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## The Importance of Population Origin and Reciprocal Heterogeneous Microhabitat on Clonal Propagation of *Iris japonica* Thunb.

(Kepentingan Populasi Asal dan Mikrohabitat Heterogen Resiprokal ke atas Perambatan Klon *Iris japonica* Thunb.)

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### ABSTRACT

A reciprocal transplant-replant experiment was carried out to investigate the clonal plasticity and local specialization of OAFE population (O type) and BF population (U type) of a clonal rhizome herb *Iris japonica* in contrasting reciprocal heterogeneous habitats on Jinyun Mountain. U Population had better performance of plant size and clonal propagation (including allocation to clonal propagation, daughter ramet and fine rhizome) in different reciprocal heterogeneous habitats than O population. Both the population origin and reciprocal spatial heterogeneous habitat had effects on clonal ramets and biomass of clonal components of experimental plants. The plasticity of clonal growth had difference in clonal components to balance High light-Low soil resources (water) (HL) or Low light-High soil resources (LH) due to the ecological isolation of the two *I. japonica* populations. Our findings indicated that two major types of patterns of spatial covariance of resources can have different effects on the growth and local variation of clonal plants.

**Keywords:** Clonal plant; clonal propagation; genotype (G) × environment (E) interaction; plasticity; reciprocal heterogeneous habitat

### ABSTRAK

Suatu eksperimen resiprokal pemindahan-tanam semula telah dijalankan untuk mengkaji keplastikan klon dan pengkhususan setempat populasi OAFE (jenis O) dan populasi BF (jenis U) daripada rizom herba klon *Iris japonica* dalam pembezaan habitat heterogen resiprokal di Gunung Jinyun. Populasi U mempunyai prestasi yang lebih baik daripada populasi O daripada segi saiz tumbuhan dan perambatan klon (termasuk peruntukan untuk perambatan klon, anak ramet dan rizom halus) dalam habitat heterogen resiprokal yang berlainan. Kedua-dua populasi asal dan ruwang habitat heterogen mempunyai kesan ke atas ramet klon dan biomassa komponen klon dalam tumbuhan yang dikaji. Keplastikan pertumbuhan klon menunjukkan perbezaan dalam komponen klon untuk mengimbangi sumber cahaya tinggi-tanah rendah (air) (HL) atau sumber tanah tinggi-cahaya rendah (LH) yang disebabkan oleh pengasingan ekologi kedua-dua populasi *I. japonica* tersebut. Hasil kajian kami menunjukkan bahawa dua corak utama kovarians ruwang sumber boleh memberikan kesan yang berbeza pada pertumbuhan dan variasi tempatan bagi tumbuhan klon.

**Kata kunci:** Habitat heterogen resiprokal; interaksi genotip (G) × persekitaran (E); keplastikan; perambatan klon; tumbuhan klon

### INTRODUCTION

Environmental gradients, even on very small scales (even within 10 m, fine-scale habitat heterogeneity), may lead to marked selection differentials (Pluess & Stöcklin 2005; Prati & Schmid 2000). Environmental conditions change with canopy density, exposure and slope, resulting in a patchy distribution of microhabitats (Hutchings & de Kroon 1994; Wang et al. 2012b). Changes in abiotic and biotic conditions may lead to major modifications in selection pressure on plant life-history traits (Pluess & Stöcklin 2005; Wang et al. 2012a). Phenotypic plasticity and genetic differentiation have been considered as two significant ways to adapt in patchy microhabitats (Hutchings & de Kroon 1994). Because local adaptation is achieved at a cost of being less well adapted to another environment, then genotype × environment interactions

may result in a trade-off in fitness-related traits across microhabitat variation that can maintain genetic variation (Prati & Schmid 2000). Morphological, physiological as well as life-history traits within populations were found to respond to heterogeneous environmental factors, e.g. competition (Prati & Schmid 2000; Rautiainen et al. 2004), water and light (Wang et al. 2012a) as well as spatial and temporal heterogeneity. Genetic differentiation also has been documented to environmental variation.

Clonal growth is among the most noticeable adaptations to heterogeneity of resources in environment. Traits and allocation of clonal propagation is commonly assumed to be significant in population expansion, survival of offsprings and seed recruitment in future (Hutchings & de Kroon 1994). Habitat heterogeneity can lead to the differences of clonal traits that accelerate local

adaptation of populations (Prati & Schmid 2000; Wilk et al. 2009). Few studies have quantified intraspecific clonal propagation variation along small-scale ecological gradients and the relative importance of population origin and microhabitat, although environmental conditions, such as spatial and temporal heterogeneity of light and soil resources in forests can strongly affect the reproduction of perennial plants.

