

Genotypic richness and phenotypic dissimilarity enhance population performance

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Abstract. Increases in biodiversity can result from an increase in species richness, as well as from a higher genetic diversity within species. Intraspecific genetic diversity, measured as the number of genotypes, can enhance plant primary productivity and have cascading effects at higher trophic levels, such as an increase in herbivore and predator richness. The positive effects of genotypic mixtures are not only determined by additive effects, but also by interactions among genotypes, such as facilitation or inhibition. However, so far there has been no effort to predict the extent of such effects. In this study, we address the question of whether the magnitude of the effect of genotype number on population performance can be explained by the extent of dissimilarity in key traits among genotypes in a mixture. We examine the relative contribution of genotype number and phenotypic dissimilarity among genotypes to population performance of the soil arthropod, *Orchesella cincta*. Nearly homogeneous genotypes were created from inbred isofemale lines. Phenotypic dissimilarity among genotypes was assessed in terms of three life-history traits that are associated with population growth rate, i.e., egg size, egg development time, and juvenile growth rate. A microcosm experiment with genotype mixtures consisting of one, two, four, and eight genotypes, showed that genotypic richness strongly increased population size and biomass production and was associated with greater net diversity effects. Most importantly, there was a positive log-linear relationship between phenotypic dissimilarity in a mixture and the net diversity effects for juvenile population size and total biomass. In other words, the degree of phenotypic dissimilarity among genotypes determined the magnitude of the genotypic richness effect, although this relationship leveled off at higher values of phenotypic dissimilarity. Although the exact mechanisms responsible for these effects are currently unknown, similar advantages of trait dissimilarity have been found among species. Hence, to better understand population performance, genotype number and phenotypic dissimilarity should be considered collectively.

Key words: biomass production; egg development time; egg size; genetic diversity; juvenile growth rate; net diversity effect; *Orchesella cincta*; phenotypic trait; population size.

INTRODUCTION

The relationship between biodiversity and community and ecosystem functioning is a central issue in ecology, because biodiversity can affect primary productivity, carbon storage, nutrient acquisition, decomposition rate, and ecosystem stability (Hector et al. 1999, Mulder et al. 2001, Hättenschwiler et al. 2005). Increases in biodiversity can result from an increase in species richness and functional diversity, as well as from a higher genetic diversity within species. The role of intraspecific genetic diversity in biodiversity has received growing attention over the past years because of the emerging synthesis of evolutionary biology and community ecology (Johnson and Stinchcombe 2007). In parallel to the community effects of species richness, a large body of experimental work has now shown several community effects of increasing genetic diversity within

species. First, a positive relationship was found between the number of genotypes and net plant primary productivity (Reusch et al. 2005, Crutsinger et al. 2006, Kotowska et al. 2010; but see Vellend et al. 2010), which is key to supporting a larger number and diversity of herbivores and predators. Second, the number of genotypes also has indirect community effects, such as an increase in coexistence of competing plant species (Booth and Grime 2003), arthropod richness (Crutsinger et al. 2006, Johnson et al. 2006), colonization success (Gamfeldt et al. 2005, Crawford and Whitney 2010), and resistance to invasion (De Meester et al. 2007). Hence, in addition to the well-known evolutionary impacts, these studies have documented the ecological impacts of intraspecific genetic diversity (reviewed in Hughes et al. 2008).

More detailed studies have proposed that the positive effect of genotypic mixtures is not only determined by properties and frequencies of constituent genotypes (additive effects) but also by interactions among genotypes (nonadditive effects; Reusch et al. 2005,

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Crutsinger et al. 2006, Crawford and Whitney 2010). Positive interactions, such as resource partitioning or facilitation, can increase population performance of genotype mixtures above that predicted from the performance of the genotype in monocultures, whereas negative interactions, such as inhibition or interference, may cause a relative decrease in mixture performance (Loreau and Hector 2001, Hughes et al. 2008). Although the existence of nonadditive effects in genotype mixtures is now generally accepted, so far there has been no effort to predict the extent of such effects. This type of interaction, positive or negative, has been hypothesized to depend on the functional dissimilarity of its components, but until now, this hypothesis has only been tested for interspecific diversity. Using microcosm experiments, at least two studies have demonstrated that the effect of species composition on key ecosystem processes is determined by dissimilarity in species traits rather than species number (Heemsbergen et al. 2004, Wojdak and Mittelbach 2007). Facilitative interactions occurred in species mixtures with high trait dissimilarity or low niche overlap, respectively. If we can extrapolate this relationship to other levels of biological organization, the extent of dissimilarity in key life-history traits among genotypes in a mixture should predict the magnitude of the effect of genotype number on population performance.

Although the variation in phenotypic traits among genotypes is likely to be smaller than that among species, it is large enough to impact population performance and community functioning. Empirical evidence shows that differences among genotypes significantly affect population performance, such as disease infection (Roscher et al. 2007), and arthropod abundance and diversity (Johnson and Agrawal 2005, Crawford et al. 2007). Genotypic identity often affects population productivity (Vellend et al. 2010), and has cascading effects on other trophic levels (Kotowska et al. 2010). For example, experiments with genetically different isolates of the arbuscular mycorrhizal fungus *Glomus intraradices*, show that AMF genotype can affect plant growth response (Munkvold et al. 2004, Koch et al. 2006), which in turn may shape diversity at higher trophic levels.

