

THE CHARACTERISTICS OF REPRODUCTIVE ALLOCATION OF *PRUNELLA VULGARIS* L. POPULATIONS IN HETEROGENEOUS ENVIRONMENTS

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Abstract

To provide a basis for the reasonable utilization and protection of natural *Prunella vulgaris* L. resources, the current study was conducted to analyze the characteristics of reproductive allocation between sexual and asexual reproduction in *P. vulgaris* populations from heterogeneous environments. Multiple comparison and correlation analyses were carried out to understand the proportions of sexual and asexual reproduction of *P. vulgaris* in heterogeneous environments. The results showed that total biomass reached its maximum in the ripening period in heterogeneous environments. Each mode of biomass allocation among the different populations was sharp; the biomass allocation to spica and cloning changed dramatically, showing gradient differences with environment changes. The volume-weight, organic matter, water content and species diversity in heterogeneous environments played very important roles in the clonal growth process of *P. vulgaris*. The intensity of *P. vulgaris* reproductive allocation was different in different heterogeneous environments, which supports a trade-off relationship between sexual and asexual reproduction.

Key words: *Prunella vulgaris* L.; Heterogeneous habitats; Reproductive allocation; Sexual reproduction; Asexual reproduction.

Introduction

Prunella vulgaris L. (Labiatae), also known as “self-heal,” is a perennial herb and an important species for medicine in China. *Prunella Spica*, the dried spica of *P. vulgaris*, is a standard medicinal material in the Chinese Pharmacopoeia (Chinese Pharmacopoeia, 2015). At present, *P. vulgaris* has been used as the raw material for nearly 500 kinds of Chinese medicine in China and is also used in the chemical industry, food, herbal tea, and many other fields (Chen *et al.*, 2018). Because of the increasing demand for *P. vulgaris* and the growing deterioration of its environment, the shortage of supply has made some pharmaceutical companies use the whole plant instead of just using the spicas (Zhang *et al.*, 2017). To protect this natural resource effectively and understand its mechanism of reproduction, it is extraordinarily necessary that we study the propagation characteristics of *P. vulgaris*.

The renewal of populations and the completion of individual life histories rely on the reproductive process, patterns of reproductive allocation, and the way organisms adjust their reproductive allocation in order to adapt to specific environments, which are the important tasks of reproductive ecology (Chen *et al.*, 2004). Plant resource allocation patterns reflect the plant's choice of environment and the plant's adaptation to the environment. Many studies have shown that changes in light intensity, soil moisture, and nutrients affect the energy allocated to vegetative growth and reproductive growth (Rautiainen *et al.*, 2004; Quan & Li, 2008; Wang *et al.*, 2009; Nie *et al.*, 2009; Chen *et al.*, 2017). Life history strategies also differ among different plants. Plants can only achieve their optimal characteristics under certain conditions (Obeso, 2002), which indicates that there are trade-offs between life-history traits and investment. Current research on *P. vulgaris* mainly focuses on germplasm resources, genetic diversity and seed germination (Guo *et al.*, 2006; Zhang *et al.*, 2008; Chen *et al.*, 2009; Liao & Guo, 2009); the

characteristics of reproductive allocation of *P. vulgaris* in heterogeneous habitats have not been reported in recent studies. On the basis of the population component of biomass, sexual reproduction and asexual reproduction, this study will discuss how the growth environment influences reproduction allocation in *P. vulgaris* and provide a basis for the rational utilization of this resource.

Materials and Methods

Study area: The test area was located in the Middle and Lower Reaches of the Yangtze River (China), the Yangtze river north and south plains area. This region belongs to the north Asia tropics; it has a warm climate and abundant rainfall, with plains, many lakes, and some mountains. The zonal soil was a yellow brown soil. Our survey samples were set in Chuzhou city in Anhui Province and Nanjing city and Zhenjiang city in Jiangsu Province. We choose six locations that have obvious differences in physiognomy, topography and soil characteristics (Table 1).

