POPULATION STUDIES IN PREDOMINANTLY SELF-POLLINATED SPECIES. VII. SURVIVAL OF A MALE-STERILITY GENE IN RELATION TO HETEROZYGOSIS IN BARLEY POPULATIONS

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RECENT studies on the population dynamics of several largely selfing species have shown that the restrictions often postulated to be imposed on the mode and extent of recombination and genetic variability by their breeding habit could be alleviated in several ways. Natural selection can regulate various factors controlling the rate of recombination (GRANT 1958), or maintain through heterozygote advantage substantial amounts of heterozygosity (JAIN and ALLARD 1960; ALLARD and HANSCH 1964a). A rather low amount of outcrossing combined with selection favoring heterozygotes of the intensities such as those observed in barley and lima bean populations can result in a highly flexible recombinational system (ALLARD and HANSCH 1964b). Alternatively, there are known several examples of species in which the breeding system has been adjusted by selection for higher degrees of outbreeding through such devices as male sterility (LEWIS and CROWE 1952, in *Origanum*), exsertion of stigma (BRESE 1959, in *Nicotiana*) and the need of tripping by insects (DRAYNER 1956, in *Vicia*).

Male sterility as a factor for increased outbreeding was apparently first discussed by MATHER (1940) in relation to the evolution of gynodioecy. That a similar role in an inbreeding species could be of even greater adaptive significance was shown by JAIN (1961) who, with the help of a simple model, demonstrated that a small proportion of male-sterile individuals having a higher rate of outcrossing than the population average permits higher levels of hybridity at equilibrium, or putting it otherwise, that the same levels of heterozygosity can be maintained with less intense selection. The present study was undertaken to determine whether male sterility has the postulated effect on heterozygosity in populations of barley in which a recessive gene (*ms*) for male sterility had been introduced to encourage recombination (SUNESON 1945). This paper presents some data on the survival of the *ms* gene in relation to the levels of heterozygosity at some other loci.

MATERIALS AND METHODS

The experimental material consisted of two composite crosses, C.C.XIV and C.C.XV, developed from intervarietal crosses made with the use of male sterility. C.C.XIV involved nine varieties of nearly common origin and C.C.XV stems from 626 parents representing wide genetic background. The hybrid populations were subsequently propagated by the bulk method from random samples of 5000 or more seeds drawn each year (SUNESON 1945). These generations

of bulk propagation are designated here as F_2 , F_3 , and so on, while S_1F_2 represents the F_2 population raised from seeds harvested on male-sterile plants of the bulk-hybrid population that would give progeny of $ms\ ms$ female $\times$ Ms male outpollinations. Male-steriles are phenotypically distinct in spikelet appearance so that the relative proportion of normals and male-steriles can be estimated directly from a census. Seed setting on male-steriles was recorded during several seasons to estimate average rate of outcrossing on $ms\ ms$ plants. Genotypic proportions at three other loci, bl (blue *vs.* white aleurone), r (rough *vs.* smooth awn) and s (long *vs.* short rachilla hairs), were determined by progeny tests.

RESULTS

Estimates of the relative proportions of pollen fertile (Ms —) and male-sterile ($ms\ ms$) individuals in various generations of C.C.XIV and C.C.XV are given in Table 1. It is interesting to note that the two populations showed like behaviour in the retention of about 20 to 25 percent male-steriles until the F_6 generation; subsequently the frequency of $ms\ ms$ decreased slowly to nearly one percent in F_{16} (C.C.XIV). The proportion of selfing (s) and outcrossing (t) in the absence of the $ms\ ms$ component were shown by JAIN and ALLARD (1960) to be of the order of .98 and .02, respectively, in barley populations grown under similar environmental conditions. Now, if k is a constant which measures the increased rate of outcrossing on male-sterile individuals, the expected percent seed set on male-steriles is simply kt which can be estimated from data of Table 2 to be

TABLE 1

*Estimates of the relative proportions of normal (Ms —) and male-sterile ($ms\ ms$) individuals and the selective values**

