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Research Article

Effects of soil moisture and floral herbivory on sexual expression in a gynodioecious orchid

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Abstract Compared to pollinator limitation and inbreeding avoidance, the role of ecological factors in sexual differentiation has received less attention in sexual dimorphic plants. The effect of soil moisture and florivory on two sexual morphs in a gynodioecious orchid, *Satyrium ciliatum,* was investigated in seven gynodioecious (with both female and hermaphrodite individuals) and 15 hermaphroditic (with only hermaphrodite individuals) populations. Our result showed that, compared to hermaphrodites, females tended to occur in drier sites in which soil water content was consistently lower than that of hermaphrodites in all gynodioecious populations. The soil water content where hermaphrodites grew was not significantly different between gynodioecious and hermaphroditic populations. We observed that females experienced less attack by insect florivores than hermaphrodites in gynodioecious populations, and hermaphroditic populations had higher insect attack than gynodioecious populations. Our results provide evidence for females being favored in stressful sites. However, the soil moisture and degree of florivory were not correlated to female frequency among populations, suggesting that the two ecological factors have not induced strong effects or other factors that may also influence the sex ratio in the facultative apomictic orchid.

Key words ecological factors, gynodioecious orchid, herbivory, *Satyrium ciliatum*, sex ratio, soil moisture.

The invasion of male-steriles (females) into hermaphrodite populations, leading to gynodioecy, has evolved many times in flowering plants. Approximate 6% of flowering plants show this dimorphic breeding system, in which both female and hermaphroditic individuals coexist in populations (Webb, 1999). Females, which save resources by not producing pollen, may obtain higher seed fitness than hermaphrodites by producing more seeds and/or higher quality seeds (reduced inbreeding depression), and can thereby be maintained in populations (see Dufay & Billard, 2012). In the past two decades, studies began to emphasize the effects of ecological context on this sexual dimorphism, including harsh environment or herbivores (Delph, 1990, 2003; Wolfe & Shmida, 1997; Ashman, 1999, 2002, 2006; Barr, 2004; Vaughton & Ramsey, 2004; Caruso & Case, 2007; Wise & Hebert, 2010).

Darwin (1877) first noted the association between sexual dimorphism and harsh environment and stated that females were more prevalent in harsh sites in gynodioecious species. A recent review showed that studies of 14 gynodioecious species all found females preferring to occur in harsh environments with lower resource availability than hermaphrodites (Ashman, 2006). To explain such an association, Delph (1990) hypothesized that the seed set of hermaphrodites would be more variable and plastic than that of females among different resource level environments. Given that hermaphrodites allocate resources to both pollen and seeds, in harsh environments resources may be inadequate for hermaphrodites to maintain both sexual functions. However, females are less costly, in that they only allocate resources to seeds. Therefore, resource-stressful environments can cause a plastic reduction of seed production in hermaphrodites, and females can be favored because of relatively higher seed fitness (Barr, 2004; Dorken & Mitchard, 2008; Bishop et al., 2010). In populations, the equilibrium frequency of females depends on their seed fitness relative to that of hermaphrodites. The effect of herbivores on sexual expression received recent attention in gynodioecious species. Several studies showed that pollen-bearing plants, such as hermaphrodites and males, are often preferred by herbivores (Marshall & Ganders, 2001; Ashman, 2002; Collin et al., 2002; Ashman et al., 2004;

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Tsuji & Sota, 2010; McCall & Barr, 2012). This male-biased herbivory is more obvious when damage is to reproductive organs such as floral buds, flowers, and seeds (Ashman, 2006). The effect of florivores on female frequency could be similar to a harsh environment. For example, male-biased florivores decreased seed fertility in hermaphrodites more than in females, and this would promote relative fitness in females and thus increase female frequency.

