

# The relative importance of host-plant genetic diversity in structuring the associated herbivore community

AYCO J. M. TACK<sup>1</sup> AND TOMAS ROSLIN<sup>2</sup>

*Metapopulation Research Group, Department of Biosciences, University of Helsinki, Viikinkaari 1, P.O. Box 65, Helsinki FI-00014, Finland*

**Abstract.** Recent studies suggest that intraspecific genetic diversity in one species may leave a substantial imprint on the surrounding community and ecosystem. Here, we test the hypothesis that genetic diversity within host-plant patches translates into consistent and ecologically important changes in the associated herbivore community. More specifically, we use potted, grafted oak saplings to construct 41 patches of four saplings each, with one, two, or four tree genotypes represented among the host plants. These patches were divided among two common gardens. Focusing first at the level of individual trees, we assess how tree-specific genotypic identity, patch-level genetic diversity, garden-level environmental variation, and their interactions affect the structure of the herbivore community. At the level of host-plant patches, we analyze whether the joint responses of herbivore species to environmental variation and genetic diversity result in differences in species diversity among tree quartets. Strikingly, both species-specific abundances and species diversity varied substantially among host-tree genotypes, among common gardens, and among specific locations within individual gardens. In contrast, the genetic diversity of the patch left a detectable imprint on local abundances of only two herbivore taxa. In both cases, the effect of genetic diversity was inconsistent among gardens and among host-plant genotypes. While the insect community differed significantly among individual host-plant genotypes, there were no interactive effects of the number of different genotypes within the patch. Overall, additive effects of intraspecific genetic diversity of the host plant explained a similar or lower proportion (7–10%) of variation in herbivore species diversity than did variation among common gardens. Combined with the few previous studies published to date, our study suggests that the impact of host-plant genetic diversity on the herbivore community can range from none to nonadditive, is generally low, and reaches its most pronounced impact at small spatial scales. Overall, our findings strengthen the emerging view that the impacts of genetic diversity are system, scale, and context dependent. As the next step in community genetics, we should then start asking not only whether genetic diversity matters, but under what circumstances its imprint is accentuated.

**Key words:** *biodiversity; community genetics; genetic diversity; genotypic diversity; intraspecific variation; plant–insect interactions; Quercus robur.*

## INTRODUCTION

The ecological consequences of intraspecific genetic diversity on the structure of associated communities and on ecosystem processes have recently received increased attention in both agricultural (Zhu et al. 2000, Mundt 2002) and natural sciences (Hughes and Stachowicz 2004, Wimp et al. 2004, Reusch et al. 2005, Crutsinger et al. 2006, 2008b, Johnson et al. 2006, Madritch et al. 2006, Hughes et al. 2008, Hughes and Stachowicz 2009). Moreover, a recent review of the field of community genetics posits that impacts of intraspecific genetic

variation on the associated community structure and on ecosystem processes are pronounced and widespread (Bailey et al. 2009). Hence, while the negative consequences of a worldwide decline in species diversity have been widely recognized for several decades (Hooper et al. 2005), community genetics holds that the loss of intraspecific genetic diversity comes with previously unrecognized consequences for a wide variety of ecosystem processes and communities (Whitham et al. 2006).

Despite this flurry of recent studies, a paper by Hughes et al. (2008) identified several major gaps in our knowledge of the ecological impact of genetic diversity. For example, there is a need for studies that compare the importance of genetic diversity to that of other ecological factors (Hughes and Stachowicz 2009). While previous studies opened up a new research frontier by demonstrating the potential importance of genetic diversity, these studies have often artificially reduced

Manuscript received 14 October 2010; revised 10 March 2011; accepted 24 March 2011. Corresponding Editor: J. J. Stachowicz.

<sup>1</sup> E-mail: ayco.tack@helsinki.fi

<sup>2</sup> Present address: Department of Agricultural Sciences, University of Helsinki, Latokartanonkaari 5, P.O. Box 27, Helsinki FI-00014, Finland.

environmental variation in their experimental setting. Hence, the next step forward is to compare the importance of intraspecific genetic diversity to that of other ecological factors, thereby establishing the relevance of a community genetics perspective in understanding community structure and ecosystem processes.

To understand the full ecological consequences of genetic diversity, we also need more knowledge on how consistent the effects of genetic diversity are in space and time. For example, Kanaga et al. (2009) showed that the ground-dwelling arthropod community associated with aspen is affected not only by genetic diversity as such, but also by an interaction between drought stress and genetic diversity. Such findings suggest that the effect of genetic diversity may vary in space (and time) as a function of environmental conditions. In that scenario, fitness differences between plants in low- and high-diversity plots may differ across the landscape, resulting in heterogeneity in natural selection and in diverging evolutionary trajectories.

A third issue that deserves more scrutiny is the mechanism behind the ecological impact of genetic diversity (Hughes et al. 2008). Here, effects of genetic diversity can be classified as either additive or nonadditive (Crutsinger et al. 2006, Johnson et al. 2006). An additive effect will emerge from mere sampling: for example, if the associated community differs between individual host genotypes, including more genotypes in a sample will automatically result in a higher species richness of the associated community. In such cases, the higher species richness associated with genetically diverse host populations can be calculated from the species richness of communities associated with host monocultures. Changes in species diversity that cannot be explained by sampling alone are called nonadditive (or interactive), and can be a direct or indirect consequence of genetic diversity. As an example of a direct effect, the genetic diversity of a host-plant patch can affect herbivore movement, colonization, and emigration (Power 1988, 1991). An example of an indirect effect is given by Crutsinger et al. (2006), who demonstrated how increased primary production in genetically diverse patches may explain a nonadditive increase in species richness in mixed patches.

Knowledge of the mechanisms behind genetic diversity effects will add fundamental insight into the factors structuring communities and controlling ecosystem processes, but it will also have clear applications in the field of conservation biology (Bangert et al. 2005, Johnson et al. 2006). For example, when the effects of genetic diversity are additive, a conservation biologist may aim for conserving those particular host-plant genotypes that come with diverse associated communities. On the other hand, when the effects are nonadditive, a conservation biologist may do best in conserving a high diversity of host-plant genotypes, since the effect of genetic diversity is then achieved only in mixtures of multiple genotypes (Johnson et al. 2006). In addition,

knowledge about the mechanisms behind genetic diversity effects in crop plants may lead to a more sustainable agriculture (Zhu et al. 2000, Mundt 2002).

To pinpoint the relative importance of intraspecific genetic diversity in a host plant on the associated herbivore community, to examine the consistency of diversity effects, and to establish the additive or nonadditive nature of the mechanisms involved, we use oak saplings (*Quercus robur*) to create 41 patches of different genetic diversity (one, two, or four genotypes), as distributed among two common gardens. More specifically, we answer two questions: (1) How do host-plant genotype, patch-level genetic diversity, and garden-level spatial variation interact to affect species-specific abundances and species richness on individual trees? (2) How important are host-plant genetic diversity, spatial location, and their interaction in structuring the associated herbivore diversity at the patch level?

## MATERIAL AND METHODS

### *Study system*

The pedunculate oak (*Quercus robur*) is the sole oak species occurring in Finland. The species sustains a diverse insect community (Southwood 1961), including over 20 specialist leaf miners, galls, and leaf folders (see Plate 1; more information on the species can be found in Tack et al. 2010). In these endophagous guilds, individual taxa can be readily identified in the field on the basis of the morphology of the leaf mine, gall, or leaf fold. Moreover, as the larval structure remains visible throughout the season (independent of the fate of the insect itself), a single survey by the end of the growing season will reveal the aggregate community of the full season. Finally, endophagous insects have been argued to respond strongly to host-plant genotype due to their intimate relationship with host-plant tissues (Mopper 1996, Stiling and Rossi 1998), rendering them a promising target group for studies on community genetics.

### *Design*

To evaluate the impact of oak genotypic diversity on the associated herbivore community, we used 5-year-old grafts of 10 large oak trees planted in 50-L plant pots ( $n = 164$ ; see Tack et al. [2010] for more details on the origin of the grafts). In early spring 2008, well before leaf flush, we constructed 41 patches, each consisting of four trees. Three treatments were established, where patches contained trees of either one, two, or four genotypes ( $n = 17$ ,  $n = 16$ , and  $n = 8$  patches, respectively). We note that the densities used by us ( $n = 4$  trees per patch) represent realistic densities at the higher end of the natural spectrum, and including more diverse patches would separate the experiment from its ecological context (Appendix A). The exact design, the distribution of tree genotypes within the experiment, and the rationale behind the treatment levels applied are described in full detail in Appendix A. Importantly, the design allows us

to disentangle whether potentially high species diversity in genetically diverse patches is due to additive (as based on genotypic identity alone) or nonadditive effects (as based on patch-level genetic diversity), since the overall response can be partitioned into the effects of a tree being of a given genotype, and into nonadditive effects of the tree being located in a patch with a given number of other genotypes (see also *Analysis*).

