Theor. Appl. Genet. 57, 11-15 (1980)

© by Springer-Verlag 1980

Gynodioecy as a Possible Populational Strategy for Increasing Reproductive Output

A. Horovitz
Department of Genetics, The Hebrew University of Jerusalem, Jerusalem (Israel)
A. Beiles
Institute of Evolution, University of Haifa, Haifa (Israel)

Summary. Cytoplasmic male steriles occur regularly in wild populations of the annual crucifer *Hirschfeldia incana* Lagr.-Foss. in Israel. In these plants numbers of ovules per flower, numbers of seed per fruit, and total seed weights per plant are slightly higher than in the hermaphrodites with which they mate. Yet their frequencies in wild populations do not exceed 2-10 per cent. There are no signs of incipient dioecy. The species is self incompatible and no mechanisms to enforce outcrossing are needed. It is argued that in this and similar cases gynodioecy functions as a pollen saving measure. Precise pollen presentation in the flower renders some of the pollen redundant and facilitates its abolition in a sector of the population. It is possible that the enhanced seed fecundity of the pollenless sector stems from a greater availability of plant resources for seed production in the unisexual than in the bisexual seed parent.

Key words: Gynodioecy – Seed yields – Reallocation of resources – *Hirschfeldia incana*

Introduction

In the mating systems of flowering plants two main roles have been ascribed to gynodioecy, the joint occurrence of female and hermaphrodite individuals in a population. Darwin's (1877) suggestion that it is an intermediate stage in the evolution of dioecy from bisexual plants has been elaborated in numerous modern studies (Charlesworth and Charlesworth 1978; Ross 1978). Alternatively, as first proposed by Mather (1940), gynodioecy may act as an outcrossing mechanism which enhances hybridity in populations of partial selfers.

Male sterile plant mutants or recombinants from wide crosses appear frequently under domestication but can maintain themselves in nature only where provisions for cross pollination are adequate. Moreover, if male sterility is transmitted by nuclear genes, females in the gynodiocious population are at a selective disadvantage since they give rise to only a single set of gametes as against the twofold set produced by their hermaphrodite counterparts. So as to fulfill the roles accorded to them, they have to be of superior fitness and make amends for their pollen deficiency by high seed fecundity and survival rates. Under cytoplasmic inheritance females suffer from no such disadvantage and can spread more easily. Here the ultimate limit to their frequency is set when the supply of fertilizing pollen reaching them falls below that reaching the hermaphrodites (Lewis 1941; Lloyd 1974). The higher seed fertility of male steriles compared to that of their bisexual mating partners, first shown by Darwin (1877) in *Thymus* is being documented increasingly in current studies of wild populations (examples reviewed by Lloyd 1976). In the current literature, various processes which can account for differences in female fecundity are proposed. Inbreeding depression may lower the fitness of selfing hermaphrodites relative to that of outcrossing females (Assouad et al. 1978; Valdeyron et al. 1973). Homozygous, often genetically recessive male steriles, initially maintained by overdominance at heterotic male sterility loci, may acquire genes promoting superior female fertility by linkage (Ross and Weir 1975, 1976). Concomitantly, genes reducing female fertility may be selected for and become linked with male sterility suppressor alleles in hermaphrodites, and pave the way to dioecy (Ross 1978).

The basis of the present paper is yet another case of higher seed fecundities in females than in hermaphrodites under gynodioecy. Botanists are well aware of the fact that plants are sensitive to pollen wastage and respond to a reduced demand for pollen by reductions of the androecium. Accordingly, the lower pollen requirements under selfing than under outcrossing are reflected in reduced pollen production by more highly selfed taxa (Cruden

0040-5752/80/0057/0011/$ 1.00

1977). Horovitz and Harding (1972) have shown that such differences may become genetically fixed within a species of *Lupinus*. Pollen redundancy does not only arise under selfing but can result from elaborate cross pollination devices in which pollen presentation is very precise. Stamen reductions in species with highly evolved pollination mechanisms are, for example, well known in the mint family. Significantly, gynodioecy is also widespread in the same family (Willis 1892). The suggestion at hand is that total abolition of pollen in a sector of the population may be selected for as an alternative to partial reductions in each individual (in *Ajuga, Marrubium, Stachys, Satureja, Thymus, Mentha*, and others) or that it may become superimposed on already present overall reductions of the androecium (e.g. in species of *Salvia, Horminum*, and *Lycopus*).

