Phenotypic Variation and Germination Behavior between Two Altitudinal Populations of Two Varieties of *Bidens pilosa* in Taiwan.

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ABSTRACT: *Bidens pilosa* L. var. *radiata*, an invasive plant in Taiwan, is distributed into mountain area. In contrast to *B. pilosa* var. *radiata*, *B. pilosa* var. *minor* is a naturalized plant and distributed mainly in mid-altitude of the island. This study aims to (1) investigate phenotypic variations between low- and mid-altitudinal populations of these two varieties of *B. pilosa*, and (2) evaluate the causes, environmental effect or genetic differentiation, of the variations. Two populations, one from low-(500 m) and the other from mid- altitude (1300 or1600 m) along an elevation gradient in central Taiwan, of both varieties were selected for the study. We compared ecophysiological traits of field populations and of progeny of these populations cultivated in a common garden.

For both varieties, mid-altitudinal populations had significantly higher chlorophyll and N contents, more positive $\delta^{13}C$ values and larger seeds than low-altitudinal populations. However, most of the phenotypic variations between altitudinal populations disappeared in common garden-grown plants. The results suggested that these variations were phenotypic plasticity in response to changes in environmental factors associated with altitudes. Comparing between populations of the same variety, seeds of the var. *minor* collected from mid-altitudinal population germinated faster. In contrast, seeds of the var. *radiata* from mid-altitudinal populations germinated slower and required more days to germinate. Thus, seeds produced by the mid-altitudinal population of the var. *radiata* had inferior germination performance, which might reduce its competitive ability at mid-altitude. However, high degree of phenotypic plasticity would allow *B. pilosa* var. *radiata* to spread into mountain area.

KEY WORDS: altitudinal variation, *Bidens pilosa* var. *radiata*, *Bidens pilosa* var. *minor*, ecophysiological traits, invasive plants, phenotypic plasticity, seed germination
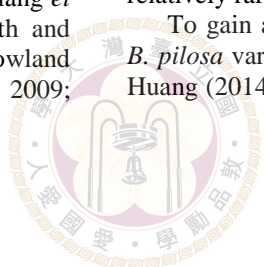
INTRODUCTION

Effects of biological invasion on biodiversity and ecosystem functions have been studied worldwide (Vilà *et al.*, 2011). The results indicated that biological invasion is a key driver for the decline of biodiversity and the changes of ecosystem functioning (Vitousek *et al.*, 1997; Mooney and Hobbs, 2000). Because of the harsher climatic condition and less human disturbance, biologists thought that mountains were less vulnerable to biological invasion than lowland ecosystems (Humphries *et al.*, 1991; Millennium Ecosystem Assessment, 2003). Therefore, most of the studies on the impact of the invasive plants focused on lowland ecosystems. However, more and more surveys showed that mountain vegetation might not be so resilient to biotic invasion (Pauchard *et al.*, 2009). Thus, it is necessary to evaluate the possibility and then prevent the dispersion of invasive plants from lowland into high altitude ecosystems.

After been introduced into Taiwan in the late 20th century, *Bidens pilosa* L. var. *radiata* has spread and becomes one of the ten most notorious plants in lowland Taiwan during the last two decades (Chiang *et al.*, 2003). Morphological, physiological, growth and reproductive traits of the invasive plant at lowland environment have been studied (Hsu and Kao, 2009;

Huang *et al.*, 2012, Huang and Kao, 2014; Hsu and Kao, 2014). Further investigation found expanded distribution of *B. pilosa* var. *radiata* from lowland ecosystems into high elevation area (Huang, 2008). In Taiwan the percentage of endemic species increases with increasing altitude, consequently, a highly positive correlation between endemism and altitude was found (Hsieh, 2002). The occurrence of *B. pilosa* var. *radiata* in the mountain suggests that the invasive plant might be able to invade high elevation ecosystems, subsequently might reduce biodiversity and threaten the survival of endemic species of Taiwan. To reduce the risk of ecosystem disrupted by invasive species, research into high-elevation plant invasions is important for developing appropriate management policies. In addition, as Körner (2007) suggested that conducting this type of research could help us understanding factors that influence spread of plant species along steep environmental gradients.

B. pilosa var. *minor* is another introduced variety of *B. pilosa* in Taiwan (Peng *et al.*, 1998). In contrast to *B. pilosa* var. *radiata*, var. *minor* is a naturalized plant and currently distributed mainly in mid-altitude whereas relatively rare in lowland Taiwan (Authors' obs.).

To gain a better understanding in the traits making *B. pilosa* var. *radiata* invasive Taiwan, Hsu (2006) and Huang (2014) have studied ecophysiological, floral and

life history traits of the plant. However, their studies focused on lowland populations. To our knowledge, characteristics of mid-altitude populations of both varieties have not been studied. Comparing changes in phenotype of populations at different altitudes between the invasive and naturalized varieties may help us evaluating which factors would favor or limit the invasive plant spread into high elevation. In this study, two populations, at low- and mid-altitudes (locations listed in Materials and Methods) along an elevation gradient in Central Taiwan, of *B. pilosa* var. *radiata* and *B. pilosa* var. *minor*, were selected. We measured ecophysiological traits of these populations in situ in three seasons. We also grew progeny of these populations in two seasons in a common garden at lowland and repeated the measurement of the ecophysiological traits. Finally, we compared germination response of seeds collected from these populations under two temperature regimes. The objectives of this study were: (1) to investigate variations in phenotype and germination behavior between low- and mid-altitude populations of these two varieties of *B. pilosa*, (2) to evaluate the causes, environmental effect or genetic differentiation, of the phenotypic variation between populations, and (3) to asses the risk of *B. pilosa* var. *radiata* invading mountain area in Taiwan.