Survey method: From April 2012 to November 2012, depending on the sample area and the *P. vulgaris* population size, we established 6–8 1 m × 1 m representative samples. We calculated the Margalef abundance index, Shannon-Wiener index (H'), Simpson diversity index (D) and Pielou evenness index (Jp) according to the species number, individual abundance of all plant species and the importance value.

Margalef abundance index (Ma): $Ma = (S-1)/\ln N$ (Ashman & Barker, 1992)

Shannon-Wiener index (H'): $H' = -\sum Pi \ln(Pi)$ (Ayyad, 2003)

Simpson diversity index (D): $D = 1 - \sum (Pi)^2$

Pielou evenness index (Jp): $Jp = -\sum [Pi \ln (Pi)] / \ln(S)$ (Barrat, 1996)

S: the number of species; N: the number of individuals of all species; Pi: $IV/\sum IV$.

Table 1. Soil and communities of *P. vulgaris* populations included in this study.

Population	Water content /%	Soil pH	Volume-weight (g·cm ⁻³)	Organic matter g·kg ⁻¹	Soil species	Cover degree (%)	Community character	Habitat	Geographic location
NSS	24.09	6.36	1.55	24.22	Thick layer of yellow sand soil	Approximately 75%	<i>P. vulgaris</i> + <i>Cynodon dactylon</i> (Linn.) Pers. For constructive species	Sunny gentle slope	31°53'16.95"N 118°43'59.16"E
LD	20.6	6.39	1.34	26.55	Chestnutcoloured soil	Approximately 50%	No obvious constructive species of weed community	Half cloudy gentle slope	31°44'23.05"N 119°17'07.62"E
WLM	30.65	6.66	1.49	20.84	Thick layer chestnut-coloured soil	Approximately 80%	No obvious constructive species of weed community	Shade slope, near bushwood	31°47'46.06"N 119°15'49.97"E
CS	24.49	6.53	1.49	30.46	Thin layer chestnutcoloured-red soil	Approximately 70%	No obvious constructive species of weed community	Sunny gentle slope	32°34'19.62"N 118°30'33.22"E
GL	19.57	6.39	1.4	14.1	Thin layer Chestnutcoloured soil	Approximately 75%	No obvious constructive species of weed community	Sunny slope, weeds	31°52'57.18"N 118°43'04.23"E
LX	33.47	6.68	1.43	14.2	Glue brown soil	Approximately 80%	Herb community under deciduous broad-leaved forest	Sparse forests	32°06'00.75"N 118°53'32.93"E

During the vegetative period (late March), flowering period (mid to late April), ripening period (mid-May) and dormant period (November), we harvested 20 cm × 20 cm samples of whole *P. vulgaris* plants from each quadrat. Samples were brought back to the laboratory and carefully washed with water; we then counted the number of plant reproductive branches and cloning branches, then separated each plant component and dried to constant weight.

By measuring the total dry weight of *P. vulgaris* in different periods and the dry weight of different plant components at the flowering and ripening periods, we were able to calculate the rhizome biomass ratio (root weight/total biomass), perennial root weight ratio (perennial root weight/total biomass), stem weight ratio (stem weight/total biomass), leaf weight ratio (leaf weight/total biomass), spica weight ratio (spica weight/total biomass), and clone biomass ratio (cloning branches/total biomass).

Statistical analysis: The data were subjected to a two-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Data regarding the interactions were reported when the interactions were statistically significant at $p < 0.05$ and $p < 0.01$. Statistical analyses were conducted using the statistical software package SPSS 18.0 for Windows (SPSS, Chicago, IL, USA).