A typical heterogeneity in forest, habitats with reciprocal patchiness (i.e. under forest and at forest edge), has low light-high soil resources (water content and nutrient) and high light-low soil resources, respectively (He et al. 2011). Here we ask if genetic differentiation of populations in clonal propagation is affected by two prominent canopy environments: Forest understory and forest edge. Hence, investigation of clonal traits (quantitative traits, biomass and allocation of clonal propagation) in *Iris japonica* populations in reciprocal patchiness of habitats (low light-high soil resources and high light-low resources) by a reciprocal transplant-replant experiment (i.e. common garden experiments) is required to explore local adaptation or specialization of understory herb populations to specific habitats.

## MATERIALS AND METHODS

### STUDY SPECIES

*Iris japonica* Thunb. (Iridaceae) is a perennial herb reproducing by both seeds and vegetative rhizomes. The clonal rhizome system includes vertical coarse rhizome and horizontal fine branched rhizome. Vegetative reproduction by clonal growth is the main method of spread and it is commonly used for pharmacy and for constructing understory horticultural landscape (Wang et al. 2012a). It occurs preferentially in the understory of sparse forest, forest gap, forest edge and the moist grassland, forming mono-dominant groups. Its geographical distribution is in Asia, mainly in south China and Japan (Wang et al. 2013). *Iris japonica* populations were mainly distributed in sparse forest and at the edge of evergreen broad-leaved forest in Jinyun Mountain of Chongqing, Southwest China.

Plants were from *Cinnamomum camphora* forest edge (O habitat, including two sites O1 and O2) ( $29^{\circ}50'15''\text{N}$ ,  $106^{\circ}23'22''\text{E}$ ) and bamboo forest (U habitat, two sites U1 and U2) ( $29^{\circ}49'38''\text{N}$ ,  $106^{\circ}23'4''\text{E}$ ). Both the habitats were 5 km parted away with sparse canopy by *Phyllostachys pubescens*. Two study sites of each habitat type have a distance of  $>800$  m. The *I. japonica* plants in U and O habitats has been living for at least five years.

### EXPERIMENTAL DETAILS

In a simulating reciprocal transplant-replant experiment, a total of 72 *I. japonica* new ramets from two U sites (U1 with 18 ramets and U2 with 18 ramets) and two O sites (O1 with 18 ramets and O2 with 18 ramets) were sampled in August 2007. The new ramets were growing well with three new leaves and at the same height, the distance between the sampled ramets was more than 5 m. These ramets from two U sites and two O sites represented U populations and O populations, respectively.

The reciprocal heterogeneous habitats were as follows (Figure 1). High light-Low soil resources (water) level (HL) was created by growing ramets without shading and without watering, simulating natural O habitat. The water content of origin soil was 5.71%. Low light-High soil resources level (LH) was created by placing ramets under black mesh that transmitted about 30% of photosynthetic photon flux density and watering often, simulating natural U habitat. The water content of origin soil was 28.69%. The high level of soil resources was due to common watering every 2-4 days depending on how fast the soil dried.

Plants grew in experimental area of Key Laboratory of Eco-environments in Three Gorges Reservoir Region, Southwest University. Ramets were planted in soil cells ( $70 \times 70$  cm, 50 cm height, total 72 cells) filled with the above two types of soil from Jinyun Mountain and the cells separated from each other with each treatment were set randomly. The following four treatments were applied: U-HL (U populations in High light-Low soil resources), U-LH (U populations in Low light-High soil resources), O-HL (O populations in High light-Low soil resources) and O-LH (O populations in Low light-High soil resources).

