



Clonal integration enhances expansion ability of *Ipomoea aquatica* in aquatic–terrestrial ecotones

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ABSTRACT

Clonal integration has been repeatedly shown to promote the performance of clonal plants in both terrestrial and aquatic heterogeneous habitats. However, only a few studies have explicitly tested the effect of clonal integration in aquatic–terrestrial ecotones. To test the role of clonal integration in aquatic–terrestrial ecotones, we conducted an experiment with an amphibious, stoloniferous clonal plant *Ipomoea aquatica*, where apical parts of stolon fragments of *I. aquatica* were planted in water and basal parts were in soil. Stolon connection between apical and basal parts was either left intact (*i.e.*, the maintenance of clonal integration) or severed (*i.e.*, the loss of clonal integration). We also set up three different nitrogen levels in water to further test the effect of increased water nitrogen on the outcome of clonal integration. Results indicated that clonal integration could facilitate the establishment of apical parts of *I. aquatica* in aquatic areas at no costs to interconnected basal ramets in terrestrial areas, and also improve the performance of basal parts at the medium- and high-nitrogen level. Besides, the maintenance of stolon connection could greatly promote the fitness of whole fragments of *I. aquatica*, irrespective of water nitrogen level. Therefore, clonal integration may be an important life history trait that contributes to the expansion of *I. aquatica* in aquatic–terrestrial ecotones.

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1. Introduction

Clonal growth is very common in amphibious plants that span a wide niche ranging from terrestrial to aquatic habitats during their entire life cycles (Barrett et al., 1993; Santamaría, 2002). One distinguishing feature of clonal growth is clonal integration between interconnected ramets within the same clone (Song et al., 2013). Studies have repeatedly reported that clonal integration can greatly improve the survival, growth and spread of clonal plants in either terrestrial (Xu et al., 2010; He et al., 2011; Roiloa et al., 2014) or aquatic habitats (Wolfer and Straile, 2012; Tuya et al., 2013a,b), due to translocation and sharing of resources (e.g., mineral nutrients, water and carbohydrates) (Xu et al., 2010; Roiloa et al., 2014). However, only a few studies have explicitly tested the effect of clonal integration on amphibious clonal plants in aquatic–terrestrial ecotones (e.g., Wang et al., 2009; Luo et al., 2014). To understand

the role of clonal integration in amphibious clonal plants is thus required to broaden the understanding of the ecological and evolutionary significance of clonal growth and adaptive strategies of amphibious clonal plants.

The outcome (or net benefit) of clonal integration for the whole clone is usually unpredictable, because clonal integration not only contributes the benefits to recipient ramets, but also potentially causes the costs to donor ramets (Caraco and Kelly, 1991). Even for the same clonal species, net benefits of clonal integration may become stronger or vice versa with the changing resource availability in heterogeneous environments (Hutchings and Wijesinghe, 2008; He et al., 2011). Because resource fluctuation in aquatic habitats is usually more intense and frequent than in terrestrial habitats, we predicted that nutrient enrichment in aquatic habitats may rapidly improve the favorability of local areas and shift contrasting levels of resource availability between water and soil, and thus influence the overall outcome of clonal integration. However, few studies have shown the effect of resource availability in aquatic habitats on the outcome of clonal integration in aquatic–terrestrial ecotones (Wang et al., 2009).

Excessive nitrogen loading in aquatic systems is a widespread phenomenon (Smith et al., 1987), which is mainly derived from both point sources of nitrogen pollution (e.g., municipal and industrial wastewater effluent) and nonpoint sources (e.g., nitrogen

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runoff from agricultural lands and atmosphere nitrogen deposition over water surface) (Smith et al., 1999; Anderson et al., 2002; Bergstrom and Jansson, 2006). Excessive nitrogen loading can cause severe environmental and ecological problems (Smith et al., 1987; Pretty et al., 2003). As it is well known, nitrogen enrichment can degrade water quality and stimulate harmful algae blooms and eutrophication (Smith et al., 1987, 2006; Johnson et al., 2007). Also, nitrogen loading could be an important factor that increases the invasibility of aquatic habitats and aquatic–terrestrial ecotones, where species richness become lower and amphibious plants are of more highly invasiveness (Zedler and Kercher, 2004; Tyler et al., 2007).

To understand the importance of clonal integration in the clonal expansion of amphibious species, we conducted an experiment with an amphibious, stoloniferous clonal plant *Ipomoea aquatica*. In the experiment, we simulated the expansion of *I. aquatica* from terrestrial to aquatic habitats, where apical parts of stolon fragments of *I. aquatica* were grown in water, and basal parts were grown in soil. Stolons connecting the two parts were either left intact (i.e., the maintenance of clonal integration) or severed (i.e., the loss of clonal integration). To examine the effect of nitrogen loading on clonal integration, we also set up three different nitrogen levels (i.e., low-, medium-, or high-nitrogen levels) in water to further test the effect of nitrogen addition on clonal integration. Specially, we tested the following hypotheses: (1) clonal integration will promote the performance of apical ramets of *I. aquatica* in aquatic areas, because the maintenance of stolon connection may allow basal ramets in terrestrial areas to translocate resources to connected apical ramets. (2) clonal integration will inhibit the performance of *I. aquatica* in terrestrial areas, because the resource translocation may cause costs for basal ramets. (3) clonal integration will increase the performance of whole fragments of *I. aquatica*, if the benefits to apical ramets are obviously higher than the costs to basal ramets. In aquatic–terrestrial ecotones, terrestrial environments are often favorable for plant growth and provide more than sufficient resources, compared to aquatic environments. If so, basal ramets may receive relatively low costs in the term of reduced growth, and the benefits to apical ramets will be expected to excess the costs to basal ramets. (4) the benefits of clonal integration at the whole-plant level will be more significant at the higher nitrogen level, if the increased water nitrogen can reduce the costs of basal ramets. As nitrogen loading in aquatic environments increases the local resource level, the dependence of apical ramets on resource sharing will become weaker and the lower costs by clonal integration will be expected to occur at higher nitrogen level.

