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Functional benefits of predator species diversity depend on prey identity

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Abstract. 1. Determining the functional significance of species diversity in natural enemy assemblages is a key step towards prediction of the likely impact of biodiversity loss on natural pest control processes. While the biological control literature contains examples in which increased natural enemy diversity hinders pest control, other studies have highlighted mechanisms where pest suppression is promoted by increased enemy diversity.

2. This study aimed to test whether increased predator species diversity results in higher rates of predation on two key, but contrasting, insect pest species commonly found in the rice ecosystems of south-east Asia.

3. Glasshouse experiments were undertaken in which four life stages of a planthopper (*Nilaparvata lugens*) and a moth (*Marasmia patnalis*) were caged with single or three-species combinations of generalist predators.

4. Generally, predation rates of the three-species assemblages exceeded expectation when attacking *M. patnalis*, but not when attacking *N. lugens*. In addition, a positive effect of increased predator species richness on overall predation rate was found with *M. patnalis* but not with *N. lugens*.

5. The results are consistent with theoretical predictions that morphological and behavioural differentiation among prey life stages promotes functional complementarity among predator species. This indicates that emergent species diversity effects in natural enemy assemblages are context dependent; they depend not only on the characteristics of the predators species, but on the identity of the species on which they prey.

Key words. Brown planthopper, ecosystem services, leaf-folder, natural pest control, rice arthropods, species diversity.

Introduction

Determining the functional significance of species diversity in natural enemy assemblages has become an important issue to those studying the ecology of predator–prey interactions and those interested in the role of biodiversity in maintaining ecosystems services (Mooney *et al.*, 1995; Rodriguez & Hawkins, 2000; Cardinale *et al.*, 2003; Montoya *et al.*, 2003). That said, the study of natural biocontrol and multiple enemy effects has remained largely distinct from the ongoing debate over how biodiversity affects ecosystem function (Cardinale *et al.*, 2003; but see Ives *et al.*, 2005). From the multiple enemy perspective, there are well-documented mechanisms whereby species diversity can be both beneficial [a positive emergent multiple predator effect (MPE)] and detrimental (negative emergent MPE) to the overall rate of predation (Sih *et al.*, 1998). For example, intra-guild predation or behavioural interference can result in a negative MPE (Polis *et al.*, 1989; Rosenheim *et al.*, 1995), reducing the effectiveness of

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introduced biological control agents under laboratory and field conditions (Rosenheim, 2001; Snyder & Ives, 2001). In contrast, a positive emergent MPE through, for example, functional synergy among natural enemy species (Soluk & Collins, 1988; Soluk, 1993; Losey & Denno, 1998; Cardinale *et al*., 2003), may result in higher than expected predation rates. This occurs, for example, when one predator induces behaviours in the prey that put them at greater risk from a second predator, or where increased diversity is associated with changes in the way prey are distributed (Cardinale *et al*., 2002). From the ecosystem function perspective, the functionality of multispecies assemblages hinges on functional complementarity among species, which arises if there is significant resource-use differentiation or facilitation among species (see Petchey, 2003). Here, functional differences among predator species in their use of prey, or synergistic interactions (as described above), result in increased functionality of more diverse predator assemblages compared with less diverse assemblages of equal abundance.

Recent theoretical studies have suggested that life-history characteristics of prey species may affect the likelihood of niche overlap among natural enemy species and, consequently, the shape of the relationship between enemy species richness and predation rate (Wilby & Thomas, 2002a, b). Furthermore, it is also likely that niche separation among natural enemies will decrease negative interactions between individuals and increase functional synergies mediated by changes in prey behaviour (Sih *et al*., 1998).

Two glasshouse experiments are reported here that test for positive diversity effects in assemblages of generalist predators in their predation of two insect herbivores with contrasting life histories. Unlike many studies that have highlighted multipredator effects, species diversity was manipulated independently of species composition allowing a valid test of the impact of predator species diversity on predation rate. The aim was to integrate insights from biodiversity–ecosystem functioning and multiple enemy effects research to investigate the effect of predator species richness on predation rate, and assess the emergent MPEs in each multispecies combination against these contrasting prey species. These experiments showed that the expression of positive diversity effects is dependent on the prey identity and the results are consistent with the hypothesis that life-stage differentiation of prey promotes functional complementarity among natural enemy species.