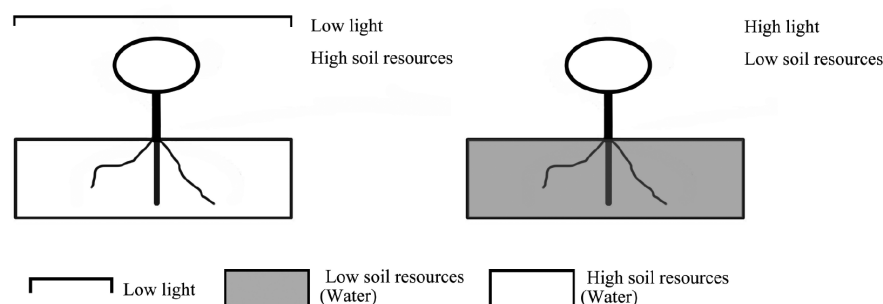


FIGURE 1. Experimental design of contrast reciprocal heterogeneous habitats. Low light-High soil resources in the left (simulated U habitat) and High light-Low soil resources in the right (simulated O habitat)

Simulated experiment was carried out on November 21, 2007, three months after transplant-replant. The ramets were grown until May 22, 2008 at the end of the natural growing season.

The number of new ramets and dead ramets in each sample was counted on December 21, 2007 (30 days after simulated experiment), January 20, 2008 (60 days), February 19 (90 days), March 5 (105 days), March 20 (120 days), March 30 (130 days), April 9 (140 days), April 19 (150 days), April 29 (160 days), May 8 (170 days) and May 18 (180 days). All ramets were then excavated and the number of primary and secondary daughter ramets and the length and diameter of coarse rhizome of ramets were measured on May 22, 2008. Length, surface area and volume of coarse and fine rhizome and root were analyzed by WinRHIZO Pro v.2004c Root Analysis System (Regent, Canada). Thus, fine rhizome length and root length per density were calculated.

One sample (in a soil cell) including mother ramet and daughter ramet can be divided into growth, sexual reproduction and clonal propagation. Growth part included leaves of mother ramets. Clonal propagation included coarse rhizome, fine rhizome, root and daughter ramets. All parts of plant were dried at 80°C for 48 h and then weighed.

#### DATA ANALYSIS

Individual plants were allocated randomly to the treatments, but we checked for relevant systematic bias in the initial state of the plants (total length of the main roots and the height of the ramet). Ramets and rhizome systems of *I. japonica* obviously contributed considerably to the plant performance in the growing season. Population origin and simulating habitat were employed as independent factors, one-Way and two-Way ANOVA were carried out using SPSS statistical package (SPSS Inc. Chicago, USA).

#### RESULTS

##### QUANTITATIVE TRAITS OF CLONAL PROPAGATION IN RESPONSE TO POPULATION ORIGIN (P) AND RECIPROCAL HETEROGENEOUS TREATMENT (T)

Genet mortality was higher in O population with Low light-High soil resources (11.11%). Clonal ratio (per genet) of *I. japonica* was higher in U population than that in O population despite reciprocal heterogeneous treatments (Table 3).

Population origin (P) had uniquely significant influence on new ramets per genet of *I. japonica* ( $p < 0.001$ ) before April 19 (fast growing season), however, both population origin (P) and reciprocal heterogeneous treatment (T) had significant effect after that time ( $p < 0.05$ ) (Table 1). The number of new ramets from U habitat was higher in High light-Low soil resources and its value in U-HL was the highest after April 19 (fast growth season) (Tables 1- 3). The number of second daughter ramets, length and

surface of fine rhizome were significantly affected by the population origin (Table 2). These parameters were significantly higher in populations from U habitat than that from O habitat (Table 3). Specific fine rhizome length significantly responded to reciprocal heterogeneous treatments, which indicated length of fine rhizome per unit weight was higher in Low light-High soil resources than in High light-Low soil resources despite population origin (Tables 2 and 3). Both the population origin and reciprocal heterogeneous treatments were significant for the number of first daughter ramets (Table 2). The parameter was higher for population from U habitat and in High light-Low soil resources (Table 3).

##### BIOMASS AND ALLOCATION OF CLONAL PROPAGATION IN RESPONSE TO POPULATION ORIGIN (P) AND RECIPROCAL HETEROGENEOUS TREATMENT (T)

Both the population origin and reciprocal heterogeneous treatments had significant influence on biomass of total plant, clonal propagation, coarse rhizome, fine rhizome and to a less, on leaves of clonal propagation (Table 2). These parameters were significantly higher in populations from U habitat than in O habitat (Table 3). Biomass of each component from U population showed no significant difference between Low light-High soil resources and High light-Low soil resources. However, plants from O population produced more these components in High light-Low soil resources than in Low light-High soil resources (Tables 2 and 3).

