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SPECIAL ISSUE: MOLECULAR DETECTION OF TROPHIC INTERACTIONS

Molecular analysis reveals high compartmentalization in aphid–primary parasitoid networks and low parasitoid sharing between crop and noncrop habitats

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Abstract

The ecosystem service of insect pest regulation by natural enemies, such as primary parasitoids, may be enhanced by the presence of uncultivated, semi-natural habitats within agro-ecosystems, although quantifying such host–parasitoid interactions is difficult. Here, we use rRNA 16S gene sequencing to assess both the level of parasitism by Aphidiinae primary parasitoids and parasitoid identity on a large sample of aphids collected in cultivated and uncultivated agricultural habitats in Western France. We used these data to construct ecological networks to assess the level of compartmentalization between aphid and parasitoid food webs of cultivated and uncultivated habitats. We evaluated the extent to which uncultivated margins provided a resource for parasitoids shared between pest and nonpest aphids. We compared the observed quantitative ecological network described by our molecular approach to an empirical qualitative network based on aphid–parasitoid interactions from traditional rearing data found in the literature. We found that the molecular network was highly compartmentalized and that parasitoid sharing is relatively rare between aphids, especially between crop and noncrop compartments. Moreover, the few cases of putative shared generalist parasitoids were questionable and could be due to the lack of discrimination of cryptic species or from intraspecific host specialization. Our results suggest that apparent competition mediated by Aphidiinae parasitoids is probably rare in agricultural areas and that the contribution of field margins as a source of these biocontrol agents is much more limited than expected. Further large-scale (spatial and temporal) studies on other crops and noncrop habitats are needed to confirm this.

Keywords: 16S rRNA, agroecosystems, Aphidiinae parasitoids, apparent competition, food webs, host–parasitoid communities

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Introduction

Host–parasitoid quantitative ecological networks, describing which species interact with which, the strength of their interaction, as well as structural properties, have become a major field of investigation in community ecology (Memmott & Godfray 1994; Tylianakis *et al.* 2007 May 2009; Pocock *et al.* 2012). Recently, the study of quantitative ecological networks (hereafter termed 'food webs') has given birth to very exciting literature that has considerable potential impact on our understanding of community ecology, both theoretically and in an applied context (Tylianakis *et al.* 2007; Bukovinszky *et al.* 2008; Pocock *et al.* 2012; Evans *et al.* 2013). For instance, in agricultural landscapes, Tylianakis *et al.* (2007) found marked changes in the structure of cavity-nesting bee, wasp and associated

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parasitoid food webs as a result of habitat modification due to agricultural intensification. Evans *et al.* (2013) also used a quantitative food web approach and found that seven farmland animal groups were generally robust to simulated habitat loss. More generally, it has been shown that food webs are far from being randomly structured and exhibit some patterns of modularity or nestedness (e.g. Thébault & Fontaine 2010).

In insect communities, the impact of a natural enemy on a focal prey species can be influenced by the availability of alternative prey (Holt & Lawton 1994). When two species share a common natural enemy, the presence of one species may negatively impact upon the dynamics of the other through an increase in predation risk. Such negative interactions mediated by common natural enemies are termed 'apparent competition' (Holt 1977). The strong influence of apparent competition based on parasitoid sharing in determining the abundance of a focal prey has been demonstrated by the experimental manipulation of a tropical food web by Morris *et al.* (2004). In agricultural landscapes, composed of heterogeneous patches of crop and noncrop habitats, a quantitative food web approach can be a reliable way to discover the possible occurrence of indirect interactions by identifying the common enemies shared by herbivorous insects (Alhmedi *et al.* 2011). More specifically, the presence of alternative hosts and prey in uncultivated habitats could result in an increased predation and parasitism pressure on pests (Langer & Hance 2004; van Veen *et al.* 2008). Even if rigorous manipulation experiments need to be conducted to confirm indirect interactions within food webs (Tack *et al.* 2011), a quantitative ecological network approach has considerable potential for pest control management. Indeed, it could be used as the first step in determining the impacts of uncultivated environments on the control of insect pests in annual arable cropping systems by rapidly identifying the species with potentially beneficial indirect interactions (Gurr *et al.* 2003; Tscharntke *et al.* 2007).

One major challenge in the study of quantitative food webs lies in the rapid and accurate identification of species within the community and of the trophic interactions between them. Host–parasitoid quantitative food webs are one of the most described, because the relationships between species can be easily detected by host and parasitoid rearing techniques. An adult parasitoid emerges from the parasitized host allowing a direct identification of the host–parasitoid interaction (e.g. Memmott *et al.* 1994; Schönrogge & Crawley 2000; Valladares *et al.* 2001; Macfadyen *et al.* 2009). However, the ecology and taxonomy of parasitoids remains largely uncertain, mainly because they are hyperdiverse and contain numerous cryptic species (Sha *et al.* 2007) and because the accurate identification of generally very small insects remains a challenging task even for specialist taxonomists.

