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INVASION NOTE

Complementarity effects do not necessarily result in significant transgressive over-performance in mixtures

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Abstract Recent empirical studies have shown that genetic factors can influence the invasion success of alien species. Like species diversity, higher genetic diversity can increase plant performance or invasion success via selection effects or complementarity effects. The latter have also been referred to as non-additive effects because in this case individual genotypes show a different performance in mixtures than in monocultures. Based on a manipulation experiment in the field, a recent study found that such non-additive effects of genotype mixing become stronger over time in an invasive plant, probably due to higher resource uptake or facilitation between genotypes. Other researchers criticized this interpretation because in their view complementarity or non-additive effects should lead to transgressive over-performance which could not occur based on selection effects alone, where simply differences of performance between genotypes would enhance the invasion ability of multiple-genotype patches. In a commentary paper we applied Monte Carlo permutation and Loreau and Hector's additive partitioning to two datasets and found that although transgressive over-performance cannot occur due to only selection effects and thus is the most stringent test for the existence of complementarity effects, complementarity effects also commonly occur without transgressive over-performance.

Keywords Complementarity effects · Genotypic diversity · Invasive ability · Non-additive genotypic effects · Selection effects

Genetic variation has been long understood as the basis for a species to improve performance in changing environments. Empirical studies in the last decade have confirmed several benefits of high genetic variation in plant populations, including increased biomass (e.g., Hughes and Stachowicz 2004), promoted tolerance to or resilience from disturbance (e.g., Reusch et al. 2005) and enhanced resistance to invasions (e.g., Crutsinger et al. 2008). Improved

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plant performance by high genetic diversity may occur via selection effects, whereby diverse populations have a higher probability of containing high performance genotype, or complementarity effect, whereby niche differentiation, facilitation or counteraction among genotypes results in increased mixture performance. The latter have also been referred to as non-additive effects (Crawford and Whitney 2010) because in this case individual genotypes show a different performance in mixtures than in monocultures.

The positive relationship between genetic variation and instantaneous or long-term performance at the population level provides important information for conservation and restoration strategies (Hughes and Stachowicz 2004). For an alien species, high genetic variation may also promote their performance, and thus increase its potential to become invasive. Although bottleneck effects during the introduction may lead to substantial loss of genetic variation, multiple introductions over time or from different source regions may restore genetic variation or and similar or even increased genetic diversity may be observed in the introduced compared with the native range. Such increases in genetic diversity by multiple introductions and reshuffling and recombination of different source regions have in fact been detected in some celebrated cases of successful invaders, e.g. *Bromus tectorum* (Novak and Mack 1993), *Phalaris arundinacea* (Lavergne and Molofsky 2007), *Ambrosia artemisiifolia* (Genton et al. 2005) and *Phragmites australis* (Kirk et al. 2011).

Spartina alterniflora Loisel (Poaceae) or smooth cordgrass is a perennial grass native to the Atlantic coast of the Americas. When it was intentionally introduced to China in 1979, seeds were collected from three sites in North America and mixed in Luoyuan Bay, Fujian Province of China (An et al. 2007). Now this species is widely distributed along coastlines of China. Although transplantation by people may accelerate the long-distance spread of this species, a number of biological properties such as fast growth, high salt tolerance and strong ability of clonal growth (Li et al. 2009) make *S. alterniflora* a superior competitor over native species. Shen (2007) analyzed genetic variation in Chinese and native populations using microsatellites. He found that populations in the invasive range had considerable levels of genetic variation comparable to native populations, suggesting that genetic factors may contribute to its invasion success.

To understand the potential role of genetic variation in the rapid spread of *S. alterniflora* in China, we compared the performance of 1-, 3- and 6-genotype patches of *S. alterniflora* in an experiment (Wang et al. 2012). We found that patches of high genotype richness did outperform patches of low genotype richness. Two nonexclusive mechanisms can be used to explain increased mixture performance leading to the observed positive relationship between species or genetic diversity and performance (Bernasconi et al. 2003). Selection (Loreau and Hector 2001) or sampling (Huston 1997) effects refer to the fact that population patches of high genotypic diversity have a high probability to contain advantageous genotypes that have a disproportionate effect on a property at the patch level. Complementarity (Loreau and Hector 2001) or non-additive (Crawford and Whitney 2010) effects refer to the fact that different genotypes may differ in their resource use or facilitate each other and thus the better performance in patches with high genotypic diversity can be due to higher resource uptake or facilitation.

