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The Consequences of Gynodioecy in Natural Populations of *Thymus vulgaris* L.

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Summary. Gene flow between the two sexual forms is asymmetrical in gynodioecious species: genes are transferred from male-fertile individuals (mF, hermaphrodites) to male-sterile individuals (mS, females) by pollen but from mS to MF individuals by diploid seeds. This situation is expected to cause differences in the genetic make up of mF and mS individuals. The expected differences were found in an experiment on a natural population of the gynodioecious species, *Thymus vulgaris* L. (thyme). The effectiveness of gynodioecy as a means of regulating heterozygosity and adaptability to various conditions of the environment is discussed.

Key words: Gynodioecy – Heterozygosity – *Thymus vulgaris*

Introduction

Among the genes which influence the genetic make up of populations, those which act directly on the reproductive system have special relations with the rest of the genome. Numerous models have been published for the conditions of maintenance of gynodioecy, a state in which some plants are female and others are hermaphrodites (Lewis 1941; Kimura 1959; Valdeyron 1967; Lloyd 1974; Ross 1978). The effect ot gynodioecy on the population structure of the remainder of the genome has been examined in a model by Gouyon (1978): one of the constant predictions was that male-fertile individuals (mF) and male-sterile individuals (mS) in the same population will differ in heterozygosity. This model was used as a basis for the present experimental work on *Thymus vulgaris* L., a species which was described as gynodioecious by Darwin (1877).

Williams (1975) and Maynard-Smith (1978) concluded from theoretical considerations that, in many cases, it is possible to explain the occurrence of sexual reproduction on the basis that it provides a short-term selective advantage by producing recombinant types. The aim of this work is to answer the question. How does gynodioecy in itself affect the general genotypic structure in the population?

Gene Flow Between Male-fertile and Male-sterile Plants

There are two sorts of individuals in populations of gynodioecious species: male-fertile individuals (mF), which are functionally hermaphrodites able to reproduce both by cross and self fertilization, and male-sterile individuals (mS), which bear no stamens and are therefore unable to self fertilize. To reproduce, mS plants must receive pollen from mF plants. This is true for most of the gynodioecious species which have been described (particularly in the Labiatae). However, in some species of the genus Plantago, the male-fertile individuals are self incompatible (Ross 1970). In this case, as Baker (1963) commented, „gynodioecism appears to be superfluous." An explanation which is often offered is that the occurrence of two distinct sexual forms results from niche differentiation rather than selection favoring allogamy. Note, however, that under this explanation niche differentiation is always associated with the allogamous reproductive system.

In a gynodioecious population, gene flow between the two sexual groups is asymmetrical: in each generation, the nuclear genes in seeds produced by mF individuals contain nuclear genes previously contained in mF individuals. In contrast, half of the nuclear genes in seeds produced on mS individuals come from mF (through the pollen) and one half from mS individuals (through the ovule). If the sexual form is determined mainly by nuclear genes, one of the consequences of

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this process is that the progeny of an mF individual will usually be mF whereas the progeny of mS individuals contain mF and mS individuals. The mS individuals are then at a disadvantage, a disadvantage that can be related to the "cost of meisosis" (Williams 1975).

Most of the models of gynodioecy cited above assume nuclear determination of male sterility. They predict, therefore, that fewer than 50 percent of the progeny of mS individuals will be mS and thus that the maximum proportion of mS individuals in the population will be 50 percent. Actual measurements in populations reveal, however, that a majority of the progeny of mS individuals are mS. This fact can be explained by interactions between cytoplasmic factors and nuclear genes in the determination of male sterility (Assouad 1972; Kheyr-Pour 1975). If male-sterile cytoplasms exist for which nuclear restorer genes do not occur in a population, the proportion of mS individuals in the progenies of mS can exceed 50 percent. If the determination of mS is exclusively cytoplasmic (i.e., there are no nuclear genes in the population that can restore the mS cytoplasms), the progenies of the mS plants will be exclusively mS and the progenies of the mF plants will be exclusively mF. Figure 1 gives the flow resulting from gynodioecy in schematic form:

mF individuals produce: pollen which self fertilizes and/or cross fertilizes other mF plants and mS plants, and seeds (self or cross fertilized) which give a large majority of mF plants;

mS individuals produce: seeds (cross fertilized) which will give the majority of the mS in the next generation.

