Full Length Article

Growth and Reproduction Characteristics of Plant's Kin Recognition in Two Different Lifestyle Species

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Abstract

Some plant species grow with closely genetically related individuals, and may benefit from an ability to recognize kin and show cooperative behavior towards neighbors. Kin recognition has been demonstrated in few plant species till now and thus constrained the understanding of significance of kin recognition. We investigated different growth and reproduction characteristics of kin recognition in two lifestyle species, the annual plant *Lolium multiflorum* Lam. and the perennial plant *Elymus sibiricus* Linn., which the main differences between are in their root system types and nutrition demand. We observed less root biomass distribution in kin groups than stranger groups for *L. multiflorum* Lam., but no indication in shoot characteristics at either the individual level or group level. For *E. sibiricus*, there were no significant differences in any measured characteristic index between kin and stranger groups at any level. Our results suggest that kin selection or plant responses to kin may depend on plant features and life-form; the most sensitive traits to competition are more likely to respond to kin selection. In addition, we considered that kin selection was tensely associated with degree of competition. It could be that the moderate competition level in our experiment could not stimulate kin cooperation activity of *E. sibiricus* © 2018 Friends Science Publishers

Keywords: Kin recognition; Kin selection; Root allocation; Shoot characteristics

Introduction

Individual plants can respond to the presence of their neighbors identity by phenotype changes both above ground (Smith, 1982, 1995; Falster and Westoby, 2003) and below ground (Casper and Jackson, 1997; Gersani *et al.*, 2001; Falik *et al.*, 2003; Holzapfel and Alpert, 2003; Gruntman and Novoplansky, 2004; O'Brien *et al.*, 2005), which could be explained as the results of kin selection. Research in *Cakile edentula* showed that individuals exhibited lower fine root allocation in groups of siblings than in groups of strangers (Dudley and File, 2007; Bhatt *et al.*, 2011), which was also found in *Ipomoea hederacea* (Biernaskie, 2011). *Arabidopsis thaliana* decreased lateral root proliferation when individuals were exposed to root exudates, which means root exudates may be kin recognition signals (Biedrzycki *et al.*, 2010), and that kin recognized each other through root interactions. However, *Impatiens pallid* provided reverse results, in which kin groups showed a higher root allocation than stranger groups (Murphy and

Dudley, 2009). Therefore, studies on how plants' phenotypic responses to kin recognition have brought equivocal conclusions and require deeper research.

Plants' phenotypic traits are a more reasonable index for kin recognition than fitness, and plants of various lifestyles may respond to kin recognition with different morphology traits (Biedrzycki *et al.*, 2010). Besides root distribution, above ground morphology traits also respond to kin recognition. For example, *Ip. hederacea* exhibited higher reproductive allocation in kin groups than in stranger groups (Biernaskie, 2011); *Im. Pallid* showed more branching and shoot elongation but less foliage biomass in kin groups, which was interpreted as a way to reduce above ground competition in the light-limiting habitats (Murphy and Dudley, 2009); *Phalaris arundinacea* was disposed by establishing hexagonal arrays with the center target plant surrounded by kin neighbors and non-kin neighbors, and without neighbors. Results showed that individuals surrounded with siblings performed better in plant height, biomass growth, and above ground biomass

(Collins *et al.*, 2010). More information and experiments can be found in a review by File *et al.* (2012).

Comparative studies in different lifestyle species, such as annual plants or perennial plants, may better demonstrate how plant traits respond to kin selection (Bais *et al.*, 2006). This is because traits are inter-dependent and plants may have trade-offs between their traits' distributions in different life stages (Schlichting, 1986), such as trade-offs between vegetative growth and reproductive growth according to different survival mechanisms (Cipollini and Schultz, 1999; Maliakal *et al.*, 1999). For annual plants, life strategies tend to rely on strong reproduction ability, such as generating more seeds. However, for perennial plants, root vigor may be the most important strategy to keep individuals alive for the next year. Thus, we propose that species with different lifestyles, i.e., annual or perennial, may respond to kin selection in their own ways and perform on respective traits. In turn, the results could aid understanding of how kin selection act on plant's phenotype plasticity.