Generation	C.C.XIV				C.C.XV			
	Ms —	$ms\ ms$	w_a	w_b	Ms —	$ms\ ms$	w_a	w_b
F_2	.7362	.2638			.7393	.2607		
			.634	.278			.848	.680
F_3	.7164	.2836			.7594	.2406		
			.450	.750			.549	.680
F_5	.8131	.1869			.8360	.1640		
			.194	.070			.243	.253
F_6	.7167	.2833			.7875	.2125		
			.504	.921			.410	.800
F_{10}	.9484	.0516			.9381	.0619		
			.385	.368			...	...
F_{13}	.9333	.0667			...	...		
			.770	.891			...	...
F_{14}	.9792	.0208			...	...		
			1.000	1.000			...	...
F_{16}	.9904	.0096			...	...		
			...	...			...	...
S_1F_2	.6342	.3658			.6087	.3913		
			...	...			...	...

*Selective values were estimated for two models: (a) $Ms\ Ms \rightleftharpoons Ms\ ms$, w_a , $ms\ ms$, 1 and (b) $Ms\ Ms \rightleftharpoons ms\ ms$, w_b , $Ms\ ms$, 1.

TABLE 2

Estimates of percent seed setting (kt) on male-sterile plants

Year	C.C.XIV	C.C.XV
1945	31	30
1946	41	27
1947	36	38
1948	49	46
1950	40	31
1952	53	33
1954	47	45
1956	42	41

42.4 ± 7.1 (C.C.XIV) and 36.4 ± 7.2 (C.C.XV). Accordingly, the values of proportionality factor k are approximately 21 and 18 for the two populations. It is apparent from data on seed setting ability that the *ms ms* genotype has lower reproductive value and would be expected to be eliminated rather rapidly from these bulk populations.

The observed changes in the frequency of the *ms ms* genotype (Table 1), however, suggest that male-steriles were retained in both populations at higher frequencies than expected so that certain other selective forces seem to be involved. To consider the simplest situation where the genotypic frequencies at the *ms* locus are changing under the effects of mating system and zygotic selection parameters only, the recursion relationships among genotypic frequencies, f_1 (*Ms Ms*), f_2 (*Ms ms*) and f_3 (*ms ms*), in the n th generation and the $(n+1)$ th are as follows:

$$\begin{aligned} f_1^{(n+1)} &\propto w_1 \{s(f_1^{(n)} + \frac{1}{4}f_2^{(n)}) + t(f_1^{(n)} + \frac{1}{2}f_2^{(n)})^2\}; \\ f_2^{(n+1)} &\propto w_2 \{\frac{1}{2}sf_2^{(n)} + t(f_1^{(n)} + \frac{1}{2}f_2^{(n)})(kf_3^{(n)} + f_2^{(n)})\}; \\ f_3^{(n+1)} &\propto w_3 \{s(\frac{1}{4}f_2^{(n)}) + \frac{1}{2}tf_2^{(n)}(kf_3^{(n)} + \frac{1}{2}f_2^{(n)})\}; \end{aligned} \quad (1)$$

where the selection coefficients (w_1, w_2, w_3) are relative fitnesses (in the sense of Darwinian fitness) of the three genotypes. At locus *ms*, selection in favor of *ms* allele might be assumed to operate in two alternative ways: (a) *Ms/Ms* and *Ms/ms* are disfavored against *ms ms* at some other stage of the life cycle with selective values ($w_a, w_a, 1$; $w_a < 1$), or (b) *Ms ms* favored over the two homozygotes such that selective values are ($w_b, 1, w_b$; $w_b < 1$). The expected proportions of *Ms ms* and *ms ms* in the S_1F_2 generation are given by

$$\begin{aligned} f_2^{(1)} &\propto kf_3^{(0)}(f_1^{(0)} + \frac{1}{2}f_2^{(0)}); \\ f_3^{(1)} &\propto kf_3^{(0)}(\frac{1}{2}f_2^{(0)}) \end{aligned} \quad (2)$$

