OPTIMAL SEX RATIO IN *SILENE ALBA* *

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1. INTRODUCTION

Silene alba (Miller) Krause in Sturm (= *Melandrium alba*) is a dioecious weed, introduced from Eurasia and common to much of the United States. A benign invader of hayfields and roadsides, the species prospers only if periodic mowing prevents the growth of more aggressive plants. It possesses heteromorphic sex chromosomes and pistillate individuals represent both the homogametic sex (Correns, 1928; Blackburn, 1923) and, contrary to Mendelian expectations, somewhat more than 50 per cent. of natural populations.

Correns (1928), working with *Silene alba*, found that crosses in which small amounts of pollen were used produced approximately equal proportions of staminate and pistillate plants but large amounts of pollen gave an excess of pistillate plants. Correns reasoned that the pollen tubes containing an X chromosome grew faster than those containing a Y chromosome. Thus if large amounts of pollen were available, the faster growing X pollen tubes could reach and enter more than half the ovules while the Y pollen tubes were still penetrating the style. With only a little pollen available, the X pollen tubes would still be first in reaching the ovules but a sufficient number of eggs would remain unfertilised to accommodate the slower Y pollen tubes, resulting in approximately equal proportions of pistillate and staminate plants. This indicates that the excess of pistillate offspring is caused primarily by gametic, rather than zygotic competition. Nevertheless, a slight excess of pistillate offspring is sometimes produced even when pollination is minimal. Therefore another factor, in addition to pollen tube competition, must be operating. Whether this factor has its effect on gametes or on zygotes is unknown (Correns, 1928; Lawrence, 1963). Differential pollen tube growth, comparable to that reported in *Silene*, was later observed in *Rumex acetosa* (Correns, 1928) and in *Humulus japonicus* (Kihara and Hirayoshi, 1932).

Lewis (1942) and Darlington (1948) have suggested that in *Silene alba*, differential pollen tube growth represents a mechanism whereby the sex ratio of natural populations can be adjusted by selective pressures. They reasoned that if pistillate flowers receive excessive amounts of pollen, the population may contain too many staminate individuals to allow maximum reproductive efficiency. Under these conditions, because of the relatively rapid growth of pollen tubes containing an

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X chromosome, the seed produced in that population will contain a greater than expected proportion of XX individuals, thus serving to increase the frequency of pistillate plants. It should also be considered that the proportion of pistillate offspring will be determined, in part, by the extent of individual excessive pollinations, *i.e.* the greater the excess of pollen, the greater the proportion of XX seeds, and, in part, by the frequency of excessive pollinations throughout the population. It is thus possible that in populations of *Silene alba* the sex ratio, by virtue of differential pollen tube growth, is maintained at some optimal value.

The optimal sex ratio is assumed to be that ratio resulting in the highest degree of seed production. This assumption rests on the fact that seed production will not only be strongly influenced by changes in the sex ratio, as is discussed below, but also, it is a factor of obvious selective significance. It must also be considered, however, that seed size is frequently inversely proportional to the number of seeds contained within individual fruits (Salisbury, 1942). This consideration could be of significance in that seed size may affect various seedling characteristics some of which could influence survival in natural population. Furthermore, Li (1955) has shown that inbreeding coefficients are in part determined by sex ratios. The assumption that the ideal sex ratio is that resulting in the largest number of seeds is thus not taken without some reservations. It seems, however, to be a reasonable point of departure.

Figure 1 represents the hypothetical relationships between the sex ratio and both the number of seeds produced by individual capsules and the total number of seeds per plot. No seed production will occur in populations which contain either 100 per cent. or 0 per cent. staminate flowers (see fig. 1). As the proportion of staminate flowers increases from 0 per cent., seed production will also increase, probably in direct proportion to the amount of pollen available, even though the proportion of pistillate flowers declines. At some point, marked "A" in fig. 1, pollen is no longer the limiting factor. Further increases in the proportion of staminate flowers will result in a decline in seed production since ovules have become limiting. The average number of seeds per capsule will also increase in proportion to the amount of pollen available. At some point, however, further increases in the flower ratio (staminate flowers/pistillate flowers) will be superfluous since all ovules will have been fertilised. Beyond this point, marked " B " in fig. 1, the number of seeds per capsule will remain constant.

