PROCEEDINGS OF THE ROYAL SOCIETY B

*Proc. R. Soc. B* (2008) **275**, 2283–2291
doi:10.1098/rspb.2008.0405

# Functional group diversity of bee pollinators increases crop yield

Patrick Hoehn[1,*], Teja Tscharntke[1], Jason M. Tylianakis[2] and Ingolf Steffan-Dewenter[3]

[1]*Agroecology, University of Göttingen, Waldweg 26, 37073 Göttingen, Germany*
[2]*School of Biological Sciences, University of Canterbury, Private bag 4800, Christchurch 8020, New Zealand*
[3]*Department of Animal Ecology I, Population Ecology, University of Bayreuth, 95440 Bayreuth, Germany*

Niche complementarity is a commonly invoked mechanism underlying the positive relationship between biodiversity and ecosystem functioning, but little empirical evidence exists for complementarity among pollinator species. This study related differences in three functional traits of pollinating bees (flower height preference, daily time of flower visitation and within-flower behaviour) to the seed set of the obligate cross-pollinated pumpkin *Cucurbita moschata* Duch. ex Poir. across a land-use intensity gradient from tropical rainforest and agroforests to grassland in Indonesia. Bee richness and abundance changed with habitat variables and we used this natural variation to test whether complementary resource use by the diverse pollinator community enhanced final yield. We found that pollinator diversity, but not abundance, was positively related to seed set of pumpkins. Bees showed species-specific spatial and temporal variation in flower visitation traits and within-flower behaviour, allowing for classification into functional guilds. Diversity of functional groups explained even more of the variance in seed set ($r^2=45\%$) than did species richness ($r^2=32\%$) highlighting the role of functional complementarity. Even though we do not provide experimental, but rather correlative evidence, we can link spatial and temporal complementarity in highly diverse pollinator communities to pollination success in the field, leading to enhanced crop yield without any managed honeybees.

**Keywords:** complementary resource use; ecosystem services; land-use management gradient; fruit set; tropical ecology; spatio-temporal variability

## 1. INTRODUCTION

Global biodiversity decline has focused attention on the implications of species losses for the maintenance of ecosystem functioning (Hooper *et al.* 2005; Tilman *et al.* 2006). Animal pollination contributes to 35% of global food production (Klein *et al.* 2007), but anthropogenic activities such as habitat loss, habitat fragmentation, land-use intensification and use of agrochemicals have adverse effects on pollinator diversity (e.g. Steffan-Dewenter *et al.* 2002; Tylianakis *et al.* 2005; Biesmeijer *et al.* 2006), placing crop pollination services at risk (Kremen *et al.* 2002; Steffan-Dewenter *et al.* 2005; Tscharntke *et al.* 2005). The most important taxon performing this service is the family Apidae (Klein *et al.* 2007), but a worldwide decline in the number of managed colonies of the European honeybee has increased reliance on diverse communities of wild bees for supplying crop pollination services (Klein *et al.* 2003*b*, 2007; Kremen *et al.* 2004; Ricketts 2004; Shuler *et al.* 2005).

Recent studies have related declining pollinator diversity to the ecosystem service of pollination (Kremen *et al.* 2002; Klein *et al.* 2003*b*), particularly when realistic (as opposed to random) extinction sequences are considered (Larsen *et al.* 2005). The literature examining the biodiversity–ecosystem functioning relationship suggests that diverse assemblages might function better due to niche complementarity (Hooper *et al.* 2005; Cardinale *et al.* 2006), whereby a combination of many different species can extract more resources in space and time than can a species-poor community (Cardinale *et al.* 2004, 2006; Fargione & Tilman 2005; Hooper *et al.* 2005; Fontaine *et al.* 2006). The importance of this mechanism in natural pollinator communities is unknown, but evidence of a stronger diversity–pollination relationship when flowers are heterogeneously distributed (Tylianakis *et al.* 2008) suggests that an analogous mechanism may operate. Pollinator species within a community may have behaviourally partitioned niches, but little is known about the mechanisms of complementarity between the bee species. Although complementarity among species niche traits is possible, pollinator abundance and flower visitation frequency have also been frequently related to pollination success (Klein *et al.* 2003*a*; Morandin & Winston 2005; Vázquez *et al.* 2005; Degrandi-Hoffman & Chambers 2006). Therefore, before testing the effect of pollinator diversity on pollination rates, it is necessary to control for the possible confounding effect of abundance that is usually correlated with diversity.

Functional diversity has been suggested to be the most important component of diversity (e.g. Tilman *et al.* 1997; Hulot *et al.* 2000; Lavorel & Garnier 2002), and a common approach to test the effects of biodiversity on ecosystem functioning is an experimental manipulation of functional guild diversity (e.g. Lanta & Leps 2006; Scherber *et al.* 2006). Species are often assigned to functional guilds

---

[*] Author for correspondence (pathoehn@web.de).

*Received* 25 March 2008
*Accepted* 9 June 2008

Table 1. Bee behaviour within flowers and pollen deposition in relation to body size (size classes: VS, very small; S, small; M, medium; L, large; VL, very large). Duration of flower visitation (mean ± s.e. in seconds) of four bee species. Pollen deposition: diameter of the pollen-transporting leg part (fe, femur; ti, tibia; ta, first tarsus; in mm) and additive pollen-transporting body parts. Different superscript letters indicate significant differences.

| body size class | duration of flower visitation | no. of visited flowers | pollen deposition | pollen distribution on the stigma |
|---|---|---|---|---|
| VS | $52\pm1.8^{b}$ ($n=19$; *Lasioglossum* sp.) | few | very low; 0.2–0.3 (*Lasioglossum*: fe, *Trigona*: ti); *Lasioglossum*: ventral abdomen, *Trigona*: none | high |
| S | $214\pm6.7^{a}$ ($n=27$; *Lasioglossum halictoides*) | few | low; 0.5 (*Ceratina*: ti, *Lasioglossum*: fe), ventral abdomen | very high |
| M | unknown | unknown | medium; 0.9–1.3 (*Apis*: ta, *Nomia*: fe), *Apis*: pronotum, *Nomia*: propodeum | medium |
| L | unknown | unknown | high; 2.1 (*Apis*: ta, *Amegilla*: ti), pronotum | low |
| VL | $21\pm0.6^{b}$ ($n=23$; *Xylocopa confusa*/*dejeani*) | many | very high; 2.5–2.8 (ta), pronotum and abdominal tip | low |

based on *a priori* expectations of complementarity due to taxonomic, physiological or morphological traits. This approach has not only been used for plant species (e.g. Tilman *et al.* 1997; Hooper & Dukes 2004; Fargione & Tilman 2005) but also for pollinators (Fontaine *et al.* 2006). Here we test the effect of pollinating bee species richness and functional diversity on the number of seeds per fruit of a crop plant, pumpkin *Cucurbita moschata* Duch. ex Poir, in different habitats (grassland, agroforest and forest). We compare the bee species in terms of their behaviour (Chagnon *et al.* 1993), and quantify the observed pollinating height, the time of day at which they are active and body size, which is strongly related to behaviour within and between pumpkin flowers (table 1). We then use statistically significant differences in these parameters to group species *post hoc* into functional guilds, and relate the effect of pollinator species and functional guild diversity to pollination success.

