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CLONAL INTEGRATION MODIFIES GROWTH AND REPRODUCTION OF THE BUNCHGRASS *Cleistogenes squarrosa* IN NUTRIENT-HETEROGENEOUS CONDITIONS

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Abstract

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Spatial heterogeneity in nutrient availability exists even at scales of centimeters. Therefore, genets of phalanx clonal plants such as bunchgrass may experience heterogeneous nutrient supply. We hypothesize that, as found in guerilla clonal plants, clonal integration may also benefit phalanx species in heterogeneous environments. We grew clonal fragments of the bunchgrass *Cleistogenes squarrosa* in both homogeneous and patchy conditions, and kept tiller ramets within a fragment either connected or disconnected. In patchy conditions, total biomass, biomass of aboveground asexual structures of tillers, root biomass, tiller production and aboveground tiller size were markedly larger in connected than in disconnected clonal fragments, whereas biomass of sexual structures were smaller. Also connected clonal fragments produced significantly more biomass (total, aboveground asexual structures, root), more and larger tillers in patchy conditions than in homogeneous ones that provided the same amount of nutrients as in the patchy treatments. We conclude that clonal integration enables phalanx clonal species to better use small-scale soil heterogeneity so that they may grow better in conditions with heterogeneous nutrient supply.

Key words: phalanx growth forms, physiological integration, resource sharing, spatial heterogeneity, tussock-forming grass

Introduction

Spatial heterogeneity in resource supply is a ubiquitous feature of ecosystems (Caldwell, Pearcy, 1994). It may occur at the scales relevant to individuals or even to plant organs (Ko-

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tliar, Wiens, 1990; Jackson, Caldwell, 1993; Stuefer et al., 1996). Theoretical and empirical studies suggest that clonal plants may have developed a series of strategies to cope with these heterogeneous environments. Clonal integration or resource sharing, i.e. transportation of water, carbon compounds and mineral nutrients between connected, asexual offspring (ramets), is proven to be very beneficial to guerrilla clonal plants suffering from local environmental stresses or disturbances (Stuefer, Hutchings, 1994; Alpert, 1996; de Kroon et al. 1996; Jónsdóttir, Watson, 1997; Wijesinghe, Hutchings, 1999). Foraging activities, i.e. selective placement of resource acquiring structures (e.g. leaves and roots) through morphological plasticity, enable guerrilla clonal plants to acquire adequate amounts of resources in heterogeneous environments and thus enhance their growth and reproduction (Hutchings, 1988; Robinson, Robinson, 1988; Hutchings, de Kroon, 1994; de Kroon, Hutchings, 1995; Stuefer et al., 1996; Wijesinghe, Hutchings, 1999).

Phalanx clonal plants lack long spacers and thus the ramets of the same genets are placed very close to one another (c.f. Lovett, 1981). One of the extremes of phalanx clonal species is bunchgrass whose tiller ramets are tightly packed together and which lack apparent spacers between ramets. Recent studies have shown that the distribution of soil nutrients is patchy even at scales of a few centimeters (e.g. Kotliar, Wiens, 1990; Jackson, Caldwell, 1993; Stuefer et al., 1996; Humphrey, Pyke, 1997; Casper, Cahill, 1998; Farley, Fitter, 1999). Therefore, like guerrilla clonal species, phalanx clonal plants may also position their ramets in contrasting nutrient patches, so that clonal integration plays a substantial role. However, the majority of studies on how clonal plants adapt to patchy environments have focused mainly on guerrilla clonal architecture. Only a few studies have dealt with phalanx clonal plants and such studies have shown that phalanx clonal plants were able to proliferate roots in the nutrient-rich microsites (Fransen et al., 1999, 2001; He et al., 2004).

We conducted a greenhouse experiment to investigate the responses of the bunchgrass *Cleistogenes squarrosa* to heterogeneous nutrient supply. We hypothesized that, as found in guerilla clonal plants, clonal integration may also enable *C. squarrosa* to cope with environmental heterogeneity in nutrient supply.

Materials and methods

Plant species

Cleistogenes squarrosa (Gramineae) is a perennial bunchgrass. It grows in clumps and is common to the shrub region in the Mu Us sandland (Commission Editorial of Inner Mongolia Flora, 1994). A single genet of *C. squarrosa* can produce many tillers and flowers in August and September.

