*Evolutionary Ecology Research*, 2010, **12**: 269–278

# Higher dimorphism and lower phenotypic integration in reproductive traits compared with vegetative traits in a gynodioecious orchid

Yang Lu and Shuang-Quan Huang

*College of Life Sciences, Wuhan University, Wuhan, China*

## ABSTRACT

**Background:** In sexually dimorphic species, vegetative traits often show minimal dimorphism compared with reproductive traits.

**Hypothesis:** Tight phenotypic integration for vegetative traits may constrain the evolution of dimorphism in those traits.

**Organism:** A gynodioecious orchid *Satyrium ciliatum*, which has one erect spike and (generally) two basal leaves without complex ramification.

**Field site:** Alpine areas in Yunnan Province, southwestern China.

**Methods:** We measured three reproductive traits and four vegetative traits in 15 hermaphroditic and seven gynodioecious populations to compare levels of trait dimorphism and phenotypic integration.

**Conclusions:** Vegetative traits exhibited less dimorphism and higher phenotypic integration than reproductive traits in this gynodioecious orchid. The level of dimorphism was negatively correlated with the level of phenotypic integration, supporting our hypothesis of constraints.

*Keywords*: gynodioecious orchid, phenotypic integration, ramification, reproductive traits, sexual dimorphism, vegetative traits.

## INTRODUCTION

Sexual dimorphism in plants refers to any differences in traits (e.g. morphology and life history) between individuals of different genders in species with gender dimorphism (Geber *et al.*, 1999). Sexual dimorphism evolves when selection within each sex favours different phenotypes of homologous traits (Geber *et al.*, 1999). However, organisms are not collections of independent traits and genetic covariation among traits will lead to selection on one trait causing correlated responses in other traits, as well as the exhibition of 'phenotypic integration' (Lande and Arnold, 1983; Pigliucci and Preston, 2004). Phenotypic integration can be the

Correspondence: S.-Q. Huang, College of Life Sciences, Wuhan University, Wuhan 430072, China. e-mail: sqhuang@whu.edu.cn

Consult the copyright statement on the inside front cover for non-commercial copying policies.

© 2010 Shuang-Quan Huang

result of the genetic, functional, and developmental relationships among traits (Schlichting and Pigliucci, 1998; Murren *et al.*, 2002; Pigliucci, 2003). In this context, dimorphism may evolve indirectly in a trait because of that trait's integration with other traits that are under selection to be dimorphic. Alternatively, the evolution of dimorphism in a trait under selection may be constrained by its integration with traits not under selection. In other words, phenotypic integration may facilitate the evolution of dimorphism in traits not under selection and constrain the evolution of dimorphism in traits under selection to be dimorphic.

Since Berg's (1960) classic work on phenotypic integration, studies have investigated the relationship between reproductive (floral) and vegetative modules across taxa under different selective pressures, for example, generalized or specialized pollination, or within species among different environmental conditions (Berg, 1960; Waitt and Levin, 1993; Conner and Sterling, 1995, 1996; Armbruster *et al.*, 1999; Anderson and Busch, 2006; Ordano *et al.*, 2008, also see review by Murren, 2002). Variation in phenotypic integration of both reproductive and vegetative traits is worth exploring in species that are dimorphic for gender and that display sexual dimorphism, to evaluate the relationship between the level of phenotypic integration and the degree of sexual dimorphism (Ashman, 2005).

Gynodioecious species, which contain both females and hermaphrodites within a population, often exhibit less dimorphism in vegetative traits than reproductive traits, but this could be confounded by differences in the degree of ramification between the sexes (reviewed in Ashman, 2005). To determine whether the difference in the degree of sexual dimorphism between reproductive and vegetative traits is related to the level of phenotypic integration, we investigated an alpine terrestrial orchid *Satyrium ciliatum*. Like other *Satyrium* species, ramification of this plant is quite simple. It has one single erect stem (i.e. spike) and generally only two basal leaves. This species is the only known gynodioecious orchid (Chen, 1979) in which some populations have both female (pollinia aborted) and hermaphrodite plants (gynodioecious populations) while other populations are hermaphroditic (containing only hermaphrodites). This provides a rare opportunity to investigate sexual dimorphism in the Orchidaceae by comparing hermaphroditic and gynodioecious populations within a species. We investigated three reproductive and four vegetative traits in 15 hermaphroditic and seven gynodioecious populations. To determine the relationship between the degree of dimorphism and phenotypic integration, we measured (1) the level of dimorphism of all measured traits and whether reproductive traits differ more in a comparison of females and hermaphrodites from gynodioecious populations versus a comparison of females and hermaphrodites from hermaphroditic populations; and (2) the level and pattern of phenotypic integration of reproductive, vegetative, and all measured traits in all three types of individuals (females, hermaphrodites in gynodioecious populations, and hermaphrodites in hermaphroditic populations).