We show that habitat variables affect species richness of pollinators, which in turn is positively related to the pollination success of pumpkin. More importantly, however, we show that quantitative species-specific differences in pollinator traits, such as species turnover in space and time, and behavioural differences during flower visitation, underlie this positive relationship between biodiversity and ecosystem functioning.

## 2. MATERIAL AND METHODS

The study was conducted on the western margin of the Lore Lindu National Park in Central Sulawesi (Indonesia) in the surroundings of the village Toro (120°2′ E, 1°30′ S, 800–1100 m above sea level) approximately 100 km south of the region's capital Palu. The land use in this area is small scale and dominated by natural and disturbed tropical forests, cacao agroforestry systems of differing management intensity, grasslands and paddy fields. As no honeybees are managed in this region, farmers completely rely on pollination services provided by the diverse native pollinator community.

We selected 18 study plots in five different habitat types, which covered a range of environmental conditions and differed in the diversity of pollinator assemblages. The five habitat types were natural forest, three different management intensities of cacao agroforestry, and grassland (open area habitat) with few trees. We refer to a plot as a site with homogeneous land-use practices of the mentioned habitat type and a minimum core area of 30 × 50 m. The cacao agroforestry systems formed a gradient according to the composition of shade tree species: low-management intensity agroforestry with natural forest trees as shade trees (low intensity); medium-intensity systems with a diverse shade tree community planted by farmers (medium intensity); and high-intensity plots with few planted shade tree species (*Gliricidia sepium* (Jacq.) and *Erythrina subumbrans* (Hassk.); high intensity). The number of shade tree species was strongly correlated with canopy cover (Spearman: $R=0.609$, $n=18$, $p=0.0073$) and corresponding microclimatic conditions such as temperature (Spearman: $R=-0.489$, $p=0.0001$) and humidity (Spearman: $R=0.705$, $p<0.0001$). Four replicates were chosen for each habitat type, but we were forced to abandon one plot in the natural forest and one plot in the open habitat, as experimental pumpkin plants failed to grow. In our study plots, farmers grew a variety of field crops between the cacao and shade trees.

Pumpkin (*C. moschata*, Fam.: Cucurbitaceae) is a common cash crop in the research area, with several advantages for studies of plant–pollinator interactions. It is cultivated in all habitat types, has local economic value and is a fast-growing plant with highly attractive flowers for bees. The plant sets seed only after cross-pollination and seed set is pollen limited so that pollination success can be directly measured (Walters & Taylor 2006). Pumpkin is a crawling and climbing plant, which allowed us to use stakes to expose flowers at different heights. This three-dimensional growth allows testing for niche differentiation in space. Finally, the opening time of flowers is restricted to approximately 4 hours in the morning, which makes it possible to record almost the entire temporal pollinator turnover during this receptive phase of flowering. We planted experimental pumpkin patches of 2 × 5 m for observations of the pollinator community and measurement of the resulting seed set in the least shaded part of each plot for standardization and to maximize plant growth. We used liquid fertilizer in a monthly cycle to prevent soil nutrient availability from limiting fruit production.

Pumpkin observations were conducted in three phases: (i) 26 April 2005–1 June 2005, (ii) 10 June 2005–21 July 2005, and (iii) 10 January 2006–3 February 2006, with each plot visited once per phase. Bee abundances in the open habitats were higher when compared with the other habitat types, so in order to classify all the occurring bee species according to body size-related flower visitation behaviour, we visited grassland plots additionally once or twice. The specimens from these fourth and fifth phases were used only for the classification of bee species into functional groups, and were not included in the calculations of seed set, pollinator richness and abundance per plot. Pollinators were recorded in a standardized way for 3.5 hours per session from 07.00 until 10.30, which encompassed most of the opening time of flowers each day (203 plot observation hours in total). We adjusted the number of flowers for each observation and removed the excess flowers or added missing flowers in small jars to keep a constant number of five flowers across replicate pumpkin patches (Ishii 2006), which resembles the approximate number of large and ephemeral pumpkin flowers on an area of $10\ \mathrm{m}^2\ \mathrm{d}^{-1}$. To add or remove flowers also allows standardization of the sex ratio per plot and control of flower height within a range of 0.1 to 1 m in each plot. To determine the factors correlated with flower visitation frequencies, single pumpkin flowers were characterized by the length of their corolla (cm), diameter of corolla edge (cm), height (m above ground) and sex (male/female). Observed bees were caught for identification and to avoid counting the same individual twice, using a simple exhauster for small species and a sweep net for larger ones. The time of flower visitation and the body size-related behaviour of the pollinator species, such as within-flower movements, were noted (table 1). We measured the diameter of the largest pollen-transporting leg part (femur, tibia or first tarsus), including hair structures and noted other pollen-transporting body parts to estimate a size-dependent hierarchy between species in terms of the amount of pollen transferred. Bee species were identified by Stephan Risch (Leverkusen, Germany). Voucher specimens are kept at the Bogor Agricultural University (IPB) in Indonesia.