However, no study has measured the extent of dissimilarity in phenotypic traits among genotypes in mixtures; instead genetic diversity is taken to be the number of genotypes present. Although chances are negligible that two genotypes are exactly identical, that still leaves a wide range of dissimilarities possible. Moreover, it is well-known that genetic variation only matters if it translates into phenotypic variation upon which natural selection can act (Merila and Crnokrak 2001, Reed and Frankham 2001). Therefore, it is necessary to quantify the degree of phenotypic dissimilarity among genotypes to predict population performance or other population properties. We hypothesize that the extent of dissimilarity in phenotypic traits can

be used to predict the extent of nonadditive effects among genotypes.

In this study, we examine the relative contribution of genotype number and phenotypic dissimilarity among genotypes to population biomass production and population growth rate in the springtail species *Orchesella cincta* (Collembola). Springtails are a globally significant group of organisms that play a major role in soil functioning (Rusek 1998), particularly through their effect on the rate of litter decomposition and nutrient fluxes (Cragg and Bardgett 2001). Presence of collembolan species has cascading effects on other trophic levels, as they enhance or reduce, for instance, plant growth by changing the functioning of mycorrhizae (Gange 2000). Moreover, collembolan community composition modifies impacts of leaf herbivory, by reducing feeding intensity or enhancing plant growth (Barker 2006). *Orchesella cincta* (see Plate 1) is found in the litter layer of forests and under bark and moss on tree trunks, where it is feeding on fungi and algae (Hopkin 1997, Berg 2007). It can reach extremely high population numbers, with annual average density up to 1500 individuals/m² (Van Straalen 1989). It is an important food source for soil predators, such as spiders, pseudoscorpions, and ground beetles; therefore *O. cincta* population size directly affects the abundance at higher trophic levels. The high predation pressure on *O. cincta* makes population growth rate a crucial parameter for population survival (Van Straalen 1985).

In our experiment, we use nearly homogeneous genotypes created from inbred isofemale lines in a microcosm experiment with genotype mixtures consisting of one, two, four, and eight genotypes. Genotypes were characterized using amplified fragment length polymorphism (AFLP) analysis to determine genetic diversity among genotypes. Phenotypic dissimilarity among genotypes was assessed in terms of three life-history traits that are associated with population growth rate: egg size, egg developmental rate, and juvenile growth rate. We show that population biomass production and growth rate is increased by the number of genotypes present, but more importantly, that the extent of phenotypic dissimilarity among genotypes determines the magnitude of the genotypic richness effect.

MATERIALS AND METHODS

Study species and experimental populations

Orchesella cincta (Linnaeus) is a bark- and soil-dwelling arthropod species widely distributed across Europe. It is a sexually reproducing species with moderately high levels of genetic diversity found within populations (Timmermans et al. 2005, Janssens et al. 2008). To study of the effect of genotypic richness, numerous identical individuals of each genotype are required. Therefore, we created inbred isofemale lines to obtain nearly identical genotypes within each line for the experiment. Each line was initiated by one parental pair from a laboratory culture that had been outbred in the

laboratory for five generations (see Driessen et al. 2007 for details). In subsequent generations, population size of the inbred lines was increased to five individuals to reduce strong deleterious inbreeding effects. Lines were kept at 20°C and a photoperiod of 12 hours light:12 hours dark. Food consisted of pieces of bark overgrown with green algae (*Desmococcus* sp.) and was always kept in excess. At the start of the experiment the lines had gone through 11 generations of inbreeding.

Genetic relatedness between lines

AFLP analysis was performed on each isofemale line to establish genetic relatedness between lines. Total DNA was extracted using Wizard SV Genomic DNA purification system (Promega, Madison, Wisconsin, USA). DNA was dissolved in 100 µL nuclease-free water. Five to 10 individuals from each isofemale line were used for the AFLP analysis (70 individuals in total). AFLP procedure followed Vos et al. (1995) with minor modifications. For digestion, 20 µL DNA was used. Instead of radioactive labeling, amplification was conducted using fluorescent-labeled primers. Genomic DNA was digested with EcoRI and MseI, and double-stranded EcoRI and MseI adapters were ligated to the sticky ends of the fragments. Preselective primers, with one selective base (EcoRI + A and MseI + C), and selective primers with additional selective bases (EcoRI + AC and MseI + CAC) were used for amplification. Preliminary experiments showed that these primer pairs resulted in banding patterns that were variable and easy to score. Amplification products were separated on 6.5% polyacrylamide gels. Up to 60 samples were loaded on one gel. Gels were run for 2 h on a LI-COR automated sequencer (LI-COR, Lincoln, Nebraska, USA). Fragments in a range of 70–350 bp size were scored by hand with image master 1-D elite 4.00 software (Amersham Pharmacia, Piscataway, New Jersey, USA). Bands showing identical size on gel were assumed to be homologous. A peak was scored as present (1) or absent (0). Using the program TFPGA (M. P. Miller, unpublished program), average heterozygosity and percentage polymorphism were calculated as an estimate for the genetic homogeneity of each isofemale line. Average heterozygosity was 0.064 ± 0.016 (mean \pm SE) and average polymorphism within each isofemale line was $16.15\% \pm 4.15\%$. This confirmed that the isofemale lines were strongly inbred.

