Genetics of Male Sterility in Gynodioecious *Plantago coronopus*. II. Nuclear Genetic Variation

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ABSTRACT

Inheritance of male sterility was studied in the gynodioecious species *Plantago coronopus* using five plants and their descendants from an area of ~50 m^2 from each of four locations. In each location, crosses between these five plants yielded the entire array of possible sex phenotypes. Both nuclear and cytoplasmic genes were involved. Emphasis is placed on the nuclear (restorer) genetics of two cytoplasmic types. For both types, multiple interacting nuclear genes were demonstrated. These genes carried either dominant or recessive restorer alleles. The exact number of genes involved could not be determined, because different genetic models could be proposed for each location and no common genetic solution could be given. At least five genes, three with dominant and two with recessive restorer allele action, were involved with both cytoplasmic types. Segregation patterns of partially male sterile plants suggested that they are due to incomplete dominance at restorer loci. Restorer genes interact in different ways. In most instances models with independent restorer gene action were sufficient to explain the crossing results. However, for one case we propose a model with epistatic restorer gene action. There was a consistent difference in the segregation of male sterility between plants from the two cytoplasmic types. Hermaphrodites of cytoplasmic type 2 hardly segregated male steriles, in contrast to plants with cytoplasmic type 1.

A major problem in understanding the gynodioecious breeding system and the occurrence of male steriles is the limited knowledge of the genetics of this system. Theoretical studies show that the existence of the breeding system is dependent on the combined action of the mode of inheritance and the extent of the disadvantage of being male sterile. ROSS (1978) surveyed most genetic studies up to 1978 and concluded that monogenic inheritance has hardly been found in *natural* populations. Inheritance of male sterility was either claimed to be digenic or complex with cytoplasmic effects. CHARLESWORTH (1981) argued that nuclear-cytoplasmic models were likely to fit most data. Her suggestion is now justified by detailed genetic studies.

Nuclear-cytoplasmic inheritance of male sterility in a species is typically thought of in terms of several cytoplasmic types: each is either unrestored (*i.e.*, determining female gender) or restored (*i.e.*, determining hermaphrodite gender) by specific alleles of nuclear genes (the so-called restorer genes). For nuclear genes of this type, one allele is called the restorer allele and the other the sterility allele. The occurrence of cytoplasmic variation within populations has now been shown in several gynodioecious species; *e.g.*, *Nemophila menziesii* (GANDERS 1978), *Origanum vulgare* (KHEYR-POUR 1980, 1981), *Plantago lanceolata* (VAN DAMME and VAN DELDEN 1982), *Thymus vulgaris* (BELHASSEN *et al.* 1991) and *P. coronopus* (KOELEWIJN and VAN DAMME 1995). Cytoplasmic variation, however, is only one side of the coin. Restorer gene variation, the reverse side, has proven to be much more difficult to solve for natural populations. It is often stated that there is variation in restorer genes, but that the genetics is complex (KHEYR-POUR 1981; BELHASSEN *et al.* 1991). Only few authors have proposed genetic models based on crossing results, but even they state that they cannot fully explain all their crossing results (VAN DAMME 1983; CONNOR and CHARLESWORTH 1989). The involvement of both dominant and recessive restorer alleles is beyond doubt. However, the number of restorer genes involved in restoring male fertility is still unknown for any species. VAN DAMME (1983) gave a minimum estimate of five genes for MS1 in *P. lanceolata*, of which three had dominant and two had recessive restorer allele action. The complexity of the inheritance in studies from *natural* populations is in contrast with that of most *cultivated* species. KAUL (1988) states that in the majority of crop plants, male sterility is determined by a single recessive gene. However, these results are often based on inbred lines, selected maintainer lines or on a few plants from known genetic background, which may mask the variation originally present in the parental plants. In studies on *natural* populations, in which plants from unknown genetic origin are used, this variation might become

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apparent in crossing studies and cause problems interpreting the results.

Some insight into the nuclear genetics is required for understanding of gynodioecious breeding systems. Most theoretical models for the maintenance of gynodioecy require variation in restorer genes within sex phenotypes to explain joint polymorphism between cytoplasmic and nuclear genes (*e.g.*, FRANK 1989). Evaluation of crossing results in terms of numbers of genes involved and their interaction is therefore indispensable and could provide a guideline for the type of models to be used. In our first article (KOELEWIJN and VAN DAMME 1995), we gave a description of anther morphology and of the subsequent distinction of three sex phenotypes: male steriles (MS), partially male steriles (PMS) and hermaphrodites (H). The involvement of cytoplasmic genes in determining male sterility was proven and shown to be limited to only two, henceforward referred to as cytoplasmic type 1 and 2. These two cytoplasmic types were present in most of the populations studied. In this paper we focus our attention on variation in restorer genes. We present detailed nuclear genetic models for four populations and address questions about the number and type of nuclear genes involved in male sterility. In addition we look for differences in restorer gene frequencies among populations and pay some attention to the role of the partially male sterile phenotypes.

MATERIALS AND METHODS

In the Netherlands *P. coronopus* is an annual or short lived perennial, mainly growing in dune grasslands along the coast. It is a wind pollinated and self-compatible species, with an average outcrossing rate of ~75% (range 20–100%; WOLFF *et al.* 1988). Plants used for crosses originated from two locations in each of two sites (Oostvoornse meer, Kwade Hoek) in the Southwest of the Netherlands. From each location three hermaphrodites (H) and two male steriles (MS) were used [see KOELEWIJN and VAN DAMME (1995) for a description of the sites and experimental procedures]. Each MS was crossed with each of the three H and the three H were crossed in a diallel design. Offspring of these crosses were grown in an experimental garden and a number of these were selected for second and third generation crosses. In a previous paper (KOELEWIJN and VAN DAMME 1995), we showed that there was cytoplasmic variation at all four locations and parental plants were classified according to their sex and cytoplasmic type (hermaphrodites: H1, H2; partial male steriles: PMS1, PMS2; male steriles: MS1 and MS2). Sex type scores were based on 20–50 flowering spikes per plant. Sex-type ratio testing was analyzed with G-tests (SOKAL and ROHLF 1981). Unless stated otherwise analyses were based on the categories MS *vs.* not-MS, *i.e.*, H and PMS were grouped together. Each cross received a family number and offspring were coded according to this number. Thus, MS-(280–1) means that the plant is a male sterile, originates from cross 280 and is the first plant used in subsequent crossing.

In 1990 a random pollen sample was collected in six populations by tapping pollen of 25 plants into a small tube. The plants were located in a square of ~10 × 10 m. Pollen was collected during peak flowering (*i.e.*, >80% of the plants were flowering). These pollen samples were used in crosses with MS of known cytoplasmic and nuclear background to look for differences in restorer allele frequencies among populations.

In agreement with the literature (KAUL 1988), we will denote dominant restorer alleles by *R* and recessives by *r*. Because cytoplasmic genes are thought to be the oldest on an evolutionary time scale, the nuclear sterility allele is considered to be the wild-type allele and will be indicated by +.

RESULTS

Restorer allele variation: Differences in restorer allele constitution among plants can be traced in several ways. Heterogeneity in male sterile segregation ratios among the offspring of selfed plants with known cytoplasmic background indicate restorer allele differences. However, often the cytoplasmic background is unknown. In that case, variation in restorer alleles can be detected by crossing several H with the same MS. Heterogeneity in segregation ratios among the H then indicates variation in restorer alleles. If the same H are crossed with several MS, and the cytoplasmic background of these MS is known, then heterogeneity among the MS of the same cytoplasmic type also indicates restorer allele variation among the MS. Within each location two MS were crossed with the same three H (Tables 1–4). There appears to be variation in restorer alleles among the H, because all eight MS * H combinations showed heterogeneity in segregation ratios among the H within each MS, as revealed by G-tests (results not shown). Moreover, the two MS of the Oostvoornse meer meadow location (Table 4) both had cytoplasmic type 2, and the between MS test showed a significant difference in segregation ratios, therefore indicating restorer allele variation between both these two MS also. In the other three locations, the MS were of different cytoplasmic types. Because there was heterogeneity among the H within each MS, this indicated variation in the restorer alleles of both cytoplasmic types at these locations.

