

NEWS AND VIEWS

OPINION

Community genetics in the time of next-generation molecular technologies

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Understanding the interactions of co-occurring species within and across trophic levels provides key information needed for understanding the ecological and evolutionary processes that underlie biological diversity. As genetics has only recently been integrated into the study of community-level interactions, the time is right for a critical evaluation of potential new, gene-based approaches to studying communities. Next-generation molecular techniques, used in parallel with field-based observations and manipulative experiments across spatio-temporal gradients, are key to expanding our understanding of community-level processes. Here, we introduce a variety of ‘-omics’ tools, with recent studies of plant–insect herbivores and of ectomycorrhizal systems providing detailed examples of how next-generation approaches can revolutionize our understanding of interspecific interactions. We suggest ways that novel technologies may convert community genetics from a field that relies on correlative inference to one that reveals causal

mechanisms of genetic co-variation and adaptations within communities.

Keywords: ectomycorrhizal symbiosis, forest ecosystems, gene-to-gene interactions, herbivorous insects, population genomics, quantitative trait analysis

Received 25 July 2011; revised 30 January 2013; accepted 16 February 2013

Community genetics aims to understand how genetic variation within and among populations of host species affects the composition of associated organisms interacting with the host (Agrawal 2003; Whitham *et al.* 2006; Johnson & Stinchcombe 2007; Rowntree *et al.* 2011; Wymore *et al.* 2011). Empirical community genetics has been stimulated by pioneering work on poplars (*Populus* spp.), their genotype-based phenotypic variation and associated communities (Whitham *et al.* 2006). However, community genetics has hitherto largely remained phenomenological, and the underlying genetic basis and processes involved in the interactions between host and associated organisms have not been studied in detail yet. Given the rapid development of molecular techniques (Rokas & Abbot 2009), it will soon be feasible to characterize the genomes of numerous members of a community. With whole-genome sequences or other types of -omics data at hand (Nadeau & Jiggins 2010), community genetics will be able to establish a solid genetic framework in which to understand the interplay between ecological and evolutionary processes (Rokas & Abbot 2009). Here, we sketch possible avenues along which research in community genetics may proceed, focussing in particular on how -omics may improve our understanding of the role of gene variants in species interactions. First, we argue for exploring spatio-temporal variation to investigate the fundamental ecological and evolutionary aspects of community genetics. Second, we describe how genomic, transcriptomic, proteomic, and metabolomic research can improve understanding of the interactions between trees as focal species and ectomycorrhizal fungi or herbivorous insects, the key players in forest ecosystems.

Community genetics in a spatio-temporal perspective

Let us consider populations of a focal species that start to diverge genetically. Genetic drift and/or selection may induce shifts in allele frequencies, leading to changes in the phenotypic traits mediating interactions with associated species that use the focal species as a host. First, these genetic changes and changes in the associated traits may lead to shifts in the occurrence and abundance of species already associated with the host. Second, the new phenotypic traits of the focal species may allow new species from

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¹See Appendix 1 for more details.

the regional species pool to colonize it. Finally, changes in the genetics of the host may induce evolutionary responses, including speciation events, in the associated organisms, which may feed back to evolutionary changes in the host.

If the above scenarios hold true, we expect the relatedness of host genotypes to co-vary with similarity among the communities of associated species (Bangert *et al.* 2006; Brändle Brandl 2006). Within species, such patterns have received considerable attention under the concept of the 'extended phenotype'. This concept was introduced by Richard Dawkins (1982) to describe the effects of genes on an individual's environment including other organisms. Whitham *et al.* (2003, 2005, 2006) adopted this concept and developed a framework for community and ecosystem genetics, which includes a feedback where an individual's phenotype is dependent on the interaction with other species.

Community assembly (Kraft *et al.* 2007; Emerson & Gillespie 2008) is shaped by successive filters, including regional species pool, habitat area and isolation (biogeographical filters), local environmental constraints (abiotic, biophysical filters) and biological interactions such as competition or predation (biotic filters; Fig. 1). The host genotype, interacting with the environment, may affect the structure of associated communities at several filtering steps by controlling phenotypic traits that allow associated organisms to locate, select and exploit resources of their host (Johnson & Agrawal 2005; Bailey *et al.* 2009) (Fig. 1). Thus, spatial variation in the composition of associated communities has a strong regional component.

