The Male-Sterility Polymorphism of *Silene vulgaris*: Analysis of Genetic Data From Two Populations and Comparison With *Thymus vulgaris*

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ABSTRACT

Results are given of genetic studies of male sterility using plants from two natural populations from Sussex, England. Both populations have substantial frequencies of females, ~0.25 in population 1 and 0.60 in population 3. As in the few other gynodioecious populations studied in detail, many genetic factors are present. In population 1, there are at least two, and more likely three, different cytoplasmic types, one of which appears to produce male sterility in progeny from any hermaphrodite pollen donor; in other words restorer alleles for this cytoplasm are rare or absent from the population. The other two populations can be carried in hermaphrodites that have the dominant restorers. In population 1, there are also probably three restorer loci with complementary recessive male-sterility alleles, as well as a locus with duplicate action, which cannot produce male sterility unless the plant is also homozygous for the recessive allele at another locus. The results from population 3 are quite similar, though there was no evidence in this population for an unrestored sterility cytoplasm. A similar joint nucleocytoplasmic model with multiple restorers fits data from *Thymus vulgaris.*

TO understand the maintenance of polymorphisms, it is necessary to know both the selective forces that act on the phenotypes and the genetics of the differences in question, because the conditions for the maintenance of male-sterility polymorphisms depend strongly on their mode of inheritance. Theoretical studies of the maintenance of gynodioecy make clear predictions about the conditions under which this type of polymorphism can be maintained. Cytoplasmic male-sterility factors can invade hermaphrodite populations if they increase female fertility and can be maintained polymorphic if the hermaphrodites are partially self-fertilizing (Lewis 1941; Lloyd 1975; Charlesworth 1981). Nuclear male-sterility factors, however, can invade only if the lowering of fitness due to the loss of male fertility is outweighed by a sufficiently large increase in realized female fertility. This could come from production of outbred, highly fit offspring, whereas hermaphrodites' offspring might be inbred and suffer from inbreeding depression. Increased female fertility could also be caused by reallocation of resources that hermaphrodites devote to male functions (Lloyd 1975; Charlesworth and Charlesworth 1978). Equilibria with both females and hermaphrodites present are possible, the frequencies depending on the relative seed outputs of the two sex phenotypes as well as on the relative quality of their offspring. Joint polymorphisms, with both cytoplasmic and nuclear factors simultaneously present in populations, are also possible. In addition to conditions similar to those for nuclear male-sterility alleles to be maintained (in particular, considerably increased female fertility, though often less than required for nuclear male sterility), there must be some disadvantage to alleles at the nuclear loci that restore male fertility to individuals carrying sterility cytoplasms, to prevent their going rapidly to fixation (Charlesworth 1981; Delannay *et al.* 1981; Frank 1989; Gouyon *et al.* 1991). At equilibrium, females are expected to have higher female fertility than hermaphrodites (Charlesworth 1981). Studies of the inheritance of male sterility and tests for the cost of restoration are thus necessary for understanding the maintenance of females.

Although there is a good deal of information about selection on male sterility polymorphisms in natural populations of several species (see, for instance, Eckhart 1992a), detailed studies of the inheritance remain few. Several gynodioecious species show complex patterns of inheritance (reviewed in Ross 1978; Charlesworth 1981), and it is often difficult to interpret the data in enough detail to be certain how many factors are involved, though more than one factor is clearly implicated in many species (*e.g.*, Kheyr-Pour 1980; Stevens and Richards 1985; Sun 1987; Connor and Charlesworth 1990; Eckhart 1992b). In the best worked-out cases, *Plantago lanceolata* (Van Damme 1983;

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De Haan *et al.* 1997a), *P. coronopus* (Koelewijn and Van Damme 1995a,b), and *Thymus vulgaris* (Belhassen *et al.* 1991), several nuclear factors, as well as several cytoplasmic types, are present in natural populations. In *P. lanceolata*, there is some evidence for fitness costs being associated with restorers of male fertility (De Haan *et al.* 1997b).

In this article, we give results of genetic studies of male sterility in *Silene vulgaris*, which exhibits polymorphism for females and hermaphrodites in most natural populations (Marsden-Jones and Turrill 1957), using plants from two English natural populations with substantial frequencies of females. We also reexamine data from *T. vulgaris*. Belhassen *et al.* (1991) published extensive segregation data in 48 lineages with the same cytoplasmic type, together with molecular analyses of mitochondrial variants, but were unable to provide a full genetic interpretation of the results. We show that the results can be interpreted under a model similar to that for *S. vulgaris*.

The genetics of male sterility in *S. vulgaris* was first studied by Correns (1906, 1908; the species was then known as *S. inflata*). Hermaphrodite maternal parents produced mostly hermaphrodite progeny, and female maternal parents produced mostly females; Correns concluded that male sterility was cytoplasmically inherited. However, segregation of the sex types within families is evident in Correns' data, so there are grounds for suspecting that the system is not as simple as Correns thought. A more recent small set of family data is similar, with some plants producing offspring of the same sex as themselves, but many segregating (Marsden-Jones and Turrill 1957). The inheritance of sex in tetraploid material from Israeli populations of this species (three plants, one from a natural population and two immediately descended from a different population) appeared to involve a single recessive nuclear male-sterility factor such that *aaaa* and *aaaA* are female, *AaAa* have both female and hermaphrodite flowers (referred to as "HF" or "polygamous plants"), and other genotypes are hermaphrodite (Horovitz and Dulberger 1983). Here, we analyze genetic results from two diploid populations of this species. Both nuclear and cytoplasmic factors influence the sex phenotype.

MATERIALS AND METHODS

Populations studied: The plants studied originated from two natural populations near the University of Sussex, England (D. Charlesworth, unpublished results). In 1979 and 1983, preliminary surveys of the sexes were done in the field. Most plants had either female or hermaphrodite flowers, but a few had flowers of both sexes. Two kinds of male-sterile flowers were observed. One kind of female had reduced, yellow anthers, while the other had aborted, white anthers.

As there is some gynomonoecy (below), sexes cannot be correctly scored without sexing multiple flowers. Surveys done in 1984 and 1985 recorded the first 15 flowers of each plant (or all flowers, on plants with <15 flowers) from the populations from which the plants studied here were derived. Plants were grouped into five sex classes: All flowers hermaphrodite (H); most ($>6/15$) flowers hermaphrodite (HF); most ($>9/15$) flowers female (FH); all flowers female with reduced anthers (Y); and all flowers female with aborted, white anthers (W). The smaller of the two populations used in the genetic study (population 1) had a female frequency of about 0.25–0.35, while in population 3 the frequency of females was close to 0.6.

Plant culture methods and pollination method: Seeds collected from maternal parents of known sex in the field in 1983 and 1984, from the two populations, form the ancestors of the families used to study the inheritance of male sterility. Seeds germinated rapidly in the greenhouse, usually within 2 wk with mild heat, though percentage germination was low ($\sim$30%) for the first 2 years' families. After 2 to 3 wk, seedlings were transplanted into individual 4-inch pots, and thereafter watered regularly and given liquid fertilizer weekly. Under these conditions, plants flowered in 4 to 6 wk.

The sexes of plants grown in the greenhouse were scored as described above. Progeny of flowers naturally pollinated in the field were grown in 1985 and 1986. In 1985, a total of 1890 plants was scored, of which 206 (0.11) had both female and hermaphrodite flowers, while the rest had flowers of just one sex; the corresponding numbers for 1986 were a total of 1672 plants scored, of which 184 (0.11) had two sexes of flowers. In the greenhouse-grown plants of mixed sex, the number of hermaphrodite flowers out of the first 15 flowers was bimodal, most having either <5 or >7 hermaphrodite flowers.

In view of the variability in sex phenotypes, it was checked that these phenotypes are stable, rather than altered by environmental differences. Six cuttings of each of 30 plants of various different sex phenotypes were grown under different conditions. Three sets were grown under the normal conditions described above, and their sex was followed for flowering bouts in two successive years; one set was grown without liquid fertilizer, so that the size at flowering was reduced, and one set was grown in shaded conditions. A final set was given a short day length (10 hr) that delayed flowering. The frequency of female flowers, out of 15 flowers scored, was recorded for each plant. Although the gender of the plants was variable, plant genotype was the only significant determinant of gender, and there was no significant effect of treatment.

