

Sex expression and fruit set in *Silene littorea* (Caryophyllaceae): variation among populations

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The flowers of *Silene littorea* may be female or hermaphrodite, and an individual may show any of three sexual phenotypes: female-flowers-only, hermaphrodite-flowers-only, or mixed-type. We estimated the relative frequencies and fruit sets of each flower type and each sexual phenotype in eleven *S. littorea* populations in northwest Spain. For all plants and sites combined, 18% of flowers were female and 82% hermaphrodite. Individuals either had only female flowers (6%), only hermaphrodite flowers (43%), or had both flower types (51%). The relative frequencies of female and hermaphrodite flowers, and of sexual phenotypes, varied among populations. There was no significant effect of flower sex on its probability of setting fruit. Differences among individual plants within populations had the most prominent role in explaining variation among flowers in probability of setting fruit, while population and its interaction with flower sex had a negligible and nonsignificant influence. Mean fruit set per plant did not differ significantly between sexual phenotypes. Neither population nor its interaction with sexual phenotype accounted for significant amounts of between-plant variance in fruit set.

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Introduction

Gynodioecy is common in many different plant groups. In most gynodioecious species, some individuals have flowers with both fertile female and fertile male organs, while other individuals have flowers with fertile female organs but atrophied male organs (Delph 1993).

A number of studies have shown that male sterility in gynodioecious plants is under genetic control, and that this control may be due to both nuclear and cytoplasmic factors, thus permitting the trait to be maintained (Maki 1992). The maintenance of sexual systems of this type appears to require female plants to have some sort of reproductive advantage over hermaphrodite plants (see Shykoff 1992).

Environmental factors may also contribute to the control of sexual phenotype, giving rise to temporal variation within individuals. Lloyd (1980) suggests that at different stages in its development the plant may "take decisions" affecting sex expression. According to Diggle (1993) such decisions are "taken" by individual flowers.

Some gynodioecious taxa have populations comprising three sexual phenotypes: individuals with hermaphrodite flowers only, individuals with female flowers only, and individuals with both types of flower (Hermanutz & Innes 1994). This population structure is a form of polygamy (Richards 1986). Polygamy is usually the result of unstable sex expression in genetically dioecious plants, due to environmental

effects. In perennial plants with complex sex expression patterns, individuals may show year-to-year variation in sexual phenotype, as has been demonstrated in various species (e.g. the genus *Acer*, Matsui 1995 and references therein; but see Schlessman 1986).

Sex expression in gynodioecious plants, and the reproductive success of each sexual phenotype, may be affected by sexual-phenotype ratios in the population and by environmental factors (see Willson 1983; Koelewijn & Van-Damme 1996; Folke & Delph 1997; Korpelainen 1998). Gynodioecious systems may thus show variation in the proportions of the different sexual phenotypes and in their respective fruit set levels (Webb 1979; see Willson 1983, and more recently Folke & Delph 1997; Puterbaugh et al. 1997, El-Keblawy & Freeman 1999; Webb 1999).

The different flower types within a gynodioecious population show morphological differences. In accordance with Bateman's principle (Bateman 1948), hermaphrodite flowers (i.e. flowers which retain male function) should invest more in attraction than female flowers; in other words, hermaphrodite flowers should be bigger and more "eye-catching" than female flowers (see however Willson et al. 1994; Delph et al. 1996). However, female flowers tend to be more fecund (Klinkhamer et al. 1994; Schultz & Ganders 1996; see Willson 1983 and references therein), although not always to the extent predicted by theory (see Shykoff 1988). Indeed, in some species fruit or seed production by female flowers is no higher than that by hermaphrodite flowers (Sugawara et al. 1994; Talavera et al. 1996), and may even be lower (Del Castillo 1993).

In the genus *Silene*, sexual polymorphism is frequent and has been investigated in a number of studies. Gynodioecy is the most common reproductive system and an ancestral character (Desfeux et al. 1996) present in various phylogenetically distant subgroups within the genus (Oxelman & Lidén 1995, Oxelman et al. 1997). The only European annual species in which gynodioecy has been studied to date is *S. stockenii*, which is related to *Silene littorea*, the species studied in this paper. The two species belong to the section *Erectorefractae* Chowdhuri, whose distribution centre is located in the southern Iberian Peninsula and comprises nine taxa (Talavera et al. 1996).