## METHODS AND MATERIALS

### Study species and populations

*Satyrium ciliatum* Lindl. is an alpine terrestrial orchid that is found on open slopes 2000–3600 m above sea level in southwestern Asia. Flowering plants have a corm, a single stem, two annual leaves (or occasionally one leaf), and an inflorescence spike with

10–35 flowers that open gradually from the bottom upwards. Flowers are pink and have a twin-spurred labellum.

All 22 investigated populations are located in three regions of Yunnan Province, southwest China:

- Dali: four populations in Cang Mountain (CM1–4) and two populations in Laojun Mountain (LJM1–2);
- Lijiang: two populations in Jade and Dragon Snow Mountain (JDSM1–2) and one in Lugu Lake (LGL); and
- Shangri-la: four populations in Gongbing country (GBC1–4); four populations in Tuguan country (TGC1–4); two populations in Shangrila Alpine Botanical Garden (ABG1–2); and one population each respectively in Heping country (HPC), Si country (SC), and Junmachang (JMC).

Populations CM3–4, GBC1–4, and ABG2 were gynodioecious and the other 15 populations were hermaphroditic.

## Traits measured

We measured three reproductive traits (i.e. spur length, bract area, and total flower production per plant) and four vegetative traits (i.e. plant height, stem length, stem diameter, and the area of the two leaves) on three types of plants after the plants had started to flower: hermaphrodites in hermaphroditic populations and hermaphrodites and females in gynodioecious populations (H-H, H-GY, and F-GY plant types respectively hereafter). Floral traits were determined using the first flower to open. We measured spur length, bract length and width, leaf length and width using a caliper micrometer to 0.1 mm. Spur length was the distance from the base of the lip to the tip of the spur tube, and stem length was the distance from the base of the flower to the top of the spike. We randomly measured 30 individuals of each sex per population.

The percentage of sexual dimorphism of the traits was calculated as $D = | M_{H\text{-}GY} - M_{F\text{-}GY} | / M_{H\text{-}GY} \times 100\%$, where $M_{H\text{-}GY}$ and $M_{F\text{-}GY}$ represent the mean value of the traits of G-H and G-Y in the population respectively (Delph *et al.*, 2002). D for each trait was calculated separately for each gynodioecious population and then was averaged across populations to obtain the mean value.

## Statistical analysis

We used one-way analyses of variance (Tukey) to determine whether all measured traits differed among hermaphrodites in hermaphroditic populations (H-H) and hermaphrodites (H-GY) and females (F-GY) in gynodioecious populations across populations and between H-GY and F-GY within populations. To investigate phenotypic integration of the three plant types, we performed two-tailed Pearson correlations among traits within each sex to establish the phenotypic correlation matrices. To determine variation in trait integration among the three types, we compared the level and pattern of the correlation matrices (Waitt and Levin, 1993). The level of phenotypic correlation represents the degree of integration of traits, determined by the number of significant correlation coefficients in the matrix

(Schlichting, 1989a, Murren *et al.*, 2002). Because the marking of individual correlation coefficients as statistically significant based on their single-test significance values often leads to spurious results, we used a sequential Bonferroni test to calculate the matrix-wide significance level (Holm, 1979; Rice, 1989). The pattern of phenotypic correlation describes the structure of the phenotypic integration among traits, which refers to the arrangements of correlations of different magnitudes in correlation matrices. We used a Mantel test to compare the pattern of phenotypic integration among the three types of plants (Mantel, 1967; Cheverud *et al.*, 1989; Schlichting and Pigliucci, 1995; Murren *et al.*, 2002). The Mantel test can be used to compute and test the linear correlation between two proximity matrices (dissimilarity or similarity) (Murren, 2002).