Subsequent to the observation of pumpkin pollinators, the diversity and density of flowers in the herb layer and understorey were measured along six transects, each 4 m wide and 30 m long. Flower density of each plant species along the transect walks was estimated on a per square metre basis, whereby 1 was equivalent to a single flower of one species and 100, a species that covers the whole area with many flowers. The transect walks per observation day and plot covered approximately half of the plot core area ($720\ \mathrm{m}^2$). Conspecific flowers act as a pollen source and may therefore enhance seed set in the experimental plot, particularly because pollinators often specialize temporarily on a single or a few plant species (Ishii 2006). To assess the number of effective pumpkin flowers in the surroundings, flowers in the plot were counted in a radius of approximately 50 m around the experimental pumpkin patch. Temperature, humidity and light intensity were measured every 30 min from 07.00 to 11.00 on each observation day at a height of 2 m using a thermo-, hygro- and luxmeter (Mavalux Digital, Gossen) in an area receiving full sun. Ripe pumpkin fruits were collected throughout the entire time the plants grew. The size of pumpkins was measured (girth in cm) and seeds were counted. We used mesh bags for 11 female flowers to test the effect of pollinator exclusion on the fruit development. Nine flowers, each from a different plot, were hand pollinated by rubbing the dry anthers of at least three male flowers from different plots against the pistil of the female flower to estimate the maximum possible seed set when pollination is not limiting. To avoid resource re-allocation to open flowers, we applied hand pollination only when a single female flower was open in a pumpkin plantation. We performed only 11 pollen exclusions and nine hand pollinations in total, because pumpkins do not set fruits without cross-pollination and there were no notable differences between hand-pollinated fruits from different habitat types in seed set (see §3).

### (a) *Statistical analysis and classification scheme*

To identify the factors that structure the pollinator community, we conducted two linear mixed-effects models using the 'nlme' package in R v. 2.3.1 (The R Development Core Team 2006), fitted using maximum likelihood rather than restricted maximum likelihood to allow comparisons of models with different fixed effects structure. Remaining analyses were carried out in STATISTICA (StatSoft, Inc. 2004, v. 7. www.statsoft.com.). Owing to collinearity of the weather conditions (temperature, humidity and light intensity), we used a principal component analysis (PCA) and reduced the number of continuous weather predictors to one component axis explaining 76% of the overall variation (henceforth, weather conditions, component relations: temperature +, humidity −, light intensity +). Similarly, we conducted a separate PCA with the collinear resource factors, flower density and diversity, reducing them to one axis explaining 74.5% of the overall variation (henceforth, resources). The maximal mixed-effects models included the number of bee species or bee individuals as response variables and season (observation phase), nested in plots as random effects, habitat type as a fixed effect and weather conditions (PCA axis), resources (PCA axis) and the number of pumpkin flowers in the plot surrounding the pumpkin plantation as continuous variables. Maximal models were then simplified based on the stepwise removal of non-significant predictors and comparison of Akaike information criterion (AIC) scores. Significance values for a predictor were obtained using a likelihood ratio test comparing a model including the predictor with one from which the predictor had been removed. Values per plot per sampling phase of response and continuous variables were used for the statistical analyses.

To analyse the effects of variables that we hypothesized to have an influence on seed set and fruit size, a general linear model (GLM) with seeds per fruit and girth as the dependent variable was conducted. Residuals in all GLMs were tested for a normal distribution and transformed if necessary. We used type I (sequential) sum of squares for each model. We give arithmetic mean ± s.e. in the text. Bee abundance and richness were used as continuous predictors of seed set per fruit. In addition, the number of pumpkin flowers surrounding the plantation, humus thickness (nutrient supply), slope (water supply) and canopy cover (light availability) were added as covariates because they are likely to affect the plant growth and thereby affect seed set. Habitat type was used as a categorical predictor. Mean values of bee observations, pumpkin flowers in the plot and measured abiotic variables per plot were used, because seed set data were available only at a plot level and not for each observation day. We could not test on the effects of specific pollinator visits to a given flower on seed set because the bee sampling on the pumpkin flower strongly influenced the flower and seed set (e.g. reduced pollen grains due to suction of smaller bees with an exhauster). Owing to a lack of female flowers in four plots (natural forest, low intensity, medium intensity and high intensity), the number of plots was reduced to $n=14$.

We tested whether species-specific spatial and temporal foraging patterns and body size-related behaviour within flowers differed significantly among species, such that this may lead to complementarity. We fitted mixed-effects models

Table 2. Mean ± s.e. of flower visiting height, flower visiting time of day (local time ± minutes) and body size (size classes: VS, very small; S, small; M, medium; L, large; VL, very large) for each of the 12 most abundant species, resulting in a classification into eight functional guilds (from A to H). (Significant differences ($p<0.05$) are indicated by different letters (figure 2). Species in one guild do not differ in any of the three traits.)

| | classification scheme | | | |
|---|---|---|---|---|
| species | flower visiting height (cm) | flower visiting time of day | body size (mm) and size class | guild |
| *Apis dorsata* | $0.8\pm0.163^{ab}$ ($n=7$) | $09.04\pm34^{abcd}$ ($n=7$) | $3.61\pm0.04$ (L; $n=7$) | A |
| *Amegilla* sp. | $0.49\pm0.049^{ab}$ ($n=56$) | $08.37\pm6^{a}$ ($n=54$) | $4.19\pm0.067$ (L; $n=7$) | A |
| *Nomia concinna* | $0.24\pm0.085^{bd}$ ($n=8$) | $09.14\pm20^{abcd}$ ($n=8$) | $2.63\pm0.037$ (M; $n=5$) | B |
| *Nomia fulvata* | $0.4\pm0.129^{ab}$ ($n=7$) | $09.24\pm9^{acd}$ ($n=7$) | $2.67\pm0.057$ (M; $n=7$) | B |
| *Ceratina cognata* | $0.46\pm0.079^{ab}$ ($n=22$) | $09.53\pm5^{c}$ ($n=22$) | $1.88\pm0.037$ (S; $n=5$) | C |
| *Lasioglossum halictoides* | $0.7\pm0.032^{ad}$ ($n=156$) | $08.55\pm3^{ad}$ ($n=156$) | $1.97\pm0.049$ (S; $n=6$) | D |
| *Lasioglossum* sp. | $0.29\pm0.027^{b}$ ($n=165$) | $09.08\pm3^{d}$ ($n=165$) | $1.49\pm0.028$ (VS; $n=9$) | E |
| *Trigona* sp. | $0.48\pm0.062^{ab}$ ($n=45$) | $09.23\pm8^{cd}$ ($n=40$) | $1.41\pm0.021$ (VS; $n=8$) | E |
| *Xylocopa confusa* | $0.63\pm0.055^{acd}$ ($n=39$) | $08.33\pm9^{ab}$ ($n=39$) | $7.59\pm0.104$ (VL; $n=8$) | F |
| *Xylocopa dejeani* | $0.4\pm0.054^{bc}$ ($n=44$) | $08.26\pm6^{ab}$ ($n=44$) | $6.52\pm0.077$ (VL; $n=9$) | F |
| *Xylocopa nobilis* | $0.9\pm0.105^{ac}$ ($n=8$) | $09.52\pm14^{cd}$ ($n=8$) | $8.03\pm0.084$ (VL; $n=8$) | G |
| *Apis cerana* | $0.38\pm0.05^{bc}$ ($n=49$) | $08.09\pm6^{b}$ ($n=49$) | $2.65\pm0.042$ (M; $n=8$) | H |

with flower visiting height, flower visiting time and flower volume as dependent variables, and habitat type (fixed effect), plot (random effect nested in habitat type) and species identity (fixed) as categorical predictors. We calculated flower volume from the diameter ($2r$) and length ($h$) of the corolla with the formula for a cone as a close approximation for flower volume: $V=(h\times\pi\times r^2)/3$. The 12 most abundant species were compared because they were abundant enough to allow statistical analyses.