To understand maintenance of gynodioecy and interpopulation variation in sex ratios within species, investigations of both the abiotic and biotic factors are needed. Here, we evaluate the effects of both harsh environment and florivores on female frequency in a gynodioecious orchid *Satyrium ciliatum* Lindl. The terrestrial herb is the only known orchid that is hermaphroditic or gynodioecious in wild populations (Chen, 1979). It wildly distributes in alpine areas in southwest China and a preliminary investigation showed that sex ratios varied largely among populations and flowers were damaged by various insect herbivores (Huang et al., 2009). Furthermore, the indirect effects of stressful environments mediated by pollinators and inbreeding depression seem to be minimal in this orchid, given that pollinators were extremely rare and seed production was largely dependent on parthenogenesis rather than sexual reproduction (Huang et al., 2009). This unusual reproductive nature of parthenogenesis in the gynodioecious orchid permits us to investigate the direct effects of soil moisture and herbivores on variation in female frequency through a comparison of hermaphroditic and gynodioecious populations. Particularly, we address the following questions: (i) Are females more prevalent in dry sites? (ii) Do florivores prefer hermaphrodites over females? (iii) Does the soil water content or the level of florivory correlate with female frequency within populations?

1 Material and methods

1.1 Study species and populations

Satyrium ciliatum is an alpine terrestrial orchid occurring on open slopes at 2000–3600 m above sea level in southwest Asia. Flowering plants have a corm, a single stem, one or two annual leaves, and an inflorescence spike with 10–35 flowers that open gradually from the bottom upward. Flowers are pink and have a twin-spurred labellum. The nectar spurs in females are significantly shorter than in hermaphrodites (Lu & Huang, 2010). Flowers of females have either aborted pollinia or no pollinia, whereas hermaphrodites have fully functional pollinia. We used these two characters to identify the sex of each individual in the field. Hermaphrodites and females were patchily distributed in gynodioecious populations. All 22 sample populations were located in three regions of Yunnan Province, China, including Diqing, Lijiang, and Shangri-la (see Huang et al., 2009). We recorded the female frequency in all populations.

1.2 Soil moisture

To measure soil moisture, we used a ND22TRSF-V fast soil moisture meter (Beijing Midwest Automation, Beijing, China) sampling 10 points at depths of 15–20 cm close to the plants in each hermaphroditic population. Given that hermaphrodites and females distributed patchily in gynodioecious populations, we collected 10 samples of soil moisture for each sex in every patch. The sample points were at least 1 m distant from each other and were taken in sunny days.

1.3 Florivore preference

Previous observations showed that flowers were damaged by various insects in the field. For example, Tenthredinidae larvae consumed all floral organs including pollinia, style, and labellum, whereas beetles and syrphid flies only consumed pollinia of *S. ciliatum* (Huang et al., 2009). To compare the flower damage by florivores in the two sexual morphs, we recorded the damage of florivores for both sexual morphs in seven gynodioecious populations and hermaphrodites in 15 hermaphroditic populations at the late stage of florescences by counting the proportion of individuals attacked and the proportion of flowers per individual attacked. The proportion of individuals predated was calculated as the number of plants predated in the population divided by the total number of plants for each morph in the population. The proportion of flowers predated was calculated as the number of flowers predated on 30 randomly sampled plants divided by the sum number of flowers of these sample plants. If there were not enough plants we surveyed all of them.

1.4 Data analysis

One-way ANOVAs were carried out to compare soil moisture between hermaphroditic and female sites within gynodioecious populations and among three sexual types among populations, that is, females (F-GY) and hermaphrodites (H-GY) in gynodioecious population and hermaphrodites in hermaphroditic population (H-H). Comparisons of proportions of individuals and flowers damaged by herbivores among the three sexual types were carried out using non-parametric analysis (Kuraskal–Wallis test), given that the data were not normally distributed. To test whether soil moisture and the degree of florivory affect sex ratios within populations, we analyzed the relationship between female frequency and soil moisture at the population level using Pearson's correlation (combining data from the samples of two sexual morphs), and the proportion of the individuals and flowers damaged by herbivores at population level among seven gynodioecious populations.

