregation of a mendelian factor of pollen lethality. This sample pollen population helped towards an understanding of the statistical characters of the complex-heterozygote pollen population.

The statistical method includes three parts:

(1) the presentation of statistical parameters;
(2) the search for a model pollen population composed of 3 classes of grains;
(3) the comparison of this model pollen population with the pollen population of complex-heterozygotes.

Materials

The pollen of some species of the Renner collection was analysed: *Oenothera biennis* L., *Oe. coronifera* Renner, *Oe. coronifera* forma *rubrisepala* Renner, *Oe. conferta* Renner, *Oe. rubricaulis* Renner, *Oe. suaveolens* Desfontaine, *Oe. syrticola* Bartlett, as well as two other species *Oe. nuda* Renner (Jean et al. 1966a, b) and *Oe. ersteinensis* Linder and Jean (1969). The heterozygote +/p was taken from our cultures of *Oe. purpurata* Klebahn.

The sample of pollen population was generally composed of about 100 grains. n is the number of samples. For *Oe. biennis*, *Oe. ersteinensis* and *Oe. syrticola*, the pollen taken off three anthers of one flower of one plant formed the subpopulation (Tables 2, 3). For all other species the sample was taken from one anther per plant and no distinction was made between the plants (Table 1). The variable x is the percentage of one class of grain. The number of samples having the same percentage is the frequency of the variable (the statistical calculations are made using a program in Algol language).

Also used for the demonstration are the results of segregations observed on corn spikes: the segregation of two independent pairs of alleles C/c and +/su, using the back-cross $\frac{c\,su}{c\,su} \times \frac{C\,+}{c\,su}$ (n = 108 spikes, Table 4, B); the segregation of two linked pairs of alleles C/c and +/wx, using the back-cross $\frac{c\,wx}{c\,wx} \times \frac{C\,wx}{c\,+}$ (n = 78 spikes, Table 4, A). As it is difficult to distinguish between normal grains and waxy grains in the grains with coloured aleuron, the phenotypes [C +] and (C wx] were grouped in one phenotype [C].

Results

A - Statistical Findings

a - The percentage distribution of the empty grains in the segregation +/p

In approaching the statistical analysis of the three classes of pollen grains of the complex-heterozygotes, we refer to a pollen population in which the mendelian factor of pollen lethality (p) is segregating.

The existence of this factor has been shown in the pollen of apple and pear trees (Gagnieu 1951; Linder 1953, 1959, 1961) and in the pollen of *Oe. fruticosa* of the sub-genus *Kneiffia* (Linder 1954). We also found the mutant +/p in *Oe. purpurata* of the sub-genus *Oenothera* (Jean and Linder 1970).

The pollen population of one flower taken from two different plants is analysed. The means and the standard deviations of the empty grains of the 8 anthers are the following (n varying between 8 and 10 for anther, and about 225 grains form a sample):

	Plant 1	Plant 2
Anther 1	50.0 ± 0.8	49.7 ± 2.3
Anther 2	50.0 ± 0.8	50.7 ± 2.1
Anther 3	50.0 ± 0.8	49.4 ± 1.2
Anther 4	50.1 ± 0.9	49.3 ± 1.5
Anther 5	49.9 ± 0.8	50.2 ± 1.6
Anther 6	50.0 ± 1.4	50.5 ± 2.3
Anther 7	49.7 ± 1.6	49.7 ± 1.6
Anther 8	49.9 ± 1.4	51.0 ± 2.1