Despite many reports demonstrating a correlation between genotypes of a focal species and the composition of associated communities, the fundamental ecological, genetic and evolutionary processes that generate this correlation remain poorly explored and require consideration in future studies. In this regard, three aspects deserve special attention: spatial variation, temporal variation and gene-to-gene interactions.

First, space needs to be better integrated into study designs. As noted above, the assembly of species depends on the regional species pool, whose phylogenetic and functional structure imposes a constraint on the emerging local communities (Fig. 1). A group of genotypes of a focal species in natural or experimental populations are embedded in a landscape context that may include forest patches, arable land, urban environment or other habitat types, each of which has different species pools that might interact with the focal species. As the associated community influences the fitness of the focal species, the relative fitness of these genotypes will vary across sites, even if the abiotic conditions are similar. However, in single common garden experiments, genotypes of a focal species are exposed only to one particular species pool. Therefore, regional replicates of such experiments are necessary to estimate the stability of relationships between genotypes of the focal species and communities of associated species. Such replicates would enable us to distinguish between mainly spatial effects and those that can be attributed to the interaction between host genotypes and associated organisms. Alternatively, one might set up more complex common gardens including particular treatments, for example, through fertilization or

irrigation. Such an approach would allow tests of the effect of genotype \times environment interactions on the assemblage of associated species for each local species pool. Furthermore, replicated common garden experiments would allow constructing reaction norms of different genotypes of the focal species. Do these genotypes respond differentially for their extended phenotypes to the changes in abiotic or biotic conditions across the testing sites? An initial step would be to identify the shape of the reaction norms (linear or quadratic) and then to estimate their variation among genotypes. Finally, the spatial context may also be dissected at the within-population level. For example, natural populations of trees usually exhibit strong spatial autocorrelation due to limited dispersal, which increases steadily over generations. On the other hand, random spatial genetic structure is observed in recently planted forests. One would therefore expect very different spatial structures of extended phenotypes among these strongly contrasting cultural regimes.

Second, community genetics should consider temporal variation in species interactions, for example, among seasons, among years along successional sequences and other types of temporal gradients. Traits involved in plant–herbivore interactions are known to change during plant ontogeny (Boege & Marquis 2005; Holeski *et al.* 2009), which is why communities of insect herbivores—and herbivory pressure—on seedlings and mature individuals may differ (Le Corff & Marquis 1999; Basset 2001). Furthermore, although associated communities may change within and between years due to fluctuations in plant phenotypes, equally they may change due to differences in weather conditions. Thus, the phenotypic traits that are important for species interactions in a particular season or year may change within and between years, and drawing conclusions from short-term experiments may be misleading. Although such traits, and the underlying genes, are genuinely involved in community interactions, their relative importance compared with other genes may vary in time and can therefore only be established in long-term experiments. Hundreds of insect generations interact with a long-living host such as a tree during its lifetime, and each generation experiences different biophysical constraints and trophic interactions with other fungi, herbivores or predators. As a consequence, even though insect populations can adapt to individual host genotypes (Mopper *et al.* 2000), the strength and direction of these adaptations are likely to change over time (moving targets; Ruhnke *et al.* 2006).

Moreover, genetic processes underlie the formation of adaptive demes and co-evolution between host and associated organisms (Fig. 1). At present, the number and type of genes involved and the associated phenotypes of interacting species are largely unknown. Recent technological advances enable researchers to sequence whole genomes and to monitor gene expression of interacting species, offering the potential to identify the candidate genes mediating the interactions between focal and associated species. Such approaches will move community genetics from studying anonymous genotype/phenotype effects to studying gene-to-organism, gene-to-gene and ultimately to