Consequently, the need for insect rearing in the laboratory (and associated biases such as failed emergences) as well as frequent identification errors currently makes the construction and analysis of reliable host–parasitoid food webs very difficult and time-consuming. To overcome these problems, molecular analyses provide an effective tool for quick and accurate identification of host–parasitoid interactions in natural communities (Greenstone 2006; Gariepy *et al.* 2008; Valentini *et al.* 2009; Traugott *et al.* 2013). Walton *et al.* (1990) were the first to use a molecular approach based on electrophoresis to assess the level of parasitism in field populations of cereal aphids. Traugott *et al.* (2008) used this approach to describe the interactions between the whole aphid, primary and secondary parasitoid community associated with wheat. Kaartinen *et al.* (2010) demonstrated that the added resolution offered by molecular information contributes to a better level of precision in food web studies. Recently, Derocles *et al.* (2012a) developed a molecular approach specifically designed to study aphid–primary parasitoid communities without any prior information on species identity, allowing a broad number of species to be distinguished. Here, we used this molecular tool to describe exhaustively for the first time the quantitative food webs linking aphids and their primary parasitoids in both crop and noncrop habitats simultaneously. We compared the quantitative food web using the molecular approach with the expected food web inferred from an exhaustive review of aphid–parasitoid interactions described in the literature (mainly Kavallieratos *et al.* 2004; Starý 2006). We considered the similarity between these two networks and whether the local indirect interactions such as apparent competition could be inferred from previous knowledge on species interactions.

The main aim of this study was to detect and quantify potential cases of apparent competition between aphids collected on crops (wheat, triticale, legumes and oilseed rape) and aphids occurring in adjacent field margins (consisting of mature hedgerows and grass strips) in spring. We tested the hypothesis that field margins contributed to enhanced biological control of pest populations by harbouring nonpest aphids sharing parasitoids with pest aphids, particularly generalist parasitoids (such as *Aphidius ervi*, *Diaeretiella rapae*, *Ephedrus plagiator* and *Praon volucre*; Starý 2006). Finally, the structure of the quantitative food web was compared to the theoretical network constructed from the literature. In both, we especially focused on the degree of compartmentalization (the degree to which a food web is divided into nonconnected subwebs) between and inside the two types of habitats in order to assess the extent to which the global network can be split into

weakly connected subsets (see van Veen *et al.* 2008). The potential of noncrop habitats to act as reservoirs of biological control agents was assessed both from the observed (molecular) and expected (literature) food webs.

Material and methods

Experimental site and field sampling

The study was conducted in the experimental site 'Zone Atelier Armorique' belonging to the Long-Term Ecological Research network (LTER- S1001201). This site is a farmland area mainly consisting of meadows, cereal and oilseed rape crops situated in the vicinity of Pleine-Fougeres, which is located in the south of Mont Saint Michel's Bay (Brittany, Western France, 48°36′N, 1°32′W). It exhibits a typical Breton 'bocage' landscape made of a mixture of cultivated fields and grassland with a dense network of hedges.

Aphids were sampled every 2 weeks from 14 April 2010 to 22 June 2010 (6 sampling dates).

Five fields were monitored during the study:

1 Field 1: Pea
2 Field 2: Pea
3 Field 3: Wheat
4 Field 4: Triticale
5 Field 5: Oilseed rape

In each field, three random sampling points of 1 m² were defined and up to 30 aphid individuals per colony were collected on the crop plant.

We considered a field margin to be a 10-m-wide uncultivated strip next to the crop. In each of the four margins sampled per field, four random quadrats of 1 m² were defined. Within each quadrat, plant species were identified at least to the family (Blamey & Grey-Wilson 2003) and up to 30 aphid individuals per aphid species and per plant species were collected. A total of 16 quadrats per field were then sampled. A higher number of quadrats were sampled in the margin compared with the field because of the anticipated higher heterogeneity of the margin and to thus maximize the number of plant and of aphid species sampled. We evaluated the accuracy of our sampling method using the 'Chao 2' estimator and the percentage asymptotic richness (Chao 2005), widely used in ecological studies (Gotelli & Colwell 2001; Chacoff *et al.* 2012; see Appendix S1, Table S1, Supporting information).

Each aphid was collected using a brush and placed in a 1.5-mL Eppendorf tube filled with 95% ethanol and then stocked at a maximum temperature of 20 °C. Aphids were identified in the laboratory using a microscope and morphological criteria (Blackman & Eastop 1994, 2000, 2006).

Molecular detection and identification of parasitoids inside the aphids' body

We used the procedure described by Derocles *et al.* (2012a) to detect and identify any parasitoids within each aphid body. We extracted DNA from each aphid individual and then performed a PCR amplification on the extracted DNA. PCR amplifications were carried out with primer pairs specific and common to the entire Aphidiinae subfamily, which amplified fragments of the mitochondrial rRNA gene 16S and of the nuclear gene LWRh (see Derocles *et al.* 2012a). A positive amplification observed with an electrophoresis gel indicates the detection of a parasitoid within its host. When a positive PCR amplification had been detected, PCR products were sequenced by Genoscreen (Lille, 59, France), using Sanger technology (Chemistry used: BIGDYE 3.1; Material used: 3730XL, Applied biosystem, 96 capillaries sequencer). The examination of sequences allowed us to identify the parasitoid species. We systematically used a 16S primer pair to detect and identify parasitoids. An LWRh primer pair was used when the 16S sequence did not provide sufficient information to allow parasitoid identification at the species level. Because of the lack of variability of rRNA 16S and LWRh genes in *Lysiphlebus* (Derocles *et al.* 2012a), the individuals of this genus could not be identified at the species level. Two different sequences in the genus *Praon* and 6 identical sequences in the genus *Trioxys* did not match those contained in our database nor in GenBank. We considered that specimens with the same sequence belong to the same species and could be designated as molecular operational taxonomic units (MOTUs).