In addition to the above traits, we sorted species into body size classes, as there was evidence that within- and between-flower behaviours are strongly influenced by body size (Stout 2000). We used four exemplary within- and between-flower pollinating traits that we could easily observe in the field and characterize different size classes (table 1). We used the same 12 species as for the spatial and temporal differences, to ensure comparability. We measured the span of the pronotum between tegulae and obtained five size classes (very small (VS), less than 1.5 mm; small (S), 1.5–2 mm; medium sized (M), 2.1–3 mm; large (L), 3.1–5 mm; and very large (VL), more than 5 mm; table 1). To test the differences in behaviour within flowers that may depend on body size, we conducted a GLM with duration (in seconds) of flower visitation at a single flower for three size classes (VS, S and VL) as the response variable and habitat type (fixed) and size class (fixed) as categorical predictors. We measured the duration of flower visitation for *Lasioglossum* sp., *Lasioglossum halictoides*, *Xylocopa dejeani* and *Xylocopa confusa*, as the behaviour of only these species from the 12 focus species (table 2) was readily observable. We described each species in terms of three further behavioural traits (number of visited flowers, pollen deposition and pollen distribution on the stigma) that depend on body size, by dividing them into categories. We recorded the number of visited pumpkin flowers and pollen distribution on the anther or pistil (due to bee movement on reproductive plant parts) during the field observations.

We used a GLM with flower visiting height and flower visiting time, respectively, as dependent variables and habitat type (fixed effect), plot (random effect nested in habitat type) and body size class (fixed) as categorical predictors to test whether bee body size influences variation in flower visiting traits in space and time. Owing to low numbers of social bee species, we did not include social status as a classification criterion.

For a classification into functional guilds, we used differences in the spatial, temporal and body size-dependent behaviour within the flowers above. Species were assigned to the same guild if they did not differ significantly in any of these three variables (table 2).

We conducted a multiple regression model with seed per fruit as the response variable and bee species richness and number of functional guilds as covariates, to have a direct comparison of the explanatory strength of richness versus functional guild diversity. To factor out abundance, we regressed seed set against abundance and used the residuals from this model as the response variable. We then adjusted appropriately the degrees of freedom for $F$ ratios in the multiple regression. As functional diversity and species richness covary (Naeem 2002), we conducted two models. In the first model, species richness was included ahead of functional diversity and vice versa in the second model (Schmid *et al.* 2002).

## 3. RESULTS

Pollinator exclusion by bagging female flowers caused plants to abort the fruit. By contrast, all hand-pollinated female flowers matured to seed-bearing fruits (419 ± 17 s.e. seeds per fruit, $n=9$). We found on average 2.72 ± 0.33 pumpkin flowers in the surroundings of each pumpkin plot ($n=14$). In total, 633 bee individuals from 25 species and nine genera were caught, belonging to the subfamilies Anthophorinae, Apinae, Halictinae, Megachilinae and Xylocopinae.

The pumpkin pollinator community was strongly determined by the habitat and environmental variables. Bee species richness increased significantly with the weather PCA axis (increasing temperature and light intensity, decreasing humidity; likelihood ratio = 7.06, $p=0.008$), and the resources PCA axis (increasing density and diversity of floral resources; likelihood ratio = 8.31, $p=0.004$). Although both of these variables differed across habitat types, habitat type itself did not significantly add any explanatory power (likelihood ratio = 3.01, $p=0.557$), and was thus removed from the minimal model.

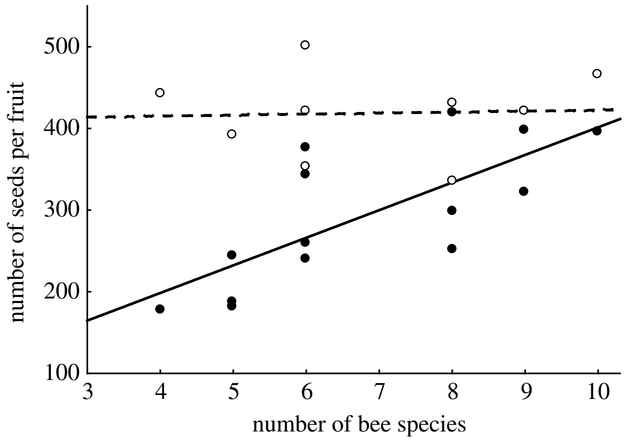

Figure 1. Mean number of seeds per fruit per pumpkin patch in relation to the number of bee species per pumpkin patch. Results for open-pollinated flowers are shown with filled circles and solid line and that for hand-pollinated bagged control flowers in nine plots are shown with filled circles and dashed line.

Conversely, the only significant predictor in the minimal model for bee abundance was habitat type (likelihood ratio$=11.26$, $p=0.024$); abundance was significantly higher in open area habitats ($21.33\pm4.06$ individuals, $n=3$) compared with all other habitat types (natural forest: $3.56\pm2.87$, $n=3$; low intensity: $6.23\pm3.86$, medium intensity: $6.08\pm3.80$, high intensity: $10.58\pm3.80$ individuals per flower and sampling (pollination rate), $n=4$).

Bee species richness was the only significant predictor variable in the model for seed set per fruit ($r^2=0.452$, $F_{1,13}=19.24$, $p=0.022$; figure 1) whereas bee abundance did not significantly correlate with seed set ($r^2<0.01$, $F_{1,13}=0.13$, $p=0.74$). Mean number of seeds per fruit from plots with high species richness (10 bee species) reached almost that of hand-pollinated control flowers (figure 1), whereas low richness (four species) led to just 50% of the seed set found in control flowers. Number of seeds per fruit was correlated with fruit size (Spearman: $R^2=0.635$, $p=0.015$), which is the economically most important trait for measuring this ecosystem service. However, we found no correlation between fruit size and the bee community (abundance: $r^2<0.001$, $F_{1,3}=0.043$, $p=0.85$; species richness: $r^2=0.05$, $F_{1,3}=8.29$, $p=0.064$) in contrast to seed set as the response variable. Habitat type, surrounding pumpkin flowers, humus thickness, slope and canopy cover did not influence seed set significantly (table 3).