Loreau and Hector (2001) provided a method to calculate the two effects and this method has been used many times in biodiversity–ecosystem functioning experiments (see e.g. Cardinale et al. 2007). This method needs information about the performance of each species or genotype in monoculture and mixture. Unlike species identity, which can be easily determined by morphological traits, genotype identity cannot be easily determined without genotyping. Because shoots of invasive species can increase to a very large number in a short time (Zhang et al. 2006), genotyping each shoot is an expensive and time-consuming work. In our study, shoot number of *S. alterniflora* increased from 18 to ~2,700 in the mixture patches in less than two growing seasons. Therefore, we used a Monte Carlo permutations test and found that both selection and non-additive effects contributed to the increased outputs of genotype mixing resulting in the positive relationship between genotypic diversity and performance of experimental patches. Furthermore, non-additive effects became stronger over time, as also observed in species mixtures (Cardinale et al. 2007; Marquard et al. 2009). Our experiment provided the first direct evidence that genotypic diversity can promote the invasion potential of an alien species.

Based on our manipulation experiment, Le Roux et al. (2014) emphasized that selection effects most likely were the main mechanism driving the results. In our view, their argumentation mixes up two issues that are not logically connected in a one-to-one relationship. First, in statistical terms non-additivity is usually defined more broadly than suggested above (Crawford and Whitney 2010), namely as a deviation of mixture performance from the mean performance of the monoculture species or genotypes making up the mixture. This includes in addition to complementarity effects also selection effects. If the deviation between mixture and average monoculture performance is positive, we speak of positive net biodiversity effects (Loreau and Hector 2001). This net effect can then be related to the sum of selection and complementarity effects. Clearly our experiment showed a positive net biodiversity effect on variables related to invasion success. Second, Le Roux et al. (2014) seemed to imply that if our net biodiversity effects were mainly or even only due to selection effects, they would not be relevant for invasion success. However, this is clearly not true, because even if after the invasion process only one genotype would remain it would still mean that starting with a larger diversity of genotypes we could increase the probability of successful invasion. Le Roux et al. (2014) then seemed to convert the logical statement 'as long as the mixture is not better than the best genotype it is *possible* that the mixture has been reduced to that single genotype' to the statement 'whenever a single genotype does have the same performance as the mixture it *must* be so that this genotype has taken over the mixture.' Therefore, under this worst-case scenario they use the strict test of transgressive over-performance (Schmid et al. 2008) to allow that complementarity effects have occurred. In the genetic analogy this would be called over-dominance, i.e. offspring vigor surpassing the vigor of even the better of two parents.

Le Roux et al. (2014) make some further very strict recommendations that are in principle or in practice not possible to fulfill in biodiversity experiments. They implicitly suggest that the confounding of the increased representation of a single genotype with increasing diversity level should be broken, but this is not possible in principle as long as all genotypes are to be tested in monoculture as well as in mixture. In fact, each genotype is represented in the single most diverse mixture that can be created using all genotypes in an experiment. Le Roux et al. (2014) also implicitly suggest that all genotypes should have no significant differences in performance in monoculture, which in practice will almost never the case. Finally, in certain places such as the final sentence of the paper, they seem to relate non-additive genotypic diversity effects, which are measured at population level, to effects of non-additive genetic variation (see Bernasconi et al. 2003 for such an analogy), which affect phenotypic variation at the individual level and where again these non-additive effects are defined as deviation of offspring values from parental means (dominance), not as over-dominance. Using the usual definitions of terms, we show in the following that, although we cannot be absolutely sure if the non-additive genotypic effects that we observed in Wang et al. (2012) were only due to selection effects, this was highly unlikely and in fact complementarity effects were probably more important and increasing with time relative to selection effects.

Le Roux et al. (2014) stated that non-significant transgressive over-performance and poorer performance in some single-genotype patches than the mixtures suggested selection effects rather than complementarity effects explaining the enhanced invasibility of genotypic mixtures. They raised an important question about how to detect the roles of selection and complementarity effects in experiments examining biodiversity–ecosystem functioning relationship. They also argued that sexual recruitment may have changed the genotypic compositions, and that studying invasion should not be restricted in plots of $0.5 \times 0.5\ \text{m}^2$. Here, we consider these issues and, specifically, test their method in detecting transgressive effects using an artificial data set of genotype richness–shoot number as well as a species richness–biomass experiment where selection and complementarity effects had been well studied (Cardinale et al. 2007).

Le Roux et al. (2014) used a non-parametric method to test the difference between monocultures of the best performing genotype and six-genotype patches, and found no significant difference, resulting in their conclusions of only selection and no complementarity effects. However, Le Roux and co-authors misunderstood the detection of complementarity effects. Their method was to test whether mixtures performed significantly better than the best monoculture because this would prove that the net biodiversity effects must be due to more than only selection effects. However, as analyzed in detail by Schmid et al.