Silene littorea, like other species of the genus (*S. vulgaris*, Jolls & Chenier 1989), shows polygamy, with populations comprising female-flower-only, hermaphrodite-flower-only and mixed-type individuals. Here, we report a study of *S. littorea* populations in northwest Spain, in which we investigated 1) the frequency of flowers of each type (female and hermaphrodite) and of individuals of each type (female-only, hermaphrodite-only and mixed-type) in each population, and 2) fruit set by the different flower types, the

different sexual phenotypes and the different populations.

Methods

The plant

Silene littorea Brot. is a low-growing annual plant, ranging in form from unbranched to highly branched. The flowers, which develop singly or on monochasia, are pink or pinkish white and have a tubular calyx that broadens markedly following fruit set. The number of flowers produced per plant is highly variable (range 1-52 in our populations), and is significantly higher in mixed-type individuals (data not shown). The fruit is an ovoid capsule that dehisces upon maturity. The seeds are kidney-shaped and finely reticulated.

Field methods

Studies were performed in 11 well-separated populations on 11 beaches on the coast of Galicia (northwest Spain), between the Praia de Trece (Coruña Province) to the north and the Cíes islands (Pontevedra Province) to the south. In each population, at the end of the 1996 flowering/fruiting period (first half of May), we collected 40 plants representative of size distribution in that population. The total number of flowers collected was 4545.

On each plant, we counted the number of female and hermaphrodite flowers, and recorded the number of flowers of each type that had set fruit. Fruit set was considered to have occurred when ovary development was clearly apparent from swelling. The capsule of *Silene littorea* (or the remains of the capsule, if it has suffered herbivory) remains intact until after seed release, so it is easy to identify flowers that have produced fruit and flowers that have not, despite the asynchrony of flowering both within and among plants. The determination of flower sex is likewise straightforward, since the remains of stamens or aborted stamens remain in place during fruit maturation.

A potential problem with this design is that the herbivory suffered by flowers and fruits may have different effects on flowers of different sex or on plants of different sexual phenotype. To rule out this possibility, we carried out a preliminary study of 75 plants from a population in the same region (Corrubedo, A Coruña Province), monitoring flower production, herbivory damage and fruit production. The results of this study (not shown) indicated that herbivory, although frequent, did not differentially affect either flowers of different sex or plants of different sexual phenotype.

Data analysis

The effects of flower sex and plant sexual phenotype on fruit set were investigated by generalized linear mixed modeling using the SAS Macro program GLIMMIX (Littell et al. 1996). Generalized linear mixed models represent an extension of ordinary generalized linear models that allow for a distinction between fixed and random effects in the model. For further details and application in the context of plant reproduction studies, see Herrera (2000).

Results

Sex structure of the different populations

Of the total of 4545 flowers examined, 813 (17.9%) were female and 3732 (82.1%) were hermaphrodite (flowers from all individuals and populations combined). The frequencies of the two flower types varied significantly among populations ($\chi^2 = 186.1$, $df = 10$, $p < 0.0001$; flowers from all individuals in the same population combined into a single sample), although hermaphrodite flowers predominated at all localities and differences between populations were relatively minor (Fig. 1).

Of the total of 440 individuals examined, 25 (5.7%) were female-only, 192 (43.6%) hermaphrodite-only, and 223 (50.7%) mixed-type. The relative frequencies of individuals with different sexual phenotypes differed significantly among populations (likelihood ratio $G = 79.8$, $df = 20$, $p < 0.001$). Hermaphrodite-only plants predominated at some sites (e.g. Areas, Cies), while mixed-type ones predominated at others (e.g. Trece,

Testal). Female-only plants were consistently rare at all sites (Fig. 2).

Fruit set

The effect of flower sex on the probability of individual flowers setting fruit was tested by fitting a generalized linear mixed model to the data, using logits as the link function and assuming a binomial error distribution. Population, individual plant (nested within population) and the interaction between population and flower sex were included as random effects in the model. After statistically accounting for these effects, there was no significant effect of flower sex on its probability of setting fruit ($F_{1,20} = 0.37$, $p = 0.55$). Differences among individual plants within populations accounted for a statistically significant amount of variance ($p < 0.001$), but neither population nor its interaction with flower sex had significant effects ($p = 0.29$ and 0.084 , respectively). The probability of a single flower setting fruit thus depended more on which plant the flower belongs to, than on any of the other factors considered.

