Effects of patch contrast and arrangement on benefits of clonal integration in a rhizomatous clonal plant

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The availabilities of light and soil water resources usually spatially co-vary in natural habitats, and the spatial pattern of such co-variation may affect the benefits of physiological integration between connected ramets of clonal plants. In a greenhouse experiment, we grew connected or disconnected ramet pairs (consisting of a proximal (relatively old) and a distal (relatively young) ramet) of a rhizomatous herb *Iris japonica* in four heterogeneous environments differing in patch arrangement (reciprocal vs. parallel patchiness of light and soil water) and patch contrast (high vs. low contrast of light and water). Biomass of the proximal part, distal part and clonal fragment of *I. japonica* were all significantly greater in the intact than in the severed treatment, in the parallel than in the reciprocal patchiness treatment and in the high than in the low contrast treatment, but the effect of severing the connection between ramet pairs did not depend on patch arrangement or contrast. Severing the connection decreased number of ramets of the distal part and the clonal fragment in the parallel patchiness arrangement, but not in the reciprocal patchiness arrangement. Therefore, the spatial arrangement of resource patches can alter the effects of clonal integration on asexual reproduction in *I. japonica*.

Light, soil water and nutrients are commonly patchily distributed in nature1,2. Many clonal plants can spread horizontally and produce connected ramets located in microhabitats differing in resource supply3–5. Due to clonal integration, these connected ramets can exchange photosynthates, water and nutrients to improve the integrative performance of the whole clonal fragment3,6–9. However, the spatial pattern of resource patchiness varies greatly in nature, which may impact the benefits of clonal integration10–12.

The availabilities of light and soil water usually spatially co-vary in natural habitats13. In some habitats such as shrublands, high light intensity under sparse vegetation is associated with low soil water availability and low light intensity under dense vegetation is accompanied with high soil water availability12–15. In such environments with reciprocal patchiness of light and soil water, neither patch alone is ideal for plant growth16,17. In some other habitats such as fixed dunes, high light intensity is accompanied with high soil water availability and low light intensity with low soil water availability18,19. In such environments with parallel patchiness of light and soil water, patches with high light and high water are ideal for plant growth, whereas those with low light and low water are not10,11. Impacts of clonal integration depend strongly on the strength of the source-sink relationship between connected ramets that are located in different resource patches, and spatial patch arrangement (reciprocal vs. parallel) may affect the strength of the source-sink relationship. Therefore, the spatial pattern of patch arrangement may affect the benefit of clonal integration between connected ramets of clonal plants10,11.

Benefits of clonal integration may also depend on resource contrast between patches where connected ramets grow4,8,20. Contrast is a basic element of environmental heterogeneity16, and a minimal contrast between two patches is required to induce resource sharing (clonal integration) between connected ramets because clonal plants will sense the environment homogeneous if patch contrast is too low6,21. Thus, patch contrast can have a profound impact on benefits of clonal integration7,8,22,23. Previous studies have indeed shown that
Clonal integration benefited ramets in low resource patches more when resource contrast between patches was higher\(^7,8,20,23\). However, little is known about the interaction effect between patch contrast and patch arrangement on benefits of clonal integration.

We grew ramet pairs of a rhizomatous herb *Iris japonica* under four heterogeneous environments differing in spatial patch arrangement (reciprocal vs. parallel patchiness of light and soil water) and patch contrast (high vs. low contrast of light and water), with the rhizome connecting the two ramets of a pair either severed or left intact (to prevent or allow clonal integration). Specifically, we addressed the following questions: (1) Does rhizome connection (i.e. clonal integration) improve the growth of *I. japonica* in terms of biomass and ramet production and their interactions on the growth of the whole clonal fragment (A), the proximal part (B) and the distal part (C) of *I. japonica*. The given are \(F\) values and significance levels (\(* * P < 0.001, \* * * P < 0.01, \* * P < 0.05\) and \(\text{ns } P > 0.05\)). Degree of freedoms were 1, 64 for all the effects.