Species differed in their spatial resource use (height of flowers: $r^2=0.142$, $F_{11,575}=9.712$, $p<0.001$), with *Nomia concinna* preferring the lowest, and *Xylocopa nobilis* preferring the highest flowers (table 2, figure 2). Pollinating height in one high-intensity plot was significantly lower ($0.26\pm0.04$) compared with two open area plots ($0.59\pm0.03$, $0.59\pm0.05$; $r^2=0.05$, $F_{13,575}=3.2$, $p=0.0001$), independent of habitat type ($r^2=0.04$, $F_{4,575}=1.9$, $p=0.177$). Temporal species turnover showed even stronger differences, as almost all species differed significantly from each other in their preferred time of visitation ($r^2=0.2$, $F_{11,567}=14.845$, $p<0.001$). The species that visited flowers the earliest were *Apis cerana*, *X. dejeani* and *X. confusa*, whereas *X. nobilis* and *Ceratina cognata* appeared significantly later (table 2, figure 2). Flower visitation in one low-intensity cacao plot was on average earlier ($08.16\pm10$) when compared with one medium-intensity ($09.19\pm11$), high-intensity ($09.14\pm6$) and open area plot ($09.05\pm5$; $r^2=0.08$, $F_{13,567}=5.123$, $p<0.001$) and habitat had no influence ($r^2=0.03$, $F_{4,567}=0.91$, $p=0.49$). Species identity explained minor variance of the overall model for preferred flower size ($r^2=0.04$, $F_{8,430}=2.82$, $p=0.0047$), and as only *A. cerana* ($233\pm37$ cm$^3$, $n=18$) differed from *C. cognata* ($134\pm20$ cm$^3$, $n=17$) and *Lasioglossum* sp. ($157\pm8$ cm$^3$, $n=137$) in preferred flower size, we did not include flower size for classification into functional guilds.

Body size was closely related to pollinating behaviour and each size class showed consistent patterns. Duration of a single flower visitation was significantly longer for small bees compared with very small and very large bees ($r^2=0.38$, $F_{2,65}=20.11$, $p<0.001$; table 1). Body size classes also differed in the number of flowers they visited. Very large bees checked two or three flowers mostly in their preferred height range, whereas small bees fed for a very long time but only on one flower. The amount of pollen transferred per flower visit was a consequence of species-specific anatomical characteristics, because larger bees had larger pollen-transporting surfaces such as the plumose ventral section of the abdomen and the dorsal part of the thorax or femur (table 1). Within-flower movements are generally responsible for pollen distribution on the stigma (Chagnon *et al.* 1993). Owing to their size, large and very large bees entered the flower directly and remained between the petal and anther or pistil, while rubbing the pollen-carrying ventral part of the abdomen on the pistil of a female flower, or picking up pollen in male flowers. High pollen transfer was restricted to a part of the pistil, as large bees could not move around the pistil as did small and very small bees (table 1). Small and very small bees landed on the petal, anther or pistil and then walked for a long time on anthers or pistil while feeding on pollen or nectar and thus distributing pollen. Very large bee species such as *X. dejeani* and *X. confusa* appeared very early in the morning, transferring large amounts of pollen, whereas *Lasioglossum* sp., *C. cognata* and *Trigona* sp. appeared significantly later, mainly providing the distribution of the pollen that was already transferred by other species (e.g. *Xylocopa*) on the stigma owing to their activity within the flower.

There was no clear pattern relating body size to pollinating height, even though very small species pollinated significantly lower flowers ($r^2=0.1$, $F_{4,582}=18.128$, $p<0.001$; 0.33 m$\pm0.026$, $n=209$) than small (0.672 m$\pm0.03$, $n=178$), large ($0.523\pm0.048$, $n=62$) or very large species ($0.544\pm0.04$, $n=91$). By contrast, small bees pollinated significantly higher flowers compared with medium-sized bees ($0.368\pm0.042$, $n=64$). We found that medium-sized ($08.25\pm6$ min, $n=64$), large ($08.40\pm7$ min, $n=60$) and very large ($08.37\pm6$ min, $n=90$) bees occurred significantly earlier compared with small ($09.02\pm3$ min, $n=178$) and very small bees ($09.11\pm3$ min, $n=203$; $r^2=0.08$, $F_{4,574}=14.99$, $p<0.001$).

According to the differences between the bee species in the three functional traits of pollination, we could identify eight functional pumpkin pollinator groups (table 2).

In a model where bee species richness was included first, after abundance was factored out, only bee species

Table 3. Seed set in relation to predictor variables tested with GLM. (Italic numbers indicate significant effects.)

| independent variable | SS | d.f. | $F$ | $p$ |
|---|---|---|---|---|
| habitat type | 32 300 | 4 | 3.6723 | 0.156840 |
| bee abundance | 296 | 1 | 0.1347 | 0.738013 |
| bee diversity | *42 311* | *1* | *19.2421* | *0.021942* |
| surrounding pumpkin flowers | 284 | 1 | 0.1289 | 0.743312 |
| humus thickness | 8357 | 1 | 3.8004 | 0.146351 |
| slope | 289 | 1 | 0.1315 | 0.740902 |
| canopy cover | 3182 | 1 | 1.4473 | 0.315250 |
| error | 6597 | 3 | | |

richness was significantly positively correlated with seed per fruit. However, when functional guild diversity was included ahead of richness in a type I (sequential) sum of squares (SS) model, species richness became non-significant. In a type I SS model, variance that is shared by two predictors is attributed to the first predictor to enter the model (Schmid *et al.* 2002). This demonstrates that richness and functional guild diversity are strongly correlated, making it impossible to attribute the shared variance to either predictor. However, functional guild diversity explained much more of the variance in seed set ($r^2=0.45$) when it was first in the model, compared with richness ($r^2=0.32$; table 4, figure 3), making functional diversity a stronger predictor of seed set.