Table 1 shows mean fruit set of plants separately by population and sexual phenotype. To investigate whether plant-level fruit set (i.e., proportion of a plant's total flowers setting fruit) was affected by the individual's sexual phenotype, we again fitted a generalized linear mixed model to the data, with sexual phenotype as a three-level (female-only, hermaphrodite-only, and mixed-type) fixed factor, and population and its interaction with sexual phenotype as random effects. The effect of sexual phenotype on fruit set was not

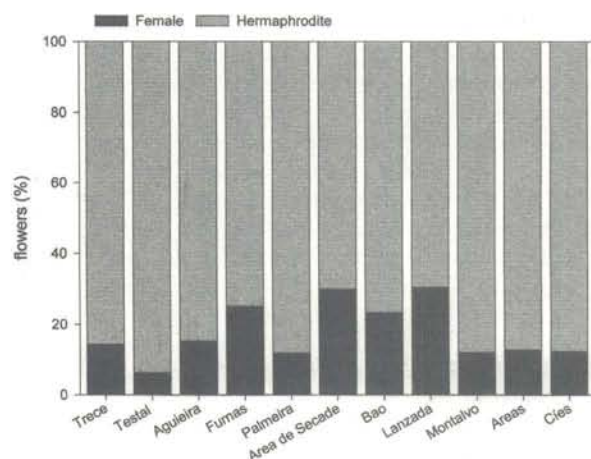


Fig. 1. Proportions of female flowers and hermaphrodite flowers in the 11 *Silene littorea* populations studied.

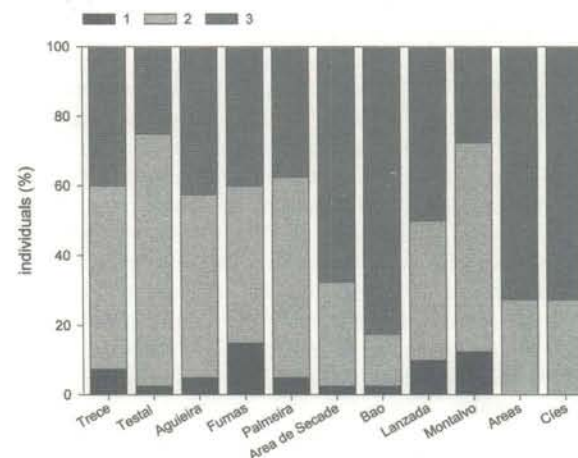


Fig. 2. Proportions of female-only ("male-sterile") (1), hermaphrodite-only (2), and mixed-type (3) individuals in the 11 *Silene littorea* populations studied.

Table 1. Mean (\pm SD) fruit set per plant (proportion of flowers setting fruit, as %), for the three sexual phenotypes (female-only, hermaphrodite-only and mixed-type) in each of the 11 *Silene littorea* populations studied. n = number of plants.

Population	(n)	All	(n)	Female-only	(n)	Hermaphrodite-only	(n)	Mixed-type
Trece	(40)	67.64 \pm 24.28	(3)	58.33 \pm 38.19	(21)	70.63 \pm 22.53	(16)	65.47 \pm 25.03
Testal	(40)	85.64 \pm 15.66	(1)	-	(29)	84.45 \pm 15.55	(10)	87.64 \pm 16.67
Aguieira	(40)	78.29 \pm 19.96	(2)	54.76 \pm 16.84	(20)	76.55 \pm 20.73	(18)	82.84 \pm 18.06
Furnas	(40)	43.67 \pm 32.30	(6)	86.82 \pm 16.20	(18)	30.65 \pm 26.79	(16)	42.14 \pm 29.35
Palmeira	(40)	35.94 \pm 27.63	(2)	73.81 \pm 37.04	(23)	31.99 \pm 25.88	(15)	36.95 \pm 27.28
Area de Secade	(39)	67.45 \pm 27.45	(1)	-	(11)	78.46 \pm 24.09	(27)	61.75 \pm 27.42
Bao	(40)	66.49 \pm 28.89	(1)	-	(6)	69.44 \pm 40.02	(33)	64.94 \pm 26.98
Lanzada	(40)	73.04 \pm 21.25	(4)	58.03 \pm 23.29	(16)	77.28 \pm 21.98	(20)	72.66 \pm 19.94
Montalvo	(40)	55.67 \pm 26.96	(5)	57.00 \pm 37.01	(24)	60.49 \pm 26.22	(11)	44.55 \pm 22.64
Areas	(40)	58.20 \pm 19.62	(0)	-	(11)	65.78 \pm 22.21	(29)	55.32 \pm 18.13
Cies	(40)	70.70 \pm 23.08	(0)	-	(11)	70.67 \pm 25.40	(29)	70.72 \pm 22.62