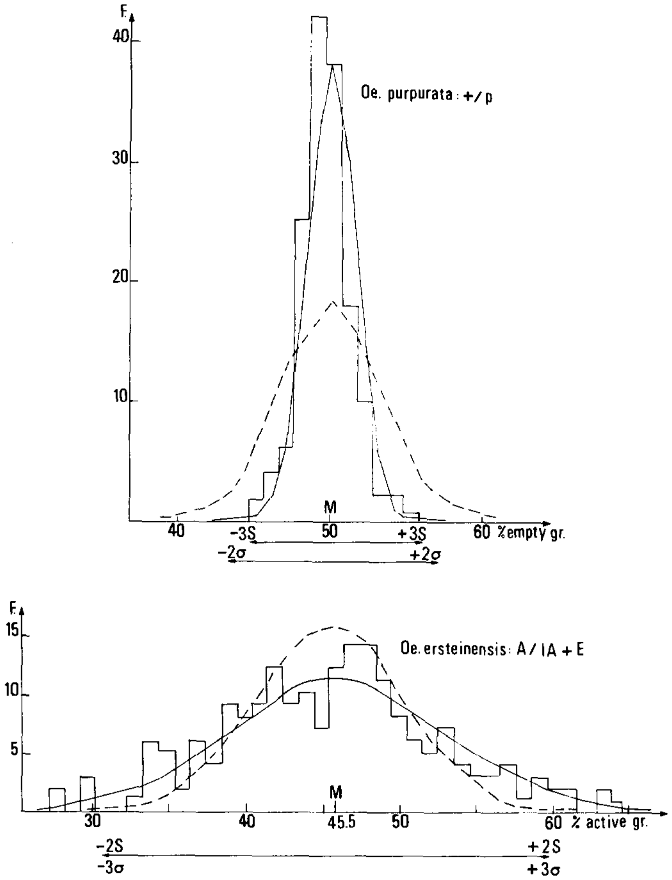

Fig. 1. This figure shows the sample fluctuation of the factorial segregation +/p in the pollen population of *Oe. purpurata* and that of the segregation "active grains/ inactive grains + empty grains" in the pollen population of *Oe. ersteinensis*. The histogram gives the experimental data. The continued line curve is the adjusted curve (*Oe. purpurata:* $\bar{x}$ = 49.9 %, σ = 1.57 % ; *Oe. ersteinensis:* $\bar{x}$ = 45.5 %, σ = 7.1 %). The dotted line curve is the theoretical curve (*Oe. purpurata:* $\bar{x}$ = 50 %, σ = 3.3 %; *Oe. ersteinensis:* $\bar{x}$ = 45.5 %, σ = 4.9 %). The two histograms are centred on the means

All the means are practically identical to the theoretical percentage of segregation. The standard deviations are very homogeneous in each plant. For plant 1, they are about 1 % and for plant 2 about 2 %. This difference is due to the fact that the second flower was plucked at a more advanced stage of anthesis. As the viscin dries when it comes into contact with air, there is less cohesion between the grains and the percentage fluctuation is slightly higher. The results show that the viscin keeps the same distribution of the grains in the sample seen with the microscope as in the pollen loculus. This gives particular statistical properties.

The pollen population of the two plants, made up of 150 samples, has a mean of 49.9 ± 1.57 % and gives the histogram of the frequencies reported in Fig. 1. The base of the histogram is narrow and its mode is very high. Comparing the histogram with its adjusted curve it can be seen that the percentages near to 50 % are preferential.

Table 1. The correlation coefficients between the percentages of the grain classes taken two by two in the pollen population of the complex-heterozygotes