### Results

**Effects on the clonal fragment.** Patch arrangement, patch contrast and rhizome severing significantly affected biomass of the clonal fragment of *I. japonica* (Table 1A). Biomass of the clonal fragment was significantly larger in the parallel than in the reciprocal arrangement, in the high than in the low contrast and in the intact than in the severed treatment (Fig. 1A). However, effects of rhizome severing on biomass of the clonal fragment did not depend significantly on patch arrangement or patch contrast (Table 1A, \(P > 0.05\) for A × S and C × S). Number of ramets of the clonal fragment was also higher in the high than in the low contrast treatment and in the intact than in the severed treatment (Table 1A, Fig. 1B), but the negative effect of rhizome severing was more pronounced in

| Arrangement (A) | Contrast (C) | Severing (S) | A × C | A × S | C × S | A × C × S |
|-----------------|-------------|--------------|-------|-------|-------|-----------|
| **A** Clonal fragment | | | | | | |
| Biomass | 7.11*** | 49.32*** | 16.53*** | 0.20ns | 1.53*** | 0.28ns | 0.34ns |
| No. of ramets | 2.70** | 5.84' | 12.80*** | 0.01ns | 4.44' | 0.76** | 0.23** |
| **B** Proximal part | | | | | | |
| Biomass | 21.29*** | 46.86*** | 10.02** | 1.80ns | 1.50*** | 1.66** | 0.65ns |
| No. of ramets | 3.21** | 5.03' | 1.51 | 1.10ns | 0.29** | 0.29** | 0.05* |
| **C** Distal part | | | | | | |
| Biomass | 3.81' | 12.05*** | 16.12*** | 0.34ns | 1.67ns | 2.89ns | 0.82ns |
| No. of ramets | 0.28ns | 19.44*** | 6.63* | 3.52ns | 6.89** | 0.69ns | 0.47** |

Table 1. Results of three-way ANOVAs for effects of patch arrangement (parallel vs. reciprocal patch), patch contrast (high vs. low) and rhizome severing (severed vs. intact) and their interactions on the growth of the whole clonal fragment (A), the proximal part (B) and the distal part (C) of *I. japonica*. The given are \(F\) values and significance levels (\(* * P < 0.001, \* * * P < 0.01, \* * P < 0.05\) and \(\text{ns } P > 0.05\)). Degree of freedoms were 1, 64 for all the effects.

**Figure 1.** Effects of patch arrangement (reciprocal vs. parallel), patch contrast (high vs. low) and rhizome severing (intact vs. severed) on the growth of the whole clonal fragment (proximal plus distal part) of *I. japonica*. Bars and vertical lines are mean and SE.
the parallel than in the reciprocal treatment, as indicated by a significant effect of patch arrangement × rhizome severing (Table 1A, Fig. 1B). There was no significant three-way interaction effect of patch arrangement, patch contrast and rhizome severing on biomass or number of ramets of the clonal fragment (Table 1A).

Effects on the proximal part. Patch arrangement, patch contrast and rhizome severing significantly affected biomass of the proximal part of *I. japonica* (Table 1B). Biomass of the proximal part was significantly larger in the parallel than in the reciprocal arrangement, in the high than in the low contrast and in the intact than in the severed treatment (Fig. 2A). However, effects of rhizome severing on biomass of the proximal part did not depend on patch arrangement or patch contrast (Table 1B, *P* > 0.05 for A × S and C × S). Number of ramets of the proximal part was higher in the high than in the low contrast treatment, but was not significantly affected by patch arrangement or rhizome severing (Table 1B, Fig. 2B). There was no significant three-way interaction effect of patch arrangement, patch contrast and rhizome severing on biomass or number of ramets of the proximal part (Table 1B).