Experimental design

Eight isofemale lines (hereafter genotypes) were selected for the experiment. First, we characterized each genotype for three life-history traits: egg size, egg development time, and juvenile growth rate (Ellers and Driessen 2011). Per genotype, five pairs were created by randomly selecting males and females from the stock population. Each pair was kept at 22°C in a plastic container of 2 cm diameter, a moistened bottom of plaster of Paris and food, and inspected daily for eggs.

When the first eggs appeared, five of them were randomly chosen, transferred to a separate container and checked twice a day for hatching. Egg development rate was calculated as the inverse of the time between laying and hatching. Emerging juveniles were reared for 20–25 days, after which their wet mass was determined to the nearest 1 µg on a microbalance (Mettler Toledo UMT2, Columbus, Ohio, USA). During this period they grow exponentially (Janssen and Joosse 1987). Because the initial juvenile mass after hatching (~5 µg) is negligible compared to the mass at the moment of measurement (average >350 µg), daily exponential growth rate can be calculated as the natural logarithm of the wet mass divided by the time between hatching and weighing (in hours). The second batch of eggs of each pair was used to measure egg size. Eggs of *O. cincta* are perfectly spherical; therefore we used diameter as an estimate of egg size. Ten randomly chosen eggs of each batch were photographed using a digital camera (Leica DC200, Allendale, New Jersey, USA) connected to a stereomicroscope. The diameter of the eggs was measured using public software ImageJ 1.30v (*available online*).²

Second, we calculated the phenotypic dissimilarity among genotypes based on the three life-history traits studied. The mean trait values were normalized between 0 and 1 for each of the three life-history traits. Phenotypic dissimilarity among pairs of genotypes was expressed as Euclidian distance, the most common metric measure (Legendre and Legendre 1998). Phenotypic dissimilarity values ranges from 0 (no dissimilarity between a given pair of genotypes) to 1.732 (maximum dissimilarity between a given pair of genotypes). When more than two genotypes were combined in a microcosm the average phenotypic distance between all possible pair-wise genotype combinations was calculated (Heemsbergen et al. 2004). We also calculated an alternative distance measure using the FunctDiv Excel-macro developed by Lepš et al. (2006) to determine the sensitivity of phenotypic dissimilarity for the distance measure selected. Lepš' method expresses phenotypic dissimilarity as the difference in mean trait value between two genotypes, averaged over all three traits (using the binary data option). Since both distance measures were strongly correlated (Pearson correlation $r = 0.992$) we only report the analysis with the Euclidian distance measure. We also calculated phenotypic dissimilarity for each of the three traits separately, and assessed the correlation of each of the single trait dissimilarity with the overall phenotypic dissimilarity. For all three single trait dissimilarities the correlation with overall similarity was very high (egg development $r = 0.930$, juvenile growth rate $r = 0.900$, egg size $r = 0.912$), therefore all three traits contributed more or less equally to the overall phenotypic dissimilarity value.

² (<http://rsb.info.nih.gov/ij/index.html>)

Third, we created microcosms with different levels of genotypic richness. Each microcosm was founded with one, two, four, or eight genotypes (Appendix A). Genotype combinations were chosen to maximize the total range of phenotypic dissimilarities, while taking into account an equal distribution of genotypes over the combinations, when possible. For each genotype, monoculture microcosms were initiated with eight males and eight females randomly sampled from our stock populations. Mixed microcosms were created in the same way but with each genotype equally represented in the founding population, keeping the total abundance in each microcosm constant. For example, in a four-genotype microcosm, the initial population consisted of two males and two females of each contributing genotype. In total there were eight monocultures, five two-genotype, four four-genotype, and one eight-genotype microcosms, all with five replicates each, adding up to a total of 90 experimental communities.

Microcosms consisted of a 20 cm diameter plastic container with a moistened bottom of plaster of Paris, and twigs and bark covered with algae for food (ad libitum). They were kept in a climate room at 22°C with a photoperiod of 12 hours light:12 hours dark and moved around three times a week to avoid any bias due to position within the climate room.

After nine weeks, which allows for two generations, the total number of individuals in each microcosm was counted. The content of the microcosms was sieved and all remaining food items were carefully removed. The live animals were spread on a white background and photographed using a digital camera (Leica DC200). When the total number of individuals was too high, they were divided over multiple photos to avoid animals clumping together in the photo. The number of individuals on each photo was counted with the software Scion Image (Scion Corporation, Frederick, Maryland, USA) using the particle analyzer procedure with threshold set at 1–17 pixels for juvenile *O. cincta* and the threshold set at 18–999 pixels for adults. A subset of photos was counted by hand as well as with Scion software, to determine the repeatability and range of error of digital analysis. Digital counts differed on average by $12.9\% \pm 6.0\%$ (mean \pm SD) from hand counts, with a consistent upward bias for the digital counts. Repeatability of digital counts was 91.4% and of hand counts 92.8%. To determine the total biomass in each microcosm, all individuals from one microcosm were placed in a plastic tube, freeze dried for 24 hours at -40°C in a desiccator, and weighed to the nearest 1 μg on a microbalance (Mettler Toledo UMT2).