As natural patterns of host-plant aggregation may affect impacts realized on the herbivore community (Hooper et al. 2005), we designed our experiment to reflect the spatial distribution of oak trees in the Southwestern archipelago, Finland (see Appendix A for a more detailed description). Within patches, trees were separated by 1 m, causing the foliage from neighboring trees to be nearly or slightly intertwined. Patches were separated by 7 m. To test whether the impact of host genetic diversity is consistent across the landscape, we divided the patches among two common gardens separated by ~100 m. Both gardens were established within 50 m from the same oak stand within the Haapastensyrjä Tree Breeding Station (60°37' N, 24°26' E), Läyliäinen, Finland. The gardens were located in similar environment (open habitat) and at a similar distance (but in opposing directions) from the oak stand. By this approach, we explicitly aimed for quantifying variation among two kindred common gardens and between treatments with different genetic diversity levels within a single experiment.

#### *Response variables*

To characterize the impact of host-plant genetic diversity on the associated herbivore community, we recorded species-specific abundances, total abundance, species richness, and the Shannon diversity index across 17 species of leaf miners, gallers, and a leaf folder (see Appendix B for the full list of species). The responses were scored between 1 and 12 September 2008.

As individual leaf-miner species within the genera *Phyllonorycter* and *Stigmella* are morphologically indistinguishable, these two taxa were treated as compound groups (see also Tack et al. 2009). A given species of galler (*Cynips divisa*) was disproportionately abundant in our study area, precluding the separate counting of every individual. Hence, we used a different scale for this taxon, by scoring the rank abundance of *C. divisa* on each of five shoots per tree using three categories: 0 = no galls; 1 = some galls, not nearly on every leaf; 2 = galls on nearly every leaf, on average 1–10 per leaf; 3 = galls on practically every leaf, abundances high, >10 per leaf.

#### *Analysis*

The insect community of an individual tree may be affected by the genotypic identity of the host, its location within the experimental setting, and the impact of the genetic diversity at the patch-level (with the latter factor capturing nonadditive effects of genetic diversity). We therefore adopted a two-tiered approach to analyze the

impact of tree-level genotypic identity, patch-level genetic diversity, and spatial variation among common gardens on local herbivore communities (for conceptually similar approaches, see Milla et al. 2009, Parker et al. 2010). First, we analyzed characteristics of the insect community (i.e., species diversity and species-specific abundances) at the tree level. Second, we analyzed how the insect community observed within a patch of four trees was affected by patch-level genetic diversity, its location within the garden, and their interaction.

*Responses at the level of individual trees.*—To detect the effect of plant genotype, patch-level genetic diversity, variation among and within common gardens, and their interactions on the herbivore community of an individual host plant, we used generalized linear mixed-effects models. Here, we built separate models of the abundance of herbivore taxa, of species richness, and of Shannon diversity on each tree as a function of tree-level genotypic identity ( $G_{id}$ ; each of 10 genotypes), patch-level genetic diversity ( $G_{div}$ ; monoculture, diculture, or tetra-culture), and garden-level spatial location (Garden; two common gardens). To allow for biologically relevant interactions among factors, we also included all two-way interactions, where  $G_{div} \times G_{id}$  captures how the effect of patch-level genetic diversity is modified by the specific genotype of the host plant,  $G_{div} \times \text{Garden}$  estimates the extent to which the effect of patch-level genetic diversity is consistent across environments, and  $G_{id} \times \text{Garden}$  indicates how differences among host-plant genotypes are affected by the environment. Importantly, a nonadditive effect of genetic diversity would be revealed by a significant effect of  $G_{div}$  (indicating a consistent effect of genetic diversity on the genotypic value) or by the interaction  $G_{div} \times G_{id}$  (indicating a change in genotypic value with a change in genetic diversity).

The variables  $G_{div}$  and Garden were treated as categorical, fixed effects, and  $G_{id}$  was treated as a random effect (reflecting variation in the host-tree population). To account for potential spatial gradients in relevant response variables within common gardens, we added the variables  $X$ -coordinate and  $Y$ -coordinate, where both coordinates were nested within Garden. Abundances of individual species and species richness followed a Poisson distribution. Hence, we fitted the model using a log-link, and, where necessary, accounted for overdispersion by adding a multiplicative scale parameter (Littell et al. 2006:450). For Shannon diversity, we assumed a normal distribution. As we had scored the abundance of *Cynips divisa* for each of five branches on an ordinal scale, we used the mean score of this metric for each tree as the response variable, and assumed a normal distribution.

To determine the factors with a real impact on respective response, we reduced each maximal GLMM to a minimum adequate GLMM by sequentially removing nonsignificant factors ( $P > 0.10$ ) from the model using backward selection. Test statistics were

TABLE 1. Generalized linear mixed models assessing the impact of spatial location of the common garden (Garden), spatial location within each garden ( $X$  and  $Y$  coordinates), patch-level genetic diversity ( $G_{div}$ ), host-plant genotype ( $G_{id}$ ), and their interactions on the abundance and species richness of herbivores.

| Species                       | Garden | $X$    | $Y$   | $G_{div}$ | $G_{id}$ | $G_{div} \times \text{Garden}$ | $G_{id} \times \text{Garden}$ | $G_{div} \times G_{id}$ |
|-------------------------------|--------|--------|-------|-----------|----------|--------------------------------|-------------------------------|-------------------------|
| Species richness              | 0.24   |        | 0.01  |           |          |                                |                               |                         |
| Shannon diversity             | 0.08   |        | 0.004 |           |          |                                |                               |                         |
| <i>Cynips divisa</i>          | 0.26   |        | 0.003 |           | <0.0001  |                                |                               |                         |
| <i>Heliozela sericiella</i>   | 0.04   | 0.0007 | 0.002 |           | 0.01     |                                |                               |                         |
| <i>Neuroterus anthracinus</i> | 0.0002 | 0.02   | 0.007 | 0.10      | <0.0001  |                                |                               |                         |
| <i>Phyllonorycter</i> spp.    | 0.76   | 0.0007 | 0.002 | 0.86      | 0.01     |                                |                               | 0.02                    |
| <i>Profenusa pygmaea</i>      | 0.01   | 0.003  | 0.009 | 0.14      | 1        | 0.07                           | 0.09                          | 0.02                    |
| <i>Stigmella</i> spp.         | 0.0003 | 0.02   | 0.001 |           | 1        |                                | 0.04                          |                         |

Note: Values are  $P$  values from  $F$  tests of fixed effects and log-likelihood ratio tests of random effects for factors included in the final model.

based on  $F$  values for fixed effects and  $\chi^2$  values (log-likelihood ratio test with one degree of freedom) for the random effects.

As analyses on species with low abundances lack statistical power, we removed species represented by fewer than 100 individuals in total from the species-specific analyses (thereby including six species in the analyses; see Table 1 and Plate 1). Community-level descriptors (total abundance, species richness and Shannon diversity) were naturally calculated across the full insect community (as listed in Appendix B). All analyses were conducted using Proc Glimmix in SAS 9.2 (SAS Institute 2008).

Two trees never flushed leaves in the experiment, and had apparently died during the previous winter. Hence, we removed all data on respective patches before the analyses.

*Responses at the level of plant patches.*—To assess the relative importance of host-plant genetic diversity, spatial location, and their interaction, we built generalized linear models of patch-level species abundances, patch-level total abundance, patch-level species richness, and patch-level Shannon diversity as a function of  $G_{div}$ , Garden, and their interaction. To normalize the residuals, we log-transformed species-specific abundances, total abundance, and species richness before analysis. Analyses were conducted using the function *lm* in R (2.11.1). The relative importance of each predictor was assessed using three metrics directly calculated from the resulting ANOVA table (Graham and Edwards 2001, Hughes and Stachowicz 2009): (1)  $\eta^2 = SS_{\text{Factor}}/SS_{\text{Total}}$ , (2) partial  $\eta^2 = SS_{\text{Factor}}/(SS_{\text{Factor}} + SS_{\text{Resid}})$ , and (3)  $\omega^2 = (SS_{\text{Factor}} - df_{\text{Factor}} \times MS_{\text{Resid}})/(MS_{\text{Resid}} + SS_{\text{Total}})$ . As each of these metrics has its own merits (Graham and Edwards 2001), we present multiple metrics to allow the reader to assess the robustness of the results.

