

Can we predict indirect interactions from quantitative food webs? – an experimental approach

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Summary

1. Shared enemies may link the dynamics of their prey. Recently, quantitative food webs have been used to infer that herbivorous insect species attacked by the same major parasitoid species will affect each other negatively through apparent competition. Nonetheless, theoretical work predicts several alternative outcomes, including positive effects.

2. In this paper, we use an experimental approach to link food web patterns to realized population dynamics. First, we construct a quantitative food web for three dominant leaf miner species on the oak *Quercus robur*. We then measure short- and long-term indirect effects by increasing leaf miner densities on individual trees. Finally, we test whether experimental results are consistent with natural leaf miner dynamics on unmanipulated trees.

3. The quantitative food web shows that all leaf miner species share a minimum of four parasitoid species. While only a small fraction of the parasitoid pool is shared among *Tischeria ekebladella* and each of two *Phyllonorycter* species, the parasitoid communities of the congeneric *Phyllonorycter* species overlap substantially.

4. Based on the structure of the food web, we predict strong short- and long-term indirect interactions between the *Phyllonorycter* species, and limited interactions between them and *T. ekebladella*. As *T. ekebladella* is the main source of its own parasitoids, we expect to find intraspecific density-dependent parasitism in this species.

5. Consistent with these predictions, parasitism in *T. ekebladella* was high on trees with high densities of conspecifics in the previous generation. Among leaf miner species sharing more parasitoids, we found positive rather than negative interactions among years. No short-term indirect interactions (i.e. indirect interactions within a single generation) were detected.

6. Overall, this study is the first to experimentally demonstrate that herbivores with overlapping parasitoid communities may exhibit independent population dynamics – or even apparent mutualism. Hence, it proves the potential for versatile indirect interactions in nature, and suggests that the link between patterns in food web structure and realized population dynamics should be verified by rigorous experiments.

Key-words: apparent competition, apparent mutualism, delayed density-dependent parasitism, density-dependent parasitism, indirect density-mediated interactions, indirect trait-mediated interactions, spatial scale, temporal scale

Introduction

The question of what structures communities of herbivorous insects is currently at the core of ecology (Morris, Lewis &

Godfray 2004; Lewinsohn & Roslin 2008). Given the ecological and economic importance of these taxa (Pimentel & Andow 1984; Strong, Lawton & Southwood 1984; Losey & Vaughan 2006), understanding the factors affecting the relative abundances of insect species is a prerequisite for understanding global ecosystem functioning. Among these factors, the idea that herbivore species may affect each other through shared natural enemies has been gaining interest over the past 10 years (e.g. van Veen, Morris & Godfray 2006). In particular, many studies have suggested a general role for apparent competition (i.e. for negative indirect

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interactions; Williamson 1957; Holt 1977) by pointing at the quantitative overlap in the parasitoid community among herbivores, as revealed by quantitative food webs (e.g. Müller *et al.* 1999; Rott & Godfray 2000; Lewis *et al.* 2002; van Veen, Morris & Godfray 2006; Hirao & Murakami 2008; Nakamura & Kimura 2009). However, the link between patterns revealed in quantitative food webs and apparent competition may be relatively weak. Theory predicts that indirect interactions by shared parasitoids can, in fact, result in outcomes ranging from apparent mutualism to apparent competition (Holt & Kotler 1987; Holt & Lawton 1994; Abrams, Holt & Roth 1998; Brassil & Abrams 2004). Moreover, empirical support for predictions based on quantitative food webs from phytophagous insects is lacking, and to our knowledge only a single study involving herbivorous insects has actually tested such quantitative predictions through experiments (Morris, Lewis & Godfray 2004). Hence, to use quantitative food webs to infer a general role for apparent competition in structuring herbivore communities may still be premature.

Another aspect deserving more experimental scrutiny is the temporal and spatial scales over which indirect interactions may occur (Holt & Lawton 1994; van Veen, Morris & Godfray 2006). Temporally, indirect interactions are frequently subdivided into short-term vs. long-term indirect interactions (Holt & Kotler 1987; Holt & Lawton 1994). Short-term indirect interactions occur within a single generation, and are typically due to behavioural responses of the parasitoids to (relative) host densities (Holt & Kotler 1987). Long-term indirect interactions take place over multiple generations, and are due to changes in densities of the hosts and parasitoids (Holt 1977). In theory, both short- and long-term effects of shared parasitoids can lead to either apparent competition or apparent mutualism. For example, short-term apparent mutualism could be caused by predator satiation, prey switching and selectivity (Holt & Kotler 1987; Holt & Lawton 1994; Abrams & Matsuda 1996), whereas cycling dynamics of hosts and parasitoids can lead to apparent mutualism in the long term (Abrams, Holt & Roth 1998; Brassil 2006). Apparent competition can occur in the short term when parasitoids select high density patches for foraging (Holt & Kotler 1987) and in the long term in stable populations (Holt 1977). From a spatial perspective, both the sign and strength of indirect interactions may change with the scale of the study (Heads & Lawton 1983; Wiens 1989; Levin 1992).

To assess the strength and consequences of indirect interactions between prey species sharing parasitoids at multiple temporal and spatial scales, we focus on three host taxa within a leaf miner-parasitoid community. We first construct a quantitative food web, and use it to generate predictions regarding how strongly different species interact through shared enemies. We then test these predictions by manipulating leaf miner densities and examining short- and long-term responses in terms of parasitism and larval survival. Finally, we compare our experimental results with patterns observed in a long-term survey of leaf miner densities and survival rates across 20 unmanipulated oak trees. To investigate

whether interactions change with spatial scale, we use three hierarchical spatial scales present in the system: the level of leaves within trees, the level of the individual tree, and the level of the landscape with multiple trees.

Based on the combined evidence, we answer the following questions: (i) To what extent does the parasitoid community overlap among the studied leaf miner species? (ii) Does the quantitative food web allow us to predict how a change in the density of one species affects the density and performance of another? (iii) Does the measured sign and strength of indirect interactions depend on the spatial and temporal scales examined?

Materials and methods

STUDY SYSTEM

For several reasons, leaf miner communities are particularly likely to be structured by indirect interactions mediated by shared parasitoids (Holt & Lawton 1993). First, the generation time of parasitoids is relatively similar to that of their host (in contrast to vertebrate predator-prey systems), allowing for a numerical response in parasitoid densities at the same time scale as host recruitment (Holt & Lawton 1993). Secondly, parasitoids often strongly reduce host densities, preventing competition among herbivores for plant resources (Hawkins 1994; Hawkins, Cornell & Hochberg 1997) and suggesting an important role of parasitoids in leaf miner population dynamics. Thirdly, parasitism is more frequently density-dependent in leaf miners than in other insect groups (Stiling 1987). Fourthly, (semi)quantitative food webs show that parasitoid communities of individual leaf miner species within a community often strongly overlap (Miller 1973; Askew & Shaw 1974; Memmott, Godfray & Gauld 1994; Rott & Godfray 2000; Hirao & Murakami 2008).

Given these considerations, we focus our study on a subset of the leaf-mining community on the pedunculate oak (*Quercus robur* L.), consisting of three genera of lepidopteran leaf miners in southwestern Finland: *Tischeria* Zeller (Lepidoptera: Tischeriidae), *Phyllonorycter* Hübner (Lepidoptera: Gracillariidae) and *Stigmella* Schrank (Lepidoptera: Nepticulidae).