This flow is asymmetrical because the path from mF to mS plants is through the gametophyte (pollen) whereas gene transmission from mS to mF plants is in pairs through sporophytes. Seeds arising from mF and from mS individuals can be qualitatively different because seedlings arising from mF plants can also result from selfing. Male-fertile individuals produce a majority of mF progeny and therefore greater heterozygosity is expected in mS than in mF plants.

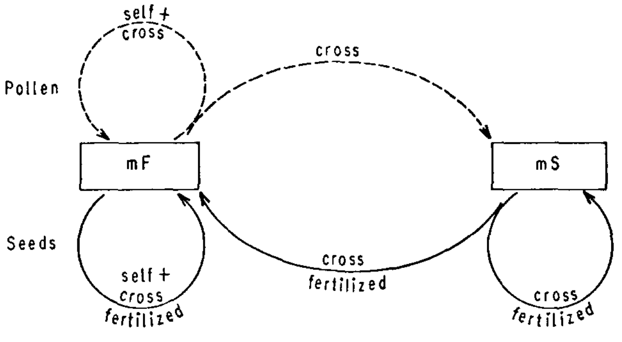

Fig. 1. Gene flow between the two sexual forms: – – – by pollen; —— by seeds

This will be illustrated with a simple example which, even though it may not correspond to any actual situation, has the advantage of simplicity (Lloyd 1974). In this model, male sterility is determined by a dominant gene *A*; the recessive allele *a* determines. when homozygous, the male-fertile form. Gene *A* cannot exist in the pollen: the genotype *AA* does not exist and the population is composed of two genotypes, *aa* (mF) and *Aa* (mS). Because pollen grains contain only *a*, it follows that the progeny of an mF individual will be composed of 100 percent *aa* (mF) and the progeny of an mS individual will be composed of 50 percent *aa* (mF) and 50 percent *Aa* (mS).

It is clear that mS plants can persist only if they produce more seedlings (Lewis 1941; Kimura 1959), or progenies of greater fitness (Valdeyron et al. 1973), than mF plants. To simplify the problem, the present study considers the case where the maintenance of mS results from greater production of seeds. The models cited above predict, for the present example, that for male sterility to persist the superiority of mS individuals in the seed production (ω = seed production of the mS individuals/seed production of the mF individuals) must be greater than two. When $\omega > 2$, the rate of mS is near 50 percent (the two sexes are separate and the population is dioecious because mS individuals are the only producers of seeds). The frequency of mS in the population can then vary from 0 to 0.5.

Theoretical Consequences

The rate of self fertilization is also an important factor influencing heterozygosity. The model cited above (Gouyon 1978), however, shows that variations in selfing do not affect the present results qualitatively, but only the intensity of the consequences. We assume complete self fertilization, so that every seed of an mF individual has resulted from selfing. This hypothesis has two advantages: (i) the calculations are simple; (ii) the consequences of the situation described are more evident.

Let P be the figure by which one must multiply 2pq to obtain the heterozygosity at a locus where two neutral alleles are in frequencies p and q. This term is equal to 1-F in an infinitely large population (Wright 1922). Assume that mS individuals represent a proportion x in the population in generation n and that this proportion is stable. In generation n + 1, these plants produce x mS progeny and mS individuals produce 1/2 mF + 1/2 mS progeny; thus the mS individuals produce x mF and the mF plants produce the rest of the mF plants, giving $1 - x - x = 1 - 2x$ mF offspring