Parasitism rate

We tested the effect of sampling date, type of field, aphid species, plant species, sampling area (field vs. margin) on the parasitism rate using generalized linear models (GLIM). All of these factors were included in the models. The best-fit model was selected using Akaike information criterion (AIC). Statistical analyses were performed using R 3.0 (R Development Core Team 2013). Statistical analyses were performed by presence–absence of parasitoids inside each aphid individual using GLIM (binomial family, link logit), with the anova.glm function in R. The statistical significance of differences in the rates of parasitoid detection was assessed with the contrast method (Hastie & Pregibon

1992) using the Esticon function in R (package DoBy, Hojsgaard, 2004).

Theoretical qualitative food web

We constructed the theoretical qualitative food web based on all known interactions involving the 28 aphid species sampled. This food web was based on binary interactions observed in Europe and described in the literature (Appendix S2, Supporting information). The 'pest' or 'nonpest' status of each aphid species was set according to Iain and Dixon (2007). To build the theoretical qualitative food web, we included only the aphid species identified in the field and collected on an identified host plant and their parasitoid interactions described in the literature. We excluded from the food web construction aphids and parasitoids identified only at the genus level.

Food web metrics

To compare the theoretical food web and the observed food web, we used several metrics. Most of the metrics proposed in the literature are designed to describe either qualitative or quantitative food webs (van Veen *et al.* 2008). For comparisons, we only considered metrics relevant for both types of food web.

The number of links is the total number of aphid–parasitoid interactions found in the food web. The link density is the total number of trophic links divided by the total number of species (Bersier *et al.* 2002). Vulnerability is calculated as the total number of trophic links divided by the number of aphid species (Bersier *et al.* 2002). Generality is calculated as the total number of trophic links divided by the number of parasitoid species (Bersier *et al.* 2002; synonymous with parasitoid host range, van Veen *et al.* 2008).

We defined connectance as the number of existing links divided by the product of host and parasitoid numbers (Müller *et al.* 1999; Lewis *et al.* 2002; van Veen *et al.* 2008). Finally, compartmentalization is the degree to which a food web is divided into unconnected subwebs (van Veen *et al.* 2008).

Food web graphics were drawn using R 3.0 software (R Development Core Team 2013) using 'Plotweb' in package 'bipartite'.

Results

Abundance and diversity of sampled aphids

A total of 535 aphids belonging to 6 widespread pest species were collected in crop fields. In margins, a total of 2097 aphids were collected belonging to 35 species (Table S2, Supporting information).

Molecular detection and identification of parasitoids

A total of 2652 aphids were tested with the parasitoid-specific 16S primer pairs; 839 aphids testing positive giving an overall parasitism rate of 32%. The parasitism rate was significantly higher in field margins (37%) than in crops (20%; glm; 1 d.f.; $P < 0.001$; Fig. 1). Parasitism rate was also significantly influenced by the sampling date (glm; 5 d.f.; $P < 0.001$; Fig. 1b). We detected parasitism earlier in field margins than in crops: the first parasitoids were found within aphids collected in margins on the 14 April and on the 10 May for aphids collected in crops.

Parasitism rate was significantly influenced by the aphid host species collected (glm; 34 d.f.; $P < 0.001$) and by the type of field sampled (glm; 4 d.f.; $P < 0.001$; Fig. 1a).

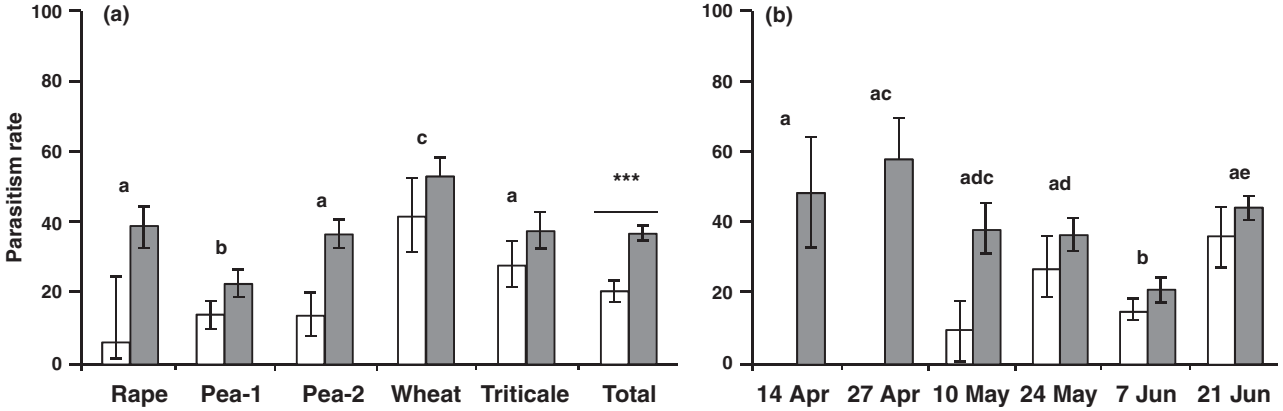


Fig. 1 Parasitism rate for (a) each sampled field and (b) each sampling date. White bars: parasitism rate in the fields; Grey bars: parasitism rate in the margins. Error bars indicate confidence intervals at 95%. Letters are applied for both field and margins parasitism rate and indicated a significant difference ($\alpha < 0.05$) between fields (glm, contrasts method; ***: $P < 0.001$).