The approach to point " B " will be asymptotic because it may be assumed that insects distribute pollen in a largely random fashion, *i.e.* flowers which have already received enough pollen to fertilise all ovules are as likely to receive additional pollen as are those flowers which have yet to be pollinated. This consideration is of little significance at low sex ratios since under those circumstances the frequency of excessive pollinations will be relatively low. As the sex ratio increases and pollen becomes more abundant, the frequency of excessive pollinations will also increase, *i.e.* the efficiency of pollen utilisation will decrease. Consequently a direct relation between the number of seeds per capsule and the sex ratio is expected to obtain only at lower sex ratios.

The ideal sex ratio, as concerns seed production, is thus among those sex ratios which will provide a proportion of staminate flowers somewhat below that represented by point " B " in fig. 1, and it may be assumed that this ideal ratio will be well below point " B " because of the asymptotic nature of the relationship and the concomitant decline in the efficiency of pollen utilisation at higher ratios.

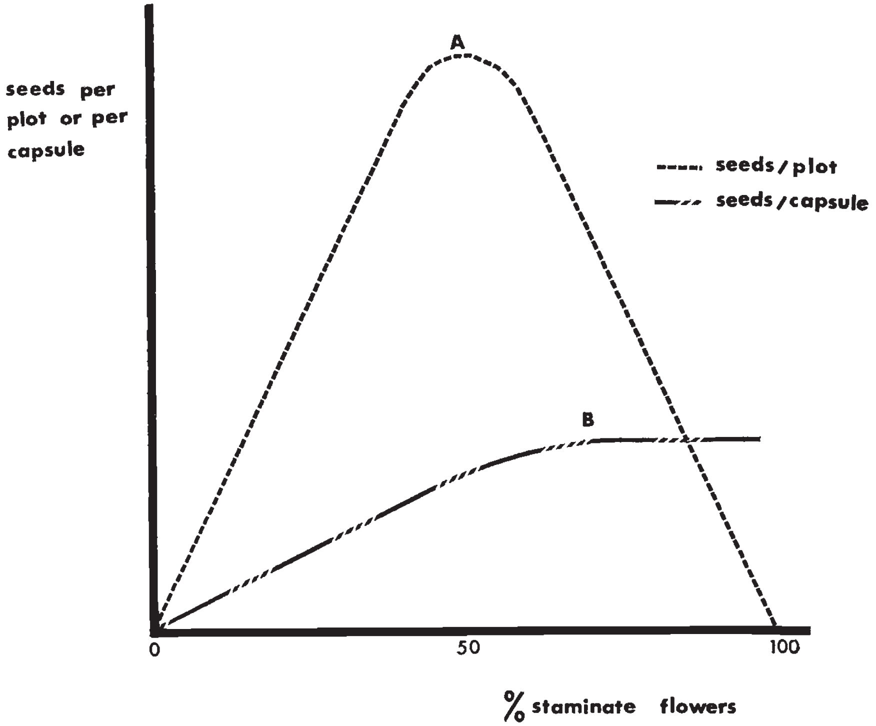

FIG. 1.—The hypothetical relationships between the proportion of staminate flowers in a population and seed production.

In order to evaluate the hypothesis that differential pollen tube growth represents a mechanism whereby the sex ratio in populations of *Silene alba* is adjusted to some optimal value, the following three questions must be answered: Firstly, what is the optimal sex ratio? Secondly, is this optimal ratio attained or approached in natural populations? Thirdly, if the non-Mendelian ratios observed in natural populations do represent the attainment of, or even an approach to, an ideal sex ratio, can this behaviour be attributed to differential pollen tube growth, *i.e.* do excess pollinations occur with sufficient frequency to be of any significance?

This study represents an attempt to answer these questions through the use of both natural and artificial populations.

2. MATERIALS AND METHODS

Because the variation in number of flowers produced by individual plants is great, it was felt that sex ratios (staminate plants/pistillate plants) would be a reasonable measure of gamete availability only in very large populations of plants. Consequently, much of the data from both natural and artificial populations are in terms of the flower ratio (staminate flowers/pistillate flowers).

