DR. FABIAN NÜRNBERGER (Orcid ID : 0000-0002-7360-3617)
DR. ALEXANDER KELLER (Orcid ID : 0000-0001-5716-3634)

Article type : Original Article

Title:

Honey bee waggle dance communication increases diversity of pollen diets in intensively managed agricultural landscapes

Running title: Honey bee communication affects pollen diets

Authors:

Fabian Nürnberger[1,2], Alexander Keller[3], Stephan Härtel[1], Ingolf Steffan-Dewenter[1]

[1] Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg, Würzburg, Germany

[2] Thünen Institute of Biodiversity, Braunschweig, Germany

[3] Department of Bioinformatics and Center for Computational and Theoretical Biology, Biocenter, University of Würzburg, Würzburg, Germany

Corresponding author:

Fabian Nürnberger

Department of Animal Ecology and Tropical Biology, Biocenter, University of Würzburg

Würzburg

Am Hubland, 97074 Würzburg, Germany

Email address: fabian.nuernberger@thuenen.de

Abstract

The benefits of honey bee dance communication for colony performance in different resource environments are still not well understood. Here we test the hypothesis that directional dance communication enables honey bee colonies to maintain a diverse pollen diet, especially in landscapes with low resource diversity. To test this hypothesis, we placed 24 *Apis mellifera* L. colonies with either intact or experimentally disrupted dance communication in eight agricultural landscapes that differed in the diversity of flowering plants and in the dominance of mass-flowering crops. Pollen from incoming foragers was collected and identified via DNA metabarcoding. Disrupting dance communication affected the way the diversity of honey bee pollen diets was impacted by the dominance of mass-flowering crops in available flower resources (p=0.04). With increasing dominance of mass-flowering crops in resource environments, foragers of colonies with intact communication foraged on an increasing proportion of available plant genera (p=0.01). This was not the case for colonies with disrupted dance communication (p=0.5). We conclude that the honey bee dance communication benefits pollen foraging on diverse plant resources and thereby contributes to high quality nutrition in environments with low-resource diversity.

Keywords (4-6): *Apis mellifera*, foraging behaviour, mass-flowering crops, plant-pollinator interactions, DNA metabarcoding, ITS2

Introduction

Honey bees of the genus *Apis* are central place foragers that collect nectar and pollen on flowering plants. To rapidly exploit rewarding flower resources foragers from a honey bee colony use waggle dances to communicate available resources (Seeley, 1986; von Frisch, 1967). This is a unique behaviour during which a successful forager conveys information about the presence and odour (and therefore identity) of a rewarding food source (motivational component) as well as its spatial position (distance and direction from nest) to nearby foragers within the nest (Menzel et al., 2011; von Frisch, 1967). While the mechanisms of the waggle dance are rather well studied, its benefits for resource exploitation and colony fitness in different resource environments are still not well understood (Dornhaus & Chittka, 2004).

Dance communication is highly effective in recruiting foragers of western honey bee (*A. mellifera* L.) colonies to small artificial nectar sources (Sherman & Visscher, 2002; von Frisch, 1967). In natural resource environments, however, the value of directional dance communication for honey bee nectar foraging success seems to be far from general and could so far only be shown under very specific conditions (Donaldson-Matasci & Dornhaus, 2012; Dornhaus & Chittka, 2004; Nürnberger, Steffan-Dewenter, & Härtel, 2017; Okada et al., 2012; Sherman & Visscher, 2002). It can even have negative effects on nectar foraging success as it is a time consuming behaviour that is not profitable if the quality of available food patches is low (I'Anson Price, Dulex, Vial, Vincent, & Grüter, 2019). Honey bee dance communication could play a larger role in pollen foraging (Nürnberger et al., 2017), which was rarely specifically addressed. While I'Anson Price et al. (2019) did not find effects of dance communication on pollen foraging, other studies demonstrated that disrupting the directional component of dance communication affects the composition of collected pollen and can reduce the amount of pollen gathered by colonies (Donaldson-Matasci & Dornhaus, 2014), even when nectar foraging is not affected (Nürnberger et al., 2017).