Experimental design

On July 1, 2004, six genets of *C. squarrosa* were collected from sandy grassland near the Ordos Sandland Ecological Station (OSES; 39°02' N, 109°51' E; Institute of Botany, Chinese Academy of Sciences), located in the SE of the Ordos Plateau in Inner Mongolia, China. The genets were transplanted into a greenhouse at OSES for cultivation. On July 27, 2004, we excised from each genet eight similar-sized clonal fragments, each consisting of two interconnected tillers. We shall call them original tillers. In half of the eight clonal fragments the tiller connections were severed (thereafter as "disconnected clonal fragment"), whereas in the other four they were left intact (thereafter as "connected clonal fragment"). Pairs of plastic pots (6 cm in diameter and 12 cm in height) were stuck together on one side and filled with river sand. On the shared side of each pair of pots, a small piece was removed and a clonal fragment of *C. squarrosa* was positioned there, with one half roots being located in one pot and the other half roots in the other pot.

After two weeks for recovery, each connected or disconnected clonal fragment was assigned to one of the four nutrient treatments: (1) both pots receiving high level of nutrient supply (coded as H-H and H/H for connected and disconnected clonal fragments, respectively), (2) both pots receiving medium level of nutrient supply (coded as M-M and M/M), (3) both pots receiving low level of nutrient supply (coded as L-L and L/L) and (4) one pot receiving high level of nutrient supply and the other pot receiving low level of nutrient supply (coded as H-L and H/L). There were six replicates and clonal fragments of each genet experienced all the eight treatments. The high nutrient level was obtained by supplying 100 ml Peters I solution which has been diluted 1000 times from its full-strength solution once every five days. The fertilizer with 20N: 20P: 20K ratio was produced by SCOTTS, an American company. In the treatments of medium (M) and low (L) levels of nutrient supply, the concentrations of nutrient solution were 50% and 0% of ones in the treatment H, respectively. All pots were supplied with 100 ml tap water every day.

Measurements and analyses

On October 11, 2004, we harvested all experimental plants. We shall call the original tiller together with the tillers it produced at the end of experiments "clonal part". Aboveground parts of each clonal part were clipped out and separated into sexual structures and asexual structures (including stem, leaf blade and sheath). Roots in each pot were harvested separately. All plant parts were then oven-dried at 85 ^{o}C and weighed.

Pairwise Ducan multiple tests were used to compare the performance of the *C. squarrosa* clonal fragments among the eight treatments and root biomass of *C. squarrosa* clonal parts among the four treatments (SPSS, 10.0).

Results

Biomass of clonal fragments

Under homogenous nutrient supply, biomass of aboveground asexual structures of tiller ramets and total biomass increased markedly with increasing nutrient supply, whereas biomass of sexual structures and root biomass did not differ among the nutrient treatments (Fig. 1).

When clonal fragments of *Cleistogenes squarrosa* were subjected to homogeneous nutrient supply, severing ramet connection did not affect biomass (Fig. 1). In contrast, when they were subjected to heterogeneous nutrient supply, biomass of aboveground asexual structures of tiller ramets (Fig. 1B), root biomass (Fig. 1C) and total biomass (Fig. 1D) were significantly larger in connected (H-L) than in disconnected (H/L) clonal fragments, whereas biomass of sexual structures was markedly smaller (Fig. 1A).

Percentage biomass allocation

Severing tiller ramets connection did not affect percentage biomass allocations when clonal fragments were subjected to homogeneous nutrient supply (Fig. 2). However, when clonal