Genetic study: To raise seeds for further generations, pollinations were done under a cage made from a wooden frame covered with window screening, which excluded insects and reduced light levels about 30%, but did not affect flowering. To pollinate the flowers of females, anthers of the hermaphrodite pollen donors were rubbed on the surface of the stigmas, using all three (or more, in some cases) stigmas of each flower. The stigmas of female flowers appeared receptive as soon as they emerged from among the petals, usually before the bud opened, and pollinations were generally successful at any time after the stigma tips could be seen. Almost all pollinations on females produced capsules. For pollinations of hermaphrodites, flowers were emasculated in the bud stage. Only about half survived this treatment in good condition; in the rest, the stigmas appeared wilted and could not be successfully pollinated. Pollinations on nonwilted hermaphrodite flowers were done when the stigmas were fully elongated, twisted at the tips, and had visible papillae (corresponding usually to the stage one or two days after both whorls of anthers had dehisced and dropped off the filaments of intact flowers). Even these flowers yielded only 53% of capsules on average. The low fruit/flower ratio of the hermaphrodites is not understood, but may be connected with differences between the stigmas of the two sex types (Horovitz and Dulberger 1983).

To test for cytoplasmic differences, reciprocal crosses between hermaphrodites were done, both within and between the two populations. Obtaining seeds from several pairs of reciprocal crosses was difficult because of the low fertility of crosses between hermaphrodite plants (see above); when one of a pair of reciprocal pollinations succeeded, the other frequently failed. In addition, females descended from five type W field females from population 1 were pollinated by hermaphrodites to raise second and third generations. By the third generation, germination had improved, presumably because of selection for an improvement, so that larger progenies could be scored.

Further results on the genetics of nuclear factors come from crosses between females and hermaphrodites and from families from hermaphrodites self-fertilized and crossed with other hermaphrodites. Crosses between descendants of the same field plant are most informative, as these plants must share cytoplasmic types. For these, more than a single cross was often done, so it was possible not only to develop genetic interpretations on the basis of examination of the ratios, but also to attempt to assign genotypes to parents. Observed ratios were tested against expected ratios of pooled hermaphroditic (H and HF) and female phenotypic categories (FH, Y, and Y) under different models were tested by *G*-tests (Sokal and Rohlf 1981). In population 1, lineages from ancestral plants in the field having one cytoplasmic type were analyzed. From population 3, four lineages were analyzed in detail. One of these probably has the same cytoplasm as one of the types found in population 1, while three carry a cytoplasm probably not found in population 1.

Notation: Families were identified by a year designation followed by a family number, and individual plants within families were designated by an identifying number (*e.g.*, 87/20-2 is plant 2 in family 20 grown in 1987). Sexes are indicated by a letter H, Y, or W (or sometimes F, when the type was intermediate between Y and W) after the plant's number. As it is reasonable to assume that cytoplasmic male-sterility factors rarely arise in populations and that populations containing them are sometimes invaded by restorer alleles (see discussion), we use the symbol + for the nonrestorer (male-sterility) alleles at the loci in our genetic interpretations and *R* or *r* for dominant and recessive restorer alleles, respectively; subscripts are used to designate different loci.

RESULTS

***S. vulgaris* progeny from naturally pollinated flowers:** The sexes of progeny grown from seeds collected after natural pollination in the field are summarized in Table 1 and Figure 1. The numbers of progeny plants were small, owing to poor germination of seeds collected in the field (on average only 10 progeny per maternal parent were scored). Of the total number of 129 maternal parents of these plants, the same parental individual may have been included in the 2 years (the number of such cases is unknown, because aboveground parts of the plants die back in the winter and permanent marking of individuals was impossible at both localities). Given these limitations and the fact that the pollen donors are unknown, segregation ratios in these families give only slight insight into the inheritance of male sterility in these populations, so they are not reported individually. However, some information can be gained.

Females often yielded all-female progenies (often from more than one capsule, which were presumably from pollination by different hermaphrodites), and many hermaphrodites produced only hermaphrodite offspring (Table 1, fourth column). These observations suggest a role for cytoplasmic inheritance, but the numerous exceptions suggest a nucleocytoplasmic system. Nearly all the families containing 100% females were of the W type, whereas the segregating families could have W females or Y females, or both. This suggests that there is some genetic basis for the difference in male-sterility phenotype. We show below that reciprocal crosses show involvement of cytoplasmic genes, and further genetic analysis of self- and cross-pollinated progenies indicates the presence of nuclear restorer loci.

Analysis of cytoplasmic sterility factors: Reciprocal crosses were done using parents of the second green-

TABLE 1

Sex phenotypes in the generation 1 progeny of naturally polinated plants from two wild populations

Year of seed collection	Maternal parent's sex	Number of maternal plants			Mean frequency of females in nonsegregating families
		Segregating	Nonsegregating	Nonsegregating and all female	
		Population 1			
1983	Hermaphrodite	9	9	0	0.278
	Female	3	5	5	0.600
1984	Hermaphrodite	7	14	1	0.233
	Female	8	6	5	0.550
		Population 3			
1983	Hermaphrodite	7	12	0	0.350
	Female	17	9	1	0.438
1984	Hermaphrodite	14	9	1	0.431
	Female	10	4	4	0.622

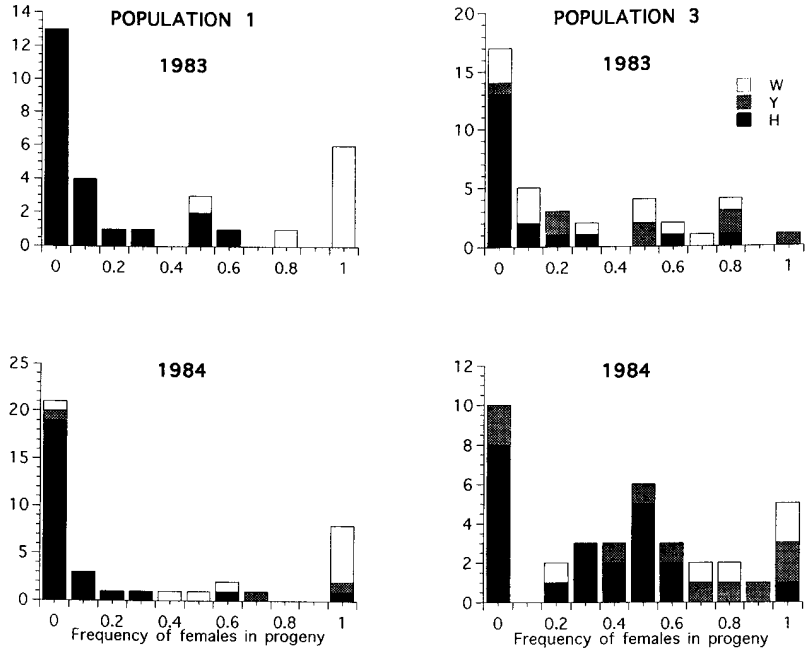

Figure 1.—Results from families of individual plants naturally pollinated in the field. H, hermaphrodite maternal plants; Y, yellow females; W, white females. Results from different capsules from the same maternal plant were pooled.

house generation, but are described here for clarity. The results are shown in Table 2 for families with at least eight progeny scored in both reciprocals. In several cases, the distribution of progeny into the five classes differed significantly in the reciprocals. The finding of a reciprocal difference is evidence for the presence of two different cytoplasmic types, though the absence of a difference does not of course imply that the parents have the same cytoplasmic type (two parents, each homozygous for a dominant restorer allele acting to restore the sterility of the other parent's cytoplasm, would produce an all-hermaphrodite progeny, for instance). However, agreement between the ratios found in two segregating progenies of a pair of reciprocal crosses is unlikely unless they have the same cytoplasm. The results can be interpreted in terms of two or more cytoplasmic genotypes in each population.