significant ($F_{2,28} = 0.63$, $p = 0.54$). Neither population ($p = 0.10$) nor its interaction with sexual phenotype ($p = 0.15$) accounted for significant amounts of between-plant variance in fruit set. These results indicate that the overwhelming influence of individual variation on fruit set at the individual flower level was not attributable to differences among plants in sexual phenotype.

Discussion

In almost all populations analysed to date, *Silene littorea* has a sexual system comprising three types of individuals: female-only ("male-sterile"), hermaphrodite-only and mixed-type. As pointed out by El-Keblawy et al. (1995), sexual systems with more than two sexual phenotypes are often grouped together under the term "gender inconstancies".

Considering all the populations studied together, nearly 20% of flowers were female. The frequency of flower types varies significantly among populations, with the proportion of female flowers ranging from about 5% (Testal) to about 30% (Lanzada). To our knowledge, similar variation among *Silene* populations in the relative frequencies of flower types has been so far reported only for *Silene alba* (Mulcahy 1967).

The values obtained for the frequencies of the different sexual phenotypes in our populations are in general similar to those reported by Talavera et al. (1996) for *S. stockenii*, with very low proportions of female plants. However, we observed significant variation among populations in the frequencies of sexual phenotypes. Among-population variation of this type has been reported for various gynodioecious species (Sun & Ganders 1986; Belhassen et al. 1989; Ågren & Wilson 1991; Maki 1992, 1993a; Hermanutz

& Innes 1994; El-Keblawy et al. 1995; Wolfe & Schmida 1997; El-Keblawy & Freeman 1999; though see also Willson 1983 and references therein). The possible causes of among-population variation in sexual phenotype frequency include environmental factors (such as pH, salinity, temperature), availability of resources (including water) (see Maki 1993a; El-Keblawy et al. 1995; Alatalo & Molau 1995; Koelewijn & Van-Damme 1996; Folke & Delph 1997; El-Keblawy & Freeman 1999; see Webb 1999 for a recent review), and levels of fungal infection (Hermanutz & Innes 1994). In *Thymelaea hirsuta*, for example, male plants tend to be more frequent in environments showing resource limitations, while females tend to be more frequent in "better" environments, which is attributable to the higher cost of adopting the female phenotype (El-Keblawy et al. 1995); males are also more common on sloping sites, while females are more common in depressions (El-Keblawy & Freeman 1999). In *Silene noctiflora*, increases in temperature and ethylene levels induce an increase in the proportion of female flowers on individual plants, though increased temperature does not have this effect in other species (Folke & Delph 1997). In some *Silene* species, the sexual phenotype of a given individual may be experimentally altered by, for example, modification of auxin levels, photoperiod changes, or exposure to pathogens, although the proximate mechanisms involved have yet not been fully elucidated (see Korpelainen 1998 for a review).

We do not yet have any data for *Silene littorea* on the relative costs of the different types of flower, or of the different sexual phenotypes; we are thus unable to confirm that the observed differences in sexual phenotype frequency are attributable to cost differences, as suggested for *T. hirsuta*. Nevertheless, it seems likely that resource limitations or microclimatic variations

play an important role in population sex structure. We have not observed fungal infection, but flower herbivory is common and has similar effects on the different sexual phenotypes (Medrano, unpublished data). The high frequency of mixed-type individuals in all of our study populations may be related to the population's ecology and growth patterns ("patchy environments") (Freeman et al. 1980, 1984; El-Keblawy et al. 1995).