Materials and methods

Study system

Experiments were conducted at the International Rice Research Institute (IRRI), Los Baños, the Philippines. The two prey species used are common insect rice pests in the region: (i) the brown planthopper (*Nilaparvata lugens* (Stål), Homoptera: Delphacidae, an exopterygote) and (ii) the rice leaf-folder (*Marasmia patnalis* Bradley, Lepidoptera: Pyralidae, an endopterygote). Four common generalist predator species were used that are known to feed on these herbivores: a wolf spider *Pardosa pseudoannulata* (Boesenberg and Strand) (Araneae: Lycosidae); a ladybird beetle *Micraspis crocea* (Mulsant) (Coleoptera: Coccinellidae); a cricket *Metioche vittaticollis* (Stål) (Orthoptera: Gryllidae); and a plant bug *Cyrtorhinus lividipennis* Rueter (Hemiptera: Miridae).

Predator assemblages were created using a substitutive design to give four single-species treatments and four three-species treatments (each with one of the four predator species absent) and all predators were starved prior to use. Control treatments contained no predators. Due to the large differences in body mass between the smallest and largest predators ($\approx$ 1:75), the numbers of individuals was standardised across species using allometric scaling of metabolic rate (B) to body mass (M) of $B = M^{3/4}$ (West *et al*., 1997). Metabolic scaling was used to standardise treatments as it is likely to give a better standardisation of feeding rate, the function in question, than the alternatives of equalising biomass or numbers of individuals across treatments. Fifteen *Cyrtorhinus*, six *Metioche*, six *Micraspis*, or three *Pardosa* were used in single-species treatments, whilst the three-species treatments used the appropriate combination of five *Cyrtorhinus*, two *Metioche*, two *Micraspis*, and one *Pardosa*. Independent evidence of daily feeding rates of 1–5 planthopper nymphs in *Cyrtorhinus* and 5–15 in *Pardosa* suggests that the ratios used were good approximate standardisations of feeding rate (Shepard *et al*., 1987).

Experimental design

Each experimental unit consisted of a 50 $\times$ 37 cm tray of 11-cm depth supporting a 83-cm tall cage constructed from Mylar® transparent sheeting (Dupont), with mesh top and side panels. Four pots (11 cm diameter, 10 cm deep), each containing four rice tillers approximately 60 cm tall, were placed in each cage and the tray filled with water. For the *N. lugens* experiment, 30 second-instar nymphs, 30 fourth-instar nymphs and 30 (non-gravid) adults were transferred to each cage. These were allowed to settle for 24 h prior to introduction of the predators. In addition, four gravid female *N. lugens* were caged individually on one of the four pots from each experimental cage to lay eggs. After 24 h the females were removed and the pot was placed back in the experimental cage.

For the leaf-folder experiment, ten adult males, 20 first-instar larvae and 20 fourth-instar larvae were introduced into the cages and allowed to settle for 24 h before introduction of the predators. In a similar way to *N. lugens*, adult females were caged on individual pots to provide egg samples. Six females were used per pot and eggs were counted under low-powered magnification before the pots were placed into the experimental cages.

In total, 30 cages were set up for each prey species and treatments were arranged in three replicate blocks, each containing the eight predator treatment combinations and

two predator-free controls arranged at random. The appropriate assemblage of predators was introduced to each cage at the start of the experiment and the cages were left undisturbed for 24 h. The short timescale was deliberately chosen to isolate complementarity of function due to the behaviour of the predators from the confounding effects of changes in predator and prey abundance, which would come into play with a longer-term experiment (see Petchey, 2003). At the end of this period, each cage was exhaustively sampled with all live and dead insects recorded and any eggs dissected under magnification to determine whether predation had occurred.

Analysis

Analysis of variance was undertaken on proportional prey mortality (arc sine transformed). Because each diversity level was represented by several species combinations, and all the combinations were themselves replicated, two error terms were used in the analysis. Predator diversity was tested against combination within diversity level as the error term, and combination against the residual error (Schmid *et al*., 2002). Predator-free controls were included in the analysis as the basal diversity level and significant diversity or combination effects were further investigated with reference to least significant differences between treatment levels (Crawley, 2002). The life stages of the prey insects were analysed individually and then overall mortality, calculated as the mean proportional mortality across the life stages, analysed for each prey species.

Emergent MPEs were defined for the multispecies treatment combinations as the residuals of the linear model $\ln(s) = b_0 + b_1n_1 + b_2n_2 + b_3n_3 + b_4n_4$, where b_i is the coefficient, and n_i the number of individuals, of predator species i, and s is the combined survival across life stages. Thus, the residuals represent departures from expected survival based on the abundances of the four predator species in each treatment. The logarithmic transformation ensures that this is equivalent to the multiplicative predation model proposed by Soluk and Collins (1988).