Statistical analysis

To show significant phenotypic differences among genotypes in the three life-history traits, a general linear models ANOVA was carried out with genotype as fixed factor and pair as random factor nested in genotype, followed by Tukey hsd post hoc tests. To check if the

assignment of genotypes to microcosms did not lead to inherent differences in average trait values of two-genotype and four-genotype microcosms, a *t* test was used. We also tested whether differences between genotypes in their phenotypic traits were related to their genetic distance. We calculated Nei's genetic distances from the AFLP data using the program TFPGA (M. P. Miller, unpublished program) to construct a genetic distance matrix of the eight genotypes. Next, we used a Mantel test with a 1000 permutations to assess the correlation between genetic distance and phenotypic dissimilarity and its statistical significance.

The gross effect of genotype number on population size and biomass was determined by log-linear regression ($Y = \beta_0 + \beta_1 \ln[x]$). Net diversity effects were obtained by subtraction of the population size of each replicate of the mixed microcosms from the predicted population size, calculated as the average of the component monocultures (c.f. Loreau and Hector 2001). The same procedure was repeated for biomass. The net diversity effect for each variable was tested against zero using a two-sided *t* test. To detect a further increase in net diversity effect between mixtures of four and eight genotypes, a two-sample *t* test was carried out. Multiple regression was used to test for the effect of number of genotypes and phenotypic dissimilarity of the mixtures on the net diversity effect. Phenotypic dissimilarity was ln-transformed because a log-linear regression gave a better fit compared to the linear model for all three population measures (biomass, number of juveniles, and number of adults). Therefore, we used $\ln(\text{phenotypic dissimilarity})$ and genotype number as variables in the multiple regression for each of the three population measures. In addition, we tested if the positive relationship between phenotypic dissimilarity and net diversity effect also holds for the higher levels of phenotypic dissimilarity. The dataset was split evenly into a lower and upper half of phenotypic dissimilarity values, and regression analysis was performed on each half.

All the analyses for net diversity effects were carried out using the average of the five replicates for each genotype mixture as data points, to avoid pseudoreplication.

RESULTS

The eight genotypes showed significant variation in egg size ($F_{7,22} = 2.93$, $P = 0.025$) and juvenile growth rate ($F_{7,37} = 2.62$, $P = 0.027$; Fig. 1). There was no significant main effect of genotype on egg development rate ($F_{7,35} = 1.41$, $P = 0.234$), although post hoc tests showed that genotype 5 and 6 differed significantly from several other genotypes (Tukey hsd, 7 out of the 28 possible pair-wise comparisons between genotypes were significant). There was no significant correlation between the average heterozygosity of a genotype and its trait values (egg development rate $r = -0.325$, $P = 0.432$; juvenile growth rate $r = 0.073$, $P = 0.864$; egg size $r = 0.157$ $P =$

0.711). The rank order of genotypes differed per life-history trait; in other words, genotypes that did best in one trait did not routinely rank high in another trait. For example, genotype 8 had the smallest eggs but it was intermediate in juvenile growth rate and egg development rate. The genotype mixtures used for the mixed microcosms showed no differences in the average values of the three life-history traits between two- and four genotype treatments (Appendix A; egg size $t = 0.28$, $df = 7$, $P = 0.79$; egg development $t = -0.52$, $df = 7$, $P = 0.62$; juvenile growth rate $t = -0.15$, $df = 7$, $P = 0.88$).

Based on the AFLP data, the average Nei's genetic distance between genotypes was 0.312. Genotype 1 and 2 were genetically most similar with a genetic distance of 0.158, whereas genotype 1 and 5 showed the largest genetic distance (0.570; Appendix B). There was no significant correlation between the genetic distance of genotypes and the dissimilarity of their phenotypes ($Z = 25.54$, $r = 0.166$, $P = 0.19$).

Effect of genotypic richness

Genotypic richness had a strong effect on average population size and total biomass of treatments. There was a positive log-linear relationship between the number of genotypes per microcosm and the number of juveniles, the number of adults, and total dry mass (respectively $r = 0.550$, $P < 0.001$; $r = 0.414$, $P < 0.001$; and $r = 0.546$, $P < 0.001$; Fig. 2).

The net genotypic richness effect was positive across all mixtures (grand mean) for the number of juveniles ($t = 5.9$, $df = 9$, $P < 0.001$), the number of adults ($t = 5.4$, $df = 9$, $P < 0.001$) and total biomass ($t = 6.0$, $df = 9$, $P < 0.001$). On average, four-genotype treatments had 256% more juveniles, 228% more adults, and 248% more dry mass than that predicted from monocultures. An additional increase in net genotypic richness effect was found between mixtures of four and eight genotypes for dry mass ($t = 2.61$, $P = 0.016$), but not for juveniles ($t = 1.20$, $P = 0.242$) and adult population size ($t = 0.30$, $P = 0.765$). A linear regression revealed a significant increase in effect size with net genotypic richness for the total dry mass, but not for the number of juveniles and number of adults (Table 1, Fig. 3a, c, e).