The larva of the univoltine *Tischeria ekebladella* Bjerkander forms a shallow, blotch-like leaf mine on the upper side of the leaf blade, whereas the bivoltine leaf miners *Phyllonorycter quercifoliella* Zeller and *Phyllonorycter harrisella* Linnaeus form tent-like mines on the underside of the leaf. The four species in the genus *Stigmella* (*S. ruficapitella* Haworth, *S. roborella* Johansson, *S. samiatella* Zeller and *S. svenssoni* Johansson) produce linear leaf mines. *Tischeria* and *Phyllonorycter* hibernate within the abscised leaves, whereas *Stigmella* exits the leaf to pupate. All three genera overlap temporally during a major part of the season (Roslin, unpublished data). The mines of individual *Phyllonorycter* and *Stigmella* species cannot be distinguished in the field surveys based on mine morphology, and the genera are hence treated as compound groups in part of the material.

Importantly, the genus *Stigmella* is relatively rare in most years (see Appendix S1), and its impact on other species hence presumably small. From a practical perspective, it is also hard to obtain adequate material of *Stigmella* for rearings and introductions. Yet, large-scale rearing from leaf mines collected across the whole island of Wättkast in 2006 and 2007 shows that *Stigmella* does share parasitoids with *T. ekebladella*, *P. quercifoliella* and *P. harrisella* (R. Kaartinen and T. Roslin, unpublished data). Therefore, we decided to exclude this taxon from the construction of quantitative food webs and from the

field manipulations, but to include it in our analyses of wild leaf miners on the observational and experimental trees.

EXPERIMENTAL DESIGN

Manipulating herbivore densities on experimental trees

To be able to address both short- and long-term indirect interactions between leaf miner species at the scale of individual host trees, we manipulated the densities of three leaf miner species on medium-sized oaks (height 4–8 m) on the island Wattkast, southwestern Finland. The manipulations took place in the summer of 2007, with the experimental trees located in an area of about ten hectares. Forty trees were randomly assigned to one of four treatments: (i) the Control treatment, i.e. unmanipulated trees ($n = 15$ trees), (ii) the *T. ekebladella*-treatment, i.e. trees on which densities of *T. ekebladella* were artificially increased ($n = 10$ trees), (iii) the *P. quercifoliella*-treatment, i.e. trees on which densities of *P. quercifoliella* were artificially increased ($n = 10$ trees) and (iv) the *P. harrisella*-treatment, i.e. trees on which densities of *P. harrisella* were artificially increased ($n = 5$ trees). The lower sample size in the latter treatment was due to a lack of moth material.

To increase leaf miner densities on the treatment trees, we introduced 15–17 moth pairs (♀♂) onto the tree in muslin bags (50 × 60 cm) tied around branches. These introductions resulted in a 3–5 fold increase in leaf mine densities of the focal species on the tree. The muslin bags prevented the adult moths from dispersing, and the adult moths and bags were removed before the larvae hatched and initiated the leaf mines. Hence, parasitoids had access to the leaf mines during the full developmental period of the mines. (No egg parasitoids are known from our focal species.) Bags were also applied to the control trees to account for any potential effects of ‘bagging’. The introductions were made for *T. ekebladella* from 10 June to 16 June, for *P. quercifoliella* from 6 July to 20 July, and for *P. harrisella* from 18 July to 7 August. These time periods closely matched the natural phenology of the univoltine *T. ekebladella* and of the second generation of the two bivoltine *Phyllonorycter* species (T. Roslin, unpublished data).

Quantitative food webs, direct density dependence and short-term indirect effects

To obtain material for constructing a quantitative food web and to test for short-term interactions, we introduced three additional *T. ekebladella* and three additional *P. quercifoliella* bags to each experimental tree. (Due to lack of moth material, *P. quercifoliella* was not introduced to the control trees; see Fig. 2a. For the same reason, we only introduced moths of *P. harrisella* to the *P. harrisella*-treatment.) These introductions were applied at the same time as the introductions for the experimental treatments (above; this material will later be referred to as the ‘sentinel material’). To limit the number of eggs oviposited on each leaf, we removed egg-laying females after *c.* 5 days. Given our control over the number of sentinel mines, the number of leaf mines added inflated local densities by *c.* 20%, compared to the 3–5 fold increase in densities caused by treatments (see above under Manipulating herbivore densities on experimental trees). Hence, we assumed the effect of these individuals on local densities to be negligible, whereas importantly, they allowed us to establish exact host-records for the parasitoids of the two *Phyllonorycter* species indistinguishable on the basis of leaf-mine morphology (Miller 1973). From each of the sentinel bags, we reared *ca.* 30 mines, resulting in 90 mines per tree (or fewer if fewer mines were available). To allow as much time as possible for natural development, these leaf

mines (total $n = 4376$) were collected in late September (2007), right before natural leaf abscission. Leaf mines were placed individually in plastic jars and stored in an underground cellar for hibernation. In early spring, the material was moved to indoor temperature to stimulate leaf miner and parasitoid emergence.

Delayed density dependence and long-term indirect effects

To quantify delayed density-dependent parasitism among conspecifics, and indirect interactions between years among heterospecifics, we revisited the experimental trees 1 year after the experimental manipulations. In autumn 2008, we assessed the performance of three taxa naturally present on the experimental trees: the first generation for the univoltine *T. ekebladella*, and the second generation for the bivoltine *Phyllonorycter* and *Stigmella*. (For the latter two genera, we treated the morphologically indistinguishable taxa within genera as compound species; cf. above.) Performance was scored by searching the foliage of 100 haphazardly selected shoots, and classifying each individual found as belonging to one of three categories: (i) parasitized larvae (parasitoid exit hole or parasitoid larva visible), (ii) larvae dead for other reasons and (iii) live larvae. This classification scheme allows us to evaluate the full extent of parasite impact, since differences in overall survival among treatments – i.e. {(all larvae) – (parasitized larvae) – (larvae dead for other reasons)} / (all larvae) – will encompass parasitoid-induced mortality beyond that detectable as parasitoid larvae or emergence holes (e.g. host feeding by adult parasitoids or mortality of parasitoid-infected host; Tack and Roslin, pers. obs.).

To increase sample size and statistical power, to distinguish between the morphologically similar *P. quercifoliella* and *P. harrisella*, and to quantify variation in parasitism and survival rates both within and among trees, we also introduced low densities of *T. ekebladella* and *P. quercifoliella* on each experimental tree in spring 2008. To spread the resulting leaf mines across the trees, for each of the species we introduced the adult moths in 7 bags per tree, and removed them as soon as more than 10 eggs were found. Importantly, this increase in mine density across trees was well within the range of natural variation in mine abundances between years (see Appendix S1) and – as it was evenly applied to all trees – it will not interfere with our assessment of treatment effects. At the end of development (midsummer for the bivoltine *Phyllonorycter* and autumn for the univoltine *T. ekebladella*), we examined all leaf mines resulting from these introductions and assigned them to the same categories as above (i.e. parasitized larva, larva dead for other reasons, or live larva).

To examine if experimentally increased densities in year t (2007) have effects that propagate through multiple generations, we revisited the experimental trees in year $t + 2$ (autumn 2009), scoring miner performance as outlined above. Due to logistic limitations, we sampled only a subset of the experimental trees in 2009, as based on the results in the previous year (see Results on Delayed density dependence and long-term indirect effects). In particular, we scored the survival and parasitism rate of *T. ekebladella* on the Control trees and trees with an increased density of *T. ekebladella* in 2007. Further, we scored the survival of *Stigmella* on the same set of trees, as the survival of *Stigmella* differed significantly among these two treatments in 2008 (see Results on Delayed density dependence and long-term indirect effects).

OBSERVATIONAL STUDIES

To test whether patterns observed in the quantitative food webs and indirect interactions detected in the experiment are reflected in

natural population dynamics, we used data from a large-scale survey of wild leaf miners. From 2003 to 2009, the spatiotemporal population dynamics of focal leaf miner taxa have been annually monitored on twenty medium-sized trees ($n = 18$ in 2008 and 2009). These trees are confined to a dense oak stand within the same part of the landscape as the experimental trees. In each year, we sampled the density (years 2003–2009) and larval survival (2004–2009) of leaf miner individuals on approximately 500 leaves per tree (mean = 535.3; SD = 159.7; see Gripenberg, Salminen & Roslin 2007 for more information on the sampling design).