To test for a linear relationship between host-plant genetic diversity and the diversity of the insect community—as commonly assumed in previous studies (e.g., Crutsinger et al. 2006)—we first extracted the residuals of species richness and Shannon diversity as a function of the categorical variable Garden (using ANOVA). We then regressed these residuals on genotypic diversity

(i.e., one, two, or four genotypes per patch). As a visual assessment suggests differences in variance among treatment levels (Fig. 2B and D), we used quantile regression instead of ordinary least squares (Cade and Noon 2003). Besides being more robust to non-constant variance and outliers, quantile regression allowed us to test whether regression slopes differ among different quantiles of the response variable (Koenker 2005). Such differences may be generated by scenarios where the “maximum” species richness stays constant among treatment levels (for example, certain genotypes may have a high species richness even in monocultures), but genetically diverse treatments have higher “minimum” species richness due to the sampling effect. More specifically, we analyzed the 80% ( $\tau = 0.8$ ), median ( $\tau = 0.5$ ), and 20% ( $\tau = 0.2$ ) quantiles using the function *rq* in the package *quantreg* version 4.50 in R (Koenker 2010). Confidence intervals were obtained using the standard  $x,y$ -pair bootstrap. Tests for differences in regression slope among the three quantiles were conducted using the Wald test implemented in *anova.rq* (Koenker 2005).

RESULTS

At the level of the individual tree, the majority of herbivore species and species diversity were affected by the spatial location (Table 1, columns Garden,  $X$ , and  $Y$ ) and by the genotypic identity of the specific oak sapling (Table 1, column  $G_{id}$ ; Fig. 1A–C). In contrast, the genetic diversity represented at the patch level only affected the species-specific abundances of two out of six herbivores (Table 1, columns including  $G_{div}$ ). In both cases, the effect was weak and dependent on the host-plant genotype and the common garden (Table 1, interaction terms; Fig. 1D).

Overall, the general lack of a separate effect of the genetic diversity represented among neighboring trees on the herbivore community of a focal tree indicates an absence (or rarity) of nonadditive effects at the patch level. However, as different host-plant genotypes differed in the abundance of the associated species, these species-specific responses may lead to an additive increase in species diversity at the patch level: a pattern tested separately by the regression analyses conducted at

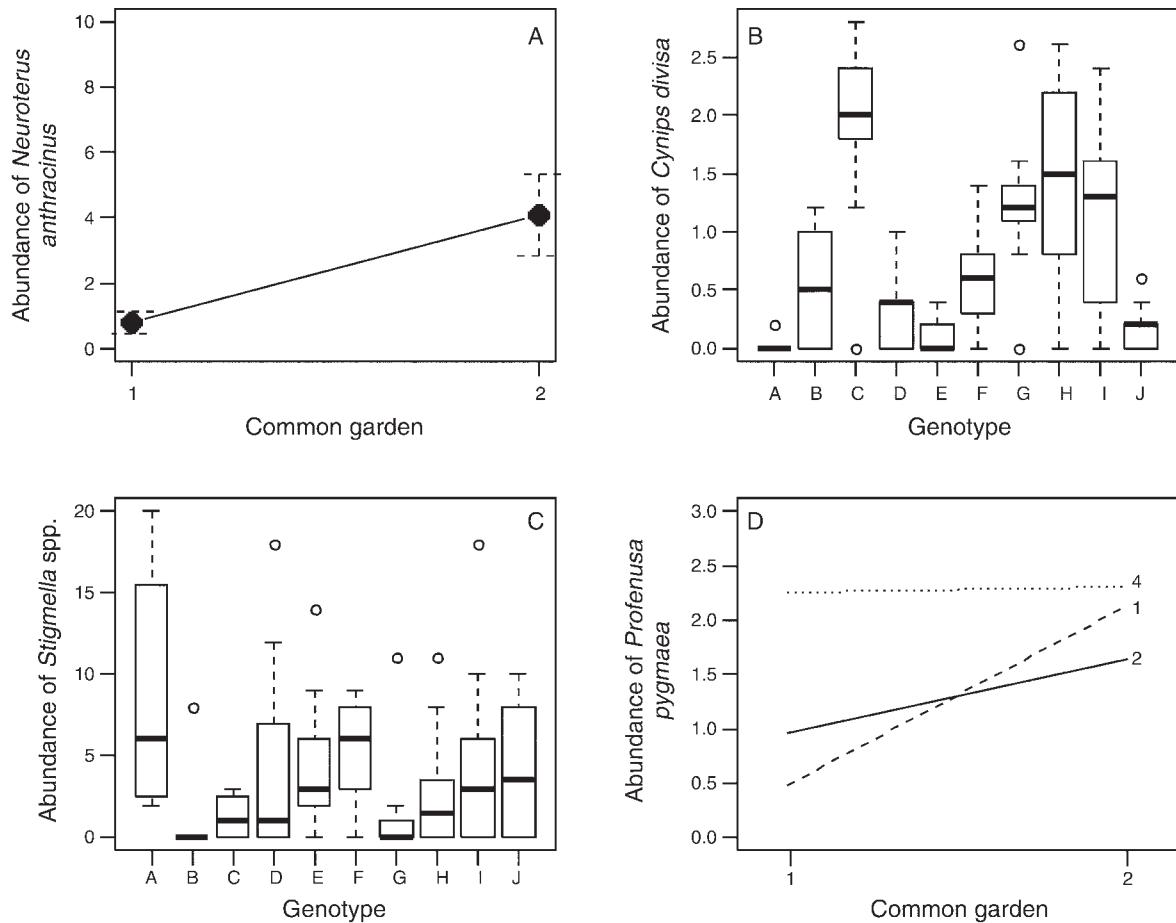


FIG. 1. Examples of herbivore responses to (A) location, (B, C) host-plant genotypic identity, and (D) the interaction between treatment and location. Panel (A) shows empirical mean values ( $\pm 95\%$  CI) of the abundance of *Neuroterus anthracinus*. Panels (B) and (C) show boxplots of herbivore abundance with mean values (heavy horizontal line), first and third quartiles (box bottom and top), whiskers (showing either the minimum and maximum value or 1.5 times the interquartile range of the data, whichever is smaller), and if present, outliers (dots); panel (D) shows the interactive effects of location and treatment on the abundance of *Profenusa pygmaea*, where lines connect treatment means across the two common gardens (treatment genotypic diversity is given to the right, next to the respective line).

the patch level. Here, median species richness and Shannon diversity at the patch level were positively related to the genotypic diversity ( $\tau = 0.5$ ,  $P = 0.07$  and  $0.01$ , respectively). Slopes did not differ among quantiles ( $P > 0.3$  for both response variables), suggesting a parallel increase in low, median, and high species diversity with increasing genotypic diversity.

The majority of variation in species-specific abundances observed at the patch level was explained by the location of the common garden (Table 2, common garden), whereas little variation was explained by genetic diversity (Table 2, treatment), or by the  $G_{\text{div}} \times$  Garden interaction (Table 2, treatment  $\times$  common garden). Likewise, in the overall insect community, most of the variation in the Shannon diversity was attributable to the common garden ( $\eta^2 = 18.1\%$ ,  $P = 0.005$ ), and considerably less could be traced to genetic diversity ( $\eta^2 = 9.6\%$ ,  $P = 0.11$ ; Table 2 and Fig. 2C and

D). In contrast, species richness was only weakly and insignificantly affected by either the common garden ( $\eta^2 = 5.6\%$ ,  $P = 0.14$ ) or by genetic diversity ( $\eta^2 = 7.9\%$ ,  $P = 0.21$ ; Table 2 and Fig. 2A and B). Neither genetic diversity nor garden had any detectable impact on total herbivore abundance. The interaction between genetic diversity and spatial location played a minor role in explaining the species-specific abundances, species richness, Shannon diversity, and total abundance ( $\eta^2 = 1-7\%$ ; Table 2).