Effect of the magnitude of phenotypic dissimilarity

The range in phenotypic dissimilarity was quite large, ranging from 0.22 to 1.06. Mean phenotypic dissimilarity among genotypes in the mixture treatments contributed significantly to the net diversity effect, independent of the number of genotypes (Table 1, Fig. 3b, d, f). We observed a significant log-linear relationship between phenotypic dissimilarity and net diversity effects for juvenile population size and total biomass (Table 1). The net diversity effect for number of adults was not significantly higher for mixtures with more dissimilar phenotypes. The magnitude of the phenotypic effect was similar or even larger than the genotypic richness effect; hence it explained a considerable part of the variation

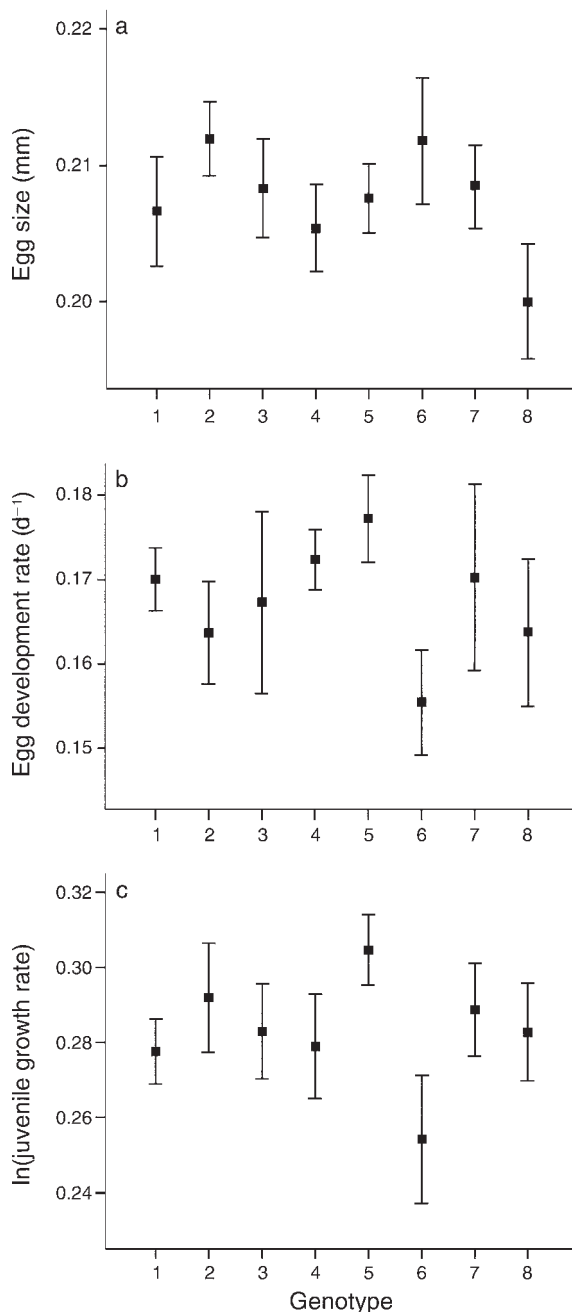


FIG. 1. The mean (\pm SE) values for (a) egg size, (b) egg development rate, and (c) juvenile growth rate (measured as $\mu\text{g}/\text{d}$) for eight genotypes of *Orchesella cincta* in monoculture.

observed (Table 1). The log-linear shape of the relationship shows that the effect of phenotypic dissimilarity levels off. For the number of juveniles, there is no significant increase in net diversity effect over the upper half of phenotypic dissimilarity values ($t = -0.152$, $df = 5$, $P = 0.893$). For total biomass the net diversity effect still shows an increase for the upper half of the dissimilarity range ($t = 6.78$, $df = 5$, $P = 0.021$).

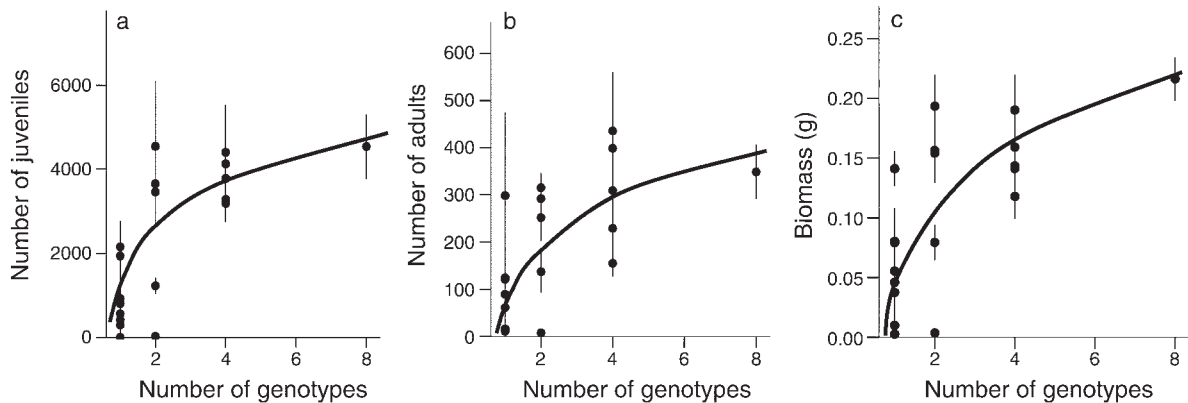


FIG. 2. The relationship between genotypic richness and population performance of *Orchesella cincta* populations: (a) the number of juveniles, (b) the number of adults, and (c) biomass production in dry mass as a function of the number of genotypes after nine weeks in microcosms. Each data point is a different genotype mixture. Data show mean (\pm SE) of five replicates.