In this experiment, we chose two species with different lifestyles: *Elymus sibiricus Linn.* (an annual plant) and *Lolium multiflorum* Lam. (a perennial plant) of monoculture in the Sichuan province. We sprouted the seeds in Petri dishes, chose shoots of similar sizes, then planted them pair-wise with a factor design of siblings and strangers from two families in one pot under a moderate competition density of each species (we tested a moderate competition density before kin selection experiment). All plants were harvested after reproduction after completion of the life span and interaction results were investigated. Traits, including plant size, whole biomass, above ground and below ground biomass, internodes and root length, and seed number and weight were measured (Lande and Arnold, 1983; Dudley and File, 2007) to estimate standards for kin selection results. We focused on the following research questions: (1) Do both lifestyle monoculture plants respond to kin recognition? (2) Do individual plant traits rank as [siblings > solitary > strangers] or as [siblings < solitary < strangers] when plants are set to grow in three treatments (neighbored with kin, neighbored with strangers, or solitary without neighbors)? (3) How do plants perform at the group and individual levels, and what phenotypic traits in each level could be specific to kin recognition?

Materials and Methods

Plant Materials

We chose two representative grazing plants species of Gramineae, *Elymus sibiricus* Linn., (hereafter referred to as *E. sibiricus*) and *L. multiflorum* Lam. (hereafter referred to as *L. multiflorum*), in the alpine meadows of the Tibetan Plateau. *E. sibiricus* is a perennial plant with a deep, developed, and drought-enduring root system; its vegetative growth occurs prior to reproductive growth in the first year of life, and both self-pollination and cross-pollination can be employed for breeding. *L. multiflorum* is an annual plant with shallow, fibrous roots, and both self-pollination and cross-pollination can be used for breeding. Both species mainly rely on seed propagation.

Testing Moderate Competition Density

Previous studies have found that plants may only cooperate when sunlight, water, nutrients or other factors affecting growth are plentiful, or when their environments become too severe for survival and it was uncertain whether kin selection would happen (Loehle, 2000; Tozer *et al.*, 2015). Relatives may cooperate when environmental resources are plentiful but compete when resources are limited or scarce (Darwin, 1859). Kin competition is more severe when individuals are planted in high-density or local soil (Tonsor, 1989; West *et al.*, 2007). In addition, plants increase biomass allocation to roots where nutrients are the most limited resource (Gersani *et al.*, 2001; O'Brien *et al.*, 2005). Kin selection analysis was affected by competition intensity caused by plant density and other above-environment conditions. Therefore, we first defined and estimated the planting competition density at a moderate level before approaching interaction results between related plants.

For the study of the moderate competition level, we designed seven degrees reliant on the distances between two plants for each species on 5 May 2012 and six density gradients for each species with planting distances arranged at 15.0, 13.0, 6.5, 4.6, 3.25, 2.52 and 1.39 cm. The seeds were germinated in Petri dishes before young seedlings were transplanted into soils with the six densities, and each replicated 3 times.

Plants were watered and weeded every day. The biomass of every plant was measured and recorded after reproduction on 28 September 2012, and the competing growth curves were drawn based on the mean value of each density. We found the moderate competition level of each species (Fig. 1), based on which the later experiment was designed.

Collecting of kin Seeds

To harvest sibling seeds for kin selection research, mother seeds were planted outdoors on 6 May 2012. They were watered daily and fertilized twice a month.

The plants began to bloom in the middle of August 2012, and we helped them self-pollinate by hand-pollinating and then isolating them, bagging the inflorescence after pollination to prevent other pollen from nearby plants.

We looked after the plants until the offspring seeds (the seeds mentioned are the self-pollination offspring) fully matured on 28 September 2012, collected the seeds from one stock plant, and marked sibling groups as families. Seeds from one stock plant are siblings, while seeds from another stock plant are strangers to them. We collected at least eight families with at least 40 seeds from one mother plant of each species for the kin selection experiment.

Kin Selection Experiment

For the kin selection study, we chose a complete factorial design with treatments consisting of root neighbors being present (with root neighbors) or absent (solitary), and siblings (kin) or non-siblings (strangers).

Within each pot, two seeds from each of the eight families (the same stock plant) were used to create combinations. The experiment consisted, for each species, of 144 pots with 72 combinations and two repeats for each combination. There are eight kin, 28 strangers and 36 solitary combinations (Fig. 1).