#### DISCUSSION

This study demonstrates how genetic diversity in the host plant affects the associated herbivore community in a specific study system: herbivorous insects on oaks. Most importantly, it sheds light on the additivity of effect, on the mechanism underlying the patterns, on the relative importance of genetic diversity of the host as

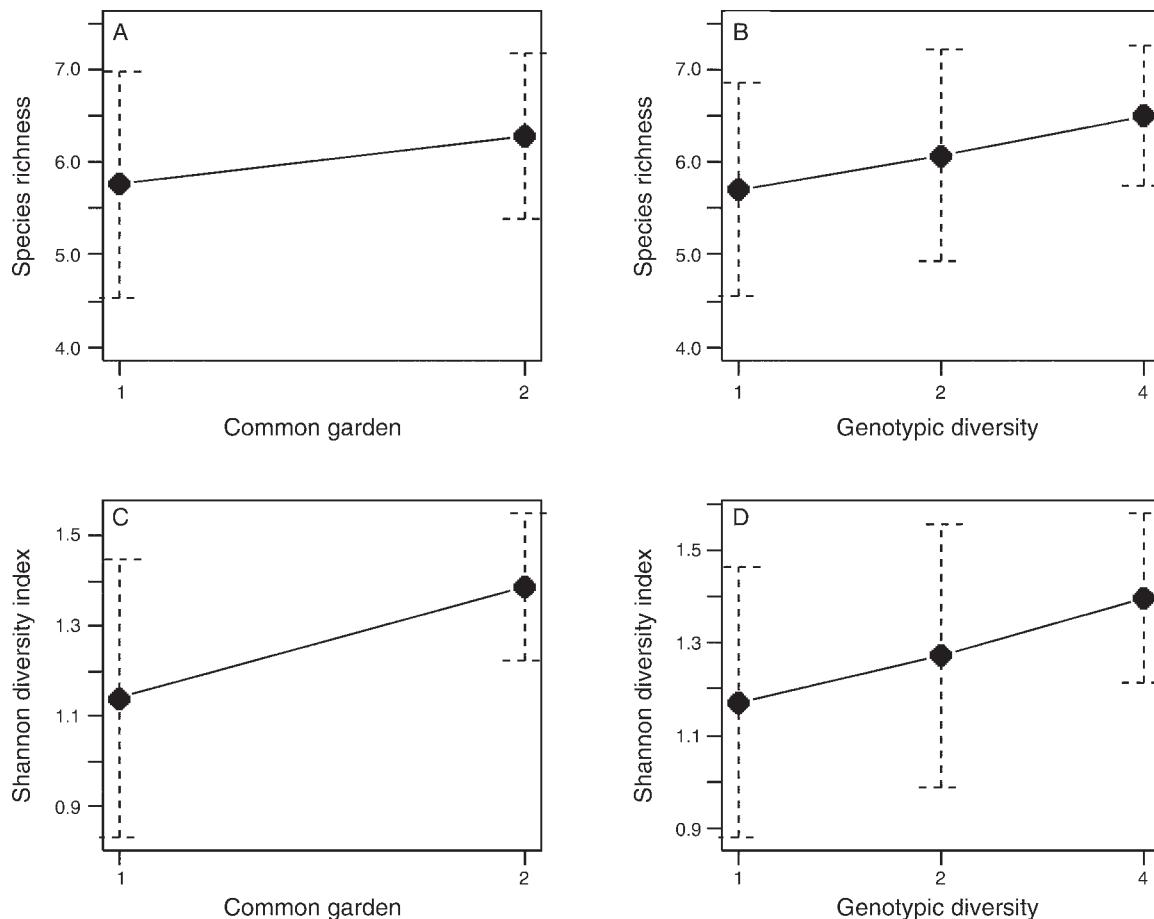


FIG. 2. (A, B) Patch-level species richness and (C, D) Shannon diversity index as functions of common garden and patch-level genetic diversity. All plots are drawn using empirical mean values, where lines connect mean values. The error bars refer to standard deviations, not to standard errors of the mean, and are therefore relatively wide.

compared to other factors in generating variation in associated communities, and on the consistency of the effects across environments. Here, we will address each aspect in turn. As study systems appear to vary widely in the impact of genetic diversity on the herbivore community, we then end by outlining general hypotheses

to explain different outcomes between study systems. to be tested in future work.

*Additivity of effect*

Additive and interactive effects of genetic diversity on herbivore communities can arise under multiple scenar-

TABLE 2. Relative importance of treatment, common garden, and their interaction in structuring the insect community at the patch level.

| Response                      | Treatment (genetic diversity levels) |                  |            | Garden       |                  |              | Treatment × Garden |                  |            |
|-------------------------------|--------------------------------------|------------------|------------|--------------|------------------|--------------|--------------------|------------------|------------|
|                               | $\eta^2$ (%)                         | Partial $\eta^2$ | $\omega^2$ | $\eta^2$ (%) | Partial $\eta^2$ | $\omega^2$   | $\eta^2$ (%)       | Partial $\eta^2$ | $\omega^2$ |
| Species richness              | 7.91                                 | 0.099            | 0.030      | 5.61         | 0.070            | 0.031        | 6.57               | 0.082            | 0.017      |
| Shannon diversity             | 9.64                                 | 0.143            | 0.054      | 18.06        | 0.268            | 0.157        | 4.89               | 0.073            | 0.008      |
| Total abundance               | 1.49                                 | 0.015            | -0.042     | 0.93         | 0.010            | -0.019       | 0.97               | 0.010            | -0.047     |
| Abundance of:                 |                                      |                  |            |              |                  |              |                    |                  |            |
| <i>Heliozela sericiella</i>   | 2.79                                 | 0.031            | -0.026     | 7.50         | 0.084            | 0.047        | 0.06               | 0.001            | -0.052     |
| <i>Neuroterus anthracinus</i> | 3.18                                 | 0.060            | 0.000      | <b>43.46</b> | <b>0.826</b>     | <b>0.412</b> | 0.73               | 0.014            | -0.024     |
| <i>Phyllonorycter</i> spp.    | 3.23                                 | 0.035            | -0.023     | 2.08         | 0.022            | -0.007       | 2.03               | 0.022            | -0.035     |
| <i>Profenusa pygmaea</i>      | 9.79                                 | 0.138            | 0.054      | <b>12.07</b> | <b>0.170</b>     | <b>0.097</b> | 7.17               | 0.101            | 0.028      |
| <i>Stigmella</i> spp.         | 0.18                                 | 0.003            | -0.039     | <b>30.80</b> | <b>0.453</b>     | <b>0.282</b> | 1.02               | 0.015            | -0.030     |

Notes: Formulas used to derive estimates of relative importance:  $\eta^2 = SS_{\text{Factor}}/SS_{\text{Total}}$ , partial  $\eta^2 = SS_{\text{Factor}}/(SS_{\text{Factor}} + SS_{\text{Resid}})$ , and  $\omega^2 = (SS_{\text{Factor}} - df_{\text{Factor}} \times MS_{\text{Resid}})/(MS_{\text{Resid}} + SS_{\text{Total}})$ . Significant factors ( $P < 0.05$ ) are shown in boldface type.

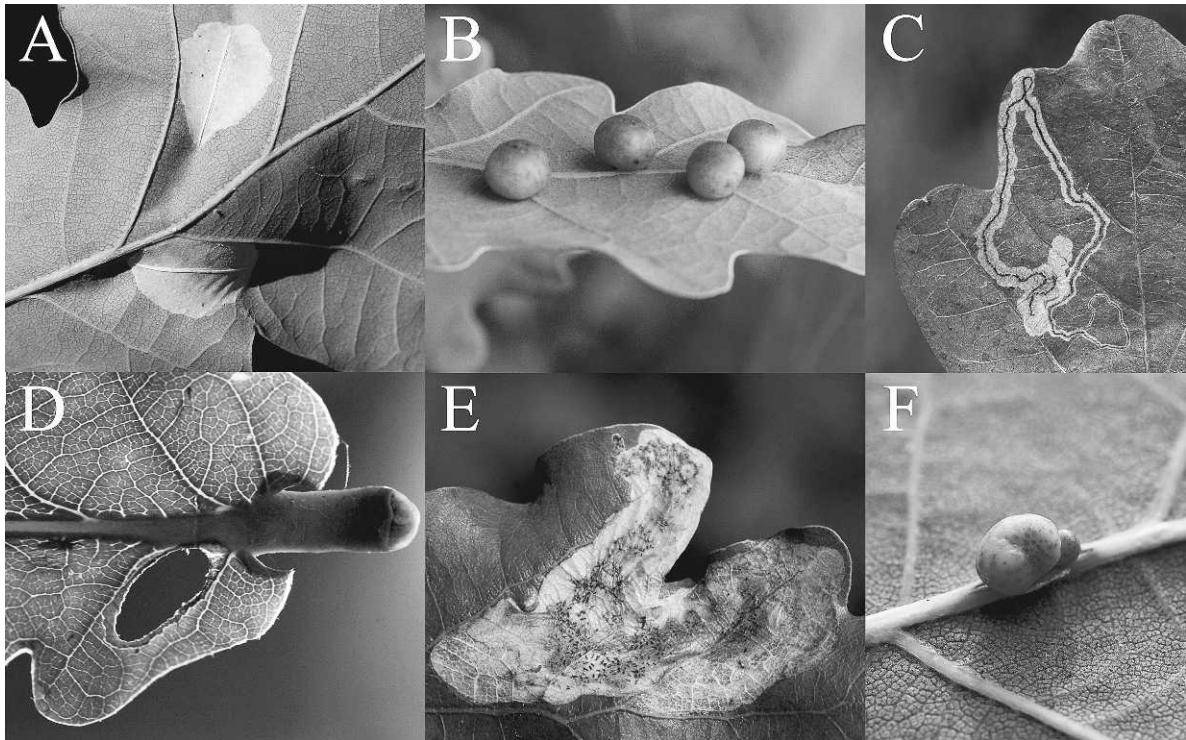


PLATE 1. Taxa included in analyses of species-specific abundances: (A) “tent”-shaped leaf mines of *Phyllonorycter* sp., (B) spherical galls of the asexual generation of *Cynips divisa*, (C) serpentine leaf mine of *Stigmella* sp., (D) leaf disk cut out by *Heliozela sericiella*, (E) leaf mine of *Profemusa pygmaea*, and (F) gall formed by the asexual generation of *Neuroterus anthracinus*. Photo credits: A. J. M. Tack and R. Kaartinen.