In cases of overall reductions, the benefits conferred by pollen economy can only be recognized vaguely, in terms of the presumed evolutionary advance they are held to represent (Hutchinson 1959). Since all members of the taxon are affected, no comparisons can be made but fitness is presumably higher than it would be under greater pollen expenditure. Under gynodioecy the quota of redundant pollen in one sector of the population is redistributed for utilization by the pollenless sector. Such a redistribution can only be advantageous to the population and raise its total reproductive output above that of a monomorphic hermaphrodite population, if females use the pollen allocated to them more efficiently than would bisexual plants. The evidence at out disposal from comparative fecundity studies in gynodioecious populations indicates that this is the case. But some of the plants studied, such as species of *Pimelea* (Burrows 1960) and *Fuchsia* (Arroyo and Raven 1975), or *Cirsium arvense* (Correns 1916; Lloyd and Myall 1976) exhibit transitions to dioecy; they exemplify reduced female functioning in hermaphrodite individuals rather than superior female functioning in male steriles. A wider review of cases of stable gynodioecy is needed. Such a review might establish that higher seed fecundities are a constant by-product of pollen abolition. This would substantiate Darwin's (1877) supposition that non-development of pollen may result in a reallocation of resources within the plant, which favours seed development.

Our suggestion is that one of the functions of gynodioecy may be to enhance population seed yields through a switch from pollen to seed expenditure in a sector of the population.

If gynodioecy can indeed be instrumental in raising seed yields, its manipulation might be of practical interest to seed growers. Hence the argument for male sterility as a means to ameliorate seed parenthood is made in this paper.

Material and Methods

Hirschfeldia incana Lagr.-Foss. is a weedy diploid annual crucifer native to the Mediterranean from where it has spread to Central and Western Europe. It has also been introduced to California, southern Australia and New Zealand. In Israel the plant inhabits mainly disturbed habitats and flowers from March to May, replacing the wild mustards, *Silene alba* and *S. arvense*. The flowers remain in anthesis for two days. They have well developed dimorphic nectaries and an elaborate system of pollen presentation which obliges pollen vectors, various bees and long tongued flies, to pass through a tunnel of pollen on their way to the nectar source. All material studied is totally self incompatible. Plants that are forcibly selfed by bud pollination show various abnormalities and inbreeding depression.

In 1972 Horovitz and Galil reported the occurrence of male sterility in local weed and wayside populations in frequencies of 2 to 10 per cent, as well as rare instances of gynomonoecious plants. Subsequent surveys confirmed this situation and repeat visits to some populations showed similar male sterile frequencies in consecutive years. Only one of the 38 populations sampled in different parts of the country revealed no male sterile or gynomonoecious plants upon repeated examinations.

Meiosis in male sterile flower buds was found to be normal but there was often a delay of microspore release from tetrads, or microspores with a defective exine separated and the degeneration of the secretory tapetum was delayed. Varying with genotype, male sterility was found to be affected by low temperatures. In controlled experiments Ilan et al. (1977) found that the exposure of premeiotic buds to temperatures between 4-6°C for a minimal period of 12 hours caused a small percentage of fertility restoration in male sterile accessions classified as stable. In unstable lines, exposures to 7-10°C, such as may be encountered by March flowering plants in nature, effected partial restoration of fertility. This means that early or out of season flowering male steriles may parade as male fertiles. Certain gynomonoecious plants were found to be temperature stable.

Progeny tests of open pollinated plants, as well as analyses of controlled diallel crosses, indicated maternal cytoplasmic inheritance with progenies exhibiting the sex type of the seed parent. In most progenies of male steriles, a small percentage of temperature unstable plants with partially fertile flowers under low temperature appeared. One of the six hermaphrodites used in the orginal diallel crosses apparently carried restorer genes and crosses involving this plant or its progenies caused changes in sex inheritance, including the production of stable gynomonoecious plants which, in turn, gave rise to segregating progeny.

Components of fecundity were measured in four wild populations and in a garden planting (Table 1). The wild populations

Table 1. Populations used for fecundity measurements

Population	Area m^2	Density plants/m^2	% MS
1 Wild	50	8	2
2 Wild	100	10	3
3 Wild	150	12	7
4 Wild next to apiary	100	8	10
5 Garden planting near apiary	270	6	23

Table 2. Means and standard arrors of fecundity estimates in male sterile (MS) and hermaphrodite (MF) plants