Seeds were germinated in Petri dishes until the seedling stage on 1 October 2012. After three days of growth in the dishes, all the healthy seedlings were planted (the sizes were similar to eliminate size effects in future experiments) based the factorial design into soil pots. All pots were moved to a warm-house as the weather became colder.

The soil comprised humus, organic silt and pearlite in a 2:2:1 ratio. We watered specimens daily to keep the soil moist and weeded as required. The temperature was adjusted from 25°C in day light to 20°C at night. Lights were turned on in daylight to provide plenty of illumination and were turned off at night.

About three months later, on 15 January 2013, the plants began to bloom, we measured and recorded plants' heights, internodes' elongation, number of leaves, leaf areas, and tiller numbers for each plant. These traits were selected because they directly relate to above ground and below ground resource acquisition (Givnish, 1986).

On 6 March 2013, after reproduction, we harvested the plants and dried them in a drying oven at 50°C for 72 h, and then weighed plants' biomass, leaf biomass, stem biomass, root biomass, and reproduction biomass (by pot) for analysis. We counted number of seeds, clusters, leaves, and branches for each plant. We measured seed cluster length, plant height, above ground biomass, leaf mass, stem mass and reproduction mass, and leaf area for each plant for individual analysis.

Statistical Analysis

All data were analyzed with SAS statistical software (version 8.02). As it was difficult to separate the roots of plants in the treatment with root neighbors present, the experimental unit for root traits and total biomass were groups of two plants (n=144). For above ground traits, the experimental unit was the individual (n=288). Data of the pots including two stands were used to test effects at group level, while data of individual stands used at individual level. We used an analysis of covariance to test differences in allocation and elongation (Coleman *et al.*, 1994; McConnaughay and Coleman, 1999). Parameters are

untransformed for clarity. Following the methods of Coleman *et al.* (1994) Cahill (2003), the data for roots were transformed $f(x) = (\log(x+1))$, so that the residual variance was homoscedastic and the distribution of the residuals did not differ too much from normality. Elongation was measured as the least square mean (LSMEAN) from an analysis of covariance with plant total height as the dependent variable (because root-to-shoot ratios are sensitive to increases in stem mass resulting from increased stem elongation) and the stem mass as the covariate. (LSMEANS option, PROC GLM). Root allocation was measured as the least square mean from an analysis of covariance with fine root mass as the dependent variable and above ground biomass as the covariate (Dudley and File, 2007). For reproduction allocation, the reproductive artifacts biomass was used as the dependent variable and supporting artifacts (leaf and stem biomass) were used as the covariate.

Results

Intermediate Competition level of Species *E. sibiricus* and *L. multiflorum*

For both species, when the planting distance was 6.5 cm, the plant biomass began to decrease sharply, which indicates competition interactions. It was reasonable to select a relatively weaker competition value, between 6.5 and 4.6 cm, as an intermediate level of competition (Masclaux *et al.*, 2010). Therefore, we selected a design with an interaction distance of 5.0 cm for easy operation in further experiments, corresponding to an intermediate level of competition.

Kin Effects at the Group level

The results showed that *E. sibiricus* had no significant difference on all measured traits under kin treatment, while *L. multiflorum* showed root distribution differences at the group level between kin and stranger groups.

For *E. sibiricus*, there was no significant difference between kin groups and stranger groups on any measured traits, including root biomass, stem biomass, leaf biomass, reproduction biomass, above ground biomass, root-to-shoot ratio, and plant whole biomass (Fig. 3 and Table 1). However, for *L. multiflorum*, kin groups averaged less root mass than stranger groups in shared pots (n=72, p=0.007), but no significant variation of other measured traits mentioned above.

Solitary groups showed no difference compared to with root neighbors for *E. Sibiricus* (n=144, p=0.343). Solitary groups showed more root mass than kin groups but no difference compared to stranger groups for *L. multiflorum* (n=144, p=0.027) (Fig. 2 and Table 1).