(2008), such transgressive over-performance is extremely rare even when complementarity effects abound in mixtures, let alone significant transgressive over-performance, due to simple statistical causes as well as sampling bias. In our case, significant non-transgressive over-performance and transgressive over-performance, though not significant, were observed, indicating the very likely role of positive complementarity effects.

Although Le Roux et al. (2014) realized that shoot number of a specific genotype was different in single-genotype and mixture patches, they did not consider this difference in their analyses. To test the usefulness of their method, we compared the outputs of their method with those of Loreau and Hector's (2001) and our methods using an artificial dataset and a classical biodiversity–productivity experiment.

The artificial data are shown in Table 1. There are two levels of genotype richness (one- and four-genotype), and each treatment has three replicates. At the beginning, each patch contained 8 shoots, and at the end, the composition and shoot number of each single genotype and mixtures were as shown in Table 1. At the end, shoot numbers of the mixtures were lower, but not significantly so, than those of the best genotype (genotype A), showing no transgressive over-performance (Fig. 1a). According to Le Roux et al.'s method, complementarity could not have played an important role, and selection effect should have dominated the output. However, the situation is totally different if we consider the performance of each genotype in mono- and polycultures. Increases in shoot numbers of *every* genotype (number of shoots at the end/number of shoots at the beginning) were significantly higher in mixtures than in monocultures (Fig. 1b). In other words, *every* genotype performed significantly better in mixtures than in monocultures, which can only be explained by complementarity effects. Using a Monte Carlo permutations test (Crawford and Whitney 2010), the actual mean fell outside the 95 % confidence interval, indicating non-additive effects. Calculated with Loreau and Hector's method, the complementarity effect was 202, while the selection effect was negative (−32). Both our and Loreau and Hector's (2001) methods indicated that complementarity effects rather than selection effects played a more critical role in explaining the non-additive outputs in the artificial dataset.

We also applied Le Roux et al.'s (2014) method to a long-term grassland experiment in Cedar Creek LTER

Table 1 An artificial data set on the relationship between genotypic diversity and shoot number

		Monoculture												Mixture		
		A			B			C			D					
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Beginning	Comp.	A8	A8	A8	B8	B8	B8	C8	C8	C8	D8	D8	D8	A2, B2, C2, D2	A2, B2, C2, D2	A2, B2, C2, D2
	Total	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
End	Comp.	A320	A360	A400	B38	B43	B48	C28	C32	C36	D80	D88	D96	A160, B36, C20, D40	A200, B28, C24, D45	A240, B32, C28, D50
	Total	320	360	400	38	43	48	28	32	36	80	88	96	256	297	350

In this data set, four genotypes (A, B, C, D) were assumed. Numerals after the codes of genotypes are shoot numbers of the corresponding genotypes

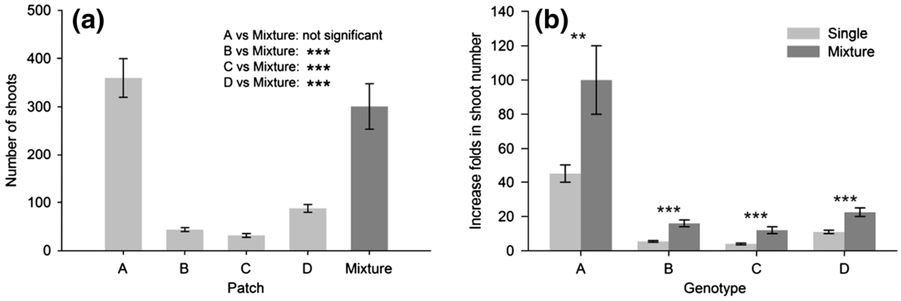

Fig. 1 Shoot number of single-genotype and mixture patches at the end (**a**) and folds of increase in shoot number of each genotype in single-genotype and mixture patches (**b**) of the artificial data set (see Table 1). **Significant level at $p < 0.01$, and ***significant level of $p < 0.001$, tested by ANOVA