Parameter		Populations				
		1	2	3	4	5
Ovules per ovary	MS		9.70 ± 0.37 (4)[a]		9.23 ± 0.50 (7)	9.82 ± 0.44 (9)
	MF		9.23 ± 0.30 (7)		8.72 ± 0.27 (5)	8.80 ± 0.31 (16)
Seeds per fruit	MS	7.73 ± 0.43 (3)	9.38 ± 0.51 (4)			8.63 ± 0.38 (9)
	MF	9.63 ± 0.15 (3)	8.56 ± 0.40 (4)			7.24 ± 0.37 (16)
Seed weight per plant (mg)	MS			578.7 ± 218.8 (7)	795.8 ± 43.7 (7)	1.191.9 ± 235.4 (10)
	MF			527.0 ± 115.5 (7)	698.0 ± 51.3 (7)	853.7 ± 107.9 (16)

[a] Number of plants in brackets

were weed colonies in the Tel Aviv area, representing the range of male sterile frequencies found in nature. The garden planting, which had served progeny tests in the analysis of sex type inheritance contained a higher proportion of male steriles than those encountered in the wild. Plants occupying random positions in these test populations were marked at the peak of the flowering season for flower and fruit collection.

The fecundity estimates compared in hermaphrodite and male sterile individuals were number of ovules per ovary, number of seeds per silique, and total seed weight. In the wild populations different individuals of the two gender components were subjected to tests. Only in the garden planting, Population 5, were the data for the three tests collected from the same individuals. Flowers for ovule counts were fixed on the first day of anthesis in Carnoy's medium and stored in 70% ethanol. Estimates for individuals were means of counts in five ovaries. The number of seeds per silique were based on ten fruits per plant. For estimation of total seed weight, fruits had to be collected repeatedly from each plant.

Results

Mean estimates of fecundity in the two gender types are shown in Table 2. The large standard errors in this table indicate considerable between plant variance. For an overall comparison between fecundities in females and hermaphrodites in the presence of such variance, the single tailed sign test, which is sensitive to uniformity in the direction of a trend, was applied to the data (Table 3). Seed number and seed weight estimates for Population 5 were pooled in Table 3, since they were based on the same sample. It is seen that values scored for male steriles were higher than those scored for hermaphrodites in seven out of eight pairs of independent estimates. The low probability of such a partition of values (3.5 per cent) allows us to infer at a 5 per cent level of significance that, in the fitness components tested, male steriles perform better than hermaphrodites. In Table 4, differences between the two gender types are set out in quantitative terms. Weighted means for the populations are presented in Table 5.

Table 3. Overall comparisons of eight independent estimates of fecundity in male sterile (MS) and hermaphrodite (MS) plants

Population	Fecundity parameter	Direction of difference	Sign
1	Seeds per fruit	MS < MF	–
2	Ovules per ovary	MS > MF	+
2	Seeds per fruit	MS > MF	+
3	Seed weight per plant	MS > MF	+
4	Ovules per ovary	MS > MF	+
4	Seed weight per plant	MS > MF	+
5	Ovules per ovary	MS > MF	+
5	Seeds per fruit Seed weight per plant	MS > MF	+

Table 4. Ratios of estimated mean fecundities in male steriles relative to hermaphrodites

Parameter	Populations				
	1	2	3	4	5
Ovules per ovary		1.051		1.058	1.114
Seeds per fruit	0.803	1.095			1.193
Seed weight per plant			1.098	1.140	1.396

Table 5. Mean fecundity values in test populations. The values are computed from estimates in Table 2 and weighted to the frequencies of gender components shown in Table 1

Parameter	Populations				
	1	2	3	4	5
Ovules per ovary		9.24		8.77	9.04
Seeds per fruit	9.60	8.59			7.56
Seed weight per plant (mg)			530.6	707.8	931.5

Ovule Numbers

Mean ovule numbers per plant revolved around 9. Table 4 shows that mean estimates in females exceeded those in hermaphrodites by 5-10 per cent.

We could not be sure whether all ovules scored contained viable embryosacs. It is possible that hermaphrodites contained larger numbers of inviable ovules, so that dissimilarities in this parameter between the two gender types were in reality as large as those between seed numbers (Tables 2 and 4). In counting higher ovule numbers in flowers of females we could have measured the direct effect of a diversion of resources away from pollen grains, which failed to undergo final phases of development a week before anthesis (Horovitz and Galil 1972), and their reallocation to embryosac development. Some ovules in the available range, doomed to abortion in bisexual plants, could have benefited in this manner in the sense of Darwin (1877). Charnov et al. (1976) have proposed that hermaphroditism is so successful in plants because the two costly processes of pollen production and seed maturation are separated by a time interval and do not compete for the same resources. However, there could be a marginal element of competition, impairing seed yields. Marginal gynodioecy could then be viewed as a measure to alleviate this competition.