Species	n	$\bar{x} \pm S$	σ	Correlation coefficients in the pairs of classes A/E	A/IA	IA/E
	$r_{A/E}$ > $r_{IA/E}$					
biennis	150	A : 23.5± 4.8	4.3	-0.801***	+0.156	-0.716***
		IA : 19.2± 4.1	3.8			
		V : 57.1± 6.7	4.3			
coronifera	66	A : 36.9± 6.3	4.8	-0.867***	+0.520***	-0.877***
		IA : 26.1± 6.6	4.4			
		V : 36.8±11.3	4.8			
nuda	62	A : 40.1±11.9	4.9	-0.849***	-0.016	-0.515***
		IA : 15.8± 7.3	3.5			
		V : 44.0±13.9	4.9			
rubricaulis	76	A : 33.8± 5.4	4.7	-0.897***	+0.593***	-0.888***
		IA : 31.0± 5.1	4.6			
		V : 35.0± 9.4	4.8			
suaveolens	40	A : 41.4± 4.8	4.9	-0.738***	-0.047	-0.640***
		IA : 35.5± 4.2	4.8			
		V : 23.0± 6.2	4.3			
syrticola	100	A : 24.4± 8.2	5.4	-0.709***	+0.024	-0.722***
		IA : 28.4± 8.3	4.5			
		V : 47.1±11.8	5.4			
	$r_{A/E}$ high					
ersteinensis	195	A : 45.5± 7.1	4.9	-0.717***	-0.382***	-0.370***
		IA : 15.3± 5.3	3.6			
		V : 39.0± 7.1	4.9			
coronifera rubrisepala	56	A : 72.1±11.2	4.0	-0.879***	-0.551***	+0.087
		IA : 6.3± 5.4	2.4			
		V : 21.5± 9.4	4.1			
	$r_{IA/E}$ > $r_{A/E}$					
conferta	81	A : 40.2± 5.8	4.9	-0.428***	-0.278**	-0.746***
		IA : 26.1± 8.0	4.4			
		V : 33.7± 8.5	4.6			

* Statistically significant $P < 0.05$
** " " $P < 0.01$
*** " " $P < 0.001$

In populations with a 50 %/50 % segregation, a theoretical standard deviation of ± 3.3 % $\left(\sigma = \sqrt{\frac{1/2 \times 1/2}{225}} \times 100\right)$ is expected. One can see that the total experimental distribution is situated within the limits of ± 2σ(± 3S = 4.71 %; ± 2σ = 6.6 %). It has been demonstrated for the +/p segregation in the pollen of the apple and the pear tree (Linder 1953 and 1959) that the sample fluctuation of a gametophytic segregation is within the limits of ± 2σ. But in *Oenothera* pollen, this fluctuation is still narrower. It seems as if the grains are taken out of an urn in which the two types of grains have the same orientated disposition as in the tetrad of two normal grains and two empty grains. The pollen image at anthesis by *Oenothera* quite accurately gives the segregation of the different potential phenotypes of the microspores in the tetrads.

b - The percentage distribution of active, inactive, and empty grains

Linder (1967, 1968) has shown that the percentage of each class of grain varies strongly within the pollen loculus, and, *a fortiori*, between anthers of the same flower or of different flowers. The histogram

Table 2. Average percentages and standard deviations of the three grain classes in the subpopulation of *Oe. biennis*, *Oe. ersteinensis*, *Oe. syrticola*

Sub-populations	n	Grain classes		
		active	inactive	empty
biennis				
1	30	22.30±4.43	18.37± 3.89	59.33± 6.05
2	30	23.07±3.75	18.83± 4.14	58.10± 6.73
3	30	24.77±5.75	19.20± 4.28	56.03± 7.93
4	30	22.67±4.37	20.77± 3.81	56.57± 6.29
5	30	25.17±4.72	18.97± 3.98	55.87± 6.04
ersteinensis				
1	30	47.63±5.93	12.20± 4.28	40.17± 7.67
2	30	43.80±7.19	20.00± 5.07	36.20± 6.45
3	30	46.87±9.46	12.93± 6.28	40.20± 9.36
4	30	47.67±6.23	13.43± 2.82	38.90± 5.56
5	30	46.40±5.66	14.30± 3.66	39.30± 5.79
6	45	42.69±6.10	17.84± 4.40	39.47± 6.48
syrticola				
1	30	23.00±8.32	27.30± 7.65	49.70±11.45
2	30	27.13±7.46	27.97± 4.63	44.90±10.07
3	40	23.42±7.74	29.60±10.59	46.97±12.95

of frequency in Fig. 1 represents an example of the percentage distribution of active grains of *Oe. ersteinensis*, the mean of which is 45.4 % ± 7.1 % for N = 100 and n = 195. This histogram is wide over the x-axis and the model class is not clearly visible. Taking the experimental mean as the theoretical percentage of segregation, the percentage distribution is given by the theoretical frequency curve with $\sigma = 4.9\,\% \left(\sigma = \sqrt{\frac{45.5 \times 54.5}{100}} \times 100\right)$.