A/A	AA	AB	AC	AD	AE	AF	AG	AH
A/B	B/B	BB	BC	BD	BE	BF	BG	BH
A/C	B/C	C/C	CC	CD	CE	CF	CG	CH
A/D	B/D	C/D	D/D	DD	DE	DF	DG	DH
A/E	B/E	C/E	D/E	E/E	EE	EF	EG	EH
A/F	B/F	C/F	D/F	E/F	F/F	FF	FG	FH
A/G	B/G	C/G	D/G	E/G	F/G	G/G	GG	GH
A/H	B/H	C/H	D/H	E/H	F/H	G/H	H/H	HH

Fig. 1: The experimental design of eight families for kin selection. The small boxes stand for pots; the diameter of each pot was 12 cm, and all 64 pots had equal volume. Letters A-H stand for seedlings derived from the same species of different maternal offspring (F1). Same-letter combinations (such as AA) represent individuals with sibling neighbors, and different letter combinations (such as AB) represent individuals with stranger neighbors. Pots separated by slashes (such as A/B) represent solitary plants. The distance between two individuals in each pot (such as AA) was 6 cm, based on the intermediate competition experiments results

Kin Effects at the Individual Level

The experiment showed that only branching number (P=0.031) and plant height (P<0.0001) were significantly different in root treatment pots of *E. sibiricus*, but there were no differences between kin and stranger groups. All other traits, such as seeds number, cluster number, leaf number, branching number, plant height, internode length, leaf area, shoot biomass, leaf biomass, stem biomass, and reproduction biomass, showed no significant differences between the three groups (Table 2). There were also no significant differences in any measured traits in *L. multiflorum* at the individual level (Table 2).

Family Genotype Influence

The contrasting analysis results between kin groups and stranger groups showed no significant difference (at the group level, Table 3). In addition, the test of different genotypes of the eight families showed no significant difference in root biomass between different families of solitary plants (at the individual level, Table 4).

Discussion

In this experiment, we chose *E. sibiricus* and *L. multiflorum*, to see how plants respond to kin selection differently through across the lifespan until reproduction. The results showed that, at the group level, only *L. multiflorum* showed
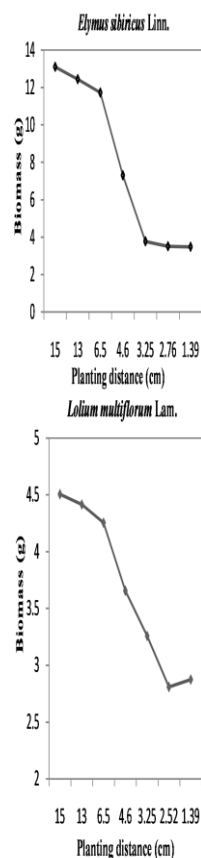

Fig. 2: Biomass change under seven planting distances of *E. sibiricus* and *L. multiflorum.* Plant biomass of both species reduced, along with adecrease inplanting distance as a whole. When the planting distance was 6.5 cm, the biomass of both species decreased sharply. It began to stabilizeat 3.25 cm for *E. sibiricus* and 2.52 cm for *L. multiflorum*, indicating that a significant competition existed between individuals when the planting distance was between 6.5 cm and 3.25 cm

lower root allocation in siblings than in strangers. *E. sibiricus* showed no significant differences in either above ground biomass or underground biomass. At the individual level, except for branching number and plant height, which differed between kin and solitary pots of *E. sibiricus*, there were no significant differences in any other measured traits for *E. sibiricus* and *L. multiflorum*. There were also no significant differences in root biomass for the eight different families of solitary plants, or for all kin groups and stranger groups.

We proposed that plants could better reduce investment in competitive traits and increase the efficiency of their resource capture and reproduction ability while living with siblings than with strangers according to kin selection theory.

Table 1: Analysis of Covariance at the Group Level for *E. sibiricus* and *L. Multiflorum*

E. sibiricus source	d.f	Log (stem+1)		Log (leaf+1)		Log (reproduction+1)		Log (shoot +1)		Log (root+1)		Log (total+1)	
		F	P	F	P	F	P	F	P	F	P	F	P
kin	1	0.071	0.791	0.121	0.729	0.025	0.857	0.033	0.857	2.085	0.153	0.241	0.625
neighbor	1	2.724	0.107	1.194	0.281	0.151	0.699	0.290	0.593	0.923	0.343	0.329	0.570
kin × neighbor	1	3.138	0.078	2.719	0.103	0.097	0.756	0.256	0.614	5.680	0.020	0.001	0.999
L. multiflorum source	d.f	Log (stem+1)		Log (leaf+1)		Log (reproduction+1)		Log (shoot+1)		Log (root+1)		Log (total+1)	
		F	P	F	P	F	P	F	P	F	P	F	P
kin	1	0.258	0.595	0.074	0.786	0.435	0.512	0.307	0.581	7.586	0.007	0.198	0.657
neighbor	1	0.049	0.827	1.453	0.236	0.165	0.687	0.359	0.533	5.302	0.027	0.034	0.856
kin × neighbor	1	1.004	0.319	2.924	0.091	0.400	0.529	1.810	0.182	0.451	0.504	0.533	0.467