ios. To truly understand the mechanisms behind effects detected, we should then try to tease apart additive and nonadditive contributions. This has rarely been done in full detail: while most studies to date (e.g., Reusch et al. 2005, Crutsinger et al. 2006, Johnson et al. 2006) consider the possibility that genetic diversity may have no effects, additive effects, and positive nonadditive effects, negative nonadditive effects may actually be equally plausible. For example, negative nonadditive effects conform to the concept of “associational resistance,” a phenomenon well documented in terrestrial insect–plant interactions (e.g., Tahvanainen and Root 1972, Hambäck et al. 2000, Barbosa et al. 2009). In addition, both additive and nonadditive effects may be present, but act in opposite directions. Pinpointing how additive and nonadditive effects jointly contribute to the relationship between genetic diversity and herbivore community structure detected will then seem like a key objective for any study of community genetics.

Overall, the patterns reported in our study are compatible with a scenario where individual insect species react to the genotype of their host tree, but do so in a species-specific way. As species responded idiosyncratically to host-plant genotype, overall species richness was not affected by the genetic identity of the host plant. Species diversity at the patch level did increase with genetic diversity (Fig. 2B and D), but this

increment was attributable to a sampling effect: when genotypes differ in their associated insect communities, a sample including more tree genotypes will include higher insect diversity. Importantly, it includes no emergent, nonadditive effects of patch-level genetic diversity.

To date, only three other studies have assessed the impact of host-plant genetic diversity on the associated herbivore community. Strikingly, each study reports a different outcome: Crutsinger et al. (2006) detected both additive and nonadditive effects of genotypic diversity on the herbivore species richness of goldenrod (*Solidago altissima*). In contrast, Johnson et al. (2006) detected neither any consistent additive nor any consistent nonadditive effects of genetic diversity in evening primrose (*Oenothera biennis*) on associated herbivore richness. Nonetheless, this particular study describes patterns indicative of negative nonadditive effects: while Johnson and Agrawal (2005) and Johnson et al. (2006) detected clear effects of genotypic identity of *Oenothera biennis* on the local arthropod communities, herbivore species richness actually increased by 20% in genetically depauperate patches relative to genetically diverse patches in late May. Interestingly, the inverse pattern (25% increase in richness in genetically diverse patches relative to monocultures) was detected in late summer. Thus, in this case, nonadditive effects may have changed direction during the season, de facto countering the

positive additive effects during the spring. Finally, Reusch et al. (2005) found no impact of genotypic diversity of seagrass on grazer diversity, whereas total grazer abundance increased in a nonadditive manner.

When combined with the current results, the evidence from the aforementioned three studies suggests that the impact of genetic diversity on the associated herbivore community may be highly variable: the effect can in fact be absent, additive, or nonadditive, and nonadditive effects may potentially be negative. Such variation shows a clear need for further dissection of how different effects of genetic diversity contribute to realized community structure.

#### *Underlying mechanisms*

The mechanism underlying nonadditive effects may be direct or indirect. As an example of an indirect effect, Crutsinger et al. (2006) showed that a nonadditive increase in herbivore species richness may be caused by increased biomass production in genetically diverse patches of host plants. In contrast, we used potted trees and hence did not expect any changes in plant quality or quantity in our study, since the plants had no or very little direct physical contact and did not compete for the same resources. Hence, the responses detected in our study will likely reflect direct effects of patch-level genetic diversity on insect movement (i.e., colonization and emigration of insects among host plants).

What our results then specifically suggest is that herbivore species do not change movement patterns in response to the genetic diversity of the host-plant patch. Here, our result is corroborated by two studies in natural study systems: Kinsman (1982; as cited in Karban 1992) detected no effect of genetic diversity on herbivore colonization of two herbivores on *Oenothera biennis*. Likewise, Schmitt and Antonovics (1986) found no evidence for an effect of genetic diversity of *Anthoxanthum odoratum* patches on aphid colonization.

These findings contrast with studies focusing on species diversity and agricultural cultivar mixtures. For example, Power (1988, 1991) showed that planthoppers and aphids changed their movement behavior in genetically diverse maize and oat patches, respectively, resulting in different abundances in monocultures and dicultures. Likewise, with respect to the interspecific genetic diversity of the host, insect colonization and emigration patterns commonly differ among plots with multiple plant species as compared to single plant species (Tahvanainen and Root 1972, Bach 1980a, b, Risch 1981, Kareiva 1985, Elmstrom et al. 1988, Coll and Bottrell 1994, Holmes and Barrett 1997).

What the combined literature then suggests is that herbivore movement behavior may commonly be affected by both species and cultivar diversity. However, in natural study systems, there may be too little variation present among conspecific plants growing close to each other to elicit an insect movement

response. Alternatively, insect colonization may play a role in only a subset of the natural communities.

#### *Relative importance of genotypic diversity*

Overall, the quantitative effect of host-plant genetic diversity on the associated herbivore community appears strongly dependent on the study system. Here, results from studies based on common gardens show ample variation in effect sizes, ranging from no consistent variation in herbivore diversity (Johnson et al. 2006) to a weak additive effect (7–9%; Table 2 in the current study) or a relatively large effect (26%; Crutsinger et al. 2006). Given the variation observed, future studies should be aimed at understanding not only whether host-plant genetic diversity matters, but also under which specific conditions it is likely to make its biggest contribution to structuring the associated herbivore community (see *Emerging hypotheses*).

Crucially, while many studies do show an impact of genetic diversity on the associated community and on ecosystem processes within common gardens (Hughes and Stachowicz 2004, Reusch et al. 2005, Schweitzer et al. 2005, Crutsinger et al. 2006, 2008a, 2009, Johnson et al. 2006, Madritch et al. 2006, Crawford et al. 2007, Hughes et al. 2008, Bailey et al. 2009, Kanaga et al. 2009, Cook-Patton et al. 2011), a major challenge remains: to appreciate the realized impact of genetic diversity in nature, we need to compare its imprint to that left by other ecological factors (Hughes et al. 2008). One factor that did explain as much as or more variation than genetic diversity in our experiment was environmental variation. This seems surprising, given the fact that the locations were selected based on similarity rather than on differences. The pattern observed then suggests that environmental factors may be even more important in other natural settings and at larger spatial scales. This latter notion is consistent with a recent experimental study showing that differences in the insect community structure among host-plant genotypes are swamped by environmental variation at large spatial scales (Tack et al. 2010). Likewise, a previous observational study showed that large-scale spatial variation outweighs the importance of genetic diversity, whereas smaller-scale spatial variation has an impact similar to that of genetic diversity (Hughes and Stachowicz 2009).

#### *Consistency of genetic diversity effects*

Importantly, both the study by Hughes and Stachowicz (2009) and the current one suggest that the effect of genetic diversity is relatively consistent across the landscape: in both studies, the interaction between genetic diversity and spatial location was weak and nonsignificant. By contrast, studies following a given system over time have revealed substantial temporal variation: Johnson et al. (2006) showed that the effect of genetic diversity on the associated herbivore community can change in strength and sign during a single season, and Crutsinger et al. (2008a) reported that the effect of

genetic diversity declined by the very end of the season. The effect of genetic diversity may then be more consistent in space than in time, but more studies are clearly needed to verify these impressions.

#### *Emerging hypotheses*

What the comparison between our study and previous work clearly brings out is large variation among studies in the effect size of plant genetic diversity on the herbivore community. This variation among study systems calls for general hypotheses to explain study-specific outcomes. At this early stage, several hypotheses can be advanced to account for the patterns emerging.

First, system-specific variation in responses to genetic diversity may reflect differences in the genetic distance between the genotypes involved. For example, hybrid species may exhibit particularly large genetic variation (Whitham et al. 1999). In such cases, genetic diversity will likely leave an imprint even at large spatial scales (Wimp et al. 2004). In contrast, our study was conducted with a non-hybridizing plant species at the northern limit of its range. Hence, the genetic variation present in our plant population may have been relatively low, as reflected in the rather weak effects of genetic diversity. However, we note that the genetic diversity of oak (*Quercus robur*; as measured by allozyme markers) is only slightly lower in Finland than at the core of the species' distribution (Vakkari et al. 2006).