Results

On the whole, the experimental protocols were successful, showing appreciable levels of predation in the treatment cages and little mortality in the control cages. In addition, few of the predators died during the course of the experiments. In the analysis of mortality of individual life stages of each prey species, predator diversity was never a significant contrast (Table 1). However, the combined mortality across life stages revealed significant differences among diversity treatments for *M. patnalis*, but not for *N. lugens*, with the three-predator species treatments causing significantly higher mortality than the single-species treatments [see Fig. 1, parameter estimates (arc sine) one species = 0.52; three species = 0.82; LSD 5% = 0.11].

Predation rates in the individual multispecies treatments reveal more about the source of overall diversity effects. Generally, MPEs were more positive in *M. patnalis*, with two predator combinations showing strongly positive deviations from the predation rate predicted from the abundance of each species (Fig. 2). In *N. lugens*, deviations were largely neutral and more variable, although one combination showed a strongly positive MPE, similar to that observed for *M. patnalis*.

Discussion

Analysis of the overall mean predation rates across all the life stages of the pests showed a positive effect of predator species richness on predation of the leaf-folder *M. patnalis*, but no significant difference between single and multiple species treatments for the planthopper *N. lugens*. Given the substitutive experimental design used, this result indicates that diverse assemblages function at a higher rate than single-species assemblages when predating *M. patnalis*. This result indicates significant functional complementarity among the predator species when attacking *M. patnalis*, which could have arisen from resource-use differentiation (e.g. utilisation of different life stages, or spatial positions) or from synergistic interspecific interactions among the predator species. The fact that diversity effects were not observed within life stages indicates that synergistic interactions, the most commonly reported mechanism of positive MPEs (Sih *et al*., 1998; but see Sokol-Hessner & Schmitz, 2002), were probably not responsible for the observed patterns in this case, although lower statistical power within life stages means that this possibility cannot be rejected absolutely. Therefore, resource-use differentiation is the most likely explanation for the positive predator diversity effect against *M. patnalis*.

What is the nature of resource-use differentiation in this context? The diversity treatment differences emerged from a substitutive design and the results indicate, therefore, increased predation by multispecies assemblages relative to single-species assemblages of similar expected feeding rate. This implies not only that there is significant resource-use differentiation when the predator assemblages attack *M. patnalis*, but also that predation rates in the single-species assemblages are limited by negative intraspecific interactions. In the absence of negative interactions among individuals of the same species there would be no advantage of resource-use differentiation and no significant difference between predator diversity treatments. The positive diversity effect can be thought of as a general relaxation of negative interactions among individual predators in multispecies compared with single-species assemblages. Importantly, the release from interference between individuals in the multispecies treatments was only apparent in the predation of *M. patnalis*, suggesting that the prey identity influences the way in which groups of predators interact. Interestingly, the results are consistent with earlier theoretical predictions concerning the effects of prey life history on the emergence of positive effects of predator

Table 1. Results on the analysis of variance of proportional predation rates (arc sine transformed) of *Nilaparvata lugens* and *Marasmia patnalis*. Block and combination are tested against the residual error, whereas diversity is tested against the combination term.

		Eggs			Small nymphs			Large nymphs			Adults			Combined		
Source	d.f.	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
(a) *N. lugens*																
Block	2	0.06	0.06	0.142	0.01	0.17	0.846	0.14	0.25	0.251	0.01	0.15	0.864	0.00	0.14	0.871
Diversity	2	0.22	0.76	0.510	0.04	0.78	0.512	0.15	1.36	0.324	0.33	1.06	0.405	0.11	3.42	0.102
Combination	6	**0.88**	**10.31**	**<0.001**	0.14	0.96	0.478	0.33	1.20	0.349	**0.94**	**3.47**	**0.019**	0.10	1.55	0.220
Residual	18	0.25			0.44			0.83			0.81			0.19		
		Eggs			Small larvae			Large larvae			Adults			Combined		
Source	d.f.	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*	SS	*F*	*P*
(b) *M. patnalis*																
Block	2	**0.51**	**5.74**	**0.011**	0.13	1.36	0.281	0.08	1.59	0.230	0.04	0.63	0.542	**0.11**	**15.81**	**<0.001**
Diversity	2	1.13	1.42	0.312	0.12	1.73	0.255	0.00	0.06	0.942	2.16	2.97	0.127	**0.41**	**8.85**	**0.016**
Combination	6	**2.37**	**8.95**	**<0.001**	0.22	0.76	0.610	0.16	1.05	0.423	**2.18**	**10.79**	**<0.001**	**0.14**	**6.92**	**<0.001**
Residual	19	0.84			0.90			0.47			0.64			0.06		

diversity (Wilby & Thomas, 2002a). Positive effects of predator diversity are found when the *M. patnalis* is the prey and, as an endopterygote, *M. patnalis* has much larger differences in morphology and behaviour between life stages than the exopterygote, *N. lugens*. It is likely that this ecological differentiation among life stages facilitated resource-use differentiation among the predator species. This is similar to the suggestion of Sih *et al.* (1998) that habitat separation or different hunting modes among predator species may increase the probability of positive relative to negative MPEs but in this case, the expression of MPEs is mediated by general life-history characteristics of the prey themselves.