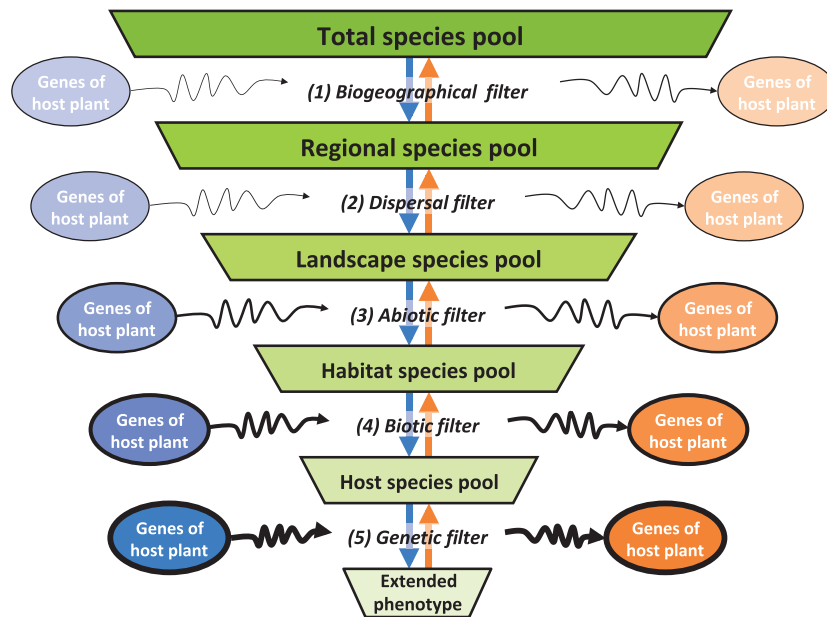


Fig. 1 How host plant genes might shape assemblages of associated organisms (blue pathway on the left). Several ecological filters drive the structure of communities associated with one host plant. Among associated species co-occurring within a region and determined by evolutionary and biogeographical processes (1, total species pool), local species assemblages depend on dispersal (2, landscape species pool) and habitat filters (3, habitat species pool). Dispersal filter refers to the ability of species to colonize the focal site. Habitat filters correspond to their capacity to develop and survive in a habitat, given abiotic constraints. Biotic interactions with the host species contribute to the shaping of a host species pool (4, biotic filter). Finally, variation among host plant genotypes may further select different associated communities, shaping the extended phenotypes. Genes of the focal host plant can interact with the four filters, as illustrated by the interaction between trees and associated insect herbivores: (1) There is evidence that pools of insect herbivore species of different tree families or genera are significantly different, probably owing to a long co-evolutionary process involving insect feeding traits and plant defence responses (Novotny *et al.* 2002); (2) insect herbivores use genetically controlled physical (e.g. shape, colour) and chemical cues (e.g. volatile organic compounds) provided by host plants to locate the plants; (3) trees can be seen as ecological engineers which can modify abiotic conditions that insects experience, for example, wind, moisture or light; (4) genes control plant phenotype and resistance traits that are deeply involved in interactions with insect herbivores (Schoonhoven *et al.* 2005); and (5) variants of host plant genes may ultimately induce quantitative changes in traits involved in plant–insect interactions with consequences for insect community structure (Crutsinger *et al.* 2008). Presumed reciprocal effects, through which associated organisms feed back to the composition of host genes, are depicted by orange colours (right side).

genome-to-genome interactions. While current research has focused on the few ‘genome-enabled’ species (Eklom & Galindo 2011), the many ongoing whole-genome projects will widen the array of study systems applying genomics data in the near future (e.g. <http://www.arthropodgenomes.org/wiki/i5K>, <http://1000.fungalgenomes.org/home/>, <http://pinenome.org/pinerefseq/>).

The following sections describe how the various types of -omics may stimulate community genetics and how they enable the genetic component of variation in community composition to be addressed at the level of variants in adaptive genes and their differential expression.

An example of functional genomics based on complete genome sequences: ectomycorrhizal symbiosis

Ectomycorrhizae, the mutualistic symbiosis between tree roots and a cortege of soil fungal partners, are the most widespread and species-rich associations in temperate and

boreal forests. Ectomycorrhizal fungi receive carbon from photosynthesis and, in turn, promote tree growth, enhance the survival of seedlings and increase the fitness of their plant partners under a wide range of environmental conditions. Despite the ecological significance of this mutualistic interaction, we have only started to explore its role for community ecology.