2. Materials and methods

2.1. Species

I. aquatica Forsk. (Water spinach) is a perennial, amphibious, stoloniferous clonal herb of Convolvulaceae family, native to southeastern Asia (Cook, 1989; Fang and Staples, 1995). It has been widely introduced outside its native ranges as a vegetative crop and is now considered to be one of the most noxious plant species in many countries and regions (Edie and Ho, 1969; Austin, 2007). *I. aquatica* can produce hollow and prostrate stolons that bear leaves, roots, and axillary stolons at each node; a node along the stolon with its leaves and roots is also referred to as a ramet (Lin et al., 2012). The species can reproduce either sexually by seeds or asexually by stolon fragments, but mainly propagate vegetatively to occupy a wide natural and anthropic habitats ranging from aquatic to adjacent open terrestrial areas, e.g., waterways, irrigation ditches, swamps and crop fields (Cook, 1989; Fang and Staples, 1995).

2.2. Experimental design

The experiment used a 3×2 factorial design with three nitrogen level treatments (i.e., low-, medium-, or high-nitrogen levels) crossed with two stolon connection treatments (i.e., intact or severed; Fig 1). Thirty-six similar-size stolon fragments of *I. aquatica* (0.98 ± 0.12 cm in diameter and 4.81 ± 0.90 cm in internode length; mean \pm SD, $N=9$) were selected for the experiment, each consisting of five ramets and one apex. Each stolon fragment was classified into two parts, one termed as “apical part” comprising the two youngest ramets and one apex, and the other termed as “basal part” comprising the remaining three oldest ramets. Nine additional similar-size stolon fragments were used for initial measurements. The initial dry mass of apical and basal parts of stolon fragments were 0.42 ± 0.12 g and 0.81 ± 0.25 g, respectively (mean \pm SD, $N=9$).

Thirty-six plastic boxes (61 cm long \times 41.5 cm wide \times 35 cm high) were used in the experiment, and each container was physically separated by a transparent plastic partition into two equal sections (30.5 cm long \times 41.5 cm wide \times 35 cm high). To simulate terrestrial areas of aquatic–terrestrial ecotones, the left section of each container was filled to a depth of 25 cm with a 1:1 (v:v) mixture (mean: 0.84 mg total Ng^{-1} and 0.95 mg total Pg^{-1}) of riverbank soil and sand. To simulate aquatic areas, the right section was firstly filled to a depth of 5 cm with the same soil mixture described above, and then to a depth of 20 cm above soil with tap water (mean: 1.84 mg total NL^{-1} and 0.088 mg total PL^{-1}). Nutrients, water and roots of plants in the two sections did not interfere with each other. Of 36 right sections (aquatic areas) of containers, 12 were additionally supplied with NH_4NO_3 at the concentration of 25 mg L^{-1} total nitrogen, corresponding to “high-nitrogen level”; another 12 were supplied at the concentration of 5 mg L^{-1} total nitrogen, corresponding to “medium-nitrogen level”; the remaining 12 were not supplied with any NH_4NO_3 , corresponding to “low-nitrogen level”. All left sections of containers were not applied with any fertilizers.

On August 18, 2012, every stolon fragment of *I. aquatica* was individually planted in one container. Three basal ramets along the stolon fragment were placed within the left section of a container, while two apical ramets were placed within the right section of a container (Fig. 1). After one week when all ramets had rooted, we randomly selected half of stolon fragments in each nitrogen treatment to keep stolons connecting the apical and basal parts disconnected or connected.

The experiment was conducted in a common garden at Forest Science Co., Ltd., of Beijing Forestry University from August 18, 2012 to October 20, 2012. During the experiment, the light intensity during the midday ranged from 524.07 to 996.85 $\mu\text{mol s}^{-1} \text{m}^{-2}$, measured by a light meter (TES 1339; TES Crop., Taiwan) and the average temperature was 21.12°C , measured hourly by two hygrochron temperature loggers (iButton DS1923; Maxim integrated products, USA). Enough distilled water was supplied every two or three days, either to keep the soil moist in the left sections or to compensate for the loss by evaporation in the right sections. Water in the right sections was completely replaced every three weeks to maintain the same initial nitrogen concentration.

On October 20, we separately harvested apical and basal parts of stolon fragments of *I. aquatica*. We measured the number of new nodes, total stolon length, leaf area (WinFOLIA Pro 2004a, regent instruments, Québec, Canada), petiole length, number of branches and stolon main axis length of different parts. The apical and basal parts of plants were then divided into petioles, stolons, leaves and roots, oven dried at 70°C for 48 h and weighed.