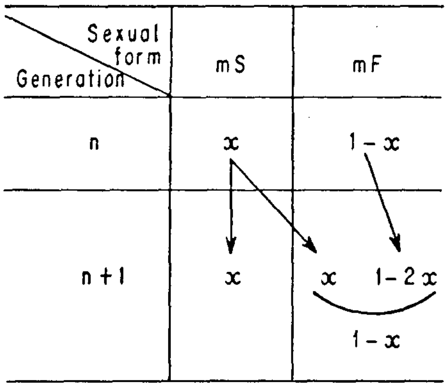

Fig. 2. Proportions produced by mS and mF in generation n + 1

(Fig. 2). It follows that mF plants in generation n + 1 arise from the mF plants of generation n in a proportion 1 − 2x/1 − x and from the mS plants of generation n in a proportion x/1 − x. The mS individuals give progenies arising from cross fertilization (P = 1) and the mF individuals give progenies arising from self fertilization. This reduces P by a factor of 1/2, and P_f (P in the mF) is

$$Pf_{n+1} = \frac{x}{1-x} \cdot 1 + \frac{1-2x}{1-x} \cdot \frac{Pf_n}{2}.$$

Because $\frac{1-2x}{2-2x}$ is smaller than one the equilibrium value, 2x, is stable. At equilibrium, P in the mF is equal to twice the proportion of mS, $Pf = 1 - F_f = 2x$. Because the mS plants arise from cross fertilization, $Ps = 1 - Fs = 1$. For the whole population, the mean P is,

$$\bar{P}_m = x \cdot Ps + (1-x) \cdot Pf,$$

$$\bar{P}_m = 1 - F_m = x(3-2x),$$

which gives the curve of Fig. 3.

Figure 3 shows that heterozygosity in the mF and the mS individuals can be quite different; any locus, say B/b, in mS individuals will be more heterozygous than in mF individuals. The difference will be greatest when the frequency of mS is intermediate. As an example, in a population in which the alleles B and b (B dominant to b) are present in equal frequencies (p = q = 1/2) and mS plants occur in 20 percent frequency, Fig. 3 shows that Pf = 0.4 and Ps = 1. The proportion of heterozygotes will be,

mF: $2pq \cdot 0.4 = 0.2$

mS: $2pq \cdot 1 \;\; = 0.5$,

and a sample of 100 mF and 100 mS is expected to have genotypic and phenotypic frequencies as shown in Tables 1 and 2. The phenotype determined by the dominant gene is more frequent in the mS than in the mF individuals.

The data obtained in an experiment involving dominance will be of the type shown in Table 2. The chi-square value calculated from the numbers in Table 2 is $\chi^2 = 5$, $P < 0.029$, which indicates a significant relationship between phenotype B and male sterility. However the χ^2 calculated from Table 1 is $\chi^2 = 20$, $P > .001$, which indicates a more highly significant relationship between heterozygosity and male sterility.

The above predictions of the model will now be tested against experimental data. Because the model is oversimplified, the numerical values given above cannot be directly compared with the experimental data.

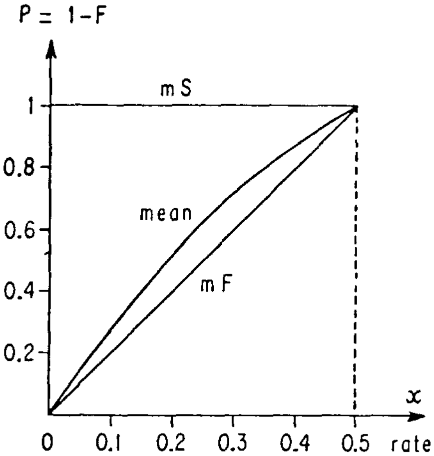

Fig. 3. Values of *P* for different rates of mS. The rate of mS influences the mean heterozygosity in the population in two ways: (i) mS are themselves more heterozygous than mF and (ii) mF are more heterozygous when the proportion of mS is high

Table 1. Genotypic frequencies in a hypothetical population (N = 200)