Four natural populations were selected for study. At intervals of three to five days, determinations were made of the number of reproductively active plants, *i.e.* those in flower and/or maturing capsules, and also the actual number of flowers on individual staminate and pistillate plants. Observations were continued until that time at which the fields are normally mown. These data provided measures of both the sex ratios and the flower ratios in natural populations.

In order to estimate the optimal flower ratio, five artificial populations were constructed, separated by approximately 30 metres. Each population contained 25 plants, of which 2, 10, 12, 18 or 24 plants were pistillate. The plots were labeled A-E, respectively. Plants were arranged within a square pattern of five plants on a side, each plant being separated from another by approximately 60 cm. The sexes were randomly distributed throughout each plot.

Since *Silene* flowers open largely during the night, the procedure used in artificial populations was as follows: Late in the afternoon all flowers which had previously opened and showed any possibility of reopening were removed from both staminate and pistillate plants, leaving large buds which would open during that night. Early in the following morning, flowers which had opened during the night were counted, tagged and all other flower buds were then removed. This had the effect of fixing the ratio of staminate to pistillate flowers at a single point. This procedure was performed three times, resulting in series 2, 3 and 4. The starting dates of these three series were May 29, June 12 and June 30, respectively. Capsules were collected when mature and seeds were counted. Seeds produced in series 4 were planted in a greenhouse and their sex ratios were determined.

3. RESULTS

(i) *Natural populations*

The results are presented in table 1 and figs. 2 and 3. At the first observations, the numbers of plants were small and staminate plants in flower outnumbered the pistillate. As the season progressed both staminate and pistillate plants increased in frequency. The increase

TABLE 1

Sex ratios (♂ plants/♀ plants) and average flower ratios (♂ flowers/♀ flowers) of natural populations

Population	Sex ratio	Average flower ratio
101 A	0·68	2·10
101 B+C	0·60	2·85
Szopa	0·32	6·00
Leavitt Hill	1·03*	4·90

* It was not possible to complete observations of this population and since ♀ plants flower later than do ♂, this ratio is unnaturally high.

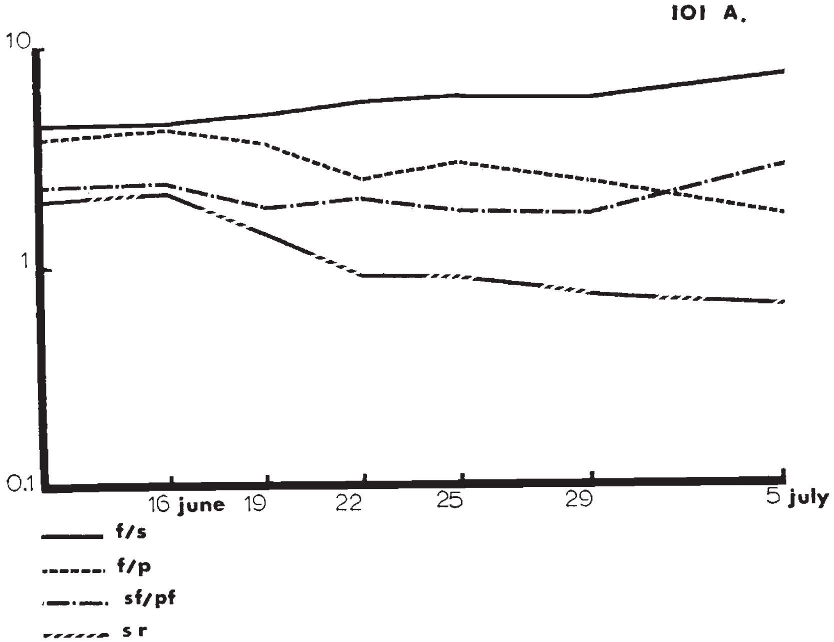

FIG. 2.