2 Results

2.1 Soil moisture

Of 22 studied populations, seven were found to be gynodioecious and 15 were hermaphroditic. In gynodioecious populations, female frequency varied largely, ranging from 0.15 to 0.84 (Table 1). The soil moisture of female sites was significantly lower than that of hermaphrodites within all seven gynodioecious populations (Table 1), indicating females were favored by drier sites. Similarly, soil moisture of female sites was lower than that of hermaphrodites in hermaphroditic populations, whereas the soil moisture of hermaphrodites was not significantly different between gynodioecious and hermaphroditic populations (Fig. 1). One may expect that more females occur in drier habitats with less soil water content. We observed that female frequencies tended to decrease with increasing soil moisture (Table 1). However, this prediction is not supported in that the soil moisture at the population level did not

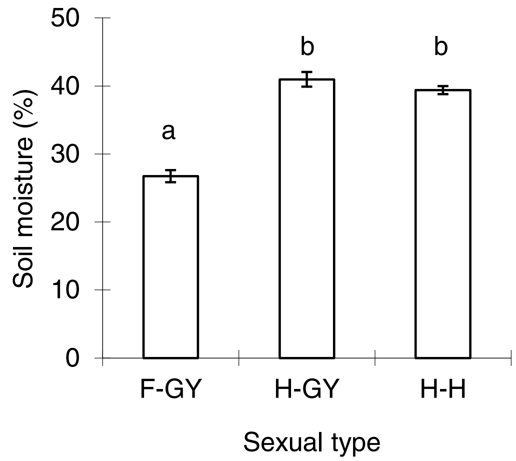

Fig. 1. Comparisons of soil moisture (mean±S.E.) of three sexual types among wild populations of *Satyrium ciliatum* in Yunnan, China. The same letters above bars indicate no significant difference. F-GY, females in gynodioecious populations; H-GY, hermaphrodites in gynodioecious populations; H-H, hermaphrodites in hermaphroditic populations.

Table 1 Female frequency in each population, soil moisture (mean±S.E.), and proportion of individual and flowers damaged by herbivores in 22 populations of *Satyrium ciliatum*, according to sexual type

Population	Female frequency	Sexual types	Soil moisture (%)	Individuals damaged (%)	Flowers damaged (%)
CM1	0.16	H-GY	32.5* (1.3)	0	0
		F-GY	23.1 (1.3)	0	0
CM2	0.15	H-GY	31.2* (2.3)	10.0	1.0
		F-GY	21.1 (2.5)	0	0
GBC1	0.84	H-GY	46.0* (0.6)	40.0	4.2
		F-GY	30.2 (2.1)	1.7	0.1
GBC2	0.74	H-GY	48.0* (0.9)	43.8	5.9
		F-GY	33.3 (1.3)	8.6	0.7
GBC3	0.55	H-GY	48.3* (1.6)	14.8	1.8
		F-GY	31.2 (0.9)	0	0
GBC4	0.35	H-GY	48.1* (1.5)	0	0
		F-GY	30.6 (1.4)	0	0
ABG1	0.8	H-GY	32.6* (1.7)	6.7	1.1
		F-GY	17.8 (1.2)	0	0
CM3	0	H-H	47.8 (1.2)	15.0	2.9
CM4	0	H-H	43.0 (1.1)	10.0	0.9
LJM1	0	H-H	41.6 (1.0)	14.3	1.1
LJM2	0	H-H	44.3 (1.5)	33.3	3.3
JDSM1	0	H-H	43.7 (1.4)	46.7	16.9
JDSM2	0	H-H	40.3 (1.4)	88.9	37.7
LGL	0	H-H	–	69.2	6.4
TGC1	0	H-H	45.7 (2.3)	10.0	1.4
TGC2	0	H-H	28.6 (1.7)	30.3	3.2
TGC3	0	H-H	37.9 (1.2)	25.0	3.1
TGC4	0	H-H	39.1 (0.7)	25.0	4.6
HPC	0	H-H	33.7 (2.1)	22.0	3.3
JMC	0	H-H	39.1 (1.0)	6.7	0.4
SC	0	H-H	31.4 (1.9)	33.3	4.3
ABG2	0	H-H	35.3 (1.2)	24.8	3.7