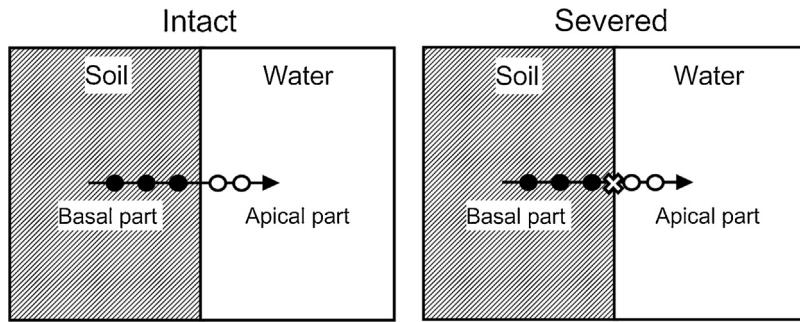


Fig. 1. Experiment design: the clonal fragment of *Ipomoea aquatica*, each consisting of three basal ramets (filled circles) grown in soil and two apical ramets (open circles) with a stolon apex (the arrow) subjected to three nitrogen level treatments. Stolons between the apical and basal ramets were either left intact (left) or severed (right).

2.3. Data analysis

To examine effects on growth of plants, we used two-way ANOVAs to test effects of stolon connection (intact vs. severed) and nitrogen levels (low vs. medium vs. high) on the total biomass, number of new nodes, total stolon length, leaf area, number of branches, number of leaves, and stolon main axis length for the apical and basal parts and for the whole fragments. The total number of new nodes, stolon length, leaf area, number of branches, number of leaves, and stolon main axis length were morphological attributes of plants, but they were used as proxies for the growth of *I. aquatica* in the present study system. To examine effects on morphological traits and root to shoot ratio of plants, we used two-way ANOVAs to test effects of stolon connection and nitrogen levels on the mean petiole length (total petiole length/number of petioles; cm), the specific petiole length (total petiole length/petiole mass; cm mg^{-1}), internode length (total stolon length/number of new nodes; cm), the specific internode length (total stolon length/stolon mass; cm g^{-1}), the specific leaf area (total leaf area/leaf mass; $\text{m}^2 \text{kg}^{-1}$) and root to shoot (leaf mass plus stolon mass) ratio for the apical and basal parts and for the whole plants. Differences between individual means were tested with post hoc Tukey tests and linear contrasts, and considered significant if $P < 0.05$. Because in four containers (one in the low-nitrogen, intact treatment, one in the medium-nitrogen, intact treatment, one in the low-nitrogen, severed treatment, and one in the high-nitrogen, severed treatment) no plants survived, they were excluded for all analyses. The number of new nodes, stolon length, leaf area, number of branches, number of leaves, and stolon main axis length of apical parts, and the leaf area, number of branches, and number of leaves of basal parts were natural-log transformed before analysis to improve homoscedasticity. All analyses were conducted using SPSS 22.0 (SPSS, Chicago, Illinois, USA).

3. Result

3.1. Performance of apical parts

Only the number of new nodes, stolon length, number of leaves, and stolon main axis length of apical parts of *I. aquatica* were significantly affected by water nitrogen level, whereas the other measures were not (Table 1). The number of new nodes, stolon length, number of leaves, and stolon main axis length of apical parts were significantly higher at the medium- and high-nitrogen level than at the low-nitrogen level (Table 1, Figs. 2B, C, F and 3F).

Most measures of apical parts were significantly affected by stolon connection, except for the internode length, specific internode length, and specific leaf area (Table 1). The total biomass, number of new nodes, stolon length, leaf area, number of branches, number of leaves, petiole length, and stolon main axis length of

apical parts were significantly higher when the stolon was connected than when it was severed (Table 1, Figs. 2 and 3). In contrast, the mean petiole length of apical parts were significantly lower when the stolon was connected than when severed (Table 1, Fig. 3B). There was a significant interaction effect of stolon connection by nitrogen level on the root to shoot ratio (Table 1). Stolon connection significantly increased the root to shoot ratio at the low-nitrogen level, but did not change it at the medium- and high-nitrogen level (Fig. 4).

3.2. Performance of basal parts

The number of new nodes, stolon length, and number of leaves of basal parts of *I. aquatica* were significantly affected by water nitrogen level, whereas the other measures were not (Table 1). Because the soil and water sections in each container were physically divided, all measures of disconnected basal parts were not affected by water nitrogen level, whereas the number of new nodes, stolon length, and number of leaves of connected basal parts were significantly greater at the medium- and high-nitrogen level than at the low-nitrogen level (Table 1, Fig. 2B, C, F).

The stolon length, stolon main axis length, and mean internode length of basal parts of *I. aquatica* were significantly affected by stolon connection, whereas the other measures were not (Table 1). The stolon length, stolon main axis length, and mean internode length of basal parts were significantly higher when the stolon was connected than when severed (Table 1, Figs. 2C and 3C, F). There were no interaction effects of stolon connection by nitrogen level on all measures of basal parts (Table 1).

3.3. Performance of the whole fragments

Only the number of new nodes, stolon length, and number of leaves of the whole fragments of *I. aquatica* were significantly affected by water nitrogen level, whereas the other measures were not (Table 1). The total number of nodes, stolon length, and number of leaves of the whole fragments were significantly higher at the medium- and high-nitrogen level than at the low-nitrogen level (Fig. 5B, C, F).

