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Community genetic interactions mediate indirect ecological effects between a parasitoid wasp and rhizobacteria

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Abstract. Indirect ecological effects (IEEs) clearly influence species dynamics and abundance, yet relatively little is known about how they influence the evolution of species involved. While genetic variation in the species causing and responding to the IEE has obvious effects, the influence of genetic variation in intermediate species remains unexamined. Given the often counterintuitive responses of populations to IEEs this seems a significant omission. Following a community genetics approach, we used a model tetra-trophic system (parasitoid wasp, aphid, barley, and rhizobacteria) to investigate the effect of genetic interactions within the two linking species (aphids and barley) on the IEE of rhizobacteria on wasps. We show that 12.4% of the variation in wasp size, a proxy for fitness, is explained by higher-order interactions between aphid genotype (A), barley genotype (B), and presence or absence of rhizobacteria (R) ($\text{Genotype}_{[B]} \times \text{Genotype}_{[A]} \times \text{Environment}_{[R]}$). Thus, the IEE of rhizobacteria on the parasitoid wasp is influenced by the specific combination of aphid and barley genotypes that mediate the interactions. In some cases changes in the genotypes of the intermediate species completely reverse the effect of rhizobacteria on wasp size. Our work demonstrates that within-species genetic variation is important in shaping IEEs in communities, an essential component of community evolutionary processes.

Key words: *community genetics; genetic interactions; genetic variation; indirect ecological effect (IEE); parasitoid wasp; rhizobacteria; species interactions.*

INTRODUCTION

Indirect interactions in a community often occur through linked chains of direct interactions. When one species influences another through mutual interactions with a third (or more) species, it is called an indirect ecological effect (IEE; Wootton 1994, Astles et al. 2005). A simple way to study IEEs is to look at trophic systems, where many species are linked through energy transfer. Trophic systems also provide a more realistic experimental approach to studying communities than using a single trophic level. Within trophic systems,

indirect interactions are known to often have strong effects and can cause unexpected results in species' responses to interactions with others (Wootton 1994, Miller and Travis 1996, Berlow 1999). For example, a predator is expected to reduce prey abundance but in some cases it can cause an increase in prey abundance, potentially through reducing competition between different prey species. Such "unexpected effects" are thought to be relatively common but will only become apparent when the positive indirect effects (increasing abundance through decreasing competition) outweigh the direct negative effects (mortality through predation) (Sih et al. 1985). Further, empirical work on interaction food webs shows that indirect interaction effects increase exponentially per species with each addition of a new species (Menge 1995). Studying indirect interactions between species will therefore develop our understanding of community composition and dynamics further than studies that focus on direct interaction effects.

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The majority of work on indirect interactions in communities has investigated how the change in abundance of a species influences the abundance of other species in the community (Wootton 1994, Muller and Godfray 1999). This work allows the impact of species extinctions or introductions to be predicted; however, it does not tell us how IEEs could influence a trophic community through evolutionary processes. Evolution within a species is a change in allele frequency over time; it can occur when there is genetic variation for a trait and selective forces act on that trait, due to some genotypes being superior in the current environment than others (Mopper 1996). The environment experienced by an individual will include biotic factors, such as the phenotype (or expressed genome) of interacting individuals, as well as abiotic factors. Indirect effects, including IEEs, are expected to be influential in the evolution of species within a community but only when they have strong ecological effects (Miller and Travis 1996). To understand more about the evolutionary influences of indirect effects within a community we must first understand how genetic variation can affect indirect interactions between species. The field of community genetics aims to understand the impact of within-species genotypic variation on species interactions and community structure.

Previous community genetics work using model communities has shown that there is genetic variation in response to an IEE in a tri-trophic system (ladybirds exhibited variation in responses when fed on clonal aphids, which were raised on two different host plants) and suggested that there is potential for IEEs to be a strong selective force in a community (Astles et al. 2005). It has also been demonstrated that within-species genetic variation is important for directly interacting species (Service 1984, Mopper 1996), and the effect can be mediated by the presence or absence of a third species in the community (Tetard-Jones et al. 2007). Thus, there is clear evidence showing that genetic variation within a species will affect directly interacting species; however, we do not know to what extent genetic variation, and genotypic interactions, within intermediate species will affect the outcome of indirect interactions. This is an important omission seeing that indirect interactions can be strong selective forces when they have a strong ecological effect.