Among crosses within population 1, those between plants 18.20 and 42.4 produce similar ratios, as expected because both carry the cytoplasm of field plant 83/348-H. The two clearly significant reciprocal differences both involved plant 97.12, a descendant of field plant 83/335-H. We refer to the cytoplasm of field hermaphrodite 83/348 as S_a, and to that of 83/335-H as S_b. The similar results from the crosses between descendants of 83/348, 83/340, and 84/060 suggest that these share S_a. The cytoplasmic types of the other plants can be deduced from these (see Table 2). A further reciprocal difference was found between plants 84/060 and 84/001. These are discussed further below.

Among the population 3 families, the crosses between two plants (281.10 and 284.19) having the maternal lineage of 84/394-H gave similar ratios, as expected. The cytoplasmic types of 274.18 and 277.29 are probably the same as the ones in these plants, as crosses with 281.10 and with 281.27, another descendant of 84/394-H, also showed no reciprocal differences. None of these lineages were successfully tested with population 1 plants, so it is not possible to say whether this cytoplasmic type is the same as S_a or S_b, and we will designate it as S_x. Reciprocal differences within population 3 are evident in the crosses involving plant 170.17, descended from field hermaphrodite 83/376 and members of the lineage descended from 84/307-H.

In interpopulation crosses, plant 170.17, but not 199.10 or 200.7, gave reciprocal differences with 3.34, whose lineage was inferred above to carry S_a; 170.17 is therefore not S_a. On the conservative assumption that population 3 has the same cytoplasmic types as population 1, 170.17 and its ancestor 83/376-H was S_b, and 199.10's ancestor (84/307-H) probably was S_a. The fact that 170.17 and 114.14 did not give a reciprocal difference is consistent with the ancestor of the latter, hermaphrodite 84/001, having S_b. This implies that 84/060 and its descendants are probably S_a. 84/001-H was also the ancestor of 57.2, which gives a reciprocal difference with a population 3 plant 168.24, descended from 83/373-H, suggesting that the latter is S_a (or else yet another type). The rest of the data suggest the cytoplasmic genotypes listed in Table 2. In general, crosses with S_b maternal parents and S_a pollen donors yielded higher female frequencies than the reciprocal crosses (Table 2). Among the population 1 plants that can be typed, S_a appears to be the commonest cytoplasmic type (five of the seven ancestral plants typed), whereas in population 3, S_a appears to be less frequent than S_x (two of five plants

TABLE 2

Sex phenotypes in the progeny of reciprocal crosses between hermaphrodite plants

Parents (maternal × paternal)	Field ancestor of first and second maternal plant	Maternal cytoplasmic type	Sex phenotypes of progeny					Reciprocal difference (χ^2, P value)
			H	HF	FH	Y	W	
Crosses involving plants from population 1								
18.20 × 42.4	83/348-H	S_a	12	0	0	4	0	NS*
42.4 × 18.20	83/348-H	S_a	19	0	1	2	1	
42.4 × 97.12	83/348-H	S_a	17	0	0	0	0	$\chi^2 = 12.02$,
97.12 × 42.4	83/335-H	S_b	12	0	1	0	11	$P = 0.025$
97.12 × 139.15	83/335-H	S_b	1	3	1	0	9	$\chi^2 = 14.85$,
139.15 × 97.12	83/305-H	S_a	22	2	0	1	0	$P = 0.01$
7.2 × 42.4	83/340-H	S_a	23	2	0	0	0	NS
42.4 × 7.2	83/348-H	S_a	23	0	1	1	0	
19.15 × 51.12	83/348-H	S_a	19	1	0	0	0	NS
51.12 × 19.15	84/060-H	S_a	8	0	0	0	0	
51.12 × 57.2	84/060-H	S_a	13	1	0	0	0	Exact test
57.2 × 51.12	84/001-H	S_b	10	1	1	3	1	$P = 0.047$
Crosses involving plants from population 3								
281.10 × 284.19	84/394-H	S_x	21	0	1	0	2	NS
284.19 × 281.10	84/394-H	S_x	10	4	1	0	1	
274.18 × 281.27	84/370-Y	S_x	25	0	0	0	0	NS
281.27 × 274.18	84/394-H	S_x	19	2	2	0	2	
277.29 × 281.10	84/381-W	S_x	11	10	1	0	2	NS
281.10 × 277.29	84/394-H	S_x	18	2	0	1	4	
170.17 × 199.10	83/376-H	S_b	16	2	3	0	4	$\chi^2 = 10.98$,
199.10 × 170.17	84/307-H	S_a	25	0	0	0	0	$P = 0.05$
170.17 × 200.7	83/376-H	S_b	12	3	2	1	6	$\chi^2 = 10.18$,
200.17 × 170.17	84/307-H	S_a	15	6	0	0	0	$P = 0.05$
Crosses between plants from populations 1 and 3[a]								
3.34 × 170.17	83/305-H	S_a	22	2	0	1	0	$\chi^2 = 17.76$,
170.17 × 3.34	83/376-H	S_b	6	5	1	0	6	$P = 0.005$
3.34 × 199.10	83/305-H	S_a	21	2	1	0	0	NS
199.10 × 3.34	84/307-H	S_a	22	2	0	1	0	
3.34 × 200.7	83/305-H	S_a	17	4	1	0	0	NS
200.7 × 3.34	84/307-H	S_a	11	0	0	0	0	
57.2 × 168.24	84/001-H	S_b	22	2	0	0	0	$\chi^2 = 35.46$,
168.24 × 57.2	83/373-H	S_b	4	0	4	0	17	$P < 0.001$
114.14 × 170.17	84/001-H	S_a	21	1	0	0	0	NS
170.17 × 114.14	83/376-H	S_b	22	1	0	0	1	
188.4 × 226.13	84/025-H	S_a	13	0	0	0	0	NS
226.13 × 188.4	84/351-H	S_a	23	1	0	0	2	
185.17 × 198.4	84/025-H	S_a	23	2	0	0	0	NS
198.4 × 185.17	84/307-H	S_a	17	1	0	0	0	

All parents' numbers refer to 1986 families.

* Not significant.

[a] In the interpopulation crosses, the population 1 parent is the left-hand parent in the upper of the pair of crosses.

that could be typed); this difference is not significant, however (2×2 $\chi^2 = 1.18$, with 1 d.f.).

Further evidence that maternally transmitted cytoplasmic male sterility factors are involved is provided by the finding that wholly female progenies were produced by females from four of the five field females whose descendants were followed further (Table 3). The alternative explanation for all-female families, dominant male sterility together with a cytoplasmic difference, is untenable. In that model, the second generation should segregate unless the pollen donors in both generations were homozygous for dominant sterility alleles that are expressed in the female's cytoplasm. Another alternative is that maternal genotype controls progeny sex, but again, while the first generation might not segregate, the second generation should do so. Given the reciprocal differences demonstrated above, cytoplasmic factors are the most reasonable interpretation. The continued all-

TABLE 3

All-female families from population 1

Parents	Cytoplasmic types of parents	Family	Sex phenotypes of progeny				
			H	HF	FH	Y	W
84/002 open	Not known	85/505	0	0	0	0	7
84/002 open	Not known	85/543	0	0	0	0	7
85/543.3 × unrelated	Not known	86/93	0	0	0	5	18
86/93.13 × 57.2	Not known, S_a	87/682	0	0	0	0	5
86/93.13 × unrelated	Not known	87/683	0	0	0	0	13
86/93.13 × unrelated	Not known	87/684	0	0	1	0	15
86/93.13 × unrelated	Not known	87/685	0	0	0	0	24
87/682.11 × unrelated	S_a, not known	88/39	0	0	0	0	30
87/682.11 × unrelated	S_a not known	88/40	0	0	0	4	26
83/464 open	Not known	84/22	0	0	0	0	4
83/464 open	Not known	85/614	0	0	0	0	6
85/614 × unrelated	Not known	86/140	0	0	0	1	30
86/138.19W × 57.2	Not known, S_a	87/685	0	0	0	0	25
83/011 open	Not known	84/23	0	0	0	0	7
83/011 open	Not known	85/25	0	0	0	0	11
85/25 × unrelated	Not known	86/123	0	0	0	10	6
86/123.6 × 185.17	Not known, S_a	87/90	0	0	0	0	20
87/90 × unrelated	S_a, not known	88/42	0	0	0	1	11
83/044 open	Not known	84/143	0	0	1	0	27
84/143 × unrelated	Not known	85/140	0	0	0	1	30
85/140 × unrelated	Not known	86/124	0	0	0	0	20
86/124 × unrelated	Not known	87/91	0	0	12	1	24
87/91 × unrelated	Not known	88/44	0	0	0	0	8

Cytoplasmic types of parents are listed for the few crosses where they are known.

female lineages also suggest the presence in population 1 of an unrestored sterility cytoplasm (or one for which restorer alleles are rare); this third type is distinct from the cytoplasms S_a and S_b discussed above, which do not behave in this manner.