The total biomass, number of new nodes, stolon length, stolon main axis length, and internode length, and specific leaf area of the whole fragments were significantly affected by stolon connection, whereas the other measures were not (Table 1). The total biomass, number of new nodes, stolon length, stolon main axis length, and internode length of the whole fragments were significantly higher when the stolon was connected than when severed (Figs. 5A–C and 6C, F). In contrast, the specific leaf area of the whole fragments were significantly lower when the stolon was connected than when severed (Fig. 6E). Besides, there were no significant interaction effects of stolon connection by nitrogen level

Table 1

ANOVA results for effects of nitrogen levels, stolon connection, and their interactions on growth, morphology traits and root to shoot ratio for apical and basal parts and for whole clonal fragments of *Ipomoea aquatica*.

Measures	Apical ramet			Basal ramet			Clonal fragment		
	Nitrogen	Connection	N × C	Nitrogen	Connection	N × C	Nitrogen	Connection	N × C
Biomass	2.091 ^{ns}	60.405***	0.514 ^{ns}	2.035 ^{ns}	0.629 ^{ns}	0.910 ^{ns}	2.980 [#]	13.502**	2.805 [#]
Number of new nodes	4.643 ^{a,*}	52.769 ^{a,***}	0.623 ^{a,ns}	6.037**	0.012 ^{ns}	1.072 ^{ns}	6.994**	6.380*	1.649 ^{ns}
Stolon length	3.989 ^{a,*}	31.496 ^{a,***}	0.719 ^{a,ns}	4.315*	9.642**	0.670 ^{ns}	3.809*	16.218***	0.791 ^{ns}
Leaf area	2.007 ^{a,ns}	126.736 ^{a,***}	0.533 ^{a,ns}	2.693 ^{a,#}	3.467 ^{a,#}	0.911 ^{a,ns}	2.600 [#]	2.203 ^{ns}	1.361 ^{ns}
Number of branches	2.740 ^{a,*}	41.922 ^{a,***}	0.621 ^{a,ns}	2.787 ^{a,#}	0.456 ^{a,ns}	2.542 ^{a,#}	2.591 [#]	1.046 ^{ns}	2.065 ^{ns}
Number of leaves	3.731 ^{a,*}	107.108 ^{a,***}	0.299 ^{a,ns}	5.063 ^{a,*}	1.073 ^{a,ns}	2.001 ^{a,ns}	4.701*	3.724 [#]	2.140 ^{ns}
Stolon main axis length	4.010 ^{a,*}	10.641 ^{a,**}	1.179 ^{a,ns}	1.139 ^{ns}	14.848**	1.044 ^{ns}	3.217 [#]	21.160***	0.367 ^{ns}
Petiole length	0.316 ^{ns}	20.369***	1.441 ^{ns}	0.454 ^{ns}	0.005 ^{ns}	3.131 [#]	0.722 ^{ns}	1.025 ^{ns}	2.037 ^{ns}
Specific petiole length	0.379 ^{ns}	31.835***	0.010 ^{ns}	1.054 ^{ns}	0.000 ^{ns}	0.008 ^{ns}	0.614 ^{ns}	0.242 ^{ns}	0.480 ^{ns}
Internode length	2.530 [#]	3.694 [#]	0.859 ^{ns}	0.157 ^{ns}	21.364***	1.014 ^{ns}	0.021 ^{ns}	15.598**	0.330 ^{ns}
Specific internode length	0.891 ^{ns}	1.565 ^{ns}	0.361 ^{ns}	2.930 ^{ns}	2.584 ^{ns}	0.162 ^{ns}	2.338 ^{ns}	1.562 ^{ns}	0.091 ^{ns}
Specific leaf area	3.102 [#]	3.224 [#]	0.084 ^{ns}	0.602 ^{ns}	1.702 ^{ns}	0.049 ^{ns}	0.296 ^{ns}	7.277*	1.249 ^{ns}
Root to shoot ratio	0.144 ^{ns}	4.320*	6.075**	0.457 ^{ns}	0.521 ^{ns}	0.381 ^{ns}	0.290 ^{ns}	1.724 ^{ns}	0.475 ^{ns}

Values give F ; symbols give P : *** <0.001 ; ** <0.01 ; * <0.05 ; # <0.1 ; ns ≥ 0.1 . Degrees of freedom for N, C and N × C are (2, 26), (1, 26) and (2, 26), respectively.

^aLn (natural-logarithmic) transformation.