A breakthrough was the release of the first two full-genome drafts of mycorrhizal fungi, namely *Laccaria bicolor* (Basidiomycota) and *Tuber melanosporum*, the Périgord truffle (Ascomycota; Martin *et al.* 2008, 2010). Comparative genomics of the two mycorrhizal fungi indicated that they use different gene networks (‘molecular toolkits’) to establish symbiosis (Martin *et al.* 2010). There are vast differences between these two ectomycorrhizal genomes. *Laccaria bicolor* has a 65-Mb genome with more than 23 000 predicted proteins, which is the largest complement of genes known for any fungus, whereas *T. melanosporum* has the largest fungal genome so far with 125 Mb, but has only 7500 predicted genes, one of the smallest complement of

proteins in any filamentous fungal genomes sequenced so far. Also, whereas the secretion of effector-like small secreted proteins seems to be crucial for the establishment of the symbiosis in *L. bicolor* (Plett *et al.* 2011), these so-called mycorrhiza-induced small secreted proteins (MiSSPs) are not present in the transcriptome of *T. melanosporum* symbiotic tissue (Martin *et al.* 2010). In spite of these differences, some common features and some novelties emerged from the comparison with genomes of saprophytic and pathogenic fungi. Besides the loss of plant cell wall-degrading enzymes in ectomycorrhizae, an increase in the diversity and expression of nutrient transporters and signalling pathways (e.g. tyrosine kinases) in symbiotic tissues are hallmarks of mycorrhizal genomes (Martin *et al.* 2008; Kosti *et al.* 2010; Martin *et al.* 2010; Plett *et al.* 2011). These symbiosis-related genes are good candidates for gene expression studies of multispecies interactions in the field. On the tree side, it is not known how the host tree selects its symbiotic associates. Plant-encoded small secreted proteins may be required, as shown for nitrogen-fixing symbioses (Van de Velde *et al.* 2010). Genomic studies will probably be the only way to elucidate the mechanisms of interaction and to understand the effect of gene variants on this interplay. Therefore, we think that this system is an exciting model for community genetics in the -omics era.

Ectomycorrhizal fungi show a continuum of specialization to the host tree from strict specialists to generalists. Differences in the expansion of multigene families, in particular dynamic repertoires of genes encoding small secreted proteins and sugar-cleaving enzymes, might be responsible for the different host ranges of specialists, such as *T. melanosporum* and generalists, such as *L. bicolor* (Martin *et al.* 2010); that is, the genome expansion observed in *L. bicolor* might be driven by selection of the symbiont to exploit diverse substrates provided by multiple potential hosts and by diverse soils. As more genomes of mycorrhizal fungi are sequenced (Martin *et al.* 2011), this hypothesis will become testable.

In addition to the genomics of host-symbiont interactions, studies of geographical patterns of co-evolution add to our knowledge of processes leading to reciprocal adaptation and specialization. There are only a handful of studies reporting the structure of geographical variation and patterns of co-evolution in mycorrhizal interactions, indicating that these patterns are geographically highly variable (Hoeksema 2010; Hoeksema *et al.* 2012). To date, mostly higher-level traits, such as intensity of mycorrhizal colonization or growth of host trees, have been studied. Several of these studies found significant genetic variation in either the host plant or the mycorrhizal fungus in its ecological effect on the other partner. For example, the relationship between the colonization intensity of the ectomycorrhizal fungus *Thelephora terrestris* and the growth of its host, Lodgepole pine (*Pinus contorta*), depends on the tree's genotype (Karst *et al.* 2009). In poplar, both the intensity of colonization and the amount of enzymes secreted by poplar root tips colonized by *L. bicolor* are under the genetic control of the host (Courty *et al.* 2011). Similar findings come from arbuscular mycorrhizal systems, where host

identity has a strong effect on the fitness of different strains of *Glomus intraradices* (Ehinger *et al.* 2009).