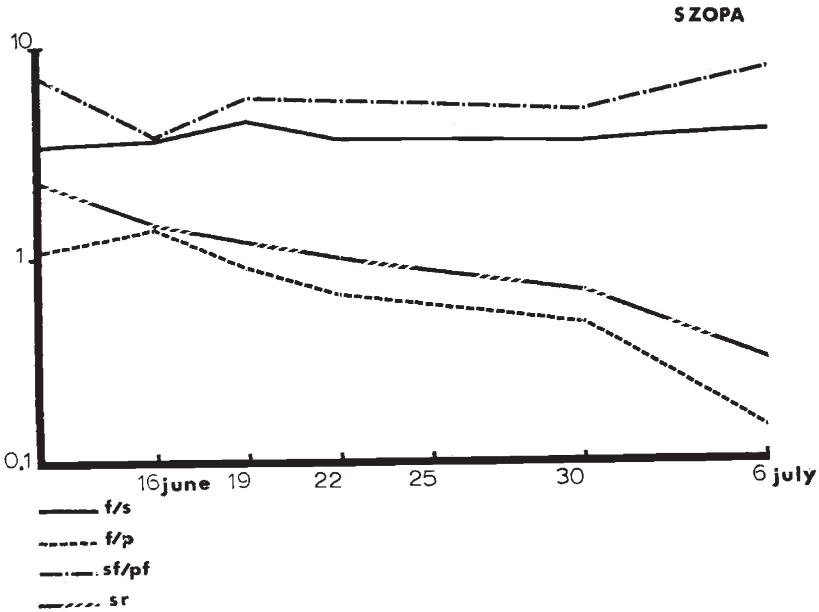

FIG. 3.

FIGS. 2 and 3.—Sex ratios and flower ratios of two natural populations. *f/s* = average number of flowers per staminate plant; *f/p* = average number of flowers per pistillate plant; *sf/pf*—total number of staminate flowers/total number of pistillate flowers = flower ratio; *sr* = staminate plants in flower/pistillate plants in flower or maturing capsules = sex ratio.

of pistillate plants, however, was relatively greater, resulting in an increased proportion of pistillate individuals. This steady decrease in the sex ratio (staminate plants/pistillate plants = S.R.) is shown in figs. 2 and 3. Eventually, a majority of the plants were pistillate. While the number of pistillate plants increased throughout the season, the average number of pistillate flowers per plants ($= f/p$) decreased. This may very likely be attributed to the inhibitory effect of capsule production on the development of subsequent flowers, a phenomenon well known to horticulturalists and shown in other studies of this species (Mulcahy, unpublished).

In staminate plants, however, where little or no inhibitory effect corresponding to capsule production exists, the number of flowers per plant $= f/s$) gradually increased. Figures 2 and 3 indicate that a balance was struck between several factors. The increasing proportion of pistillate plants in the populations apparently compensated for the decrease in number of flowers per pistillate plant, the increase in number of staminate plants, and the increase in flowers per staminate plant. This balance maintained the relative proportions of staminate and pistillate flowers ($= sf/pf$) at a fairly constant value, a value differing from one population to another but usually within the range of 2-6 staminate flowers for each pistillate flower.

(ii) *Artificial populations*

The average numbers of seeds produced in capsules of series 2, 3 and 4 are shown in figs. 4, 5 and 6, respectively. Ranges are shown in fig. 7. The data are presented in table 2. Sex ratio of progenies from series 4 are shown in table 3.

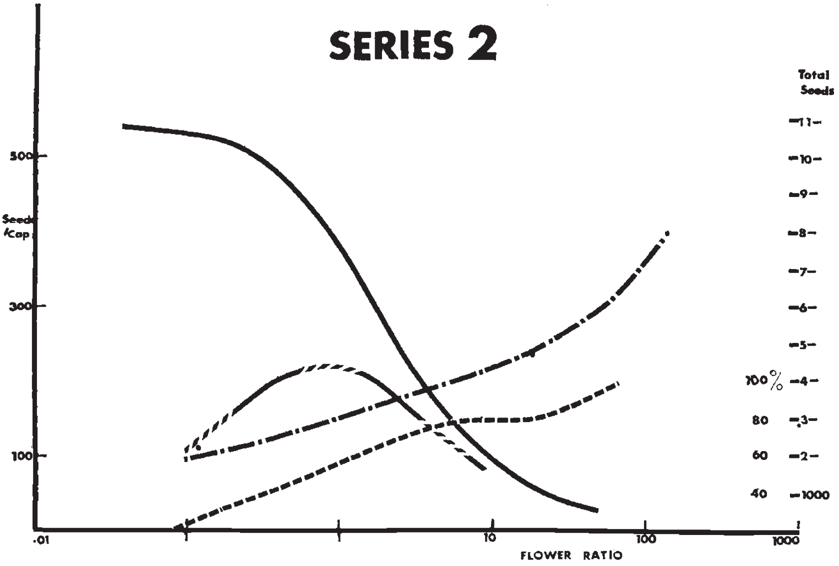

FIG. 4.