Results

The dynamic substance accumulation of *P. vulgaris* in different habitats: Biomass is one of the most basic characteristics of a plant. Biomass dynamics reflect the interaction of the plant with the environment and indicate a plant's environmental adaptability and embodiment of the law of growth and development. During the whole growth period, plant biomass in different populations showed a trend of initial increase then decrease; the variation coefficients of different population yields were larger, with the highest variation coefficient at 94.30% (Table 2). The biomass in the NSS population was greater than in other populations. Especially in the ripening period, the per plant yield could be up to 9.22 g; in the dormancy period, it was also quite high (approximately 0.61 g per plant). The yield was smallest in the GL population, where the vegetative, seed and dormant period yields were also relatively small, with per plant yields of 0.28 g, 1.57 g and 0.09 g, respectively. Only in the vegetative and dormant periods did per plant biomass in different habitats reach an extremely significant difference ($p < 0.01$). In addition to the GL and LX populations, per plant biomass in the other populations reached its maximum in the ripening period.

Table 3 shows that reproductive allocation was a dynamic process: the growth of plant spending on sexual reproduction was different in different periods. In the flowering period, the stem and leaf biomass percentages were larger, and in the ripening period, the spica and stem biomass percentages were higher. Compared with the flowering period, the root, perennial root, stem and leaf biomass percentages in the ripening period showed a reducing tendency, and the percentage of leaf biomass was sharply reduced, but the biomass percentage of the spica and cloning branches increased significantly. This

indicated that the growth of *P. vulgaris* began to shift to the reproductive organs. In the ripening period, as the light intensity and community coverage decreased, biomass of root, perennial root and cloning branches increased with the change in habitat conditions, while the stem, leaf and spica biomass percentages decreased (in addition to the stem and leaf biomass percentages in CS). The spica biomass percentage in the LX population was the minimum (20.12%), while the cloning branch biomass percentage (48.44%) was the maximum. These results indicated that *P. vulgaris* reproductive allocation had great ecological plasticity; the reproductive tiller of *P. vulgaris* in different habitats and at different times in the growth process could carry out the appropriate adjustments to reproductive allocation.

Principal component analysis of environmental factors in different environments: Eigenvalues and contribution rates of principal components were based on the principal

component analysis. The cumulative contribution of the first three principal components was 96.085%, so our analysis focused on the first three principal components (Tables 4 and 5). The Eigenvector of each principal component factor reflects the relative contribution of each factor to the principal component. The first principal component contribution rate was 58.882%; the amount of information was largest in the first principal component, which included the Shannon-Wiener index, Pielou evenness index, Simpson diversity index and water content (0.971, 0.973, 0.914 and 0.939, respectively) which showed that the four factors that influenced the reproductive allocation were maximum. In the second principal component, volume-weight was the largest factor (0.973), which showed that the effect of the one factor on the reproductive allocation was maximum; in the third principal component, organic matter was the largest factor (0.919), showing that the effect of this factor on the reproductive allocation was maximum.

Table 2. The biomass multiple comparison analysis of *P. vulgaris* in different populations /g.

Population	Vegetative period	Flowering period	Ripening period	Dormancy period
NSS	1.28aA	3.53abA	9.22aA	0.61aA
LD	0.30cAB	3.38abA	4.77abAB	0.09cC
WLM	0.88abA	2.32abA	4.53abAB	0.18bcBC
CS	0.52bcAB	1.50bA	3.52abAB	0.46aAB
GL	0.28cAB	2.18abA	1.57bB	0.09cC
LX	0.83abA	4.32aA	1.90bAB	0.23bcBC
RSD	94.30%	91.67%	89.60%	76.25%

Note: Values within a column sharing an uppercase or lowercase letter are not significantly different at the thresholds of alpha = 0.05 (lowercase letters) or alpha = 0.01 (capital letters)

Table 3. The comparison of *P. vulgaris* biomass allocation in different populations at flowing and ripening.