To test the hypothesis that reproductive traits are more integrated than vegetative traits, we used one-way analysis of variance to compare means of absolute correlation coefficients that were calculated for relationships among reproductive traits and among vegetative traits (Armbruster *et al.*, 1999). Furthermore, we used two-tailed Pearson correlation analysis to test whether the degree of sexual dimorphism of traits was correlated with the mean absolute correlation coefficient that was calculated from the correlation coefficients between the trait and the other traits.

## RESULTS

### Sexual dimorphism in reproductive and vegetative traits

Females had shorter spur lengths and larger bract areas than hermaphrodites, exhibiting sexual dimorphism among seven gynodioecious populations (Table 1). Four traits – spur length, bract area, flower number and leaf area – were found to be dimorphic within gynodioecious populations (Table 2). Only spur length exhibited dimorphism in all gynodioecious populations. Bract area was dimorphic only within GBC2, GBC4, and ABG2, flower number within CM3 and ABG2, and leaf area within CM4 and ABG2. The D of seven traits ranged from 2% to 75% (Table 1). Spur length was the most sexually dimorphic trait. Furthermore, differences between H-H and H-GY plants were not significant for any of the seven traits.

**Table 1.** Comparisons of means (± standard errors) for seven traits among hermaphrodites in 15 hermaphroditic (H-H) populations, hermaphrodites (H-GY) and females (F-GY) in seven gynodioecious populations, and the percentage of dimorphism (D) of traits in *Satyrium ciliatum*

| Types | Spur length (mm) | Bract area ($mm^2$) | Flower number | Leaf area ($cm^2$) | Plant height (cm) | Stem length (cm) | Stem diameter (mm) |
|---|---|---|---|---|---|---|---|
| H-H | $4.56 \pm 0.04^a$ | $178.86 \pm 3.37^a$ | $20.4 \pm 0.4^a$ | $59.1 \pm 2.1^a$ | $21.9 \pm 0.4^a$ | $7.3 \pm 0.1^a$ | $3.07 \pm 0.05^a$ |
| H-GY | $4.09 \pm 0.10^a$ | $193.68 \pm 5.86^a$ | $21.5 \pm 0.7^a$ | $64.8 \pm 2.5^a$ | $22.5 \pm 0.6^a$ | $7.6 \pm 0.2^a$ | $3.33 \pm 0.04^a$ |
| F-GY | $1.01 \pm 0.09^b$ | $228.25 \pm 4.84^b$ | $18.8 \pm 0.5^a$ | $77.8 \pm 3.1^a$ | $21.4 \pm 0.5^a$ | $7.4 \pm 0.2^a$ | $3.36 \pm 0.04^a$ |
| $F_{2,28}$ | **135.50** | **5.219** | 0.393 | 1.662 | 0.005 | 0.062 | 1.898 |
| $P$ | <0.001 | 0.012 | 0.697 | 0.209 | 0.995 | 0.940 | 0.170 |
| D (%) | 75 | 18 | 14 | 20 | 5 | 2 | 5 |

*Note*: For each trait, means with a different superscript differ significantly.

**Table 2.** The mean (and standard error) of reproductive and vegetative traits of three plant types in 15 hermaphroditic (H-H) and seven gynodioecious (GY) populations