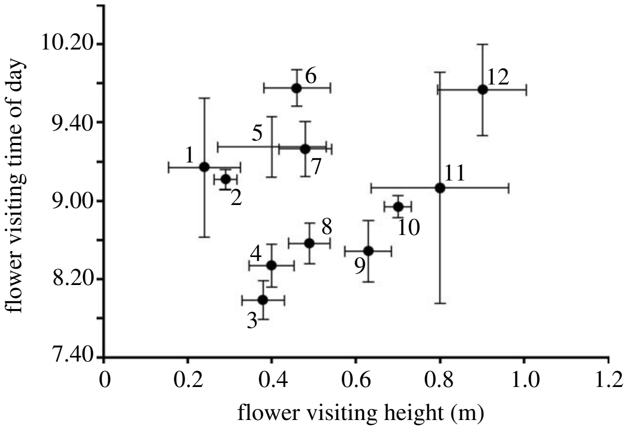

Figure 2. Height and time of flowers preferred by each bee species. Arithmetic means ± s.e. are given. For mean values, standard error and significance levels, see table 2. Numbers represent species identity: 1, *N. concinna*; 2, *Lasioglossum* sp.; 3, *A. cerana*; 4, *X. dejeani*; 5, *N. fulvata*; 6, *C. cognata*; 7, *Trigona* sp.; 8, *Amegilla* sp.; 9, *X. confusa*; 10, *L. halictoides*; 11, *A. dorsata*; 12, *X. nobilis*.

Table 4. Bee species richness and functional guild diversity in relation to the residuals of seed set after correlation with bee abundance. (Italic numbers indicate significant effects.)

| | $r^2$ | $F_{1,10}$ | $p$ |
|---|---|---|---|
| *model 1* | | | |
| bee species richness | *0.32* | *6.08* | *0.033* |
| functional guild diversity | 0.15 | 2.87 | 0.121 |
| *model 2* | | | |
| functional guild diversity | *0.45* | *8.47* | *0.015* |
| bee species richness | 0.02 | 0.47 | 0.507 |

## 4. DISCUSSION

Our results show that the ecosystem service of pollination was closely related to functional pollinator diversity, based on the species-specific traits that appeared to drive complementary use of floral resources. Seed set increased strongly with bee richness, supporting previous studies that showed positive correlations between pollinator diversity and pollen deposition (Kremen *et al.* 2002) or fruit set (Klein *et al.* 2003*b*). We quantified species-specific and complementary resource use in pollination and then related this to final crop yield. Complementarity was assessed by classifying species into functional guilds, as this method allows for objective grouping based on the quantified morphological/behavioural differences. In our study, bee species strongly differed in their preferred flower height, time of flower visitation and within-flower behaviour, which were related to body size. This field study relating bee diversity to seed set is necessarily based on correlative, not experimental, evidence although we kept constant potential confounding factors such as soil nutrient availability and shading. Furthermore, we used seed set instead of fruit size as a response variable for testing the effect of functional group diversity, because seed set is more directly and quantitatively related to pollen deposition. Fruit size was less sensitive to pollination, which might be due to the numerous abiotic factors influencing the fruit size in addition to pollination and seed set (Bos *et al.* 2007).

Spatial niche partitioning of pollinator communities (e.g. Willmer & Corbet 1981; Tylianakis *et al.* 2005) results in certain bee species being observed foraging at certain heights, which may have been selected to minimize energy expenditure (Hambäck 2001; Dafni & Potts 2004). Pumpkin plants produce flowers at very different heights, thereby attracting pollinator species with different height preferences. Reduced species richness may therefore cause a lack of pollination at certain heights, thereby reducing the average seed set within a plot.

Willmer (1983) and Stone (1994) discussed the relationship between behavioural and physiological determinants of circadian species-specific activity patterns, such as temporally structured foraging activity of hymenopterans, showing that certain species have precise daily times of foraging activity (Stone *et al.* 1999). Most studies hold not only morphological traits (such as body size and colouring) responsible for the circadian niche partitioning of bees (Pereboom & Biesmeijer 2003), but also time of pollen release from principal food sources in the case of specialized bee species (Stone *et al.* 1999). In fact, we found that most bees of the three larger pollinator

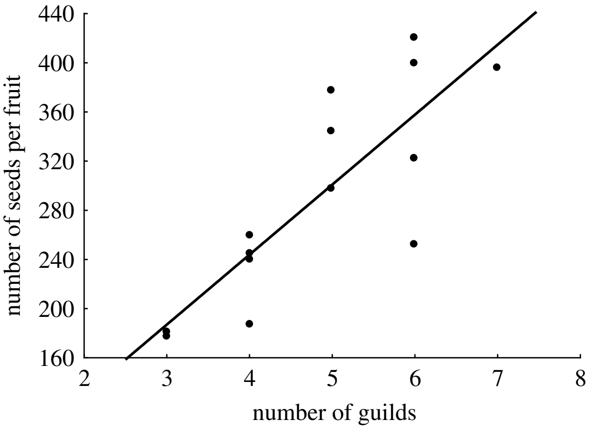

Figure 3. Number of guilds per plot (based on differences in table 2) in relation to the number of pumpkin seeds. Seed set increases with increasing number of functional groups.

size classes appeared significantly earlier (during cooler morning hours) compared with smaller sized bees. Hence, bee body size appeared to affect flower visitation, but other traits may also influence circadian bee activity. Gimenes *et al.* (1996) hold the species' response to the environmental light/dark cycle responsible for a coupling of circadian bee activity to the flower's anthesis and withering. We expect that temporal species turnover is beneficial for pollination success, as not just the amount of transferred pollen will increase additively through time, but also the distribution of pollen. The early and large bees transported more pollen, while the late and small bees appeared to enhance within-flower pollen transfer to the stigma, due to their characteristic movements (Chagnon *et al.* 1993). The temporal change of pollinator species from large pollen-transferring to small pollen-distributing species may improve seed set due to a combination of both traits. Furthermore, for many species, stigma receptiveness of female flowers is known to vary temporally. Owing to the short opening phase of pumpkin flowers, we expect a very short receptive phase for female pumpkin flowers. Our study showed temporal differences in the activity of bees during a day, but bee communities are also known to show temporal turnover at longer time-scales (between months; Tylianakis *et al.* 2005), and this may further promote complementarity in pollen transfer throughout the season. Our field observations revealed body size-dependent as well as within- and between-flower behavioural traits supporting the inclusion of body size as a functionally important classification criterion (table 1). We rarely observed body size-dependent competition between bees (Pinkus-Rendon *et al.* 2005) and only when pollinator densities were high, with smaller bees having been subordinate when they met larger species at the pumpkin flowers. However, due to low pollinator densities in almost all plots, competition was unlikely to have played an important role.