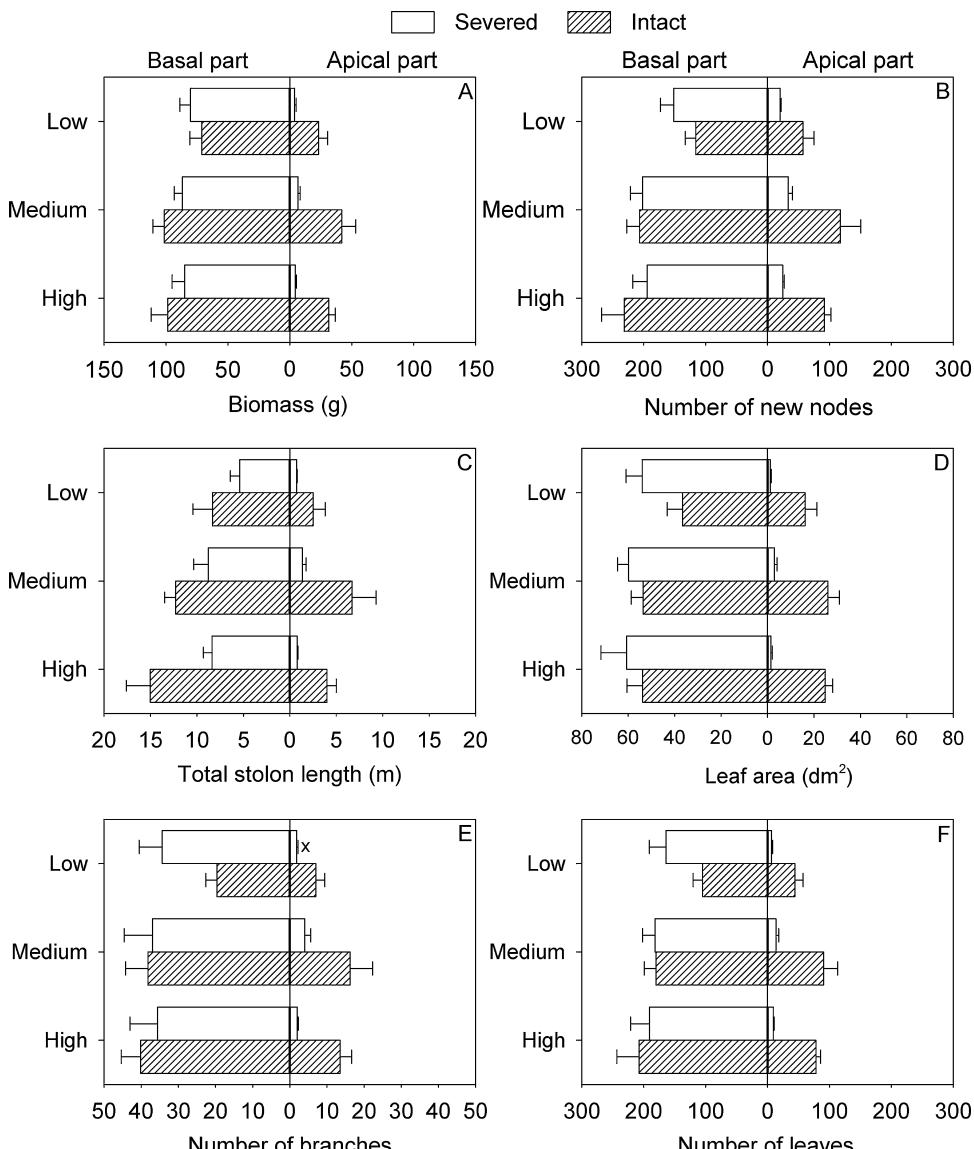


Fig. 2. (A) Biomass, (B) number of new nodes, (C) total stolon length, (D) leaf area, (E) number of branches, and (F) number of leaves of apical and basal parts of *Ipomoea aquatica* subjected to three nitrogen levels. Stolons connecting apical and basal parts were either left intact or severed. Bars show means + 1 SE.