| Population | Type | Spur length (mm) | Bract area ($mm^2$) | Flower number | Leaf area ($mm^2$) | Plant height (cm) | Stem height (cm) | Stem diameter (mm) |
|---|---|---|---|---|---|---|---|---|
| CM1 | H-H | 4.95 (0.11) | 175.77 (11.94) | 22.7 (1.6) | 81.3 (6.3) | 32.3 (1.3) | 10.0 (0.7) | 3.06 (0.14) |
| CM2 | H-H | 4.71 (0.12) | 194.63 (10.21) | 27.1 (1.3) | 104.4 (8.3) | 31.8 (1.1) | 10.2 (0.5) | 3.39 (0.11) |
| LJM1 | H-H | 3.91 (0.15) | 172.71 (6.64) | 18.3 (0.9) | 40.8 (3.0) | 21.5 (1.1) | 8.6 (0.3) | 2.56 (0.07) |
| LJM2 | H-H | 4.28 (0.16) | 187.60 (7.81) | 20.1 (1.1) | 52.4 (4.1) | 22.9 (1.0) | 8.4 (0.4) | 3.01 (0.08) |
| JDSM1 | H-H | 4.64 (0.13) | 243.12 (23.1) | 24.9 (2.3) | 109.1 (13.3) | 30.5 (1.8) | 8.6 (0.7) | 3.67 (0.16) |
| JDSM2 | H-H | 4.17 (0.14) | 186.34 (10.50) | 17.1 (1.7) | 54.5 (5.8) | 21.3 (1.1) | 6.3 (0.5) | 3.12 (0.12) |
| LGL | H-H | 4.91 (0.12) | 239.21 (14.72) | 25.4 (1.5) | 74.1 (2.4) | 22.3 (0.8) | 7.7 (0.4) | 3.61 (0.10) |
| TGC1 | H-H | 4.70 (0.22) | 163.62 (10.30) | 18.4 (1.3) | 52.6 (4.3) | 20.9 (0.9) | 6.3 (0.5) | 2.73 (0.09) |
| TGC2 | H-H | 4.57 (0.09) | 136.94 (3.70) | 15.3 (0.7) | 33.0 (1.8) | 16.5 (0.4) | 5.3 (0.2) | 2.43 (0.07) |
| TGC3 | H-H | 4.48 (0.11) | 161.50 (6.65) | 14.4 (1.0) | 38.5 (2.6) | 16.1 (0.6) | 5.5 (0.3) | 2.58 (0.09) |
| TGC4 | H-H | 4.72 (0.12) | 188.54 (8.19) | 19.2 (1.1) | 63.9 (5.4) | 22.4 (0.7) | 6.8 (0.3) | 3.09 (0.08) |
| HPC | H-H | 4.25 (0.12) | 138.14 (7.05) | 20.1 (1.3) | 35.4 (2.4) | 16.7 (0.5) | 7.4 (0.3) | 3.06 (0.10) |
| JMC | H-H | 4.81 (0.26) | 139.44 (6.11) | 18.7 (1.5) | 47.0 (4.0) | 18.1 (1.0) | 5.7 (0.3) | 3.84 (0.07) |
| SC | H-H | 4.21 (0.10) | 147.79 (6.26) | 20.8 (1.4) | 22.4 (1.6) | 14.3 (0.5) | 7.2 (0.3) | 2.86 (0.07) |
| ABG1 | H-H | 4.79 (0.08) | 233.18 (13.98) | 23.3 (1.1) | 81.5 (5.3) | 23.2 (1.0) | 6.0 (0.3) | 3.45 (0.10) |
| CM3 | H-GY | **4.25***** (0.16) | 195.93 (14.39) | **25.3*** (1.8) | 76.0 (5.8) | 27.9 (1.2) | 9.3 (0.7) | 3.23 (0.13) |
| | F-GY | **1.63** (0.20) | 207.47 (7.21) | **20.7** (1.1) | 69.8 (4.6) | 25.4 (0.7) | 8.9 (0.4) | 3.00 (0.09) |
| CM4 | H-GY | **3.99***** (0.17) | 229.92 (14.73) | 25.7 (1.6) | **70.2***** (5.7) | 29.1 (1.0) | 9.9 (0.5) | 3.31 (0.13) |
| | F-GY | **0.56** (0.21) | 247.26 (12.16) | 29.6 (1.5) | **108.1** (7.0) | 31.6 (1.3) | 11.9 (0.5) | 3.56 (0.13) |
| GBC1 | H-GY | **3.32***** (0.19) | 233.39 (10.78) | 20.2 (1.3) | 67.9 (5.8) | 19.0 (0.7) | 6.8 (0.3) | 3.47 (0.09) |
| | F-GY | **0.94** (0.20) | 232.96 (11.18) | 19.8 (0.9) | 64.9 (5.1) | 18.9 (0.9) | 7.0 (0.3) | 3.46 (0.09) |
| GBC2 | H-GY | **5.12***** (0.20) | **168.33***** (14.72) | 20.1 (1.6) | 52.9 (5.9) | 17.9 (1.0) | 6.9 (0.6) | 3.32 (0.13) |
| | F-GY | **1.13** (0.24) | **238.08** (12.68) | 17.6 (0.9) | 72.0 (6.1) | 18.5 (0.8) | 6.8 (0.4) | 3.53 (0.09) |
| GBC3 | H-GY | **3.16***** (0.23) | 180.71 (15.75) | 16.8 (1.0) | 53.6 (6.9) | 18.0 (1.1) | 5.8 (0.4) | 3.29 (0.09) |
| | F-GY | **0.68** (0.23) | 196.29 (12.00) | 13.9 (0.9) | 52.6 (7.8) | 16.8 (1.0) | 6.0 (0.4) | 3.08 (0.11) |
| GBC4 | H-GY | **3.69***** (0.33) | **148.69**** (13.42) | 15.9 (1.4) | 47.5 (9.2) | 16.8 (1.5) | 5.1 (0.5) | 3.17 (0.15) |
| | F-GY | **1.06** (0.27) | **206.26** (13.36) | 14.9 (1.0) | 63.1 (4.9) | 18.2 (0.8) | 5.8 (0.3) | 3.24 (0.09) |
| ABG2 | H-GY | **5.30***** (0.17) | **171.67***** (10.67) | **24.1**** (1.5) | **78.6***** (4.3) | 24.7 (1.2) | 7.9 (0.3) | 3.46 (0.12) |
| | F-GY | **0.99** (0.23) | **274.16** (11.78) | **18.0** (1.0) | **121.3** (7.6) | 23.2 (1.1) | 6.9 (0.4) | 3.68 (0.09) |