An increasing body of evidence shows that subtle intraspecific differences in the genome of host plants determine the composition of interacting communities in mycorrhizal fungi (e.g. Korkama *et al.* 2006; Whitham *et al.* 2006; Sthultz *et al.* 2009; Karliński *et al.* 2010; Leski *et al.* 2010; Hoeksema *et al.* 2012). We have experimental evidence that such an intraspecific genetic variation in the host also affects the composition of interacting mycorrhizal populations (Hoeksema & Thompson 2007), but this has not yet been tested under natural conditions. To understand the links between structure and diversity of communities and ecosystem functioning, we need to know more about spatio-temporal patterns of genetic variation. There are indications that both interspecific (e.g. van der Heijden *et al.* 1998; Maherali & Klironomos 2007) and intraspecific (e.g. Johnson *et al.* 2012) diversity of mycorrhizal fungi can regulate productivity and ecosystem functioning. We advocate studies of community and population diversity in forests and combining them with functional field studies, involving both partners of ectomycorrhizal symbioses. Numerous new techniques are emerging for gene expression studies, marker gene evaluation using comparative genomics and enzyme activity profiling of whole ectomycorrhizal assemblages (Courty *et al.* 2010). The rapid development of high-throughput sequencing technologies facilitates the survey and comparison of whole microbial communities (Buée *et al.* 2009), although analysis, interpretation and publication of data still need to be optimized (Henrik Nilsson *et al.* 2012). Nevertheless, combined genotypic and functional studies are now feasible and may be expanded to natural and experimental gradients. Several reports indicate that soil microbe and mycorrhizal diversity differentially affect ecosystem functioning under different environmental conditions, for example, nutrient status (van der Heijden *et al.* 2008). We also know that plant-associated microorganisms are an important factor influencing plant responses to climate change (Courty *et al.* 2010; Pickles *et al.* 2012). Combined genotypic and functional studies in diverse environments will help to understand current patterns and to predict changes and effects in the future.

Associations between genes and traits: potential of next-generation approaches in community genetics

An essential part of future studies in community genetics will be to identify the genes that underlie the traits of hosts that affect associated organisms. For this, sequencing of the complete genome of a host species is not sufficient. Rather, it is essential to link the presence or action of particular variants of genes or genomic regions of a host plant to the presence or abundance of associated organisms or arrays of their genes. There are basically two strategies for this, namely quantitative trait analysis (QTL) mapping and genome-wide association studies (GWAS). We briefly outline and illustrate below the pros and cons of these two approaches for community genetics.

An example of QTL mapping of community traits of poplar is a study aimed at identifying genomic regions associated with susceptibility to insects (DeWoody *et al.* unpublished data). Parents and progeny of a poplar (*Populus trichocarpa* × *P. deltoides*) F2 mapping population were assessed for various categories of leaf damage, including chewers and skeletonizers. The damage levels significantly varied among offspring genotypes. Each category was treated as a quantitative trait in a QTL mapping approach, and more than ten QTLs were detected. QTLs also varied seasonally, suggesting that the insect community responds to traits and the underlying genetic variation over time. This underlines the importance of considering temporal variation in studies of community genetics, as noted above.

Another example is a study on QTLs affecting ectomycorrhizal symbiosis in a *P. deltoides* × *P. trichocarpa* F1 population (Labbé *et al.* 2011). Four identified QTLs were associated with candidate genes, and differential transcript levels were assessed with the help of a whole-genome microarray. The transcripts with the highest over-representation were, based on their gene ontology, in the repress defence mechanisms and in pathogen resistance.

Relatively few mapping populations have been produced for long-lived tree species, due to the length of time needed to maintain and study them and the high costs associated with it. As a single cross will not contain all alleles present in a large population of an outcrossing species, not all QTLs can be detected in a single cross, and most QTL interactions will go unnoticed. Hence, several populations are necessary, and producing them would be an important investment. Next to full-sib families, it may be possible to use full or partial diallel designs with multiple parents, so that more alleles are included and many more allele combinations can be studied, similar to MAGIC populations (Kover *et al.* 2009) but without the need for selfing to multiply and maintain the population.

In the meantime, an elegant alternative for forest trees is to use existing progeny trials. Many of these have been established and often replicated at different locations, and phenotypic data are usually available for extensive periods of time. Many trials consist of half-sib families, in which the alleles from the mother segregate in the progeny. If only a limited number of fathers were involved, genotyping may even allow them to be split into a few interconnected full-sib families. Common garden experiments often include a sample of the diversity of an area. When these experiments are replicated at multiple sites, it may be possible to perform genome-wide association mapping with the advantage of multisite/multiyear data.