Inheritance of male sterility types: In each location all possible crosses between the MS and H were made. Second and third generation crosses focused mainly on plants that showed a reciprocal difference, but additional crosses were made with the offspring of the MS * H crosses. Inspection of Tables 1–4 shows a variety of ratios of MS *vs.* the other phenotypes. The results can be explained by a multiple gene system, consisting of a number of restorer genes with either recessive or dominant action, which can interact in different ways. We will assume independent gene action as the basic model (but see FRANK 1989), *i.e.*, restoration at only one of the loci is sufficient to restore male fertility and show the PMS or the H phenotype. We will, however, also provide evidence for interaction between restorer genes. To avoid confusion we will treat each location

TABLE 1

Crosses between Kwade Hoek (dune area) plants of *Plantago coronopus*

Family no./cross	Offspring MS	Offspring PMS	Offspring H	MS:(PMS + H) Cytoplasm 1 Ratio[a]	Cytoplasm 1 G(1)[b]	Cytoplasm 2 Ratio	Cytoplasm 2 G(1)
A. First generation crosses Kwade Hoek (dune area)							
Selfing							
14 (kh69)	0	41	41	0:1			
15 (kh80)[c]	0	13	59			0:1	
16 (kh120)	28	19	53	1:3	0.47		
H * H							
107 (kh69 * kh80)	1	9	33	0:1			
109 (kh80 * kh69)	0	6	69			0:1	
108 (kh69 * kh120)	0	11	64	0:1			
111 (kh120 * kh69)	0	5	57	0:1			
110 (kh80 * kh120)	0	11	68			0:1	
112 (kh120 * kh80)	4	14	41	1:15	0.03		
MS * H							
35 (kh89 * kh69)	0	22	48	0:1			
36 (kh89 * kh80)	1	6	63	1:15	3.97*		
37 (kh89 * kh120)	40	15	16	1:1	1.14		
38 (kh115 * kh69)	0	5	67			0:1	
39 (kh115 * kh80)	14	11	47			1:3	1.25
40 (kh115 * kh120)	13	34	21			1:3	1.33
B. Second generation families with females having cytoplasmic type 1							
112 (kh120 * kh80)							
Selfing							
112-1	14	18	22	3:13	1.68		
H1 * H2							
112-1 * 110-1	23	19	14	3:5	0.31		
112-1 * kh80	2	22	21	1:31	0.24		
110 (kh80 * kh120)							
H1 * H2							
kh120 * 110-1[d]	0	10	12				
37 (kh89 * kh120)							
H1 * H1							
37-4 * kh120	16	23	16	1:3	0.48		
kh120 * 37-4	12	18	13	1:3	0.19		
C. Second generation families with females having cytoplasmic type 2							
110 (kh80 * kh120)							
Selfing							
110-1	0	10	33			0:1	
H2 * H1							
110-1 * 112-1	0	10	18			0:1	
110-1 * kh120	0	13	41			0:1	
112 (kh120 * kh80)							
H2 * H1							
kh80 * 112-1	0	26	40			0:1	

[a] Ratio is the expected ratio of MS:not-MS, according to the proposed genetic models in the text.
[b] G(1) is the value of the G-test (with 1 d.f.) that evaluates the difference between expected and observed ratio.
[c] Underscore denotes plants having cytoplasmic type 2.
[d] 50% of seedlings died due to drought in petri dish.
* $P < 0.05$.

TABLE 2

Crosses between Kwade Hoek (salt marsh) plants of *Plantago coronopus*

Family no./cross	Offspring			MS:(PMS + H)			
				Cytoplasm 1		Cytoplasm 2	
	MS	PMS	H	Ratio[a]	G(1)[b]	Ratio	G(1)
A. First generation crosses Kwade Hoek (salt marsh)							
Selfing							
11 (kh6)[c]	0	13	56			1:63	2.17
12 (kh41)	0	31	50			0:1	
13 (kh51)	16	24	39	9:55	2.24		
H * H							
101 (kh6 * kh41)	0	3	63			0:1	
103 (kh41 * kh6)	0	11	44			0:1	
102 (kh6 * kh51)	0	7	52			0:1	
105 (kh51 * kh6)	1	1	8	1:3	1.45		
104 (kh41 * kh51)	0	17	44			0:1	
106 (kh51 * kh41)	42	56	83	9:23	2.25		
MS * H							
29 (kh2 * kh6)	27	35	0	1:1	0.06		
30 (kh2 * kh41)	42	26	0	9:7	0.85		
31 (kh2 * kh51)	16	28	29	9:23	1.46		
32 (kh56 * kh6)	12	28	32			1:7	1.05
33 (kh56 * kh41)	0	6	66			0:1	
34 (kh56 * kh51)	0	13	57			0:1	
B. Second generation families with females having cytoplasmic type 1							
106 (kh51 * kh41)							
Selfing							
106-1	24	18	36	1:3	1.32		
H1 * H2							
106-1 * 104-1	17	23	30	1:3	0.02		
106-1 * kh41	36	13	21	1:1	0.12		
MS1 * H2							
106-2 * kh41	66	4	0	1:0			
106-3 * kh41	11	0	0	1:0			
280 (106-2 * kh41)							
MS1 * H2							
280-1 * kh41	120	2	0	1:0			
104 (kh41 * kh51)							
H1 * H2							
kh51 * 104-1	14	26	29	3:13	0.11		
30 (kh2 * kh41)							
H1 * H2							
30-1 * kh41	46	23	7	1:1	3.39		
30-1 * 33-1	9	17	35	1:3	3.82		
31 (kh2 * kh51)							
H1 * H1							
31-1 * kh51	11	17	57	1:7	0.02		
31-2 * kh51	35	28	52	1:3	1.73		
33 (kh56 * k41)							
H1 * H2							
kh51 * 33-1	7	23	21	3:29	1.01		

TABLE 2

Continued

Family no./cross	Offspring MS	Offspring PMS	Offspring H	MS:(PMS + H) Cytoplasm 1 Ratio[a]	Cytoplasm 1 G(1)[b]	Cytoplasm 2 Ratio	Cytoplasm 2 G(1)
C. Second generation families with females having cytoplasmic type 2							
104 (kh41 * kh51)							
Selfing							
104-1	5	20	44			1:15	0.11
H2 * H1							
104-1 * 106-1	2	22	42			1:15	1.43
104-1 * kh51	0	14	42			0:1	
106 (kh51 * kh41)							
H2 * H1							
kh41 * 106-1	0	14	53			0:1	
30 (kh2 * kh41)							
H2 * H1							
kh41 * 30-1	4	18	43			0:1	
33 (kh56 * kh41)							
H2 * H1							
33-1 * 30-1	0	11	57			0:1	
H2 * H2							
33-1 * kh41	0	8	60			0:1	
kh41 * 33-1	0	6	52			0:1	

[a] Ratio is the expected ratio of MS:not-MS, according to the proposed genetic models in the text.
[b] G(1) is the value of the G-test (with 1 d.f.) that evaluates the difference between expected and observed ratio.
[c] Underscore denotes plants having cytoplasmic type 2.

separately. In each case, we will start with an analysis of cytoplasmic type 1, using the H * H crosses; later the MS * H crosses will be incorporated.

Kwade Hoek (dune area): First generation crosses with cytoplasmic type 1 segregated few MS, except for MS-kh89 * H-kh120 (Table 1A). Selfing of H-kh120 gave a 1:3 ratio, indicating the presence of a gene with a dominant restorer allele. Because H-kh120 * H-kh80 gave a 1:15 ratio, at least two other restorer genes must be involved, with H-kh120 heterozygous at one locus and homozygous for sterility at the other two and H-kh80 heterozygous at all three loci. Because crosses with H-kh69 did not segregate any MS at all, this plant should be fully restored at at least one locus. We cannot make a definite statement about the state of the other two loci of H-kh69, because of the lack of subsequent crosses. The second generation crosses (Table 1B) also fitted into the scheme of three restorer genes with dominant restorer alleles, with plant H-(112–1) being *R1*/+ +/+ +/+ and H-(110–1) being +/+ +/+ +/+. Contrary to expectations kh120 * 110–1 did not segregate MS. However, ~50% of the seedlings died, which might have obscured the ratio (see KOELEWIJN and VAN DAMME 1995). The shortage of MS, due to drought in the seedling stage, is in line with the results of VAN DAMME (1991). Male sterile kh89 should be homozygous sterile at all three loci. When crossed with H-kh120 a 1:1 ratio is in line with this, and with H-kh69, a plant fully restored at at least one locus, there is no segregation. However, we would expect a 1:7 ratio for MS-kh89 * H-kh80, which was not observed. Invoking another locus decreased the ratio to 1:15, but this still gave a *P* value of 0.046, indicating that at least one other restorer locus must be involved in order to explain this and previous results, in addition to the three genes already needed to fit the data. This new restorer allele must be recessive. The final model for this location therefore requires four restorer genes, three with dominant and one with recessive restorer alleles (Table 1, A and B). The proposed genotypes are shown in Figure 1A. The proposed model is a "minimum" one, because using more plants would probably result in a more complicated model (*cf.*, VAN DAMME 1983).