If genetic variation within a species influences IEEs, this will lead to different outcomes in different populations potentially influencing the evolutionary trajectory of a species differently among populations. The number of genotypes in a population is only a subset of the available genotypes for a particular species, leading to differences in genotypic diversity between populations. Because this is true for every species in a community, it means that the genotype combinations among interacting species will be different between populations producing a number of different responses to an IEE. The overall response of a population (positive, negative, or negligible) is determined by the

frequency of the genotypes within a population and the resulting frequency of each interspecific genotypic interaction. For example, two populations of a species may reside in different communities with similar species diversity, but every species within this community has a different subset of genotypes than other communities. The resulting response of the species to an IEE is likely to differ in both direction and magnitude depending on which genotypes of each species are present.

Here, we have used a quantitative community genetics approach to quantify the magnitude of within-species genetic variation on an IEE. By using quantitative genetics methods, phenotypic variation in a species can be partitioned out into the effects of its own genotype, the genotype of other species, and the interactions between these genotypes, assuming the abiotic environment is controlled. We use a multispecies model ecosystem, which allows manipulation of numerous genotypes of different species, through use of clonal species and homozygous lines. We investigated the effect of indirect interactions using a tetra-trophic system of a plant-growth-promoting rhizobacteria, barley, an aphid, and a parasitoid wasp. We show that the IEE between the rhizobacteria and the parasitoid is mediated by genotypic interactions between the two intermediate species (barley and aphids). This study is the first to focus on the influence of genotypic interactions using a system involving four trophic levels.

METHODS

Experimental design

A fully factorial design was used consisting of four barley genotypes, four aphid genotypes, rhizobacterial treatment (addition of rhizobacteria vs. no addition of rhizobacteria), producing 32 treatments. Each treatment was replicated five times, giving a total of 160 plants. Plants were grown in a randomized design.

Rhizobacteria preparation

Single colonies of *Pseudomonas aeruginosa* 7NSK2, known to have plant-growth-promoting effects (Iswandi et al. 1987), were chosen from a streak plate and grown aerobically in LB medium (Sambrook et al. 1989) for 24 hours at 30°C. The bacteria were harvested by centrifugation (10 000 gravities for 13 minutes; one gravity = 9.80665 m/s²), washed three times in 10 mmol/L MgSO₄, and resuspended at A₆₀₀ = 1, using 10 mmol/L MgSO₄ as a diluent. The suspension was used directly for root inoculation of seedlings.

Plant material

Hordeum vulgare accessions Morex, Steptoe, BCD47, and Baroness (originally from P. Hayes, Oregon State University, USA) were used, which had been grown in a common environment with genotypes isolated from each other to ensure selfing occurred prior to the experiment. The seeds were sterilized by soaking in 10% NaOCl for 10 minutes, on a rotary shaker, and then washed six

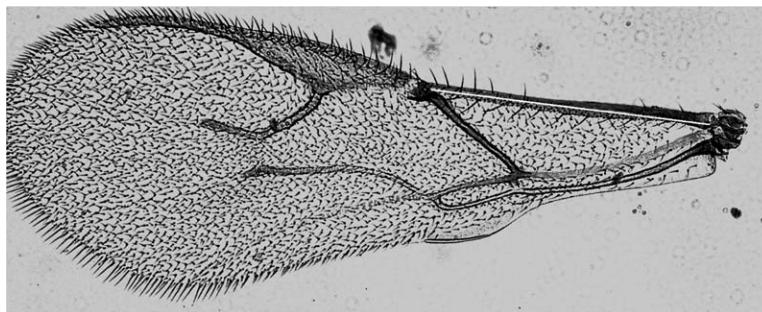


FIG. 1. Diagram indicating measured costal vein on a parasitoid wasp wing (white line).