	BB	Bb	bb	Total
mF	40	20	40	100
mS	25	50	25	100

Table 2. Phenotypic frequencies in a hypothetical population (N = 200)

	[B-]	[bb]	Total
mF	60	40	100
mS	75	25	100

Experimental Results

The Laboratory of Ecological Genetics of the C.E.P.E. in Montpellier has studied two gynodioecious species, *T. vulgaris* L. and *Origanum vulgare* L. Elena-Rossello et al. (1976) studied two populations of *Origanum vulgare* L., one in the Cevennes mountains (Valleraugue, Gard, France) where the species is abundant, and a second small population in woods near Montpellier. An electrophoretic marker, the esterase B locus, which is polymorphic for three alleles, was used to estimate the proportion of heterozygotes in progenies from open-pollinated mF and mS individuals. In both of the two populations studied, heterozygotes were more frequent in the progenies of mS than in the progenies of mF individuals: 83 percent (53/64) in the progeny of mS and 72 percent (69/124) in a progeny of mF in the Cevennes population, and 74 percent (74/100) and 55 percent (66/119) in the Montpellier population. Because the primary purpose of the above study was to estimate selfing rates, seeds for electrophoretic assay were collected from individuals which carried rare alleles. This sampling procedure may have led to an upward bias in the estimate of heterozygosity reported above.

In both *T. vulgaris* and *O. vulgare,* the genetic determination of male sterility appears to be complex; according to Assouad (1972) several loci may interact with cytoplasmic information in the determination of male sterility. In the context of the present study, it is not necessary to know the exact genetic determination of male sterility because Assouad (1972) found that a large majority of individuals in progenies of mF are mF and that the progeny of mS include a mixture of mF and mS (cf. Table 3). Moreover, we know that substantial self fertilization occurs in mF individuals. Valdeyron et al. (1977) reported values which ranged from 0.08 to 0.64 in the populations studied.

Table 3. Proportions of mF and mS in progenies obtained from open pollination (from Assouad 1972)

Parents	Progenies	
	mF	mS
mF (13 individuals tested)	500	11
mS (21 individuals tested)	144	528

In *T. vulgaris* L. the *Ph* locus (Vernet et al. 1977) can be used as a marker to test the predictions of the model. Plants carrying the dominant *C* allele accumulate carvacrol, a phenolic terpene which can be distinguished by vapor phase chromatography from thymol, which accumlates when allele *c* is homozygous.

Phenotype frequencies observed in a natural population of *T. vulgaris*, in La Jasse (St. Martin de Londres, near Montpellier, Herault, France) are given in Table 4. There are more individuals of the dominant phenotype in mS than in mF plants. However the difference between the two distributions is not significant, perhaps because the test on phenotypes is much less sensitive than the test on the proportions of heterozygotes, as shown above. The estimate of the proportion of mS (x) is 0.61 in this population.

Seeds were harvested from the [*C*-] individuals, and each progeny (about 30 individuals) was grown and analyzed for its chemotype. Those progenies which included both [*C*-] and [*cc*] individuals were classified as heterozygous *Cc* and those progenies which contained only [*C*-] individuals were classified as homozygous *CC*. Four mF individuals which did not produce enough seedlings for an adequate progeny test contained only *CC* individuals. The absence of *cc* individuals might be due to small sample size. The fact that these rather unfertile individuals were mF is not surprising, because, as noted above, mS individuals must produce more seeds than mF individuals to be maintained in the population. Observed genotype numbers are given in Table 5. Allelic frequencies in mF and mS individuals do not differ significantly ($P_c = 0.37 \pm 0.14$ in mF and $P_c = 0.43 \pm 0.10$ in mS). However, the proportions of heterozygotes are highly different ($p(\chi_1^2 = 6.28) < 0.02$), which is in conformity with the model.