MATERIALS AND METHODS

Materials

The two study plants belong to the species *Bidens pilosa*, a member of the Asteraceae family. *B. pilosa* var. *radiata* is an annual or perennial herb, while *B. pilosa* var. *minor* is an annual herb (Peng, 1998). The two varieties, sometimes growing sympatrically, can be distinguished by their ray florets, which are usually longer than 10 mm in var. *radiata* but shorter than 8 mm in var. *minor*.

Field study site and measurements

Populations of *B. pilosa* var. *radiata* at two altitudes (120°36′E, 23°26′N alt. 500 m and 120°41′E, 23°28′N alt.1300 m) and those of *B. pilosa* var. *minor* at two altitudes (120°36′E, 23°26′N alt. 500 m and 120°43′E, 23°24′N alt.1600 m) in Central Taiwan were selected for the investigation. Plants of the two varieties at the lowland site grew sympatrically. The mean monthly air temperature and mean monthly precipitation (Fig. 1) close to the study area in 2007 were provided by two weather stations, Chiayi (23°29′52″E, 120°25′28″ N, alt. 28 m) and Fanchihu (23°29′45″E, 120°41′28″N, alt. 1385 m), of Central Climate Bureau, Taiwan.

Chlorophyll content of leaves from plants (n = 8) growing at each altitude were estimated with a chlorophyll meter (SPAD-502, Minolta Co., Japan).
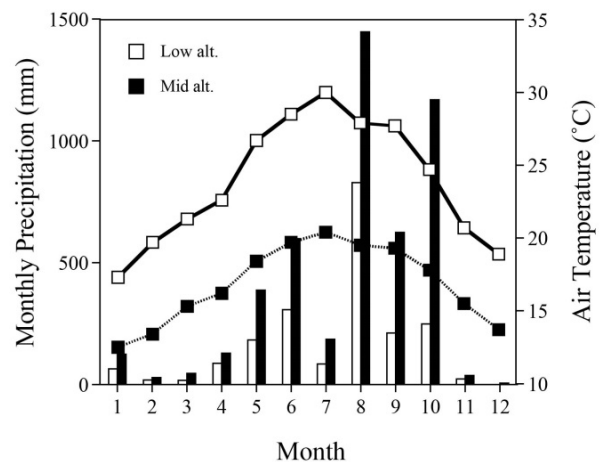

Fig. 1. Mean monthly precipitation (bars) and air temperature (square) during the year of 2007. Data are from Chiayi (23°29′52″E, 120°25′28″N, alt. 28 m) (Low alt.) and Fan-Chi Lake (23°29′52″E, 120°25′28″N, alt. 1405 m) (Mid alt.) weather stations located at central Taiwan.

After the chlorophyll contents recorded, leaves were excised, leaf area was measured using a leaf area meter (LI-3100, Licor, Lincoln, NE, USA). The leaf was then dried at 60 °C for at least 48 h and its dry mass weighted with an electronic balance (Mettler AB104). Leaf mass per area (LMA= mg cm^{-2}) was then calculated as leaf dry mass/leaf area. The leaf material was then ground and subsequently its nitrogen (N) content and stable carbon isotopes ratio (δ^{13}C) were determined using continuous flow isotope mass spectrometry (CF-IRMS)consisting an elementary analyzer (FlashEA1112, ThermoFisher, Italy) connected to an isotopic ratio mass spectrometer (delta V, Finnigan, Germany). δ^{13}C values were calculated as: δ^{13}C (‰) = $[(R_{sample}/R_{standard}) -1] \times 1000$, where R is the corresponding ratio of δ^{13}C: δ^{12}C of a standard (PDB, $R_{standard}$ = 0.0112372) or the measured sample (R_{sample}) (Ehleringer and Osmond, 1989).

To calculate the mean weight of a seed for an individual plant, total seeds of the individual plant were collected, counted and weighted. Seeds collected from 10 individual plants of each population in April and Dec. of 2007 were compared.

Measurements of common garden-grown plants

Seeds collected from populations at two altitudes were germinated in Petri dishes (diameter of 9 cm), followed by transplanting of the seedlings to the experimental farm of National Taiwan University, Taipei. Two transplants were conducted, one from spring to fall (Apr. to Oct. 2007) and the other from fall to spring (Oct. 2007 to May 2008). Measurements of ecophysiological traits, above mentioned, were taken in August 2007 and in March 2008, respectively, for both experiments.