Importantly, different plant species produce pollen with different quality, quantity and composition of proteins, lipids, sterols, and vitamins (Roulston & Cane, 2000) and thus vary strongly in quality for honey bee nutrition (Haydak, 1970). The identity and diversity of gathered pollen is of high importance for honey bee health (C. Alaux et al., 2017; Cédric Alaux, Ducloz, Crauser, & Le Conte, 2010; Di Pasquale et al., 2016; Di Pasquale et al., 2013). Under laboratory conditions, honey bee foragers preferred pollen that complements colony diets (Hendriksma & Shafir, 2016) and performed faster dances for complementing pollen species to indicate the perceived higher quality (Zarchin, Dag, Salomon, Hendriksma, & Shafir, 2017). So far it is not known if and how honey bees actively arrange composition of pollen diets on colony level under field conditions, but bumble bee foragers were shown be actively seek out pollen sources that provide a favourable protein-to-lipid ratio, both in the field and in laboratory tests (Vaudo, Patch, Mortensen, Tooker, & Grozinger, 2016). In many bee species, composition of pollen diets appears to be strongly affected by the resource distribution in a landscape. Recent studies showed that the local resource diversity and the presence of large, highly rewarding monofloral food patches, like mass-flowering crop fields, can have strong effects on pollen diets: in a non-dancing social stingless bee (*Tetragonula carbonaria*) local resource diversity was positively correlated with pollen diet diversity (Kaluza et al., 2017), while bumble bees and solitary bees were observed to concentrate foraging efforts on mass-flowering crops, if available, and neglected alternative resources (Holzschuh et al., 2016). Remarkably, this seems not to be true for honey bees in temperate landscapes. In field studies, local and landscape level flower richness had little effect on the diversity of pollen harvest in honey bees (Colwell, Williams, Evans, & Shutler, 2017; Danner, Keller, Härtel, & Steffan-Dewenter, 2017) and even in the presence of mass-flowering crops, honey bees were more likely to forage on alternative resources (Garbuzov, Couvillon, Schürch, & Ratnieks, 2015; Holzschuh et al., 2016). In fact, honey bees actively recruit nest mates to distant, but diverse semi-natural resource patches, even when mass-flowering crops in the vicinity of hives are available (Danner et al., 2017; Danner, Molitor, Schiele, Härtel, & Steffan-Dewenter, 2016).

Here, we test the hypothesis that honey bees rely on dance communication to apply a foraging strategy that ensures a non-random and diverse pollen diet in landscapes with low local resource diversity and dominance of mass-flowering crops. We used molecular tools to study the effects of local resource availability, resource diversity and dominance of mass-flowering crops on the diversity of foraged pollen and similarity between pollen composition and pollen availability in honey bee colonies with intact *vs.* experimentally disrupted directional dance communication. Further, we tested if disruption of dance communication affects the abundance of mass-flowering crops in pollen samples.

Methods

This study is based on the same field experiment as Nürnberger et al. (2017). Here we present results on the diversity of collected pollen resources, based on pollen identification via next generation sequencing, whereas in the previous study we could only analyse the amount of collected pollen (Fig. 1).

Study organism and study sites

We established 24 equally sized colonies of *Apis mellifera carnica* with sister queens from a professional breeder (Schüler, Münster, Germany) in nine frame hive boxes. The experimental colonies were placed into eight different landscapes around Würzburg, Germany, from 17 July 2013 to 18 August 2013.