The same is observed in the other two pollen classes of *Oe. ersteinensis* and in the three classes of the other analysed species (Table 1, column 3, and Table 2). In the majority of distributions S is greatly superior to σ. This is the complete inverse of the segregation of a couple of mendelian alleles. From this it can be deduced that several segregation proportions exist for active grains, inactive grains and empty grains. It seems that the segregation of active, inactive and empty microspores varies from one tetrad to another. The pollen population sample comes from different kinds of tetrads; the high number of their combinations explains the high value of the standard-deviation.

From this hypothesis, two modes of distribution of the three microspore phenotypes in the tetrad can be advanced. These modes of distribution are either haphazard or preferential. In the latter case certain tetrads could give microspores of only one phenotype. Consequently, in the pollen population at anthesis, a high percentage of one grain phenotype could correspond quite regularly to a low percentage of the other two phenotypes, and vice-versa. In this way it can be seen that the coefficients of linear correlation between the percentages of the grain classes can have a descriptive value for the tetrad population.

c - Correlation between the percentages of the three grain classes

Before calculating the coefficients of correlation, the normality of the distribution of the percentages of the three grain classes for each species is analysed by the χ^2 test. On the whole the normality is respected. Only in two cases does the percentage distribution depart from the normal law for the active grain class of *Oe. biennis* ($\chi^2_{(20)} = 35.8$, $P = 0.02$) and for the inactive grain class of *Oe. coronifera rubrisepala* ($\chi^2_{(13)} = 119$, $P < 0.000$). But this does not seem to change in any way the value of the coefficients r, which fit in well with the global result.

Table 3. The correlation coefficients between the percentages of the grain classes taken two by two in the pollen subpopulation of *Oe. biennis, Oe. ersteinensis, Oe. syrticola*

Sub-populations	n	Correlation coefficients in the pairs of classes A/E	 A/IA	 IA/E
Oe. biennis				
1	30	-0.766***	+0.054	-0.683***
2	30	-0.837***	+0.456**	-0.869***
3	30	-0.851***	+0.233	-0.709***
4	30	-0.803***	+0.178	-0.729***
5	30	-0.753***	+0.042	-0.625***
Oe. syrticola				
1	30	-0.744***	-0.039	-0.638***
2	30	-0.903***	+0.352*	-0.721***
3	40	-0.576***	-0.027	-0.802***
Oe. ersteinensis				
1	30	-0.832***	+0.105	-0.639***
2	30	-0.778***	-0.348*	-0.319
3	30	-0.728***	-0.491**	-0.240
4	30	-0.891***	-0.450**	-0.004
5	30	-0.796***	-0.289	-0.350*
6	45	-0.757***	-0.272	-0.423**

The binary combinations of the three grain classes are: active grain percentages/empty grain percentages (abbreviation:A/E), active grain percentages/inactive grain percentages (abbreviation : A/IA), and inactive grain percentages/empty grain percentages (abbreviation : IA/E). The values for the coefficients r are given in the Table 1 for all of the species, and in Table 3, by plant, for *Oe. biennis, Oe. ersteinensis* and *Oe. syrticola* (the sub-population is the pollen population of one plant).

Each species is characterised by a fixed combination of the three coefficients. In Table 1 the species are regrouped according to identical combination. The following combinations are observed: the combination called *biennis:* A/E has a high negative r; A/IA has a low negative or positive r, or a high positive r; IA/E has a high negative r; the combination called *ersteinensis:* A/E has a high negative r; A/IA and IA/E have a medium to low negative or positive r; the combination called *conferta:* A/E and A/IA have a medium to low negative r, I/AE has a high negative r.