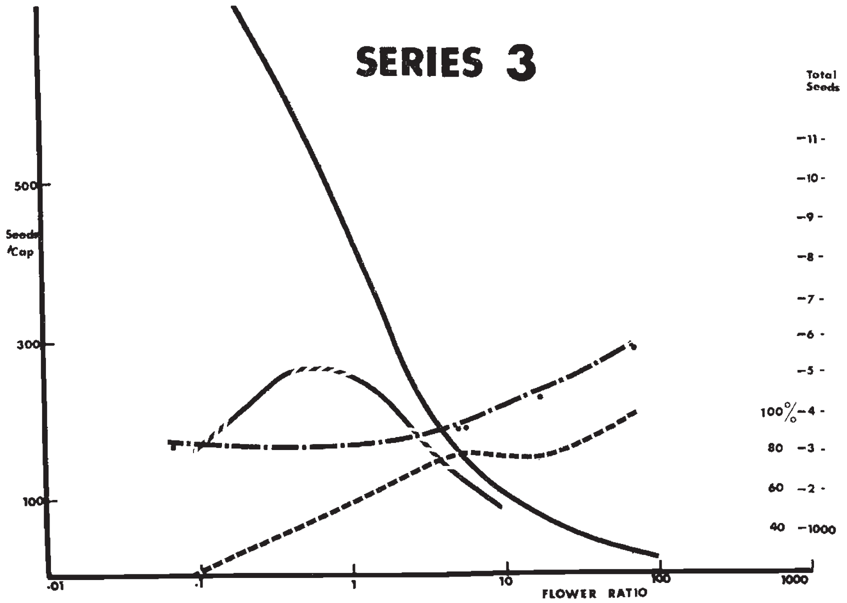

Fig. 5.

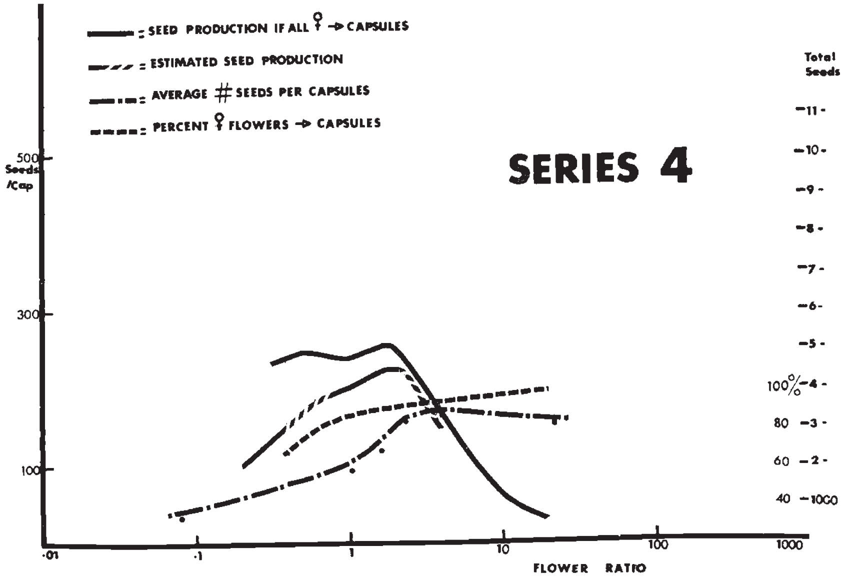

Fig. 6.

Figs. 4, 5 and 6.—Relationships between seed production and flower ratio in artificial populations. Figure 6, series 4, contains legend.

In series 2, the highest average number of seeds per capsule was obtained with a flower ratio of 106·00. The correlation between flower ratio and seeds per capsule was good (multiple r^2 = 0·985).