An issue for community genetics, as mentioned above, is that the local species pool may be different between the locations of the trials. This can be tackled efficiently by replicating the populations and planting them in different locations. Replicated populations will also spread the risk of losing individual members of the populations.

After finding a QTL region based on the presence of an associated organism or, for example, damage caused by an insect species, the underlying mechanism can be unravelled, in this case by measuring the secondary compound

composition of all progeny trees and locating such traits on the genetic map. Co-localization of a compound with a QTL would suggest that it was responsible for the effect on the insects and that a structural or regulatory gene involved in its synthesis is located in that genomic region. In some species, this can be tested by mutant analysis, but it is not practical with trees. Alternatively, one could analyse the naturally occurring genetic variation in a large set of unrelated trees with different combinations of compounds and conduct association tests (i.e. GWAS).

Genome-wide association studies assume that, in the absence of population substructure, markers that are physically linked to a gene associated with a phenotype of a trait can be distinguished from markers that are not linked, as the latter are assumed to occur randomly in individuals of the population regardless of the phenotype (Nordborg & Weigel 2008). There is no need to construct a mapping population as in QTL detection, but a reference genome or a dense genetic map in combination with sufficient linkage disequilibrium (LD) is required (Kim *et al.* 2007). LD appears to be limited in tree species (Ingvarsson 2005; Heuertz *et al.* 2006; Pyhäjärvi *et al.* 2007), which implies that high-density genetic marker arrays are needed for applying association mapping and that many more individuals need to be studied. For instance, Fournier-Level *et al.* (2009) tested target candidate genes and identified the functional variation responsible for the observed variation in anthocyanin variation in grape by association analysis. The very low LD often encountered in natural tree populations (Neale & Savolainen 2004) will assist in finding many of the possible combinations of compounds, thus increasing the power of the association study. A new approach, becoming feasible because of high-throughput sequencing technology, is to pool and sequence DNA from multiple individuals within a population with clearly distinct phenotypes or habitat conditions (Turner *et al.* 2010) and to identify those markers across the genome that display a large difference in allelic frequency between the pooled groups (Holderegger *et al.* 2008). The advantage of this 'population resequencing' approach, which vaguely resembles bulked segregant analysis (BSA), is that no mapping population or extensive LD is necessary; the drawback is that an annotated genome is still needed for reference. As annotated genome sequences are increasingly becoming available, this will be less of a problem in the future. The approach can be readily extended to polygenic traits (Heard *et al.* 2010). A potential application to community genetics in trees would be to pool the DNA from trees that host a particular insect with DNA from those that do not and compare the sequenced genomes of the two groups.

Next-generation methods now enable genotyping by sequencing (Baird *et al.* 2008). In the context of segregating populations, restriction-site associated DNA (RAD) markers or transcriptome sequencing permit direct mapping-by-sequencing, thus skipping marker development altogether (Hartwig *et al.* 2012; Zhu *et al.* 2012). In QTL mapping, this solves the problem of generating dense maps, so that the limiting factor for high resolution is the number of recombinations or the size of the segregating

Table 1 From genes of focal species to traits of the extended phenotype—and back: questions and experimental considerations, related to (a) spatio-temporal variation, (b) the application of -omics approaches and (c) reciprocal effects to stimulate future studies in community genetics