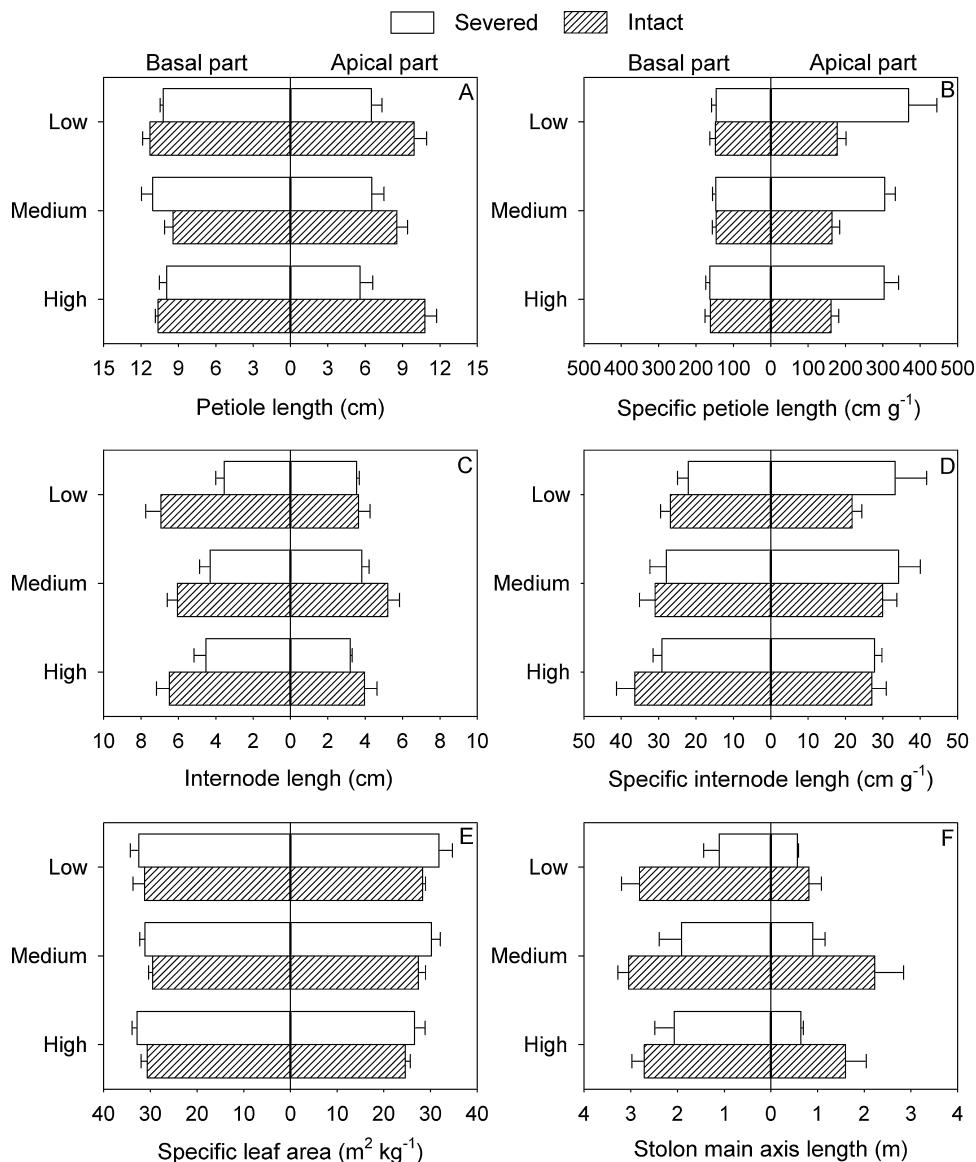


Fig. 3. (A) Petiole length, (B) specific petiole length, (C) internode length, (D) specific internode length, (E) specific leaf area, and (F) stolon main axis length of apical and basal parts of *Ipomoea aquatica* subjected to three nitrogen levels. Stolons connecting apical and basal parts were either left intact or severed. Bars show means \pm 1 SE.

on all measures of the whole fragments (Table 1). However, there was a marginal interaction effect on the biomass of the whole fragments, and linear contrast indicated that the positive effect of clonal integration on the biomass tended to be more obvious at the medium- and high-nitrogen level than at the low-nitrogen level (Fig. 5A).

4. Discussion

Irrespective of the nitrogen level in water, the maintenance of stolon connection could markedly improve the growth (measured by biomass, leaf area and number of leaves), spread (measured by total stolon length, number of branches and main axis length) and clonal reproduction ability (measured by number of new nodes) of apical ramets of *I. aquatica* in aquatic habitats. These results are consistent with the first hypothesis, showing that the maintenance of stolon connection may be very important for the early growth and establishment of juvenile ramets of *I. aquatica* in aquatic habitats. One plausible reason is that the maintenance of stolon connection may guarantee continuous export of resources from basal

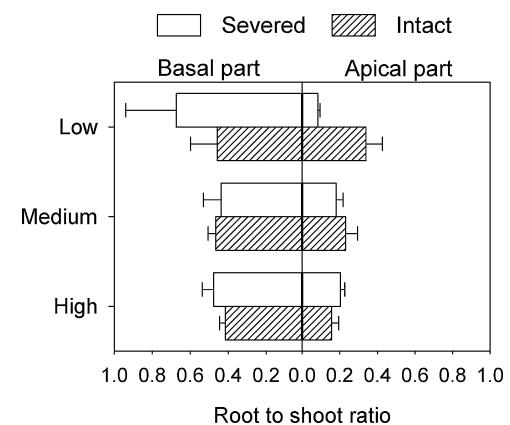


Fig. 4. Root to shoot ratio of apical and basal parts of *Ipomoea aquatica* subjected to three nitrogen levels. Stolons connecting apical and basal parts were either left intact or severed. Bars show means \pm 1 SE.