*Note*: Values are in **bold** if they are significantly different between female (Y-GY) and hermaphrodite (H-GY) morphs within gynodioecious populations. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

**Table 3.** The correlation coefficients for reproductive and vegetative traits of *Satyrium ciliatum*

| | Spur length | Bract area | Flower number | Leaf area | Plant height | Stem length |
|---|---|---|---|---|---|---|
| **H-H** | | | | | | |
| Bract area | 0.130* | | | | | |
| Flower number | 0.117 | 0.618*** | | | | |
| Leaf area | 0.302*** | 0.631*** | 0.656*** | | | |
| Plant height | 0.244*** | 0.552*** | 0.635*** | 0.862*** | | |
| Stem length | 0.021 | 0.524*** | 0.747*** | 0.547*** | 0.707*** | |
| Stem diameter | 0.367*** | 0.427*** | 0.497*** | 0.458*** | 0.340*** | 0.334*** |
| **H-GY** | | | | | | |
| Bract area | –0.237** | | | | | |
| Flower number | 0.252** | 0.533*** | | | | |
| Leaf area | 0.023 | 0.702*** | 0.568*** | | | |
| Plant height | 0.179 | 0.539*** | 0.708*** | 0.730*** | | |
| Stem length | 0.096 | 0.646*** | 0.828*** | 0.708*** | 0.846*** | |
| Stem diameter | 0.102 | 0.644*** | 0.641*** | 0.645*** | 0.402*** | 0.588*** |
| **F-GY** | | | | | | |
| Bract area | 0.064 | | | | | |
| Flower number | 0.052 | 0.548*** | | | | |
| Leaf area | 0.030 | 0.718*** | 0.573*** | | | |
| Plant height | 0.103 | 0.501*** | 0.771*** | 0.743*** | | |
| Stem length | 0.035 | 0.541*** | 0.912*** | 0.609*** | 0.873*** | |
| Stem diameter | 0.091 | 0.768*** | 0.575*** | 0.733*** | 0.470*** | 0.523*** |

*Abbreviations*: H-H, H-GY, and F-GY – as in Table 1. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

## Phenotypic integration of the three plant types

For the three plant types, all traits except spur length were positively correlated with each other (Table 3). The patterns of correlation matrices of the three types were similar among each other (H-H and H-GY: Mantel test, $r = 0.822$, $P = 0.004$; H-H and F-GY, $r = 0.846$, $P < 0.0001$; H-GY and F-GY, $r = 0.941$, $P < 0.0001$). However, the number of significant correlations in the matrices decreased from H-H to H-GY to F-GY. In H-H, spur length was significantly correlated with all other traits except flower number and stem length; in H-GY, spur length was significantly correlated with the two reproductive traits only; and in F-GY, spur length was not correlated with any other traits (Table 3).