Nuclear restorers of male sterility: general features of the genetics: As a basis for examining the nuclear genetics of male sterility, a model with two cytoplasmic types, each with a single restorer of male fertility, was initially tested. This can fit many of the results of crosses between second generation plants, which are given in the appendix for families with nine or more progeny. Ratios in families from the different generations were mostly either ~3H:1F or 1H:1F (Tables 5 and 6 and appendix).

Under the model fitted, recessive restoration is unlikely because hermaphrodites should not segregate on selfing, but only one selfed family failed to segregate. Furthermore, selfings should not produce 3H:1F, which often occurs (see appendix). With dominant restoration of male fertility, females must be homozygous for the nonrestorer (sterility, or +) allele for their own

TABLE 4

Summary of the genetic results, showing numbers of families with nine or more progeny from within-population crosses that yield significant differences from a model with two cytoplasmic types, each with a dominant restorer of male fertility

Sexes of parents model of cross	Number of families		Ratio tested	Numbers of segregating families differing significantly from	
	Total	Segregating		Population 1	Population 3
F × H	39	31	1H:1F	6	7
H self	25	24	3H:1F	3	8
H × H	40	37	3H:1F or 1:1	5	3

cytoplasm. They should thus segregate 1H:1F ($+/+ \times R/+$) and can also produce all-hermaphrodite families ($+/+ \times R/R$) and, given a cytoplasmic difference, all-female families (*e.g.*, using subscripts for the sterility cytoplasm restored by each locus, $S_a+_a/+_a \times S_b+_a/+_a$). Considering all the families from all generations, 13 segregating families differ significantly from 1:1 (Table 4), 5 with excess females and 8 with excess hermaphrodites.

For hermaphrodite maternal plants, 3H:1F or 1H:0F ratios are expected from selfing. Crossing to other hermaphrodites should yield the same ratios, and also 1:1 (from $S_a\ R_a/+_a\ -/- \times S_b\ +_a/+_aR_b/-$). Again, many families cannot be fitted by this model: seven of the eight segregating families from selfing that differ significantly from 3H:1F have excess females (Table 4). Families that result from crossing different hermaphrodites often fit this model, not surprisingly given the range of ratios possible when the cytoplasmic types of the parents are unknown and may differ. Nevertheless, several segregating families from within-population crosses differed from both possible ratios, as did 11/12 of the segregating between-population crosses (Table 2). The model in its simplest form is thus disproved, and a more complex hypothesis is necessary.

Although there is no reason to assume the same genes in both populations, such an assumption can give us the minimum genetic complexity, so we provisionally adopt it here. The progenies from selfing hermaphrodites with either S_a or S_x cytoplasms, taken as a whole, require at least two loci with dominant epistatic restorer alleles, in addition to a cytoplasmic difference (equivalent to two recessive sterility alleles with independent action within one cytoplasmic genotype). Such a model can explain most of the discrepant families. 3H:1F ratios can then occur if a $+/+\ +/+$ female is mated to an $R1/+\ R2/+$ hermaphrodite. This fits all the F × H families with excess hermaphrodites (see appendix). The interpretation of at least two loci is supported by the recurring result that selfing hermaphrodites yielded too many females, compared to single locus expectations. Five of the 11 selfed families that do not fit 3H:1F can be fitted assuming 9H:7F ratios, which is expected with two loci with dominant restorers, but other families (discussed below, including 86/226 and 87/784) suggest a yet more complex model. Moreover, most of the discrepant crosses between hermaphrodites require two independently acting dominant restorers, as they appear to be 15H:1F ratios (also seen in one progeny from selfing). As mentioned above, crosses between hermaphrodites are less informative than other kinds of families and can be fitted in various ways; in this instance, a model with one dominant and one recessive restorer can also fit.

Results from further generations and genetic interpretations: The results just discussed make clear the general features of the inheritance of male sterility in these populations. The results from smaller-sized families (not shown) were consistent. There is little power to discriminate ratios in such families, but it is clear that further genetic factors are involved, in addition to those assumed in the simplest model tested above. The models given below fit most of the other family data well, so the results are not all given in detail, but a few families demonstrating particular conclusions are next discussed. Tables 6 and 7 show the most informative crosses, with the genotypes assigned to as many plants as possible, and the results of tests of genetic ratios.

Population 1: A model with two dominant epistatic restorers fits all the data from the small families grown from population 1 field plants with cytoplasmic type S_b (not shown) and from most of the families interpreted above as carrying cytoplasmic type S_a. The descendants of the field hermaphrodite 83/348 yielded the only data from this population that could not be fitted by this two-locus model. The interpretation in Table 5 assumes two epistatic loci with dominant restorers (*i.e.*, restorers at loci 1 and 2 must both be present for male fertility) and an additional independent restorer at locus 3 (also dominant); a model with three loci with dominant male-epistatic restorers is also possible, but fits slightly less well. Seeds were grown from three capsules from 83/348 (first generation families 84/21, 84/265, and 85/581) and all 36 were hermaphrodite. Two hermaphrodites from one family (84/265) were selfed and pollinated by unrelated plants. One selfed family is shown in Table 5. Its ratio suggested that hermaphrodite 84/265.9 was heterozygous for a recessive male-sterility allele ($R_a/+$, yielding 3:1 on selfing, $G = 2.22$). Two small families from a sib (not shown) were consistent. When 84/265.9's progeny 85/632.7 and 85/631.7 were selfed, however, the ratios obtained differed significantly from 3H:1F (Table 5, families 3 and 4, $G = 4.99$ and 11.5, respectively); approximately equal numbers of hermaphrodite and female plants were produced, suggesting that these plants were heterozygous for two epistatic dominant restorers. But hermaphrodite 85/632.7 was a product of selfing so, on the basis of this interpretation, its parent must also have been heterozygous for both these alleles, which is inconsistent with its producing a quite different ratio on selfing.

These findings can be reconciled by introducing a third locus heterozygous in plant 86/265.9 with an independently acting dominant restorer (*R3*), such that genotypes *R1*/–*R2*/–/– and –/–/–*R3*/– are hermaphrodite. This interpretation is supported by the observation of families that do not fit 3:1 but can be fitted by 7:1 ratios on this model (Table 5, nos. 7, 10, and 14). The reciprocal crosses of plant 86/42.4 with 86/7.2 and with 86/18.20, both with cytoplasm S_a (Table 5, families 12–14), are also consistent, and the interpretations of their genotypes given in Table 5 are consistent with their behavior in the crosses in Table 3.