Table 1. Results of *P* value from the two-way ANOVA (general linear model) assessing the effect of season, population (altitude) and the interactions of both on leaf mass per unit area (LAM), chlorophyll content (Chl), nitrogen content (N), stable carbon isotope ratio (δ¹³C) and seed weight of *Bidens pilosa* var. *minor* growing in field or in a common garden. NA: not available.

Variable	Field growing plants			Garden cultivated plants		
	Season	Altitude	Season × Altitude	Season	Altitude	Season × Altitude
LMA	<0.01	0.09	0.06	<0.01	0.24	0.87
Chl	0.13	<0.01	0.38	<0.01	<0.01	0.39
N	<0.01	<0.01	0.13	<0.01	0.79	0.35
δ¹³C	0.78	<0.01	0.02	<0.01	0.64	0.26
Seed weight	<0.01	<0.01	0.01	NA	0.47	NA

Table 2. Mean weight of a seed (mg) (Mean ± SE, n = 10) collected from plants of *B. pilosa* var. *minor* and *B. pilosa* var. *radiata* growing in field at two altitudes and from their progeny grown in a common garden.

		B. pilosa var. *minor*		*B. pilosa* var. *radiata*	
		500 m	1600 m	500 m	1300 m
field	Apr., 2007	1.37±0.02^c*	1.58±0.03^b	0.95±0.02^e	1.16±0.02^d
	Dec.,2007	1.46±0.02^b	1.79±0.03^a	0.92±0.02^e	1.15±0.02^d
garden	Apr., 2008	1.39±0.06^c	1.47±0.03^bc	1.33±0.11^cd	1.11±0.07^d

* Means within the same raw followed by different letters are significantly different (LSMEAN, P < 0.05).

Measurements of germination behavior

Seeds collected from field growing plants were placed on water-saturated filter paper in a Petri dish (diameter × height = 90 mm × 15 mm), with 25 seeds per Petri dish and 5 Petri dishes per species per treatment. These Petri dishes were then transferred to two growth chambers controlled at relative humidity of 70%, light/dark cycle of 12/12 h and PFD (photosynthetic photon photon flux density) of 100-150 μmol m^{-2} s^{-1} at light period and air temperature (light/dark period) of 30/25 and 18/13 °C, respectively, simulating summer and winter temperature in lowland (< 500 m) Taiwan. Seed germination was recorded every day for 14 days. Seeds were considered germinated when radicals could be observed by naked eyes. After 14 days of treatment, cumulative germination percentage, speed of germination and mean days to geminate were calculated following Hou *et al.* (2000).

Cumulative germination percentage = seed germinated / 25 seeds sowed

$$\text{Mean days to geminate} = \sum_{d=1}^{14} f_d / N$$

$$\text{Speed of germination} = \sum_{d=1}^{14} f_d / d$$

d: days after germination (1-14), f_d: number of seeds germinated on the d day, N: total number of seeds germinated on each Petri-dish within 14 days

Statistical and data analysis

Two ways analyses of variance (general linear model procedure of SAS, release 9.1, SAS Inst. Inc.) were used to determine differences in parameters

between populations and among seasons and the interaction.

RESULTS

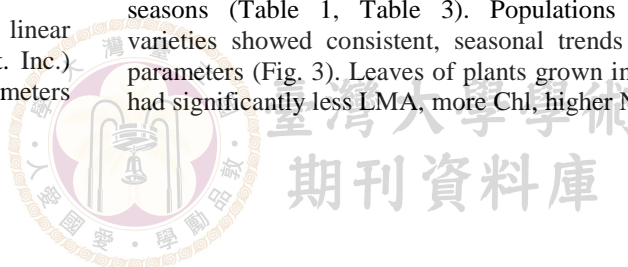**Phenotypic variation between altitudinal populations**

In *B. pilosa* var. *minor*, seasonal effect on LMA was detected, however, no significant difference was found in LMA between populations (Fig. 2a, Table 1). In contrast, significant differences were found in chlorophyll (Chl) and N contents (per leaf area), δ¹³C and seed weight between populations (Table 1). In general, plants at 1600 m had significantly higher Chl (ca. 5 μg cm^{-2} more, Fig. 2c) and N content (Fig. 2e), more positive δ¹³C values (ca. 1-1.5‰, Fig. 2g) and heavier mean dry weight of seeds (ca. 20%, Table 2) than those at 500 m.

Similar to *B. pilosa* var. *minor*, pattern of differences in Chl (Fig. 2d) and N contents (Fig. 2f), δ¹³C values (Fig. 2h) and mean seed weight was found in *B. pilosa* var. *radiata* at two altitudes. In contrast to no difference in LMA between populations of *B. pilosa* var. *minor* (Table 1), *B. pilosa* var. *radiata* plants at 1300 m had significantly higher LMA (about 30%) than those at 500 m (Fig. 2b).

Values of leaf δ¹³C (-32 to -29‰, Fig.2g, 2h) confirm that both varieties are C3 plants.