times in sterilized distilled water. The seeds were germinated by placing them between two pieces of filter paper that had been soaked in sterilized distilled water, and then kept in the dark at 23°C for 5 days. Seedlings of uniform shoot and root length were chosen for the experiment. Seedling roots were inoculated with bacteria by submerging in bacterial suspension for 1 hour (control plants were submerged in 10 mmol/L MgSO₄), and then seedlings were transplanted into 10-cm pots containing heat-sterilized horticultural sand, which had been watered with tap water the previous day. Each pot contained a single plant for the duration of the experiment. The experiment was undertaken in a glasshouse at the Firs Experimental Research Station, University of Manchester, UK, using supplemental mercury lights to provide a 16:8 L:D regime and with a daily temperature range of 15–25°C. Plants were watered with 40-mL full strength Hoagland's solution (Hoagland and Arnon 1950) twice a week for the first three weeks and once a week until the end of the experiment. If the sand was dry between Hoagland's applications, tap water was given. Three days prior to aphid introduction, plastic tubes with mesh tops and mesh windows were placed around each plant to isolate it from the others (described in Tetard-Jones et al. 2007).

Aphids

Sitobion avenae genotypes HF92a, H1, CLO7, and DAV95 were supplied by Rothamsted Research, Harpenden, UK. Aphids were reared on *Hordeum vulgare* accession B83 prior to the study in isolation cages. Eleven days post-transplantation of the barley, one adult aphid was placed on the corresponding plants. When nymphs were produced, all aphids but two nymphs were removed from the plant and the nymphs allowed to develop and reproduce. This process was done to remove the effect of maternal rearing environment. Aphid numbers were counted to assess aphid fitness but those data are not reported here as we found a similar effect to that already described in Tetard-Jones et al. (2007).

Parasitoid measurements

Aphidius rhopalosiphii wasps were supplied by M. Torrance, Rothamsted Research, Harpenden, UK. The

parasitoid wasps were reared, in a large population, on aphid genotype DAV95 (also used as an experimental genotype) and barley genotype B83 (not used as an experimental genotype) prior to the study, grown in John Innes compost number 3 (John Innes Centre, Norwich, UK). The results show no bias toward aphid DAV95 used to rear the parasitoid wasps, and therefore we assume there are negligible conditioning effects from using this genotype in the experiment. A single randomly mated female parasitoid was placed onto each plant 40 days after seedling transplantation (29 days after aphid introduction). Mummified aphids were collected from each plant, within one week of releasing the parasitoid, and kept in glass vials until the new parasitoids emerged. Up to eight emerged female parasitoids were dropped in ethanol and then the wings removed using tweezers and mounted on glass slides to allow measurements to be taken. Wing morphological measurements were taken of costal vein length (Fig. 1) by taking digital photographs using a light microscope with mounted camera (Leica, Wetzlar, Germany). Costal vein length was measured using software ImageJ (Abramoff et al. 2004). Wing size is used as a measure of parasitoid fitness because it is a general measure of body size that correlates positively with fitness traits such as egg load and fat reserves (Visser 1994, Ellers et al. 1998, Kolliker-Ott et al. 2003).

Data analysis

A generalized linear model (GLM) was used to analyze the effect of variation within the rhizobacteria–barley–aphid system on costal vein length in emerged parasitoids. Mean parasitoid wing sizes among the treatments were calculated and analysis was performed on this data. A model with linear scale response, identity link function, normal distribution, maximum likelihood scale parameter estimate, and a robust covariance matrix estimator was used. The Wald chi-square statistic was used to analyze the significance of each term in the model. Variance component analysis (VCA; maximum likelihood method) was used to determine the amount of variance explained by each term in the model. For the VCA we used each parasitoid as an individual data point, in order to further

TABLE 1. Results from a generalized linear model (GZLM) and variance component analysis of the effect of aphid genotype, barley genotype, rhizobacteria environment, and interactions between these factors, on parasitoid wing costal vein length.