F-GY, females in gynodioecious populations; H-GY, hermaphrodites in gynodioecious populations; H-H, hermaphrodites in hermaphroditic populations. Detailed population information is available in Huang et al. (2009). –, Not available. *Significant difference ($P<0.0001$) between F-GY and H-GY in gynodioecious populations.

correlate with female frequency among seven gynodioecious populations ($R^2 = 0.383$, $P = 0.396$).

2.2 Florivore preference

The 22 populations experienced various degrees of florivory, ranging from 0% to 88.9% individuals attacked. Within gynodioecious populations, the proportion of flowers and individuals of hermaphrodites damaged by florivores was significantly higher than that of females (Table 1). Among populations, both the proportion of individuals and flowers damaged by florivores were higher in H-GY than in F-GY, but there was no significant difference between H-H and H-GY (proportion of individuals damaged, $\chi^2 = 13.877$, $P = 0.001$; proportion of flowers damaged, $\chi^2 = 14.098$, $P = 0.001$; Fig. 2). If florivores prefer large flowers (or hermaphrodites) over small flowers (females), one may predict that populations with more hermaphrodites involve a higher degree of florivory. However, the proportion of individuals and flowers predated at population level did not correlate with female frequency ($R^2 = 0.367$, $P = 0.418$; $R^2 = 0.119$, $P = 0.800$, respectively).

3 Discussion

Our data indicated that the soil moisture of females was lower than that of hermaphrodites in gynodioecious populations. Soil moisture of hermaphrodites in gynodioecious populations did not differ from that of hermaphrodites in hermaphroditic populations. Our comparisons of three sexual types (F-GY, H-GY, and H-H) confirmed that females are favored by a relatively drier habitat given that previous investigations generally showed females more popular in harsh sites through comparisons between females and hermaphrodites in gynodioecious populations (Barr, 2004; Case & Barrett, 2004; Vaughton & Ramsey, 2004). The investigation of florivores indicated that insect damage on flowers and individuals of hermaphrodites was significantly higher than in females. However, female frequency did not correlate with either the soil moisture or flower predation at population level. This suggests that the effects of soil moisture and florivory do not translate into strong effects on sex ratio, or other factors may contribute to the sex ratio in the gynodioecious orchid.

Several factors can cause variation in the relative fitness of the two morphs in gynodioecious species, including variation in pollen limitation, the amount of self-fertilization, site quality, and sex-differential herbivory (Lewis, 1941; Lloyd, 1974; Sun & Ganders, 1986; Delph, 1990; Delph & Lloyd, 1991; Maurice & Fleming, 1995; McCauley & Taylor, 1997; Marshall & Ganders, 2001; Ashman, 2002, 2006). Previous theoretical and experimental studies have shown that if female frequency is too high, pollen limitation of seed production increases, limiting the seed production of females and consequently leading to a decrease in female frequency (Lewis, 1941; Lloyd, 1974;