Parasitoids belonged to at least 32 species. 705 parasitoids were identified at least to the genus level (168 to the genus level; 537 to the species level). For 134 samples in which a positive PCR amplification was detected, no sequence was obtained because of the poor quality of the DNA amplicon (a second PCR and a new sequencing did not improve the quality of the sequences obtained).

Aphid–parasitoid food web

Parasitoid detection and identification inside the aphids' bodies enabled us to construct a quantitative food web between aphids and primary parasitoids in the five sampled crop fields and margins (Fig. S1, Supporting information), and to identify parasitoid species shared by aphids collected on crops and on noncrops (Fig. 2). Quantitative data are presented in Table S2 (Supporting information).

Three aphid species were found both in fields and margins: *Acyrthosiphon*, *Metopolophium dirhodum* and *Sitobion avenae*. Very few cases of parasitoid sharing between aphids collected in fields and in margins were observed (Fig. 2).

Overall, only three parasitoid species were found parasitizing aphids in both crops and uncultivated habitats (Fig. 2). The shared parasitoid species were *Aphidius ervi*, *Ephedrus plagiator* and *Praon volucre.* These parasitoids were found in five aphid species collected in cultivated crops (*Brevicoryne brassicae*, *Acyrthosiphon pisum*, *M. dirhodum*, *S. avenae* and *Sitobion fragariae*) and in six aphid species collected in noncultivated habitats (*Macrosiphum euphorbiae*, *Macrosiphum rosae*, *Hyperomyzus lactucae*, *Brachycaudus sp.*, *Myzus cerasi* and *Myzocallis coryllii*). We found *A. ervi* in 64 individuals collected in crop habitats and in three individuals collected in noncrop habitats, *E. plagiator* in 21 crop-dwelling individuals and in five noncrop dwelling individuals and *P. volucre* in 16 crop-collected individuals and in seven noncrop collected individuals (Table S2, Supporting information).

Theoretical qualitative food web

The theoretical food web contained 61 parasitoid and 27 aphid species. Among them, seven aphid species are considered as important crop pests: *A. pisum*, *Aphis fabae*, *B. brassicae*, *M. euphorbiae*, *M. dirhodum*, *Myzus persicae* and *S. avenae* (Iain & Dixon 2007). Twenty-five parasitoid species were associated with economically important aphid species that exploited host plants in both cultivated host plants and uncultivated habitats. We did not find any aphid–parasitoid interactions for the aphid species *Cavariella pastinaceae* in the literature.

Comparisons between the molecular and theoretical food webs

The density of the molecular food web is considerably lower than those of the theoretical food web (Figs 2 and 3): we found many more aphid–parasitoid interactions in the theoretical food web than in the molecular food web (Table 1). The linkage density, vulnerability, generality and connectance were also higher for the theoretical food web than for the molecular food web (Table 1). As a direct consequence of these results, we found that the molecular food web was much more compartmentalized than the theoretical food web, especially in field margins (12 compartments in molecular food web vs. 4 compartments in theoretical food web; Table 1; Figs 2 and 3).

Discussion

Applying molecular approaches to study host–parasitoid food webs

Using molecular methods, we were able to examine the trophic interactions between aphids (collected on crops and on uncultivated habitats) and their associated parasitoids in different farmland habitats. Moreover, our molecular approach allowed us to identify and quantify interactions between parasitoids and their hosts without any dissection or laboratory rearing and also for an unprecedented large number of individuals and species. This approach allowed us to describe food webs in an agricultural area including both cultivated and noncultivated environments without any prior knowledge of the community's composition and without the help of an expert in taxonomic identification of Hymenoptera parasitoids, a very complex group (Tomanovic *et al.* 2003). Molecular approaches avoid erroneous distortion of the actual distribution of trophic links across the network due to the high frequency of species misidentification (e.g. Bridge *et al.* 2003), with probable knock-on effects on the inference of potential indirect interactions mediated by shared natural enemies (see Holt 1977; Holt & Lawton 1993, 1994; Kaartinen *et al.* 2010). Additionally, this method has the potential to reveal unknown interactions. For example, we found two aphid–parasitoid interactions between the aphid *Cavariella pastinacea* and the Aphidiinae parasitoid *Ephedrus hellini* and *Aphidius salicis* that were previously not mentioned in the literature. Some closely related species were not accurately identified (e.g. in the genus *Lysiphlebus).* Alternative genes need to be considered to overcome the lack of the 16S fragment used in this study. Cytochrome C oxidase I is probably not a suitable alternative as previously discussed in Derocles