Source of variation	Effect	Wald chi-square	df	P	Variance explained (%)
Rhizobacteria	Main	1.703	1	0.192 (NS)	
Barley	Main	11.232	1	0.011*	0.0
Aphid	Main	51.224	3	<0.001***	11.4
Barley × rhizobacteria	G × E	16.474	3	0.001***	0.8
Aphid × rhizobacteria	G × E	6.405	3	0.094 (NS)	0.0
Barley × aphid	G × G	26.161	9	0.002**	0.0
Barley × aphid × rhizobacteria	G × G × E	37.574	9	<0.001***	12.4

Notes: Results for the full GZLM model are shown. Variance component analysis was performed on data using individual parasitoids and a maximum-likelihood method. Key to abbreviations: G, genotype or the specific genetic makeup of a particular individual, e.g., a particular clone of aphid; E, environment, the environment experienced by an individual, e.g., the presence or absence of rhizobacteria.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, nonsignificant ($P > 0.05$).

understand the effect the $G \times G \times E$ interaction (G, genotype or the specific genetic makeup of a particular individual, e.g., a particular clone of aphid; E, environment, the environment experienced by an individual, e.g., the presence or absence of rhizobacteria) is having on the parasitoid population; rhizobacteria was a fixed effect, with barley and aphid being random effects in the analysis. Analyses were performed using SPSS version 14.0 for Windows (SPSS 2006). Pixel length was used in the analysis to reduce the error associated with converting the data into length measurements. However, pixel data were converted into millimeters using a micrometer calibration slide on the microscope for result interpretation.

RESULTS

A total of 338 female parasitoid wasp wings, across all 32 treatment groups, were collected and measured. The wasps measured had a mean costal vein length of 0.808 mm and length ranged from 0.529 to 0.983 mm, following a normal distribution. Within each treatment the results followed a normal distribution. The costal vein length (wing size) of the parasitoid was significantly affected by the $G \times G \times E$ interaction term between the aphid, barley, and rhizobacteria treatments (Wald chi-square = 37.574, $P < 0.001$; Table 1). Parasitoid wing size is influenced by whether or not rhizobacteria are present in the system, with the direction of the indirect ecological effects (IEEs) being dependent on the combination of aphid genotype and barley genotype (Fig. 2). For example, parasitoids emerging from CLO7 aphids grown on Morex barley are larger when rhizobacteria are present than when absent. However, parasitoids emerging from CLO7 aphids grown on BCD47 barley are smaller when rhizobacteria are present than when absent. The largest negative effect of the presence of rhizobacteria on parasitoid wing size was when parasitoids emerged from HF92a aphids raised on Baroness barley; the mean parasitoid wasp costal vein length was 0.190 mm greater when no rhizobacteria were present than when rhizobacteria were present. The largest positive effect of rhizobacterial presence on parasitoid wasp size was with DAV95

aphids reared on BCD47 barley, when the presence of rhizobacteria increased costal vein length by 0.125 mm. The variance component analysis shows that the $G \times G \times E$ interaction between rhizobacteria, aphid, and barley is explaining 12.4% of the variation in parasitoid wing size (Table 1).

DISCUSSION

Indirect ecological effects can be highly influential on the phenotype of higher organisms in a trophic system. In this study, we showed that the wing costal vein length of a parasitoid wasp is affected by $G \times G \times E$ interactions between the three species below it in a tetratrophic system (an aphid, barley, and rhizobacteria). This means that the interaction between aphid genotype, barley genotype, and presence or absence of rhizobacteria is having a strong effect on the phenotypic variation of parasitoid wing size in our model system; we show that 12.4% of the variation in parasitoid wing size is explained by the $G \times G \times E$ interaction. Both the aphid and barley genotypes we used are clonal, and thus natural sexually reproducing populations may show smaller interaction effects because of greater heterozygosity within species and a reduced genetic covariance between species.