Population	Root biomass ratio /%		perennial biomass ratio /%		Stem biomass ratio /%		Leaf biomass ratio /%		Spica biomass ratio%		Clone biomass ratio%	
	FP	RP	FP	RP	FP	RP	FP	RP	FP	RP	FP	RP
NSS	10.94a	3.83ab	7.47bc	7.06b	36.08a	32.16a	24.14d	12.58a	21.36b	40.93a		3.44c
LD	6.13b	6.02ab	8.1ab	7.19b	21.99d	20.21b	27.93c	7.04b	35.86a	31.42ab		28.12b
WLM	9.57a	4.57ab	9.57a	6.22b	36.27a	24.69b	34.27bc	7.77b	10.33c	38.22ab		18.53b
CS	9.19a	8.32ab	8.87ab	8.82ab	28.02bc	27.94ab	42.88a	12.37a	11.05c	31.06ab		11.49b
GL	7.58ab	9.84a	6.34c	10.44a	20.84d	20.36b	46.49a	6.73b	18.75b	32.98ab		19.65b
LX	8.5ab	6.46ab	6.69bc	5.64b	25.81cd	12.21c	39.85ab	7.14b	19.14b	20.12c		48.44a

Note: Different letters indicate significant differences at alpha = 0.05. The blank column represents a value which did not exist for the clonal architecture. FP=flowering period, RP=ripening period

Table 4. The Eigenvalues and cumulatives of different environment factors.

Principal component	Eigenvalues	Devotion /%	Cumulatives /%
Principal component 1	5.299	58.882	58.882
Principal component 2	2.033	22.591	81.472
Principal component 3	1.315	14.612	96.085
Principal component 4	0.207	2.297	98.382
Principal component 5	0.146	1.618	100.000
Principal component 6	1.54E-16	1.72E-15	100.000
Principal component 7	4.77E-17	5.30E-16	100.000
Principal component 8	-6.81E-17	-7.56E-16	100.000
Principal component 9	-1.99E-16	-2.21E-15	100.000

Table 5. Principal component values of different environment factors.

Principal component Environment factor	Principal component 1	Principal component 2	Principal component 3
Water content	0.939	0.276	0.086
pH	0.907	0.101	0.089
Volume-weight	0.099	0.973	0.068
Organic matter	0.269	0.207	0.919
Cover degree	0.580	0.763	0.070
Margarlef abundance index	0.615	0.458	0.595
Simpson diversity index	0.914	0.386	0.085
Shannon-Wiener index	0.971	0.102	0.208
Pielou evenness index	0.973	0.078	0.204

The correlation analysis (Table 6) showed that soil volume-weight and root biomass ratio were positively correlated in the flowering period, and soil volume-weight and stem weight showed a significant positive correlation. Organic matter, soil water content, Simpson diversity index, Shannon-Wiener diversity index and Pielou evenness index were positively correlated with the root biomass ratio, perennial biomass ratio and stem biomass ratio; these same factors had a negative correlation with the whole plant weight, leaf biomass ratio and spica biomass ratio. This illustrates that *P. vulgaris* growth in the flowering period was mainly concentrated in the growth of root, perennial root and shoot.

In the ripening period, the water content was positively and significantly correlated with the Simpson diversity index, Pielou evenness index, Shannon Wiener index and pH and negatively correlated with the total plant weight and spica biomass ratio. Water content was also positively correlated with clone biomass ratio, which illustrates that in the environment with high soil water content, species diversity was abundant and limited the sexual reproduction of *P. vulgaris* to a low proportion of reproductive allocation. pH was significantly positively correlated with the Simpson diversity index and Shannon-Wiener index and was very significantly positively correlated with the Pielou evenness index. On the other hand, pH was negatively correlated with total plant weight, spica biomass ratio and clone biomass, which indicates that the neutral and acidic soil environments were not conducive to the growth of *P. vulgaris* but were advantageous to the growth of accompanying plant species. The spica biomass ratio was significantly positively correlated with cover degree and positively correlated with volume-weight and organic matter; it was negatively correlated with the Margarlef abundance index, Simpson diversity index, Shannon-Wiener index and Pielou evenness index. In the environment high in organic matter and soil compaction, the proportion of allocation for sexual reproduction was larger because intense interspecific competition inhibited sexual reproduction of *P. vulgaris*. Volume-weight, organic matter, but positively correlated with the Margarlef abundance index, Simpson diversity index, Shannon-Wiener index and Pielou evenness index, which indicated that in the absence of organic matter, *P. vulgaris* mainly tends toward clonal reproduction in the environment with large population coverage and intense interspecific competition. Spica biomass ratio had a significant negative correlation with clone biomass ratio, which illustrates that an antagonistic effect exists between sexual and asexual reproduction.