Table 2: Analysis of Covariance at the Individual Level for *E. sibiricus* and *L. Multiflorum*

Species	*E. sibiricus*				*L. multiflorum*			
Source	kin		neighbor		kin		neighbor	
d.f	1		1		1		1	
F/P- value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
seed number	0.044	0.834	0.935	0.337	0.145	0.704	0.059	0.809
cluster number	0.192	0.662	1.063	0.306	0.062	0.804	0.760	0.386
leaf number	0.318	0.573	3.083	0.078	2.908	0.090	2.501	0.118
branching number	0.062	0.803	4.841	0.031	2.389	0.124	1.786	0.186
plant height	3.406	0.607	23.011	<0.0001	3.814	0.053	2.475	0.119
internode length	0.266	0.607	1.063	0.306	0.296	0.587	0.797	0.375
leaf area	1.446	0.231	0.445	0.507	1.834	0.178	0.663	0.418
Log (shoot biomass)	0.104	0.748	1.194	0.278	0.428	0.514	0.007	0.935
Log (leaf biomass)	0.003	0.957	3.473	0.066	0.542	0.463	0.049	0.826
Log (stem biomass)	0.001	0.957	0.104	0.748	0.717	0.398	0.078	0.781
Log (reproduction biomass)	0.526	0.469	2.533	0.116	0.266	0.607	0.109	0.743

Table 3: Analysis of variance of difference between kin groups of 8 families, stranger groups of 8 families, and solitary plantings of 8 families of *E. sibiricus* and *L. multiflorum*

E. sibiricus source	d.f	Log (stem+1)		Log (leaf+1)		Log (reproduction+1)		Log (aboveground+1)		Log (root+1)		Log (shoot/root+1)		Log (total+1)	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
kin	7	0.158	0.989	1.005	0.473	0.198	0.980	0.253	0.961	0.399	0.885	0.987	0.484	0.210	0.976
strangers	27	0.347	0.969	1.540	0.149	0.535	0.869	0.380	0.958	0.507	0.889	0.397	0.951	0.576	0.838
genotypes	7	0.476	0.834	2.930	0.051	0.755	0.634	0.849	0.569	0.278	0.951	0.277	0.951	0.615	0.734
L. multiflorum source	d.f	Log (stem+1)		Log (leaf+1)		Log (reproduction+1)		Log (aboveground+1)		Log (root+1)		Log (shoot/root+1)		Log (total+1)	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
kin	7	0.936	0.504	1.310	0.324	0.826	0.571	1.206	0.367	3.943	0.055	0.813	0.580	1.260	0.367
strangers	27	1.377	0.210	1.913	0.057	1.092	0.389	1.744	0.088	1.315	0.242	0.418	0.949	1.823	0.072
genotypes	7	1.286	0.335	1.192	0.376	1.560	0.238	1.434	0.278	0.375	0.900	0.524	0.800	1.623	0.220

Table 4: Analysis of variance of difference between solitary plantings of individual plants of *E. sibiricus* and *L. multiflorum*

Source	d.f	F/P	seeds number	clusters number	branching number	plant height	clusters length	leaf area	Log (shoot+1)	Log (leaf+1)	Log (stem+)	Log (reproduction+)
E. sibiricus	7	F	2.481	0.633	0.905	0.517	0.639	0.199	0.260	0.262	1.420	1.325
		P	0.054	0.702	0.983	0.814	0.720	0.983	0.964	0.964	0.235	0.276
L. multiflorum	7	F	1.604	0.737	0.922	0.963	0.375	0.818	1.505	1.093	1.492	2.310
		P	0.175	0.643	0.503	0.466	0.909	0.580	0.203	0.393	0.208	0.053

Our data show that only root biomass distribution was different between kin groups and stranger groups of *L. multiflorum* (kin<strangers=solitary), which is consistent with previous studies of kin cooperation (Gersani *et al.*, 2001; Maina *et al.*, 2002; O'Brien *et al.*, 2005). However, we saw no significant differences on above ground biomass, such as stems, leaves, and reproduction biomass, in the three groups, which indicated that plants may respond to kin and strangers with competition traits only in limiting conditions. For example, in another study,