Use of (2) allows us to determine initial gene frequencies in the F_2 generation. Knowing the relative proportions of *Ms*— and *ms ms* in subsequent generations, and the values of s , t and k , the recursions (1) can be used iteratively to estimate selective values w_a or w_b depending on the model. These estimates are given in Table 1 which indicates that under both models selection is rather intense and fluctuates widely from generation to generation (C.C.XIV: $w_a = .551 \pm .232$, $w_b = .680 \pm .317$; C.C.XV: $w_a = .479 \pm .278$, $w_b = .687 \pm .377$). Thus, the ob-

served rates of decrease in frequency of male-steriles seem to involve a strong selective force acting to compensate for their lower reproductive potential. The selection differential among the three genotypes is less with the second model based on heterozygote advantage at this locus. The present data are, however, inadequate to allow a choice between these alternative forms of selection. The variations in selective values might be due to seasonal fluctuations encountered during successive generations, or due to cumulative errors associated with the estimators of various parameters (e.g. t, k).

The genotypic frequencies at equilibrium are given by the relationship $f_1^{(n+1)}/f_1^{(n)} = f_2^{(n+1)}/f_2^{(n)} = f_3^{(n+1)}/f_3^{(n)}$, which can be solved to give w_a or w_b in terms of expected genotypic frequencies at equilibrium, viz.

$$w_a = \frac{f_1\{s(1/4f_2) + t(1/2f_2)(kf_3 + 1/2f_2)\}}{f_3\{s(f_1 + 1/4f_2) + t(f_1 + 1/2f_2)^2\}} ; \quad (3)$$

$$k = \frac{1/2f_2(s + tf_2)}{tf_1f_3} ;$$

so that for given equilibrium frequencies (f_1, f_2, f_3), w_a and k are uniquely determined. Table 3 lists some numerical examples that show the relative magnitudes of w_a and k necessary for maintaining a given frequency of *ms ms*. Clearly, the values of w_a obtained from the data were too high to retain any *ms ms* at equilibrium.

Two other factors can significantly influence the genotypic changes at locus *ms*, namely, (1) increase in k as male-steriles become rarer and (2) heterozygote advantage at other loci segregating in these populations. There is some evidence that seed set on male-sterile plants had improved during the later generations which one might in fact expect in terms of the relative abundance of normal pollen. Assuming $t = .02$ for the entire period under study, the value of k seemed to increase from 21 to nearly 30 in the F_{18} - F_{21} generations of C.C.XIV (SUNESON, unpublished data). Such a change in k can perhaps retain a low frequency of *ms ms* for longer periods. The presence of male-steriles results in effectively higher rates of outcrossing (JAIN and SUNESON 1963) so that if there are loci with heterozygote advantage, a selective differential would arise in favor of the *ms* allele, owing to the interfamily type of selection. Thus, it is relevant to estimate

TABLE 3

Some numerical examples of equilibria (see text)

$\hat{f}_3$	k	w_a	$\hat{f}_3$	k	w_a
.05	27.2	.258	.005	21	.216
.02	25.5	.254	.010	21	.209
.02	38.7	.377	.020	21	.204
.10	30.7	.268	.100	21	.138

TABLE 4

Estimates of genotypic frequencies (f_1, f_2, f_3) at loci bl, r and s

Locus	Genotype	C.C.XIV				C.C.XV		
		F_2	F_6	F_{14}	F_{19}	F_2	F_6	F_{10}
<i>bl</i>	f_1	.082	.125	.165	.170	.083	.157	.288
	f_2	.349	.125	.104	.108	.221	.195	.118
	f_3	.569	.750	.731	.722	.696	.648	.594
<i>r</i>	f_1	...	.750	.813	.846	...	.773	.800
	f_2	...	.021	.008	.008	...	.065	.005
	f_3	.100	.229	.179	.146	...	.162	.195
<i>s</i>	f_1	...	.208	.178	...	...	.283	.268
	f_2	...	.012	.000	...	...	.000	.000
	f_3	...	.780	.822	...	...	.717	.732

heterozygosity at some other loci in these populations. Estimates of genotypic frequencies (f_1, f_2, f_3) at three other loci, *bl*, *r* and *s*, are given in Table 4. The estimates of selective values for the dominant and recessive homozygotes (w_1, w_3), taking $w_2 = 1$ for heterozygotes, are given in Table 5. Clearly, there is involved a striking heterozygote advantage for the chromosome segment carrying locus *bl*. Loci *r* and *s*, in contrast, showed a slight advantage for the homozygotes as w_1 and w_3 were estimated to be near or above unity. Similar analysis of genotypic changes at many more loci will be needed for studying the overall effects of the male sterility gene on heterozygosis, and on the breeding system in general for these bulk populations.