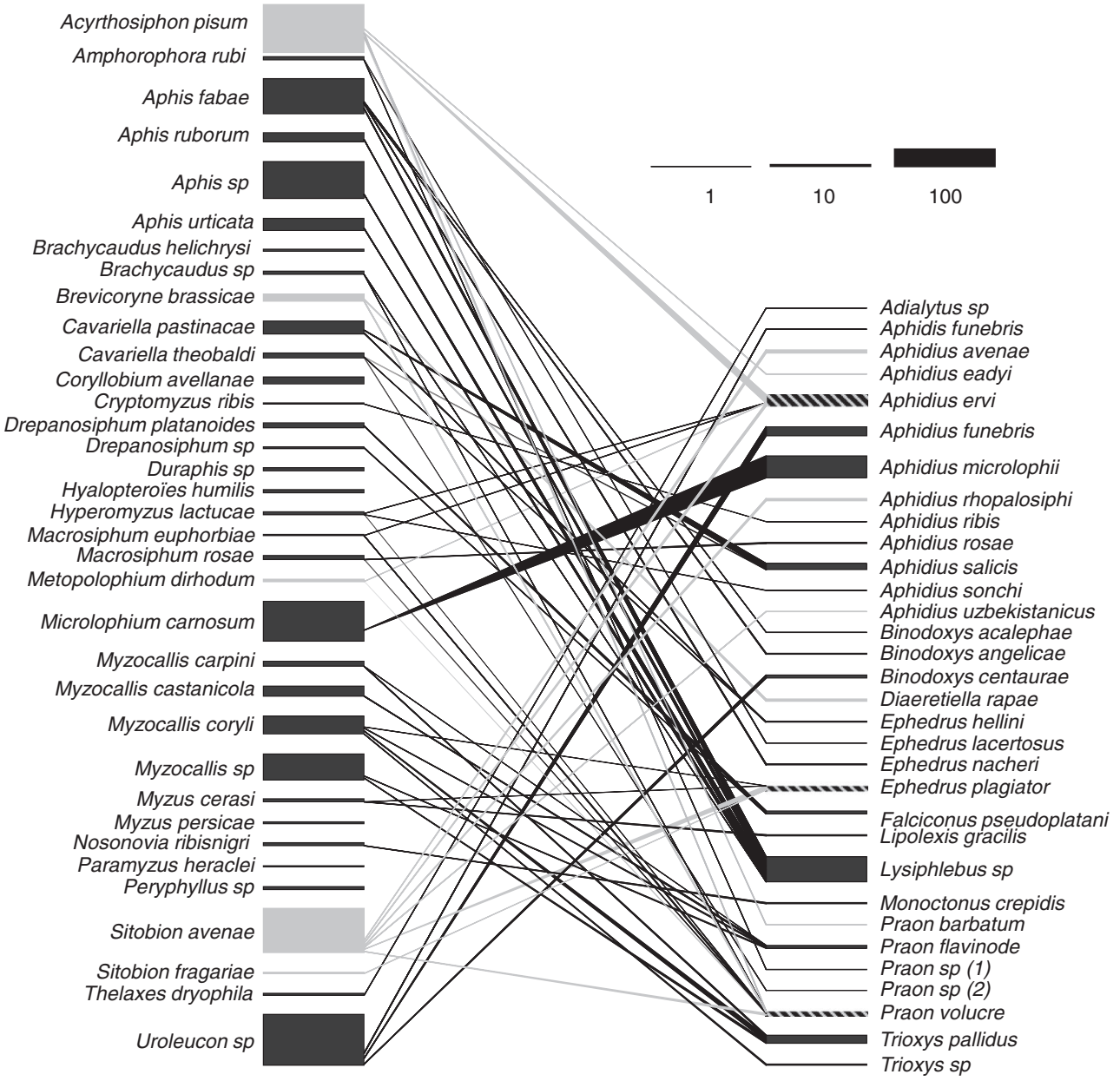


Fig. 2 Molecular quantitative food web between aphids and their parasitoids constructed from data of five fields and their margins in Pleine-Fougères (Brittany, France). The series of bars represent aphid abundance (left) and parasitoid abundance (frequency of positive detections using a molecular approach; right). The width of edge links between aphids and parasitoids illustrates the relative strength of each aphid–parasitoid interaction. Grey bars are for aphids and parasitoids found inside crops at least once, black bars are for aphids and parasitoids only found inside margins (Bars above graphics represent thickness for 1, 10 and 100 aphids or parasitoids.

et al. (2012a). Other genes, such as ITS genes, could be considered.