The selfed progeny of hermaphrodite plant 86/19.15

TABLE 5

Segregations in families descended from population 1 plants with the S_a cytoplasmic type arranged in order of the generations

Family number and parentage (Female × male parent)	Family	Proposed genotypes	Progeny sexes H	Progeny sexes F	Predicted ratio (H:F)	*G*
		Second generation				
1. 84/265.9-H self	85/632	$\frac{R_a1}{+}\frac{R_a2}{+}\frac{R_a3}{+}$	11	1	57:7	0.09
2. 84/265.9-H × unrelated	85/631	$\frac{R_a1}{+}\frac{R_a2}{+}\frac{R_a3}{+} \times \frac{R_a1}{+}\frac{R_a2}{+}\frac{R_a3}{+}$	14	1	7:1	0.47
		Third generation				
3. 85/632.7-H self	86/42	$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+}$	11	10	9:7	0.12
4. 85/631.7-H self	86/18	$\frac{+}{+}\frac{+}{+}\frac{+}{+}$	9	13	9:7	2.08
5. 85/632.7-H × unrelated	86/45	$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+} \times$, *e.g.*, $\frac{R_a1}{+}\frac{R_a2}{R_a2}\frac{+}{+}$	13	4	3:1	0.02
6. 85/631.4-H × unrelated	86/19	$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+} \times$, *e.g.*, $\frac{R_a1}{+}\frac{R_a2}{R_a2}\frac{+}{+}$	13	6	3:1	0.42
7. 85/631.4-H × unrelated	86/20	$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+} \times$, *e.g.*, $\frac{R_a1}{+}\frac{R_a2}{R_a2}\frac{R_a3}{+}$	11	1	7:1	0.22
		Fourth generation				
8. 86/45.9-H self	87/664	$\frac{R_a1}{R_a1}\frac{R_a2}{+}\frac{+}{+}$	9	5	3:1	0.79
9. 86/45.2-Y × 86/45.9-H	87/680	$\frac{+}{+}\frac{R_a2}{+}\frac{+}{+} \times \frac{R_a1}{R_a1}\frac{R_a2}{+}\frac{+}{+}$	16	2	3:1	2.19
10. 86/45.9-H × 86/20.5-H	87/650	$\frac{R_a1}{R_a1}\frac{R_a2}{+}\frac{+}{+} \times \frac{-}{-}\frac{R_a2}{+}\frac{R_a3}{+}$	21	1	7:1	1.63
11. 86/19.15-H self	87/733	Not interpreted	1	7	Not tested	
12. 86/42.4-H × 86/18.20-H		$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+} \times \frac{R_a1}{+}\frac{R_a2}{R_a2}\frac{+}{+}$	12	4	3:1	0
13. reciprocal of 12			19	4	3:1	0.76
14. 86/42.4-H × 86/7.2-H		$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+} \times \frac{R_a1}{R_a2}\frac{R_a2}{+}\frac{R_a3}{+}$	23	2	7:1	0.52
15. 86/42.4-H × 86/97.12-H		$\frac{R_a1}{+}\frac{R_a2}{+}\frac{+}{+} \times$, *e.g.*, $\frac{R_a1}{R_a1}\frac{R_a2}{R_a2}\frac{-}{-}$	17	0	1:0	0

The genetic model assumes that restorers at loci 1 and 2 are dominant and must both be present to restore male fertility and that plants with dominant restorer at locus 3 are also male fertile.

(Table 5, no. 11) had a high frequency of females, suggesting heterozygosity for several dominant restorers. Family 86/19 included many plants with intermediate (HF and FH) sex phenotypes, which is consistent with its having several different sterility factors. It is also consistent with the idea that this family was produced by an outcross to an unrelated plant, which could have introduced alleles different from those in family 18. However, it is difficult to place much reliance on this isolated case. Overall, the population 1 data thus require a minimum of three loci for the S_a cytoplasm, as well as the two loci for S_b discussed above.

Another possible explanation for high female frequencies in progeny of hermaphrodites is labile sex expression, such that a plant whose genotype usually produces male sterility can sometimes be hermaphrodite, which does occasionally occur in *S. vulgaris* (see above). An example is a set of four crosses involving plants derived from one field female, 84/351, that was assigned to cytoplasmic type S_a. Two progenies derived from selfing hermaphrodite plants in this lineage yielded high female frequencies (one with >75% females), and when this plant was pollinated by an unrelated hermaphrodite it yielded a 1H:3F ratio, though its female sibling crossed with the same unrelated hermaphrodite gave 3H:1F. Such families are difficult to explain in any other way than by labile sex expression.

Population 3: Descendants of two females and two hermaphrodites from this population were followed in detail. No families from females remained wholly female in all generations, in contrast to the results from population 1.

For the plants with cytoplasmic type S_x (families 1–15 in Table 6), the hypothesis of a single nuclear locus for restoration of male fertility is ruled out by our findings, and indeed no simple genetic model accounts for all the observed segregation ratios. On the one hand, high frequencies of females from selfing require epistatic action of several dominant restorer alleles at two or three loci (families 4 and 5) or even four loci (cross 15). On the other hand, several restorer alleles acting independently are required to account for high fre-

TABLE 6

Families descended from population 3, showing results that cannot be explained with two loci

Family number and parentage (Female × male parent)	Family	Proposed genotypes	Progeny sexes H	Progeny sexes F	Predicted ratio (H:F)	*G*
Plants with cytoplasm S_x (Model: two loci, *R1* dominant and *r2* recessive, independent action)						
Descendants of hermaphrodite 84/394						
1. 84/394-H open-pollinated	85/791	Not interpreted (several possible)	6	7		
2. 85/791.1-Y × unrelated	86/281	*e.g.*, $\frac{+}{+}\frac{r_x2}{+} \times \frac{R_x1}{+}\frac{r_x2}{r_x2}$	22	5	3:1	0.64
3. 85/791.6-Y × unrelated	86/284	*e.g.*, $\frac{+}{+}\frac{r_x2}{+} \times \frac{R_x1}{+}\frac{+}{+}$	13	10	≈1:1	0.39
4. 86/281.10-H selfed	87/830		5	18	9:7	
5. 86/281.27-H selfed	87/789		8	14	9:7	3.51
6. 86/284.19-H × 86/263.3-H	87/794	$\frac{R_x1}{+}\frac{r_x2}{+} \times \frac{R_x1}{+}\frac{r_x2}{r_x2}$	21	4	7:1	0.28
7. 86/284.19-H × 86/284.7-Y	87/796	$\frac{R_x1}{+}\frac{r_x2}{+} \times \frac{+}{+}\frac{+}{+}$	14	10	1:1	0.67
8. 86/263.3-H × 86/284.7-Y	87/797	$\frac{R_x1}{+}\frac{r_x2}{+} \times \frac{+}{+}\frac{+}{+}$	11	14	1:1	0.36
9. 86/284.19-H selfed	87/793	$\frac{R_x1}{+}\frac{r_x2}{+}$	24	1	13:3	2.67
Descendants of female 84/381						
10. 84/381-W open-pollinated	85/783	Various possibilities	1	3	Not tested	
11. 85/783.1-W × unrelated	86/277	$\frac{+}{+} \times \frac{R_x1}{+}$	18	13	1:1	0.81
12. 86/277.29-H selfed	87/799	$\frac{R_x1}{+}\frac{r_x2}{+}$	3	5	13.3	7.40
Descendants of female 84/370						
13. 84/370-Y open-pollenated	85/769	Various possibilities	3	2	Not tested	
14. 85/769.5-W × unrelated	86/274	$\frac{+}{+}\frac{r_x2}{+} \times \frac{R_x1}{R_x1}\frac{r_x2}{+}$	23	8	3:1	0.01
15. 86/274.18-H selfed	87/780		6	15	Not tested	
Plants of unknown cytoplasmic type (Model: multiple loci, dominant restoration of male fertility, epistatic interaction)						
Descendants of hermaphrodite 84/319						
16. 85/732.1-H selfed	86/203	$\frac{R1}{+}\frac{R2}{+}$	17	15	9:7	0.13
					27.37	1.55
17. 86/203.10-H selfed	87/744	$\frac{R1}{+}\frac{R2}{+}$	8	5	9:7	0.15
					27:37	1.97
18. 86/203.10-H × 86/203.20-W	87/748	$\frac{R1}{+}\frac{R2}{+}\frac{R3}{+} \times \frac{+}{+}\frac{+}{+}\frac{+}{+}$	2	23	1:7	0.52
19. 86/203.10-H × 86/185.17-H	87/819	$\frac{R1}{+}\frac{R2}{+}\frac{R3}{+} \times \frac{R1}{+}\frac{R2}{R2}\frac{R3}{+}$	18	6	9:7	3.64
20. 86/203.10-H × 86/182.12-H	87/745	$\frac{R1}{+}\frac{R2}{+}\frac{R3}{+} \times \frac{R1}{+}\frac{R2}{+}\frac{R3}{R3}$	18	7	3:1	0.12
21. 86/203.20-W × 86/185.17-H	87/821	$\frac{+}{+}\frac{+}{+}\frac{+}{+} \quad \frac{R1}{+}\frac{R2}{R2}\frac{R3}{+}$	3	22	1:3	2.63
22. 86/203.20-W × 86/182.12-H	87/750	$\frac{+}{+}\frac{+}{+}\frac{+}{+} \times \frac{R1}{+}\frac{R2}{+}\frac{R3}{R3}$	0	25	1:7	6.68