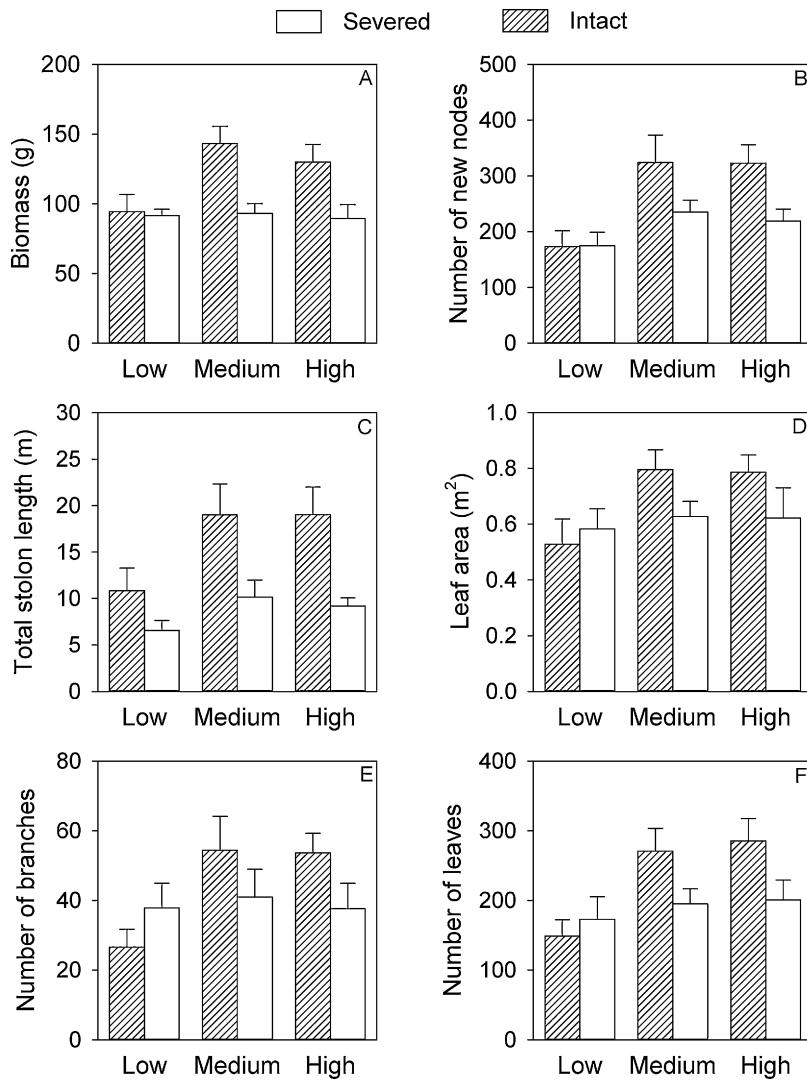


Fig. 5. (A) Biomass, (B) number of new nodes, (C) total stolon length, (D) leaf area, (E) number of branches, and (F) number of leaves of the whole fragments of *Ipomoea aquatica* subjected to three nitrogen levels. Stolons connecting apical and basal parts were either left intact or severed. Bars show means + 1 SE.

ramets of *I. aquatica* in terrestrial habitats (i.e., clonal integration) to support the survival and early growth of apical ramets in aquatic habitats (Ooi et al., 2011; Tuya et al., 2013a; Roiloa et al., 2014). Such benefits to recipient ramets are also common in many other amphibious clonal species, such as *Alternanthera philoxeroides*, *Carpobrotus edulis* and *Spartina alterniflora* (Wang et al., 2008; Roiloa et al., 2010; Xiao et al., 2011). For instance, the maintenance of stolon (or rhizome) connection can significantly improve the survival and growth of *C. edulis* (Roiloa et al., 2010), the photosynthetic efficiency and competitive ability of *A. philoxeroides* (Wang et al., 2008; Yu et al., 2009) and the sexual reproduction of *S. alterniflora* (Xiao et al., 2011). To some extent, clonal integration will potentially contribute to the expansion of amphibious clonal species in stressful habitats.

Furthermore, we found that the maintenance of stolon connection could alter the petiole morphology of apical ramets of *I. aquatica* to make them thinner and longer and stimulate the root growth of apical ramets from 50% rooted ramets in the severed treatment to 94% in the intact treatment. The results implied that the maintenance of stolon connection may bring both direct benefits via the resource translocation to apical ramets mentioned above, and indirect benefits via the petiole morphological modification and rapid root growth to heighten the resource uptake

capacity of juvenile apical ramets themselves (Dong, 1995; Alpert, 1999).

Previous studies have shown that clonal integration is a double-edged sword that often contributes the benefits to recipient ramets at the costs to donor ramets (Pauliukonis and Gough, 2004; Wang et al., 2009). These costs to donor ramets may be caused by export of resources (Xu et al., 2010), increased respiration consumption via maintaining stolon or rhizome connection (Pauliukonis and Gough, 2004), transmission of diseases and pathogen (Stuefer et al., 2004) and transference of phytotoxic elements (Outridge and Hutchinson, 1990). However, our results showed that the maintenance of stolon connection did not cause any costs to basal ramets of *I. aquatica* in terrestrial habitats, although it tended to influence leaf production and stolon morphology. These results are inconsistent with the second hypothesis. The plausible reason for lack of costs incurred by basal ramets in the experiment may be the fact that only surplus resources may be transported from donor ramets to recipient ramets (Yu et al., 2002), so the resource supply for apical ramets of *I. aquatica* may not limit the normal growth of basal ramets, even when the nitrogen level in water is relatively low.

Our results further showed that as the increased nitrogen concentration in water, the maintenance of stolon connection could improve not only the ramet, leaf and stolon productions of apical