In addition to not being able to distinguish between some species, another limitation of our approach is that it neglects multiparasitism. Indeed, it is possible to identify only one parasitoid species in a single aphid (Derocles *et al.* 2012a). A molecular approach with species-specific primer pairs is able to detect multiparasitism and identify the species present in a single host (e.g. Traugott *et al.* 2008). However, this method requires a good knowledge of the communities and, in the case of this study, needs at least thirty-five different primer pairs. A poor-quality DNA sequence with ambiguous base calls or an erroneous identification can

Table 1 Comparison of molecular food web and theoretical food web structure: Number of links, linkage density, vulnerability, generality, connectance and compartmentalization of the food webs

	Molecular food web	Theoretical food web
Number of links	57	190
Linkage density	0.84	2.159
Vulnerability	1.583	7.037
Generality	1.781	3.115
Connectance	0.047	0.115
Compartmentalization	12	4

result from two or more parasitoid species being present in the same aphid. This may be the case for 134 of our samples that provided a positive PCR amplification but no readable sequences. Such limitations could be overcome using molecular methods that allow several different DNA fragments to be sequenced simultaneously, as permitted by next-generation sequencing tools (Shokralla *et al.* 2012). Another very efficient method would be to combine our approach with the molecular approach developed by Traugott *et al.* (2008) in order to study whole aphid–parasitoid communities.

Differences in rates of parasitism between field and margins of field

We found that parasitism was earlier, and levels higher, in field margins than in crops. This phenomenon may be linked to differences in the stability of environmental conditions between these two habitats (Rodriguez & Hawkins 2000; Gurr *et al.* 2003). Indeed, agricultural practices such as the application of pesticides and harvesting imposed upon cultivated habitats may result in rapid extinction–colonization dynamics (margins can also be affected by agricultural practices but should, in general, be more stable). Such instability induces a rapid turnover in aphid pest populations that regularly colonize new enemy-free spaces. This rapid turnover tends to maintain pest–parasitoid interactions in an immature state characterized by a low and delayed rate of parasitism.

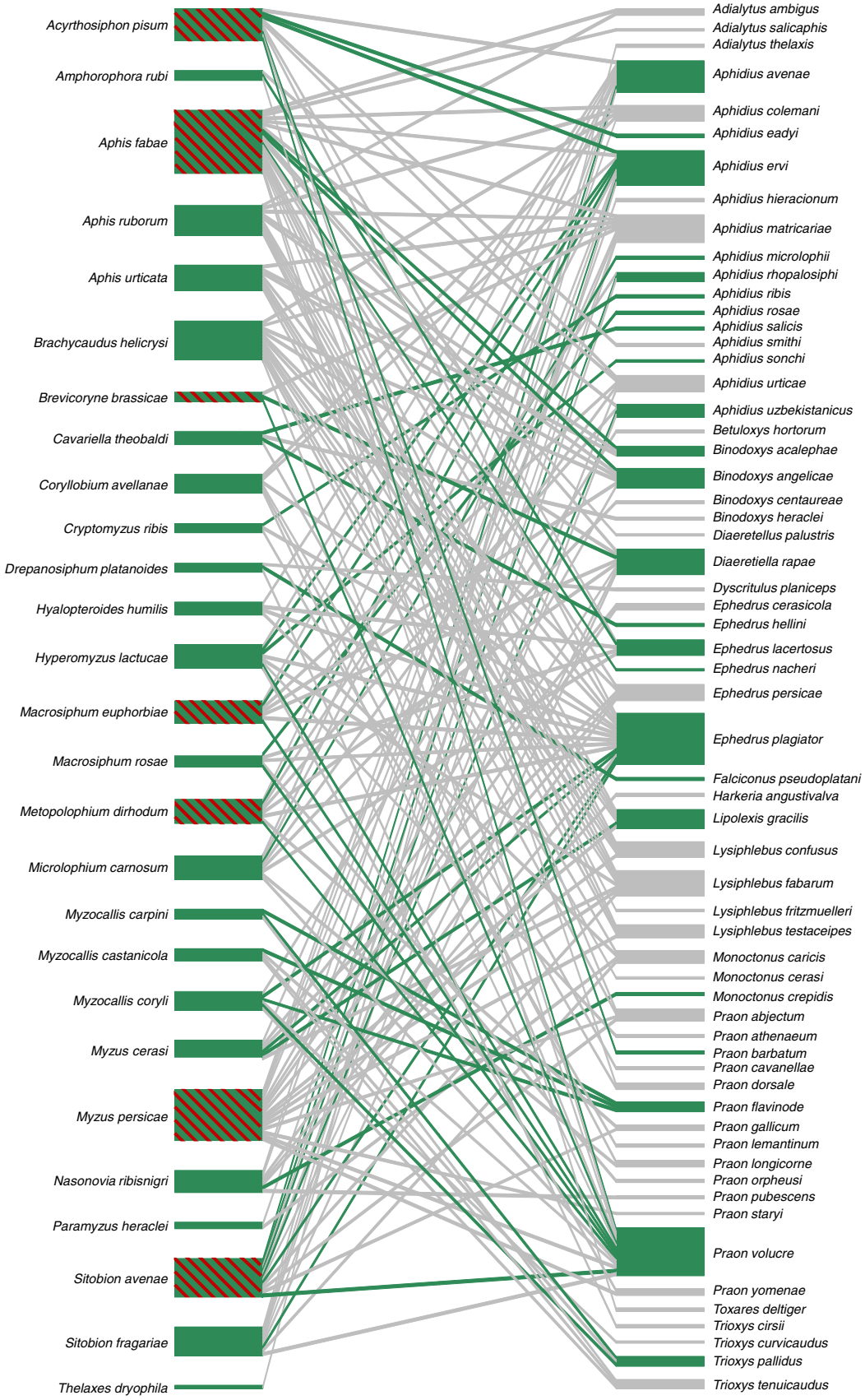
Comparisons between the theoretical qualitative food web and the molecular quantitative food web