## Difference in phenotypic correlations between reproductive and vegetative traits

The mean absolute correlation of reproductive traits did not differ significantly from that of vegetative traits in H-H ($F_{1,8} = 2.109$, $P = 0.190$). The mean absolute correlation of reproductive traits was significantly lower than that of vegetative traits in both H-GY ($F_{1,8} = 8.085$, $P = 0.025$) and F-GY ($F_{1,8} = 9.723$, $P = 0.017$) (Fig. 1). The degree of sexual dimorphism for a trait was negatively correlated with the mean absolute correlation

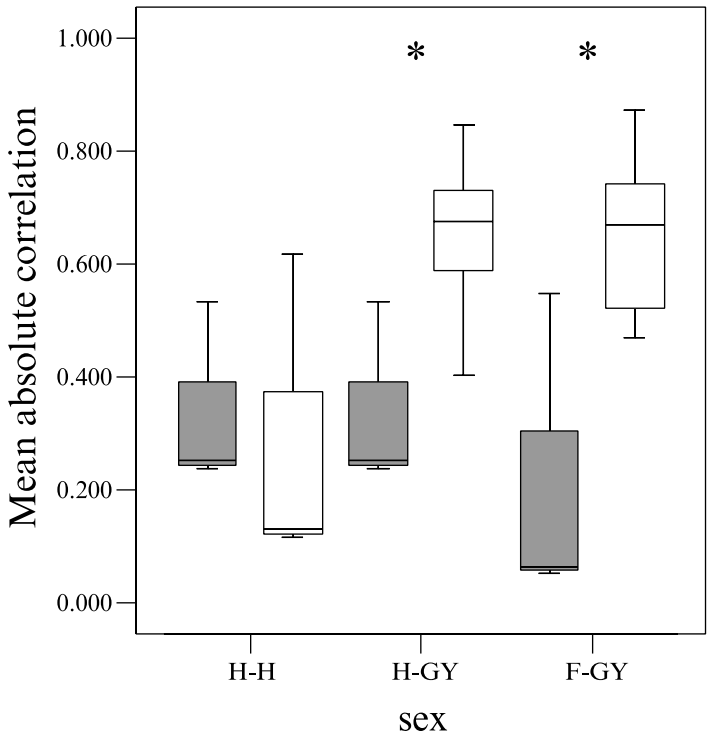


**Fig. 1.** Comparisons of the mean absolute correlation coefficient between reproductive (grey bars) and vegetative (open bars) traits among three types of plant. *Abbreviations*: H-H, H-GY, and F-GY – as in Table 1. Asterisks indicate significant differences ($P < 0.05$) between two groups of traits.

coefficient – that is, the mean of the correlation coefficients of the trait and the other six traits – in both H-GY ($r = -0.932$, $P = 0.002$) and F-GY ($r = -0.957$, $P = 0.001$).

## DISCUSSION

Two of three measured reproductive traits exhibited sexual dimorphism, but none of the vegetative traits were dimorphic among gynodioecious populations in *Satyrium ciliatum*. Although leaf area was dimorphic within two gynodioecious populations, the average degree of dimorphism of reproductive traits was higher than that of vegetative traits. These results demonstrate that vegetative traits exhibit modest dimorphism, a pattern observed in another gynodioecious species (Ashman, 2005). The spur length of H-GY was greater than that of F-GY, which is in line with hermaphrodites having greater nectar production than females in this orchid. Long spurs could be favoured by bumble bee pollinator-mediated sexual reproduction in hermaphrodites, but be diminished by greater parthenogenesis in females (Huang *et al.*, 2009). Sexual dimorphism in traits did not vary between morphs of H-H and H-GY, suggesting that females may have been under past selection to differ from hermaphrodites and/or there are pleiotropic effects of male sterility (e.g. smaller flowers because of the lack of pollen).