Published studies dealing with niche partitioning of pollinators focus on the relationship of complex flowers to the proboscis length affecting resource use by pollinators (Graham & Jones 1996; Fontaine *et al.* 2006). The pumpkin flowers studied here offered nectar and pollen to a broad spectrum of bee pollinators with spatial and temporal resource partitioning in case of high species numbers, which was related to maximum seed set, equal to that observed in hand-pollinated fruits (figure 1). Diversity of functional pollinator guilds, based on the significant differences in activity at different flower heights, time of flower visitation and body size-related within-flower behaviour, explained variation in seed set better than did species richness. The models (table 4) suggest that interspecific differences in these functional traits allow high complementarity resulting in the enhanced fruit set.

In conclusion, we show how a native bee community may sustain pollination services without any managed honeybees. The species-rich bee community appeared to enhance pollination efficiency through spatial and temporal complementarity in its pollination behaviour, suggesting that a single or a few efficient species may not provide the same benefits as a rich community of functionally distinct species. Owing to species-specific niche partitioning, a single functional group may exploit only a portion of the overall resource.

Our field studies revealed real-world patterns in pollination up to final crop yield, while the experimental manipulation of this species-rich pollinator community or of its species-specific traits was impossible. Our results provide a first step towards a mechanistic understanding of how pollinator diversity affects food production. Global changes greatly affect bee diversity (Biesmeijer *et al.* 2006) and the associated loss of functional diversity threatens sustainable crop production (Klein *et al.* 2007). Conservation initiatives will profit from realistic studies showing economic benefits of maintaining high biodiversity in agricultural landscapes.

We thank the Deutsche Forschungsgemeinschaft for financing the Collaborative Research Centre STORMA, LIPI for the research permit and supporting fieldwork, Damayanti Buchori for collaboration and Stephan Risch and Leverkusen for identification of the bee species. We also thank Saul A. Cunningham, Bernhard Schmid and Peter Hambäck for their valuable suggestions and ideas on the manuscript.

## REFERENCES

Biesmeijer, J. C. *et al.* 2006 Parallel declines in pollinators and insect-pollinated plants in Britain and The Netherlands. *Science* **313**, 351–354. (doi:10.1126/science.1127863)

Bos, M. M., Veddeler, D., Bogdanski, A. K., Klein, A.-M., Tscharntke, T., Steffan-Dewenter, I. & Tylianakis, J. M. 2007 Caveats to quantifying ecosystem services: fruit abortion blurs benefits from crop pollination. *Ecol. Appl.* **17**, 1841–1849.

Cardinale, B. J., Ives, A. R. & Inchausti, P. 2004 Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. *Oikos* **104**, 437–450. (doi:10.1111/j.0030-1299.2004.13254.x)

Cardinale, B. J., Weis, J. J., Forbes, A. E., Tilmon, K. J. & Ives, A. R. 2006 Biodiversity as both a cause and consequence of resource availability: a study of reciprocal causality in a predator–prey system. *J. Anim. Ecol.* **75**, 497–505. (doi:10.1111/j.1365-2656.2006.01070.x)

Chagnon, M., Gingras, J. & Deoliveira, D. 1993 Complementary aspects of strawberry pollination by honey and indigenous bees (Hymenoptera). *J. Econ. Entomol.* **86**, 416–420.

Dafni, A. & Potts, S. G. 2004 The role of flower inclination, depth, and height in the preferences of a pollinating beetle (Coleoptera: Glaphyridae). *J. Insect Behav.* **17**, 823–834. (doi:10.1023/B:JOIR.0000048991.45453.73)

Degrandi-Hoffman, G. & Chambers, M. 2006 Effects of honey bee (Hymenoptera: Apidae) foraging on seed set in self-fertile sunflowers (*Helianthus annuus* L.). *Environ. Entomol.* **35**, 1103–1108.

Fargione, J. E. & Tilman, D. 2005 Diversity decreases invasion via both sampling and complementarity effects. *Ecol. Lett.* **8**, 604–611. (doi:10.1111/j.1461-0248.2005.00753.x)

Fontaine, C., Dajoz, I., Meriguet, J. & Loreau, M. 2006 Functional diversity of plant–pollinator interaction webs enhances the persistence of plant communities. *PLoS Biol.* **4**, 129–135. (doi:10.1371/journal.pbio.0040001)

Gimenes, M., Benedito-Silva, A. A. & Marques, M. D. 1996 Circadian rhythms of pollen and nectar collection by bees on the flowers of *Ludwigia elegans* (Onagraceae). *Biol. Rhythm. Res.* **27**, 281–290. (doi:10.1076/brhm.27.3.281.12971)

Graham, L. & Jones, K. N. 1996 Resource partitioning and per-flower foraging efficiency in two bumble bee species. *Am. Midl. Nat.* **136**, 401–406. (doi:10.2307/2426743)

Hambäck, P. A. 2001 Direct and indirect effects of herbivory: feeding by spittlebugs affects pollinator visitation rates and seedset of *Rudbeckia hirta*. *Ecoscience* **8**, 45–50.

Hooper, D. U. & Dukes, J. S. 2004 Overyielding among plant functional groups in a long term experiment. *Ecol. Lett.* **7**, 95–105. (doi:10.1046/j.1461-0248.2003.00555.x)

Hooper, D. U. *et al.* 2005 Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* **75**, 3–35. (doi:10.1890/04-0922)

Hulot, F. D., Lacroix, G., Lescher-Moutoue, F. O. & Loreau, M. 2000 Functional diversity governs ecosystem response to nutrient enrichment. *Nature* **405**, 340–344. (doi:10.1038/35012591)

Ishii, H. S. 2006 Floral display size influences subsequent plant choice by bumble bees. *Funct. Ecol.* **20**, 233–238. (doi:10.1111/j.1365-2435.2006.01106.x)

Klein, A.-M., Steffan-Dewenter, I. & Tscharntke, T. 2003*a* Pollination of *Coffea canephora* in relation to local and regional agroforestry management. *J. Appl. Ecol.* **40**, 837–845. (doi:10.1046/j.1365-2664.2003.00847.x)

Klein, A.-M., Steffan-Dewenter, I. & Tscharntke, T. 2003*b* Fruit set of highland coffee increases with the diversity of pollinating bees. *Proc. R. Soc. B* **270**, 955–961. (doi:10.1098/rspb.2002.2306)

Klein, A.-M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. & Tscharntke, T. 2007 Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. B* **274**, 303–313. (doi:10.1098/rspb.2006.3721)