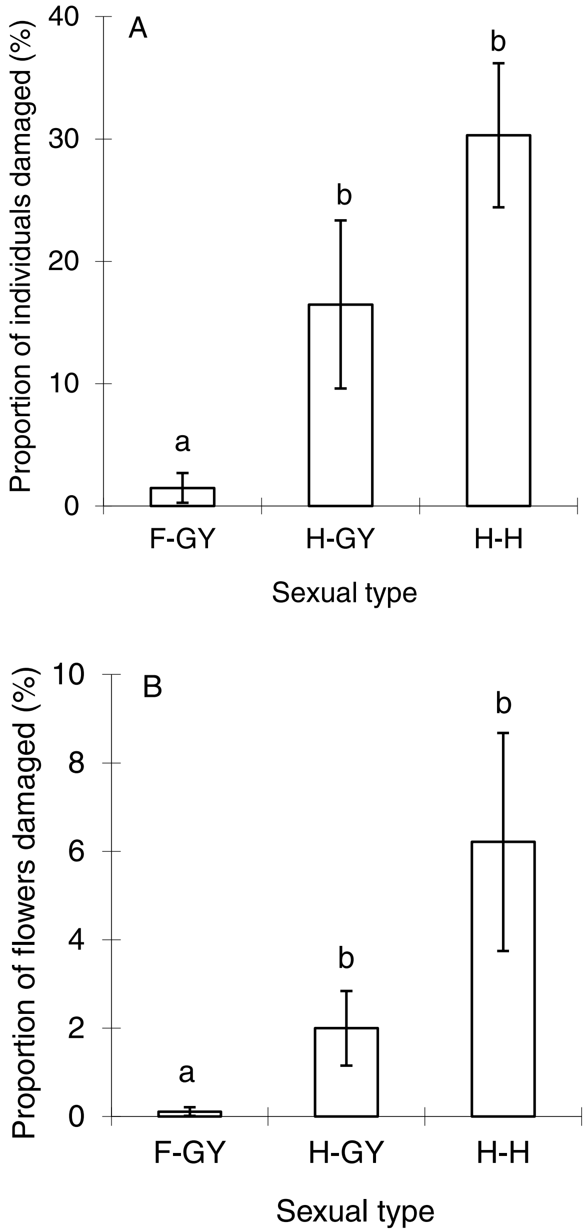

Fig. 2. Comparison of proportion of individuals (**A**) and flowers (**B**) damaged by florivores (mean±S.E.) among three sexual types of *Satyrium ciliatum* in Yunnan, China. The same letter above bars represents no significant differences. F-GY, females in gynodioecious populations; H-GY, hermaphrodites in gynodioecious populations; H-H, hermaphrodites in hermaphroditic populations.

Maurice & Fleming, 1995; McCauley & Taylor, 1997). The gynodioecious orchid *Satyrium ciliatum* could maintain high frequencies of females because females produce more seeds than hermaphrodites through facultative parthenogenesis in the absence of pollinia (Huang et al., 2009). One bumblebee pollinator was observed to carry pollinia on its tongue but its visitation to the orchid was extremely rare in these 22 field populations, in which the average percentage of flowers that received pollinia was only 1% (Huang et al., 2009). However, the fruit set could be 100% if flowers were not damaged by herbivores because both females and hermaphrodites were able to be apomictic.

We found evidence of sex-biased floral damage, in that florivores preferred hermaphrodites over females in the gynodioecious populations. The proportion of damaged individuals and flowers in hermaphrodites was higher in pure hermaphroditic populations than gynodioecious populations, although the difference was not significant (Fig. 2). It seems difficult to determine which floral traits are attractive to florivores without experimental manipulations and behavioral tests (McCall & Barr, 2012). The plant height and flower number were similar between hermaphrodites and females, but female flowers were significantly smaller with short spurs in the orchid (Lu & Huang, 2010). Flower size (corolla diameter) was shown to be the main determinant of florivore preference in a gynodioecious plant *Nemophila menziesii* H. & A. (see McCall & Barr, 2012). We observed that larvae of Tenthredinidae usually consumed flowers in hermaphrodites rather than females (see Tsuji & Sota, 2010) and pollen-eating beetles consumed pollinia in *S. ciliatum*. Such male-biased florivory may favor more females in the populations, given that they experienced less insect attack.