The results by plant (Table 3) show that the combinations of the three coefficients vary slightly within the species. In the active/empty pair with high correlation the coefficients r are remarkably homogeneous. The 95% confidence limit is theoretically between -0.3 and -1. In fact the total fluctuation of r is between -0.6 and -0.9. The correlation coefficients demonstrate that there are preferential segregations by tetrad. But they do not indicate which grains the tetrads are composed of. In order to understand them, it is necessary to calculate the correlation coefficients for a pollen population of which the tetrad population is well known and which presents a certain analogy with the tetrad population of the complex heterozygote *Oenothera.*

B - Models of the tetrad population of three grain classes

The pollen population which in our opinion answers to these conditions is that in which two allelic linked pairs segregate. It is the inequality of the proportion between the parental ditype tetrads and the recombined ditype tetrads which allows this analogy. We use the classical segregation of the alleles C, c and t, wx which is observed on corn spikes. The value of the correlation (Table 4, A) differs from one pair of classes to another, and it remains remarkably stable in any two parts of the total population. When considering the origin of the grains in the tetrads, it can be seen that the highest correlation appears in a pair in which one phenotype comes from a parental tetrad (PA) [c +] and the other [c wx] from a recombined tetrad (RE). By comparison, in the segregation of two independent allelic pairs $\frac{C+}{c\,su}$ (Table 4, B), the correlations in the total population are low and practically of the same value (the $\chi^2_{(5)} = 4.66$; $P = 0.46$). The low correlations are found in the sub-populations 1 and 3, but the sub-population 2 shows the same gradation of the correlation coefficient as that of the segregation of two allelic linked pairs.

To obtain exact similarity between the pollen population of three grain classes and that of four classes, it is necessary to foresee, for this latter population, the different possibilities of grouping two phenotypes in one. But as the results of the segregation C wx/c + are already given in a grouped form, which makes any other grouping impossible, we continue to work

Table 4. Correlation between the percentage of grain classes taken two by two in the pollen of the *Zea mays* (coefficient r calculated from segregation on spikes coming from a testcross)

A. Bifactorially linked segregation $\frac{C\ wx}{c\ +}$

		Correlation coefficients in the pairs of classes		
		c +/c wx	C /c +	C /c wx
Sub-populations	n	tetrad: Pa/Re	Pa+Re/Pa	Pa+Re/Re
1	30	-0.825***	-0.540***	-0.031
2	46	-0.688***	-0.488***	-0.298*
Total population	76	-0.748***	-0.515***	-0.184

B. Bifactorially independent segregation $\frac{C\ +}{c\ su}$

		Correlation coefficients in the Intra-tetrad pairs	Inter-tetrad pairs	
Sub-populations	n	C +/c su	C +/c +	c su/c +
1	30	-0.398*	-0.371*	-0.292
2	30	-0.086	-0.217	-0.639***
3	48	-0.229	-0.429***	-0.150
Total population	108	-0.264**	-0.372***	-0.288**
		c +/C su	C su/c su	C +/C su
1	30	-0.515***	-0.451	-0.319
2	30	-0.158	-0.240	-0.658***
3	48	-0.353**	-0.345*	-0.450***
Total population	108	-0.400***	-0.229*	-0.423***

on the sub-population 2 of the independent segregation, which is statistically similar to the linked segregation. Six model populations of tetrads (Fig.2) are obtained, each of which is characterised by 3 correlation coefficients. Inversely the value of these 3 coefficients enables a definition of the 3 types of tetrads to be made.

C - The organisation of tetrads

Applying these model populations to the pollen populations of *Oenothera*, it can be seen that the three combinations of the coefficients r, analysed above, give three population-types of tetrads, which are shown in Fig.3. These latter are successively described and empty (E) microspores in the tetrads of *biennis type*, *ersteinensis type*, *conferta type* (successively presented by line).