STATISTICS

Quantitative food webs

To generate predictions regarding the strength of indirect interactions among our focal herbivore species, we constructed both a quantitative food web and corresponding matrices of quantitative parasitoid overlap. For constructing the quantitative food web, we used data on the rearings of sentinel larvae introduced to the trees in 2007. For deriving *a priori* predictions regarding long-term indirect interactions, we subsequently adjusted the quantitative structure of the web to match host densities imposed by experimental treatments.

Overall, our approach was then built on using data on host abundances and interaction frequencies between specific host and parasitoid species in one year to predict overall parasitism rates in the next. In doing so, we explicitly used data on the same local food webs (oak trees) across years, for two clear-cut reasons: First, our null hypothesis posits the entity predicted (overall parasitism rate) to be independent of the variable manipulated in the experiment (conspecific and heterospecific host density). Only if the host species are linked by their natural enemies should we find a match between *a priori* predictions and the experimental outcome. Secondly, parasitism rates and interaction structure vary substantially in space and in time (Müller *et al.* 1999; Gripenberg & Roslin 2008; see also Appendix S1 of current paper). Hence, to offer a strong test of the hypothesis, specific predictions for what should occur in a given place at a given time must be based on what occurred there before (If based on an independent system, any mismatch between prediction and outcome might be attributed to spatiotemporal variation in actual interaction structure).

For predicting long-term interactions, we calculated the quantitative parasitoid overlap d_{ij} , which represents the fraction of parasitoid individuals that emerge from host species j , and that subsequently attacks host species i , and is defined as $d_{ij} = \sum_k \left[\frac{\alpha_{ik} \alpha_{jk}}{\sum_l \alpha_{il} \sum_m \alpha_{mk}} \right]$ (Müller *et al.* 1999). Here, α_{ik} is the interaction frequency among host i and parasitoid k in the interaction matrix, the sums of k and l both include all parasitoids, and the sum of m includes all hosts. The first quantity within the square brackets represents the fraction of parasitoids of host i that belongs to species k , whereas the second quantity is the fraction of parasitoids of species k that develops on host species j . Hence, d_{ij} equals zero when no parasitoids are shared among species i and j , and equals one when all parasitoids on species i develop on species j . As such, d_{ij} has frequently been interpreted as the ‘potential for apparent competition’ (e.g. Müller *et al.* 1999; Nakamura *et al.* 2008), but will hereafter be referred to as ‘potential for indirect interactions’. In addition, d_{ii} gives the fraction of parasitoids that emerges from leaf miner i and continues to attack leaf miner i in the next generation, indicating the ‘potential for delayed density-dependent parasitism’.

As the potential for indirect effects depends upon host densities, we calculated the d_{ij} values separately for each of the four treatments

(Control treatment, increased density of *P. harrisella*, increased density of *P. quercifoliella*, and increased density of *T. ekebladella*; note that the d_{ij} -values are calculated across trees within each of these treatments). In doing so, we used data on the observed natural abundance of the leaf miner individuals in year 2007 (Appendix S1), the realized abundances of the introduced larvae in each treatment, and the fraction of leaf mines of each species from which parasitoids emerged (as derived from the same data used for the construction of the quantitative food web). As the parasitoid communities and parasitism rates were similar across treatments (see Results on Density-dependent parasitism and short-term indirect interactions), we merged the rearing data from the different treatments for each leaf miner species before calculating the ‘potential for indirect interactions’ and assessing the species-specific parasitism rates. Hence, differences in d_{ij} -values across treatments are caused by the experimental manipulation of leaf miner densities. All matrices were generated using the package bipartite (Dormann, Gruber & Fründ 2008), version 1.12, in R (R Development Core Team 2009).

Direct density dependence and short-term indirect interactions

Direct density-dependent parasitism and short-term indirect interactions were assessed by examining parasitism of the sentinel individuals introduced in 2007. To test for an effect of density dependence and indirect interactions within a single season, we first assessed whether the parasitoid community structure (i.e. the relative abundance of parasitoid species) was affected by the treatments. For this, we used a GLMM to model the fraction of parasitized leaf mines as a function of ‘parasitoid species’, ‘treatment’ and the interaction ‘treatment × species’ (all treated as fixed effects). Here, the term ‘parasitoid species’ adjusts for differences in mean abundance among parasitoid species, ‘treatment’ estimates differences in parasitism rate among trees with different densities of host species, and the interaction ‘treatment × species’ captures the effect of real interest: whether parasitoids respond differently to different treatments. Since we were modelling a proportion (parasitism rate), we assumed a binomial distribution and a logit link function. A separate model was constructed for each leaf miner species.

To quantify differences in total parasitism rate among treatments, we built species-specific models of larval parasitism (0/1) as a function of ‘treatment’ (fixed effect). To account for density-dependent parasitism at the leaf-level, we included the number of conspecific mines on the leaves as a covariate. To account for (and quantify) variation among trees and among branches, we included the random variables ‘tree’ (nested within ‘treatment’) and ‘branch’ (nested within ‘tree × treatment’). The response variable was assumed to follow a binomial distribution with a logit link, and we used Kenward–Roger’s approximation to estimate relevant degrees of freedom. Whether or not the variance associated with a given random effect significantly exceeded zero was evaluated using log-likelihood ratio tests (Littell *et al.* 2006). All models were fitted and tests conducted using proc GLIMMIX in SAS 9.2.

Delayed density dependence and long-term indirect interactions

To assess whether the densities of conspecifics and heterospecifics in 1 year affected a leaf miner species in the following year, we modelled both survival and parasitism of sentinel individuals introduced in 2008 (*T. ekebladella* and *P. quercifoliella*) as a function of the variable ‘treatment’ (fixed effect). Separate models were constructed for each

species and response. To quantify variation among trees and among branches within trees, we included the random variables ‘tree’ (as nested within ‘treatment’) and ‘branch’ (as nested within ‘tree × treatment’).

To verify the results derived from introduced larvae, we constructed similar models for two other data sets: individuals that occurred naturally on the experimental trees in 2008 and 2009. Here, we also included individuals of the genus *Stigmella*, which had strongly increased in density from 2007 to 2008 (see Fig. 1 in Appendix S1). For each taxon separately (*T. ekebladella*, *Phyllonorycter*, *Stigmella*), we built a model of the fraction of parasitized individuals at the tree-level as a function of treatment, and another model of the fraction of individuals alive.

For both survival and parasitism, we assumed a binomial distribution and a logit link. We used Kenward–Roger approximation to estimate relevant degrees of freedom, log-likelihood ratio tests for assessing the significance of random effects (see above), and Proc GLIMMIX in SAS 9.2 for implementation.

Observational studies

To compare the strength of indirect interactions observed in our experiments with community structure and population dynamics observed in the wild, we assessed whether there is any evidence for short- or long-term indirect effects among species in our long-term observational data. As the sign and strength of indirect interactions may depend on the spatial scale, we selected two hierarchical levels for our analyses: at a small scale, we assumed that each tree sustained a separate, local insect community, whereas at a landscape scale, we assumed that all trees were linked by frequent movements of parasitoids and together formed a single community. (Unfortunately, the scale of parasitoid dispersal is still unknown in our study system.)