Measurements of common garden-grown plants

For both varieties the values of LMA, Chl and N content and δ¹³C showed significant difference between seasons (Table 1, Table 3). Populations of both varieties showed consistent, seasonal trends in these parameters (Fig. 3). Leaves of plants grown in summer had significantly less LMA, more Chl, higher N content
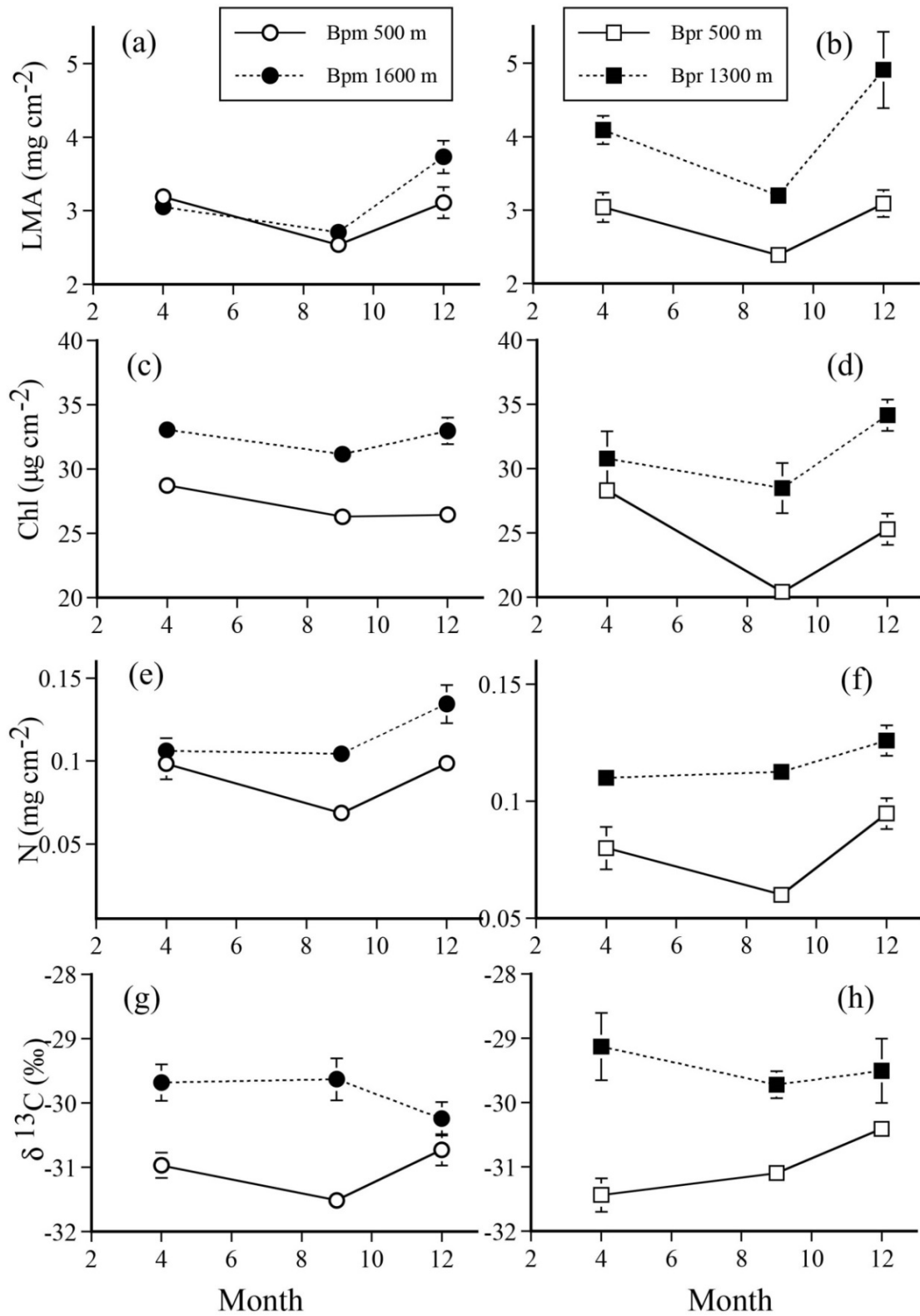
Fig. 2. Leaf mass per area (LMA) (a, b), chlorophyll content (Chl) (c, d), nitrogen content (N) (e, f) and stable carbon isotope ratio (δ^{13}C) (g, h) of leaves of *Bidens pilosa* var. *minor* (Bpm) (a, c, e, f) and *B. pilosa* var. *radiata* (Bpr) (b, d, f, h) growing in low-(open symbols) and mid-(closed symbols) altitudes in different month of 2007.

Table 3. Results of *P* value from the two-way ANOVA assessing the effect of season, population (altitude) and the interactions of both on leaf mass per unit area (LAM), chlorophyll content (Chl), nitrogen content (N), stable carbon isotope ratio (δ^{13}C) and seed weight of *Bidens pilosa* var. *radiata* growing in field or in a common garden. NA: not available.