The molecular quantitative food web described in this study is relatively simple, with a low diversity of interactions between aphids and parasitoids. Within this molecular food web, we found a higher number of independent compartments. Moreover, according to this molecular network, the communities of aphids and associated parasitoids appear strongly segregated between crops and uncultivated field margins. Aphids and parasitoids of crops seem to belong to a unique compartment, whereas communities of uncultivated habitats are separated in several very small compartments frequently including a single aphid species and a single parasitoid species. These results are even more accentuated when we observe aphid–parasitoid food webs at the scale of a single field and its margins (Fig. S1, Supporting information). Moreover, the number of compartments in the molecular food web may not be entirely accurate due to our inability to identify members of the genus *Lysiphlebus* to species level, and therefore, the molecular food web may be more compartmentalized than demonstrated here. Obviously, this strong compartmentalization could also result from an insufficient sampling effort that fails to detect rare species.

In contrast, the theoretical qualitative food web is dense, with a broad diversity of aphid–parasitoid interactions, and, as a consequence, has a very low level of compartmentalization. A first explanation of this result is that studies dealing with parasitoid host range or qualitative food web do not distinguish rare from frequent interactions. Even generalist parasitoids can have strong host preferences (e.g. *Aphidius colemani*; Storeck *et al.* 2000), and these preferences can affect the density of the food web. Moreover, we have based the theoretical food web on the data collected at the scale of Europe, mainly because the data specific to the studied region are rare. It is possible that some interactions are specific to some particular geographical area or to a particular period of time, which might not be found in the West of France in spring. Consequently, our molecular food web is likely a subsample of the theoretical food web, possibly explaining structural differences between these two food webs. Nevertheless, our study confirms the importance of dealing with quantitative data in food web studies as demonstrated in previous work, especially in the search for indirect interactions (Memmott *et al.* 1994; Müller *et al.* 1999; Rott & Godfray 2000; Valladares *et al.* 2001; Lewis *et al.* 2002; Hirao & Murakami 2008; van Veen *et al.* 2008; Tack *et al.* 2011).

Apparent competition and biological control of pest species

Despite the scarcity of parasitoid species shared by two or more aphid species, we were able to identify some potential cases of apparent competition between aphid species that occur in cultivated vs. noncultivated habitat. As previously demonstrated, aphid parasitoids are able to move from uncultivated habitats to crops and



then apply a higher pressure on pest aphids (Langer & Hance 2004).

As expected, parasitoid species involved in these potential indirect interactions (*Aphidius ervi*, *Ephedrus plagiator* and *Praon volucre*) are well known to be generalists (Kavallieratos *et al.* 2004; Starý 2006). However, these species are essentially found on aphids from crops (nearly on 100 aphids collected on crops vs. 15 aphids collected on noncrop environments) but could theoretically be found in several aphids species sampled in margins. This could result from host preferences of these generalist parasitoids, but also from host availability. Indeed, when hosts within the crops are more abundant than hosts in uncultivated areas, we can hypothesize that they are easier to detect by parasitoid females and then more likely to be parasitized.

Alternatively, some evidence based on molecular and biological data suggests that *E. plagiator* and *P. volucre* are actually composed of cryptic specialist species (Gardenfors 1986; Kavallieratos *et al.* 2004; Tomic *et al.* 2005; Derocles *et al.* 2012b). The molecular approach used in this study is not able to identify cryptic species within the groups *E. plagiator* and *P. volucre*. Consequently, apparent competition between aphids of crops and aphids of noncrops could be rarer still than observed in our study area. For further study, it is desirable to improve the resolution of the quantitative food webs by designing new primer pairs that would provide more resolution within these groups. With such an approach, we would be able to distinguish cryptic species within *E. plagiator*, *P. volucre* and in other generalist parasitoids which are often considered as a group of cryptic species (e.g. *Lysiphlebus fabarum*; Barahoei *et al.* 2011; Derocles *et al.* 2012b).

We conclude that in the agro-ecosystem studied, aphid–parasitoid interaction networks are sparse and that cases of parasitoids sharing the same resource are limited to a few aphid species sharing the same or closely related habitats (crops for *Sitobion*, trees for *Myzocallis*, weeds for *Aphis*). This segregation by habitat or by host species can be explained by the fact that this situation reduces interspecific competition and facilitates the coexistence between parasitoid species (English-Loeb *et al.* 1993; Lei & Hanski 1998; von Zeipel *et al.* 2006; Elzinga *et al.* 2007; Klapwijk & Lewis 2011). Nevertheless, a single parasitoid species shared by two aphid species could be enough to control pest populations, and the efficiency of this parasitoid to mediate indirect interactions between species needs to be assessed by experimental manipulations in the field (e.g. Langer & Hance 2004).