If indirect interactions play a key role in population dynamics of the leaf-miner species on individual trees, we expect the survival rate (and hence population growth) to reflect differences in parasitoid build-up in the previous year. Therefore, we examined whether the fraction of individuals alive in a given year (2008) was detectably influenced by the log (density + 1) of conspecific and heterospecific individuals in the current year (2008, reflecting contemporary density dependence) and/or in the previous year (2007, reflecting parasitoid build-up from the previous year). Separate models were built for each herbivore taxon. Since we were dealing with three taxa (*T. ekebladella*, *Phyllonorycter* and *Stigmella*), each model contained six explanatory variables: conspecific density in year 2007, conspecific density in year 2008, heterospecific density of species *j* in 2007, heterospecific density of species *j* in 2008, heterospecific density of species *k* in 2007 and heterospecific density of species *k* in 2008.

As we used many explanatory variables, we selected a more compact model using the *stepAIC* procedure of R, where each maximal GLM with six predictors was reduced to a minimum adequate GLM by choosing between fits by minimizing the Akaike Information Criterion (Crawley 2007). As we were modelling proportions, we assumed that the errors were distributed either binomially or quasi-binomially (the latter indicating overdispersion), and applied the corresponding link function (Crawley 2007). As it is currently not possible to use *stepAIC* with quasi-binomial errors, binomial errors were used for model selection and the final model was reanalysed with quasi-binomial errors to derive the reported results.

To test whether indirect interactions change when extending the spatial scale, we modelled larval survival rate of our focal leaf miner taxa during the period 2004–2009 as a function of landscape-level

densities (average density across all 20 trees; $n = 18$ trees in 2008 and 2009) of conspecifics and heterospecifics in the current and previous year. As we had observational data for only 7 years (hence, six transitions), we could not simultaneously enter variables reflecting the landscape-level densities of conspecifics and heterospecifics in both the current and the previous year (cf. above), since that would have produced an over-saturated model. Instead, we modelled landscape-level survival rates of each focal leaf miner taxa in the form of multiple single regressions.

In theory, there may be a time lag of more than 1 year among the growth of the leaf miner population and the increase in parasitism rate. However, as visual examination of the population dynamics at both the landscape scale and tree scale (see Fig. 1 in Appendix S1) did not reveal any clear patterns, we refrained from increasing the number of tests (e.g. correlating survival rate with densities of conspecifics in year $t - 2$ or $t - 3$).

Results

QUANTITATIVE FOOD WEB

Each of our three focal leaf miner species was attacked by multiple parasitoid species ($n = 7$ –8; Fig. 1a and Appendix S2). A large fraction of these parasitoid species attacked more than one host species, with each pair of leaf-miner species sharing a minimum of four parasitoid species (Fig. 1a). Quantitatively, interaction frequencies varied strongly among host-parasitoid pairs (Fig. 1a). *Tischeria ekebladella* was most frequently attacked by the parasitoid species *Chrysocharis nautius* (Walker), which relatively rarely attacked *P. quercifoliella*, and was never found on *P. harrisella*. In contrast to *T. ekebladella*, the parasitoid complex of the two *Phyllonorycter* species was not dominated by any single species. Among themselves, *P. quercifoliella* and *P. harrisella* shared a relatively similar parasitoid community, and the same two species (*Cirrospilus lynceus* Walker and *Cirrospilus diallus* Walker) were the dominant parasitoids attacking both *Phyllonorycter* species. As a result, the ‘potential for indirect interactions’ was high among *P. harrisella* and *P. quercifoliella*, while it was lower among each *Phyllonorycter* species and *T. ekebladella* (Fig. 1b).

Based on the patterns identified above, we predict that of the host density manipulations performed in the experiment, changes in *Phyllonorycter* densities should strongly affect long-term indirect interactions among *P. harrisella* and *P. quercifoliella* – whereas the parasitism rate of *T. ekebladella* should only be affected by manipulations of conspecific densities. We further predict that long-term indirect interactions should be asymmetric, with the degree of asymmetry reflecting the relative densities and parasitism rates of each leaf miner species.

With respect to short-term indirect interactions, the patterns observed do not allow us to generate quantitative predictions. Nonetheless, if parasitoids respond behaviourally to the density of their host, we would expect overall parasitism rates to be correlated among species sharing the same parasitoid taxa. Qualitatively, we then predict that parasitism rates should be coupled between the two species sharing