All three plant types in *S. ciliatum* showed significant phenotypic correlations among the seven traits investigated. The pattern of phenotypic correlation of the traits was very similar

among the three types, and indicated that the structure of correlations among traits did not vary with gender dimorphism. Although previous studies did not compare the pattern of phenotypic integration between two types within species, it was shown that the pattern of phenotypic integration was similar within populations across environments and among populations within environments in *Phlox drummondii* (Waitt and Levin, 1993). This similar pattern of phenotypic integration within species may be because individuals of a species share a similar genetic basis and developmental mode. On the other hand, the extent of phenotypic correlation decreased from H-H to F-GY, indicating that the degree of trait correlation varied among the three types (i.e. they were plastic). This plasticity of phenotypic integration was also found in *P. drummondii* (Waitt and Levin, 1993). The strength of correlation can be altered by certain environmental factors (Marshall *et al.*, 1986; Schlichting, 1989b; Stearns *et al.*, 1991). One possible reason for variation in the level of phenotypic correlation in *S. ciliatum* may be that gynodioecious populations, and hence females, occur in relatively arid habitats compared with those of hermaphroditic populations (Y. Lu and S.-Q. Huang, unpublished data), something that is found in numerous gynodioecious species (Delph, 2003). Using three closely related species of *Phlox* grown under different water/nutrient treatments, Schlichting (1989b) found that the phenotypic correlations differed among the species and among environments. Schlichting and Pigliucci (1995) suggested differential changes across environments might confer appropriate phenotypic changes among functionally related traits to enable the maintenance of fitness across environments.

We found that the level of integration did not differ between reproductive and vegetative traits in hermaphroditic populations, whereas in gynodioecious populations vegetative traits were more tightly integrated than reproductive traits within the two gender morphs, indicating that variation of integration was different between reproductive and vegetative traits. Although vegetative traits were not dimorphic among gynodioecious populations, leaf area was dimorphic within two gynodioecious populations. Furthermore, leaf area was not correlated with spur length in the two morphs in gynodioecious populations. Thus, dimorphism in leaf area may not be a consequence of integration with reproductive traits, but rather both reproductive and vegetative traits may be under selection. Combined with the result that reproductive traits were less phenotypically integrated, our results suggest that differences in phenotypic integration contributed to the differences in the degree of sexual dimorphism between reproductive and vegetative traits. Furthermore, the degree of sexual dimorphism in the traits was negatively correlated with the mean absolute correlation coefficient within both morphs in gynodioecious populations, implying that the degree of dimorphism was constrained by the level of phenotypic integration.

Why do vegetative traits show less sexual dimorphism than reproductive traits? Geber (1999) suggested dimorphism in vegetative traits might evolve after the evolution of separate genders in response to sex-differential selection. Ashman (2005) evaluated the roles of limited variation, negative genetic covariation, and lack of gender-differential selection in helping to constrain the sexual dimorphism of vegetative and phenological traits in *Fragaria virginiana*. The results indicated that minimal sexual dimorphism in these traits was caused by limited disruptive selection for dimorphism, low genetic variation, and extensive genetic covariance both within and between sexual morphs. In this study, we showed that tight phenotypic integration constrained the evolution of sexual dimorphism in vegetative traits, providing additional perspectives on this phenomenon.

## ACKNOWLEDGEMENTS

We thank Kai-Xiong Ye and Chang-Qiu Liu for help in the field, Zhi-Gang Zhao for help with data analysis and discussion on the manuscript, and Lynda Delph for providing helpful suggestions on this work. A grant from the National Science Foundation of China (#30770135) to S.Q.H supported this work.

## REFERENCES

Anderson, I.M. and Busch, J.W. 2006. Relaxed pollinator-mediated selection weakens floral integration in self-compatible taxa of *Leavenworthia* (Brassicaceae). *Am. J. Bot.*, **93**: 860–867.

Armbruster, W.S., Di Stilio, V.S., Tuxill, J.D., Flores, T.C. and Velasquez Runk, J.L. 1999. Covariation and decoupling of floral and vegetative traits in nine neotropical plants: a re-evaluation of Berg's correlation-pleiades concept. *Am. J. Bot.*, **86**: 38–55.