It is very difficult to make a definite statement about the nuclear genetics of cytoplasmic type 2, because only MS-kh115 segregated MS (Table 1A). MS-kh115 * H-kh80 and MS-kh115 * H-kh120 both gave a 1:3 ratio, indicating that at least one dominant restorer allele is involved. Because neither the cross H-kh80 * H-kh120 nor selfing of H-kh80 segregated, while both hermaphrodites did when crossed with MS-kh115, a second gene with a recessive restorer allele is necessary. Therefore, H-kh80 and H-kh120 have the same genotype. Although this is true with respect to segregation of MS,

TABLE 3

Crosses between Oostvoornse meer (dune area) plants of *Plantago coronopus*

Family no./cross	Offspring			MS:(PMS + H) Cytoplasm 1		MS:(PMS + H) Cytoplasm 2	
	MS	PMS	H	Ratio[a]	G(1)[b]	Ratio	G(1)
A. First generation crosses Oostvoornse meer (dune area)							
Selfing							
4 (om80)[c]	1	20	53				
5 (om90)	22	24	18	1:3	2.80		
6 (om120)	8	21	33			0:1	
H * H							
83 (om80 * om90)	0	12	64				
85 (om90 * om80)	18	25	90	1:7	0.13		
84 (om80 * om120)	0	34	32				
87 (om120 * om80)	0	8	70				
86 (om90 * om120)	20	23	32	1:3	0.11		
88 (om120 * om90)	0	2	62			0:1	
MS * H							
23 (om64 * om80)	2	16	50	1:7	7.90*		
24 (om64 * om90)	33	20	7	1:1	0.61		
25 (om64 * om120)	26	11	30	3:5	0.05		
26 (om76 * om80)	4	34	34				
27 (om76 * om90)	44	8	2			3:1	1.29
28 (om76 * om120)	30	22	12			1:1	0.25
B. Second generation families with females having cytoplasmic type 1							
86 (om90 * om1200)							
Selfing							
86-2	14	33	18	3:13	0.32		
H1 * H2							
86-2 * 88-1	16	41	14	1:3	7.32*		
86-2 * om120	11	20	41	3:13	0.60		
MS1 * H2							
86-3 * om120	16	24	18	3:5	2.54		
88 (om120 * om90)							
H1 * H2							
om90 * 88-1	49	21	12	1:1	3.14		
24 (om64 * om90)							
H1 * H1							
24-2 * om90	16	27	19	1:3	0.02		
H1 * H2							
24-2 * 27-3	13	25	11	1:3	0.06		
27 (om76 * om90)							
H1 * H2							
om90 * 27-3	16	29	22	1:3	0.05		
C. Second generation families with females having cytoplasmic type 2							
88 (om120 * om90)							
Selfing							
88-1	0	11	18			0:1	
H2 * H1							
88-1 * 86-2	0	19	50			0:1	
88-1 * om90	4	18	42			0:1	
86 (om90 * om120)							
H2 * H1							
om120 * 86-2	0	13	47			0:1	

Because of uncertainties no ratios are given for several crosses where om80 is involved and the females have cytoplasmic type 2.

[a] Ratio is the expected ratio of MS:not-MS, according to the proposed genetic models in the text.

[b] G(1) is the value of the G-test (with 1 d.f.) that evaluates the difference between expected and observed ratio.

[c] Underscore denotes plants having cytoplasmic type 2.

* $P < 0.05$.

TABLE 4

Crosses between Oostvoornse meer (meadow) plants of *Plantago coronopus*

Family no./cross	Offspring MS	Offspring PMS	Offspring H	MS:(PMS + H) Cytoplasm 1 Model 1 Ratio[a]	Cytoplasm 1 Model 1 G(1)[b]	Cytoplasm 1 Model 2 Ratio	Cytoplasm 1 Model 2 G(1)	Cytoplasm 2 Ratio	Cytoplasm 2 G(1)
A. First generation crosses Oostvoornse meer (meadow)									
Selfing									
1 (om2)	31	18	38	1:3	4.85*	21:43	0.31		
2 (om8)[c]	0	19	52					0:1	
3 (om17)	0	29	28					0:1	
H * H									
77 (om2 * om8)	43	17	21	1:1	0.31	15:17	1.25		
79 (om8 * om2)	0	6	63					0:1	
78 (om2 * om17)	0	5	73	0:1		0:1			
81 (om17 * om2)	0	1	61					0:1	
80 (om8 * om17)	1	23	48					0:1	
82 (om17 * om8)	1	14	61					0:1	
MS * H									
17 (om46 * om2)	73	2	0				1:0		
18 (om46 * om8)	0	51	20					0:1	
19 (om46 * om17)	0	49	27					0:1	
20 (om60 * om2)	12	18	39					3:13	0.09
21 (om60 * om8)	0	38	37					0:1	
22 (om60 * om17)	7	26	38					0:1	
B. Second generation families with females having cytoplasmic type 1									
77 (om2 * om8)									
Selfing									
77-5	48	21	0	3:1	1.04	3:1	1.04		
77-6	32	25	17	1:3	11.66**	7:9	0.01		
H1 * H2									
77-5 * 79-1	35	21	14	1:1	0.00	15:17	0.27		
77-6 * 79-1	29	21	22	1:3	8.07**	7:9	0.36		
77-5 * om8	59	17	0	3:1	0.29	3:1	0.29		
77-6 * om8	44	24	12	1:1	2.74	5:3	0.24		
MS1 * H2									
77-1 * om8	36	0	0	1:0		1:0			
77-2 * om8	48	2	0	1:0		1:0			
77-2 * 79-4	27	2	0	1:0		1:0			
278 (77-1 * om8)									
MS1 * H2									
278-1 * om8	98	3	0	1:0		1:0			
79 (om8 * om2)									
H1 * H2									
om2 * 79-1	26	34	18	1:3	2.71	21:43	0.01		
18 (om46 * om8)									
H1 * H2									
om2 * 18-2	19	22	30	1:3	0.12	1:3	0.12		
om2 * 18-4	25	18	8	1:1	0.02	1:1	0.02		
21 (om60 * om8)									
H1 * H2									
om2 * 21-1	25	41	21	1:3	0.63	21:43	0.67		

TABLE 4

Continued

Family no./cross	Offspring MS	Offspring PMS	Offspring H	MS:(PMS + H) Cytoplasm 1 Model 1 Ratio[a]	Cytoplasm 1 Model 1 G(1)[b]	Cytoplasm 1 Model 2 Ratio	Cytoplasm 1 Model 2 G(1)	Cytoplasm 2 Ratio	Cytoplasm 2 G(1)
C. Second generation families with females having cytoplasmic type 2									
79 (om8 * om2)									
Selfing									
79-1	2	9	47					3:61	0.22
H2 * H1									
79-1 * 77-5	0	8	60					0:1	
79-1 * 77-6	0	16	44					0:1	
79-1 * om2	6	7	38					3:13	1.83
77 (om2 * om8)									
H2 * H1									
om8 * 77-5	0	33	36					0:1	
18 (om46 * om8)									
H2 * H1									
18-2 * om2	14	15	12					3:5	0.20
18-4 * om2	11	18	19					3:13	0.52
H2 * H2									
18-2 * 21-1	0	53	19					0:1	
21 (om60 * om8)									
H2 * H1									
21-1 * om2	7	33	32					1:15	1.28
H2 * H2									
21-1 * 18-2	0	44	24					0:1	
17 (om46 * om2)									
MS2 * H1									
17-1 * om2	36	0	0					1:0	
17-3 * om2	58	6	0					1:0	
17-4 * om2	39	3	0					1:0	
17-5 * om2	41	6	1					3:1	3.12
155 (17-3 * om2)									
MS2 * H1									
155-1 * om2	56	3	0					1:0	
302 (155-1 * om2)									
MS2 * H1									
302-1 * om2	51	0	0					1:0	

[a] Ratio is the expected ratio of MS:not-MS, according to the proposed genetic models in the text.
[b] G(1) is the value of the G-test (with 1 d.f.) that evaluates the difference between expected and observed ratio.
[c] Underscore denotes plants having cytoplasmic type 2.
* $P < 0.05$.
** $P < 0.01$.

they segregated different frequencies of PMS plants. At present we are unable to fit another model. Plant H-kh69 should be fully restored for the dominant gene, because it never segregates MS. The second generation crosses (Table 1C) are in agreement with the model, because it predicts no segregation of MS. Proposed genotypes are shown in Figure 1B.