Although parasitoid genetics were not included in our model, it is expected that this will explain much of the remaining variation in wing length because body size in a related parasitoid species, *Aphidius ervi*, has been shown to have a broad sense heritability of 30% in females and 60% in males (Gilchrist 1996). There are many possible mechanisms for the influence of the $G \times G \times E$ interaction between rhizobacteria, barley, and aphid on parasitoid wing size. The rhizobacterium we used is a plant-growth-promoting bacterium and may differentially affect the barley growth across genotypes; also the different barley genotypes may respond differently to aphid attack and differently between aphid genotypes. This may all influence the aphid population through aphid size, number, or aphid physiology, which can in turn affect the growth of the parasitoid in the aphid host. However, the mechanisms for the effect of

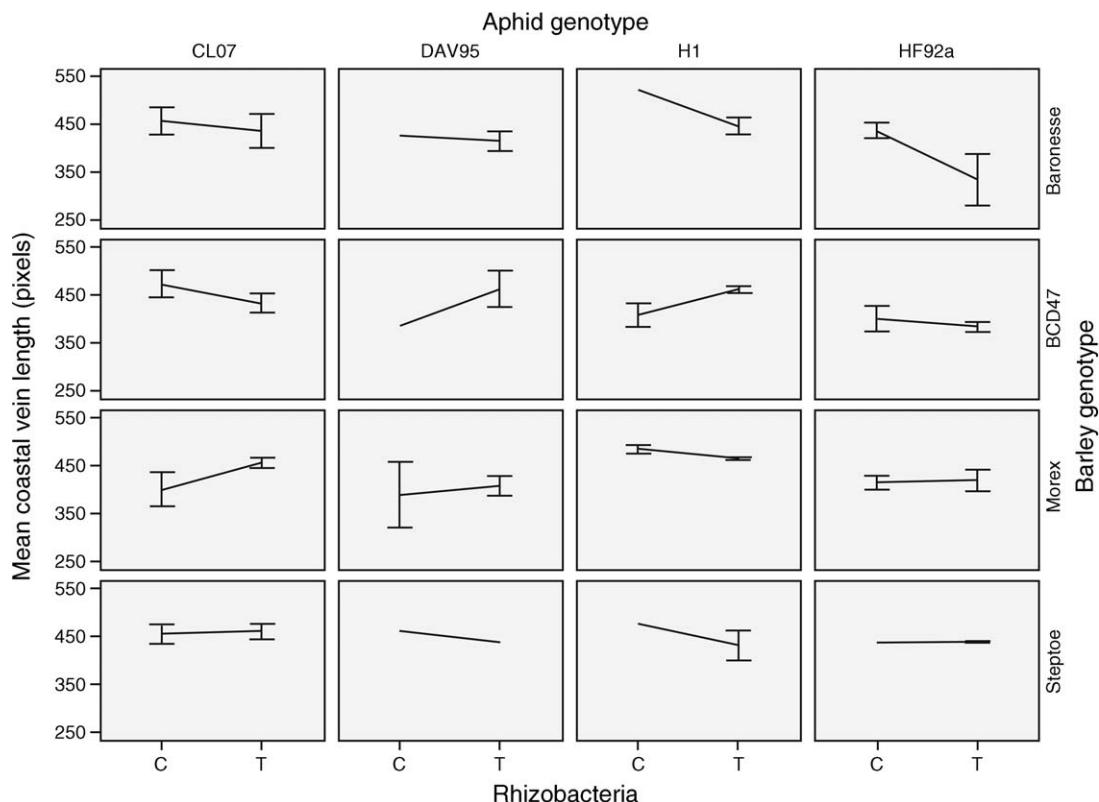


FIG. 2. Phenotypic response of the parasitoid wasp to the indirect ecological effects (IEEs) of rhizobacteria is dependent on the combination of aphid and barley genotypes in the model. The measured trait is parasitoid wing costal vein length (pixels); each separate graph shows the mean vein length for the untreated control with rhizobacteria on the left (C) and the mean for the treatment on the right (T). Each row is the result for a different barley genotype, and each column is the result for a different aphid genotype. Error bars represent \pm SE. For each treatment with a single plant replicate, the data are the average of either four or five parasitoids emerged from the one plant, except for treatment Control-H1-Baroness, which is from a single parasitoid.

the three-way interaction on parasitoid wasp size have not been determined.