However, the variation in the net diversity effects is large, and the number of combinations in the low range of phenotypic dissimilarity values was low. The small diversity effect observed in the mixture with the lowest phenotypic dissimilarity is an important driver of the relationship.

DISCUSSION

Our results demonstrate that intraspecific diversity in *Orchesella cincta* is a key factor determining several measures of population performance. Two crucial results support this conclusion. First, genotype number strongly increased population size and biomass production and was associated with greater net diversity effects. Second, the extent of phenotypic dissimilarity between genotypes enhanced the diversity effect, independent of the number of genotypes present in the population. The notion that phenotypic dissimilarity between genotypes is an essential component of biodiversity emphasizes that the number of genotypes and their phenotypic dissimilarity collectively affect productivity in *O. cincta*.

Positive effects of genotypic richness

In *O. cincta*, all measures of population productivity increased with genotypic richness within microcosms. The benefits of additional genotypes were diminished in mixtures with more than four genotypes, but the population productivity was still significantly higher in

eight-genotype mixtures than in four-genotype mixtures. Similar effects of genotypic richness on population productivity have been documented for various plant populations (Reusch et al. 2005, Crutsinger et al. 2006), but in other studies these effects are absent (see Fowler and Partridge 1986, Bell 1991). Genotypic mixtures have been suggested to outyield single genotype populations because of complementary interactions between genotypes (Martin et al. 1988, Lopez-Suarez et al. 1993, Santos 1997). As a preface to discussing positive interactions between genotypes, we start by considering alternative explanations for the increase in productivity in genotype mixtures in our study.

Part of the difference between the monocultures and genotype mixtures may be due to the inbred nature of our isofemale lines, and the potential for heterosis in genotype mixtures. Mating between multiple genotypes may alleviate inbreeding depression through the production of more-fit heterozygous offspring. This may account for the larger effect of genotype number in our experiments compared to previous studies. For example, nearly all combinations of genotypes gave positive net diversity effects. Also, benefits of additional genotypes accrued up to eight genotypes, whereas previous studies found saturation at lower numbers of genotypes (Reusch et al. 2005, Crutsinger et al. 2006). Using AFLP markers, the average percentage of polymorphic loci within the lines was estimated to be between 1.54%

TABLE 1. Multiple linear regression of genotype number and phenotypic dissimilarity on net diversity effect measured for number of juveniles, number of adults, and biomass.

Parameter	r	Genotype number			$\ln(\text{phenotypic dissimilarity})$		
		B (SE)	Beta	P	B (SE)	Beta	P
Number of juveniles	0.766	277.4 (157.7)	0.431	0.122	1450.2 (611.0)	0.581	0.049
Number of adults	0.568	25.39 (16.95)	0.470	0.178	55.50 (65.67)	0.265	0.426
Biomass	0.868	0.014 (0.004)	0.587	0.017	0.052 (0.017)	0.569	0.020

Notes: The analyses takes genotype mixture as the level of replication ($n = 10$ replicates). The Beta coefficients, obtained on standardized variables (mean = 0 and SD = 1), show the relative contribution of each independent variable in the prediction of the dependent variable. B is the slope of the regression.

