Physiological integration helps a clonal macrophyte spread into competitive environments and coexist with other species

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ABSTRACT
Physiological integration may help clonal macrophytes invade or escape from existing communities. No studies have tested the above hypothesis in aquatic plants. In an outdoor pond experiment, we subjected clonal fragments of the submerged macrophyte Vallisneria spiralis to heterogeneous environments in which V. spiralis spread from bare habitats towards vegetated habitats occupied by Myriophyllum spicatum L. or V. spiralis spread from vegetated habitats towards bare habitats. V. spiralis stolons between ramets in bare habitats and in vegetated habitats were either intact or severed. We investigated the habitat selection of V. spiralis by examining the allocation of biomass and ramets to heterogeneous habitats during its vegetative spread phase. Results showed that the stolon connection had different effects on the habitat selection of V. spiralis with regard to invasion and escape. When V. spiralis spread from bare to vegetated habitats, in comparison to severing the stolon, the stolon connection eventually facilitated a 49% increase in biomass and a 27% increase in number of ramets allocated to vegetated habitats. However, when V. spiralis spread from vegetated to bare habitats, biomass and ramets allocated to bare habitats were not significantly changed by the stolon connection (only a 5% increase in biomass and a 6% increase in number of ramets). These results indicate that clonal integration facilitated V. spiralis not to escape from but invade into vegetated habitats. The study provides evidence that physiological integration is important for survival and tolerance of ramets in competitively stressful environments and can help clonal macrophytes coexist with other species.

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1. Introduction

A great number of studies have shown that connected ramets of clonal plants can share photosynthesize, water, and nutrients through clonal integration (Alpert and Mooney, 1986; Stuefer and Hutchings, 1994; de Kroon et al., 1998; Alpert et al., 2003; Marbà et al., 2006). Clonal integration can improve the survival, growth, and reproduction of ramets in stressful environments and help genets occupy open space (Hutchings and Wijesinghe, 1997; Amsberrry et al., 2000; Wang et al., 2008). These positive effects of clonal integration may provide clonal plants with a competitive advantage by improving the performance of ramets in communities and reducing the growth and reproduction of their competitors. Therefore, clonal integration may influence species coexistence and community structure (Pennings and Callaway, 2000; Peltzer, 2002; Brezina et al., 2006; Yu et al., 2009).

Genets or fragments of clonal plants can be established initially in bare or vegetated habitats because of the mosaic structures of natural communities (Hutchings and Wijesinghe, 1997; Oborny et al., 2000). When clonal plants, especially the guerrilla growth forms (i.e. clonal plants with relatively long stolons or rhizomes and a strong tendency to grow in a single direction with little branching, see Lovett Doust, 1981), begin their vegetative spread across borders in heterogeneous communities, two possible directions exist: escape away from or invasion into interspecific neighbours. However, previous experiments dealing with the physiological integration of clonal plants during competition with neighbours have not considered the direction of clonal growth as an important variable. Whether invasion into a competitive habitat or escape to an open habitat is more dependent on clonal integration remains to be clarified.

Clonal growth is very common in wetland and aquatic plant species (Grace, 1993; Sosnová et al., 2011). After initial colonization of a site by seedlings or asexual propagules, clonal growth seems to be the primary method of maintaining and expanding natural populations for aquatic clonal species (Marbà and Duarte, 1998; Santamaria, 2002). For understanding the role of clonal growth of aquatic plants, it is necessary to research the clonal integration, one of the important characteristics of clonal growth. There are
2. Materials and methods

2.1. Plant materials

V. spiralis is an important freshwater submerged macrophyte in many regions of the world (Lowned, 1982). It can produce plagiotropic stolons, spread horizontally above the sediment surface, and form ramets at the nodes. Each ramet has a basal rosette of leaves which may extend to the surface but do not form a canopy. These ramets are interconnected by stolons and form a large clonal system across heterogeneous environments (Xiao et al., 2007). At the study site, V. spiralis generally began clonal growth in April and continued to September, flowered and fruited in autumn, and overwintered by tubers. Its leaves died down at the end of autumn. In the field, V. spiralis and its congener Vallesineria americana Michx. often coexist with M. spicatum (Titus and Adams, 1979; Zhan et al., 2001). Whereas, V. spiralis occupies horizontal space over the sediment by clonal growth, M. spicatum generally occupies vertical space in the water column by shoot branching.