Second, the strong nonadditive effects of genetic diversity observed in some systems may be mediated by a specific mechanism: by increased plant biomass in genetically diverse host-plant patches. Augmented biomass in genetically diverse patches has been observed in a wide variety of plant species (Reusch et al. 2005, Crutsinger et al. 2006, Milla et al. 2009, Crawford and Whitney 2010, Kotowska et al. 2010, Cook-Patton et al. 2011; but see Dudley and File 2007, Vellend et al. 2010) and may have important cascading effects on higher trophic levels (Reusch et al. 2005, Crutsinger et al. 2006, but see Johnson et al. 2006). That such effects were lacking in our study may be due to the fact that we used potted plants. Using both potted and planted host plants within the same experiment could then offer important insights into the relative roles of nonadditive effects mediated by the host plant, and effects mediated by other mechanisms (see Stachowicz et al. [2008] for a demonstration of how combining multiple experimental designs within a single study system may clarify the mechanisms underlying effects of species-level diversity). We further note that if nonadditive effects mediated by the host plant turn out to be the rule, we may then expect nonadditive effects to be most common in species occurring at high densities (with many interactions among nearby conspecific neighbors, e.g., *Zostera* and *Solidago*). In contrast, when individuals are sparsely distributed throughout the landscape, as is the case for *Quercus robur* in southwestern Finland, nonadditive genetic effects may be weak or absent.

Third, the study by Johnson et al. (2006) on the arthropod community of evening primrose provides strong evidence for a specific hypothesis: that herbivores may sort themselves differentially among plant individuals as based on genotype-specific plant phenotypes. In contrast, higher trophic levels may respond in a nonadditive fashion to the increased herbivore diversity or to structural complexity present in polycultures. If this is the case, then nonadditive effects of genetic diversity are likely confined to higher trophic levels responding to the broader niche space available in genetically diverse plant patches. Here, our study corroborates the notion that genotypic identity (and not genetic diversity) structures the herbivore community. However, more studies are needed to demonstrate the general notion that the effects of genetic diversity are more pronounced on higher trophic levels.

Fourth, variation among studies may be related to differences in the extent to which the herbivore response measured reflects short-term behavioral choices on the one hand, and longer-term population dynamics on the other. As our study primarily measured movement of univoltine and bivoltine insect herbivores, the behavioral basis of the response may explain the weak effect as compared to studies focusing on multivoltine species. For example, Underwood (2004, 2009) has suggested that source-sink dynamics in genotype mixtures could explain differences in aphid population dynamics in strawberry monocultures and polycultures. Likewise, a higher growth rate in genetically diverse patches (e.g., due to diet mixing; Mody et al. 2007) may in the long term lead to differing community structures among monocultures and polycultures. However, little support for the latter hypothesis exists to date. Hughes et al. (2010) detected no effect of genetic diversity on the fecundity of three grazer species. Likewise, Harris and Brock (1972) found no evidence for an effect of either clover or ryegrass genetic diversity on the survival and weight of porina caterpillars (*Wiseana* spp.).

A final hypothesis states that the effects of genetic diversity may only appear under, or be enhanced by, stressful conditions (Hughes and Stachowicz 2004, Reusch et al. 2005, Kanaga et al. 2009). While little or no experimental evidence exists to date with regard to herbivore communities, this hypothesis certainly deserves more experimental attention.

As will be evident at this stage, current ideas regarding when genetic diversity is likely to play its biggest role are both diverse and many. Much more work across multiple study systems will then be needed to resolve the ultimate explanatory power of competing hypotheses. We hope that this brief overview of the options will catalyze future synthesis. Most importantly, we feel it is time to move from the question of whether genetic diversity matters to the next step of asking under what circumstances genetic diversity leaves an important imprint on the herbivore community.

## ACKNOWLEDGMENTS

We gratefully acknowledge the Haapastensyrjä Tree Breeding Station for providing the large space needed for this experiment, and its personnel for help with distributing each tree to its designated location within either garden. In particular, we thank Piritta Lohela for taking care of the trees during the field season. Moreover, we are indebted to many fieldworkers, and in particular Janne Haaksluoto, for surveying the trees for insects during the hectic autumn months.

## LITERATURE CITED

- Bach, C. E. 1980a. Effects of plant density and diversity on the population dynamics of a specialist herbivore, the striped cucumber beetle, *Acalymma vittata* (Fab.). *Ecology* 61:1515–1530.
- Bach, C. E. 1980b. Effects of plant diversity and time of colonization on an herbivore-plant interaction. *Oecologia* 44:319–326.
- Bailey, J. K., J. A. Schweitzer, F. Úbeda, J. Koricheva, C. J. LeRoy, M. D. Madritch, B. J. Rehill, R. K. Bangert, D. G. Fischer, G. J. Allan, and T. G. Whitham. 2009. From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philosophical Transactions of the Royal Society B* 364:1607–1616.
- Bangert, R. K., R. J. Turek, G. D. Martinsen, G. M. Wimp, J. K. Bailey, and T. G. Whitham. 2005. Benefits of conservation of plant genetic diversity to arthropod diversity. *Conservation Biology* 19:379–390.
- Barbosa, P., J. Hines, I. Kaplan, H. Martinson, A. Szczepaniec, and Z. Szendrei. 2009. Associational resistance and associational susceptibility: having right or wrong neighbors. *Annual Review of Ecology, Evolution, and Systematics* 40:1–20.
- Cade, B. S., and B. R. Noon. 2003. A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment* 1:412–420.
- Coll, M., and D. G. Bortrell. 1994. Effects of nonhost plants on an insect herbivore in diverse habitats. *Ecology* 75:723–731.
- Cook-Patton, S. C., S. H. McArt, A. L. Parachnowitsch, J. S. Thaler, and A. A. Agrawal. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. *Ecology* 92:915–923.
- Crawford, K. M., G. M. Crutsinger, and N. J. Sanders. 2007. Host-plant genotypic diversity mediates the distribution of an ecosystem engineer. *Ecology* 88:2114–2120.
- Crawford, K. M., and K. D. Whitney. 2010. Population genetic diversity influences colonization success. *Molecular Ecology* 19:1253–1263.
- Crutsinger, G. M., M. W. Cadotte, and N. J. Sanders. 2009. Plant genetics shapes inquiline community structure across spatial scales. *Ecology Letters* 12:285–292.
- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* 313:966–968.
- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, and N. J. Sanders. 2008a. Temporal dynamics in non-additive responses of arthropods to host-plant genotypic diversity. *Oikos* 117:255–264.
- Crutsinger, G. M., L. Souza, and N. J. Sanders. 2008b. Intraspecific diversity and dominant genotypes resist plant invasions. *Ecology Letters* 11:16–23.
- Dudley, S. A., and A. L. File. 2007. Kin recognition in an annual plant. *Biology Letters* 3:435–438.
- Elmstrom, K. M., D. A. Andow, and W. W. Barclay. 1988. Flea beetle movement in a broccoli monoculture and diculture. *Environmental Entomology* 17:299–305.
- Graham, M. H., and M. S. Edwards. 2001. Statistical significance versus fit: estimating the importance of individual factors in ecological analysis of variance. *Oikos* 93:505–513.
- Hambäck, P. A., J. Ågren, and L. Ericson. 2000. Associational resistance: insect damage to purple loosestrife reduced in thickets of sweet gale. *Ecology* 81:1784–1794.
- Harris, W., and J. L. Brock. 1972. Effect of porina caterpillar (*Wiseana* spp.) infestation on yield and competitive interactions of ryegrass and white clover varieties. *New Zealand Journal of Agricultural Research* 15:723–740.
- Holmes, D. M., and G. W. Barrett. 1997. Japanese beetle (*Popillia japonica*) dispersal behavior in intercropped vs. monoculture soybean agroecosystems. *American Midland Naturalist* 137:312–319.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* 75:3–35.
- Hughes, A. R., R. J. Best, and J. J. Stachowicz. 2010. Genotypic diversity and grazer identity interactively influence seagrass and grazer biomass. *Marine Ecology Progress Series* 403:43–51.
- Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11:609–623.
- Hughes, A. R., and J. J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proceedings of the National Academy of Sciences USA* 101:8998–9002.
- Hughes, A. R., and J. J. Stachowicz. 2009. Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. *Ecology* 90:1412–1419.
- Johnson, M. T. J., and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* 86:874–885.
- Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters* 9:24–34.
- Kanaga, M. K., L. C. Latta IV, K. E. Mock, R. J. Ryel, R. L. Lindroth, and M. E. Pfrender. 2009. Plant genotypic diversity and environmental stress interact to negatively affect arthropod community diversity. *Arthropod-Plant Interactions* 3:249–258.
- Karban, R. 1992. Plant variation: its effects on populations of herbivorous insects. Pages 195–215 in R. S. Fritz and E. L. Simms, editors. *Plant resistance to herbivores and pathogens: ecology, evolution, and genetics*. University of Chicago Press, Chicago, Illinois, USA.
- Kareiva, P. 1985. Finding and losing host plants by *Phyllotreta*: patch size and surrounding habitat. *Ecology* 66:1809–1816.
- Koenker, R. 2005. *Quantile regression*. Cambridge University Press, Cambridge, UK.
- Koenker, R. 2010. *quantreg: quantile regression*. R package version 4.50. (<http://CRAN.R-project.org/package=quantreg>)
- Kotowska, A. M., J. F. Cahill, Jr., and B. A. Keddie. 2010. Plant genetic diversity yields increased plant productivity and herbivore performance. *Journal of Ecology* 98:237–245.
- Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. *SAS for mixed models*. Second edition. SAS Institute, Cary, North Carolina, USA.
- Madritch, M., J. R. Donaldson, and R. L. Lindroth. 2006. Genetic identity of *Populus tremuloides* litter influences decomposition and nutrient release in a mixed forest stand. *Ecosystems* 9:528–537.
- Milla, R., D. M. Forero, A. Escudero, and J. M. Iriondo. 2009. Growing with siblings: a common ground for cooperation or for fiercer competition among plants? *Proceedings of the Royal Society B* 276:2531–2540.
- Mody, K., S. B. Unsicker, and K. E. Linsenmair. 2007. Fitness related diet-mixing by intraspecific host-plant-switching of specialist insect herbivores. *Ecology* 88:1012–1020.