Variable	Field growing plants			Garden transplants		
	Season	Altitude	Season × Altitude	Season	Altitude	Season × Altitude
LMA	<0.01	<0.01	0.08	<0.01	0.61	0.65
Chl	<0.01	<0.01	0.08	<0.01	0.79	0.34
N	<0.01	<0.01	0.08	0.01	0.08	0.80
δ^{13}C	0.25	<0.01	0.09	0.02	0.42	0.20
Seed weight	0.54	<0.01	0.80	NA	0.15	NA

but more negative δ^{13}C values than those in spring. Consistently higher LMA, Chl and N content and more negative δ^{13}C values was found in *B. pilosa* var. *minor* plants of 1600 m source population than those of 500 m source one (Fig. 3). However, among these parameters, significant difference was detected only in Chl content between populations (Table 1). In *B. pilosa* var. *radiata*, the phenotypic differences in LMA, Chl and N content between field populations growing at alt. of 500 and 1300m was not found in common garden grown plants (Table 3, Fig. 3).

Seed germination behavior

In comparison between seeds of the two altitudinal populations of *B. pilosa* var. *minor* germinated at the same temperature regime, similar cumulative germination percentage (ca. 97%) was found after 14 days of sowing, however, seeds collected from 1600 m population germinated faster and required significant less days to germinate (Fig. 4a, Table 4).

Comparing at two temperature regimes, seeds produced by *B. pilosa* var. *minor* population at the same altitude showed no significant difference in cumulative germination percentage after 14 days of sowing (Table 4). However, seeds at 30/25 °C had faster germinated speed and required significantly less days to germinate than those at 18/13°C require (Table 4).
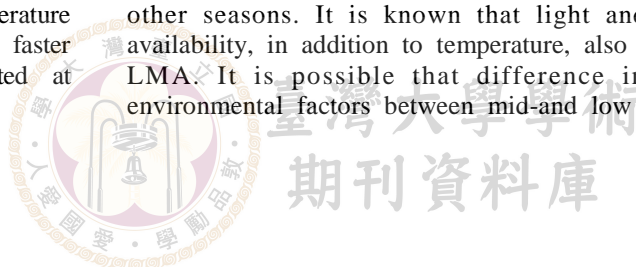
In comparison between seeds of the two altitudinal populations of *B. pilosa* var. *radiata* germinated at 30/25°C, no significant difference was found in cumulative germination percentage (ca. 90%) after 14 days of sowing, but seeds of 500 m populations had significantly faster germination speed and required less days to germinate (Fig. 4b, Table 5). In comparisons of seeds between the two altitudinal populations germinated at 18/13°C, seeds produced by 500 m population had significantly higher germination percentage (ca. 20% more), faster germination speed and required less days to germinate (Fig. 4b, Table 5). In addition, significant difference in germination behavior was found between seeds of *B. pilosa* var. *radiata* collected from the same altitudinal population but grown at different temperature regimes, higher germination percentage, faster germination rate and less mean days to germinated at 30/25°C than at 18/13°C.

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Note: Only a small population of about 10 individuals were found. In the *Platanthera* genus in Taiwan, the labella have sidelobes only found in three species: *P. devolii*, *P. sonoharae* and *P. nantousylvatica*. This species differs from the other two in having elliptic or oblong leaves, in contrast to linear ones. The specific epithet refers to the location where it was found.

DISCUSSION

This study measured ecophysiological traits of plants of two varieties of *B. pilosa* growing in two different altitudes in natural condition and those of their progeny grown in a common garden. Results of the measurements not only provide information on the degree of phenotypic variation between the altitudinal populations but also provide opportunity to determine whether the variation is under environmental and/or genetic control.