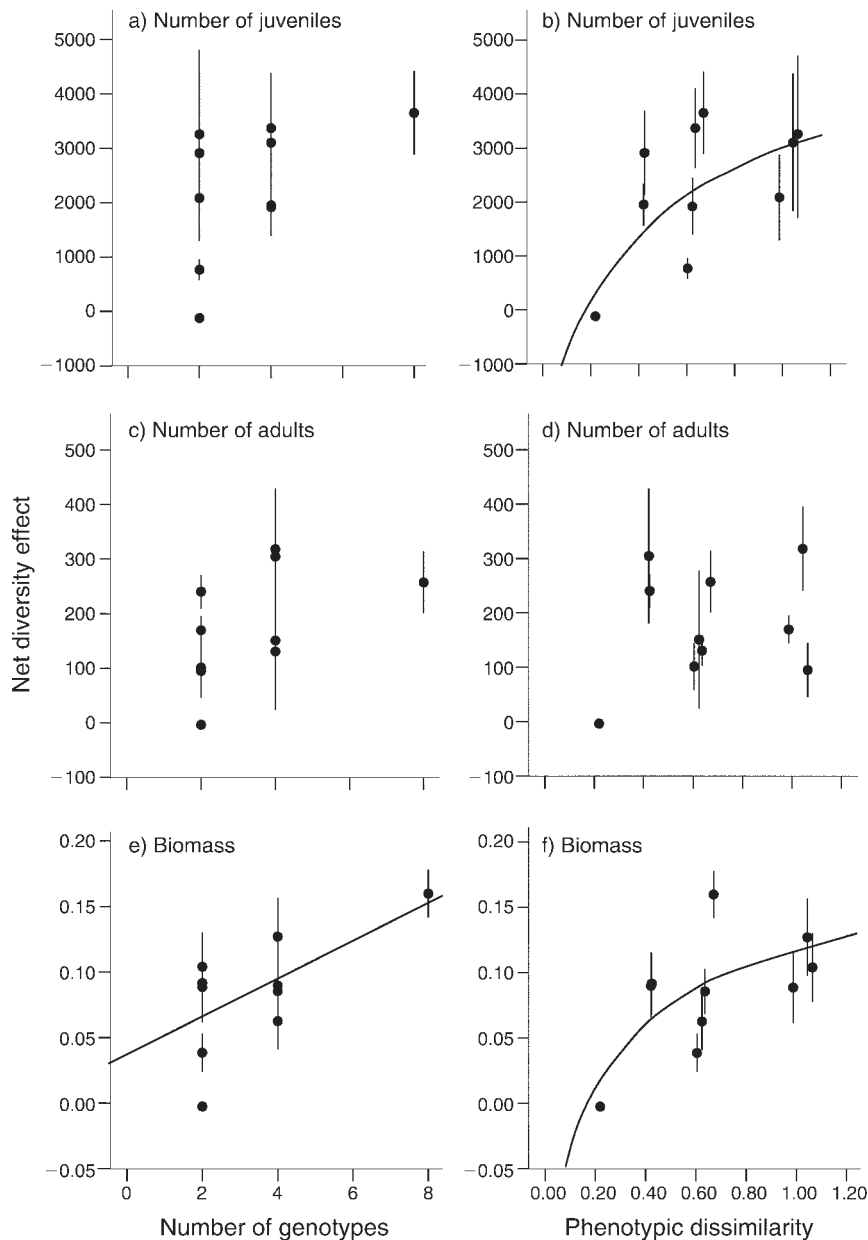


FIG. 3. The effect of increasing number of genotypes and phenotypic dissimilarity on the net diversity effect in *Orchesella cincta* populations. Net diversity effect is shown for (a, b) number of juveniles, (c, d) the number of adults, and (e, f) biomass production in dry mass after nine weeks in microcosms. The calculation of net diversity effect is explained in *Materials and Methods: Statistical analyses*.

and 38.5%, whereas outbred populations of *O. cincta* typically have estimates of around 80% (Timmermans et al. 2005).

However, two lines of evidence suggest that the fitness effects of inbreeding in our isofemale lines, although not absent, were not very severe. First, there was no correlation between the level of inbreeding and the trait values for any of the three life-history traits considered in this study, while inbreeding is known to cause

deleterious effects on several life-history parameters (DeRose and Roff 1999). Second, a previous study with these lines also showed no change in a stress resistance nor heat shock protein (Hsp70) induction (Bahrndorff et al. 2010), traits usually associated with inbreeding depression (Kristensen et al. 2002). The relative lack of inbreeding effects may be due to the slow rate of inbreeding, as minimal population size was never smaller than five individuals. A slow rate of inbreeding



PLATE 1. An adult individual of the springtail *Orchesella cincta* foraging on algae-covered pieces of bark. Photo credit: Theodoor Heijermans.

allows deleterious alleles to be purged, hence reducing the fitness effects of inbreeding (Pedersen et al. 2005). Nevertheless, we cannot exclude that a significant part of the increase in performance in genotype mixtures compared to monocultures could be due to outbreeding and the associated increase in heterozygosity in the offspring. Yet, even if heterosis enlarged the effect of genotypic richness on population performance, it does not change our main result, which is the importance of phenotypic dissimilarity among genotypes.

An additional concern when testing genotypic richness effects is the possibility of sampling artifacts, because mixtures with more genotypes have a higher chance of containing one or more highly productive genotypes. This is known as the selection effect (Huston 1997), which can potentially produce erroneous correlations between genotypic richness and productivity. The contribution of the selection effect and a complementarity effect can be distinguished based on the relative productivity of each genotype in mixtures compared to productivity of monocultures of the same genotype (Loreau and Hector 2001). Unfortunately, in this study the selection–complementarity partitioning was not possible due to a lack of information on how each genotype actually performed in mixtures. Despite this limitation, we found that the mixture performance exceeded that of the most productive monoculture, which suggests complementarity or facilitation among genotypes, rather than selection in *O. cincta*.

One of the key differences between this study and previous plant-based studies is that this species was followed through multiple sexual generations. On one hand, this is an improvement in terms of temporal scale and realism, but it also comes at the cost of making it more difficult to get at underlying mechanisms. For example, as noted above, the selection–complementarity analysis is impossible due to recombination of genotypes. Recombination can also change trait values and, consequently, the extent of phenotypic dissimilarity in genotype combinations. If complementarity among trait combinations is a major factor in determining population performance, diversifying and/or frequency-dependent selection on these traits would be expected. A comparison of the magnitude of genetic variation for the traits of interest before and at the end of the experiment could elucidate if differences in trait variation will be maintained throughout the experiment, possibly through complementarity, or if recombination and selection will cause a convergence in trait variation across combinations. Incorporating these kinds of measurements into future studies should substantially deepen the insights into the mechanisms underlying the diversity effect.