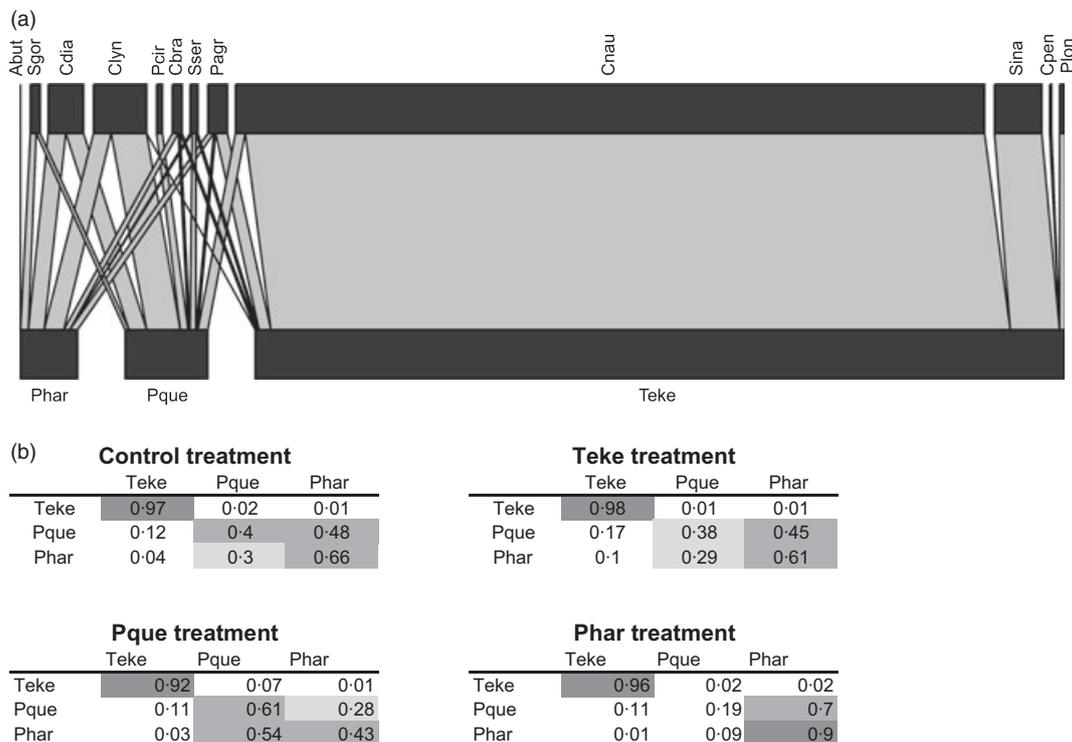


Fig. 1. Parasitoid communities of the three studied leaf miner species. Panel (a) shows a consensus version of quantitative food web structure, as derived from the rearing of sentinel larvae in 2007 (merged across treatments). Here, the width of the upper bars reflects the relative densities of each parasitoid species, whereas the width of the lower bars reflects the relative density of respective leaf miner species. The width of the grey lines connecting parasitoids and hosts indicates the frequency with which a given leaf miner species is attacked by a given parasitoid species. To facilitate visual assessment, parasitoid and host species are shown in an order minimizing the number of crossing connectors. Panel (b) shows the ‘potential for indirect interactions’ d_{ij} among the leaf miner species, calculated separately for each of the four treatments applied on the basis of realized host densities. The entries in each column (d_{ij}) refer to the proportion of parasitoid individuals attacking species i (row) that have developed on each of the host species j (columns). Hence, the values in each row sum up to 1. For example, in the control treatment, 97% of parasitoids attacking Teke are likely to have developed on Teke, 2% on Pque, and 1% on Phar. Shading ranges from white (‘low potential for indirect interactions’) to dark grey (‘high potential for indirect interactions’). Species names are abbreviated by combining the first letter of the genus name with the first three letters of the species name. Host species: Phar – *Phyllonorycter harrisella*; Pque – *Phyllonorycter quercifoliella*; Teke – *Tischeria ekebladella*. Parasitoid species: Abut – *Achrysocharis butus* (Walker, 1839); Cbra – *Colastes braconius* Haliday, 1833; Cdia – *Cirrospilus diallus* Walker, 1838; Clyn – *Cirrospilus lyncus* Walker, 1838; Cnau – *Chrysocharis nautius* (Walker, 1846); Cpen – *Chrysocharis pentheus* (Walker, 1839); Pagr – *Pnigalio agraulis* (Walker, 1839); Pcir – *Pholetesor circumscriptus* (Nees, 1834); Plon – *Pnigalio longulus* (Zetterstedt, 1838); Sgor – *Sympiesis gordius* (Walker, 1839); Sina – *Scambus inanis* (Schrank, 1802); Sser – *Sympiesis sericeicornis* (Nees, 1834). In total, the rearings are based on 2361 rearings of *T. ekebladella*, 1641 mines of *P. quercifoliella* and 375 mines of *P. harrisella*.

major parasitoids (the congeneric *Phyllonorycter* species), but uncoupled between *Phyllonorycter* and *Tischeria*. In *Tischeria*, parasitism rates should largely reflect the density of the species proper.

DENSITY-DEPENDENT PARASITISM AND SHORT-TERM INDIRECT INTERACTIONS

We did not detect any density-dependent parasitism or short-term indirect interactions. Parasitoid community composition was not affected by treatment for either *T. ekebladella* or *P. quercifoliella* ($F_{21,192} = 0.67$, $P = 0.86$ and $F_{14,168} = 0.79$, $P = 0.67$ respectively), suggesting that the relative abundances of individual parasitoid species do not differ between trees with high and low densities of hosts. Furthermore, no difference in overall parasitism rates could be detected among treatments for either *T. ekebladella* or *P.*

quercifoliella ($F_{3,20-17} = 0.78$, $P = 0.52$ and $F_{2,23-23} = 0.33$, $P = 0.72$, respectively; Fig. 2a). In addition, we did not detect any sign of density-dependent parasitism at the leaf-level (*T. ekebladella* $F_{1,2355} = 2.92$ and $P = 0.09$; *P. quercifoliella* $F_{1,1587} = 2.72$ and $P = 0.10$). Notably, for both taxa, there was large variation in parasitism rate both among trees and branches within trees (all log-likelihood ratio tests $P < 0.01$).

DELAYED DENSITY DEPENDENCE AND LONG-TERM INDIRECT EFFECTS

In 2008, 1 year after the experimental manipulations of host densities, we found slightly different patterns in parasitism and survival rates among wild individuals (i.e. leaf mines emerging from the background population of moths on the experimental trees) and the sentinel individuals introduced

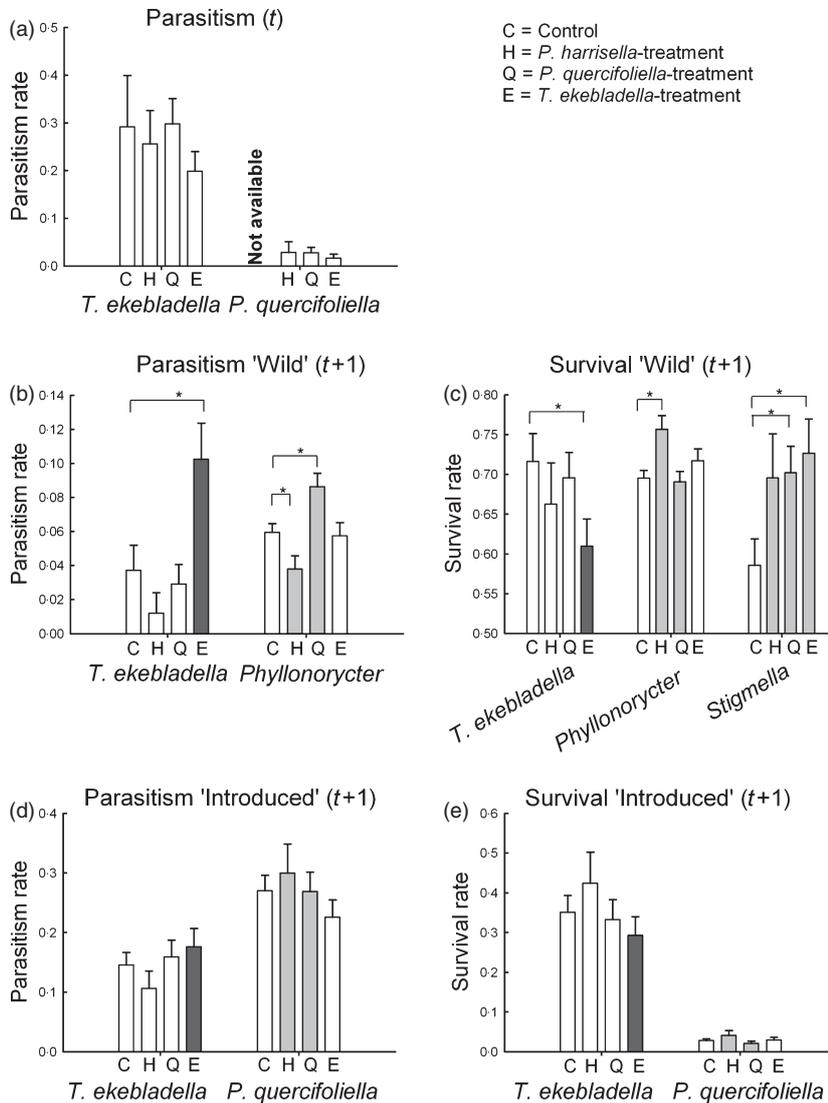


Fig. 2. Survival and parasitism across experimental treatments, species and years. Shown in panel (a) is the parasitism rate in year t (2007) of individuals of *Tischeria ekebladella* and *Phyllonorycter quercifoliella* that were introduced on trees assigned to different treatments: C = Control trees, which were not manipulated ($n = 15$ trees); H = *Phyllonorycter harrisella*-treatment, where *P. harrisella* densities were artificially increased ($n = 5$ trees); Q = *P. quercifoliella*-treatment, where *P. quercifoliella* densities were artificially increased ($n = 10$ trees); and E = *T. ekebladella*-treatment, where *T. ekebladella* densities were artificially increased ($n = 10$ trees). Due to lack of moth material, individuals of *P. quercifoliella* could not be introduced in all experimental treatments. Bars show average values across the studied trees (SE). Panel (b) and (c) show parasitism rate and survival of wild individuals in year $t + 1$ (2008) on the same set of trees. In the 'wild' material, the two *Phyllonorycter* species could not be distinguished from each other and have therefore been pooled. The shading emphasizes treatments that are expected to reveal strong indirect interactions as based on the predictions (and corresponding colours) shown in Fig. 1b. Likewise, panel (d) and (e) show parasitism rate and survival of introduced individuals on the same set of trees in year $t + 1$ (2008). Note that for *Phyllonorycter*, the 'wild' material includes mines from the second generation, whereas the 'introduced' mines are from the first generation. Asterisks denote significant differences ($P < 0.05$) between the control and specific treatments.

by ourselves (i.e. the offspring of females transplanted to the trees).