The main source of parasitoids involved in the control of agricultural aphid pests could be generalist species of aphids found on wild plants in field margins. Of course, those wild plants could also act as a source of aphid pests, and their relative value in reducing pest abundance in the field depends on their relative contribution to aphid and parasitoid population dynamics. However, studies on aphid and parasitoids intraspecific host specialization, although still rare, suggest a higher level of intraspecific host specialization in aphids (*Sitobion avenae*, Vialatte *et al.* 2005; *Acyrthosiphon pisum*, Peccoud *et al.* 2009; *Aphis gossypii*, Vanlerberghe-Masutti & Chavigny 1998; but see Guillemaud *et al.* 2003 for *Myzus persicae*) than in parasitoids (*A. ervi*, Bilodeau *et al.* 2013; *Diaeretiella rapae*, Baer *et al.* 2004). In general, this feature would result in potentially large transfers of parasitoid populations between cultivated and uncultivated compartments but a low contribution of uncultivated compartments to crop colonization by aphid pests.

Perspectives

DNA barcoding and other molecular approaches give considerable advantages for the study of host–parasitoid communities, mainly because these methods are less time-consuming than classical methods and can be used by scientists lacking taxonomist expertise (Gariepy *et al.* 2008). Consequently, large-scale studies could potentially become easier with molecular approaches and open several exciting prospects. To our mind, it is essential to study aphid–parasitoid food webs across temporal and larger spatial-scales. Other wild environments, for instance, can host a different diversity of aphids and parasitoids, possibly contributing to an increase in the density of the ecological network and therefore the frequency of apparent competition as well. Concerning the temporal scale, very few data exist on aphid–parasitoid interactions during autumn and winter. During these seasons, pest species are rarer and the food web structure can consequently be highly affected. Moreover, apparent competition can arise as a long-term interaction, not only in the short-term (Lawton 1986; Holt & Lawton 1993, 1994; Bonsall & Hassell 1998): this supports the need for the long-term study of aphid–parasitoid interactions.

Fig. 3 Theoretical qualitative food web between aphids and their parasitoids constructed from European aphid–parasitoid interactions described in the literature. The thickness of bars represents the number of interactions found for each species in the literature (i.e. specificity's degree of parasitoid species or the number of known parasitoid species for each aphid species). Grey bars and links are for aphid–parasitoid interactions found only in the literature. Green bars and links are for aphid and parasitoid species found in both the literature and in the field (present study). Red hatched bars are for pest aphid species (Iain & Dixon 2007).

To define the actual functioning of aphid–parasitoid food webs, complementary studies on host specialization and parasitoid behaviour are also required. Obviously, the existence of cryptic species within generalist species has to be investigated, and any interactions that arise within these cryptic species need to be partitioned appropriately to reflect host utilization patterns. Specialization can also occur at the intraspecific level, as has been observed in aphids (e.g. Vialatte *et al.* 2005; Peccoud *et al.* 2009). Finally, dispersal behaviour and the tendency of parasitoids to exploit aphids of the same species on which they have developed should be considered. Finally, to get a better understanding of the aphid–parasitoid network functioning, it would be interesting to enlarge our focus to other groups of organisms. First, the Aphelinidae, a second group of aphid parasitoids, has been neglected in this study despite their possible impact on aphid communities. This group was not studied because it is largely in a minority and our molecular tool (i.e. primer pairs) is not able to detect and identify these species. Second, other trophic levels have been ignored in this first study. The first trophic level, plants, should be identified more precisely to study bottom-up effects and particularly the impact of plants structure and richness on higher trophic levels (e.g. Petermann *et al.* 2010). The fourth trophic level, especially the community of hyperparasitoids, must be considered too because of its top-down effects (Rosenheim *et al.* 1995; Rosenheim 1999). Studies on hyperparasitoids could be of importance as previous studies (e.g. Müller *et al.* 1999) revealed less specialization at this trophic level. Rand *et al.* (2012) confirm that the fourth trophic level plays an important role in linking insect population dynamics across habitat types. Consequently, cases of apparent competition could be more frequent between primary parasitoids than between their aphid hosts, and the interplay of interaction networks between cultivated and uncultivated compartments could be more pronounced at this trophic level. Investigation of these interactions is now possible using new molecular methods as developed by Traugott *et al.* (2008) and Gariepy and Messing (2012) who designed molecular tools to study aphid hyperparasitoid communities. These approaches promise to considerably improve our understanding of the structure of ecological networks and pave the way for new and exciting studies in the field of community ecology.

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Data accessibility

DNA sequences: 16S sequences of *Trioxys* and *Praon* were assigned GenBank accessions: KJ209798 to KJ209800.

Sampling location, aphid–parasitoid interactions data, 16S sequences: Dryad doi:10.5061/dryad.v1q8j.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Estimation of the sampling completeness.

Appendix S2 References used to create the theoretical qualitative aphid–parasitoid food web.

Table S1 Number of species observed (S_O), number of species found in only one quadrate (L), in exactly two quadrates (M), Chao estimator (S_E) and percentage asymptotic richness ($\%S_O$) for the four levels analysed.

Table S2 Quantification of interactions found between aphids and parasitoids for five fields and their margins in Pleine-Fougères (Brittany, France).

Fig. S1 Quantitative food webs between aphids and their parasitoids constructed from each field and its margins in Pleine-Fougères (Brittany, France) for (a) Wheat (b) Rapeseed (c) Pea (1) (d) Pea (2) (e) Triticale.