We placed each group of three colonies on one hive stand in the center of each landscape at the start of the experiment. We characterized the landscapes within a circular area of 2km radius (i.e. 1265.64 ha area) around the hive stands as in comparable landscape types more than 90% of all pollen sources advertised by dancing bees are within this distance (Danner, Härtel, & Steffan-Dewenter, 2014). The selected landscapes covered gradients in the amount and diversity of floral resources as well as the contribution of mass-flowering crops to available floral resources (Tab. 1). Mass-flowering crops that flowered within the study sites and period included maize, legumes (alfalfa, white and red clover and legume mixtures) and sunflowers. The most common wild flower genera within the study sites and period were *Lotus*, *Daucus*, *Achillea*, *Crepis* and *Trifolium* (see Online Resource 1 for a full list of genera mapped within the landscapes). We used available digital land-use data in a geographical information system (Arc-GIS) to identify and quantify land-use types within the landscapes. We mapped identity and abundance of flowering plant species within the different relevant land-use types. To calculate flower diversity and flower cover on landscape level, we mapped flowering resources for at least three randomly selected 100m² plots per land-use type (Scheper et al., 2015) twice during the study period (once between 17-Jul-2013 and 2-Aug-2013, and once between 3-Aug-2013 and 18-Aug-2013). Within each of these plots we identified present flowering plants to species level and determined the area covered by flowers for each species. To match the precision of pollen identification via DNA metabarcoding, we used taxa richness on the genus level to quantify local resource diversity in each landscape. As a measure for local resource abundance, we extrapolated the total flower cover of landscapes by summing the estimated mean flower cover per area multiplied by the area covered for each land-use type for both mapping periods (Nürnberger et al., 2017). We calculated the proportion of mass-flowering crops in available resources for each landscape and mapping period by dividing the flower cover of mass-flowering crop species by total flower cover within the respective landscape, to study communication dependent effects of mass-flowering crops on pollen foraging.

We checked colonies every eight days to confirm presence of brood and queen. One queen was lost during the experiment and had to be replaced with a spare sister-queen. Removing this colony from statistics did not have significant effects on model outcomes and the colony was therefore included in the final models.

All applicable institutional and national guidelines for the care and use of animals were followed.

Communication treatments

We used a well-established method to disrupt the directionality of waggle dances in colonies to identify the role of directional waggle dance communication for pollen foraging in honey bee colonies (Dornhaus & Chittka, 2004; Sherman & Visscher, 2002; von Frisch, 1967). In normal dark colonies, honey bee dancers on the vertical combs surface use the gravitational cue to orient the waggle-run component of dances used to encode the direction to an advertised resource patch. By placing colonies on levelled tables and rotating them by 90° so that combs were now horizontally aligned, dancers can no longer orient their dances in a specific angle but dance in a random direction for each waggle run (Nürnberger et al., 2017). Dance followers are not able to use the directional component of such disoriented dances to find resource patches.

At each time we paced one of three colonies in each landscape in horizontal comb position, to disrupt dance orientation while one colony was left unmanipulated to serve as a control and the third colony was assigned to a treatment that was not incorporated in this study. In order to test each set of three colonies in each landscape and prevent carry-over effects which might obscure the role of communication (Schürch & Grüter, 2014), we moved each set of colonies to another landscape after four days during night time for a new test round and randomly reassigned treatments to colonies within each set at this point (Nürnberger et al., 2017). By the end of the experimentation period after eight test rounds, each set of colonies had been tested in each landscape. Consecutive colony sites were always more than 10 km apart from previous sites (mean ± *SD*: 19.2 ± 6.9 km) to prevent foragers from returning to old sites.

Pollen collection

We used pollen traps to collect pollen of incoming foragers at the hive entrance (Danner et al., 2017; I. Keller, Fluri, & Imdorf, 2005). When pollen traps were active, foragers were forced to squeeze through 5 mm diameter holes when entering or leaving the hive whereby most of the gathered pollen is stripped off and collected in a storage tray that was not accessible by honey bees. Pollen traps were activated during the nights when colonies were placed for a new test round in a new landscape, and deactivated and emptied during the consecutive night. This way most of the pollen gathered during the first day which colonies spent in a new landscape was collected for further analysis. After collection, we stored the pollen from each colony individually in a -20°C freezer and dried it later in a vacuum-dryer and cleaned from contaminations to prepare them for DNA-barcoding.