Allocation to clonal propagation as well as to leaves of clonal propagation significantly responded to population origin (Table 2). The two parameters were significantly higher in populations from U habitat than in O habitat (Table 3). However, both the population origin and light treatment had no significant influence on allocation to coarse rhizome and fine rhizome (Tables 2 and 3).

#### DISCUSSION

Significant differences in growth and clonal propagation among populations of *I. japonica* and some evidence for adaptive population differentiation in contrasting habitats were found. The results partly supported the effects of population origin and habitat on the performance of clonal plants, depending on reciprocal experience of above- and below-ground resources. In general, plants grew under environmental stress (limited resources, e.g. light, water and nutrients) varied in size and fewer plants reproduced simultaneously with both reproductive modes (Guo et al. 2011; Rautiainen et al. 2004). However, in a reciprocal spatial heterogeneous habitat (Low light-High soil resources and High light-Low soil resources), environmental stress had different influences on plants in above-ground and under-ground. The effect of micro-habitat on both population size and clonal propagation can be divided into above-ground resource (light) and below-ground resource (water) components. Clonal propagation

TABLE 1. F-values of ANOVAs of effect of population origin (P) and reciprocal heterogeneous treatment (T) on number of new ramets per genet in different time of *I. japonica*

Source	df	Number of new ramets per genet in different time									
		Jan 20, 2008 (60 d)	Feb 19 (90 d)	Mar 5 (105 d)	Mar 20 (120 d)	Mar 30 (130 d)	Apr 9 (140 d)	Apr 19 (150 d)	Apr 29 (160 d)	May 8 (170 d)	May 18 (180 d)
P	1, 68	22.51***	30.88***	34.45***	33.50***	50.62***	46.25***	52.29***	44.90***	36.06***	61.18***
T	1, 68	2.50	1.60	1.16	0.28	2.26	2.51	6.67*	10.08**	13.93***	25.32***
P×T	1, 68	1.28	0.58	1.74	1.87	0.09	0.01	0.267	0.01	0.00	1.10

\*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ . P: population origin, and T: reciprocal heterogeneous treatment

TABLE 2. ANOVA summary of the effect of population origin, reciprocal heterogeneous treatment and interaction of treatments on clonal propagation in stimulated experiment

Quantitative traits	df	P-value					
		Number of 1st daughter ramet	Number of 2nd daughter ramet	Length of fine rhizome	Surface area of fine rhizome	Specific fine rhizome length	
P	1	<0.001***	<0.001***	<0.001***	<0.001***	0.150	
T	1	<0.001***	0.083	0.987	0.403	<0.001***	
P×T	1	0.499	0.280	0.796	0.702	0.976	
Biomass	df	Total plant	Clonal propagation	Leaves of clonal propagation	Coarse rhizome	Fine rhizome	
P	1	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***	
T	1	0.002**	0.008**	0.057	0.012*	0.001**	
P×T	1	0.838	0.958	0.759	0.377	0.944	
Allocation	df	Clonal propagation	Leaves of clonal propagation	Coarse rhizome	Fine rhizome		
P	1	<0.001***	0.001**	0.598	0.148		
T	1	0.337	0.822	0.505	0.124		
P×T	1	0.477	0.516	0.986	0.829		

\*\*\*  $p < 0.001$ , \*\*  $p < 0.01$  and \*  $p < 0.05$ . P: population origin, and T: reciprocal heterogeneous treatment

TABLE 3. Traits of clonal propagation in *Iris japonica* populations from O and U habitats in a reciprocal heterogeneous experiment with High light-Low soil resources (HL) and Low light-High soil resources (LH) (mean±s.e.)