In terms of wild individuals, overall densities of leaf mines of *T. ekebladella* and *Phyllonorycter* did not differ among the treatments ($F_{3,36} = 1.67$, $P = 0.19$ and $F_{3,36} = 0.34$, $P = 0.79$ respectively). Yet, in terms of parasitism rates, patterns in *T. ekebladella* confirmed our *a priori* expectation that parasitism rate should increase with increased densities of this very species: wild individuals of *T. ekebladella* showed higher parasitism and lower survival rates on trees where conspecific densities had been elevated in the previous year than on control trees ($t_{35} = 2.29$, $P = 0.03$ and $t_{35} = 2.12$, $P = 0.04$; Fig. 2b,c). In *Phyllonorycter*, we also detected treatment effects, but they were largely positive: here, parasitism rates were lower in the *P. harrisella* treatment compared to the control treatment, whereas the parasitism rate increased in the *P. quercifoliella* treatment ($t_{36} = 2.05$, $P = 0.05$ and $t_{36} = 3.72$, $P = 0.006$, respectively; Fig. 2b). Survival of *Phyllonorycter* was significantly higher in the *P. harrisella* treatment ($t_{35} = 2.12$, $P = 0.04$;

Fig. 2c) than in the control treatment. In *Stigmella*, survival rates of naturally occurring individuals were higher on trees where the densities of *T. ekebladella* and *P. quercifoliella* had been elevated in the previous year than on control trees ($t_{34} = 2.45$, $P = 0.02$ and $t_{34} = 2.44$, $P = 0.02$, respectively; Fig. 2c).

In terms of introduced sentinel individuals, increased host densities in 2007 had no significant effect on the parasitism rate in year 2008 ($P > 0.05$ for both *T. ekebladella* and *P. quercifoliella*; Fig. 2d). With respect to survival rates, the difference in survival of *P. quercifoliella* between the control treatment and the treatment with increased densities of *P. harrisella* was close to significant ($t_{36-63} = 1.73$ and $P = 0.09$). Interestingly, this trend was towards higher survival of *P. quercifoliella* in the *P. harrisella*-treatment (Fig. 2e). For both *T. ekebladella* and *P. quercifoliella*, variation among trees and branches within trees was large (all but one log-likelihood ratio test $P < 0.01$, where the exception is the tree-level variation in parasitism rate of *P. quercifoliella*: $P = 0.12$).

Two years after the treatments, no effects were further detectable: In the follow-up study in 2009, we detected no differences among treatments in the survival and parasitism rate of *T. ekebladella* ($F_{1,17} = 0.06$, $P = 0.81$ and $F_{1,17} = 1.73$, $P = 0.20$ respectively), or survival of *Stigmella* ($F_{1,17} = 0.97$, $P = 0.34$).

REALIZED POPULATION DYNAMICS

At the scale of individual trees, the observational data did not reveal any indirect interactions among leaf miner taxa. However, there was a trend towards lower survival of both *Phyllonorycter* and *Stigmella* with increasing densities of conspecifics in the previous year ($F_{1,14} = 3.27$, $P = 0.09$ and $F_{1,16} = 3.61$, $P = 0.06$, respectively; Fig. 3a,b). In contrast with the data obtained from the experiment, we did not detect any effect of conspecific density in the previous year on the survival of *T. ekebladella* ($P > 0.1$).

At the landscape scale, we detected further evidence for density-dependent parasitism and negative indirect interactions. Here, the survival of *T. ekebladella* showed a weak negative correlation with the density of conspecifics in the previous year ($F_{1,4} = 7.60$ and $P = 0.05$; Fig. 3c). In

contrast, the survival of *Phyllonorycter* and *Stigmella* was not affected by conspecific densities in the current or previous year (all $P > 0.1$). Interestingly, in this data set, *Tischeria* and *Stigmella* revealed signs of negative interactions: Here, the survival of *T. ekebladella* was negatively correlated with the density of *Stigmella* in the previous year ($F_{1,4} = 9.41$, $P = 0.04$; Fig. 3d). Likewise, the survival of *Stigmella* was negatively correlated with the density of *T. ekebladella* in both the current and previous year ($F_{1,4} = 11.67$, $P = 0.03$ and $F_{1,4} = 18.76$, $P = 0.01$ respectively).

Discussion

This study reveals versatile consequences of indirect interactions through shared natural enemies. Our quantification of food web structure for oak-specific herbivores and their parasitoids yielded two prime predictions: that parasitoid-mediated indirect interactions among two congeneric *Phyllonorycter* species would be strong, whereas parasitism in *T. ekebladella* would mostly be affected by the density of conspecific individuals. In our experiment, we found support for the predicted delayed intraspecific density-dependent parasitism in *T. ekebladella*. Interestingly, long-term indirect

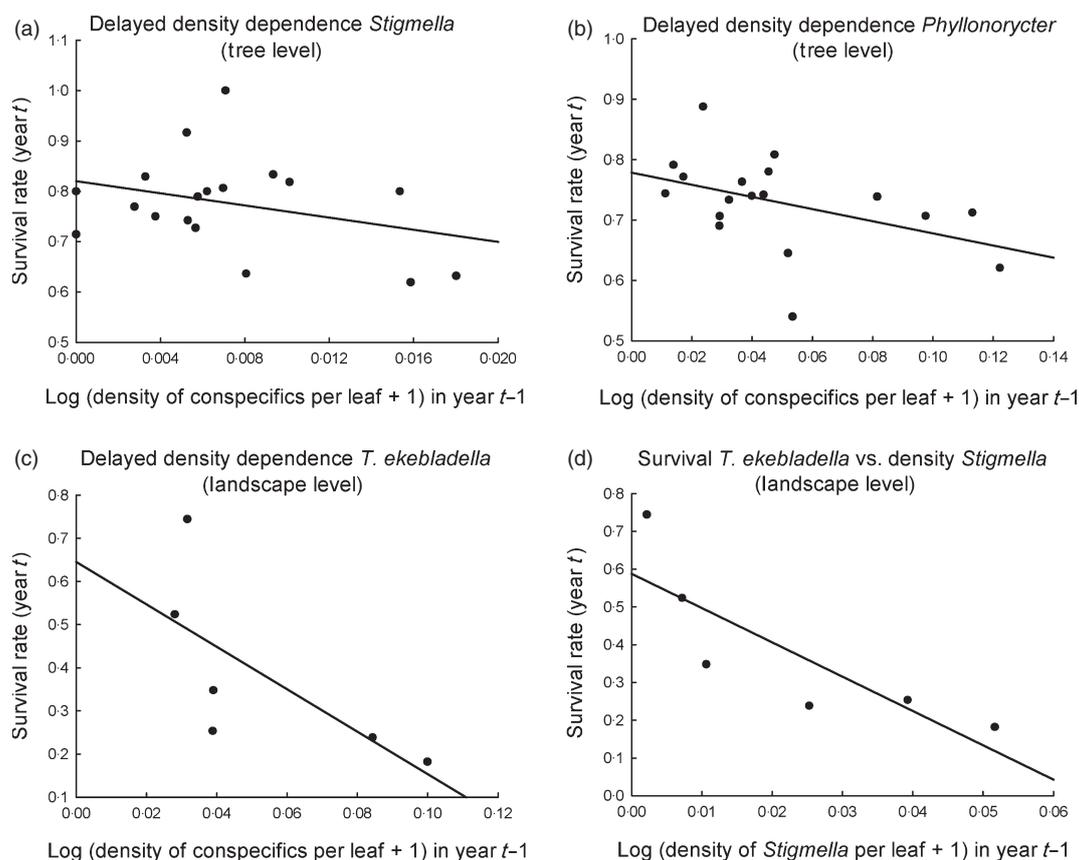


Fig. 3. Survival of wild leaf miners on trees as a function of conspecific and heterospecific densities in the previous year. Panel (a) and (b) show the relationship between leaf miner survival in 2008 and conspecific density in 2007 for *Stigmella* and *Phyllonorycter* respectively. Each dot represents an individual tree ($n = 18$). Panels (c) and (d) show the mean survival of *Tischeria ekebladella* (summed over 20 trees within the same landscape; $n = 18$ in 2008 and 2009) as a function of conspecific (panel c) and heterospecific (panel d) density in the previous year. Here, each dot ($n = 6$) reflects the mean survival in a given year (2004–2009).

interactions among leaf miner species were largely revealed as positive effects. Adding further complexity, the strength and sign of indirect interactions changed with increasing spatial scale.