Kwade Hoek (salt marsh): Every cross involving plants with cytoplasmic type 1 as maternal plants segregated MS (Table 2, A and B). Especially striking were some crosses involving family 106, which produced a 100% MS progeny (Table 2B). Because the cross H-kh51 * H-kh41, and selfing of H-kh51, both gave ~1:3 ratios, at least one dominant restorer allele must be involved. The difference between the crosses MS-(106–2) * H-kh41 and H-(106–1) * H-kh41 indicates the presence of another restorer gene. Therefore, there must be at least two genes, one having a dominant and the other

a recessive restorer allele. H-kh51 must be heterozygous for both loci (*R1*/+ +/*r2*), giving rise to a 3:13 ratio, MS-(106–2) should be +/+ +/+ and H-(106–1) is *R1*/+ +/+. However, H-kh41 must then be +/+ +/*r2* and when crossed with H-kh51 should give a 3:5 ratio, which is clearly not the case ($G_{(1)} = 16.9$, $P < 0.001$). We thus invoke at least one other locus. The model giving the best fit consisted of three genes, one with a dominant and two with a recessive restorer allele (Table 2, A and B). Proposed genotypes are shown in Figure 1A. Plant H-kh51 is heterozygous at all three loci. Hermaphrodite H-kh41, having cytoplasmic type 2, is nonrestored at the loci that control male fertility in cytoplasmic type 1, as is MS-kh2. The other hermaphrodite with cytoplasmic type 2 (kh6), however, is nearly completely restored for cytoplasmic type 1. From the crosses H-(30–1) * H-(33–1) and H-kh51 * H-(33–1) we can also propose a nuclear genotype with regard to cytoplasmic type 1 for MS-kh56, which is a male sterile having cytoplasmic type 2. This plant appears to be nearly restored for cytoplasmic type 1. The second and third generation crosses were in line with the model, although crosses involving family 30 barely fitted expectations. Plant MS-(106–2) had the sterile genotype at all three loci.

As for the other Kwade Hoek (dune area) location, plants with cytoplasmic type 2 hardly ever segregated MS. This difference in restoration level between the hermaphrodites of different cytoplasmic type was also described in our previous paper (KOELEWIJN and VAN DAMME 1995) and hampers interpretation because of a lack of clear ratios. The simplest model that fitted the data had three dominant restorer alleles. The only cross that gave a little information was MS-kh56 * H-kh6. Plant H-kh6 was heterozygous, and MS-kh56 homozygous sterile, at all three loci. Other proposed genotypes are shown in Figure 1B. Because H-kh41 and H-kh51 are restored at one of the dominant loci, they do not segregate MS. However, they have to be restored at different loci, or their descendant H-(104–1) could not segregate MS when selfed (Table 2C). One cross in Table 2C, H-kh41 * H-(30–1), did not confirm our expectations because it segregated MS. This indicates that there might be more variation and illustrates the difficulties in explaining all the results of all the crosses.

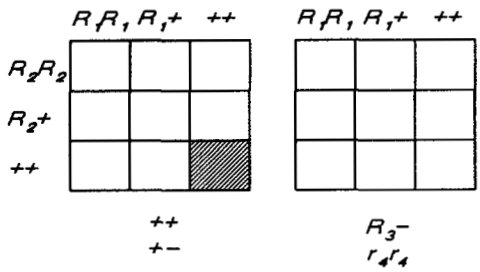
Oostvoornse meer (dune area): This set of crosses posed several problems, and we did not succeed in finding a completely satisfactory model of the first generation crosses for cytoplasmic type 1 (Table 3A). Selfing of H-om90 resulted in a 1:3 ratio, implying the involvement of a dominant restorer allele. Excluding the crosses in which H-om80 was involved, the results could be explained by a two gene model, one with a dominant and the other with a recessive restorer allele. The genotype for H-om90 is then *R1*/+ +/+, H-om120 is doubly heterozygous (*R1*/+ +/*r2*) and MS-om64 homozygously sterile at both loci (+/+ +/+). Second generation crosses (Table 4B) confirmed this model, except for H-(86–2) * H-(88–1) where there was a shortage of MS plants. The crosses with H-om80, however, imply the need for at least one other restorer locus, for this plant only. H-om90 and H-om120 showed no variation for the new locus, therefore the fitted ratios of the model proposed above are unchanged. The extra locus was necessary to explain the results of H-om90 * H-om80. It further changes the ratio of MS-om64 * H-om80 from 1:3 to 1:7, but although the shift is in the right direction, this last cross still departs from expectations (Table 3A). Therefore, more loci are probably involved. Proposed genotypes are shown in Figure 1A. For MS-om76, a male sterile with cytoplasmic type 2, it was not possible to infer the complete allelic state of the genotype with respect to cytoplasmic type 1, but unlike MS-kh56 one locus was homozygous for a sterility allele.

It was not possible to present a definite model for cytoplasmic type 2. As in the previous two locations, there is again hardly ever any segregation of MS in the H * H crosses. Only the MS * H crosses gave information. To explain the 3:1 and 1:1 ratios, two recessive restorer alleles are necessary (Table 3A). Proposed genotypes are shown in Figure 1B. Crosses supporting this model are MS-om76 * H-om90, MS-om76 * H-om120 and H-om120 * H-om90. Selfing of H-om120 should not segregate MS, but it does. Three of the four second generation crosses conform to the model, but contrary to expectations H-(88–1) * H-om90 did segregate MS (Table 3C). H-om80 was excluded because we had no good idea of its genotype. H-om80 * H-om90 and H-om80 * H-om120 did not segregate MS; therefore H-om80 should be *r1*/*r1* *r2*/*r2* (similar to H-om120). However, MS-om76 * H-om80 should then give a 1:1 ratio, which was not the case. Therefore, no definite model can be proposed beyond the conclusion that, based on the MS * H crosses, at least two genes with recessive restorer alleles are involved.

Oostvoornse meer (meadow): This location is the most interesting one. It showed the problems that can arise from environmentally dependent sex-expression and contained indications for an alternative model, implying epistatic interaction between restorer genes. Firstly, we were confronted with MS-H ratios up to 3:1 after selfing of a hermaphrodite (Table 4B). Moreover, we already noted that sex expression in *P. coronopus* is to a certain extent labile. Within the group of PMS plants, changes in the degree of male fertility are often observed (H. P. KOELEWIJN, unpublished results). To explain a 3:1 ratio after selfing, the genotype of 77–5 must either be male sterile, or else gamete selection or selective abortion need to be invoked. However, germination was 92%, pollen stainability ~80% and seed set did not differ from other crosses. The original sex classi-

A - Nuclear genetic constitution for cytoplasmic type 1

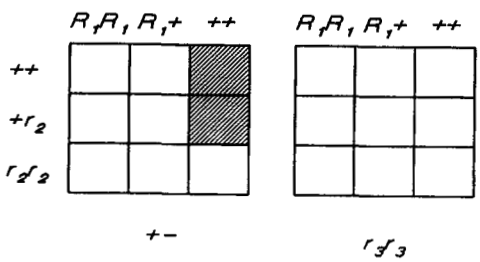
Kwade hoek (Dune area)



3 dominant, 1 recessive allele

H1 - kh69	*R1*	*R1*	*?*	*?*	*?*	*?*	*?*	*?*
H2 - kh80	*R1*	+	*R2*	+	*R3*	+	*r4*	*r4*
MS1 - kh89	+	+	+	+	+	+	+	*r4*
H1 - kh120	*R1*	+	+	+	+	+	+	+

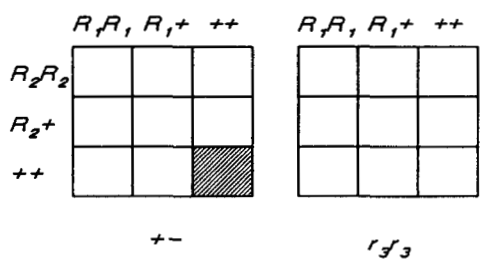
Kwade hoek (Salt marsh)



1 dominant, 2 recessive alleles

MS1 - kh2	+	+	+	*r2*	+	*r3*
H2 - kh6	*R1*	+	*r2*	*r2*	*r3*	*r3*
H2 - kh41	+	+	+	*r2*	+	*r3*
H1 - kh51	*R1*	+	+	*r2*	+	*r3*
MS2 - kh56	*R1*	*?*	*r2*	*r2*	*r3*	*r3*

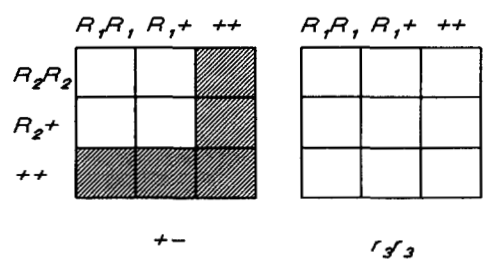
Oostvoornse meer (Dune area)