2.2. Experimental design

Two hundred tubers of V. spiralis were buried into pots with sand covered by 10 cm water in a greenhouse and sprouted in mid-April. After approximately 3 weeks, each shoot formed a ramet and began producing a stolon. Ninety-six ramets having an initial stolon and uniform size were selected as testing plants (15–20 cm in height and three to four leaves). At the same time, about 800 shoots of M. spicatum, each 40–50 cm high, were collected from Liangzi Lake. On 8 May, we conducted the experiment in 24 outdoor concrete ponds in the National Field Station for Lake Ecosystem in Liangzi Lake, Hubei Province, China (30°15′N, 114°33′E; 17 m asl.). The ponds were the same size (200 cm in length, 200 cm in width, 120 cm in height), were oriented equivalently towards the sun, and each of them was filled with a 20 cm depth of lake clay. The lake clay was homogenized before being placed in the ponds. Each pond was divided into four strips of equal size (200 cm in length, 50 cm in width), and M. spicatum was then transplanted into half of each strip (100 cm in length, 50 cm in width, vegetated habitats) at a density of 16 shoots m^-2. The initial planting density of M. spicatum shoots was consistent with shoot density in the field. The other half of the strip was left bare (100 cm in length, 50 cm in width, bare habitats). The four vegetated habitats or four bare habitats were located side by side in the same side of each pond (Fig. 1). One V. spiralis ramet having an initial stolon was then transplanted into each strip. Of the four strips in each pond, two ramets were transplanted randomly into two bare habitats with their initial stolons pointing towards two vegetated habitats, and the other two ramets were transplanted randomly into two vegetated habitats with their initial stolons pointing towards two bare habitats. After transplanting the plants, all of the ponds were filled with lake water and maintained full water during the experiment. On 31 May, we found that all transplanted plants survived. Each V. spiralis initial ramet had elongated its stolon and produced rooted offspring ramets in the other habitats. We then randomly selected one of the two V. spiralis plants oriented in the same growth direction in each pond and severed the stolons between the mother ramets and the offspring ramets in different habitats. Therefore, there were four treatments were set up randomly in each pond, which were designated as follows: B0V (stolons spreading from bare to vegetated habitats were severed), B–V (stolons spreading from bare to vegetated habitats remained intact), V0B (stolons spreading from vegetated to bare habitats were severed), and V–B (stolons spreading from vegetated to bare habitats remained intact) (Fig. 1).

2.3. Measurements

The experiment was harvested on 21 June, 12 July, and 4 August. Eight ponds were used for each harvest, and each treatment was replicated with eight V. spiralis clones for each harvest. For each part of the V. spiralis clonal fragment in different habitats, we counted the number of ramets, oven-dried the plants at 80°C for 72 h, and measured their weight. A complete unit of
V. spiralis ramets originating from the initially transplanted ramet was defined as one whole clonal fragment. At a cloudless midday before the final harvest, we used a solar monitor to measure irradiance at the sediment surface in each bare and vegetated patch. The measured photosynthetic photon flux density was 1512 ± 21 μmol m⁻² s⁻¹ (means ± SD) in bare habitats and 453 ± 14 μmol m⁻² s⁻¹ in vegetated habitats. The average biomass and density of M. spicatum in vegetated habitats was 117 ± 18 g (mean ± SD) and 131 ± 12 shoots m⁻² (mean ± SD) at the final harvest, respectively.