Together, these findings suggest the outcome of indirect interactions to be context-dependent and hard to predict from food web structure alone. Hence, they add an important caveat to the published literature, where direct extrapolations from quantitative food web structure to population dynamics are common. Of twelve studies on natural host-parasitoid webs, nine conclude that apparent competition may play an important role in structuring the herbivore community – based on the existence of shared predators alone (Memmott, Godfray & Gauld 1994; Müller *et al.* 1999; Rott & Godfray 2000; Valladares, Salvo & Godfray 2001; Lewis *et al.* 2002; Barbosa, Caldas & Godfray 2007; Hirao & Murakami 2008; van Veen *et al.* 2008; Nakamura & Kimura 2009). The two exceptions are studies of parasitoids on gall makers, where parasitoid overlap among species was relatively small (Schönrogge & Crawley 2000; Paniagua, Medianero & Lewis 2009). A single study refrains from speculation (Henneman & Memmott 2001). In striking contrast, not a single study explicitly mentions apparent mutualism as a potential structuring force. Furthermore, despite these common inferences from quantitative food webs, only one study provides experimental support for a hypothesis generated from a quantitative food web (Morris, Lewis & Godfray 2004): when experimentally removing a single host plant (with its associated leaf miner species) from a tropical host-parasitoid food web in Belize, a group of dipteran leaf miners sharing parasitoids with the removed species increased in density and sustained lower parasitism.

This strong focus of the empirical literature on apparent competition, as opposed to apparent mutualism, stands in bright contrast with earlier theoretical work, which demonstrates that depending on system-specific features, indirect interactions may result in outcomes ranging from apparent mutualism to apparent competition (e.g. Holt 1984; Holt & Lawton 1994; Abrams & Matsuda 1996; Abrams, Holt & Roth 1998; Brassil & Abrams 2004; Brassil 2006). Hence, as far as we are aware, our study is the second to actually use food web based predictions to test for apparent competition between plant-feeding insects, and the first one to find evidence of positive interactions. Moreover, apparent mutualism has recently been suggested in two compelling observational studies (Teder & Tammaru 2003; Hambäck, Stenberg & Ericson 2006), supporting the notion that apparent mutualism may be more widespread within herbivore communities than commonly envisioned.

When dissected into short- and long-term components, we found little evidence for short-term density-dependent interactions either within or across species: within a single leaf miner generation neither parasitoid community structure nor parasitism rate differed detectably between trees with different densities of conspecific or heterospecific leaf mines. While the effect of conspecific density was nearly significant ($P = 0.09\text{--}0.10$), the lack of true significance seems more

indicative of a real lack of an effect than of lack of evidence for an effect. The statistical tests applied were powerful (see degrees of freedom) and the estimated effect small by biological standards: While a minority of leaf mines will occur singly on the leaf or share it with very few individuals (Tack *et al.* 2009), an increase of conspecifics on the leaf from zero to two is expected to increase the parasitism rate by 1.6% and 1% for *T. ekebladella* and *P. quercifoliella* respectively.

The lack of any difference in the proportion of parasitized leaf mines among trees with high and low conspecific densities, or between single and multiple-mined leaves, suggests a rapid behavioural response of the parasitoids to high-density areas within a single generation, in the form of an individual response (higher efficiency per parasitoid, corresponding to a type I functional response) and/or an aggregative response (i.e. dispersal of parasitoids into high-density areas; Hassell 1966). Unfortunately, the current data cannot distinguish between these two mechanisms.

Density-dependent effects were more evident in long-term responses, where we found clear signs of delayed density-dependent parasitism within species in both observational and experimental data. Here, the density of conspecifics in 1 year increased the parasitism rate (and lowered the survival rate) in the next. Across species, we did detect some indirect interactions – and importantly, these interactions were all positive. Specifically, the survival of *Stigmella* was higher on the trees with high densities of *P. quercifoliella* and *T. ekebladella* in the previous year, and survival of *P. quercifoliella* was higher on trees with increased densities of *P. harrisella*.

Multiple mechanisms could cause positive interactions among species. We may, for instance, have failed to observe important interactions between herbivores, or between the parasitoids of our food web and host species beyond it (Holt & Lawton 1994). However, previous work suggests that both direct resource competition and indirect competition mediated by the host plant have little impact on the leaf miners in our study system (Tack *et al.* 2009). With respect to interactions beyond the present food web, a major sampling effort in 2006 and 2007 revealed only a few weak links to other leaf miner species on oak (Kaartinen and Roslin, in preparation) – a pattern consistent with that described by Rott & Godfray (2000), who found limited potential for indirect interactions among *Phyllonorycter* species on different tree species.

A perhaps more likely explanation for positive indirect interactions is (pseudo-)interference among parasitoid individuals. Such interference may weaken the numerical response of the predators to changes in the herbivore densities, with consequences for the strength and sign of indirect interactions (Holt 1977; Polis & Holt 1992; Holt & Lawton 1994; van Veen, Morris & Godfray 2006). One option is that different parasitoids attack each other. In our study system, both parasitoid species dominant on *Phyllonorycter* (*Cirrospilus diallus* and *Cirrospilus lyncus*) are known to be facultative hyperparasitoids (Bouček & Askew 1968; Sternlicht 1968; Askew & Shaw 1979b), and may attack each other

more frequently with increasing densities. Another, more common mechanism for interference among parasitoids may be host feeding (e.g. Askew & Shaw 1979a,b; Grabenweger *et al.* 2009), where high parasitoid densities may lead to resource depletion (in terms of live hosts) and host-feeding on parasitized host larvae – or even on parasitoid larvae. That host-feeding might be relatively common in our system is suggested by the discrepancy between the relatively low parasitism rates detected here (see Fig. 2a) and the high parasitism rates reported in the literature (e.g. Hawkins 1994), the relatively large proportion of dead hosts yielding no observable parasitoid but dying for other reasons, and the fact that overall survival (i.e. $1 - (\text{mortality attributable to parasitism}) - (\text{mortality due to unknown reasons})$) reacted to the treatments in much the same way as did parasitism *per se* (e.g. Fig. 2b vs. c). Overall, we emphasize that a more detailed understanding of the life-history of the species is needed to disentangle the exact mechanisms behind the observed positive interactions.

In addition to the consistent imprint of positive indirect interactions discussed above, we also detected some differences among individual data sets. In the introduced sentinel larvae, we found weaker responses to the density manipulations than in the wild larvae (Fig. 2). This difference may in part be linked to a difference in the aggregation of individuals at the branch level across the two materials: for *T. ekebladella* the branch-level densities differed 3–4 fold, whereas the number of multiple-mined leaves increased only 1.2-fold (Tack, unpublished data). Thus, differences in intraspecific host aggregation may potentially affect the strength of density-dependent parasitism. For the bivoltine species, a key difference among the sentinel larvae and wild larvae was that for the sentinel larvae, we scored survival and parasitism in the first generation, whereas for the wild larvae, we scored the same responses in the second generation. Here, the effect of treatment on population dynamics may differ among these consecutive generations.