Table 4. Phenotypic frequencies in the La Jasse population of thyme

Sexual form	Chemotype			
	[C-]	[cc]	Total	
mF	16	11	27	
mS	32	11	43	x = 43/70 = 0.61
Total	48	22	70	

Table 5. Genotypic frequencies in the La Jasse population of thyme

Sexual form	Genotype			
	CC	Cc	cc	Total
mF	5	7	11	23
mS	5	27	11	43
Total	10	34	22	66

Discussion

It was found that mS individuals are more heterozygous than mF individuals for most polymorphic loci of the genome. This has two consequences: (i) if dominance occurs, there is a sort of "linkage disequilibrium," without genetic linkage or selection, between male sterility and the phenotypes determined by dominant alleles; (ii) if heterozygotes are at an advantage (overdominance) at some loci, the mS should have greater fitness than the mF because they are more heterozygous.

Bonnemaison et al. (1979) grew mixtures of mF and mS in pots at different planting densities. She observed that increases in density had greater adverse affect on the dry weight of mF than mS plants. In situations where competition is important, dry weight may be a measure of the ability of a plant to take what it needs from the environment, despite the presence of other individuals, i. e., dry weight may be a good indicator of fitness under competition. The conclusion which was first drawn from these results was that the male sterility genes give vegetative superiority to the individuals which carry them. The present study indicates that the superiority may result from heterosis associated with increases in the heterozygosity of mS individuals. It would be interesting to repeat this experiment with plants obtained from controlled crosses. In a progeny where both sexes are present, the greater genetic homogeneity of the offspring would permit a direct comparison of the vigor resulting from the sexual genes.

Thyme is a plant that is adapted to xeric conditions of the Mediterranean region. It is found in only a few situations of deep soil and dense herbaceous vegetation. The situations are believed to represent generally more or less immature post cultural stages. Dommee (1978) and Assouad et al. (1978) state "that the areas occupied by populations having high percentages of mS individuals are more disturbed than the others. They are frequently located on old cultivated sites, which are periodically reploughed, where the ground is amost flat and the soil is deep. Sheep grazing which is quite intensive in the Mediterranean region, especially in grasslands, is also a factor of disturbance [...]. In contrast, no such effect has been noticed on rocky sites (where high percentages of mF can be found). These seem more stable than grasslands and old fields are predominantly colonized by phanerotypes ...".

The phytoecological analyses made in the region of Montpellier shows the same trend; the highest percentages of mS are found in associations containing mostly herbaceous species whereas the highest percentages of mF are found in the cocciferetum association growing on poor rocky sites (Braun-Blanquet et al. 1952) and nearer the climax than the former association.

This can be related to the work of Allard et al. (1972) on *Avena barbata.* They found that restriction of recombination due to high levels of selfing promotes the maintenance of favored coadapted complexes of alleles on a multilocus basis. In the xeric conditions, thyme is in its normal environment, the competition is mainly intraspecific, and disturbance is low. Stable genetic combinations might here be selected and maintained by selfing. On the contrary, in the situations of higher interspecific competition, which correspond to rather marginal conditions of life for this species, higher outcrossing rates (given by a greater mS frequency) may be selected because they promote the formation of new genetic combinations.

The results presented here show that a high rate of mS is found when heterozygosity (and new recombinant types) is at a selective advantage. If, in some environment, heterozygotes (or new recombinant types) are favored by natural selection, then mS individuals which are more heterozygous and are able to produce more new genetic combinations than mF individuals, may be favored. And, over time, their proportion would be expected to increase (Fig. 3). On the contrary, if stable coadapted combinations are selectively advantageous, mF individuals, which are able to maintain these combinations by selfing, are favored and they are expected to increase in the population.

The main effect of male sterility in natural population may therefore be associated with regulation of the level of heterozygosity. Any selection favoring heterozygotes or new recombinant types favors the mS and any increase in the mS proportion increases the heterozygosity of the whole population (mS and mF). This process, acting by individual selection, gives to the population a great adaptability by a rapid and economic response to the environmental pressure for or against genetic diversity.

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