Effects on the distal part. Biomass of the distal part was significantly larger in the parallel than in the reciprocal arrangement, in the high than in the low contrast and in the intact than in the severed treatment (Fig. 3A). However, effects of rhizome severing on biomass of the distal part did not depend on patch arrangement or patch contrast (Table 1C, *P* > 0.05 for A × S and C × S). Number of ramets of the distal part was higher in the high than in the low contrast treatment (Table 1C, Fig. 3B). In the reciprocal patch arrangement, rhizome severing had no effect on number of ramets of the distal part, but in the parallel patch arrangement rhizome severing decreased it, as indicated by a significant interaction effect of patch arrangement × rhizome severing (Table 1C, Fig. 3B). There was no significant three-way interaction effect of patch arrangement, patch contrast and rhizome severing on biomass or number of ramets of the distal part (Table 1C).

Discussion

In heterogeneous environments, clonal integration significantly improved the growth of both the proximal and the distal part of *I. japonica*, thereby markedly increasing the growth of the whole clonal fragment. This result agrees with the finding of a previous meta-analysis demonstrating that clonal integration generally increases the growth of whole clonal fragments in heterogeneous environments. While the effects of clonal integration on biomass did not depend on patch arrangement, its effects on number of ramets of both the distal part and the whole clonal fragment depended significantly on patch arrangement. These results suggest that the spatial arrangement of resource patches can alter the effects of clonal integration on asexual reproduction.

Clonal integration increased number of ramets of both the distal part and the whole clonal fragment of *I. japonica* in the parallel patch arrangement but had little impacts in the reciprocal patch arrangement. Under parallel patchiness, the gradients of both resources (light and water) were in the same direction so that the internal source-sink relationships of photosynthates and water between the proximal and the distal part were very likely to be in the same direction. By contrast, under reciprocal patchiness, the gradients of light and water were in the opposite directions so that the internal source-sink relationships of photosynthates and water between the proximal and the distal part were likely to be in the opposite directions. Resource transportation may be more efficient when the source-sink resources of both photosynthates and water are in the same direction than when they are in the opposite directions. This increased efficiency of resource transportation may have contributed to the production of more but smaller ramets in the parallel patch arrangement.
The benefits of clonal integration on biomass or number of ramets of *I. japonica* did not depend significantly on patch contrast that was used in our experiment. However, Friedman & Alpert (1991) found that clonal integration increased growth of *Fragaria chiloensis* when patch contrast of light and nutrients was high, but not when patch contrast was low20. Similarly, effects of clonal integration on growth of *Diplopterygium glaucum* also depended on the degree of patch contrast and were significant only under relatively high patch contrast22. In our study, the possible reason for lack of an effect of patch contrast is that the resource contrast in the low patch contrast treatment already exceeded the minimum required by clonal integration of *I. japonica*, and that the resource contrast in the high patch contrast treatment was not large enough to change the effect of clonal integration.

Regardless of clonal integration, biomass of the proximal part, the distal part and the whole clonal fragment of *I. japonica* were significantly larger in the parallel than in the reciprocal arrangement. Previous study also showed that biomass of the whole clonal fragment of *F. orientalis* was larger in parallel patchiness than in reciprocal patchiness11. In the parallel arrangement, high light was always accompanied with high water, and medium light was always accompanied with medium water. Thus, disconnected ramets can grow much better in these resource-rich patches24–28. Furthermore, the high efficiency of resource transportation between connected ramets when the source-sink relationships of both photosynthates and water are in the same direction may have also promoted growth of *I. japonica*.

Growth of the whole clonal fragment of *I. japonica* was higher under the high than under the low patch contrast treatment. This is very likely because the overall resource levels of light and water for the whole clonal fragment differed between the two patch contrast treatments. In our experiment, the low contrast was between the medium and the low level of light or water, but the high contrast was between the high and the low level of light and water. As a result, the overall levels of light and water were higher in the the high contrast than in the low contrast treatment. The higher overall resource levels in the high patch contrast treatment may have contributed to the increased growth of the whole clonal fragment of *I. japonica*.