2.4. Data analysis

Prior to analysis, variables were transformed as necessary to meet the assumptions of normality and homoscedasticity for ANOVA. Block effects (the position effect of four treatments in each pond) had been examined in the analysis. Two-way ANOVA was used to investigate the effects of clonal growth direction, stolon connection and their interaction on dry weight and ramet number of the whole clonal fragments, the mother and offspring ramets of V. spiralis at the last harvest time, respectively. A post-hoc test (Duncan) at the 0.05 significance level was performed for difference between size of clonal fragments among the four treatments at the last harvest time. All statistical tests were performed with SPSS 15.0 (SPSS, Chicago, IL, USA).

3. Results

During the experimental period, V. spiralis showed a strong capacity for acropetal stolon growth. Substantial biomass and ramets were produced in offspring ramets of the clonal fragment whether or not it was in vegetated habitats (Fig. 2). Severing the stolon resulted in an increase in biomass and number of ramets in the mother ramets of the clonal fragments at the expense of offspring ramets. When V. spiralis grew from vegetated to bare habitats, the biomass and ramet number of the whole clonal fragment were not significantly changed by severing stolon. Harvest time had a noticeable and significant interaction with stolon connection on the growth of ramets in vegetated habitats (for dry weight: \( F = 12.698, P < 0.001 \); for ramet number: \( F = 77.177, P < 0.001 \)). Especially when separated from ramets in bare habitats, the biomass and number of ramets in vegetated habitats, even the offspring ramets of the clonal fragment, significantly decreased from 12 July to 4 August (Fig. 2).

The clonal direction and stolon connection had significant interaction on the growth of V. spiralis (Table 1). When V. spiralis invaded vegetated habitats, the stolon connection significantly enhanced the biomass and number of ramets of the whole clonal fragment, especially the offspring ramets in vegetated habitats (for biomass: \( F = 56.716, P < 0.001 \); for ramet number: \( F = 212.286, P < 0.001 \); Fig. 3). In comparison to severing the stolon, the stolon connection eventually facilitated a 49% increase in the total biomass and a 27% increase in number of ramets allocated to vegetated habitats. However, no significant effect of stolon connection was observed with regard to the size of whole clonal fragment in the opposite direction. When V. spiralis grew from vegetated to bare habitats, the biomass and number of ramets in bare habitats were not significantly changed by the stolon connection (for biomass: \( F = 3.044, P = 0.088 \); for number of ramets: \( F = 0.808, P = 0.374 \); Fig. 3). The stolon connection only helped V. spiralis a 5% increase

![Fig. 2. Dry weight (means ± SE) and ramet number (means ± SE) of the mother and offspring ramets of Vallisneria spiralis over the four harvest times in the four treatments. See Fig. 1 for the treatment codes.](image)

### Table 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Whole clonal fragments</th>
<th>Mother ramets</th>
<th>Offspring ramets</th>
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<tr>
<td></td>
<td></td>
<td>Dry weight</td>
<td>Ramet number</td>
<td>Dry weight</td>
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<td>Block</td>
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<td>2.130&lt;sup&gt;ns&lt;/sup&gt;</td>
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<tr>
<td>Clonal direction (D)</td>
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<td>31.015&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>5.510&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stolon connection (C)</td>
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<td>24.065&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>62.246&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>19.810&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>D × C</td>
<td>1.31</td>
<td>0.075&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>42.215&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>7.088&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
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</table>

<sup>ns</sup>, not significant.
<sup>*</sup> \( P < 0.05 \).
<sup>**</sup> \( P < 0.01 \).
<sup>***</sup> \( P < 0.001 \).
offspring ramets in vegetated habitats (Stuefer et al., 1994; Roiloa and Retuerto, 2005; Xiao et al., 2007). Therefore, clonal integration enhanced the performance and tolerance of ramets in vegetated habitats. This result confirms the first hypothesis.