C. edentula responded to kin and strangers by changing root distribution while *I. cf. palliida* increased allocation for competitive traits above ground instead of for root biomass by changing light quality (Murphy and Dudley, 2009), *Trifolium repens* siblings increased investment inseed production at the expense of allocation to competitive organs with increasing neighbor density (Lepik *et al.*, 2012). *L. multiflorum* was given enough illumination and above ground living space in this study; the only limiting condition was root nutrition and root space.
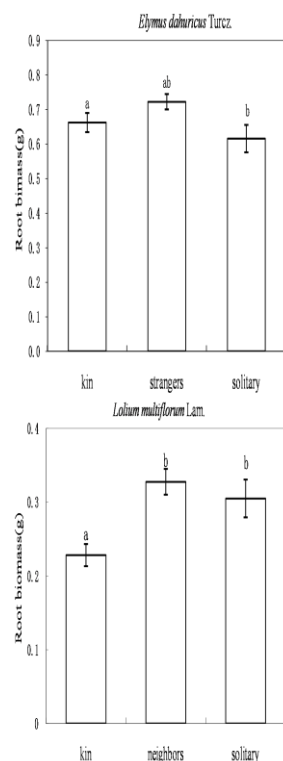
Fig. 3: Root biomass of *E. sibiricus* and *L. multiflorum* grown either with kin or strangers, and grown solitary as a reference. The vertical coordinates refer to the total root biomass of two individuals from a kin pair, a stranger pair, and solitary pair

Therefore, a result of different root distribution, but not above ground biomass, could be expected. Traits responding to kin and strangers may be consistent with plant's living conditions and competition mode (Dudley and File, 2007).

Kin selection may rely on plant's lifestyle and survival mechanisms. According to our results, *L. multiflorum* responded to kin recognition, but *E. sibiricus* did not. While previous consequences of kin competition usually focused on only one species at a time (Dudley and File, 2007; Murphy and Dudley, 2009; Biedrzycki *et al.*, 2010; Bhatt *et al.*, 2011; Biernaskie, 2011), previously reported positive results may be due to the careful selection of study species with life histories that could be predicted to promote kin selection (e.g., formation of mono-specific stands and high rates of self-fertilization plants be selected) (S. Dudley, pers. comm.). Because of differences in lifestyle and survival mechanisms, our comparative study of these two species may be more representative of the kin selection effect in similar controlled conditions.

A recent study in eight plant species from temperate grasslands showed that kin recognition may not be a common phenomenon in temperate grassland plants, with only a small proportion of plants altering their morphology and biomass allocation when growing next to kin (Lepik *et al.*, 2012). We supposed *E. sibiricus* not responding to kin could be due to its lifestyle. The main difference between *E. sibiricus* and *L. multiflorum* is that *E. sibiricus* is a perennial plant, its root system was deep and developed, and vegetative growth occurred prior to reproductive growth in the first year life, which could adapt to the poor nutrition environment. *L. multiflorum* is an annual plant, with fibrous roots and shallow soil location, favoring nutrient-rich soil. Thus, under the experimental condition, the main competition for *L. multiflorum* may be nutrient absorption to safeguard the lifecycle. Root biomass distribution changes responding to neighbors' identity was expected. But for *E. sibiricus*, the main living strategy in the first year was to maintain root vigor rather than root biomass quantity, as it was a species that could adapt to the poor nutrition environment.

In this experiment, we prepared the same soil formula for each species, and it could be that the soil nutrients were enough for *E. sibiricus* to maintain the first year's lifetime. The main competition mechanism did not rely on root biomass and above ground traits, but on the resilience of root systems and the reproduction ability of the next year's lifetime. We should consider the whole lifecycle of perennials to discover the corresponding traits, such as root vigor, to see how species react to kin neighbors (Lepik *et al.*, 2012). Previous studies mainly chose species of annual plants, and more research is still needed on how different lifestyles of species respond to kin recognition.