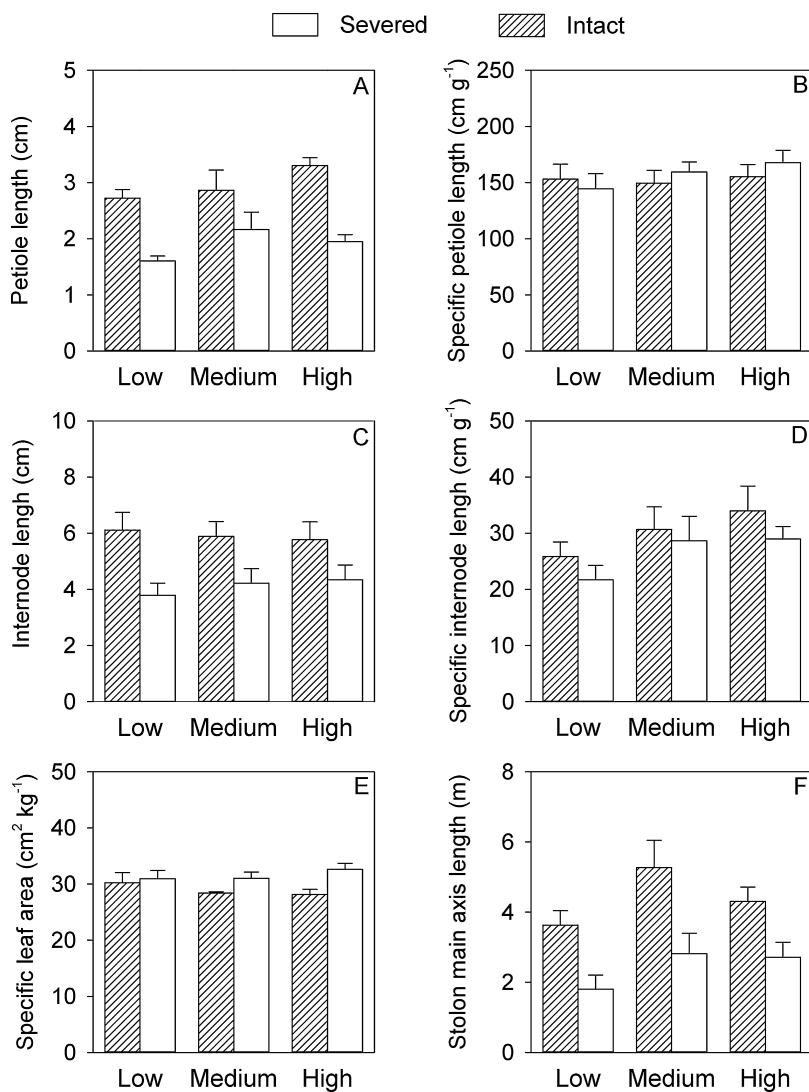


Fig. 6. (A) Petiole length, (B) specific petiole length, (C) internode length, (D) specific internode length, (E) specific leaf area, and (F) stolon main axis length of the whole fragments of *Ipomoea aquatica* subjected to three nitrogen levels. Stolons connecting apical and basal parts were either left intact or severed. Bars show means + 1 SE.

ramets of *I. aquatica*, but also the performance of connected basal ramets. One possible reason is that the increased nitrogen in water may accelerate the root growth of apical ramets in aquatic areas, and thus alleviate the negative influence from clonal integration on basal ramets. The other possible reason is that apical ramets at the high nitrogen level may replace basal ramets as the new donor ramets after the establishment, and transport resources (e.g., photosynthates) to support the growth of basal ramets, although the direction of resource transportation within the same clone is commonly acropetal from older to younger ramets (Tomasko and Dawes, 1989; Xiao et al., 2007).

The outcome of clonal integration in the whole fragments is determined by the interaction between the apical and basal parts. Because of the high benefits to apical parts and no apparent costs to basal parts, the maintenance of stolon connection significantly improved the performance of the whole fragments of *I. aquatica*. The results were consistent with the third hypothesis, implying that clonal integration may be an importance life history trait that contributes to the expansion of *I. aquatica* in aquatic–terrestrial ecotones. However, the positive consequence of clonal integration was not found in some other amphibious clonal species (Wang et al., 2008; Luo et al., 2014). For instance, clonal integration did not affect the growth and clonal production of the whole fragments of

A. philoxeroides in aquatic–terrestrial ecotones, because the costs of clonal integration to donor ramets completely counterbalanced the benefits to recipient ramets (Wang et al., 2009). Therefore, the general conclusion about whether clonal integration is the advantageous trait for most amphioxous clonal species in aquatic–terrestrial ecotones should be further tested.

Besides, our results showed that there was only an additive effect between clonal integration and water nitrogen level, rather than a synergistic interaction. The results were inconsistent with the fourth hypothesis, suggesting that the impact of clonal integration at the whole-plant level did not depend on water nitrogen level, in the current range of nitrogen concentration. However, based on the result of the biomass of the whole fragments, we also found a slight positive relationship between clonal integration and water nitrogen level. We predict that if the experiment used a wider range of nitrogen concentration, it was possible to detect the more obvious positive relationship between clonal integration and water nitrogen level.

In sum, as a typical amphibious clonal plant, clonal integration could improve not only the performance of apical ramets of *I. aquatica* in aquatic habitats, but also basal ramets at the relatively high nitrogen level. Clonal integration also increased the growth of the whole fragments of *I. aquatica* in aquatic–terrestrial ecotones,

irrespective of water nitrogen concentration. Therefore, clonal integration may be an important trait that allows the support to apical ramets under current environments or even more unfavorable environments, if conditions are ideal enough for basal ramets, and that contributes to the expansion of the whole clone of *I. aquatica* in aquatic–terrestrial ecotones. Besides, there are some limitations in the current experiment. One is that the conclusions mentioned above are mainly deduced from the changes in morphological traits, and the physiological evidence of resource translocation between adjacent ramets by tracing carbon and nitrogen movements should be needed in future studies to better elucidate the underlying mechanism. The other is that the current experiment setup cannot examine the relationship between clonal integration and plant invasiveness, and comparison of the capacity of clonal integration between *I. aquatica* and other native species in aquatic–terrestrial ecotones may solve the problem.

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