The two varieties showed differential response in leaf mass per area (LMA) to altitudinal variation (Fig. 2a, 2b). Mid-altitude population of the var. *radiata* had significantly higher LMA than low-altitude population indicating that mid-altitude population had thicker and/or denser leaves than the low-altitude population. The difference in LMA between two altitudinal populations of the var. *radiata* was not found in common garden-grown plants, which suggested that the population variation in LMA was due to phenotypic plasticity and not from genetic differentiation. It has been found that lower temperature could reduce leaf expansion and result in thicker leaves (Woodward, 1979). The pattern of seasonal changes in LMA, low in summer and high in winter, found in field populations (Fig. 2) and in plants grown at the common garden (Fig. 3) suggest that high LMA found in the mid-altitude population of the var. *radiata* may be a consequence of the effect of lower temperatures. Seasonal effect on LMA was also found in common garden-grown populations of the var. *minor* (Fig. 3, Table 1). However, difference in LMA between field populations of *var. minor* was only detected in Dec. 2007 but not in other seasons. It is known that light and water availability, in addition to temperature, also affected LMA. It is possible that difference in other environmental factors between mid-and low altitude
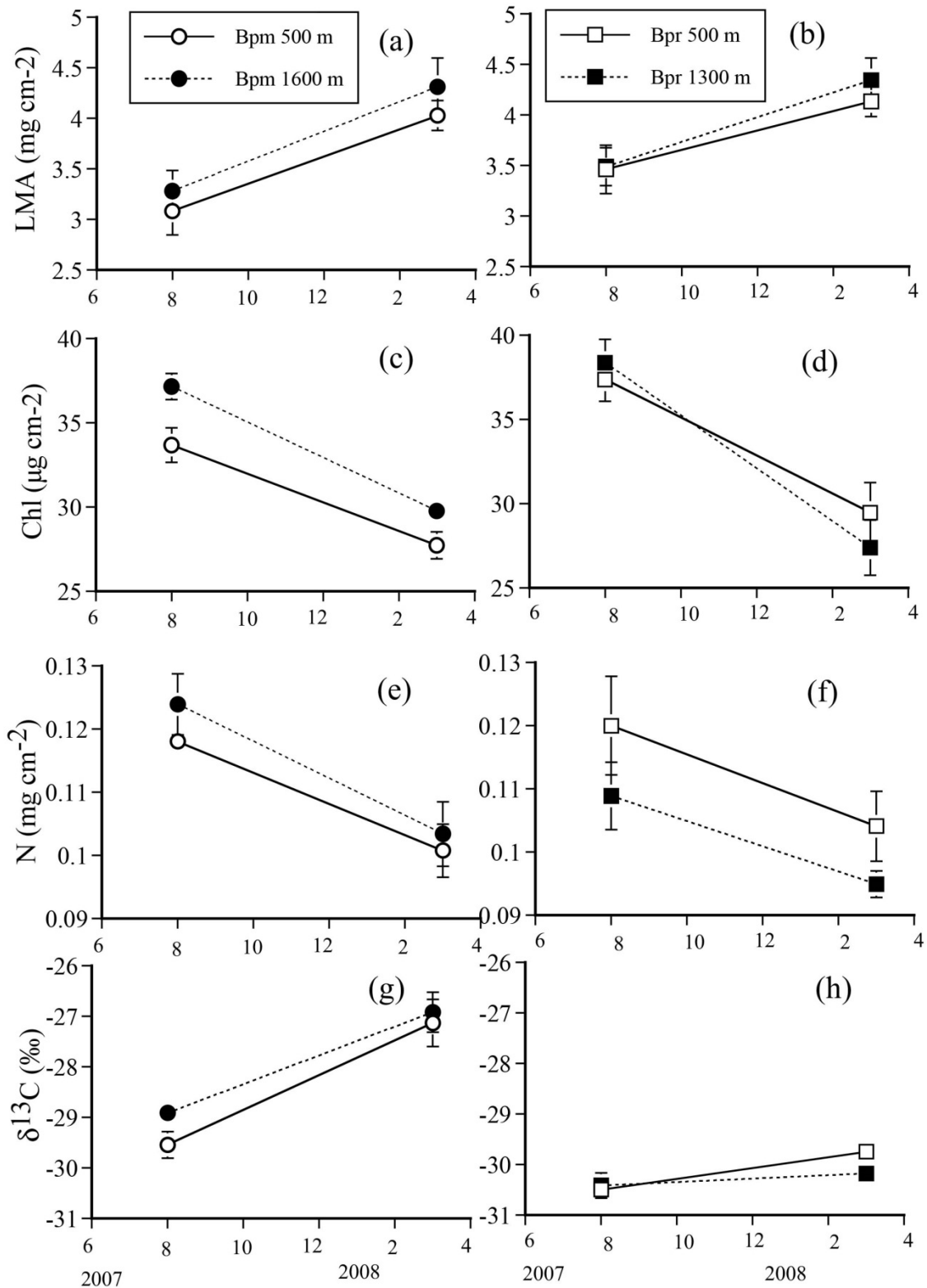
Fig. 3. Leaf mass per area (LMA) (a, b), chlorophyll content (Chl) (c, d), nitrogen content (N) (e, f) and stable carbon isotope ratio (δ^{13}C) (g, h) of leaves of *Bidens pilosa* var. *minor* (Bpm) (a, c, e, f) and *B. pilosa* var. *radiata* (Bpr) (b, d, f, h) plants, origins of low-(open symbols) and mid-(closed symbols) altitudes, grown at a common garden from April to Oct. of 2007 and from Oct. to May of 2008, respectively. Data were taken in August 2007 and March 2008.
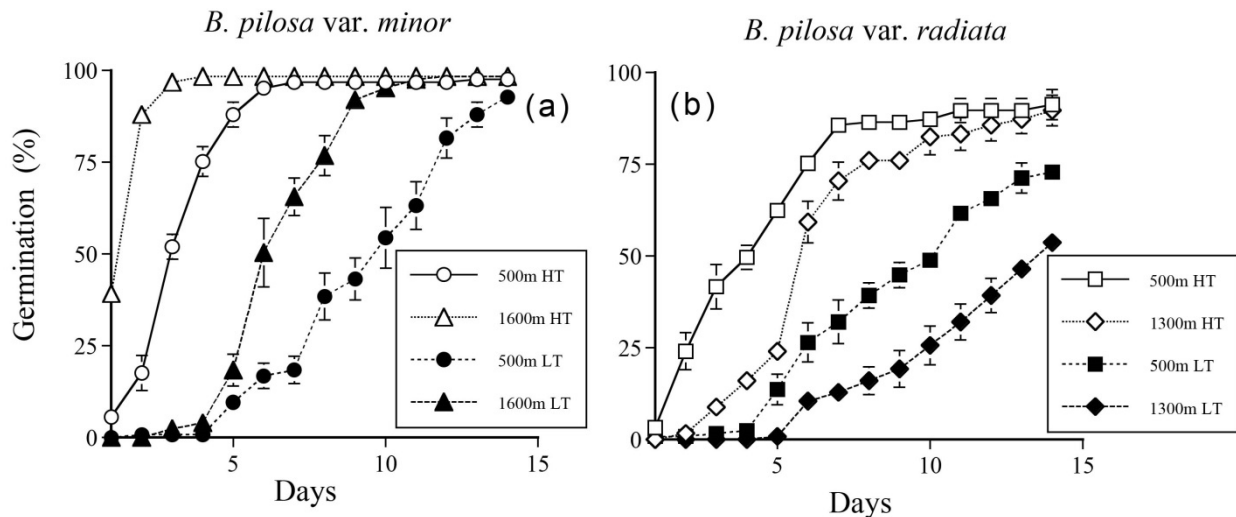
Fig. 4. Cumulative germination percentage of *Bidens pilosa* var. *minor* (a) and *B. pilosa* var. *radiata* (b) seeds, collected from low- (500 m) and mid- (1600 m or 1300 m) altitudinal populations, under two temperature regimes, 30/25 (HT) and 18/13℃ (LT),for 14 days.