Theme	Questions	Experimental considerations
(a) Spatio-temporal variation	To what degree do regional species pools determine the composition of organisms associated to particular genotypes?	Assess naturally occurring spatial replicates of particular genotypes, for example, agricultural, horticultural or silvicultural clones, and perform regionally replicated experiments using the same (set of) genotypes exposed to various regional species pools of potentially associated organisms
	What is the relevance of phylogeographical structure in host species for the composition of associated communities?	Consider genetic structure and evolutionary lineages of the focal species
	How do relationships between genotypes and associated organisms vary among seasons or among life stages?	Perform temporally replicated experiments or monitor natural communities across >1 year; establish long-term experiments with host plants from seedlings to mature adults
	How does landscape configuration, for example, differences in the relative abundance of, or connectivity among, particular habitat types, affect regional species pools and thus, the communities of associated organisms in a focal species?	Include landscape characteristics when setting up experimental plots or assessing natural communities
(b) -omics approaches	To which degree does phenotypic plasticity shape extended phenotypes?	Set up common garden experiments along ecological gradients including reciprocal transplants to test for genotype-by-environment interactions and reaction norms
	Which QTL relate to particular groups of associated organisms?	Establish various full-sib families or diallel crosses to include a wide range of allele variants
	What (classes of) compounds differ among host genotypes that are differentially affected by groups of associated organisms?	Genome/transcriptome sequencing of pools of host plants differing in their associated communities
	Do traits affecting community composition of associated species rely on single or multiple genes, and how large is their allelic variation within host populations?	Identify genes directly involved in the interaction, for example, through QTL mapping and quantify the degree of polymorphism using high-throughput, reduced-representation sequencing
(c) Reciprocal effects	Does one gene of a focal species influence a single, a group of, or all associated species?	Use feeding (herbivores) or inoculation (ectomycorrhizae) experiments and perform co-expression profiling and subsequent protein annotation
	How many such genes exist, given that a focal species may interact with hundreds of associated species?	Perform gene expression studies of focal species that are experimentally associated with different single species or groups of species of associated organisms
	How do different groups of associated species induce changes in the phenotypic traits (and the underlying allele frequencies) of the host?	Expose the same (set of) hosts to different (sets of) associated species and test for changes in traits and allele frequencies over time
	What genes in host and associated species determine whether they interact as generalists or specialists?	Combine comparative genomics and expression profiling among generalists and specialists in both hosts and associated species

QTL, quantitative trait analysis.

population. As forest trees have very small LD, the ability to generate high volumes of genomic data is a very promising development for GWAS.

Gene expression profiling, a complementary approach to association genomics as a strategy for functional genomics, is also being revolutionized by developments in next-generation technologies. Gene expression profiling has been applied to study stress response in trees, for example, following insect attack where transcript analyses by cDNA microarray profiles have been combined with 2D protein and protein spectrometric analyses (Lippert *et al.* 2007). In this pioneering work on pines and pine weevils, the authors demonstrated that transcripts and their proteins were complementary. Next-generation sequencing of tagged cDNA ends now enables researchers to quantify the number of transcripts from different subsets of individuals (Xu *et al.* 2009). Given the availability of gene annotations, the transcripts will be associated with gene models and their regulators using publicly available databases. We expect that co-expression profiling will become feasible for populations as well as for individual ontogenetic stages of interacting species. Such an approach may also be scaled up from two-species interactions to multiple-species interactions, that is, a true 'community transcriptome' approach.

Proteomic approaches allow for an efficient and simultaneous detection of the proteins in a sample. The proteome composition to some extent integrates fluctuations in expression over a period of time, thus potentially being robust with regard to sampling time in the field. The identification of peptides relies on either a large, high-quality RNA-seq data set, a complete set of alleles from a multi-gene family, or the genome sequence. An example is the use of peptide identification (Q-TOF LC-MS^E) for fast screening of Bet v 1 isoforms in pollen of various birch species, as it was possible to determine both presence and relative abundances of individual isoforms (Schenk *et al.* 2009). For this, the mass spectra obtained from the pollen were compared with a set of predicted peaks based on a complete set of isoforms obtained by sequencing the genes. In species for which the genome sequence or a large amount of transcriptome data are available, this prediction becomes a relatively simple bioinformatics exercise.

Other -omics techniques, such as metabolomics, may be employed in similar experimental schemes. Recent advances have increased the sensitivity and throughput of metabolomics and proteomics assays ('next-gen biochem'). Now, one can directly map QTL controlling the metabolic profile of all offspring of a cross. For instance, untargeted GC-TOF-MS metabolite profiling allowed mapping of 100 mQTLs (Carreno-Quintero *et al.* 2012). The main drawbacks of metabolomics are the higher costs and the problem of interfering factors due to the different growing conditions of the trees included in the association analysis. Moreover, the samples cannot be all taken at the same time. On the other hand, the compounds measured are also the ones that affect the interaction with associated insect species. So if genetic variation in multiple genes affects the content of

one important compound, the association of the compound with presence or absence of one or more insect species will be stronger than that of each of the underlying genes, and the association will also be more informative on the mechanism of the interaction. Even GWAS could be carried out in this way. In our example using a pool of trees including those that host a particular insect and those that do not, a comparison of compounds may be more straightforward than comparing DNA markers. In particular, if the insect is not always present on the same trees across years, the compounds present in each tree in each year could reveal a strong correlation, whereas the genes that enable the tree to produce the compounds would not.