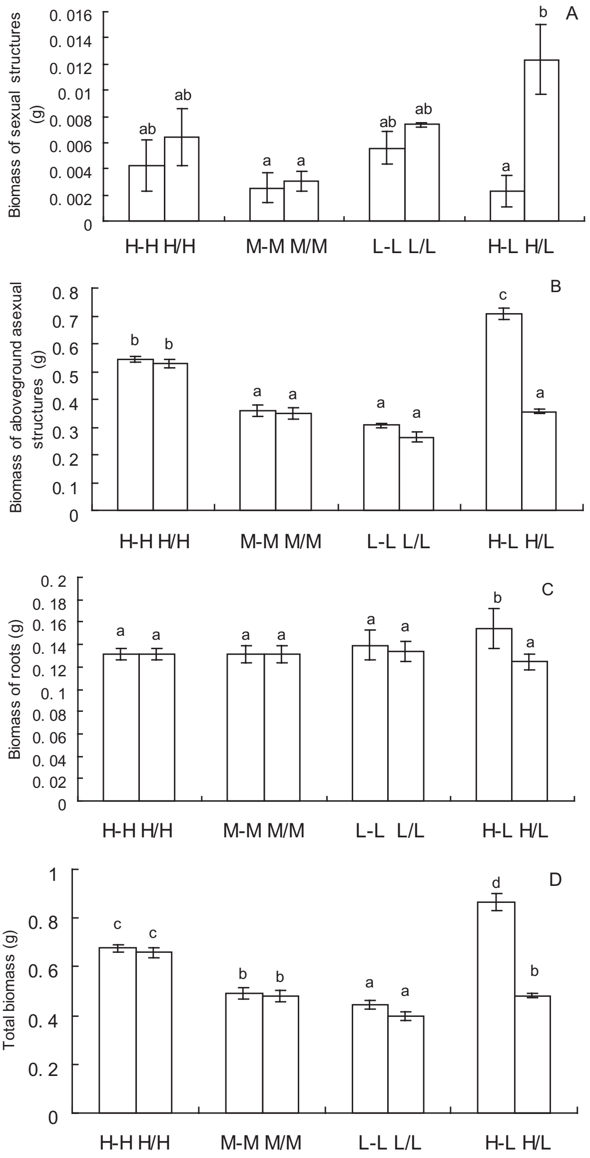

Fig. 1. Biomass of sexual structures (A), of aboveground asexual structures of tiller ramets (B), of roots (C) and (D) total biomass of the *Cleistogenes squarrosa* clonal fragments under the eight treatments. Bars and vertical lines stand for mean and standard error, respectively. Bars sharing the same letter are not different at $P = 0.05$. Meanings of the treatment codes: (1) both pots receiving high level of nutrient supply (coded as H-H and H/H for connected and disconnected clonal fragments, respectively), (2) both pots receiving medium level of nutrient supply (coded as M-M and M/M), (3) both pots receiving low level of nutrient supply (coded as L-L and L/L) and (4) one pot receiving high level of nutrient supply and the other pot receiving low level of nutrient supply (coded as H-L and H/L)..

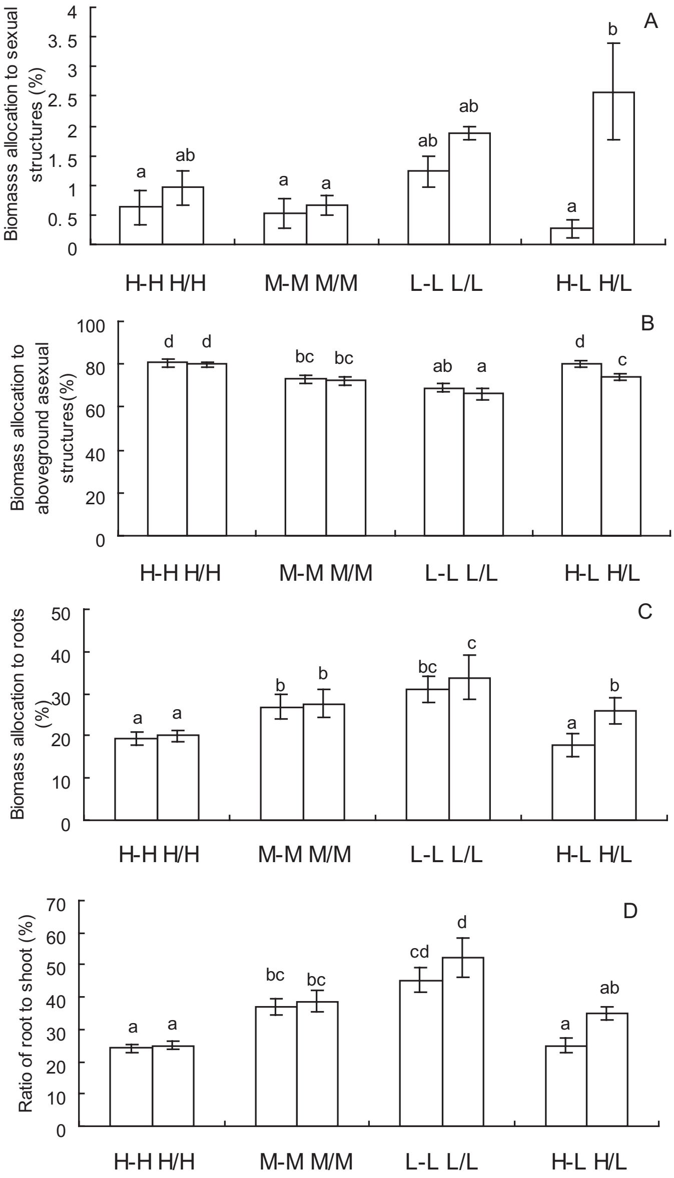

Fig. 2. Percentage biomass allocation to sexual structure (A), to aboveground asexual structures of tiller ramets (B), to roots (C) and root to shoot ratio (D) of the *C. squarrosa* clonal fragments under the eight treatments. Bars and vertical lines stand for mean and standard error, respectively. Bars sharing the same letter are not different at $P = 0.05$. Meanings of the treatment codes see Fig. 1.