Alternatively, higher mean ovule numbers in male steriles could be a genetically fixed feature of the female plasma type.

Number of Seeds per Fruit

Seed numbers had a mean value per plant of about 8.6. In Population 1, an exceptional female to hermaphrodite seed number ratio of below one was obtained (Table 4). In the other populations this ratio was higher than in ovules.

Unless it was based on larger numbers of inviable ovules in the hermaphrodite parent, this post-floral phenomenon could signify a better supply of resources to seed development in the unisexual seed parent. It could also be the result of a better use of pollen. Because of self incompatibility, wasteful self pollinations are bound to occur in heavily visited bisexual parents. The abolition of male organs can be a measure to reduce such wasteful self pollinations in self incompatible species with clustered inflorescences. Such a function has been attributed to gynomonoecy in beetle pollinated self incompatible Compositae by Burtt (1978) citing Gottsberger. The reduction of wasteful self pollinations is not among the generally recognized roles of gynodioecy. Yet the low seed set in hermaphrodites in the self incompatible *Rhus* populations studied by Young (1972) may be the result of ineffective intra-plant pollinations and gynomonoecy and gynodioecy may be counter-devices to such waste. Another case in point may be that of self incompatible *Cortarderia selloana* in which male steriles have more numerous florets per spikelet than hermaphrodites. In *Cortaderia richardii*, in which self pollinations are viable, floret numbers in the two genders are equal (Connor 1973).

With rising proportions of male steriles in the population, seed numbers per fruit decline (Table 5). This appears to be a symptom for decreasing pollen loads deposited on the flower as the number of pollen donor plants drops below that found in stable wild populations. As shown in Table 6, the seed set relative to fertilizable ovules in a wild population with few male steriles is 10 per cent higher than that in the garden population in which male steriles exceed their frequency in nature. The less severe seed set reductions in male steriles compared with hermaphrodites (Tables 2, 6) confirm better use of diminishing pollen loads.

The effects of reductions of seed numbers per fruit on total seed yields cannot be gauged fully from the comparisons that were made. We have no data on numbers of flowers and fruits produced by the two gender types. Where pollen is limiting, the Brewbaker (1963) effect may result in only partially fertilized flowers.

Seed Weights

Tables 2 and 5 show a rising seed weight per plant from Populations 3 to 5, notwithstanding rising frequencies of male steriles. This may reflect increasingly higher frequencies of pollen vectors in the vicinity of apiaries (Table 1), as well as better growing conditions under irrigation and lack of competition in the garden. With the general increase in seed weights, the extent to which females exceed hermaphrodites rises from 10 to 40 per cent (Table 4). The data may thus substantiate a superiority of females under improving conditions. The differences noted between gender types and between populations either represent differences in total numbers of seeds per plant or weight differences in individual seeds, or both.

Table 6. Seed set per fruit under different frequencies of male steriles in the population

Population	% MS	Ratio of mean seed no. to mean ovule no.	
		in Male steriles	in Hermaphrodites
2	3	0.967	0.928
5	23	0.879	0.823

Discussion

Importance of Restorer Genes

The data presented are favourable for the spread of females. Cytoplasmic inheritance coupled with fecundity advantages in females and self incompatibility in hermaphrodites should lead to a total replacement of hermaphrodites by females (Lewis 1941; Lloyd 1974, 1976) and hence to the extinction of pollenless populations. The potential spread of females and even their persistence after the extinction of normal hermaphrodites could be further favoured by interbreeding between plants which produce fertile anthers temporarily, under low temperatures (Ilan et al. 1977). However, no unstable largely female populations were encountered in our surveys. Equilibrium frequencies of females are maintained at 2-10 per cent.

The restorer genes observed in some crosses are the only known antagonistic force which halts the spread of unisexuality. Increases in male steriles in accordance with their fecundity and their diminishment caused by an accumulation of restorer genes appear to be held in equilibrium, and equilibrium frequencies appear to be dictated by amounts of useable surplus pollen produced in the population.

We suggest that restorer genes are key factors in gynodioecious populations in which male sterility is inherited through the cytoplasm. As such they warrant serious research and incorporation into models of population dynamics.

Manipulation of Gynodioecy to Increase Crop Seed Yields

If gynodioecy can contribute to seed yields in nature, its manipulation could also be advantageous in crop production. It is likely that some cases of vigour noted in cultivated F_1 seed progenies from male steriles are not entirely manifestations of heterosis but evidence for enhanced maternal aid accorded to them by their seed parents. To produce such an effect, the parent population could conceivably be of a single genotype in characters other than those determining sex. A comparative survey of seed yields in male steriles and hermaphrodites in crop plants known to be abundant pollen producers should show whether benefits can be reaped from applying the measures of pollen economy, which we find in nature, to seed production plots.