When V. spiralis grew from vegetated to bare habitats, the stolon connection did not significantly help offspring ramets escaping from vegetated habitats at the beginning of experiment, and that the growth of mother ramets in vegetated habitats was not improved by connections with offspring ramets in bare habitats at the end of experiment. Therefore, the second hypothesis was not confirmed. These results imply that the reciprocal physiological integration did not occur or was too small to have a significant effect on growth. This is consistent with the author’s former study, in which V. spiralis only benefited from acropetal clonal integration in heterogeneous light environments (Xiao et al., 2007). However, several studies demonstrated extensive physiological integration in some clonal plants such that, regardless of the direction of clonal growth, ramets in a disadvantageous environment can be supported by those in a favourable environment (Jónsdóttir and Watson, 1997; D’Hertefeldt and Falkengren-Gruner, 2002). Stuefer and Hutchings (1994) considered acropetal transport to be the predominant reason for the loss of reciprocal physiological integration. Although no transpiration occurs under water, submerged plants still have the ability of acropetal transport (Terrados et al., 1997; Marba et al., 2002). Some studies have shown that material transfer between interconnected ramets of clonal plants is closely related to water transport (de Kroon et al., 1998; D’Hertefeldt and Falkengren-Gruner, 2002). Because basipetal integration is often observed in clonal plants located in very dry environments, basipetal transport might result from the uneven distribution of water. Since water transport is acropetal in submerged plants (Pedersen and Sand-Jensen, 1993), we presume that physiological integration would also be acropetal under water, although this hypothesis requires further study.

4. Discussion

4.1. Neighbour competition

When separated from ramets in bare habitats, the biomass and number of ramets of V. spiralis in vegetated habitats significantly decreased at the end of experiment. Even though the connection significantly increased the biomass and number of ramets in vegetated habitats, their values were still smaller than those observed for bare habitats, especially for the final biomass. These results suggest that competition with M. spicatum suppressed the clonal growth of V. spiralis. The reason might be that the competition between M. spicatum and V. spiralis was asymmetrical because of the low density of V. spiralis in this experiment. In addition, larger individuals may reduce the light available to smaller individuals and thus suppress their growth (Weiner, 1990; de Kroon et al., 1992). The loss in biomass and number of V. spiralis in vegetated habitats from 12 July to 4 August might be due to the suppression by M. spicatum. As we measured, reduced light levels resulting from M. spicatum over-topping could be the main reason for the reduction in growth parameters of V. spiralis in vegetated habitats.

4.2. Effects of physiological integration

When V. spiralis invaded the vegetated habitats occupied by M. spicatum, stolon connection significantly enhanced the biomass and number of ramets in vegetated habitats and resulted in a larger-sized clonal fragment. This may be due to promotion of photosynthetic capacity of ramets in bare habitats by the strong carbon demand of sinks created by connected ramets in vegetated habitats. Furthermore, more material was produced and transported to in its total biomass and a 6% increase in its number of ramets into bare habitats at the end of experiment.

4.3. Habitat selection in heterogeneous communities

One growth strategy for clonal species in heterogeneous environments is the selective placement of ramets in favourable habitats. This is expected to enhance resource acquisition and has been interpreted as habitat selection or foraging behaviour (Hutchings and de Kroon, 1994). When spread from vegetated to bare habitats, V. spiralis placed more ramets into bare habitats in spite of severing connections with basal ramets. This indicates that V. spiralis can escape an adverse environment through clonal growth. This habitat selection has been demonstrated in some terrestrial clonal plants (Evans and Cain, 1995; Macek and Lepš, 2003; Sampaio et al., 2004), and in some aquatic clonal plants (Titus and Stephens, 1983; Xiao et al., 2006). Because resource availability in bare habitats was sufficient for offspring ramets, they no longer depended on support from mother ramets. Therefore, clonal integration between connected ramets in different habitats contributed little to escape of V. spiralis away from vegetated habitats occupied by M. spicatum. In the process of competition between V. spiralis and M. spicatum, physiological integration greatly increased the interaction between V. spiralis and M. spicatum. We speculate that V. spiralis can continually invade into the area occupied by M. spicatum, leading to coexistence.

In conclusion, the present study showed that clonal integration did not facilitate V. spiralis to escape away from vegetated habitats at the temporal resolution of this study, but significantly helped V. spiralis invasion into vegetated habitats. This provides evidence that clonal integration is important for survival and tolerance of ramets in competitively stressful environments and can help clonal plants to coexist with other species.
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References