Reciprocal heterogeneous treatments Population origin	High light-Low soil resources		Low light-High soil resources	
	U habitat	O habitat	U habitat	O habitat
Quantitative traits				
Genet mortality (%)	0	0	0	11.11
Clonal ratio (%)	100	100	77.78	61.11
Number of first daughter ramet	5.8±0.3 a	2.8±0.6 b	3.3±0.3 b	0.9±0.2 c
Number of second daughter ramet	2.3±0.6 a	0.2±0.2 b	1.3±0.3 a	0 b
Length of fine rhizome (cm)	1223.9±200.4 a	400.8±63.7 b	1253.8±92.8 a	374.3±39.4 b
Surface area of fine rhizome (cm <sup>2</sup> )	663.5±105.0 a	243.9±43.8 b	637.0±47.1 a	172.7±20.0 b
Specific fine rhizome length (cm/g)	578.6±53.1 b	478.8±47.8 b	1010.3±81.5 a	906.3±76.6 a
Biomass and allocation				
Total plant (g)	24.0±2.3 a	11.4±1.7 b	19.4±1.0 a	6.1±0.7 c
Biomass of clonal propagation (g)	15.3±1.8 a	5.8±1.1 b	12.0±1.0 a	2.6±0.4 c
Biomass of leaves of CP (g)	7.8±1.2 a	2.1±0.4 b	6.3±0.7 a	0.9±0.3 c
Biomass of coarse rhizome (g)	2.7±0.3 a	1.4±0.2 b	2.4±0.2 a	0.7±0.1 c
Biomass of fine rhizome (g)	4.3±0.4 a	2.0±0.3 b	3.2±0.3 a	0.9±0.1 c
Allocation to clonal propagation (%)	61.3±2.4 a	49.0±4.5 b	60.4±2.2 a	42.9±4.6 b
Allocation to leaves of CP (%)	29.5±2.4 a	18.6.0±4.5 b	31.2±2.0 a	15.1±5.7 b
Allocation to coarse rhizome (%)	11.4±0.3	12.1±0.7	12.3±0.5	13.0±2.5
Allocation to fine rhizome (%)	18.4±1.5	16.8±1.2	16.7±0.8	14.6±1.5

Leaves of CP: Leaves of clonal propagation. Different letters mean significant differences (a, b and c:  $p < 0.05$ ) among different treatments

of *I. japonica* was mainly influenced by population origin and partly by habitat after fast growing season. *I. japonica* with high clonality from U population whose traits of clonal propagation can complement in HL or LH reciprocal heterogeneous resources compared to O population, indicating a differential response of two populations to reciprocal patchiness and a link between this response and an ecological range in the species.

A multi-scale spatial pattern of resources (light and nutrients) in the forest community and the distribution of resources in space can determine the increase in clonal traits and regional adaptation to sharing of resources (Hutchings & Wijesinghe 2008). The results from this study showed that the levels of two or more resources occur in the same habitat can determine the performance of clonal growth. It has been recognized that clonal growth of clonal plants can be viewed as an extension of the basic modularity of non-clonal plants, a second-order modularity in which ramets themselves composed of modules, become modules of clones (He et al. 2011). Genetic differences among populations were explained by habitat contrasts and were probably mainly a result of drift (Pluess & Stöcklin 2004), as populations of *I. japonica* were spatially isolated and gene flow was limited. Plants in U population might occupy habitat and accumulate nutrients through the ability of exploring new ramets and rhizomes in understorey with adequate growing space (Kanno & Seiwa 2004). However, long-term intense competition (above and below-ground space) of genets in forest edge was not helpful to clonal growth of O population, which might promote seed reproduction or increase the storage structure investment (Rautiainen et al. 2004).

U population was better adapted to changed environment than O population, probably because they competed better for balance between above- and below-ground resources by obtaining scarce light and water by physiological integration of connecting ramets. The differential effect of pattern of resources on accumulation of biomass in *I. japonica* was due largely to the growth of above part (leaves) in low light to benefit from below part (rhizome and root) in high levels of water. However, high light could not complement low level of water. One interpretation is that ramets in low light were unable to utilize these imported photosynthates for growth unless they were accompanied by imports of water. Previous studies have shown some associations between characteristics of clonal propagation and ecological range, such as between relatively extensive clonal growth and occurrence in heterogeneous habitats that was in reciprocal resources (Schenk et al. 2008). Moreover, differential effect of reciprocal habitat on performance may be degree of physiological or morphological plasticity. Based on measurements of clonal traits, biomass to clonal propagation, *I. japonica* generally showed obviously phenotypic plasticity and was better able to benefit from patterns of resources.

Both the population origin and reciprocal spatial heterogeneous habitat had significant effects on clonal ramets and biomass of clonal components of experimental plants, to a lesser, which also indicated clonal plasticity of *I. japonica*. Due to the ecological isolation of the two *I. japonica* populations, the plasticity of clonal growth had difference in clonal components to balance HL or LH resources. Our findings indicated that two major types



of patterns of spatial covariance of resources can have different effects on the growth and local variation of clonal plants. This should provide a basis for further research to understand survival and spread of clonal species based on interactions between traits of species and patterns of resource patchiness.

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