- Mopper, S. 1996. Adaptive genetic structure in phytophagous insect populations. *Trends in Ecology and Evolution* 11:235–238.
- Mundt, C. C. 2002. Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology* 40:381–410.
- Parker, J. D., J.-P. Salminen, and A. A. Agrawal. 2010. Herbivory enhances positive effects of plant genotypic diversity. *Ecology Letters* 13:553–563.
- Power, A. G. 1988. Leafhopper response to genetically diverse maize stands. *Entomologia Experimentalis et Applicata* 49:213–219.
- Power, A. G. 1991. Virus spread and vector dynamics in genetically diverse plant populations. *Ecology* 72:232–241.
- R Development Core Team. 2010. R version 2.11.1. R Foundation for Statistical Computing, Vienna, Austria. ([www.R-project.org](http://www.R-project.org))
- Reusch, T. B. H., A. Ehlers, A. Hämmerli, and B. Worm. 2005. Ecosystem recovery after climatic extremes enhanced by genotypic diversity. *Proceedings of the National Academy of Sciences USA* 102:2826–2831.
- Risch, S. J. 1981. Insect herbivore abundance in tropical monocultures and polycultures: an experimental test of two hypotheses. *Ecology* 62:1325–1340.
- SAS Institute. 2008. SAS 9.2. SAS Institute, Cary, North Carolina, USA.
- Schmitt, J., and J. Antonovics. 1986. Experimental studies of the evolutionary significance of sexual reproduction. IV. Effect of neighbor relatedness and aphid infestation on seedling performance. *Evolution* 40:830–836.
- Schweitzer, J. A., J. K. Bailey, S. C. Hart, and T. G. Whitham. 2005. Nonadditive effects of mixing cottonwood genotypes on litter decomposition and nutrient dynamics. *Ecology* 86:2834–2840.
- Southwood, T. R. E. 1961. The number of species of insect associated with various trees. *Journal of Animal Ecology* 30:1–8.
- Stachowicz, J. J., R. J. Best, M. E. S. Bracken, and M. H. Graham. 2008. Complementarity in marine biodiversity manipulations: reconciling divergent evidence from field and mesocosm experiments. *Proceedings of the National Academy of Sciences USA* 105:18842–18847.
- Stiling, P., and A. M. Rossi. 1998. Deme formation in a dispersive gall-forming midge. Pages 22–36 in S. Mopper and S. Y. Strauss, editors. *Genetic structure and local adaptation in natural insect populations*. Chapman and Hall, New York, USA.
- Tack, A. J. M., O. Ovaskainen, P. J. Harrison, and T. Roslin. 2009. Competition as a structuring force in leaf miner communities. *Oikos* 118:809–818.
- Tack, A. J. M., O. Ovaskainen, P. Pulkkinen, and T. Roslin. 2010. Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology* 91:2660–2672.
- Tahvanainen, J. O., and R. B. Root. 1972. The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). *Oecologia (Berlin)* 10:321–346.
- Underwood, N. 2004. Variance and skew of the distribution of plant quality influence herbivore population dynamics. *Ecology* 85:686–693.
- Underwood, N. 2009. Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. *Journal of Animal Ecology* 78:839–847.
- Vakkari, P., A. Blom, M. Rusanen, J. Raisio, and H. Toivonen. 2006. Genetic variability of fragmented stands of pedunculate oak (*Quercus robur*) in Finland. *Genetica* 127:231–241.
- Vellend, M., E. B. M. Drummond, and H. Tomimatsu. 2010. Effects of genotype identity and diversity on the invasiveness and invisibility of plant populations. *Oecologia* 162:371–381.
- Whitham, T. G., et al. 2006. A framework for community and ecosystem genetics: from genes to ecosystems. *Nature Reviews Genetics* 7:510–523.
- Whitham, T. G., G. D. Martinsen, K. D. Floate, H. S. Dungey, B. M. Potts, and P. Keim. 1999. Plant hybrid zones affect biodiversity: tools for a genetic-based understanding of community structure. *Ecology* 80:416–428.
- Wimp, G. M., W. P. Young, S. A. Woolbright, G. D. Martinsen, P. Keim, and T. G. Whitham. 2004. Conserving plant genetic diversity for dependent animal communities. *Ecology Letters* 7:776–780.
- Zhu, Y., et al. 2000. Genetic diversity and disease control in rice. *Nature* 406:718–722.

#### APPENDIX A

Detailed experimental design (*Ecological Archives* E092-134-A1).

#### APPENDIX B

List of studied species (*Ecological Archives* E092-134-A2).

## Appendix A. Detailed experimental design.

This appendix provides detailed information on the rationale behind the experimental design, the tree material used, and the set-up of the experiment.

### *Rationale behind the experimental design*

As pointed out by Hooper et al (2005), the natural patterns of spatial aggregation of species may be an important factor affecting the associated community and ecosystem properties. Hence, we based our experimental design on the natural distribution of oak trees in Southwestern Finland. Here, the majority of trees are saplings (e.g. Gripenberg et al. 2008) – and thus, most insects will be faced with young trees, justifying a focus on this relatively easy-to-manipulate subset of trees.

In the study area, oak saplings are relatively uncommon and their occurrence often aggregated at several spatial scales. More specifically, a sapling is often surrounded by a few nearby small trees, which can be offspring of the same tree (low diversity treatment) or offspring of different trees (high diversity treatment). Several such aggregates can often be found within a few tens of meters (unpublished data, Tomas Roslin). At a larger spatial scale, these larger aggregates are scattered across the landscape (Fig. 1b in Tack et al. 2010; unpublished data, Tomas Roslin). Based on this natural distribution, we focused on three spatial scales: trees within patches were separated by 1 meter, patches within common gardens were separated by seven meters, and the two common gardens were separated by 100 meter.

Given the distances chosen between patches (7 m; see above), the genetic diversity present within any single patch should be matched to the number of genotypes likely encountered within a radius of 3.5 m. Data on the distribution of oak trees (n=1868) within an intensively studied area, the island of Wattkast (Gripenberg and Roslin 2005, Gripenberg et al. 2007, Gripenberg et al. 2008, Tack et al. 2009, Tack et al. 2011), show that only 12.2% of the trees have three or more neighbours within this radius (for a map of all trees on the island, see Figure 1 in Gripenberg et al 2008 or Tack and Roslin 2010). Only 2.7% of the trees have more than five neighbours within a 3.5 meter radius. Hence, the densities used by us (n=4 trees per patch) represent realistic densities at the higher end of the natural spectrum, and including more diverse patches would separate the experiment from its ecological context.

Based on the selected spatial scales and on dispersal estimates for selected herbivore species, we can estimate the role that herbivore movement can play in creating community patterns within this system. Here, the empirically-based expected lifetime displacement for the leaf miner moth *T. ekebladella* is roughly 120 meters<sup>1</sup>. Hence, we expect frequent movement of mobile adult insects among trees within patches; less but still regular movement among patches; and only infrequent movement among the two common gardens. The large majority of species in the current community is likely to show dispersal limitation comparable to that of *T. ekebladella* at the landscape scale (5km<sup>2</sup>; Tack et al. 2010). Based on these considerations, we expect that patch immigration, patch emigration, or within-patch movement may be affected by host plant genetic diversity. Under such a scenario, differences in insect movement parameters between patches with low and high genetic diversity are likely to create a non-additive effect of patch-level genetic diversity on the insect community structure (Elmstrom et al. 1988, Power 1988, 1991).