In order to evaluate the amount of selective advantage for the *ms ms* component that may result from heterozygote advantage at another locus, say *c*, linked with locus *ms*, a two-locus model was used to simulate the observed data on frequency of *ms ms* with the help of a digital computer. Initial populations were taken to be the F_2 of cross *Ms C/Ms C* $\times$ *ms c/ms c* in all runs, and several sets of recombination values (p) and selective values (w) were used. The value of k was made frequency dependent such that $k = (0.6 - \text{freq.}_{ms\ ms})/t$, with $t = .02$. Results from eight different runs are given in Table 6 in order to illustrate the relative

TABLE 5

Estimates of selective values (w_1, w_3) at loci bl, r and s

Locus		C.C.XIV			C.C.XV	
		F_2-F_6	F_6-F_{14}	$F_{14}-F_{19}$	F_2-F_6	F_6-F_{10}
<i>bl</i>	w_1	.48	.47	.48	.44	.56
	w_3	.66	.53	.53	.49	.59
<i>r</i>	w_1	..	.92	1.25	..	1.15
	w_3	..	.89	1.20	..	1.17
<i>w</i>	w_1	..	..	1.18	..	.00
	w_3	..	..	1.21	..	..

TABLE 6

Expected proportions of ms ms and Cc genotypes under a two-locus model

Selective values*	Recombination value, p	Genotype	Generation							
			0	5	10	20	30	40	50	
1.0,1.0,1.0	.50	<i>ms ms</i>	.250	.039	.007	.000	.000	.000	.000	
		<i>Cc</i>	.500	.064	.027	.020	.020	.020	.020	
0.8,0.8,1.0	.10	<i>ms ms</i>	.250	.047	.012	.001	.000	.000	.000	
		<i>Cc</i>	.500	.130	.053	.033	.032	.032	.032	
0.6,0.6,1.0	.10	<i>ms ms</i>	.250	.060	.026	.007	.002	.001	.000	
		<i>Cc</i>	.500	.236	.149	.096	.083	.079	.078	
0.6,0.6,1.0	.50	<i>ms ms</i>	.250	.049	.014	.002	.000	.000	.000	
		<i>Cc</i>	.500	.212	.128	.085	.078	.078	.077	
0.8,0.8,0.8	.50	<i>ms ms</i>	.250	.088	.046	.018	.008	.004	.002	
		<i>Cc</i>	.500	.140	.088	.054	.043	.037	.035	
0.8,0.8,0.8	.10	<i>ms ms</i>	.250	.094	.053	.025	.014	.009	.006	
		<i>Cc</i>	.500	.172	.112	.071	.054	.046	.041	
0.8,0.8,0.6	.10	<i>ms ms</i>	.250	.170	.156	.151	.151	.151	.150	
		<i>Cc</i>	.500	.246	.228	.224	.223	.223	.223	
0.6,0.6,0.8	.10	<i>ms ms</i>	.250	.145	.126	.120	.121	.122	.122	
		<i>Cc</i>	.500	.307	.274	.260	.260	.261	.261	

* The three selective values are for genotypes *CC*, *cc* and *M_s M_s* respectively. Assuming all other w 's to be unity, multiplicative model was used to obtain w_{ij} -matrix for selection at both loci.

effects of selection and linkage on the survival of *ms ms* and *Cc* genotypes. Three points to note are: (1) although selection at a linked locus is inadequate to retain *ms ms* indefinitely, it results in much lower rates of loss of *ms ms* than in a case without linkage; (2) linkage is more important with stronger selection and tends to spread effects of overdominance; and (3) frequency dependency of k can ensure stability of equilibrium with the maintenance of a few male-steriles in the population. In Figure 1 are shown the observed frequency curve (C.C.XIV) and several theoretical curves for *ms ms* obtained from the computer simulations. It is apparent from the above results that heterozygote advantage at several such loci can indeed be very effective in the adaptive survival of male-sterility factors despite their low reproductive potential under a predominant selfing system.