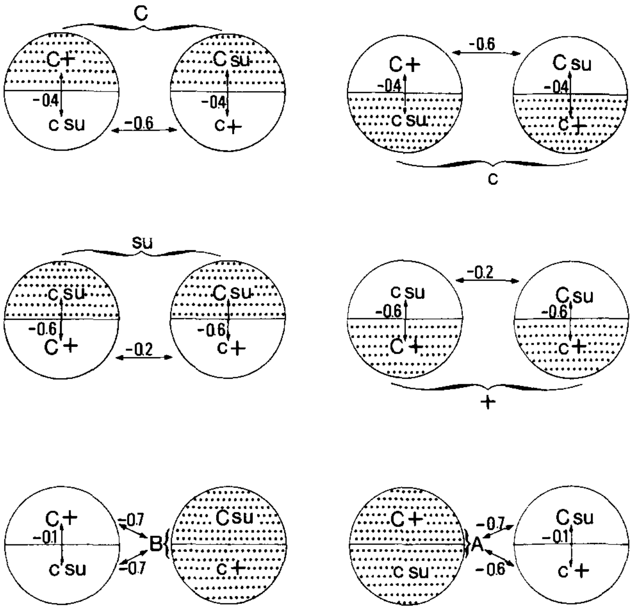

Fig.2. Diagram showing the model pollen populations 1, 2, 3 with three grain classes. The grouped microspores are shown by the dots, the class pairs are shown by the arrows together with the coefficient r

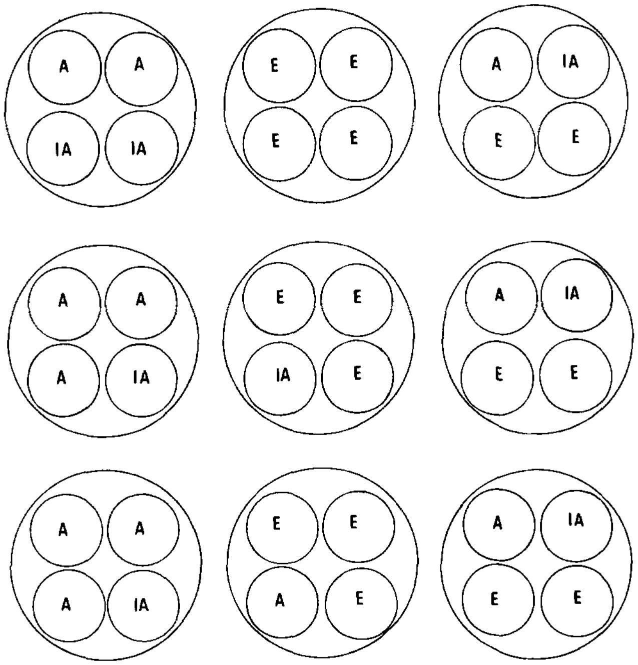

Fig.3. Distribution of the potentially active (A) inactive (IA) and empty (E) microspores in the tetrads

a - The *biennis* type

The *biennis* combination follows the model population 3: the population of tetrads is composed of ditype tetrads in which active and inactive microspores segregate, and monotype tetrads in which only empty microspores are found. In the *biennis* species set, the pollen population of *Oe. rubricaulis* is note worthy because of its high positive coefficient r(+ 0.7) in the pair A/IA. The value of the coefficient shows that the percentages of the active and inactive grains increase and decrease at the same time from one sample to another. This can only be if the active grains and the inactive grains come from the same tetrad. The variance of each grain class is therefore the sum of two variations: a) the variation of the number of tetrads giving active and inactive grains; b) the sample fluctuation of the segregation active, inactive grains in the tetrad. The estimation of this fluctuation is given by calculating the ratio of each sample $\frac{\%\text{ active grains}}{\%\text{active grains} + \%\text{inactive grains}}$. Following this transformation, the mean of the active grains is 52 % ± 3.7 %. The new standard deviation is weaker than that observed. In the case of a segregation 1/1 the standard deviation should have a value of 2 % (cf. pollen population +/p of *Oe. purpurata*). Therefore the segregation active/inactive grains approaches the segregation 1/1. The surplus of ± 1.7 % is explained by supposing that a few tetrads of empty microspores contain one active microspore or one inactive microspore or both. Thus the tetrad population of *rubricaulis* is composed principally of ditype tetrads with active and inactive microspores, and of monotype tetrads with empty microspores in which can be seen here and there active or inactive microspores.