quencies of hermaphrodites in other crosses (for example, the >50% of hermaphrodites observed in a progeny of some females, as in families 2 and 14, or >75% in the progeny of an hermaphrodite, as in family 9). A two-locus model with one dominant restorer allele and one recessive restorer (*i.e.*, females must be *R1*/*R1 r2*/–, where the restorer allele at locus 1 is dominant and the one at locus 2 is recessive) works well for these data (Table 6). This model also agrees well with the pedigree analysis below. It was possible to assign consistent genotypes for all the individuals crossed, with the exception that the three selfed families containing high female frequencies remain unexplained (crosses 4, 5, and 15). These results may be caused by labile sex expression (see above), or by low frequency male-sterility alleles carried in hermaphrodites and expressed on selfing (see discussion).

Further progenies with very high female frequencies occurred among the descendants of field hermaphrodite 84/319, for which the cytoplasmic type is unknown. That plant yielded only two progeny: one hermaphrodite and one female. Both the selfed progenies of her-

maphrodites 85/732.1 and 86/203.10 (crosses 16 and 17) required a minimum of two loci with epistatic dominant restorer alleles. A cross between 86/203.10 and a female sibling required a third locus with a dominant restorer allele acting epistatically with both previous ones (Table 6, cross 18). Such a model is also consistent with 27:37 ratios in the two families produced by selfings in the previous generation (though this ratio fits the data slightly less well than 9:7). The crosses with the two hermaphrodites of population 1 origin, 86/182.12 and 86/185.17 (descended from plant 84/025 that has been assigned cytoplasmic type S_a), can also mostly be reconciled with such an interpretation (crosses 20 and 21). The genotypes are consistent with those deduced for these two population 1 plants from independent crosses (which require them to have different genotypes from one another; not shown). It is thus likely that the descendants of plant 84/319 have the S_a cytoplasm.

The few results from the one plant classified as cytoplasmic type S_b, hermaphrodite 83/376 (not shown), fit a model with a single recessive sterility factor. Thus the results from population 3 suggest at least three recessive sterility factors expressed in cytoplasmic type S_a, as in population 1 (though with a different form of interaction), one expressed in type S_b (compared with two for this cytoplasmic type in population 1), and two restorers that act independently in S_x.

Interpretation of the genetics of male-sterility in *T. vulgaris*: Another data set that should be reexamined in this light is that of *T. vulgaris*. The lineages studied by Belhassen *et al.* (1991) were derived from females descended from female J1-44 (nine "isofemales," thus all having the same cytoplasmic type) pollinated by six hermaphrodites from the same or a nearby population. Molecular analyses of mitochondrial types of the isofemale F125 and three hermaphrodites (H168, H142, and H174) suggested that H174, unlike H142 and H168, carried the same sterility cytoplasm as the females, and therefore must also carry a nuclear restorer. This is supported by the higher frequency of restoration in its descendants, compared to other hermaphrodites. The family data of Belhassen *et al.* (1991) permit a genetic interpretation of the nuclear restoration in the cytoplasm of the isofemales. We first examine the data in their Table 6, using a simple model first to infer from observed segregation ratios the rules of inheritance and mode of action of the restorer genes. Then, as in the analysis above, the simplest model is tested by attempt-

TABLE 7

Analysis of the ratios observed in the 24 related *T. vulgaris* families with the same cytoplasmic male sterility type

Family number	H	F	Observed ratio H:F	Single-locus model (G)[a]	Two-locus model, epistatic restorers (G)[b]
1	H168	F107	0:44	0:1	
2	H168	F115	2:20	1:3 (3.648)	3:13 (1.597), 1:7 (0.255)
3	H168	F117	0:9	0:1	
4	H168	F124	1:31	***	1:7 (3,558), 1:15 (0.347)
5	H174	F108	11:18	1:3 (2.359), 1:1 (1.706)	3:5 (0.002)
6	H174	F115	11:30	1:3 (0.072)	
7	H174	F118	11:18	1:3 (2.359), 1:1 (1.706)	3:5 (0.002)
8	H174	F125	14:20	1:1 (1.064)	3:5 (0.194)
9	H169	F108	3:18	1:3 (1.449)	3:13 (0.294)
10	H169	F115	2:5	1:3	
11	H169	F118	7:17	1:3 (0.215)	
12	H169	F125	4:16	1:3 (0.280)	3:13 (0.020)
13	H143	F107	2:7	1:3	
14	H143	F116	0:41	0:1	
15	H143	F117	2:9	1:3	
16	H143	F124	9:22	1:3 (0.260)	
17	H171	F107	1:1	1:1	
18	H171	F114	47:4	***	3:13 (0.913), 1:7 (0.068), 1:15 (3.701)
19	H171	F117	4:53	***	1:7 (1.823), 1:15 (0.055)
20	H171	F124	3:28	***	3:13 (1.960), 1:7 (0.242)
21	H142	F108	0:13	0:1	
22	H142	F116	4:40	***	3:13 (3.195), 1:7 (0.510)
23	H142	F118	22:25	1:1 (0.196)	
24	H142	F125	11:27	1:3 (0.306)	

For segregating families, the 1:3 and 1:1 ratios (H:F) expected under a single-locus hypothesis were tested.

[a] Only theoretical ratios that agree with the data by *G*-tests are given. *** Neither of the two ratios fits the observations, at the 5% significance level.

[b] Alternative ratios under a two-locus hypothesis (3:5, 3:13, 1:7, and 1:15) were also tested. When this model gives a better fit than the single-locus one, this is indicated by the value of the *G*-test.

ing to assign consistent genotypes for each parent, resulting in the introduction of more complexity.

Taking into account all families except five that had fewer than 13 members (Table 7), a single locus model cannot account for the ratios, as five families have too many females. The data can be fitted with a two-locus model with epistasis such that, to be hermaphrodite, an individual must have the restorer genotype at both loci. The results are most clear-cut in large families containing high female frequencies, and genotypes were assigned under such a model, after which we examined the smaller families to check for consistency.

Three models are possible. Table 7 shows tests of expected segregation ratios for these models. The critical families (numbers 4, 18–20, and 22) include 1H:7F, 1:15, and 1:13 ratios. These are inconsistent with a model with both restorer alleles dominant, but do not distinguish between a model with one restorer dominant and one recessive and a model with two loci with recessive restorer alleles. However, a model with one restorer dominant and one recessive appears most probable, as it yields 3:5 and 3:13 ratios, which agree better than alternative ratios for families 5, 7–9, and 12. This model was thus adopted. On this basis, we have assigned genotypes to all 15 parents.

Because all parents segregate hermaphrodites in at least one set of offspring, none of them can be homozygous for the dominant sterility allele at locus 2, but all must be *r2*/–. The other genotypes can be deduced as follows (Table 8): All-female families require both parents to be homozygous for the recessive alleles at locus 1 under this model. The genotypes of H168 and F107 and of H143 and F116 must thus be +/+ *r2*/−. The families of females F115 and F125 pollinated by H168 are consistent with a 1:7 ratio, suggesting the genotype *R1*/+ *r2*/+ for these females and +/+ *r2*/+ for H168. The four families from H174 fit a 3:5 ratio, suggesting that all four females involved were *R1*/+ *r2*/+ and that H174 was *R1*/+ *r2*/*r2*. This agrees with the molecular data for H174, which has the same type as the isofemales. The same four females crossed to H169 all yielded 3:13 or 1:3 ratios, confirming the females' genotype, and suggesting that the pollen donor was +/+ *r2*/*r2* or *R1*/+ *r2*/+. The cross between H143 and F116 yielded only females, so both parents must be recessive homozygotes at locus 1: +/+ *r2*/–. Crossed with H143, female F124 gave a 1:3 ratio, implying that these parents were +/+ *r2*/+ and *R1*/+ *r2*/+, respectively. Crossed with H171, females F124, F114, and F117 all gave 1:7 ratios, so their genotypes must have been identical, and that of H171 was probably +/+ *r2*/+.