2 dominant, 1 recessive allele

MS1 - om64	+	+	+	+	+	*r3*
MS2 - om76	*R1*	*?*	*R2*	*?*	+	+
H2 - om80	*R1*	+	*R2*	+	*r3*	*r3*
H1 - om90	*R1*	+	+	+	+	+
H2 - om120	*R1*	+	+	+	+	*r3*

Oostvoornse meer (Meadow)



2 dominant, 1 recessive allele

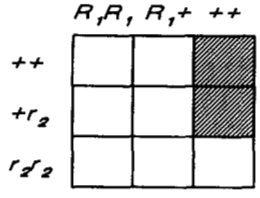
H1 - om2	*R1*	+	*R2*	+	+	*r3*
H2 - om8	*R1*	+	+	+	+	*r3*
H2 - om17	*R1*	*R1*	*R2*	*R2*	*?*	*?*
MS2 - om46	*R1*	*?*	*R2*	*?*	*r3*	*?*
MS2 - om60	*?*	*?*	*R2*	*?*	*?*	*?*

FIGURE 1.—Proposed genotypes for the restorer genes of the parents of the crosses in Tables 1A–4A. Genes having dominant or recessive restorer allele action are denoted by *R* and *r*. +, sterility allele; ?, the exact allelic state at that locus could not be inferred from the crosses; −, the state of the second allele did not matter. Plants having cytoplasmic type 2 are marked by an underline. ▨, allele combinations giving MS. For the Oostvoornse meer meadow location for cytoplasmic type 1, the results from model 2 are presented.

fication of the plants took place in the garden. Plant 77–5 was a PMS plant, having 95% male sterile anthers, and 77–6 was a hermaphrodite. In the greenhouse, where plants selected for further crossing were kept, 77–6 was still a hermaphrodite, but 77–5 became a real PMS plant with 30% fertile anthers, enough for pollination. Because 77–6 died during the crossing program, crosses were continued with 77–5. After 77–5

B - Nuclear genetic constitution for cytoplasmic type 2

Kwade hoek (Dune area)



at least 1 dominant, 1 recessive allele

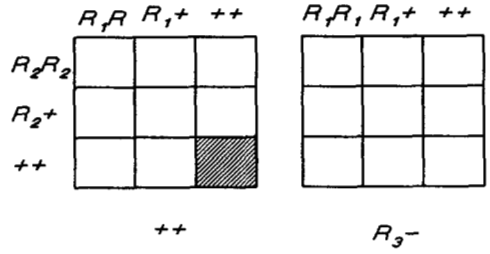
H1 - kh69 *R1 R1 ? ?*

H2 - kh80 *R1 + r2 r2*

MS2 - kh115 + + + *r2*

H1 - kh120 *R1 + r2 r2*

Kwade hoek (Salt marsh)



3 dominant alleles

MS1 - kh2 *R1 + R2 + ? ?*

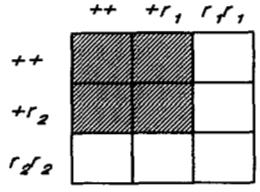
H2 - kh6 *R1 + R2 + R3 +*

H2 - kh41 + + *R2 R2 ? ?*

H1 - kh51 *R1 R1 + + ? ?*

MS2 - kh56 + + + + + +

Oostvoornse meer (Dune area)



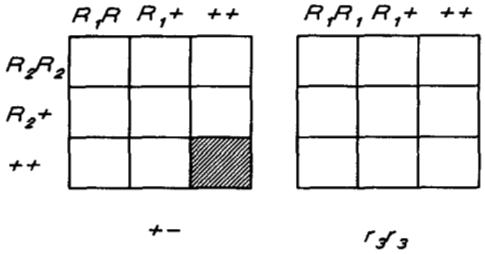
no satisfactory solution, at least 2 recessive alleles involved

MS2 - om76 + + + *r2*

H1 - om90 *r1 r1 + r2*

H2 - om120 *r1 r1 r2 r2*

Oostvoornse meer (Meadow)



2 dominant, 1 recessive allele

H1 - om2 + + + + + *r3*

H2 - om8 *R1 R1 R2 + r3 r3*

H2 - om17 *R1 R1 + + r3 r3*

MS2 - om46 + + + + + +

MS2 - om60 + + + + + *r3*

FIGURE 1.—*Continued*

had been used, the plant was neglected and became male sterile. We therefore propose that 77–5 has a male sterile genotype, but that at least some loci are heterozygous, giving rise to labile sex expression (see paragraph on partial male sterility). Assuming 77–5 to be MS, a reasonable fit was obtained with a model of one dominant and two recessive restorer alleles. This is model one in Table 4, A and B. Plant H-om2 was classified as *R1*/+ +/+ +/+ and H-om8 as +/+ +/*r2* +/*r3*. The selfing of H-om2 and H-(77–6) and the cross H-(77–6) * H-om8, however, still resulted in a significant shortage of MS plants. Although other second generation crosses fell into line with the model, a systematic shortage of MS plants remains. Still assuming 77–5 to

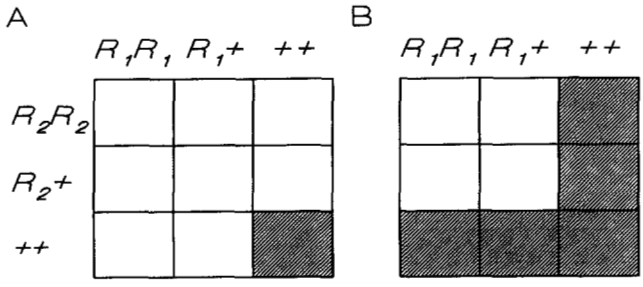


FIGURE 2.—Illustration of independent (A) and epistatic (B) restorer gene action for a model with two genes having dominant restorer alleles. ▨, allele combinations that give male sterility.

have a male sterile genotype, we propose a model with epistatic restorer gene action (model two in Table 4, A and B), such that plants restored at just one of the two loci are still male sterile (Figure 2). The model proposed involves three restorer genes, two being epistatic and dominant and one independent and recessive. Proposed genotypes are shown in Figure 1A. A plant is MS, when it is sterile at the recessive locus and at least *one* of the dominant loci. Hermaphrodite require either restoration at both dominant loci, or restoration at the recessive locus. As can be seen from Table 4, A and B, the model fits almost perfectly. It explains high MS ratios after selfing and in other crosses such as H-(77–6) * H-om8 and H-om2 * H-(79–1). For H-om2 and H-om8, a complete genotypic solution could be given (Figure 1A). H-om17 must be restored at both dominant loci, because it does not segregate MS when crossed with either H-om2 or H-om8. For the MS plants, no complete solution could be given, but MS-om46, having cytoplasmic type 2, must be restored to a large extent for cytoplasmic type 1.

Cytoplasmic type 2 was also interesting because of the difference between the two MS plants (Table 4A). Again, H * H crosses did not segregate many MS plants. On the other hand, MS-om46 * H-om2 gave a 1:0 ratio, indicating that om2 must be sterile for cytoplasmic type 2. When MS-om60 is omitted, a model with two dominant and one recessive allele is sufficient. Second generation crosses of MS-om46 * H-om2 (family 155, Table 4C) should also give 1:0 ratios, although there were always a few doubtful PMS plants. When transferred to the greenhouse most of these PMS plants became MS, except for a few individuals from MS-(17–5) * H-om2. Because of this, a 3:1 ratio is fitted for this cross and H-om2 is assumed to be heterozygous at one of the loci. Because H-om8 and H-om17 do not segregate male steriles with MS-om46, they must be restored to a large extent. Proposed genotypes are shown in Figure 1B (loci *R1*, *R2*, *r3*). MS-om46 turned out to be the perfect male sterile, being homozygous sterile at all five loci. Including the crosses with MS-om60 requires at least two other recessive restorer loci (*r4*, *r5*) for which MS-om60 should be heterozygous. The results of MS-om60 * H-om2 and MS-om60 * H-om8 can then be explained, but not the segregation of MS with MS-om60 * H-om17. Thus, no completely satisfactory solution for MS-om60 can be given.

Integration: In the previous sections we tried to solve the restorer genetics for male sterility of plants from four different locations. However, no uniform model was obtained (Figure 1). For both cytoplasmic types, restorer genes with either dominant or recessive restorer alleles are involved, but their numbers vary between the plants sampled from the different locations. The number of genes involved is probably large and the proposed models provide minimum estimates. Including more plants would probably increase the number of genes and using different plants from the same location might result in another model. If one assumes the absence of population-specific restorer genes, we can formulate "minimum overall" models for each cytoplasmic type based on crosses between plants with known genetic background from different locations. For each cytoplasmic type the minimum model would then be five genes with three having a dominant and two a recessive restorer allele. Therefore, at least five restorer genes will be involved for each cytoplasmic type (Figure 1).