Interestingly, the imprint of indirect interactions also varied with spatial scale. At the level of individual trees, we did not detect negative interactions among species, whereas we did detect negative interactions among species at the landscape scale. This finding is consistent with the classical observation of Heads & Lawton (1983), showing that patterns of parasitism in the holly leaf miner are likely to change in strength and sign across spatial scales. However, we caution that the patterns at the landscape scale are correlational, and that landscape-level manipulations will hence be needed to corroborate the current results.

Overall, our results show that interactions among herbivores sharing natural enemies may range from mutualistic to competitive, and that the strength and sign of the effect may be modified by a plethora of factors beyond contemporary food web structure. We also note that quantitative food webs offer but snapshots of interaction structure in any natural community – and that this structure will vary in both space and time. Hence, we caution against simple extrapolations from estimates of quantitative food web structure to realized

population dynamics, and propose that rigorous experiments should be conducted to validate the strength and sign of indirect interactions in the field.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Population dynamics of three leaf miner taxa from 2003 to 2009.

Appendix S2. Host–parasitoid interaction matrix.

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1 **Appendix S1. Population dynamics of three leaf miner taxa from 2003 to 2009**

2

3 From 2003 to 2009, the spatiotemporal population dynamics of focal leaf miner taxa
4 has been annually monitored on twenty medium-sized trees (n=18 in 2008 and 2009).

5 These trees are confined to a dense oak stand (ca. 5 ha) within the same landscape
6 as the experimental trees referred to in the body of the article. In each year, we

7 sampled the density and larval survival of leaf miner individuals on ca. 500 leaves per
8 tree (mean=535.3; SD=159.7; see Gripenberg, Salminen and Roslin 2007 for more

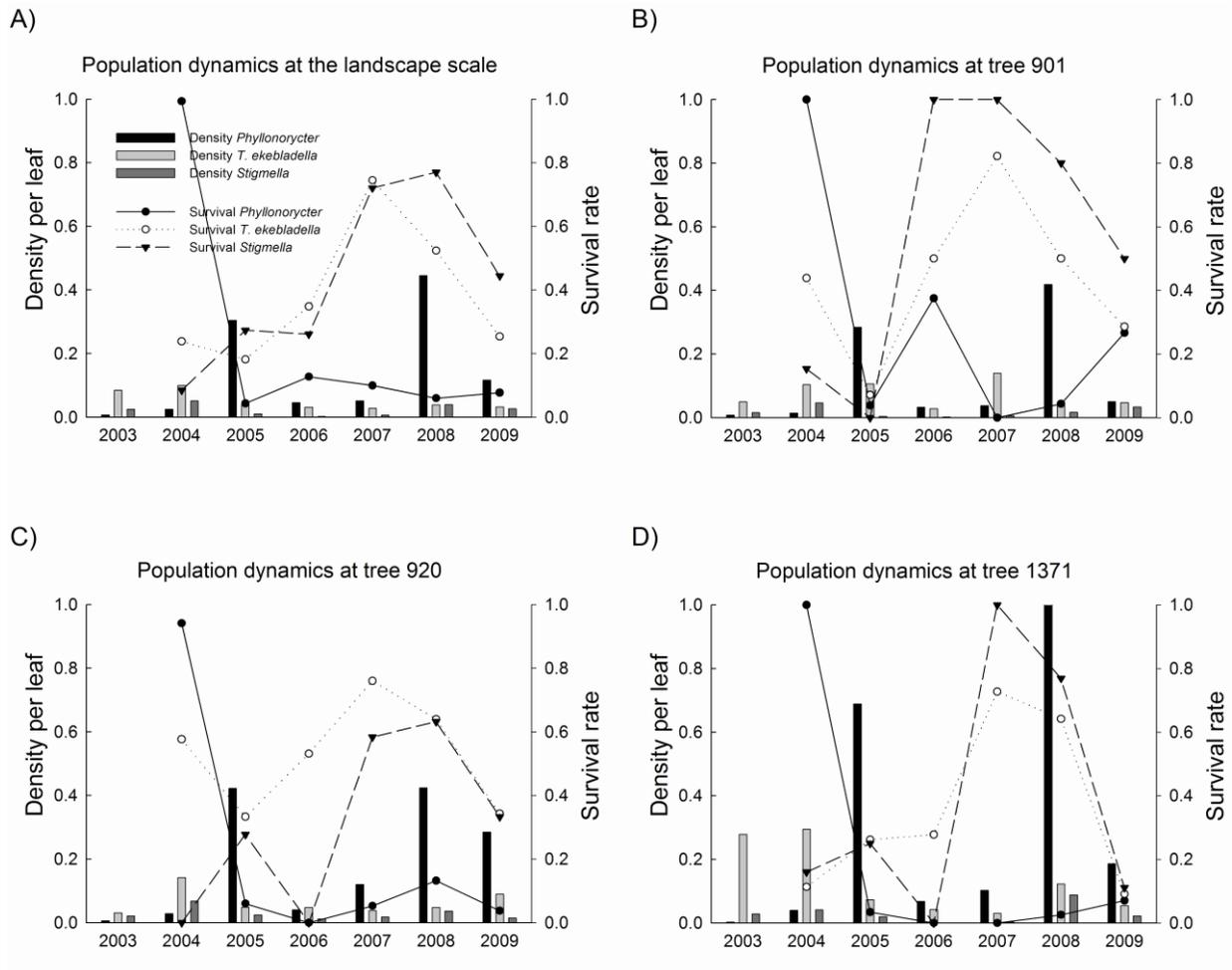
9 information on the sampling design). In the figure below, vertical bars indicate the

10 density of each leaf miner species in each year, and lines connect the yearly survival

11 rates for each species. In panel A, densities and survival rates are averaged across 18

12 trees growing in a relatively dense oak stand, whereas panels B-D show the dynamics

13 of the leaf miner species on three individual trees.



Appendix S2. Host-parasitoid interaction matrix

The figure below shows the number of individuals of each parasitoid species (columns) reared from each of the three leaf miner species (rows), a metric often referred to as interaction frequency. Shading reflects the frequency of the interaction and ranges from white (parasitoid never reared from leaf miner species) to dark grey (parasitoid species frequently attacks leaf miner species). Parasitoid species are ordered in a way which facilitates visual assessment of parasitoid overlap among species. Species names are abbreviated by combining the first letter of the genus name with the first three letters of the species name. Host species: Phar – *Phyllonorycter harrisella*; Pque - *Phyllonorycter quercifoliella*; Teke – *Tischeria ekebladella*. Parasitoid species: Abut – *Achrysocharoides butus* (Walker, 1839); Cbra – *Colastes braconius* Haliday, 1833; Cdia – *Cirrospilus diallus* Walker, 1838; Clyn – *Cirrospilus lynceus* Walker, 1838; Cnau – *Chrysocharis nautius* (Walker, 1846); Cpen – *Chrysocharis pentheus* (Walker, 1839); Pagr – *Pnigalio agraulis* (Walker, 1839); Pcir – *Pholetesor circumscriptus* (Nees, 1834); Plon – *Pnigalio longulus* (Zetterstedt, 1838); Sgor – *Sympiesis gordius* (Walker, 1839); Sina – *Scambus inanis* (Schrank, 1802); Sser – *Sympiesis sericeicornis* (Nees, 1834). In total, the rearings are based on 2361 rearings of *T. ekebladella*, 1641 mines of *P. quercifoliella* and 375 mines of *P. harrisella*.

		PARASITOIDS											
		Abut	Sgor	Cdia	Clyn	Pcir	Cbra	Sser	Pagr	Cnau	Sina	Cpen	Plon
HOSTS	Phar	1	5	14	15		5	1	5				
	Pque		3	15	28	6	2	4	2	7			
	Teke				1		1	2	9	601	39	1	4