offset the temperature effect on LMA of the var. *minor*.

C3 plants growing at higher altitudes might compensate partially for reduced partial pressure of CO_2 by increasing the concentrations of RuBp carboxylase per unit leaf area, thus leaf N concentrations per area have been found to increase with altitudes in both herbaceous and woody species (Morecroft and Woodward, 1996; Weih and Karlsson, 2001; van de Weg *et al.*, 2009). In this study, we also found that mid-altitudinal populations of both varieties had significantly higher leaf N content per unit leaf area than the low- altitudinal populations (Fig. 2e, 2f). In addition, mid altitudinal populations also had higher Chl contents than at low- altitudinal populations (Fig. 2c, 2d). An increased in leaf nitrogen and chlorophyll contents suggested that mid-altitudinal populations had higher photosynthetic capacity than low-altitudinal populations. In the common garden experiment, progeny of mid-altitudinal population of var. *minor*, but not var. *radiata*, also had significantly higher Chl contents than those of low-altitude population (Fig. 3c, 3d). Accordingly, our results evidenced both genetic and environmental effects on Chl. contents of the var. *minor* but mainly environmental effects on that of the var. *radiata*.

In vascular plants, increase leaf $\delta^{13}C$ with elevation has been reported widely in interspecific and intraspecific comparison (Morecroft, Woodward and Marrs, 1992; Hultine and Marshall 2000; Qiang *et al.*, 2003; Takahashi and Miyajima, 2008). In this study, for both varieties growing in field, mid-altitudinal populations also had more positive $\delta^{13}C$ (Fig. 2g, 2h), indicating a lower ratio of CO_2 partial pressure inside the leaf to that in the atmosphere and a higher ratio of carboxylation efficiency to stomatal conductance (Farquhar *et al.*, 1982), than low

altitudinal populations. It has been found that the increase in leaf $\delta^{13}C$ with elevation was associated with higher leaf mass per area (LMA) at lower temperatures (Körner *et al.*, 1991; Vitousek *et al.*, 1990; Cordell *et al.*, 1999). Though mid-altitudinal population of var. *radiata* also had higher LMA than low-altitudinal population (Fig. 2b), however, no significant difference was found in LMA between the two populations of var. *minor* (except in Dec. 2007, Fig. 2a). The result suggests that the difference in $\delta^{13}C$ between populations of var. *minor* was not resulted from their differences in LMA; other factors (probably N content) were involved. In contrast to field growing populations, no significant difference was found in $\delta^{13}C$ of plants with different altitude origins grown in a common garden (Fig. 3e. 3f). Accordingly, environmental effect contributed to phenotypic variation in $\delta^{13}C$ between the two-altitudinal populations of both varieties.

Seed weight is a critical trait of a plant's life history. In general, larger seeds can have a higher establishment success, as they provide more reserves for seedlings (Moles and Westoby, 2004). As favorable conditions for seed recruitment decrease in higher altitudes, selection might favor populations producing large seeds at higher altitudes. Indeed, increasing seed weight of the same species with increasing altitude has been reported (Lord, 1994; Ayana and Bekele, 2000). In consistent with these reports, our results also showed that in both varieties mid-altitudinal populations did produce heavier seeds than low-altitudinal populations (Table 2). However, in common garden experiment, no significant difference was found in seed weight between seeds produced by progeny from different altitudes. Accordingly, environmental effects caused phenotypic variation in seed weight between field populations. It was reported that in *Aegilops ovata*
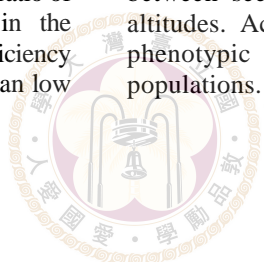
Table 4. Germination percentage, means days to germinate and the speed of germination (no. of seed germinate per day) of *Bidens pilosa* var. *minor* seeds, collected from two altitudinal (500 m and 1600 m) populations, after germination at two temperature regimes (30/25 and 18/13℃),for 14 days. Values are means ± standard errors (n = 5). Means within the same category followed by different letters were significantly different (LSMEAN, P < 0.05).