Molecular analysis of pollen samples

We added double distilled H2O (4 mL) to 2g of pollen from each colony and sampling day (after thorough mixing) and homogenized this sample with an electronic pestle. After that, we ground 200 µL (~30 mg pollen) of the emulsion in a bead mill (Tissue Lyser II, Qiagen) as described by A. Keller et al. (2015). DNA was then extracted using the Macherey-Nagel Food Kit (Düren, Germany) (A. Keller et al., 2015). We created two mock "negative" controls to account for potential contamination by similarly extracting pure *Lolium perenne* tissue, which we can exclude as a foraged plant for honey bees. We performed pollen ITS2-metabarcoding according to Sickel et al. (2015): we performed PCR in three separate 10µL reactions in order to avoid PCR bias and combined after PCR. Each reaction contained 5µL 2x Phusion Master Mix (New England Biolabs, Ipswich, MA, USA), 0.33µM each of the forward and reverse primers, 3.34µL PCR grade water and 1µL DNA template. PCR conditions were as follows: initial denaturation at 95°C for 4 min, 37 cycles of denaturation at 95°C for 40 sec, annealing at 49°C for 40 sec and elongation at 72°C for 40 sec; followed by a final extension step at 72°C for 5 min. Each sample was assigned a different forward/reverse index combination for sample-specific labelling. We did between-sample normalization with the SequalPrep™ Normalization Plate Kit (Invitrogen GmbH, Darmstadt, Germany) and pooled normalized indexed samples. These pools were quality controlled using a Bioanalyzer High Sensitivity DNA Chip (Agilent Technologies, Santa Clara, CA, USA), quantified with the dsDNA High Sensitivity Assay (Life Technologies GmbH, Darmstadt, Germany), diluted to 8pM, denatured and spiked with 5% Phix Control Kit v3 (Illumina Inc., San Diego, CA, USA). We performed sequencing on the Illumina MiSeq using 2x250 cycles v2 chemistry (Illumina Inc., San Diego, CA, USA). Raw filters were joined and quality filtered (>Q20, >150bp, no ambiguous base-pairs) with VSEARCH (v2.8.1) (Rognes, Flouri, Nichols, Quince, & Mahé, 2016) similarly to Sickel et al. (2015). We downloaded all ITS2 sequences available at the ITS2 database v5 (Ankenbrand, Keller, Wolf, Schultz, & Förster, 2015) and Genbank (Benson, Karsch-Mizrachi, Lipman, Ostell, & Wheeler, 2008) (accessed May 2018) for German plants (excluding algae) and classified our reads by direct global alignment matches (identity > 0.97) against these. We removed mock control *Lolium perenne* hits from the dataset. Other taxa were not conspicuous in the mock controls. We used number of reads of individual pollen genus divided by total number of reads in each sample to quantify the relative abundance of individual pollen genera within samples (A. Keller et al., 2015).

Statistics

We used the statistical software R version 3.4.0 (R Core Team, 2017) for data analysis. We used generic richness as indicator of floral resource diversity, which is based on presences and absences of taxa to counter potential biases in the metabarcoding data.

To account for the count data character of the response variable and for overdispersion, we used generalized mixed effects models with negative binomial-distribution to identify effects of the dance communication treatment and landscape parameters on diversity of pollen samples. Based on visual inspection of model residuals and AIC-comparison, the identity link function was given preference over the log link function.

(1) We used a negative binomial model with c*olony identity* (categorial with 24 levels), *test round* (categorial with eight levels) and *landscape identity* (categorial with eight levels) as random intercepts was test for effects of *total flower cover* (continuous) as well as *communication treatment* (categorial with two levels), *proportion of mass-flowering crops* in available flower cover (continuous), and the interaction terms *communication treatment* ×

total flower cover and *communication treatment* × *proportion of mass flowering crops* on *generic richness of pollen samples* (continuous; count data):

generic richness of pollen samples ~ *communication treatment* × *total flower cover* + *communication treatment* × *proportion of mass flowering crops* + (1|c*olony identity*) + (1|*test round*) + (1|*landscape identity*)*;* Family = negative binomial (link = identity).

The correlation between *proportion of mass-flowering crops* and *total flower cover* was small and considered tolerable (r=0.18; t=2.06, df=127, p=0.041)

(2) As local diversity of flowering species was strongly correlated with total flower cover, we used a separate negative binomial model with the identity link function and the same random terms to test for effects of the interaction term local *generic richness of flowering plants* (continuous) × *communication treatment* on *generic richness of pollen samples* :

generic richness of pollen samples ~ *generic richness of flowering plants* × *communication treatment* + (1|c*olony identity*) + (1|*test round*) + (1|*landscape identity*)*;* Family = negative binomial (link = identity).