DISCUSSION

The observed high rates of differential survival of a male-sterility gene particularly during early generations suggested rather intense selective forces to be involved. The relative efficiency of out-pollination on *ms ms* individuals and the degree of heterozygote advantage at various other loci were found to be critical for the maintenance of a certain proportion of male-steriles in populations at equilibrium. The present populations seemed to be rather borderline cases resulting in transient survival of this gene. Under a predominantly selfing system, even the observed rates of occurrence of male-steriles in successive generations could be important in terms of the recombinational potential of such populations.

FIGURE 1.—Observed and expected frequency curves for *ms ms* individuals. Curves b—e were derived from computer simulations.

Control of segregation and recombination through the regulation of outcrossing and the occurrence of overdominant loci (marked segments) are interrelated factors (JAIN and ALLARD 1960). Experimental work in several other highly inbreeding populations showed evidence for rather widespread occurrence of marked chromosome segments showing heterozygote superiority in fitness (IMAM 1961; ALLARD and JAIN 1962; ALLARD and WORKMAN 1963). That selection intensities at such loci vary among different populations is not surprising since the adaptive value of a genotype might well be dependent on such factors as population density, its genetic composition, and the stochastic processes of shifting gene and genotypic frequencies. As pointed out by ALLARD and WORKMAN (1963), changes in genotypic frequencies might not be accurately predicted by using the estimated mean values of parameters in a deterministic population model.

The overall population fitness as measured in terms of the agronomic productivity and stability of performance was found to improve steadily in the barley populations carrying the *ms ms* component to a higher level than the check variety Atlas 46 and two other composite crosses (SUNESON 1956). The effect of continued recombination in the presence of male steriles might be significant in terms of both heterozygosis and total genetic variability. In terms of genetic loads, there are two counteradjusted components of fitness involved, namely, the mutational load due to the lower fertility of male-steriles and an increment in population fitness due to increased recombination potential. Appropriate selective forces can be specified, at least in theory, that would bring about a balance between them. Such "individually disfavored but socially valuable" characters

were briefly discussed by HALDANE (1932) and WRIGHT (1945). In fact, factors governing changes in a breeding system are likely to be often of this sort and it is largely in terms of interpopulational fitness that their effects should be studied. An interesting example is provided by BODMER (1960) who showed the amount of selfing versus random mating in some natural populations of *Primula vulgaris* to be controlled by the relative frequencies of heterostyles and homostyles. Similarly, in *Vicia faba* a mixed selfing (70 percent) and outbreeding (30 percent) system was found to be maintained by certain self-fertility genes, and the added requirement of tripping by insect pollinators (DRAYNER 1956; HOLDEN and BOND 1960).

The population genetic studies on devices controlling transitions between inbreeding and outbreeding systems such as male sterility, heterostyly and self-incompatibility should be of considerable evolutionary interest in relation to genetic systems. DARLINGTON (1939) discussed several interesting examples about which quantitative studies can further elucidate evolutionary implications. There is very little known, for instance, about the rates of occurrence of male sterility in natural populations, their competing ability or efficiency of outpollination, etc. RICK (1950, *et seq.*) reported the occurrence of several male sterile mutants in tomato populations and also variation in exertion of stigma to result in higher rates of outcrossing. Further work on such materials in terms of genotypic frequency changes should provide results bearing on our model.

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SUMMARY

Two bulk-hybrid populations of barley were investigated for changes in genotypic frequencies at a locus for male sterility and at three other loci. The male-steriles (*ms ms*) seemed to be adaptively favored owing to their effect on increased outbreeding and higher levels of hybridity. Heterozygote advantage at loci *ms* and *bl* reinforced by the increasing rates of outpollination on male-steriles could account for the observed low rate of loss of *ms ms* and *Bl bl* genotypes in both populations. A comparison with some computer simulations of the model showed that selection intensity, linkage and the rate of outcrossing are all critical for determining the genotypic frequencies at equilibrium points. These findings were briefly discussed in relation to the adaptive role of male sterility as a factor regulating the recombination system in predominantly selfing species.

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