This accounts for all plants other than H142. Its segregation with F116 was 1:7, suggesting the genotypes *R1*/+ *r2*/+ and +/+ *r2*/+, respectively, for these two plants. Crossed with the double heterozygote, female F125, the expected 3:13 ratio was seen, unlike that in the cross with F118. But these two females were assigned the same genotype above, on the basis of crosses with H174 and H169. A third locus is therefore necessary to explain these data. To give the high female frequency observed in the progeny of F118, this must restore fertility independently of the first system. Furthermore, *r3* must be recessive, so that the ratios in other families are unaffected. F118 and H142 must both be triple heterozygotes, giving an expected ratio of 25:39, consistent with the data.

The addition of a third locus can also explain the appearance of hermaphrodites in the offspring of F107 crossed with H171 and H142. Without this, this female should be homozygous for the nonrestorer allele at locus 1 and would produce no hermaphrodites. The genotypes and ratios in Table 7 are consistent, assuming that all other plants are homozygotes for the dominant allele at this third locus. A model with two epistatic loci with dominant restorer alleles and a third locus acting independently with a recessive restorer can fit all progenies of all 15 parents.

However, some families derived from isofemales with the same cytoplasmic type can be fitted only by 1:15 ratios (see Belhassen *et al.* 1991). Such segregations imply two loci with recessive restorers and cannot be explained under the above model. One can, however, modify the above model by assuming that *r3* is not independent of the other loci, but interacts with *r2*. Genotypes at locus 3 can be assigned without altering the proposed ratios, except for the cross of H171 and F107 (not shown), which changes to 1:3, and remains consistent with the data. A model in which a recessive restorer *r2* interacts with, most likely, a dominant restorer *R1* or a recessive restorer *r3*, to produce hermaphrodites, can thus explain all Belhassen *et al.'s* (1991) data.

DISCUSSION

Mode of action of genes affecting male fertility: The similarity in the two species analyzed here is striking. Both involve multiple loci, and epistasis appears in both sets of data. Our results suggest that in both *S. vulgaris* populations studied there are multiple dominant restorers of cytoplasmic male sterility and probably also a recessive factor in one population. In thyme, at least two recessive restorers and one dominant restorer are required to explain families within a single-sterility cytoplasm. With such complex sets of data, the results could be interpreted in many ways, but we have excluded several other models. Recessive restorers fail to explain the observed high frequencies of females in families produced by selfing *S. vulgaris* hermaphrodites and by crossing between different hermaphrodites having the same cytoplasmic type. An interpretation involving dominant epistatic factors seems, therefore, to be necessary. Recessive restorers have been proposed for other gynodioecious species, but less commonly than dominant ones. In *P. lanceolata*, 4 of the 9 restorers proposed for the three cytoplasms taken together are recessive (Van Damme 1983; De Haan *et al.* 1997a), and for the two

TABLE 8

Interpretation of the segregation ratios (H:F) observed (upper numbers in each cell) in families with the cytoplasm of the isofemales

	Hermaphrodites (*G* ratios)					
Females	H168 +/+ *r2*/+ +/+	H174 *R1*/+ *r2*/*r2* +/+	H169 +/+ *r2*/*r2* +/+ or *R1*/+ *r2*/+ +/+	H143 +/+ *r2*/*r2* *r3*/+	H171 +/+ *r2*/+ *r3*/*r3*	H142 *R1*/+ *r2*/+ *r3*/+
F107	0:44			2:7	1:1	
+/+ *r2*/− *r3*/+	(0:1) 0			(1:3)	(1:1)	
F108		11:18	3:18			0:13
R1/+ *r2*/+ +/+		(3:5) 0.002	(3:13) 0.29			(3:13)
			(1:3) 1.449			
F114					0:7	
R1/+ *r2*/+ +/+					(1:7) 0.068	
F115	2:20	11:30	2:5			
R1/+ *r2*/+ +/+	(1:7) 0.255	(3:5) 2.091	(1:3 or 3:13)			
F116				0:41		4:40
+/+ *r2*/+ +/+				(0:1) 0		(1:7) 0.510
F117	0:9			2:9	4:53	
R1/+ *r2*/+ +/+	(1:7) 2.40			(1:3)	(1:7) 1.823	
F118		11:18	7:17			22:25
R1/+ *r2*/+ *r3*/+		(3:5) 0.002	(1:3) 0.215			(25:39) 1.162
			(3:13) 1.52			
F124	1:31			9:22	3:28	
R1/+ *r2*/+ +/+	(1:7) 3.538			(1:3) 0.260	(1:7) 0.242	
F125		14.20	4:16			11:27
R1/+ *r2*/+ +/+		(3:5) 0.194	(3:13) 0.020			(3:13) 2.312
			(1:3) 0.280			

Observed ratios and genotypes assigned under a three-locus model in which epistatic restorers at loci 1 and 2 interact to produce the hermaphrodite phenotype, while a restorer at a third locus restores male fertility independently of the genotypes at the first two. The genotypes assigned to the females and hermaphrodites that fit best on this model are shown, together with expected ratios (numbers in parentheses) and *G*-test results. Note that the hermaphrodites do not necessarily have restored genotypes, because they come from independent lineages from those of the isofemales.

cytoplasms of *P. coronopus* 4 out of 10 are recessive (Koelewijn and Van Damme 1995a,b).

Epistasis of the kind proposed here has rarely been reported, and most data can be fitted with several loci with independently acting alleles, *i.e.*, a restorer at a single locus suffices for male fertility. In *P. coronopus*, with five plants sampled per population, the interpretation involves two to four independent restoration systems, both dominant and recessive, for each cytoplasmic sterility type, including some evidence for epistasis (Koelewijn and Van Damme 1995a,b). In *P. lanceolata*, on the basis of a sample of 12 plants, the simplest restoration model (for *ms3*) involves two independently acting dominant restorers, while the most complex (for *ms1*) invokes five such loci, two with dominant restorers and three with recessive ones (Van Damme 1983). In species, such as *P. lanceolata*, in which progeny cannot be derived by self-fertilization because of self-incompatibility, it is hard to distinguish between multiple epistatic factors and recessive restorers, but epistasis could be an alternative explanation for some of the data (De Haan *et al.* 1997a). In such species, it is important to include crosses between related individuals to help distinguish these possibilities (see Van Damme 1983).

A problem in working out the genetics of male-sterility polymorphisms in a self-compatible species, however, is that sterility alleles could be present in different plants simply as a result of mutational load, and these would of course be detected on self-fertilization, in addition to sterility alleles maintained as part of the polymorphism for females in gynodioecious populations. These alleles would most likely appear as complementary sterility factors or, equivalently, epistatic restorers, different in different individual lines of descent, and might in part explain the observed sporadic very high female frequencies in progenies derived from selfing hermaphrodites. However, the frequent appearance of females when unrelated hermaphrodite plants are crossed shows that the frequencies of the nonrestorer (*i.e.*, sterility) alleles detected are quite high in these populations.