Consistent with previous investigations, we found evidence of dry sites apparently favoring the presence for females rather than hermaphrodites (Delph, 2003; Ashman, 2006). Hermaphrodite and female individuals of the orchid were distributed patchily on slopes of alpine meadows in southwest China, generally females occurring at higher elevations in gynodioecious populations which facilitated our measurement of soil moisture. We found that soil moisture of female sites was significantly lower than that of hermaphrodites in all gynodioecious populations and in hermaphroditic populations.

Comparisons between three sexual types in the gynodioecious orchid indicated that females would be favored by the harsh environment and florivory, but the two factors could not explain the sex ratio variation among populations. Neither the soil moisture nor the proportion of floral damage at population level correlated with female frequency. Only seven gynodioecious populations were investigated for this report, and the limited samples may have weakened the power of the significance test. For example, there was no florivory in two gynodioecious populations. However, the soil moisture of the same sexual type was considerably different among the seven populations (Table 1). Although theoretical models predict that sex ratio can be correlated with ecological factors, that higher frequencies of females should be usually found in poorer quality sites (Delph & Lloyd, 1991), no direct correlation between habitat quality and female frequency was reported in *Silene acaulis* (L.) Jacq. (Delph & Carroll, 2001) or *Nemophila menziesii* (Barr, 2004). Variation in female frequency (23% to 39%) and variation in site quality may not have varied as much across 10 sites, which could have contributed to a non-significant relationship in *S. acaulis* (Delph & Carroll, 2001). Barr (2004) found that soil moisture affected the relative fitness of females and hermaphrodites, reporting that watered hermaphrodites produced approximately twice as many seeds as unwatered hermaphrodites, with little treatment effect on female seed production in the dry site. However, regression of soil moisture on the female frequency (0%–50%) of 23 populations was not significant. These results in *N. menziesii* suggested that the effect of soil moisture did not translate into a strong effect on sex ratio, and environmental effects were not the sole determinant of the sex ratio (Barr, 2004).

Female frequency in *S. ciliatum* populations can be very high (Table 1). We found one population in Shangri-La, southwest China, that consisted solely of females (Huang et al., 2009). Such extremely high female frequency can be maintained in wild populations in that the orchid can be asexually reproduced by parthenogenesis. Our investigation of the effects of ecological contexts on sexual differentiation provides additional evidence in support that females are favored by lower soil moisture and florivory in an apomictic orchid.

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References

Ashman TL. 1999. Determinants of sex allocation in a gynodioecious wild strawberry: Implications for the evolution of dioecy and sexual dimorphism. Journal of Evolutionary Ecology 12: 648–661.

Ashman TL. 2002. The role of herbivores in the evolution of separate sexes from hermaphroditism. Ecology 83: 1175–1184.

Ashman TL. 2006. The evolution of separate sexes: A focus on the ecological context. In: Harder LD, Barrett SCH eds. Ecology and evolution of flowers. New York: Oxford University Press. 204–222.

Ashman TL, Cole D, Bradburn M. 2004. Sex differential resistance and tolerance to florivory in a gynodioecious wild strawberry: Implications for floral and sexual system evolution. Ecology 85: 2550–2559.

Barr CM. 2004. Soil moisture and sex ratio in a plant with nuclear-cytoplasmic sex inheritance. Proceedings of the Royal Society B: Biological Sciences 271: 1935–1939.

Bishop EJ, Spigler RB, Ashman TL. 2010. Sex-allocation plasticity in hermaphrodites of sexually dimorphic *Fragaria virginiana* (Rosaceae). Botany 88: 231–240.

Caruso CM, Case AL. 2007. Sex ratio variation in gynodioecious *Lobelia siphilitica*: Effects of population size and geographic location. Journal of Evolutionary Ecology 20: 1396–1405.

Case AL, Barrett SCH. 2004. Environmental stress and the evolution of dioecy: *Wurmbea dioica* (Colchicaceae) in Western Australia. Evolutionary Ecology 18: 145–164.