The relevance of the magnitude of phenotypic dissimilarity for the diversity effect

Our results show that the magnitude of net diversity effects significantly correlates with the extent of dissimilarity in phenotypic traits. In the range of two to eight

genotypes, higher phenotypic dissimilarity was associated with an increased net diversity effect in biomass and number of juveniles, independent of the mere number of genotypes present. The extent of phenotypic dissimilarity was not determined by genetic distance between genotypes; therefore it is not an indirect genetic effect.

While other population genetic studies have shown positive effects of genotype number (Perez Tome et al. 1982, Lopez-Suarez et al. 1993, Gamfeldt et al. 2005, Reusch et al. 2005), few studies have looked at the factors influencing the magnitude of this effect. Fitness enhancements in genetically diverse populations have been attributed to competitive release experienced by individuals due to micro-niche differentiation (Semlitsch et al. 1997, Tagg et al. 2005). Indeed, comparison among genotypes has shown divergence in functional traits (e.g., Madritch et al. 2006), but the degree of trait divergence has not been linked to population performance. The strength of a trait-based approach has been shown in interspecific studies, looking at the effects of dissimilarity among species traits (Heemsbergen et al. 2004, Wojdak and Mittelbach 2007). Communities consisting of a species composition with highly dissimilar traits showed a significant increase in community functioning measured as decomposition rates, or soil respiration. Given that the range of variation in traits among species is usually much larger than within species (Heemsbergen et al. 2004), it is surprising that the more subtle intraspecific trait dissimilarity can provide similar advantages. However, the exact shape of the relationship between intraspecific trait dissimilarity and the net diversity effect remains to be determined.

In this study, we only considered three life-history traits: egg size, growth rate, and egg development time. How could divergence in these variables cause positive diversity effects? We hypothesize that these traits are indicative of contrasting life-history strategies enabling nonadditive effects because of competitive release or resource specialization. Ernsting et al. (1993) found evidence for a negative relationship between growth rate and size at maturity in *O. cineta* in an experiment that manipulated food availability. The broad range of life-history strategies in a field-derived population of *O. cineta* indicates that these genotypes can coexist under natural conditions. Genetic variation for the ability to cope with differences in food nutrient content has been found to be correlated to growth rate and to facilitate coexistence of *Daphnia* genotypes (Weider et al. 2005). Also, larger eggs give rise to larger offspring which may have a competitive advantage in size-based competitive interactions (Fielding 2004). We only considered a limited number of life-history traits in this study and it is possible that including other traits may enhance the predictive power of the phenotypic dissimilarity approach.

In addition to an increase in the number of traits, it may also be important to consider the performance of genotypes and their interactions under different envi-

ronmental conditions. In our experiment, abiotic variables such as humidity, temperature, and photoperiod were kept constant, whereas under more natural situations these would show strong fluctuations. Phenotypic traits can be moderated by the environment through genotype by environment interactions or phenotypic plasticity. Especially life-history traits, such as considered here, are highly sensitive to temperature and nutritional conditions (Ernsting and Isaaks 2000, Ellers et al. 2008, Liefting and Ellers 2008, Liefting et al. 2010). The sensitivity of genotypic interactions to environmental conditions can be expected to be as important as species interactions.

Community consequences of the extent of phenotypic dissimilarity

Earlier studies on the interface between evolutionary biology and community ecology have mainly focused on the community effects of genotypic richness of plants. Relatively few studies have looked at the community effects of intraspecific variation in animal populations, but genotype number is known to increase resistance to invasion in *Daphnia* (De Meester et al. 2007) and enhance settling success in marine invertebrates (Gamfeldt et al. 2005). Together with the present results, this suggests that the positive effects of genotypic richness are unspecific to taxon or phylogeny, but instead are generally applicable. Increased population productivity is important to community composition because it can influence the abundance of higher trophic levels; in the case of primary productivity it increases abundance of herbivores and their associated predators (Cardinale et al. 2006). In much the same way, *O. cineta* is an important food source for many predators, which strongly suggests that productivity mediated by the number of genotypes in *O. cineta* also affects diversity at higher trophic levels. Vice versa, our study indicates that the community effects of genotype number of plants can be predicted more accurately if the degree of phenotypic dissimilarity among plant genotypes is taken into account.

The question remains to be answered if the positive effects of genotypic richness and the extent of phenotypic dissimilarity are additive to the positive effects of species diversity or if they are mutually exclusive. It has been proposed that a positive correlation exists between genetic diversity and species diversity in natural populations, which would suggest that processes are acting in parallel at the two levels (Vellend and Geber 2005). Future research using an experimental setup that includes both genotypic richness and species diversity may determine the relative importance of both.

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APPENDIX A

Key variables of the genotype mixtures used for the mixed microcosms (*Ecological Archives* E092-135-A1).

APPENDIX B

UPGMA dendrogram based on Nei's genetic distance between genotypes (*Ecological Archives* E092-135-A2).