For plants for which the original classification is based on fewer restorer genes, we can hypothesize their allelic state at these extra genes, by fitting the between-location crosses. Because we assume common loci across locations and do not know which loci are the common ones, we tried several arrangements of the loci to explain the between-location crosses and adopted the model with the lowest overall G-value. A uniform solution for cytoplasmic type 1 was not found. First, different models of gene action were necessary in the different localities (epistatic *vs.* independent), and second, within the group of plants with independent gene action a common model based on three dominant and two recessive restorer alleles gave a bad fit. Leaving out H-om2 and H-om8, because they have epistatic restorer gene action, only 4 of 12 crosses with cytoplasmic type 1 as a maternal parent fitted the overall minimum model (by including H-om2 and H-om8 this became 9 of 13) (Table 5). Because H-om90, H-kh51 and H-kh120 are heterozygous at a locus with a dominant restorer allele (Figure 1A, locus *R1*) and also have sterility alleles at other restorer loci, crosses between these plants are likely to segregate MS. There is a large variation in segregation ratios (Table 5). Interestingly, segregation ratios for the between-location crosses show the same pattern as observed in the separate locations. Plant H-kh120 segregates few MS whereas H-om8 gives ratios even higher than 1:1, when used as a paternal parent (H-om2 * H-om8, H-om90 * H-om8, H-kh51 *

TABLE 5

Reciprocal crosses between hermaphrodites from four locations and crosses between these hermaphrodites and plant MS-om46 (cytoplasmic type 2, homozygous sterile for all loci (Figure 1B)

	Offspring			Offspring of reciprocal cross			Reciprocal difference	Fitted ratio, MS:(PMS + H)		Expected ratio, MS:(PMS + H)			
										Cyto 1		Cyto 2	
Cross	MS	PMS	H	MS	PMS	H	G(2)	Cyto 1	Cyto 2	Ratio	G(1)	Ratio	G(1)
H1 * H1													
om2 * om90	38	15	33	41	17	18	4.04[b]	1:1		1:3	42.3***		
om2 * kh51	29	23	20	14	17	17	1.63[b]	3:5		1:3	6.9**		
om2 * kh120	12	18	35	19	23	21	5.62[b]	1:3		1.3	0.1[b]		
om90 * kh51	7	13	22	2	9	13	0.99[b]	1:7		1:3	5.2*		
om90 * kh120	4	31	37	8	38	27	3.54[b]	1:15		1:3	27.0***		
kh51 * kh120	9	16	13	6	12	10	0.05[b]	1:3		1:3	0.2[b]		
H2 * H2													
om8 * om120	0	22	49	0	23	44	0.17[b]		0:1			0:1	
om8 * kh41	0	13	59	0	8	59	1.00[b]		0:1			0:1	
om8 * kh80	0	30	27	0	29	34	0.52[b]		0:1			0:1	
om120 * kh41	0	8	37	0	10	61	0.28[b]		0:1			0:1	
om120 * kh80	0	15	37	0	14	57	1.35[b]		0:1			0:1	
kh41 * kh80	0	22	48	0	17	53	0.88[b]		0:1			0:1	
H1 * H2													
om2 * om8	43	17	21	0	6	63	84.6***	1:1	0:1	1:1	0.3[b]	0:1	
om2 * om120	13	19	34	0	7	56	28.3***	3:13	0:1	1:3	1.1[b]	0:1	
om2 * kh41	32	23	13	0	2	54	89.1***	1:1	0:1	1:1	0.2[b]	0:1	
om2 * kh80	14	46	12	0	9	57	76.4***	3:13	0:1	1:3	1.3[b]	0:1	
om90 * om8	39	18	5	0	23	49	94.0***	5:3	0:1	1:1	4.2*	0:1	
om90 * om120	20	23	32	0	2	62	56.0***	1:3	0:1	1:3	0.1[b]	0:1	
om90 * kh41	21	21	25	0	5	66	57.5***	5:11	0:1	1:1	9.6**	0:1	
om90 * kh80	20	38	22	0	15	57	52.7***	1:3	0:1	1:15	28.7**	0:1	
kh51 * om8	28	18	5	0	14	42	70.1***	9:7	0:1	9:23	19.1***	0:1	
kh51 * om120	6	16	44	0	16	61	9.70**	1:15	0:1	3:13	4.8*	0:1	
kh51 * kh41	42	56	83	0	17	44	29.7***	1:3	0:1	9:23	2.3[b]	0:1	
kh51 * kh80	10	20	11	0	12	58	41.8***	1:3	0:1	1:15	13.9***	0:1	
kh120 * om8[a]	0	11	25	0	16	56	0.85[b]	0:1	0:1	1:1	49.9***	0:1	
kh120 * om120	6	24	19	0	14	58	25.9***	1:7	0:1	1:3	4.9*	0:1	
kh120 * kh41	9	26	19	0	10	50	32.8***	3:13	0:1	1:1	26.2***	0:1	
kh120 * kh80	4	14	41	0	11	68	9.10**	1:15	0:1	1:15	0.1[b]	0:1	
MS2 * H1													
om46 * om2	73	2	0						1:0			1:0	
om46 * om90	30	21	38						5:11			3:5	0.6[b]
om46 * kh51	28	17	25						3:5			0:1	
om46 * kh120	64	11	27						5:3			1:1	6.7**
MS2 * H2													
om46 * om8	0	51	20						0:1			0:1	
om46 * om120	0	16	53						0:1			0:1	
om46 * kh41	0	8	60						0:1			0:1	
om46 * kh80	0	15	54						0:1			0:1	

The G-test for reciprocal differences is based on a three groups comparison (MS:PMS:H, d.f. = 2). The fitted ratio is the ratio which gave the lowest G-value, as determined by an iterative fitting of a G-test (with a stepsize of 1/16). The expected ratio is based on the "overall minimum" models presented in the text. Cyto 1 and cyto 2 are abbreviations for the two cytoplasmic types.

[a] Due to drought in petri dish 50% of seedlings died. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; [b] not significant.

H-om8). Because the minimum overall model gave a bad fit, we have to assume the involvement of further (population specific) genes. All possible crosses can than be explained, but for every deviant cross a new locus has to be assumed. The number of genes involved thus increases rapidly, approaching polygenic determination of sex.

For cytoplasmic type 2 all crosses with hermaphro-

dites as maternal parents (Table 5) could be explained by a model with three dominant and two recessive alleles. None of the 22 crosses segregated MS. Because H-om8 and H-kh41 were homozygous and H-kh80 heterozygous at at least one locus with a dominant restorer allele for cytoplasmic type 2 (Figure 1B), the genetic constitution at other loci was irrelevant, which resulted in no segregation of MS and a good fit of the model. Since MS-om46 was sterile at all loci with respect to cytoplasmic type 2 (Figure 1B), it was crossed with all other hermaphrodites, to act as a tester. The results of crosses with fathers having cytoplasmic type 2 (H-om8, H-om120, H-kh41 and H-kh80) can be explained, because no segregation of MS is expected. However, crosses with fathers having cytoplasmic type 1 cannot be explained using the minimum model for cytoplasmic type 2. The cross MS-om46 * H-kh51 should segregate no MS and the highest ratio possible for MS-om46 * H-kh120 is 1:1. Therefore, the involvement of other population-specific loci is again necessary. Moreover, crosses MS-om46 * H-om120 and MS-om46 * H-kh80 do not segregate MS, but these plants do segregate MS when crossed with male steriles having cytoplasmic type 2 from their own locations (MS-om76 * H-om120 (Table 3A) and MS-kh115 * H-kh80 (Table 1A), also indicating the presence of other genes. Thus, no completely satisfactory overall model for cytoplasmic type 2 was achieved, although the fit was much better than for cytoplasmic type 1.