If, as indicated above, a compound affects the presence of insect species, then one would expect, reciprocally, the presence of catabolites of the compound in insect species that tolerate the compound, when these insects are sampled on the trees that produce it. This can be used to experimentally validate the statistical associations between compounds in the tree and the presence of insect species or guilds, and for a starting point for understanding the mechanisms behind the interactions between trees and insects.

Perspectives

A suite of -omics approaches are available to pave the way for studying entire communities. Accordingly, we need to refine hypotheses and develop suitable study designs and statistical tools (Augustin *et al.* 2010; Ovaskainen *et al.* 2010), which will improve implementation once reduced costs make these tools applicable to large-scale sampling of community-level interactions (Table 1).

As outlined above, we see two main directions that should be followed in community genetics to substantiate inference on the interplay of genes, organisms, communities and their respective environments. First, joint descriptive and experimental studies should include spatial and temporal gradients to account for environmental variation in these dimensions (Thompson 2005; Crutsinger *et al.* 2009; Tack *et al.* 2010). Second, researchers in community genetics should make better use of the exponentially increasing genomic information becoming available, which will require solid expertise in bioinformatics. If this is achieved, gene-to-gene interactions can be explored in individual-based associations and at the level of entire communities and shift community genetics towards becoming community genomics.

Moreover, community genetics goes beyond the effects of genotypes in one species on the community of associated organisms. We also need to consider the reciprocal effects of how associated communities shape the genotypic composition of their hosts and of how the genotypes of associated species affect host communities (Fig. 1). There are virtually no studies available on this aspect of community interactions, which leaves a wide-open field of empirical research for the future. Exploring reciprocal interactions might help to extrapolate population genomics

and quantitative genomics of focal species. We will then need to adopt a community-based understanding of selection and drift as well as to include G x G x E interactions into reaction norm calculations. However, elaborating on this subject goes beyond the scope of the present article.

In conclusion, we believe that the amalgamation of traditional population genetics, quantitative genetics and ecology, fostered by the advent of new genomic technologies, will revolutionize our perception of community and ecosystem processes and push community genetics into a new era.

Acknowledgements

Our sincere thanks go to Rolf Holderegger and the anonymous reviewers for helpful comments on earlier versions of the manuscript. Karen A. Brune and Stephen Cavers provided linguistic editing. This work was financed through the EC-supported Network of Excellence Evoltree (GOCE-016322).

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All authors have conceptually contributed to the content of the present article through their active involvement and the many discussions held during the EvolTree Jointly Executed Research Activity 3 (JERA 3) on community ecology, which was managed by B.Z., F.G. designed and organized the article preparation, F.G., R.B., B.C., H.J., M.P., M.J.M.S. and B.Z. drafted the various sections, and all main authors participated in the writing of the final text.

doi: 10.1111/mec.12300

Appendix 1

The EC-supported Network of Excellence Evoltree (<http://www.evoltree.eu>) formed a group of scientists involved in and actively contributing to Jointly Executed Research Activities on community genetics in forest ecosystems. In addition to the main authors, the group includes S. Augustin, M. Brändle, C. Burban, J. Burczyk, S. Cavers, I. Chybicki, C. Conord, E. Cremer, J. DeWoody, K. Donges, B. Fady, L. Karlinski, C. Kerdelhué, B. Kieliszewska-Rokicka, G. Kost, M. Kulczyk-Skrzeszewska, F. Lakatos, F. Lefèvre, S. Liepelt, S. Oddou-Muratorio, K.-H. Rexer, M. Rudawska, M. Schädler, G. Taylor, K. Tuba, M. Viger, F. Villani, M. Villar.