Ashman, T.-L. 2005. The limits on sexual dimorphism in vegetative traits in a gynodioecious plant. *Am. Nat.*, **166** (suppl.): S5–S16.

Berg, R.L. 1960. The ecological significance of correlation pleiades. *Evolution*, **14**: 171–180.

Chen, X.Q. 1979. Notes on bisexual and unisexual forms of *Satyrium ciliatum* Ldl. *J. Syst. Evol.*, **17**: 54–60.

Cheverud, J.M., Wagner, G.P. and Dow, M.M. 1989. Methods for the comparative analysis of variation patterns. *Syst. Zool.*, **38**: 201–213.

Conner, J.K. and Sterling, A. 1995. Testing hypotheses of functional relationships: a comparative study of correlation patterns among floral traits in five insect pollinated species. *Am. J. Bot.*, **82**: 1399–1406.

Conner, J.K. and Sterling, A. 1996. Selective for independence of floral and vegetative traits: evidence from correlation patterns in five species. *Can. J. Bot.*, **74**: 642–644.

Delph, L.F. 2002. Among-population variation and correlation in sexually dimorphic traits of *Silene latifolia*. *J. Evol. Biol.*, **15**: 1011–1020.

Delph, L.F. 2003. Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. *Evol. Develop.*, **5**: 34–39.

Geber, M.A. 1999. Theories of the evolution of sexual dimorphism. In *Gender and Sexual Dimorphism in Flowering Plants* (M.A. Geber, T.E. Dawson, and L.F. Delph, eds.), pp. 97–122. Berlin: Springer.

Geber, M.A., Dawson, T.E. and Delph, L.F., eds. 1999. *Gender and Sexual Dimorphism in Flowering Plants*. Berlin: Spring-Verlag.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. *Scand. J. Stat.*, **6**: 65–70.

Huang, S.-Q., Lu, Y., Chen, Y.-Z., Luo, Y.-B. and Delph, L.F. 2009. Parthenogenesis maintains male sterility in a gynodioecious orchid. *Am. Nat.*, **174**: 578–584.

Lande, R. and Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution*, **37**: 1210–1226.

Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Res.*, **27**: 209–220.

Marshall, D.L., Levin D.A. and Fowler, N.L. 1986. Plasticity of yield components in response to stress in *Sesbania macrocarpa* and *Sesbania vesicaria* (Leuminosae). *Am. Nat.*, **127**: 508–521.

Murren, C.J. 2002. Phenotypic integration in plants. *Plant Species Biol.*, **17**: 89–99.

Murren, C.J., Pendleton, N. and Pigliucci, M. 2002. Evolution of phenotypic integration in *Brassica* (Brassicaceae). *Am. J. Bot.*, **89**: 655–663.

Ordano, M., Fornoni, J., Boege, K. and Domínguez, C.A. 2008. The adaptive value of phenotypic floral integration. *New Phytol.*, **179**: 1183–1192.

Pigliucci, M. 2003. Phenotypic integration: studying the ecology and evolution of complex phenotypes. *Ecol. Lett*., **6**: 265–272.

Pigliucci, M. and Preston, K. 2004. *Phenotypic Integration: Studying the Ecology and Evolution of Complex Phenotypes*. New York: Oxford University Press.

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**: 223–225.

Schlichting, C.D. 1989a. Phenotypic integration and environmental change. *Bioscience*, **39**: 460–464.

Schlichting, C.D. 1989b. Phenotypic plasticity in *Phlox*. II. Plasticity of character correlation. *Oecologia*, **78**: 496–501.

Schlichting, C.D. and Pigliucci, M. 1995. Lost in phenotypic space: environmental-dependent morphology in *Phlox drummondii* (Polemoniaceae). *Int. J. Plant Sci.*, **156**: 542–546.

Schlichting, C.D. and Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland, MA: Sinauer Associates.

Stearns, S., de Jong, G. and Newman, B. 1991. The effects of phenotypic plasticity on genetic correlation. *Trends Ecol. Evol.*, **6**: 122–126.

Waitt, D.E. and Levin, D.A. 1993. Phenotypic integration and plastic correlations in *Phlox drummondii* (Polemoniaceae). *Am. J. Bot.*, **80**: 1224–1233.