To understand the evolution of complex sexual systems it is important to consider not only population structure but also the reproductive success rates of the different types of flower and individual. In *Silene littorea*, female-only plants were rare but occurred in almost all of the populations studied. In accordance with the "sexual allocation" theory, female-only plants will be maintained in the population only if the benefits of dedication to seed production outweigh the costs of not producing pollen. However, in the present study we did not detect differences in fruit set between the different sexual phenotypes.

Fruit and seed set patterns in gynodioecious plants have been considered in a number of studies. Many such studies have considered the fruit and seed sets of individual plants, generally finding that female-only plants are more fertile (Shykoff 1988; Maki 1993b; Hermanutz & Innes 1994; Klinkhamer et al. 1994; Koelewijn & Van-Damme 1996; Wolfe & Schmida 1997; Sakay et al. 1997, Delph et al. 1999; for a recent review see Webb 1999), though this is not always the case (Talavera et al. 1996). In the genus *Silene*, female-only plants have been found to be more fertile in *S. vulgaris* (Pettersson, 1992) and *S. acaulis* (Shykoff 1988; Delph et al. 1999).

The fact that we did not detect statistically significant differences in fruit set between the different sexual phenotypes of *S. littorea* may simply be due to type-II error (i.e., erroneous acceptance of the null hypothesis of no difference), because of the very small number of female-only plants. However, the maintenance of female-only plants in the population may in fact be attributable not to the higher fruit set (as predicted by the sexual allocation hypothesis; see above), but to seed characteristics, as reported for *S. vulgaris* (Pettersson 1992). Note, however, that studies of *S. acaulis* have argued against the possibility that female-only plants persist because of "better" seed production (Shykoff 1988; Delph et al. 1999).

Our results suggest that the observed among-plant differences in fruit set are not due to sexual phenotype, and that the probability that an individual flower will set fruit is independent of that flower's sex and dependent on the characteristics of the plant to which that flower belongs. This may be related to individual characteristics other than sexual phenotype (and number of

flowers per plant, which varies among sexual phenotypes), such as environmental characteristics (microclimate, pollinator availability, etc.), population structure (abundance or density of individuals), etc. Models in which the fitness of different sexual phenotypes is a function of local sex ratio have recently been developed by McCauley & Taylor (1997).

Female and hermaphrodite flowers of *S. littorea* differ in numerous morphological respects, notably attraction area and size of floral parts (Medrano, unpublished data). Factors like flower size or pollinator abundance (Del Castillo 1993) may influence the fruit set of the different flower types. In this study, there were no differences in fruit set between female and hermaphrodite flowers when differences between plants and populations were statistically accounted for.

The stigmas of female flowers of *S. littorea* may be more effective for pollen capture than the stigmas of hermaphrodite flowers, as occurs in other species of this genus (Bock 1976; Dulberger & Horovitz 1984; see however Shykoff 1992). On the other hand, hermaphrodite flowers may be more successful under conditions of pollen limitation if they are self-compatible (see for example Del Castillo 1993, and references therein), as is the case in *S. littorea*.

In conclusion, our results suggest that, for *S. littorea*, the probability of a single flower setting fruit depends more on which particular plant the flower belongs to than on flower type or plant sexual phenotype.

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References

- Agren, J. & Willson, M. F. 1991. Gender variation and sexual differences in reproductive characters and seed production in gynodioecious *Geranium maculatum*. – *Am. J. Bot.* 78: 470-480.
- Alatalo, J. M. & Molau, U. 1995. Effect of altitude on the sex ratio in populations of *Silene acaulis* (Caryophyllaceae). – *Nord. J. Bot.* 15: 251-256.
- Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. – *Heredity* 2: 349-368.
- Belhassen, E., Traband, L., Couvet, D. & Gouyon, P. H. 1989. An example of nonequilibrium processes: gynodioecy of