Effect of genetic variation on IEEs

We have shown that an IEE can span across two species but, importantly, we show that the effect of the IEE is mediated by the genotypic interactions within the intermediate species. It is notable that genotypic changes in the intermediate species can completely reverse the effect of rhizobacteria on the parasitoid wasp. This suggests a mechanism by which IEEs might produce differential results among a number of different populations. As we show, IEEs can produce positive and negative responses dependent on which genotypes are interacting, and the relative frequencies of these responses (determined by the relative frequencies of the genotypes) will produce the overall response of a population. In our system, if the majority of IEEs in a population produced positive effects, this would result in a larger mean parasitoid costal vein length than in a population dominated by negative effects. Indirect interactions often strengthen community structure (Menge 1995, Berlow 1999), and we show they may be highly influenced by genetic variation within intermedi-

ate species; therefore we argue that understanding genetic diversity at all levels is highly important when considering community structure or dynamics.

Potential evolutionary effect of IEEs

It has been shown that there is genetic variation for a response to an IEE (Astles et al. 2005), which means that there is the potential for IEEs to alter the evolutionary trajectory of a species. In addition, we found that genetic variation in the intermediate species mediated the effect of the IEE, so potentially the interspecific genotypic interactions between the aphids and barley, in our system, will affect the IEE of the rhizobacteria and alter the evolutionary trajectory of the parasitoid wasp. Within a population, if the majority of aphid–barley combinations influenced the IEE to produce a positive response in the parasitoid (larger wing size), then the mean size of parasitoid wing may be expected to increase over the next generations. However, irrespective of the fact that interactions between the aphids and barley strongly influence the morphology, and thus the fitness of the parasitoid (Visser 1994, Ellers et al. 1998, Kolliker-Ott et al. 2003), if there is no selective effect, there will be no resulting change in the species over time.

For an evolutionary trajectory to be altered by these interspecific interactions there would need to be a nonrandom association of genotypes in the community. Random associations of genotypes would cause any effects to average out over time, although they might occasionally have a strong effect. A nonrandom association of genotypes might occur through differential survival or through assortative association. For example, nonrandom association of aphid and barley genotypes might occur either due to differential survival of aphid genotypes on different barley genotypes (Tetard-Jones et al. 2007), through aphid genotypes preferring different barley genotypes as hosts or as an influence of parasitoids attacking different aphid genotypes depending on barley host. In either case a genetic correlation would be established between the two species. Given current agricultural strategies of planting large areas with a small number of barley genotypes such associations appear entirely plausible. The extent to which such associations occur in more complex natural communities is unclear as they are rarely examined. However, random association in natural communities cannot be assumed, as demonstrated by the preference or host-associated differences (HAD) for a number of phytophagous insects to different goldenrod genotypes (Stireman et al. 2005). Furthermore, the recent discovery that insect-host genetic differences promote parasitoid cryptic diversity, through cascading HAD, suggests that the evolutionary trajectory of the parasitoid will be influenced by genotypic interactions between interacting species (Stireman et al. 2006).

In conclusion, our study shows that parasitoid morphology, a surrogate for fitness, is dependent to a considerable extent on the $G \times G \times E$ interaction between the aphid, barley, and rhizobacteria. The response of the parasitoid to the IEE of rhizobacteria presence or absence differs due to the genotypic combinations of the intermediate species, and in a natural population this means the response will be dependent on the frequency of different genotypes. However, for this to have an evolutionary effect there needs to be a selective force acting on the parasitoid, determined by the biotic environment of the expressed genomes of interacting species and a nonrandom association of the genotypes. There is the potential for nonrandom association of genotypes in certain systems; however, it is still unclear to what extent this can be generalized.

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