Discussion

In different habitats, variation in the ratio of sexual reproduction and asexual reproduction in clonal plants mainly depends on changes in the three ecological factors of moisture, light and temperature (Wang *et al.*, 2005). Under conditions of restricted resources, clonal plants tend to distribute a larger proportion of their biomass to asexual reproduction (Verburg *et al.*, 2000). As the environmental conditions changed, *P. vulgaris* populations revealed certain differences in sexual reproduction and asexual reproduction.

In an environment enriched in organic matter and with compacted soil (NSS), the reproductive allocation for sexual reproduction in *P. vulgaris* was the biggest among all the populations. While clonal reproduction accounted for a small proportion of reproductive allocation, in this suitable habitat, *P. vulgaris* reproduction was mainly composed of sexual reproduction. The results of Macek's research (Macek *et al.*, 2003) are basically identical to our findings: cloning and seed breeding plants of *P. vulgaris* can perform asexual reproduction with stolons. In a favorable environment, shortening the internode length increases the number of branches to expand populations.

In the environments of LD and WLM, the sexual reproduction allocation of *P. vulgaris* was lower than at NSS, but the asexual reproduction allocation was much higher than at NSS. This might be because of intense interspecific competition which forced *P. vulgaris* to contribute its limited resources toward clonal propagation to improve its offspring's reproductive success.

In an arid mountain environment poor in organic matter (GL, CS), *P. vulgaris* was at a disadvantage; growth was limited, sharply reducing the whole biomass and the allocation for sexual reproduction. These results illustrate that soil moisture and organic matter were the main factors limiting sexual reproduction of *P. vulgaris*.

In the understory environment, the volume-weight and organic matter were rather low and interspecific competition was very fierce, resulting in a level of clonal reproductive allocation that was much higher than in the other populations. We found that *P. vulgaris* seedlings were rather scarce in the understory habitat; this was because the forest's environment was not conducive to seed germination and seedling growth. Seedlings in the forest could not easily get enough light, space and soil nutrients, causing insufficient assimilation and slow growth, so the plants need to reproduce clonally (Wang *et al.*, 2005).

Table 6. Correlation analysis between *P. vulgaris* reproductive allocation and environmental factors in different life history periods.