(3) We calculated the Jaccard index to quantify the similarity between available genera in a landscape and genera identified in respective pollen samples. The Jaccard index is a similarity index, which is based on presence and absence of species (Krebs, 1999). For this analysis, we only considered genera that were identified in both, the surrounding landscapes as well as the pollen samples gathered from colonies. We used a linear mixed-effects model with c*olony identity*, *test round* and *landscape identity* as random intercepts (categorial) to test for effects of *communication treatment*, *proportion of mass-flowering crops* in flower

cover, and the interaction term *communication treatment* × *proportion of mass-flowering crops* on the *Jaccard index* (continuous):

Jaccard index ~ *communication treatment* × *proportion of mass flowering crops* + (1|*colony identity*) + (1|*test round*) + (1|*landscape identity*)*;* Family = gaussian.

(4) We used separate linear mixed-effects models with the same random factors to test if the *communication treatment* affected the *relative abundance* (continuous) of the mass-flowering crop genera *Helianthus* and *Trifolium* in pollen samples, based on numbers of sequence reads:

relative abundance ~ *communication treatment* + (1|*colony identity*) + (1|*test round*) + (1|*landscape identity*)*;* Family = gaussian.

We only compared abundances within taxa to avoid taxon specific issues with quantification based on sequencing reads (Richardson et al., 2015).

We used the R-package glmmTMB to calculate the generalized mixed-effects models and lme4 (Bates, Maechler, Bolker, & Walker, 2014) for the linear mixed-effects models. To determine whether fixed factors significantly affected the respective response variables we applied Type 2 Wald Chisquare-tests for the generalized models and ANOVA-tests using R-package lmerTest with the Satterthwaite approximation for degrees of freedom (Kuznetsova, Brockhoff, & Christensen, 2017) for the linear models. Insignificant terms were removed from models before the calculation of test statistics of remaining fixed factors. To investigate the nature of conditional effects of landscape parameters that interacted with the communication treatment on the respective response variable, we used post hoc tests based on contrast matrices with Benjamini-Hochberg correction for multiple testing (Benjamini & Yekutieli, 2001).

Results

Pollen foraging success

By analysing the dry-weight of pollen foraged within one day for each combination of colony and landscape in this experiment, we have shown earlier that disrupting dance communication decreased the pollen foraging success by 40.2% (Fig. 1). See Nürnberger et al. (2017) for details on the analysis.

Identified pollen genera

DNA sequencing identified a total of 153 plant taxa on the genus level in the pollen samples. Single pollen samples from honey bee colonies, which resembled a representative subset of the pollen collected within a single day, contained on average 16.1 (±0.6 *SE*; range: 3 to 35) plant genera. During field surveys of plants within the study landscapes, we identified 52 flowering plant taxa on the genus level. Of the identified plant genera 40 overlapped with the plant genera detected by metabarcoding. Individual landscapes contained in mean 29.6 (±0.9 *SE*; range: 23 and 35) overlapping genera at a given time. A full list of identified genera can be found in the electronic supplement (Online Resource 1). Availability of mass-flowering crops was negatively correlated with the generic richness of available pollen species (r=-0.19; t=-2.18, df=127, p=0.031). Although sporadic visual inspection of pollen samples revealed presence of maize pollen, the meta-barcoding method failed to identify maize (genus *Zea*) in pollen samples. Following this, we performed regular PCRs with maize tissue from our region and beyond and did not receive amplification bands for our regional crops, yet for others. This indicates that our primers were unfortunately not successful for hybrids cultivated in our study area.

Genus richness of foraged pollen

We hypothesized that dance communication allows a more targeted exploitation of high-quality resources and thereby decouples local resource diversity from diversity of foraged pollen. Generic richness of locally available flowers did not significantly affect generic richness of pollen foraged by colonies, regardless of communication treatment (main effect: $\chi^2(1, n=129)=1.92$, $p=0.17$; interaction with communication treatment: $\chi^2(1, n=129)=1.41$, $p=0.24$). Further, the number of foraged pollen genera declined with general flower availability, which did not depend on communication treatment (main effect: $\chi^2(1, n=129)=6.46$; $p=0.011$; interaction term: $\chi^2(1, n=129)=0.24$; $p=0.63$; Fig. 2).