	Seed source	Germination temperature	
		30/25℃	18/13℃
Germination(%)	500m	97.6 ± 0.98	92.8 ± 2.90
	1600m	98.4 ± 1.60	98.4 ± 0.98
Mean days to germinate	500m	3.6 ± 0.2[c]	9.5 ± 0.4[a]
	1600m	1.7 ± 0.1[d]	6.9 ± 0.3[b]
Speed of germination	500m	8.2 ± 0.5[b]	2.7 ± 0.2[c]
	1600m	16.7 ± 0.5[a]	3.8 ± 0.1[c]

Table 5. Germination percentage, means days to germinate and the speed of germination (no. of seed germinate per day) of *Bidens pilosa* var. *radiata* seeds, collected from two altitudinal (500 m and 1600 m) populations, after germination at two temperature regimes, 30/25 and 18/13 ℃,for 14 days. Values are means ± standard errors (n = 5). Means within the same category followed by different letters were significantly different (LSMEAN, P < 0.05).

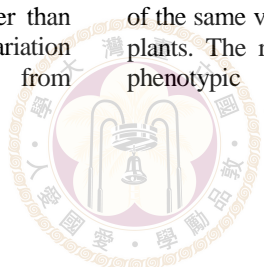|	Seed source	Germination temperature	
		30/25℃	18/13℃
Germination(%)	500m	91.2 ± 4.1[a]	72.8 ± 3.2[b]
	1600m	89.6 ± 4.1[a]	53.6 ± 3.0[c]
Mean days to germinate	500m	4.4 ± 0.3[d]	8.4 ± 0.2[b]
	1600m	6.5 ± 0.3[c]	10.3 ± 0.4[a]
Speed of germination	500m	7.0 ± 0.5[a]	2.6 ± 0.2[c]
	1600m	4.0 ± 0.3[b]	1.4 ± 0.1[d]

(Datta *et al.*, 1972) and in *Plantago lanceolata* (Alexander and Wulff, 1985; Lacey, 1996) the seeds produced under lower temperatures were heavier than those developed at warmer conditions. Thus, the phenotypic variation in seed weight of *B. pilosa* varieties might be also caused by growth temperature.

Seeds of both varieties did not show apparent dormancy, but their germination is sensitive to temperature (Fig. 4). Regardless of their origins, seeds of both varieties germinated significantly more rapidly at 30/25 than at 18/13°C. The result suggests that seeds of both varieties produced by mid-altitudinal populations were not more adapted to germinate at 18/13°C than those by low-altitudinal populations.

Though seed germination of both varieties showed similar response to temperature, difference was detected in germination speed between populations of the same variety at the same temperature regime (Table 4). In var. *radiata*, seeds produced by the mid-altitudinal population germination slower, either at 30/25 or at 18/13°C, than those by low-altitudinal population. Similar results were reported in *Chenopodium bonus-henricus* L. (Dorne, 1981) and in *Festuca novae-zelandiae* (Lord, 1994), in which slower germination rates were observed with increasing seed size. A different result was detected in the var. *minor*, in which seeds collected from mid-altitudinal population were also heavier; however, they geminated faster than those from low-altitudinal population (Table 4). Variation in germination characteristics between seeds from

different environments may be due to heritable differences (ecotypic differentiation) and/or phenotypic difference resulting from differences in the environment experienced by maternal parent during seed maturation (maternal environment effect). Numerous studies have shown maternal effect on seed germinability (Alexander and Wulff, 1985; Gutterman, 2000; Figueroa *et al.*, 2010). Ecotypic differentiation in seed germination was also reported (Meyer and Pendleton, 2005; Shin and Kim, 2013). Results of current study cannot distinguish the extent of these two factors contributing to the behavior of seed germination in these two varieties. Whatever the causes of the phenomenon, the result suggest that the var. *minor* is more adapted to mid-altitude than the var. *radiata* in term of their germination behavior. Though the spread of *B. pilosa* var. *radiata* into higher elevation might be potentially limited by its germination behavior, however, it is known that the plant is capable of vegetative reproduction (Hsu, 2006; Huang 2008) which might compensate for its inferiority in seed germination behavior at higher elevations.

In conclusion, results of this study highlights high levels of phenotypic variability for leaf functional traits between two altitudinal populations of both varieties of *B. pilosa*. However, most of the variations, except Chl content of the var. minor, found between field populations of the same variety disappeared in common garden-grown plants. The results suggested that these variations were phenotypic plasticity in response to changes in
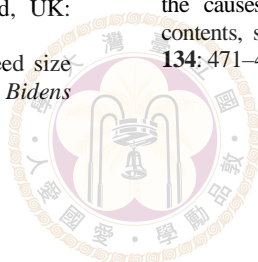
environmental factors associated with altitudes. Seeds of the var. *radiata* had inferior germination performance than those of the var. *minor* at lower temperature, which might reduce the competitive ability of the var. *radiata* at mid-altitude. However, high degree of phenotypic plasticity in combination with its ability of vegetative reproduction (Hsu and Kao, 2014) might contribute to the expansion of *B. pilosa* var. *radiata* into mountain area.

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