fragments were subjected to heterogeneous nutrient supply, percentage biomass allocation to sexual structures was larger in disconnected than in connected clonal fragments, whereas percentage biomass allocation to aboveground asexual structures of tiller ramets and to roots were smaller (Fig. 2 A, B, C). Severing tiller ramets connection did not affect root to shoot ratio of clonal fragments subjected to either homogeneous or heterogeneous nutrient supply (Fig. 2D).

Tiller ramets number and size

When receiving homogeneous nutrient supply, clonal fragments produced markedly less tiller ramets with decreasing nutrient supply (Fig. 3A), and connected and disconnected clonal fragments produced similar number of and sized tiller ramets (Fig. 3). When receiving heterogeneous nutrient supply, however, disconnected clonal fragments produced both less and smaller tiller ramets than connected clonal fragments (Fig. 3).

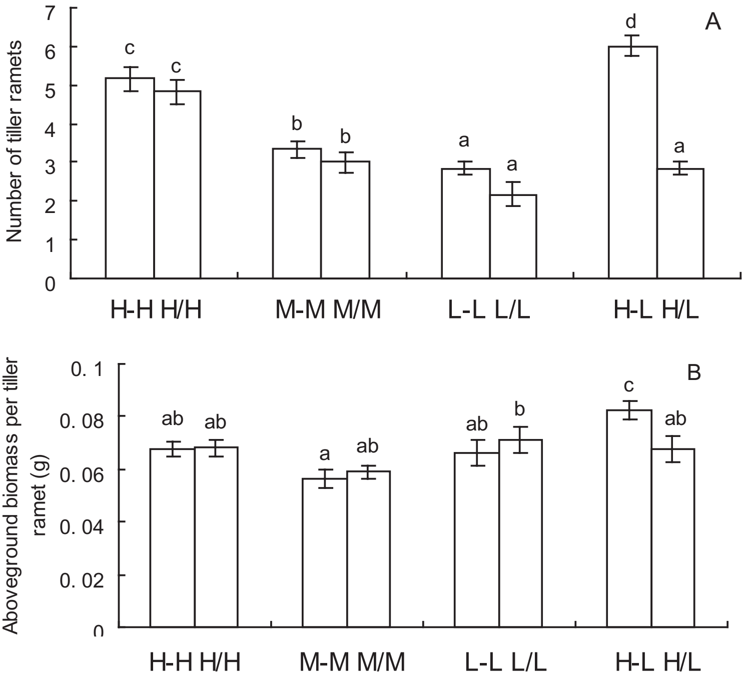

Fig. 3. Number of tiller ramets (A) and aboveground biomass per tiller ramet (B) of the *Cleistogenes squarrosa* clonal fragments under the eight treatments. Bars and vertical lines stand for mean and standard error, respectively. Bars sharing the same letter are not different at $P = 0.05$. Meanings of the treatment codes see Fig. 1.

Root biomass of clonal parts

When clonal fragments were subjected to heterogeneous nutrient supply, root biomass in pots receiving high nutrient supply was markedly larger in the connected than in the disconnected clonal fragments, whereas that in pots receiving low nutrient supply did not differ between the connected and the disconnected clonal fragments (Fig. 4).