Partial male sterility: Up to this point in this paper partially male sterile plants have been grouped together with the hermaphrodites. The number of PMS plants segregated, however, is too high to be ignored. In other plants, partial male sterility is often associated with incomplete restoration at loci having dominant restorer alleles (LOUIS and DURAND 1978; WICKERHAM and PATTERSON 1980). We therefore expect that segregation of PMS plants would follow a regular pattern that relates to the segregation of male steriles. If this is true, reciprocal crosses within a cytoplasmic type should render the same frequency of PMS plants. Despite the environmentally dependent sex expression of PMS plants (H. P. KOELEWIJN, unpublished results), for cytoplasmic type 1 none of the 8 and for cytoplasmic type 2 only 1 of 11 reciprocal crosses that were planted randomly in the garden and in different years showed a significant difference in the segregation pattern of PMS *vs.* (MS + H) (crosses presented in Tables 1–5). Partial male sterility might therefore have a genetic basis. From studies involving single gene inheritance of male sterility, the heterozygote is often found to be partially male sterile (WICKERHAM and PATTERSON 1980; KAUL 1988). When more loci are involved, plants that are heterozygous at a locus with a dominant restorer allele may therefore have the PMS phenotype. Predictions based on this model have been fitted to the data of Tables 1–4, although not all data are suitable because of uncertainties concerning the genotypes of some plants (Figure 1). We will not present detailed genetics. Using the plants H-kh80, MS-kh89 and H-kh120 for the Kwade Hoek dune area location (Table 1), only 4 of the 8 crosses fitted the model for cytoplasmic type 1. No attempt was made for cytoplasmic type 2 from this location. In case of the Kwade Hoek salt marsh location (Table 2), 11 of 15 crosses fitted the model for cytoplasmic type 1. Of the four deviant crosses, three had H-(106–1) as a mother. For cytoplasmic type 2 at this location, 7 of 13 crosses gave a good fit. For the Oostvoornse meer dune area location (Table 3) no attempt was made to test cytoplasmic type 2, for cytoplasmic type 1, 10 of the 14 crosses fitted the model. At the Oostvoornse meer meadow location the results for cytoplasmic type 2 gave a poor fit. Leaving out MS-om60, 5 of the 10 crosses fitted the model. For cytoplasmic type 1 another model is needed, due to the epistatic restorer gene action. The basic idea is the same, however, we now assume that a plant is a partial male sterile when restored at both dominant loci (*i.e.*, double heterozygous). This model fitted 9 of the 12 crosses.

Thus, a moderately good fit of the model was obtained for PMS plants having cytoplasmic type 1, but a bad fit for PMS2. Because we do not know anything about the relationships between loci and their interaction, the proposed models are only tentative, assuming equal contributions of the various loci. However, this exercise does show that the inheritance of the PMS phenotype is probably largely determined by the same genes that also determine male sterility.

Differences between cytoplasmic types: Tables 1–4 reveal a marked difference in MS segregation patterns between the two cytoplasmic types. Crosses with cytoplasmic type 2 as maternal parent hardly ever segregate MS. Of these progenies, only crosses where MS2 is involved segregate MS; segregation rarely happens with selfings of H2 or crosses H2 * H2. Table 6 summarizes all our crosses. All combinations with cytoplasmic type 1 as a maternal parent are capable of segregating MS, irrespective of the father. However, for cytoplasmic type 2 the status of the maternal parent (MS or H) and the status of the paternal parent (cytoplasmic type 1 or 2) is important. Table 6 also shows that H1 and H2 plants are equally variable for cytoplasmic type 1 restorer genes, as there are no large differences between the MS1 * H1, MS1 * H2, H1 * H1 and H1 * H2 crosses. This is not the case for cytoplasmic type 2. There is no difference between H2 * H2 and H2 * H1 crosses, but results for MS2 * H1 and MS2 * H2 differ. This indicates a difference in the presence of cytoplasmic type 2 restorer genes between H1 and H2 plants. Moreover, H2 plants seem to be much more restored for their own cytoplasmic type, compared with H1 plants. Comparison of the genotypes of the plants most used in the

TABLE 6

Summary of the crossing program of all plants that were involved in elucidating nuclear and cytoplasmic differences

Parental genotypes	No. of crosses	Offspring distribution			Sample size
		MS	PMS	H	
MS1 * H1	4	33.7	35.5	30.8	276
MS1 * H2	10	41.8	24.5	33.7	481
MS2 * H1	12	48.7	17.6	33.7	823
MS2 * H2	15	9.1	35.2	55.7	1049
H1 * H1	30	26.0	34.0	40.0	1806
H1 * H2	42	30.0	34.4	35.6	2658
H2 * H1	38	2.6	20.7	76.7	2411
H2 * H2	34	2.1	29.3	68.6	2125

Crosses are grouped according to cytoplasmic background. Sample size is the total number of plants for which a sex score was available. Results are presented as a percentage of the total.

crosses (*e.g.*, om2, om90, kh51, kh120 (all H1) and om8, om120, kh41, kh80 (all H2)) (Figure 1) shows that H2 plants are at least homozygous at one (dominant) restorer locus for their own cytoplasmic type, on the other hand, H1 plants are more likely to be heterozygous at restorer loci of their own cytoplasmic type.

Restorer gene frequencies: The frequency of male steriles differs among populations of *P. coronopus* (range 0–31%; KOELEWIJN and VAN DAMME 1995). They may therefore differ in the frequency of restorer alleles and display different restoring capabilities. Without knowing anything about the genetics, differences in frequencies of restorer alleles can be detected by crossing the same male steriles, preferably homozygous for the sterility alleles of the different cytoplasmic types, with a random sample of pollen from several populations. We compared the pollen pool during peak flowering of the populations we had studied in 1990. Table 7 shows a large difference in restoring capabilities between the populations. There is a significant heterogeneity in the segregation of male steriles between the populations within all three MS plants (G-values are 78.4, 38.0 and 17.7 for MS-(278–1), MS-(280–1) and MS-om76 with 5 d.f., respectively) and, strikingly, there is also a difference between the two MS1 plants. Although both MS1 plants are capable of producing 100% male sterile offspring (Tables 2B and 4B), they must still differ at certain loci, otherwise no differences in their segregation ratios could occur. This suggests population-specific differentiation and stresses again the difficulty in trying to define a uniform genetic model for all populations. For cytoplasmic type 2 there is also a difference in the restoring capabilities of populations, although less pronounced than for cytoplasmic type 1.

TABLE 7

Frequency distributions of the offspring of three male steriles

MS * Population	Offspring distribution			*n*
	MS	PMS	H	
278-1				
Kwade Hoek	38.6	31.4	30.0	70
Kleistee	85.6	14.4	—	69
Oostvoornse meer	22.9	18.8	58.3	96
Westduinen	55.6	28.4	16.0	81
Westplaat 1	31.2	37.6	31.2	93
Westplaat 2	55.6	24.4	20.0	45
Total	45.6	26.2	28.2	454
280-1				
Kwade Hoek	17.6	25.9	55.6	108
Kleistee	51.6	39.8	8.6	93
Oostvoornse meer	17.5	23.7	58.8	97
Westduinen	31.6	26.3	42.1	95
Westplaat 1	26.6	23.4	50.0	94
Westplaat 2	24.2	30.5	45.3	95
Total	27.9	28.2	43.9	581
om76				
Kwade Hoek	40.1	34.5	24.4	49
Kleistee	47.4	25.0	27.6	115
Oostvoornse meer	38.5	21.4	40.1	70
Westduinen	52.8	27.5	19.8	91
Westplaat 1	30.8	16.9	52.3	61
Westplaat 2	26.1	29.0	44.9	69
Total	30.9	25.4	33.7	460

MS 278-1 and 280-1 have cytoplasmic type 1 (see Table 4B and table 2B) and om76 has cytoplasmic type 2 (Table 3A). Each MS is crossed with a pollen sample collected in each of 6 populations during peakflowering in 1990. Population of origin of the male sterile is marked by underlining. Values are percentages; *n* is the number of plants involved.

DISCUSSION

This article presents evidence of complex nuclear inheritance of two male sterility types in *P. coronopus*. Both sterility types appear to result from a number of nuclear genes interacting with particular cytoplasmic types. Variation in these nuclear genes between individuals within a population is large: in all eight cases where the one male sterile was crossed with several hermaphrodites, significant heterogeneity in segregation ratios was detected. The impression from agricultural research that recessive restorer alleles are rare (GOTTSCHALK and KAUL 1974; KAUL 1988) was not confirmed. The large genetic variation for male sterility and the resulting phenotypic variation were found in each location, using only five plants from an area of ~50 m^2. In solving the nuclear genetics, we tried to use simple genetic models. Therefore, we restricted ourselves in the first instance to a model with independent restorer gene action. Even then, a variety of models involved in determining male sterility were possible (Figure 1). The number of re-

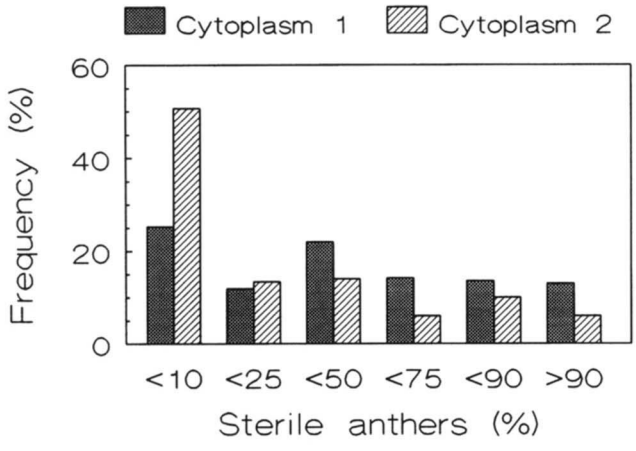


FIGURE 3.—Frequency distribution of the PMS plants from Table 6. Plants have been grouped according to cytoplasmic type and their percentage of sterile anthers. Distributions are significantly different from each other ($G = 277.9$; d.f. = 5; $P < 0.001$.)

storer genes ranges from two to five, but is probably larger. No single genetic model could explain the results from all of the localities. This indicates that population-specific restorer genes are involved and that the number of restorers is much larger than would be inferred from each location separately. For the Oostvoornse meer (meadow) location we had to invoke epistatic restorer gene action (Figure 2), which has not previously been suggested, although FRANK (1989) in his thorough theoretical study of cytoplasmic male sterility used a mixture of epistatic and independent restorer genes.