Chen S-C. 1979. Notes on bisexual and unisexual forms of *Satyrium ciliatum* Ldl. Acta Phytotaxonomica Sinica 17 (4): 54–60.

Collin CL, Pennings PS, Rueffler C, Widmer A, Shykoff JA. 2002. Natural enemies and sex: How seed predators and pathogens contributes to sex-differential reproductive success in a gynodioecious plant. Oecologia 131: 94–102.

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

Delph LF. 1990. Sex-ratio variation in the gynodioecious shrub *Hebe strictissima* (Scrophulariaceae). Evolution 44: 134–142.

Delph LF. 2003. Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. Evolution and Development 5: 34–39.

Delph LF, Carroll SB. 2001. Factors affecting relative seed fitness and female frequency in a gynodioecious species, *Silene acaulis*. Evolutionary Ecology Research 3: 487–505.

Delph LF, Lloyd DG. 1991. Environmental and genetic control of gender in the dimorphic shrub *Hebe subalpina*. Evolution 45: 1957–1964.

Dorken ME, Mitchard ETA. 2008. Phenotypic plasticity of hermaphrodite sex allocation promotes the evolution of separate sexes: An experimental test of the sex-differential plasticity hypothesis using *Sagittaria latifolia* (Alismataceae). Evolution 62: 971–978.

Dufay M, Billard E. 2012. How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species. Annals of Botany 109: 505–519.

Huang SQ, Lu Y, Chen YZ, Luo YB, Delph LF. 2009. Parthenogenesis maintains male sterility in a gynodioecious orchid. The American Naturalist 174: 578–584.

Lewis D. 1941. Male sterility in natural populations of hermaphrodite plants. New Phytologist 40: 56–63.

Lloyd DG. 1974. Theoretical sex ratios of dioecious and gynodioecious angiosperms. Heredity 32: 11–31.

Lu Y, Huang SQ. 2010. Higher dimorphism and lower phenotypic integration in reproductive traits compared to vegetative traits in a gynodioecious orchid. Evolutionary Ecology Research 12: 269–278.

Marshall M, Ganders FR. 2001. Sex-biased seed predation and the maintenance of females in a gynodioecious plants. American Journal of Botany 88: 1437–1443.

Maurice S, Fleming TH. 1995. The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms. Oikos 74: 55–60.

McCall AC, Barr CM. 2012. Why do florivores prefer hermaphrodites over females in *Nemophila menziesii* (Boraginaceae)? Oecologia [online]. Available from http://dx.doi.org/10.1007/s00442-012-2278-z [accessed 21 May 2012].

McCauley DE, Taylor DR. 1997. Local population structure and sex ratio: Evolution in gynodioecious plants. The American Naturalist 150: 406–419.

Sun M, Ganders FR. 1986. Female frequencies in gynodioecious populations correlated with selfing rates in hermaphrodites. American Journal of Botany 73: 1645–1648.

Tsuji K, Sota T. 2010. Sexual differences in flower defense and correlated male-biased florivory in a plant-florivore system. Oikos 119: 1848–1853.

Vaughton G, Ramsey M. 2004. Dry environments promote the establishment of females in monomorphic populations of *Wurmbea biglandulosa* (Colchicaceae). Evolutionary Ecology 18: 323–341.

Webb CJ. 1999. Empirical studies: Evolution and maintenance of dimorphism breeding system. In: Geber MA, Dawson TE, Delph LF eds. Gender and sexual dimorphism in flowering plants. Berlin: Springer-Verlag. 61–96.

Wise MJ, Hebert JB. 2010. Herbivores affect natural selection for floral-sex ratio in a field population of horsenettle, *Solanum carolinense*. Ecology 91: 937–943.

Wolfe LM, Shmida A. 1997. The ecology of sex expression in a gynodioecious Israeli desert shrub (*Ochradenus baccatus*). Ecology 78: 101–110.