The source of the positive effect of predator diversity on predation is further elucidated through the analysis of MPEs in the experiments. In the case of *M. patnalis*, two of the four three-species predator combinations resulted in strongly positive emergent MPEs, whereas for *N. lugens* only one showed a positive MPE and the others tended to be neutral and variable. While the significant diversity effect signifies a general tendency to positive diversity effects against *N. lugens*, examination of the MPEs against both prey species reveals that different combinations of predators can display very different behaviours. This emphasises the importance of replication of diversity in these types of studies; unless different species combinations are used within diversity levels, it is not possible to test general diversity effects, merely the MPEs of the particular species combinations used. In addition, the fact that the same predator assemblages can lead to such contrasting effects between prey species indicates that emergent MPEs are not simply a function of the predators themselves, but also the ecological context (in this case prey type) in which they interact (cf. Cardinale *et al.*, 2003).

Understanding the relationship between predator species diversity and predation rate is of great importance for the development of biological control strategies and in determining whether there are likely to be conflicts between conservation of biodiversity and biological pest control (Finke & Denno, 2004). This study highlights a mechanism for positive diversity effects on predation rate which may vary among prey types. There was limited evidence for net negative MPEs in these experiments, unlike many other studies of MPEs (e.g. Snyder & Ives, 2001; Rosenheim, 2001), but this is probably due to the short timescale of the experiments. Mechanisms of negative diversity effects, such as intra-guild predation, occur at a population level and usually involve abundance changes, which may take some time to manifest (Rosenheim, 2001). An important

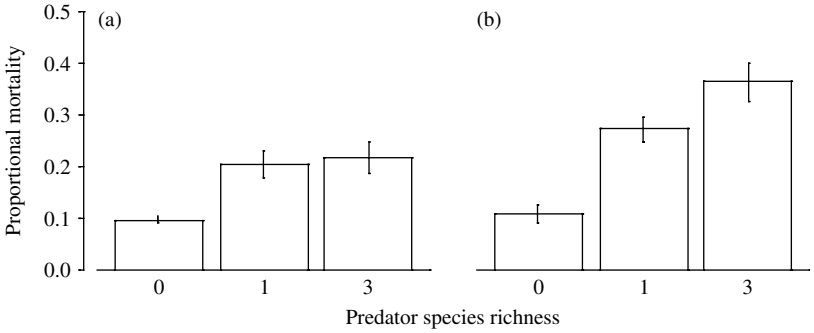

Fig. 1. Parameter estimates for the mean proportional mortality across all life stages of (a) *Nilaparvata lugens* and (b) *Marasmia patnalis*. Bars denote standard errors of the mean.

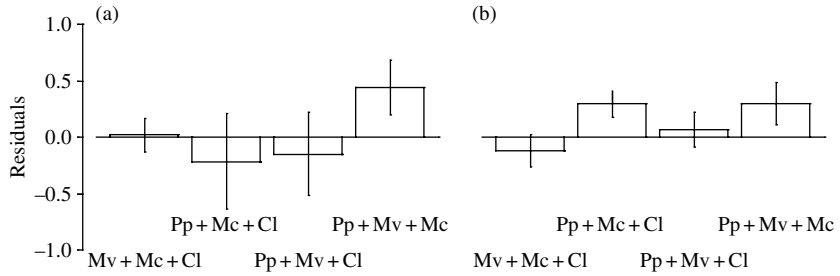

Fig. 2. Multi-predator effects in the three-species treatments exhibited as residuals from the model, $\ln(s) = b_0 + b_1n_1 + b_2n_2 + b_3n_3 + b_4n_4$, where b_i is the coefficient, and n_i the number of individuals, of predator species i, and s is the combined survival across life stages for (a) *Nilaparvata lugens* and (b) *Marasmia patnalis*. Predator species are labelled as follows: Pp, *Pardosa pseudoannulata*; Mv, *Metioche vittaticollis*; Mc, *Micraspis crocea*; Cl, *Cyrtorhinus lividipennis*. Bars denote standard errors of the mean.

development of the current research will be to take replicated experiments into the field to determine whether the reported mechanisms behind positive diversity effects hold over longer timescales with dynamic populations.

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