TABLE 2

Seed production at different flower ratios (staminate/pistillate) of series 2, 3, and 4. Plots of each series are arranged in order of decreasing flower ratio

	Flower ratio	Seeds produced	Capsules produced	Average of seeds per capsule	Range
Series 2					
A	212/2 = 106·00	792	2	396·0	(349-443)
C	151/5 = 30·20	1185	4	296·2	(202-407)
D	72/4 = 18·00	481	2	240·5	(216-265)
B	63/11 = 5·73	764	4	191·0	(110-322)
E	4/34 = 0·12	1430	12	119·2	(20-319)
Series 3					
A	410/6 = 68·30	1738	6	289·6	(253-331)
C	329/19 = 17·32	2977	15	212·6	(86-368)
B	153/29 = 5·28	3419	24	142·0	(42-217)
D	103/20 = 5·15	4333	17	270·8	(73-495)
E	21/312 = 0·07	5996	42	142·7	(9-359)
Series 4					
A	401/18 = 22·30	2774	18	154·1	(20-263)
C	146/63 = 2·32	8897	54	164·7	(13-380)
D	101/66 = 1·68	7148	58	123·2	(16-286)
B	59/57 = 1·04	4806	52	92·3	(1-328)
E	9/111 = 0·08	1430	42	34·3	(2-224)

In series 3, this correlation was only fair (multiple r^2 = 0·483). In series 4, the highest average number of seeds per capsule was obtained at a flower ratio of 2·32. The correlation between flower ratio and seeds per capsule was good (r = 0·989). Thus, in all three series, a positive correlation was found between the flower ratio and the average number of seeds per capsule. The data also show a similar correlation

TABLE 3

The sex ratio of seeds produced in series 4, arranged in order of descending flower ratios of parental populations

Plot	Flower ratio	Number of pistillate offspring	Number of staminate offspring	Staminate/pistillate × 100
A	22·30	217	168	77·4*
C	2·32	665	628	94·4
D	1·68	916	796	86·9†
B	1·04	689	631	91·5
E	0·08	61	76	124·5

* Significant excess of pistillate offspring.
† Highly significant excess of pistillate offspring.

between flower ratio and the proportion of pistillate flowers producing capsules (see table 2 and figs. 4, 5 and 6).

An inverse relation was found to exist between the number of seeds per capsule and both the size of seeds ($r = -0{\cdot}57$) and the percentage of germination ($r = -0{\cdot}22$). Regression analysis of the data showed that germination declined 0·137 per cent. for each unit increase in flower ratio. Germination data was available for series 4 only.

The sex ratio of seeds produced in plots C, B and E of series 4 did not deviate significantly from the expected 1:1 ratio. Progenies from plots A and D, however, showed, respectively, significant and highly significant excesses of pistillate individuals (table 3).

4. DISCUSSION AND CONCLUSIONS

Although the data show an inverse relationship between flower ratio and germination percentage of seeds produced, the decline in germinability is quite small and perhaps may safely be ignored. Any doubts on this point should be settled by the fact that the effect of reduced germinability would be to lower the optimal flower ratio. As will be shown below, this will only strengthen the general conclusions derived from this study. These data, therefore, support the assumption that the optimal sex ratio or flower ratio is that ratio resulting in maximal seed production.

The data from artificial populations show that, as expected, the average number of seeds per capsule declines with the flower ratio. It should be recalled, however, that the number of pistillate flowers in the population is inversely proportional to the flower ratio. These two considerations have been used to plot the number of seeds which would be produced within a series of populations differing in only flower ratios, but not total flower numbers, and assuming all pistillate flowers produced capsules (see figs. 4, 5 and 6). These curves obviously reach peaks at relatively low flower ratios but must be corrected for the fact that the proportion of pistillate flowers producing capsules declines with decreasing flower ratio. When this is done, the curves obtained represent the estimated seed production at different flower ratios (see figs. 4, 5 and 6). The peaks in these curves are considered to be the optimal flower ratios, and occur at values of 0·66, 0·43 and 2·30 for series 2, 3 and 4 respectively.