We conclude that the spatial arrangement of resource patches can alter the effects of clonal integration on asexual reproduction of *I. japonica*. Because the overall resource levels in the two patch contrast treatments were not the same, we cannot fully eliminate the confounding effect of resource levels from those of patch contrast. Thus, further studies testing effects of patch contrast should consider to set the overall resource levels for the whole clonal fragment the same across all the treatments. In our study, we considered only the effects of clonal integration during the vegetative growth period of *I. japonica*, and did not test those on sexual reproduction, i.e. the real fitness measures. Studies examining the effects on both asexual reproduction (clonal growth) and sexual reproduction (flowering and fruiting) are more valuable.

**Materials and Methods**

**The species and material preparation.** *Iris japonica* Thunb. (Iridaceae), a perennial clonal herb, is widely distributed in Asia29,30. This species produces long rhizomes with rooted ramets on its nodes. Most rhizomes are distributed in the topsoil of less than 5 cm deep30. The distance between adjacent ramets varies between 5 to 15 cm, and population density can be higher than 20 ramets m⁻². Ramets of the same clone are often located in heterogeneous light and water environments from forest understories to open area11,20. Without disturbance, rhizome connections between ramets can last up to one year in natural conditions. Disturbance such as animal trampling and rodent grazing can easily break rhizome connections. It flowers from March to April and fruits from May to June. Viable seeds are produced each year, but seedlings occur at very low densities in established populations. Clonal growth is the main method for its population spread12.
In early January 2014, more than 100 connected ramet pairs of *I. japonica* were collected from five locations in an evergreen broad-leaved forest on Shizi Mountain in Hubei Province, China. The distance between any two locations was at least 100 m to increase the chance of sampling different genets. The ramet pairs were cultivated in a greenhouse at Huazhong Agricultural University in Hubei Province, China. One ramet in each pair was recognized as the initial proximal part, indicating its relative proximity to the mother ramet, while the other as the initial distal part. After two weeks of cultivation, we selected 84 similar-sized ramet pairs of *I. japonica*, and each ramet of a pair had three leaves and some roots. Of them, 20 ramet pairs were randomly selected for measuring initial dry mass (mean ± SE: 0.842 ± 0.113 g for the proximal ramet and 0.733 ± 0.053 g for the distal ramet), and the other 64 pairs were used for the experiment described below.

**Experimental design.** The experiment used a factorial design with two levels of rhizome severing (intact vs. severed), two patch contrast treatments (high vs. low) and two patch arrangement treatments (reciprocal vs. parallel). In the high contrast treatment, the proximal ramet of a pair was subjected to the high water and/or high light treatment, while the distal ramet was subjected to the low water and/or low light treatment; in the low contrast treatment, the proximal ramet was subjected to the moderate water and/or moderate light treatment, while the distal ramet was subjected to the low water and/or low light treatment. In the parallel arrangement, high light was always accompanied with high water, moderate light with moderate water and low light with low water; in the reciprocal arrangement, high and moderate light were accompanied with low water and low light with high water or moderate water.

Figure 4. Experimental design. The experiment used a factorial design with two rhizome severing treatments (intact vs. severed), two patch contrast treatments (high vs. low) and two patch arrangement treatments (reciprocal vs. parallel). In the high contrast treatment, the proximal ramet of a pair was subjected to the high water and/or high light treatment, while the distal ramet was subjected to the low water and/or low light treatment; in the low contrast treatment, the proximal ramet was subjected to the moderate water and/or moderate light treatment, while the distal ramet was subjected to the low water and/or low light treatment. In the parallel arrangement, high light was always accompanied with high water, moderate light with moderate water and low light with low water; in the reciprocal arrangement, high and moderate light were accompanied with low water and low light with high water or moderate water.
treatment during the experimental period (by Soil Moisture Meter TZS-II, HEB Biotechnology Co., Xi'an, China). Ramet pairs were randomly assigned to positions on a bench in the greenhouse.