Another possible reason for our results that the two species did not respond to kin recognition consistently may be planting density, as fierce competition between neighbors may affect kin reaction results. Previous studies found that plants only cooperated when sunlight, water, nutrients, and other factor were plentiful; when living situations become threateningly severe, it is uncertain that kin selection will happen (Loehle, 2000; Gersani *et al.,* 2001; O'Brien *et al.*, 2005; Tozer *et al.*, 2015). It has also been reported that significant dependence of plant performance on neighbor phenotype or genetic relatedness only occurs at high neighbor densities (Escarré *et al.*, 1994; Donohue *et al.*, 2000). Moreover, experiments also found that a perennial species *T. repens* increased allocation to inflorescent mass among siblings more at high neighbor density compared with low density (Lepik *et al.*, 2012). Therefore, alternative conclusions might be presented if only one neighbor density is chosen. For our experiment, under the chosen intermediate competition level, the planting density for *L. multiflorum* was compatible with kin recognition, while the density may have been too high or too low for *E. sibiricus* to show kin reaction results. Thus, it is important to recognize that kin recognition may trigger different responses depending on whether a plant experiences little competition or intense competition with its neighbors; a density gradient

design to test kin selection of *E. sibiricus* and other species in future studies is necessary.

Kin recognition should be predicted by both group level and individual level according to kin selection theory. Previous work on plant responses to relatives has focused on individuals, but fitness of the focal individual is determined by natural selection on its own traits, which could be increased by having more competitive capacity (Kelly, 1996). If a trait under selection is altruistic, a negative individual selection gradient is predicted to measure the cost of cooperation, while a positive group selection gradient could measure the cooperation benefit of neighbors to the focal individual. Therefore, selfish and altruistic traits mean positive individual selection and negative group selection, respectively (Goodnight, 2005). In addition, frequency-dependent natural selection (usually at the group level) could confuse kin selection or niche partitioning results. For example, positive, frequency-dependent natural selection favors similarity phenotypes, and negative frequency-dependent natural selection results in phenotype diversity (Kerkhoff, 2004; Xiao *et al.*, 2009). Furthermore, to justify whether plants or a community have kin selection, it is insufficient to test only the focal plant; neighbors should also be considered (Masclaux *et al.*, 2010).

Considering these factors, we used a pair-wise experimental design, growing only pairs of seedlings together to provide a robust test for the prediction of kin recognition, because local plants are affected by their neighbor's identities only under the same environmental control. Reversely, we could treat the neighbors as the focal plant and test their reactions and kin recognition at both the group and individual levels. Our results indicate that *L. multiflorum* responded to siblings only at the group level because those traits are interdependent, and plants may have trade-offs between their phenotypes (Schlichting, 1986). The most sensitive organs to limiting resources may finally cause phenotype or distribution changes to a neighbor's reaction. For *L. multiflorum,* the most limiting conditions were root space and soil nutrients. Thus, integrated individual phenotypic changes are also a group-level index of kin selection, and it is necessary for further study to test relative phenotypic traits as much as possible to sift some peculiar traits in direct response to kin recognition.

Competition differences among genotypes and families could certainly affect plants' phenotype changes, which could confuse the results in kin selection experiments (Masclaux *et al.*, 2010). For example, if a family type of neighbors predominates, fixed behavior toward neighbors is likely to be favored (Alpert and Simms, 2002; Givnish, 2002). Therefore, we analyzed competition ability of all individuals living in solitary conditions and the variance between all kin groups of eight different families, and both sets of results showed no significant differences. Previous studies also suggested the size inequality of pairs before a competitive reaction could affect lateral root allocation

(Klemens, 2008). To avoid the influences of plant size and seed vitality, we set all the seeds to sprout and grow to similar sizes in a Petri dish before transplanting them to the soil. To some extent, it implied that genotype competition ability doesn't affect the biomass distribution of *L. multiflorum*. However, our results did not deny the existence of competition-based interactions in each span of the plant's life cycles, and we supposed that both kin selection and competition may happen in plant's lifetimes. However, the one performed that more tensely may cover up the effect of the other one and dominate the interaction results, which may cause responses in plants, such as biomass distribution and other phenotypic changes.

Conclusion

It indicates that kin recognition may be species-specific and trait-specific, and plants may have different response modes to kin selection; more candidate traits should be investigated and observed at both the group and individual levels. More different lifestyle species should be introduced to discover which specific characteristics are required for kin selection to occur. It appears that kin selection is related to planting density, while further research on different density gradients is needed to improve our understanding of how kin recognition will evolve.

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