- Thymus vulgaris* L. in burned habitats. – *Evolution* 43: 662-667.
- Bock, C. 1976. Polymorphisme floral et stérilité mâle chez le *Silene acaulis* (L.) Jacq. – *Comptes Rendues de l'Académie des Sciences (Paris)* 282: 1865-1868.
- Del Castillo, R. 1993. Consequences of male sterility in *Phacelia dubia*. – *Evol. Trends Pl.* 7: 15-22.
- Delph, L. F. 1993. Factors affecting intraplant variation in flowering and fruiting in the gynodioecious species *Hebe subalpina*. – *J. Ecol.* 81: 287-297.
- , Galloway, L. F. & Stanton, M. L. 1996. Sexual dimorphism in flower size. – *Am. Nat.* 148: 299-320.
- , Bailey, M. F. & Marr, D. L. 1999. Seed provisioning in gynodioecious *Silene acaulis* (Caryophyllaceae). – *Am. J. Bot.* 86: 140-144.
- Desfeux, C., Maurice, S., Henry, J. P., Lejeune, B. & Gouyon, P. H. 1996. Evolution of reproductive systems in the genus *Silene*. – *Proc. Roy. Soc. London, Ser. B. Biol. Sci.* 263: 409-414.
- Diggle, P. M. 1993. Developmental plasticity, genetic variation, and the evolution of andromonoecy in *Solanum hirtum* (Solanaceae). – *Am. J. Bot.* 80: 967-973.
- Dulberger, R. & Horovitz, A. 1984. Gender polymorphism in flowers of *Silene vulgaris* (Moench) Garcke (Caryophyllaceae). – *Bot. J. Linn. Soc.* 89: 101-117.
- El-Keblawy, A. A., Lovett-Doust, J., Lovett-Doust, L. & Shaltout, K. H. 1995. Labile sex expression and dynamics of gender in *Thymelaea hirsuta*. – *Écoscience* 2: 55-66.
- & Freeman, D. C. 1999. Spatial segregation by gender of the subdioecious shrub *Thymelaea hirsuta* in the Egyptian desert. – *Int. J. Plant Sci.* 160: 341-350.
- Folke, S. H. & Delph, L. F. 1997. Environmental and physiological effects on pistillate flower production in *Silene noctiflora* L. (Caryophyllaceae). – *Int. J. Plant Sci.* 158: 501-509.
- Freeman, D. C., Harper, K. T. & Charnov, E. L. 1980. Sex change in plants: old and new observations and new hypotheses. – *Oecologia* 47: 222-232.
- , McArthur, E. D. & Harper, K. T. 1984. The adaptive significance of sexual lability in plants using *Atriplex canescens* as a principal example. – *Ann. Missouri Bot. Gard.* 71: 265-277.
- Hermanutz, L. A. & Innes, J. D. 1994. Gender variation in *Silene acaulis* (Caryophyllaceae). – *Plant. Syst. Evol.* 191: 69-81.
- Herrera, C. M. 2000. Flower-to-seedling consequences of different pollination regimes in an insect-pollinated shrub. – *Ecology* 81: 15-29.
- Jolls, C. L. & Chenier, T. C. 1989. Gynodioecy in *Silene vulgaris* (Caryophyllaceae): progeny success, experimental design, and maternal effects. – *Am. J. Bot.* 76: 1360-1367.
- Klinkhamer, P. G. L., De Jong, T. J. & Nell, H. W. 1994. Limiting factors for seed production and phenotypic gender in the gynodioecious species *Echium vulgare* (Boraginaceae). – *Oikos* 71: 469-478.
- Koelewijn, H. P. & Van-Damme, J. M. M. 1996. Gender variation, partial male sterility and labile sex expression in gynodioecious *Plantago coronopus*. – *New Phytol.* 132: 67-76.
- Korpelainen, H. 1998. Labile sex expression in plants. – *Biol. Rev.* 73: 157-180.
- Littell, R. C., Milliken, G. A., Stroup, W. W. & Wolfinger, R. D. 1996. SAS System for mixed models. – SAS Institute, Cary, North Carolina, USA.
- Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. – *New Phytol.* 86: 69-79.
- Maki, M. 1992. Fixation index and genetic diversity in hermaphroditic and gynodioecious populations of Japanese *Chionographis* (Liliaceae). – *Heredity* 68: 329-336.
- 1993a. Floral sex ratio variation in hermaphrodites of gynodioecious *Chionographis japonica* var. *kurohimensis* Ajima et Satomi (Liliaceae). – *J. Plant Res.* 106: 181-186.