Literature

Arroyo, K.M.T.; Raven, P.H. (1975): The evolution of subdioecy in morphologically gynodioecious species of *Fuchsia* sect. *Encliandra* (Onagraceae). Evolution **29**, 500-511

Assouad, W.B.; Dommée, B.; Loumaret, R.; Valdeyron, G. (1978): Reproductive capacities in the sexual forms in the gynodioecious species *Thymus vulgaris* L. Bot. J. Linnean Soc. 77, 29-30

Brewbaker, J.L.; Kwack, B.H. (1963): The essential role of calcium ion in pollen germination and pollen tube growth. Amer. J. Bot. **50**, 859-865

Burrows, C.J. (1960): Studies in *Pimelea*. 1: The breeding system. Transact. Roy. Soc. New Zeal. **88**, 29-45

Burtt, B.L. (1978): Notes on the evolution of Compositae: Compositae Newsl. 7, 6-7

Charlesworth, B.; Charlesworth, D. (1978): A model for the evolution of dioecy and gynodioecy. Am. Nat. **112**, 975-997

Charnov, E.L.; Maynard Smith, J.; Bull, J.J. (1976): Why be a hermaphrodite? Nature **263**, 125-126

Connor, H.E. (1973): Breeding systems in *Cortaderia*. Evolution **20**, 433-455

Correns, C. (1916): Untersuchungen über Geschlechtsbestimmung bei Distelarten, Sber.-Kgl. Preuß. Akad. Wiss. **20**, 448-477

Cruden, R.W. (1977): Pollen-ovule ratios; a conservative indicator of breeding systems in flowering plants. Evolution **31**, 32-46

Darwin, C.R. (1877): The different forms of flowers on plants of the same species. London: John Murray

Horovitz, A.; Galil, J. (1972): Gynodioecism in East Mediterranean *Hirsfeldia incana*. Bot. Gaz. **133**, 127-131

Horovitz, A.; Harding, J. (1972): Genetics of *Lupinus*. 5: Intraspecific variability for reproductive traits in *Lupinus nanus*. Bot. Gaz. **133**, 155-165

Hutchinson, J. (1959): The families of flowering plants. Oxford: Clarendon Press

Ilan, R.; Galil, J.; Horovitz, A. (1977): Effects of low temperatures and short days on sex expression in male sterile *Hirschfeldia incana*. Isr. J. Bot. **26**, 51-52

Lewis, D. (1941): Male sterility in natural populations of hermaphrodite plants. New Phytol. **40**, 56-63

Lloyd, D.G. (1974): Theoretical sex ratios of dioecious and gynodioecious angiosperms. Heredity **32**, 11-34

Lloyd, D.G. (1976): The transmission of genes via pollen and ovules in gynodioecious angiosperms. Theor. Pop. Biol. **9**, 299-316

Lloyd, D.G.; Myall, A.J. (1976): Sexual dimorphism in *Cirsium arvense* L. Scop. Ann. Bot. **40**, 115-123

Mather, K. (1940): Outbreeding and separation of the sexes. Nature **145**, 484-486

Ross, M.D. (1978): The evolution of gynodioecy and subdioecy. Evolution **32**, 174-188

Ross, M.D.; Weir, B.S. (1975): Maintenance of male sterility in plant populations. 3: Mixed selfing and random mating. Heredity **35**, 21-29

Ross, M.D.; Weir, B.S. (1976): Maintenance of males and females in hermaphrodite populations and the evolution of dioecy. Evolution **30**, 425-441

Valdeyron, G.; Dommée, B., Valdeyron, R. (1973): Gynodioecy: another computer simulation model. Am. Nat. **107**, 454-459

Willis, J.C. (1892): On gynodioecism in the Labiatae. Proc. Cambridge Philos. Soc. 7, 348-351

Young, D.A. (1972): The reproductive biology of *Rhus integrifolia* and *Rhus ovata* (Anacardiaceae). Evolution **26**, 406-414

Received September 5, 1979. Accepted November 12, 1979
Communicated by P. Spiegel-Roy

Dr. A. Horovitz
Department of Genetics, The Hebrew University of Jerusalem
Jerusalem (Israel)

Dr. A. Beiles
Institute of Evolution, University of Haifa
Haifa (Israel)