---

<sup>1</sup> As calculated from the estimate of  $1/\alpha$  reported in Supplement 2 of Gripenberg et al. 2008. Following Turchin (1998), we calculated expected lifetime displacement as  $(1/\alpha) * (\pi/2)$ .

### *Tree material*

For the diversity experiment, we used potted five-year old grafted oak saplings (n=164). The origin of these grafts is described in full detail in Appendix A of Tack et al (2010). In short, shoots were collected from ten randomly selected large oak trees on the island of Wattkast (60°11'35"N, 21°37'50"E) in Southwestern Finland, and subsequently grafted onto root stocks at the Haapastensyrjä Tree Breeding Station in Läyliäinen (60°37'N, 24°26'E), Finland. Mainly due to differential mortality during the first two years, each 'mother tree' was represented by a different number of grafts (n=6-24 grafts per 'mother tree').

### *Distribution of trees*

In the spring of 2008, we used the potted oak trees to create patches consisting of four trees each. Here, each patch consisted of one, two or four different host plant genotypes. The number of replicates of each treatment were fixed, with a lower number of patches with high genetic diversity relative to the number of monocultures (n=17, n=16, and n=8, respectively; for a similar solution, see Crutsinger et al. 2006). We then randomized tree individuals to patches, with the constraint that each tree genotype had to be represented at least once in each monoculture. Here, random assignment of genotypes prevents confounding community composition with genetic diversity (Hooper et al. 2005), whereas the presence of each genotype in monoculture allows for direct assessment of the additivity of the response: if the response is purely additive, then the expected value of the polyculture can be derived from the value of each genotype in its monoculture. Finally, we randomly distributed the patches between the two common gardens. Due to space limitation, the number of patches slightly differed between common gardens (n = 22 and n = 19). The exact distribution of trees across patches and gardens is identified in Table A1.

**Table A1.** The location and genotypic composition of each patch. The design incorporated 41 patches, as divided between two common gardens. Each patch contained four trees of either one, two or four genotypes. The last column gives the exact genotypic composition of each patch.

| Garden | Patch | Diversity | Genotypes  |
|--------|-------|-----------|------------|
| 1      | 1     | 4         | A, D, H, I |
| 1      | 2     | 2         | G, J       |
| 1      | 3     | 1         | H          |
| 1      | 4     | 1         | D          |
| 1      | 5     | 2         | D, H       |
| 1      | 6     | 1         | A          |
| 1      | 7     | 1         | E, I       |
| 1      | 8     | 1         | I          |
| 1      | 9     | 1         | F          |
| 1      | 10    | 4         | D, E, F, H |
| 1      | 11    | 1         | C          |
| 1      | 12    | 1         | D          |
| 1      | 13    | 2         | E, H       |
| 1      | 14    | 1         | E          |
| 1      | 15    | 2         | D, I       |
| 1      | 16    | 2         | D, H       |
| 1      | 17    | 2         | A, I       |
| 1      | 18    | 4         | C, H, I, J |
| 1      | 19    | 1         | J          |
| 1      | 20    | 1         | H          |
| 1      | 21    | 4         | A, D, E, F |
| 1      | 22    | 2         | F, I       |
| 2      | 23    | 4         | D, G, H, I |
| 2      | 24    | 2         | C, H       |
| 2      | 25    | 1         | D          |
| 2      | 26    | 2         | C, D       |
| 2      | 27    | 1         | B          |
| 2      | 28    | 4         | D, E, G, I |
| 2      | 29    | 1         | E          |
| 2      | 30    | 4         | F, H, I, J |
| 2      | 31    | 1         | G          |
| 2      | 32    | 2         | H, I       |
| 2      | 33    | 4         | D, G, H, I |
| 2      | 34    | 2         | D, E       |
| 2      | 35    | 2         | B, J       |
| 2      | 36    | 2         | A, G       |
| 2      | 37    | 1         | I          |
| 2      | 38    | 1         | J          |
| 2      | 39    | 1         | H          |
| 2      | 40    | 2         | C, G       |
| 2      | 41    | 2         | F, H       |

## References

- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* **313**:966-968.
- Elmstrom, K. M., D. A. Andow, and W. W. Barclay. 1988. Flea beetle movement in a broccoli monoculture and diculture. *Environmental Entomology* **17**:299-305.
- Gripenberg, S., E. Morriën, A. Cudmore, J.-P. Salminen, and T. Roslin. 2007. Resource selection by female moths in a heterogeneous environment: what is a poor girl to do? *Journal of Animal Ecology* **76**:854-865.
- Gripenberg, S., O. Ovaskainen, E. Morriën, and T. Roslin. 2008. Spatial population structure of a specialist leaf-mining moth. *Journal of Animal Ecology* **77**:757-767.
- Gripenberg, S. and T. Roslin. 2005. Host plants as islands: resource quality and spatial setting as determinants of insect distribution. *Annales Zoologici Fennici* **42**:335-345.
- Hooper, D. U., F. S. Chapin III, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs* **75**:3-35.
- Power, A. G. 1988. Leafhopper response to genetically diverse maize stands. *Entomologia Experimentalis et Applicata* **49**:213-219.
- Power, A. G. 1991. Virus spread and vector dynamics in genetically diverse plant populations. *Ecology* **72**:232-241.
- Tack, A. J. M., S. Gripenberg, and T. Roslin. 2011. Can we predict indirect interactions from quantitative food webs? – an experimental approach. *Journal of Animal Ecology* **80**:108-118.
- Tack, A. J. M., O. Ovaskainen, P. J. Harrison, and T. Roslin. 2009. Competition as a structuring force in leaf miner communities. *Oikos* **118**:809-818.
- Tack, A. J. M., O. Ovaskainen, P. Pulkkinen, and T. Roslin. 2010. Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology* **91**:2660-2672.
- Turchin, P. 1998. *Quantitative analysis of movement: measuring and modelling population redistribution in animals and plants*. Sinauer Associates, Sunderland, MA, USA.

**Appendix B.** List of studied species.

**Table B1.** Systematic affinity and guild membership of the studied species. Taxa included in species-specific analyses are shown in bold.

| Genus: Species  | Order: Family               | Guild                    |
|---|-----------------------------|--------------------------|
| <i>Ancylis mitterbacheriana</i>   | Lepidoptera: Tortricidae    | Leaf folder              |
| <i>Andricus curvator</i>  | Hymenoptera: Cynipidae      | Galler                   |
| <i>Andricus fecundator</i>  | Hymenoptera: Cynipidae      | Galler                   |
| <i>Andricus inflator</i>  | Hymenoptera: Cynipidae      | Galler                   |
| <i>Caloptilia alchimiella</i>   | Lepidoptera: Gracillariidae | Leaf miner / Leaf folder |
| <i>Coleophora flavipennella</i> /<br><i>C. kuehnella</i>  | Lepidoptera: Coleophoridae  | Leaf miner               |
| <b><i>Cynips divisa</i></b>   | Hymenoptera: Cynipidae      | Galler                   |
| <i>Cynips longiventris</i>  | Hymenoptera: Cynipidae      | Galler                   |
| <i>Dyseriocrania subpurpurella</i>  | Lepidoptera: Eriocraniidae  | Leaf miner               |
| <i>Ectoedemia albifasciella</i>   | Lepidoptera: Nepticulidae   | Leaf miner               |
| <b><i>Heliozela sericiella</i></b>  | Lepidoptera: Heliozelidae   | Leaf miner / Borer       |
| <b><i>Neuroterus anthracinus</i></b>  | Hymenoptera: Cynipidae      | Galler                   |
| <i>Neuroterus quercusbaccarum</i>   | Hymenoptera: Cynipidae      | Galler                   |
| <b><i>Phyllonorycter harrisella</i></b> /<br><b><i>P. quercifoliella</i></b>  | Lepidoptera: Gracillariidae | Leaf miner               |
| <b><i>Profenusa pygmaea</i></b>   | Hymenoptera: Tenthredinidae | Leaf miner               |
| <b><i>Stigmella svenssoni</i></b> /<br><b><i>S. ruficapitella</i></b> / <b><i>S. roborella</i></b> /<br><b><i>S. samiatella</i></b> | Lepidoptera: Nepticulidae   | Leaf miner               |
| <i>Tischeria ekebladella</i>  | Lepidoptera: Tischeriidae   | Leaf miner               |