Although not the primary object of interest in the present study, partially male sterile plants were numerous and comprised on average 30% of the offspring. This is considerably higher than those for most other gynodioecious species (*e.g.*, CORRENS 1928; FRÖST 1963; VAARAMA and JAASKELAINEN 1967; H. P. KOELEWIJN, unpublished results). We have evidence that PMS has a genetic basis (see above). The occurrence of both PMS1 and PMS2 can largely be explained by incomplete restoration at the several loci. Plants might therefore become more or less sterile, depending on the number of restorer genes, giving rise to a continuum in male fertility at the morphological level. Circumstantial evidence for this comes from the distribution of the percentage of male sterility within the group of PMS plants. Plants having cytoplasmic type 2, especially the hermaphrodites, were found to carry more restorer alleles for their own cytoplasmic type than plants having cytoplasmic type 1. Crosses having cytoplasmic type 2 plants as maternal parents are therefore expected to segregate more PMS plants with a low percentage of sterile anthers. Figure 3 shows that the distribution of sterile anthers within the class of PMS plants is different for the two cytoplasmic types. Within cytoplasmic type 2, more than 50% of the PMS plants have less than 10% male sterile anthers. For cytoplasmic type 1 there is a more even distribution between the classes. The predictions also hold for individual crosses. For example, cross MS-om76 * H-om90 (Table 3A) segregated only MS and PMS plants. These PMS plants turned out to be mostly sterile (~80–90% of the flowers were male sterile). This was expected because of the large number of male steriles that segregated, indicating the presence of few restorer alleles. In our genetic interpretation partial male sterility is a fundamental property of the gynodioecious breeding system, rather than an abnormality. Due to partial restoration PMS plants produce some pollen, the amount dependent on the number and quality of restorer genes. Within the group of hermaphrodites there is also variation in male fertility (H. P. KOELEWIJN, unpublished results), although it is not visible by eye, unlike the distinction of MS and PMS. It may well be possible that these differences in male fertility are also related to differences in the number of restorer genes between hermaphrodites.

In our first article we showed that in all four locations there was polymorphism for cytoplasmic genes. In the present article we showed that in each location a variety of restorer genes for both cytoplasmic types are present. We therefore have evidence for the presence of joint polymorphisms, *i.e.*, variation for both nuclear genes and cytoplasmic types. Earlier theoretical models did not allow a stable joint polymorphism (CASPARI *et al.* 1966; CONSTANTINO 1971; CHARLESWORTH and GANDERS 1979). However, these studies either dealt with single gene inheritance or assumed fitness differences between sex phenotypes only. CHARLESWORTH (1981) showed that stable joint polymorphisms were possible when there were not only fitness differences between sex phenotypes, but also within sex phenotypes associated with the genetic factors causing male sterility. Thus, there must be multiple genotypes within sex phenotypes, which differ in fitness. From Figure 1 it appears that there are indeed multiple genotypes within the group of MS and H plants.

Based on our studies of *P. coronopus* and *P. lanceolata* the most striking characteristics of cytoplasmic male sterility in natural populations are: (1) the maintenance of a distinct but limited number of cytoplasmic types each with their own set of restorer alleles and (2) a large array of nuclear restorer loci. Elucidating the genetics of male sterility is the first and necessary step for insight into the gynodioecious breeding system. The next step in evaluating the results is to combine the genetical results with (field) experiments on fitness parameters of plants with known cytoplasmic and nuclear background.

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LITERATURE CITED

BELHASSEN, E., B. DOMMEE, A. ATLAN, P. H. GOUYON, D. POMENTE *et al.*, 1991 Complex determination of male sterility in *Thymus-Vulgaris* L—genetic and molecular analysis. Theor. Appl. Gen. **82:** 137–143.

CASPARI, E., G. S. WATSON and W. SMITH, 1966 The influence of cytoplasmic pollen sterility on gene exchange between populations. Genetics **53:** 741–746.

CHARLESWORTH, D., 1981 A further study of the problem of the maintenance of females in gynodioecious species. Heredity **46:** 27–39.

CHARLESWORTH, D., and F. R. GANDERS, 1979 The population genetics of gynodioecy with cytoplasmic-genic male sterility. Heredity **43:** 213–218.

CONNOR, H. E., and D. CHARLESWORTH, 1989 Genetics of male sterility in gynodioecious *Cortaderia* (Gramineae). Heredity **63:** 373–382.

CORRENS, C., 1928 *Bestimmung, Vererbung und Verteilung des Geschlechts bei den hoheren Pflanzen.* Handb. Vererb. Wiss. Vol. 2C, Borntraeger, Berlin.

COSTANTINO, R. F., 1971 Genetic consequences of the couplet cytoplasmic pollen sterility and pollen migration. Genetics **68:** 313–321.

FRANK, S. A., 1989 The evolutionary dynamics of cytoplasmic male sterility. Am. Nat. **133:** 345–376.

FRÖST, S., 1963 Cytoplasmic pollen sterility in Centaurea scabiosa. Hereditas **49:** 454–456.

GANDERS, F. R., 1978 The genetics and evolution of gynodioecy in *Nemophila menziesii* (Hydrophyllaceae). Can. J. Bot. **56:** 1400–1408.

GOTTSCHALK, W., and M. L. H. KAUL, 1974 The genetic control of microsporogenesis in higher plants. Nucleus (Calcutta) **17:** 133–166.

KAUL, M. L. H., 1988 *Male Sterility in Higher Plants.* Springer-Verlag, Berlin.

KHEYR-POUR, A., 1980 Nucleo-cytoplasmic polymorphism for male sterility in *Origanum vulgare* L. J. Hered. **71:** 253–260.

KHEYR-POUR, A., 1981 Wide nucleo-cytoplasmic polymorphism for male sterility in *Origanum vulgare* L. J. Hered. **72:** 45–52.

KOELEWIJN, H. P., and J. M. M. VAN DAMME, 1995 Genetics of male sterility in gynodioecious *Plantago coronopus*. I. Cytoplasmic variation. Genetics **139:** 1749–1758.

LOUIS, J. P., and B. DURAND, 1978 Studies with the dioecious Angiosperm *Mercurialis annua* L. (2n=16). Correlation between genic and cytoplasmic male sterility, sex segregation and feminizing hormones (cytokinins). Mol. Genet. **165:** 309–322.

ROSS, M. D., 1978 The evolution of gynodioecy and subdioecy. Evolution **32:** 174–188.

SOKAL, R. R., and F. J. ROHLF, 1981 *Biometry.* Freeman & Co., San Fransisco.

VAARAMA, A., and O. JAASKELAINEN, 1967 Studies on gynodioecism in the Finnish populations of *Geranium sylvaticum* L. Ann. Acad. Sci. Fenn. Ser. A Biol. **108:** 3–39.

VAN DAMME, J. M. M., 1983 Gynodioecy in *Plantago lanceolata* L. II. Inheritance of three male sterility types. Heredity **50:** 253–273.

VAN DAMME, J. M. M., 1991 A restorer gene in gynodioecious *Plantago coronopus* L. subject to selection in the gametophytic and seedling stage. Heredity **66:** 19–27.

VAN DAMME, J. M. M., and W. VAN DELDEN, 1982 Gynodioecy in *Plantago lanceolata* L. I. Polymorphism for plasmon type. Heredity **49:** 303–318.

WICKERHAM, D. S., and F. L. PATTERSON, 1980 Male fertility restoration in crosses of R5 with soft red winter wheats (*Triticum aestivum*). Crop. Sci. **20:** 100–102.

WOLFF, K., B. FRISO and J. M. M. VAN DAMME, 1988 Outcrossing rates and male sterility in natural populations of *Plantago coronopus*. Theor. Appl. Genet. **76:** 190–196.

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