These figures are generally lower than the flower ratios observed in natural populations, despite the fact that the flower ratio of 2·30 for series 4 exceeded the average ratio (2·10) observed in natural population 101A. This indicates that natural populations contain a greater proportion of staminate flowers (and therefore staminate plants) than is permissible for maximum seed production. However, since natural populations contain a smaller than expected proportion of staminate individuals, it would seem that the aberrant sex ratios observed in nature represent an approach to, but not the attainment of, the optimal sex ratio. Thus the excess of pistillate individuals observed in natural populations should be considered as a positive selective advantage in that its effect is to increase the number of offspring produced.

The selective advantage of a greater than expected proportion of pistillate individuals in natural populations, however, indicates nothing about the selective significance of differential pollen tube growth, unless these aberrant sex ratios can be reasonably attributed to the functioning of the differential growth. Some of the data obtained in the artificial populations suggest that this can be done.

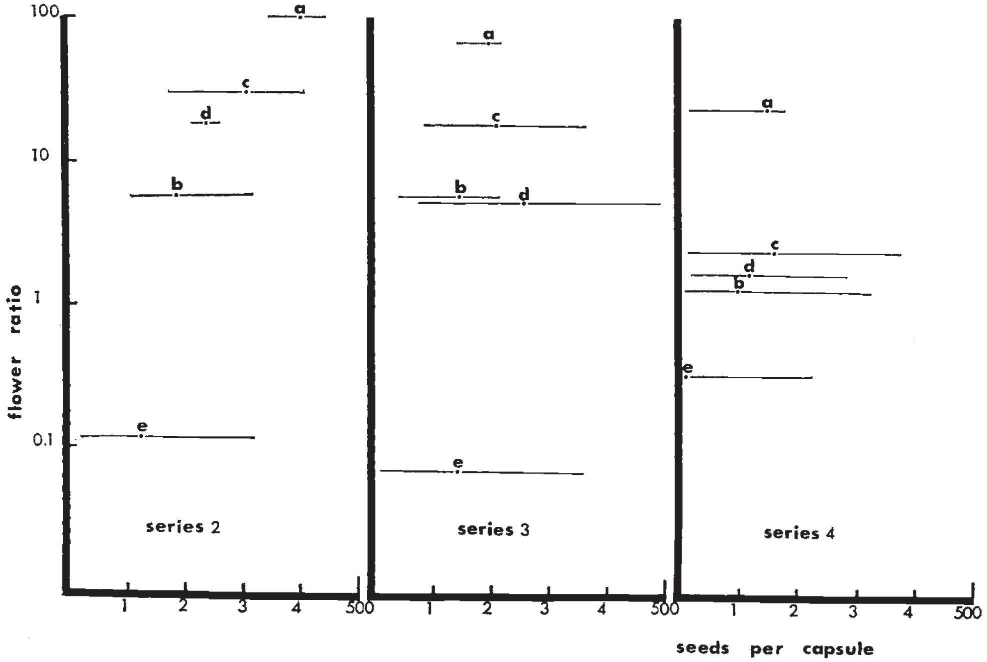

FIG. 7.—Range in number of seeds per capsule produced in artificial populations. Dots indicate means, horizontal extensions the ranges. Lower-case letters over each mean identify the population in which capsules were collected.

In series 2, with the exception of plot D, the maximum number of seeds per capsule as well as the average number per capsule, is obviously correlated with the flower ratio (see fig. 7). This indicates that pollen availability in series 2 is the primary factor in determining the number of seeds per capsule. Furthermore, the steady increase in number of seeds per capsule even up to a flower ratio of 106·0, indicates that fertilisation of all ovules will occur only at flower ratios far above those observed in natural populations. Thus, excessive pollinations must have been very infrequent in series 2. In series 4, however, the maximum number of seeds per capsule fail to exhibit a high degree of correlation with the flower ratio (see fig. 7). This failure applies in particular to plots A and D, in both of which the maxima are obviously low. Since pollen was obviously not the limiting factor, it seems reasonable to assume that ovules were, *i.e.* a significant number of excessive pollinations must have occurred. This assumption is supported by the fact that progeny from plots 4A and 4D showed, respectively, significant and highly significant proportions of pistillate individuals (see table 3). In the remaining three plots, none of which showed any indication of excessive pollinations, the progenies all failed to show any significant deviations from the expected 1:1 sex ratio. Thus it may be reasonably concluded that in plots 4A and 4D excessive pollinations occurred and resulted in greater than expected proportions of pistillate offspring. This is of particular interest since the flower ratio of plot 4D, 1·68, resulting in a highly significant excess of pistillate offspring, was lower than that observed in natural populations. Thus similar excesses of pistillate offspring may be expected to occur in natural populations. This provides supporting evidence for the hypothesis that the greater than expected proportion of pistillate individuals in natural populations is the result of differential pollen tube growth, following excessive pollinations.