Oe. coronifera presents the same coefficient r combination as *Oe. rubricaulis*, but the means of the inactive and active grains differ from the segregation 1/1, the inactive grains being generally weaker in proportion in the ditype tetrads.

Oe. biennis, *Oe. syrticola* and *Oe. suaveolens* have an independent active/inactive pair. The ratio $\frac{\%\text{active grains}}{\%\text{active grains} + \%\text{inactive grains}}$ calculated for the *Oe. syrticola* population gives the following parameters: 46 % ± 11.6 %. By this transformation the standard deviation has greatly increased. This means that the active grains and the inactive grains do not only come from ditype tetrads but also from a third tetrad type, tritype tetrads in which active, inactive and empty grains segregate. This organisation is found in all sub-populations (Table 3). The tritype tetrad, which is rare in the tetrad population of *Oe. rubricaulis*, is regularly present in the tetrad population of *Oe. syrticola* and in that of all other species of the *biennis* set.

The results are peculiar to *Oe. nuda*, in that the correlation coefficient indicates a tetrad population of *biennis* type but the standard deviations of the active grains and the inactive grains have different values, which is not the case in the other species of the *biennis* set. This is explained by the low percentage of inactive grains. Thus the majority of the tetrads with active microspores are monotype and they follow the fluctuation of the tetrads with empty microspores.

Finally, the statistical results are coherent for all the species with tetrads of the *biennis* type, when the means and the standard deviations are compared with the significance of the correlation coefficient. The basic tetrads are those which have active microspores and those which have empty microspores. According to the species, the former tetrads show the active/inactive segregation in proportions which generally depart from 1/1, while the latter tetrads show empty grains and can also show active and inactive grains.

b - The *ersteinensis* type

The combination of the three correlation coefficients follows the model population 1. This means that certain tetrads contain active microspores while other tetrads contain empty microspores, the inactive grains being distributed randomly in one or the other tetrad. It is probable that tritype tetrads appear among the tetrad-population. The fluctuation in number of the two ditype tetrads may be estimated by the standard deviation of the active grains or of the empty grains. In this way their identical value is explained. As the inactive grains can appear in the two tetrads, the fluctuation in their percentages is lower.

It can be seen that the tetrad population of the *ersteinensis* type is quite close to that of the *biennis* type. The essential difference between the two types is that, for the *ersteinensis* type, the inactive microspores do not segregate only with the active microspores in a tetrad. In the sub-populations (Table 3) the combinations of the three r coefficients represent the fluctuation of the combination of the whole population. The sub-population 1 is the exception in that if follows the model population 3 and is of the *biennis* type. Knowing the two tetrad populations, it is interesting to compare the parameters of *Oe. nuda* and *Oe. ersteinensis:* the means of the three classes are approximately identical, but the combination of the correlation coefficients is not the same, which shows that their tetrad populations are different.

In Table 1, *Oe. coronifera rubrisepala* is placed after *ersteinensis*. The tetrad population of this species cannot be stated with any certainty. As the mean of the inactive grains is very low, the percentage distribution of this class is dissymetrical and therefore departs from the normal law. If we consider only the classes of active and empty grains with normal distribution, their very high correlation (r near to -0.9) shows two tetrads, one with active microspores and the other with empty microspores. If, despite the absence of normality, we gave a descriptive value to the r coefficients of the two pairs A/IA and IA/E, we could arrive at a tetrad population of the *ersteinensis* type.

c - The *conferta* type

Among the species analysed, *Oe. conferta* is the only one which shows the highest r coefficient for the inactive/empty pair. The combination of the coefficients approximately follows model population 1: one high coefficient, two medium coefficients. This signifies that there are two types of tetrads, one with inactive microspores, the other with empty microspores; the active microspores appear randomly in the two tetrads. In comparison with the *biennis* type we will admit the presence of the tritype tetrad. We find in the *conferta* type another form of the population of the *ersteinensis* type, in that only one phenotype of microspore is distributed randomly between the two ditype tetrads.