The experiment ran for four months, from 1 March to 1 July 2014. During the experiment, the mean temperature was 26.0 °C and the mean relative humidity was 70.5% (by Amprobe TR300, Amprobe, Everett, WA, USA). The light intensity in the greenhouse was about 85% of that outside.

**Measurements and analyses.** We counted number of ramets in the proximal and the distal parts (patches) separately. Then, all ramets in each part were harvested, dried at 80 °C for 48 h and weighed. Biomass and number of ramets of a clonal fragment were the sum of those of the proximal and the distal part.

We used three-way ANOVAs to test effects of rhizome severing (severed vs. intact), patch arrangement (reciprocal vs. parallel), patch contrast (high vs. low) and their interactions on biomass and number of ramets of the clonal fragment, the proximal part and the distal part. All statistical analyses were carried out with SPSS 13.0 (SPSS, Chicago, IL, USA). Prior to ANOVAs, data of biomass was transformed to the square root to increase normality and homogeneity of variance. The differences were considered significant if $P < 0.05$.

**References**

1. Hutchings, M. J. & Wijesinghe, D. K. Patchy habitats, division of labour and growth dividends in clonal plants. *Trends Ecol. Evol.* **12**, 390–394 (1997).

2. Hutchings, M. J. & John, E. A. The effects of environmental heterogeneity on root growth and root/shoot partitioning. *Ann. Bot.* **94**, 1–8 (2004).

3. Roiloa, S. R. & Retuerto, R. Responses of the clonal *Fragaria vesca* to microtopographic heterogeneity under different water and light conditions. *Environ. Exp. Bot.* **61**, 1–9 (2007).

4. Hutchings, M. J. & Wijesinghe, D. K. Performance of a clonal species in patchy environments: effects of environmental context on yield at local and whole-plant scales. *Evol. Ecol.* **22**, 313–324 (2008).

5. Roiloa, S. R. & Retuerto, R. Clonal integration in *Fragaria vesca* growing in metal-polluted soils: parents face penalties for establishing their offspring in unsuitable environments. *Ecol. Res.* **27**, 95–106 (2012).

6. Song, Y. B. et al. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* **171**, 317–327 (2013).

7. Zhang, Y. C., Zhang, Q. Y., Yirdaw, E., Luo, P. & Wu, N. Clonal integration of *Fragaria orientalis* driven by contrasting water availability between adjacent patches. *Bot. Stud.* **49**, 373–383 (2008).

8. Zhang, Y. C., Zhang, Q. Y., Luo, P. & Wu, N. Photosynthetic response of *Fragaria orientalis* in different water contrast clonal integration. *Ecol. Res.* **24**, 617–625 (2009).

9. Yu, F. H., Chen, Y. F. & Dong, M. Clonal integration enhances survival and performance of *Potentilla anserina*, suffering from partial sand burial on Ordos plateau, China. *Evol. Ecol.* **15**, 303–318 (2002).

10. He, W. M., Alpert, P., Yu, F. H., Zhang, I. L. & Dong, M. Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants. *J. Ecol.* **99**, 1202–1210 (2011).

11. Zhang, Y. & Zhang, Q. Clonal integration of *Fragaria orientalis* in reciprocal and coincident patchiness resources: cost-benefit analysis. *Plos One* **8**, e60263 (2013).

12. Li, Q. Y., Tao, J. P., Zhong, Z. C. & Wang, Y. J. Growth performance, sexual reproduction and clonal propagation of *Ranunculus reptans* in heterogeneous habitats. *Ecol. Res.* **32**, 328–334 (2011).

13. Alpert, P. & Mooney, H. A. Resource heterogeneity generated by shrubs and topography on coastal sand dunes. *Vegetatio* **122**, 83–93 (1996).