It is necessary to consider why flower ratios of 1·68 and 22·30 in plots D and A, respectively, of series 4 resulted in excessive pollinations, even though a flower ratio of 106·0 in plot A of series 2 failed to do so. The explanation is based on the fact that, as was shown in natural populations, capsule production by individual pistillate plants retards the development of subsequent flowers. While this is known to reduce only the quantity of later capsules, it may be reasonable to assume that the quality of these capsules, *i.e.* the number of ovules contained in them, is also lowered. This assumption is supported by the fact that in plot A of series 2, the average number of seeds per capsule was 396·0, with the range extending up to 443·0 seeds per capsule with no indication of excess pollination. In contrast, plot A of series 4 showed definite indications of excessive pollinations even though the average number of seeds per capsule was 154·1 and the observed maximum of 263·0 was less than the average for series 2 of the same plot. The reduced maximum of plot D, series 2, indicates excessive pollinations but, without checking the sex ratio of progeny, this is uncertain. The data from plot A, however, definitely suggest that the number of ovules contained in an ovary declines throughout the season. This explains the greater tendency toward excessive pollinations in the late season.

Since natural populations possess a mechanism, very likely differential pollen tube growth, whereby a selectively significant reduction in the flower ratio has been produced, it is reasonable to ask why there has been only an approach to, but not the attainment of, the optimal flower ratio. Two possible hypotheses can be suggested.

If it can be assumed that these non-Mendelian sex ratios are the result of excessive pollinations and subsequent differential pollen tube growth, lower sex ratios, and thus lower flower ratios, would be dependent upon a greater frequency of excess pollinations. As the sex and flower ratios decline, however, excess pollinations should become more and more infrequent. Beyond some point, the rarity of excessive pollinations would prohibit further decreases in the ratios.

A second, and perhaps more significant, hypothesis is based on a concept published by Shaw and Mohler (1953). On theoretical grounds, they demonstrated that the more the sex ratio deviates from equality, the greater is the selective advantage conferred upon the minority sex. Thus natural selection will favour those genotypes which produce a relatively high proportion of the minority sex in their progeny. The net result is that selective pressures drive the sex ratio toward equality from either direction. In applying these concepts to populations of *Silene alba* it would seem that as the sex ratio is lowered, there is increased competition among pistillate plants for pollen while the staminate plants experience a reduction in reproductive competition. This confers a selective advantage upon genotypes which produce a relatively high proportion of staminate offspring, thus retarding any tendency toward lower sex ratios. This example is of particular interest in that it represents a conflict between the selective advantages of the population as a whole and those of individuals within the population. That is, the population will benefit by decreased sex ratios and the resultant increased seed production while any reduction in sex ratios will confer a particular selective advantage upon those very genotypes which will tend to increase the sex ratio. It thus would seem that the sex ratio observed in natural populations represents a balance between these two conflicting forces.

5. SUMMARY

1. A series of five artificial populations was constructed, each with a different sex ratio.

2. Seed size and per cent. germination decline with an increase in number of seeds per capsule. The decline in germination percentage is relatively small and more than compensated for by the increased number of seeds.

3. The number of seeds per capsule and the total number of seeds per population are closely correlated with the relative proportion of staminate and pistillate flowers in the population. The ratio of staminate to pistillate flowers which results in maximum seed production (0·43 to 2·30) is estimated to be generally lower than that observed in natural populations (2-6).

4. The ratios observed in natural population, however, would deviate even further from the optimal value were it not for an observed excess of pistillate individuals.

5. The non-Mendelian sex ratios of natural populations thus are considered to be of positive selective value to the species. The aberrant sex ratios result from differential pollen tube growth following excess pollinations.

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