- 1993b. Outcrossing and fecundity advantage of females in gynodioecious *Chionographis japonica* var. *kurohimensis* (Liliaceae). – *Am. J. Bot.* 80: 629-634.
- Matsui, K. 1995. Sex expression, sex change and fruiting habit in an *Acer rufinerve* population. – *Ecol. Res.* 10: 65-74.
- McCauley, D. E. & Taylor, D. R. 1997. Local population structure and sex ratio: Evolution in gynodioecious plants. – *Am. Nat.* 150: 406-419.
- Mulcahy, D. L. 1967. Optimal sex-ratio in *Silene alba*. – *Heredity* 22: 411-423.
- Oxelman, B. & Lidén, M. 1995. Generic boundaries in the tribe *Sileneae* (Caryophyllaceae) as inferred from nuclear rDNA sequences. – *Taxon* 44: 525-542.
- , Lidén, M. & Berglund, D. 1997. Chloroplast *rps16* intron phylogeny of the tribe *Sileneae* (Caryophyllaceae). – *Pl. Syst. Evol.* 206: 393-410.
- Pettersson, M. W. 1992. Advantages of being a specialist female in the gynodioecious *Silene vulgaris* s.l. (Caryophyllaceae). – *Am. J. Bot.* 79: 1389-1395.
- Puterbaugh, M. N., Wied, A. & Galen, C. 1997. The functional ecology of gynodioecy in *Eritrichum aretioides* (Boraginaceae), the alpine forget-me-not. – *Am. J. Bot.* 84: 393-400.
- Richards, A. J. 1986. *Plant Breeding Systems*. – George Allen and Unwin Publishers, London.
- Sakay, A. K., Weller, S. G., Chen, M. L., Chou, S. Y. & Tسانوت, C. 1997. Evolution of gynodioecy and maintenance of females: The role of inbreeding depression, outcrossing rates, and resource allocation in *Schiedea adamantis* (Caryophyllaceae). – *Evolution* 51: 724-736.
- Schlessman, M. A. 1986. Gender diphasy (“sex choice”). – In: Lovett-Doust, J. & Lovett-Doust, L. (eds), *Plant Reproductive Ecology. Patterns and strategies*. Oxford University Press, New York, pp. 139-153.
- Schultz, S. T. & Ganders, F. R. 1996. Evolution of unisexuality in the Hawaiian flora: a test of microevolutionary theory. – *Evolution* 50: 842-855.
- Shykoff, J. A. 1988. Maintenance of gynodioecy in *Silene acaulis* (Caryophyllaceae): stage-specific fecundity and viability selection. – *Am. J. Bot.* 75: 844-850.
- 1992. Sex polymorphism in *Silene acaulis* (Caryophyllaceae) and the possible role of sexual selection in maintaining females. – *Am. J. Bot.* 79: 138-143.
- Sugawara, T., Nakamura, A., Kanbayashi, M. & Mikami, M. 1994. Floral and reproductive biology of gynodioecious *Dianthus superbus* L. var. *superbus* (Caryophyllaceae). – *Acta Phytotax. Geobot.* 45: 23-31.
- Sun, M. & Ganders, F. R. 1986. Female frequencies in gynodioecious populations correlated with selfing rates in hermaphrodites. – *Am. J. Bot.* 73: 1645-1648.
- Talavera, S., Arista, M. & Salgueiro, F. J. 1996. Population size, pollination and breeding system of *Silene stockenii*

- Chater (Caryophyllaceae), an annual gynodioecious species of southern Spain. – Bot. Acta 109: 333-339.
- Webb, C. J. 1979. Breeding systems and the evolution of dioecy in New Zealand apioid Umbelliferae. – Evolution 33: 662-672.
- 1999. Empirical studies: Evolution and Maintenance of Dimorphic Breeding Systems. – In: Geber, M. A., Dawson, T. E. & Delph, L. F. (eds), Gender and Sexual Dimorphism in Flowering Plants. Springer-Berlag, Berlin Heidelberg, pp. 61-95.
- Willson, M. F. 1983. Plant reproductive ecology. – John Wiley & Sons, New York.
- Willson, P., Thomson, J. D., Stanton, M. L. & Rigney, L. 1994. Beyond floral batemanian: gender biases in selection for pollination success. – Am. Nat. 143: 283-296.
- Wolfe, L. M. & Shmida, A. 1997. The ecology of sex expression in a gynodioecious Israeli desert shrub (*Ochradenus baccatus*). – Ecology 78: 101-110.