Kremen, C., Williams, N. M. & Thorp, R. W. 2002 Crop pollination from native bees at risk from agricultural intensification. *Proc. Natl Acad. Sci. USA* **99**, 16 812–16 816. (doi:10.1073/pnas.262413599)

Kremen, C., Williams, N. M., Bugg, R. L., Fay, J. P. & Thorp, R. W. 2004 The area requirements of an ecosystem service: crop pollination by native bee communities in California. *Ecol. Lett.* **7**, 1109–1119. (doi:10.1111/j.1461-0248.2004.00662.x)

Lanta, V. & Leps, J. 2006 Effect of functional group richness and species richness in manipulated productivity–diversity studies: a glasshouse pot experiment. *Acta Oecol.* **29**, 85–96. (doi:10.1016/j.actao.2005.08.003)

Larsen, T. H., Williams, N. M. & Kremen, C. 2005 Extinction order and altered community structure rapidly disrupt ecosystem functioning. *Ecol. Lett.* **8**, 538–547. (doi:10.1111/j.1461-0248.2005.00749.x)

Lavorel, S. & Garnier, E. 2002 Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* **16**, 545–556. (doi:10.1046/j.1365-2435.2002.00664.x)

Morandin, L. A. & Winston, M. L. 2005 Wild bee abundance and seed production in conventional, organic, and genetically modified canola. *Ecol. Appl.* **15**, 871–881. (doi:10.1890/03-5271)

Naeem, S. 2002 Disentangling the impacts of diversity on ecosystem functioning in combinatorial experiments. *Ecology* **83**, 2925–2935.

Pereboom, J. J. M. & Biesmeijer, J. C. 2003 Thermal constraints for stingless bee foragers: the importance of body size and coloration. *Oecologia* **137**, 42–50. (doi:10.1007/s00442-003-1324-2)

Pinkus-Rendon, M. A., Parra-Tabla, V. & Melendez-Ramirez, V. 2005 Floral resource use and interactions between *Apis mellifera* and native bees in cucurbit crops in Yucatan, Mexico. *Can. Entomol.* **137**, 441–449.

Ricketts, T. H. 2004 Tropical forest fragments enhance pollinator activity in nearby coffee crops. *Conserv. Biol.* **18**, 1262–1271. (doi:10.1111/j.1523-1739.2004.00227.x)

Scherber, C., Milcu, A., Partsch, S., Scheu, S. & Weiser, W. W. 2006 The effects of plant diversity and insect herbivory on performance of individual plant species in experimental grassland. *J. Ecol.* **94**, 922–931. (doi:10.1111/j.1365-2745.2006.01144.x)

Schmid, B., Hector, A., Huston, M. A., Inchausti, P., Nijs, I., Leadley, P. W. & Tilman, D. 2002 The design and analysis of biodiversity experiments. In *Biodiversity and ecosystem functioning: synthesis and perspectives* (eds M. Loreau, S. Naeem & P. Inchausti), pp. 61–75. Oxford, UK: Oxford University Press.

Shuler, R. E., Roulston, T. H. & Farris, G. E. 2005 Farming practices influence wild pollinator populations on squash and pumpkin. *J. Econ. Entomol.* **98**, 790–795.

Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C. & Tscharntke, T. 2002 Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* **83**, 1421–1432.

Steffan-Dewenter, I., Potts, S. G. & Packer, L. 2005 Pollinator diversity and crop pollination services are at risk. *Trends Ecol. Evol.* **20**, 651–652. (doi:10.1016/j.tree.2005.09.004)

Stone, G. N. 1994 Activity patterns of females of the solitary bee *Anthophora plumipes* in relation to temperature, nectar supplies and body-size. *Ecol. Entomol.* **19**, 177–189. (doi:10.1111/j.1365-2311.1994.tb00408.x)

Stone, G. N., Gilbert, F., Willmer, P., Potts, S., Semida, F. & Zalat, S. 1999 Windows of opportunity and the temporal structuring of foraging activity in a desert solitary bee. *Ecol. Entomol.* **24**, 208–221. (doi:10.1046/j.1365-2311.1999.00181.x)

Stout, J. C. 2000 Does size matter? Bumblebee behaviour and the pollination of *Cytisus scoparius* L. (Fabaceae). *Apidologie* **31**, 129–139. (doi:10.1051/apido:2000111)

The R Development Core Team 2006 *R: a language and environment*, v. 2.3.1. Vienna, Austria: R Foundation for Statistical Computing

Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M. & Siemann, E. 1997 The influence of functional diversity and composition on ecosystem processes. *Science* **277**, 1300–1302. (doi:10.1126/science.277.5330.1300)

Tilman, D., Reich, P. B. & Knops, J. M. H. 2006 Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* **441**, 629–632. (doi:10.1038/nature04742)

Tscharntke, T., Klein, A.-M., Kruess, A., Steffan-Dewenter, I. & Thies, C. 2005 Landscape perspectives on agricultural intensification and biodiversity—ecosystem service management. *Ecol. Lett.* **8**, 857–874. (doi:10.1111/j.1461-0248.2005.00782.x)

Tylianakis, J. M., Klein, A.-M. & Tscharntke, T. 2005 Spatiotemporal variation in the effects of a tropical habitat gradient on Hymenoptera diversity. *Ecology* **86**, 3296–3302. (doi:10.1890/05-0371)

Tylianakis, J. M., Rand, T. A., Kahmen, A., Klein, A.-M., Buchmann, N., Perner, J. & Tscharntke, T. 2008 Resource heterogeneity moderates the biodiversity–function relationship in real world ecosystems. *PLoS Biol.* **6**, e122. (doi:10.1371/journal.pbio.0060122)

Vázquez, D. P., Morris, W. F. & Jordano, P. 2005 Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol. Lett.* **8**, 1088–1094. (doi:10.1111/j.1461-0248.2005.00810.x)

Walters, S. A. & Taylor, B. H. 2006 Effect of honey bee pollination on pumpkin fruit and seed yield. *Hortscience* **41**, 370–373.

Willmer, P. G. 1983 Thermal constraints on activity patterns in nectar-feeding insects. *Ecol. Entomol.* **8**, 455–469. (doi:10.1111/j.1365-2311.1983.tb00524.x)

Willmer, P. G. & Corbet, S. A. 1981 Temporal and microclimatic partitioning of the floral resources of *Justicia aurea* amongst a concourse of pollen vectors and nectar robbers. *Oecologia* **51**, 67–78. (doi:10.1007/BF00344655)