Differences between populations: A genetic difference between the two *S. vulgaris* populations, such that allelism is rare between their most frequent male-sterility genes, is shown by the low frequency of females in crosses between populations 1 and 3. The populations may also differ in the cytoplasms they contain (or in their frequencies) such that plants from one population frequently carry alleles that restore male fertility of carriers of the most common sterility cytoplasm of the other population. The difference between the segregation ratios in the within- and between-population crosses is the opposite of the interpopulation effect on the segregation ratios found in *T. vulgaris* (Couvet *et al.* 1985). The difference found in Thymus suggests that different populations contain different sterility cytoplasms, with the restorer alleles for each cytoplasm tending to be found at highest frequency in populations that have that cytoplasm, as might be expected if restorer alleles tend to have deleterious effects on fitness, keeping them rare except in the presence of the appropriate sterility cytoplasm, as occurs in the model system studied by Couvet *et al.* (1998). A difference in the direction found here would be surprising if the populations differ in their cytoplasmic types but, as discussed above, there is no evidence for this in the two populations studied, though the frequencies of restorer alleles differ. Because populations with nucleocytoplasmic gynodioecy are expected to undergo extreme changes in the frequencies of the genetic factors (Charlesworth 1981; Delannay *et al.* 1981; Frank 1989; Gouyon *et al.* 1991), frequency differences are quite possible. The finding of the same cytoplasmic type in different populations is in accord with what is found in Plantago (Koelewijn and Van Damme 1995a; De Haan *et al.* 1997a).

Maintenance of cytonuclear male-sterility polymorphisms: Even assuming that the *S. vulgaris* populations studied here do not differ in their male-sterility genes, several cytoplasmic and nuclear factors are clearly involved. The same is true in *T. vulgaris* and in most other gynodioecious species studied in detail (see above). The maintenance of this genetic variation is still not understood in any species, although many of the conditions necessary for stable polymorphisms are found in gynodioecious populations (*e.g.*, Perrot *et al.* 1982; Kohn 1988, 1989; Sakai *et al.* 1989; Belhassen *et al.* 1990; Willson and Ågren 1991; Eckhart 1992a,b). In particular, we have no good understanding of the reason for the existence of so many different genetic factors or the involvement of cytoplasmic variants as well as nuclear loci in most cases of male sterility studied. The fact that even a slight increase in female fertility allows a cytoplasmic variant to invade a population (Lewis 1941) may be sufficient explanation for the presence of cytoplasmic factors.

In theoretical models, polymorphism for cytoplasmic sterility is often readily maintained (see above), and, even under conditions that do not permit stable maintenance in a single population, structured populations may maintain such variants (McCauley and Taylor 1997; Couvet *et al.* 1998). There would thus be time for restorer alleles to arise by mutation, so it is plausible that the cytoplasmic variation is the first to arise.

Restorers are readily selected in the presence of cytoplasmic sterility factors, though they often rise to fixationand therefore gynodioecy is lost (*e.g.*, Charlesworth 1981; Frank 1989; Gouyon *et al.* 1991; McCauley and Taylor 1997). The allele frequency dynamics and maintenance of the cytoplasmic and nuclear genetic variation in gynodioecious populations depend on the restorers' mode of action. Recessive alleles are expressed only when homozygous, so they should spread slowly in relatively outcrossing populations such as populations with gynodioecy; there is a risk of stochastic loss while they are rare, and also in small populations

where genetic drift occurs. Dominant restorers, however, should spread rapidly, and are thus expected to be found in populations. In numerical models of annual outcrossing metapopulations of many demes (Couvet *et al.* 1998) nucleocytoplasmic gynodioecy can be maintained (even without a cost of restoration) when each cytoplasmic type has a dominant restorer. That model assumes that females have a high (but not necessarily more than twofold) seed output advantage over hermaphrodites, so that newly founded demes containing high female frequencies grow faster than other demes and produce more seeds that colonize empty sites. Limit cycles of the frequencies of the genetic factors are maintained indefinitely, and mean female fequencies of up to 50% are found, with dominant but not with recessive restorers.

Epistatic restorers face the worst situation, because two or more factors must be present at appreciable frequency to produce hermaphrodites and be selected, whereas restorers that act independently can always be selected. It is therefore surprising that they should be found. Epistatic gene action has not yet been studied theoretically in models of gynodioecy, but it seems consistent with the observation that recently founded small populations of thyme often have very high female frequencies, implying slow selection for restoration.

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APPENDIX

Sex phenotypes in the second generation, *i.e.*, families from crosses between the first generation of plants grown from field-collected seeds

	Sex phenotypes of progeny					*G*-test value for	
Family	H	HF	FH	Y	W	1H:1F	3H:1F
			Crosses between females and hermaphrodites				
Population 1							
86/89	0	0	0	0	9	12.5	25
86/143	0	0	1	0	27	38	78
86/92	0	0	0	2	13	20.8	42
86/123	0	0	0	10	6	22.2	44
86/87	3	0	0	1	17	13.0	36.9
86/114	12	0	0	4	8	0 NS*	6.9
86/130	11	0	1	8	0	0.2 NS	3.8
86/88	0	0	0	1	9	13.9	28
86/93	0	0	0	5	18	31.9	4
86/140	0	0	0	1	30	43	86
86/122	4	2	3	17	22	30.4	84
85/644	8	0	0	4	5	0.06 NS	6.05
86/115	10	0	0	2	3	1.07 NS	0.52 NS
86/121	18	0	0	7	5	1.2 NS	3.2
86/144	1	2	0	0	9	3.1 NS	13.2
86/113	27	0	0	0	4	19.1	2.8 NS
86/182	14	3	1	3	0	8.7	0.42 NS
86/185	18	2	4	1	0	9.6	0.35 NS
Population 3							
86/221	0	0	2	16	3	29.1	58
86/267	1	2	0	6	2	2.35 NS	11.0
86/293	8	0	3	0	1	1.35 NS	0.42 NS
85/719	0	0	0	10	0	13.9	28
86/278	3	0	0	2	9	4.9	17.7
85/660	6	1	1	2	2	0.33 NS	1.76 NS
85/684	5	0	1	0	7	0.69 NS	7.7
85/687	3	1	1	0	6	0.82 NS	7.3
86/296	13	0	0	8	0	1.20 NS	1.75 NS
86/277	18	0	1	0	12	0.81 NS	4.23 NS
86/284	12	1	0	10	0	0.39 NS	3.71
86/292	6	1	2	2	6	0.53 NS	8.7
87/274	22	1	3	0	5	7.57	0.01 NS
86/281	22	0	1	4	0	13.7	1.42 NS
86/290	25	0	1	4	7	4.67	1.02 NS
86/263	20	1	0	0	0	29	12.1
			Selfing of hermaphrodite progeny				
Population 1							
86/56	11	2	0	0	3	6.74	0.36 NS
85/630	11	0	0	4	1	2.31 NS	0.32 NS
85/632	11	0	0	0	1	9.75	2.22 NS
85/635	6	2	0	2	0	3.85	0.14 NS
86/90	13	0	0	0	0	18	7.5
86/68	3	1	1	0	6	0.82 NS	7.29
Population 3							
85/651	7	0	1	0	1	2.94 NS	0.04 NS
86/226	2	0	0	7	0	2.94 NS	11.0
86/227	1	0	0	7	7	13.4	32
85/662	9	0	0	0	0	12.5	5.2
85/701	4	1	2	3	0	0 NS	2.88 NS
85/721	7	2	0	0	5	1.16 NS	0.79 NS
86/193	5	0	1	1	3	0 NS	2.88 NS
86/230	7	3	1	0	0	8.54	1.28 NS
86/251	10	5	0	6	1	2.97 NS	0.52 NS
86/203	13	4	0	12	3	0.13 NS	7.13
86/306	4	1	3	3	1	0.33 NS	5.98

(*continued*)

APPENDIX

Continued

Family	Sex phenotypes of progeny					*G*-test value for	
	H	HF	FH	Y	W	1H:1F	3H:1F
	Crosses between different hermaphrodites						
Population 1							
85/631	14	0	0	1	0	11.3	2.69
86/78	32	1	0	2	0	27.5	6.90
86/312	10	4	2	4	1	2.3 NS	0.78 NS
86/57	14	3	0	0	0	17	5.7
86/65	22	0	0	0	0	22	7.3
86/51	18	0	0	1	1	12.8	3.94
Population 3							
86/199	12	1	0	0	0	13	4.33
86/200	8	0	0	0	1	5.44	0.93 NS
86/259	9	1	1	4	3	0.22 NS	3.63
86/253	19	2	0	1	2	13.5	2.0 NS
86/231	9	3	0	10	2	0 NS	8.0

Only families with at least nine progeny are shown.
* Not significant.