	Water content	pH	Volume-w eight	Organic matter	Cover degree	Margalef abundance index	Simpson diversity index	Shannon-Wiener index	Pielou evenness index	Total plant weight	Root biomass ratio	perennial biomass ratio	Stem biomass ratio	Leaf biomass ratio	Spica biomass ratio
Water content	1.000	0.915	0.338	-0.301	0.621	0.434	0.941	0.874	0.938	0.374	0.401	0.198	0.462	0.037	-0.470
pH	0.915	1.000	0.141	-0.268	0.537	0.430	0.892	0.876	0.948	0.079	0.176	0.322	0.259	0.323	-0.568
Volume-weight	0.338	0.141	1.000	0.226	0.627	-0.336	0.485	0.014	0.168	-0.157	0.992	0.304	0.873	-0.212	-0.655
Organic material	-0.301	-0.268	0.226	1.000	-0.551	0.238	-0.081	-0.104	-0.424	-0.375	0.122	0.670	0.246	-0.399	0.086
Cover degree	-0.290	-0.473	0.653	0.350	1.000	-0.382	0.622	0.253	0.636	-0.023	0.684	-0.124	0.486	0.359	-0.800
Margalef abundance index	0.434	0.430	-0.336	0.238	0.624	1.000	0.424	0.782	0.428	0.488	-0.330	0.160	-0.186	-0.184	0.346
Simpson diversity index	0.941	0.892	0.485	-0.081	0.424	0.424	1.000	0.863	0.909	0.161	0.515	0.304	0.495	0.140	-0.626
Shannon-Wiener index	0.874	0.876	0.014	-0.104	-0.256	0.424	1.000	0.891	0.891	0.383	0.050	0.194	0.116	0.113	-0.226
Pielou evenness index	0.938	0.948	0.168	-0.424	-0.541	0.428	0.909	0.891	1.000	0.263	0.225	0.039	0.186	0.361	-0.522
Total plant weight	-0.134	-0.433	0.580	0.504	0.838	-0.143	-0.116	-0.316	-0.447	1.000	-0.057	-0.475	-0.016	-0.484	0.543
Root biomass ratio	-0.413	-0.125	-0.453	-0.220	-0.516	-0.210	-0.300	-0.212	-0.075	-0.775	1.000	0.232	0.879	-0.227	-0.637
perennial biomass ratio	-0.777	-0.573	-0.177	0.028	0.081	-0.631	-0.636	-0.724	-0.562	-0.292	0.802	1.000	0.548	-0.252	-0.336
Stem biomass ratio	-0.343	-0.443	0.702	0.658	0.892	-0.481	-0.163	-0.528	-0.532	0.762	-0.328	0.219	1.000	-0.494	-0.493
Leaf biomass ratio	-0.086	-0.235	0.781	0.667	0.570	-0.130	0.179	-0.145	-0.206	0.639	-0.244	0.076	0.821	1.000	-0.510
Spica biomass ratio	-0.388	-0.478	0.501	0.365	0.963	-0.669	-0.366	-0.687	-0.613	0.719	-0.411	0.185	0.856	0.425	1.000
Clone biomass ratio	0.498	0.546	-0.600	-0.554	-0.883	0.652	0.328	0.695	0.634	-0.643	0.144	-0.428	-0.969	-0.716	-0.885

Note: The upper triangular matrix correlation coefficients were reproductive allocation and environmental factors in the flowering period, the lower triangle matrix correlation coefficients were reproductive allocation and environmental factors in the ripening period

In summary, the volume-weight, organic matter, water content and species diversity in heterogeneous environments play very important roles in the clonal growth process of *P. vulgaris*.

In different habitats, *P. vulgaris* can choose both sexual and asexual reproduction. Through field investigation, we found that wild *P. vulgaris* resources are endangered by a society in thirsty demand for wild resources and the production, life and grazing interference of humans. *P. vulgaris* habitats were seriously destroyed; some groups exhibited the "island" or "strip" distribution, the area of the populations was small, and groups were very far apart. Small populations are more vulnerable than large populations to environmental fluctuations, disasters and other natural factors and human factors such as farming and grazing (Primack, 1993). Therefore, protection of the *P. vulgaris* resource is extremely urgent at present. For effective conservation of this species, some suggestions can be made based on its characteristics described above. First, measures such as artificial cultivation and transplantation should be taken to increase the number of individuals in the populations. This will accelerate seed production and generative propagation, which is essential for species sexual reproduction. Second, some plantations and gardens should be established near the reserves specifically for medicinal purposes, because *P. vulgaris* is important medicinal plant species in China (Chen *et al.*, 2009). This may be important for the local economy and will also be beneficial for conservation of the natural populations of this species (Liao *et al.*, 2009). The plantation techniques for this species are already available (Chen *et al.*, 2009, 2012, 2013).

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