14. Maron, J. L. & Jeffries, R. L. Bush lupine mortality, altered resource availability and alternative vegetation states. *Ecology* **80**, 443–454 (1999).

15. Griffiths, A. B. Positive effects of native shrubs on *Bromus tectorum* demography. *Ecology* **91**, 141–154 (2010).

16. Stuefer, J. F., de Kroon, H. & During, H. J. Exploitation of environmental heterogeneity by spatial division of labour in a clonal plant. *Funct. Ecol.* **10**, 328–334 (1996).

17. Alpert, P. & Stuefer, J. F. In *The Ecology and Evolution of Clonal Plants*. (ed. de Kroon, H. & van Groenendael, J.) 137–154 (Backbayus, 1997).

18. Dyer, A. R. et al. The role of adaptive trans-generational plasticity in biological invasions of plants. *Evol. Appl.* **3**, 179–192 (2010).

19. Prati, D. & Schmid, B. Genetic differentiation of life-history traits within populations of the clonal plant *Ramunculus reptans*. *Oikos* **90**, 442–456 (2000).

20. Friedman, D. & Alpert, P. Reciprocal transport between ramets increases growth of *Fragaria chiloensis* when light and nitrogen occur in separate patches but only if patches are rich. *Oecologia* **86**, 76–80 (1991).

21. Wijesinghe, D. K. & Hutchings, M. J. The effects of environmental heterogeneity on the performance of *Glehuma hederaeae*: the interactions between patch contrast and patch scale. *J. Ecol.* **87**, 860–872 (1999).

22. Guo, W., Song, Y. B. & Yu, F. H. Heterogeneous light supply affects growth and biomass allocation of the understory fern *Diplolpterygium glaucum* at high patch contrast. *J. Ecol.* **87**, 860–872 (1999).

23. Wang, Z., Li, Y., During, H. J. & Li, D. Do clonal plants show greater division of labour morphologically and physiologically at higher patch contrasts? *Plos One* **6**, e25401 (2011).

24. Wang, Y. J., Shi, X. P., Meng, X. F., Wu, X. J., Luo, F. L. & Yu, F. H. Effects of spatial patch arrangement and scale of covarying resources on growth and intraspecific competition of a clonal plant. *Front. Plant Sci.* **7**, 753 (2016).

25. Wijesinghe, D. K. & Hutchings, M. J. The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glehoma hederaeae*. *J. Ecol.* **85**, 17–28 (1997).

26. He, W. M., Yu, F. H. & Zhang, L. L. Physiological integration impacts nutrient use and stoichiometry in three clonal plants under heterogeneous habitats. *Ecol. Res.* **25**, 967–972 (2010).

27. Wang, Z., van Kleunen, M., During, H. J. & Werger, M. J. A. Root foraging increases performance of the clonal plant *Potentilla reptans* in heterogeneous nutrient environments. *Plos One* **8**, e58602 (2013).

28. Dong, B. C. et al. Soil heterogeneity affects ramet placement of *Hydrocotyle vulgaris*. *J. Plant Ecol.* **8**, 91–100 (2015).

29. The Editorial Board of the Flora of China. *Flora of China*. 176–177 (Beijing, Science Press, 1985).

30. Wang, Y. J., Shi, X. P. & Zhong, Z. C. The relative importance of sexual reproduction and clonal propagation in rhizomatous herb *Iris japonica* Thunb. from two habitats of Jinyun Mountain, Southwest China. *Russ. J. Ecol.* **44**, 199–206 (2013).

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Author Contributions
Y.-J.W., X.-P.S. and F.-H.Y. designed the experiment, X.-J.W. and X.-F.M. performed the experiment, Y.-J.W., P.-C.W., Z.-X.Z. and F.-H.Y. did the statistical analysis, F.-L.L. and Y.-J.W., contributed to data collection and making the figures, Y.-J.W. and F.-H.Y. wrote the manuscript.

Additional Information
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