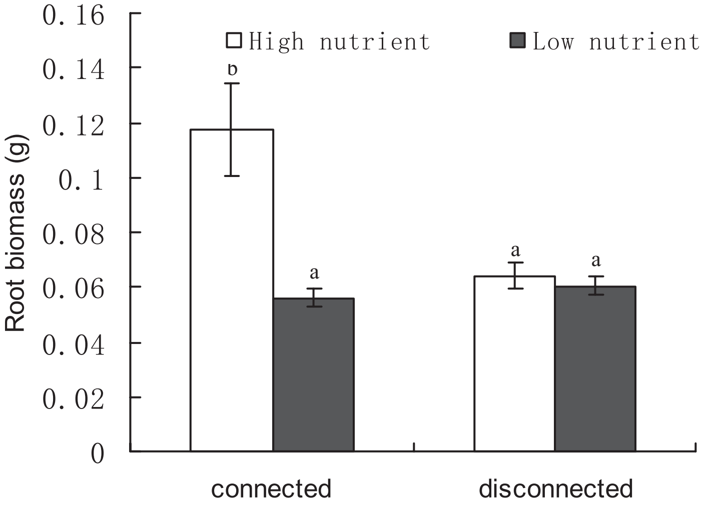

Fig. 4. Root biomass of *Cleistogenes squarrosa* clonal parts when the connected and disconnected clonal fragments were in heterogeneous soil nutrients. Bars and vertical lines stand for mean and standard error, respectively. Bars sharing the same letter are not different at $P = 0.05$. Meanings of the treatment codes see Fig. 1.

Discussion

In many plants with guerrilla growth form, clonal integration have been proven to be very beneficial to the growth and/or reproduction of the ramets facing local stress or disturbance (e.g. Slade, Hutching, 1987a, b; Evans, 1991; D'Hertefeldt, van der Putten, 1998; van Kleunen, Stuefer, 1999; Yu et al., 2002). In phalanx clonal plants, although isotope studies have shown that nutrients can be transported within connected tillers (Derner, Briske, 1998), the effects of such nutrient translocation on the growth and reproduction of the plants have not been widely tested. In homogeneous environments tiller connections did not affect the growth and reproduction of the bunchgrass *C. squarrosa*, whereas in heterogeneous soil nutrient conditions connected clonal fragments produced more biomass, more and larger tiller ramets than disconnected ones (Figs 1–3). These results support the hypothesis that clonal integration may also benefit greatly to the growth and clonal reproduction of phalanx species experiencing fine-scale spatial heterogeneity in resource supply. In a recent study, Bullock et al. (1994) also found that clonal integration helped the young tillers (2 wk of tillers) of the phalanx grass *Holcus lanatus* to withstand simulated herbivore.

Many studies have demonstrated that clonal plant species may actively or passively place more roots in nutrient-rich patches when growing in heterogeneous environments (reviewed by Robinson, 1994; Hodge, 2004). Due to such localized plastic responses of root system, plants may grow equally well or even better when similar amounts of nutrients are supplied patchily rather than evenly (e.g. Birch, Hutchings, 1994; Fransen et al., 1999). In the present study, when clonal fragments of *C. squarrosa* grew in the heterogeneous soil conditions, the connected clonal fragments produced more root biomass in pots receiving high nutrient supply than disconnected ones (Fig. 4). Clonal integration, therefore, modified the growth responses of roots receiving high nutrient supply. Theories predict that resource acquisition efficiency will be markedly enhanced in resource-rich patches (Bloom, 1985). Therefore, even when the same amount of nutrients was supplied, *C. squarrosa* produced significantly more biomass in heterogeneous than in homogeneous nutrient treatments when tiller ramets connections were maintained (Fig. 1). This result suggests that phalanx clonal species such as the bunchgrass *C. squarrosa* may also make good use of soil heterogeneity, as demonstrated in many guerilla clonal species (Birch, Hutchings, 1994; Fransen et al., 1999, 2001).

Trade-offs between sexual reproduction and clonal growth have been observed in some plant species. For instance, an increase in sexual investment reduced the rhizome branching intensity in *Tipularia discolor* (Snow, Whigham, 1989) and the tuber production in *Helianthus tuberosus* (Westley, 1993). According to the model of Gardner and Mangel (1999), investment in asexual reproduction may favor foraging locally. In heterogeneous conditions, the connected clonal fragments of *Cleistogenes squarrosa* allocated relatively more resources to asexual offspring, but less to sexual structures, than the disconnected clonal fragments. Such trade-offs between sexual and asexual reproduction induced by clonal integration, therefore, may favor *C. squarrosa* to forage in the spatially heterogeneous environments. No matter whether the tiller ramets were connected or not, clonal fragment of *C. squarrosa* in high nutrient conditions tended to allocate more biomass to asexual reproduction (Fig.1), agreeing with the findings of Tomlinson and O' Connor (2004) that the availability of soil nitrogen plays a crucial role in ramet (tiller) recruitment in bunchgrasses.

From this experiment we conclude that phalanx clonal species such as the bunchgrass *C. squarrosa* may also make good use of the fine-scale spatial heterogeneity in resource supply and clonal integration and root plasticity play a substantial role during this process.

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