Conclusion: Genetical significance of the tetrad populations

The species analysed are characterised by three types of tetrads: one which gives active microspores, another empty microspores and a third the three types of microspores. It is known that active microspores transmit the complex intact (Renner 1919) and we have shown that an empty microspore carries a recombined complex (Jean 1974). In this way the tetrad with active microspores is the result of a meiosis without any exchange, the tetrad with empty microspores is the result of a meiosis with exchange between the four homologous chromatids, and the tritype tetrad is the result of a meiosis with exchange between two chromatids. It can be seen that the tetrad population of *Oenothera* is comparable with that observed in mendelian genetics: the tetrad with active microspores is the homologue of the parental tetrad, the tetrad with empty microspores is the homologue of the ditype recombined tetrad and the tritype tetrad is the homologue of the tetratype tetrad.

This basic structure varies with the partition of the inactive microspores. In the *biennis* type species, which are only heterogamous, the active microspores and the inactive microspores appear with preference in the same tetrad. The two complexes segregate in this tetrad, but as the means and the standard deviation do not correspond to the segregation 1/1, the morphological segregation active grains/inactive grains is not super-imposed on the genetical segregation of the complexes. The active grains, which are generally predominant, carry the two complexes. The inactive grains carry the inactive complex but, given the large standard deviation, it is probable that the inactive grain will be able to carry the two complexes. This means that the segregation "active microspores, inactive microspores" in the tetrad occurs according to the five combinations 4/0, 3/1, 2/2, 1/3 and 0/4.

In the *ersteinensis* type and the *conferta* type, which group the isogamous species (*coronifera rubrisepala* is heterogamous, but belongs to the translocation mutants which are generally isogamous), the active microspores and the inactive microspores do not segregate with preference in the same tetrad. The morphological segregation "active microspores, inactive microspores" is not connected to the genetical segregation of the two complexes. This means that the inactive grain is genetically heterogeneous: it carries the complex intact or recombined.

Our results can be considered parallel to those of W. Stubbe (1959, 1960) and to those of Göpel (1970), both of which treat the inactivity of the complexes, and to those of Harte (1969a und b) which treat the pollen competition between the complexes, all works which followed the discovery of cytoplasmic heredity made by Renner (1936). These authors showed that pollen fertility is determined both by the sporophyte and by the gametophyte. This conclusion was made from analysis of the hybrid generations. Basing our work on the pollen analysis of the plant species, we have arrived at the same conclusion, that is to say, the gametophyte according to the complex type and the sporophyte according to isogamous or heterogamous plant give together the tetrad population.

As we know the segregation of the potentially active, inactive and empty microspores in the tetrads, we are led to introduce a correction in the Darlington hypothesis (1931) which was taken up by Catcheside (1940). These authors show that exchanges at the level of the homologous differential segments do not take place. This is confirmed by the existence of the ditype tetrads with active and inactive microspores which originate from meiosis without exchange on any of the fourteen chromosome arms. But our tetrad analysis shows that the hindrance to the exchange is not absolute, as the monotype tetrads with potentially lethal microspores originate from mother cells in which, at the pachytene stage, exchanges are made between the homologous differentiated segments. If the recombined complexes were not eliminated in the empty grains, the cytological mechanism would be insufficient by itself to avoid the breaking up of the complexes. Therefore the pollen lethality together with the cytological mechanism maintains the factorial linkage of the complex.

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