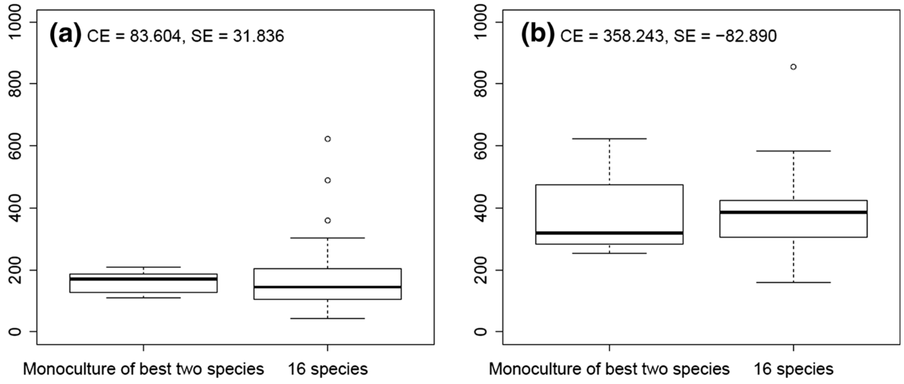

Fig. 2 Boxplots of aboveground plant biomass (m^{-2}) for monocultures of the best two species and polycultures of 16 species of Cedar Creek Biodiversity II (E120) in **a** 1996 and **b** 2003. Data were downloaded from Cedar Creek LTER website (www.cedarcreek.umn.edu). Complementarity effects (CE) and selection effects (SE) were extracted from Cardinale et al. (2007)

(Tilman et al. 2001). We used data of 1996 and 2003. Both complementarity and selection effects played positive roles in aboveground plant biomass in 1996, while complementarity effects were positive and selection effects were negative in 2003 (Cardinale et al. 2007) (Fig. 2). Because replicates of monocultures of each species were less than 3, we combined data of the best two species and compared with those of 16-species mixtures. For the two years, no significant differences in yield between the best two species and mixtures of 16 species (Fig. 2) suggest no complementarity effect according to Le Roux et al.'s (2014) approach, contrasting to conclusions drawn from Loreau and Hector's (2001) method.

In the above two cases, Le Roux et al.'s method cannot obtain correct conclusions. Although significant transgressive over-performance can not only be caused by selection effects, complementarity effects among genotypes not necessarily result in significant transgressive over-performance. Therefore, Le Roux et al. (2014) method is not suitable for separating selection from complementarity effects.

We agree that sexual reproduction is important in invasive species, for colonizing unoccupied areas, but may be less important for recruitment in dense patches of species with strong ability of clonal growth. *Spartina alterniflora* has a very strong capacity of clonal growth, and can form dense patches consisting of a single genotype. Nevertheless, potential impact of seedling recruitment was considered in our experiment, which lasted less than two growth seasons. However, in the first year, due to a late start of the experiment (June 2006), no seeds could reach maturity even though a few individuals flowered. In the second growing season, the plants flowered and set seeds in autumn. We ended the experiment in winter, and thus the impact of seedling recruitment can be neglected. Furthermore, our experimental site was relatively isolated from other *S. alterniflora* patches. If there was seedling recruitment, seedlings had a high probability to survive in the gaps between *S. alterniflora* patches. However, no scattered individuals were observed around the experimental patches in the first and second years. Thus, the impact of seedling recruitment at the study site could not have been large.

Le Roux et al. (2014) also claimed that our plots were too small (0.5 × 0.5 m) and were not suitable for conducting an invasion experiment. However, the author misunderstood the patch size. In fact, 0.25 m^2 was the size when 18 ramets were planted to make sure each patch having the same size at the beginning. We did not set up any barrier to restrict the expansion of individuals, and the size of each patch was monitored as a variable of invasion ability. As found in our paper as well as in the commentary paper, both patch size and spread distance were used to evaluate the invasion. Nevertheless, Le Roux and co-authors had pointed out an important difference in manipulation experiments between invasion ecology and biodiversity–ecosystem functioning.

Roles of genetic factors in the invasive success of alien species have been widely recognized. However, the potential mechanisms may be different. Some species have very low genetic diversity, even only one genotype in the extreme case, in populations in the invasive range, while in some other aliens high genetic diversity was observed (Dlugosch and Parker 2008; Roman and Darling 2007). Therefore, understanding underlying mechanisms is critical for management and controls of specific invasive species. Like in species diversity–ecosystem functioning relationship, we found that selection effects played an important role in the early stages of invasion, and complementarity effects played a more important role as time passed. However, more studies in other invasive species are needed to understand the generality of such a pattern. We agree with Le Roux et al.'s opinion that well-designed manipulation experiments are needed to estimate the roles of selection and complementarity effects. Because invasive plants can have a great increase in shoot number in a short time, and genotyping each shoot takes a lot of time and money, we suggest to genotype a subset of shoots randomly sampled from the patches. Then we can have an estimation of the performance of each genotype. In addition, we may detect new genotypes other than the planted ones to understand the impact of seedling recruitment and resistance of each patch to invasion of alien genotypes. Furthermore, like various designs have been used to test the relationship between species diversity and ecosystem functioning, different specific designs are needed to test specific mechanisms in the genetic diversity–invasion relation. We believe, as manipulation experiments are accumulating, the roles of selection and complementarity effects in species invasions will become clearer in the future.

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