However, the communication treatment had a significant effect on the generic richness of pollen foraged by honey bee colonies in relation to the proportion of mass-flowering crops in available resources ($\chi^2(1, n=129) =4.42$; $p=0.036$; Fig. 3). At low dominance of mass-flowering crops, colonies with disrupted communication foraged on a higher number of plant genera than colonies with intact communication, while the opposite was true at high dominance of mass-flowering crops. In landscapes with a high dominance of mass-flowering crops, colonies with intact communication foraged on an increased number of different plant genera, despite a decreasing number of available plant genera, while colonies with disrupted communication foraged on a reduced number of plant genera. However, post hoc-testing did not reveal the slopes to be significantly different from zero for either the disrupted ($z=-1.30$; $p=0.20$) or the intact communication treatment ($z=1.27$; $p=0.$ 20).

Similarity between foraged and available pollen

To analyse if communication enables honey bee foragers to discriminate for certain pollen genera instead of gathering a random subset of available pollen and if this is affected by the availability of resources within the landscape, we calculated the similarity between available pollen genera and foraged pollen genera. The communication treatment affected the relation between the Jaccard index and the availability of mass-flowering crops ($F_{1,\ 120}$=7.24; p=0.0082; Fig. 4). In colonies with disrupted communication the proportion of foraged pollen genera (i.e. Jaccard index) was not significantly affected by mass-flowering crops in available floral resources (z=-0.71; p=0.48). In colonies with intact communication, however, the proportion of foraged pollen genera was positively correlated with the dominance of mass-flowering crops in a landscape (z=-2.64, p=0.012).

Effect of communication treatment on abundance of mass-flowering crops in pollen samples

Based on numbers of sequence reads, sunflower pollen (*Helianthus)* was the most frequent taxon detected in the pollen samples. There was no effect of communication treatment on the amount of *Helianthus* pollen harvested by colonies ($F_{1,\ 114}$=0.08; p=0.78, Fig. 5a). Beside maize and sunflower, plants from the genus *Trifolium* were commonly but not exclusively grown as mass-flowering crops in the studied landscapes. Honey bee colonies with unimpaired communication foraged less on *Trifolium* than colonies in which directional communication was disrupted ($F_{1,\ 114.2}$=4.50; p=0.036, Fig. 5b). Numbers of sequence reads for all pollen genera can be found in Online Resource 2.

Discussion

We analysed plant genus composition of foraged pollen in colonies with either disrupted or intact directional waggle dance communication in a landscape experiment. Our experiment revealed that, regardless of communication treatment, total flower availability, but not resource diversity affected the diversity of foraged pollen on colony level. Importantly, disrupted directional communication reduced the generic richness of foraged pollen in landscapes with high dominance of mass-flowering crops when compared to colonies with intact communication.

We applied DNA metabarcoding to identify pollen genera which was previously successfully used to tackle ecological questions in honey bee pollen foraging (Danner et al., 2017). The high-throughput sequencing method allowed us to directly identify genus composition of mixed pollen samples foraged by honey bee colonies with high precision and relate them to local resource diversity on genus level, which would be challenging using traditional palynological methods (A. Keller et al., 2015). Unfortunately, maize pollen was not well identifiable with this approach. The reasons for this are unclear and may be related to genomic properties of cultivated hybrids or pollen architecture. As other Poaceae were identifiable without issues, a systematic failure of primers for this group can be excluded.

Our data showed that, regardless of the ability to communicate resource locations, colonies foraged on a smaller number of pollen resources in landscapes with high local resource abundance. Flower rich landscapes provide more options for choice and might allow honey bee colonies to compose an optimal pollen diet with a mix of fewer plant species. In contrast, in landscapes with low overall flower cover, exploitation of more plant species could be necessary to fulfil nutritional requirements of colonies in terms of quantity, as individual species are less abundant and hence more quickly depleted. Our model predicted the number of pollen genera foraged by individual colonies during one day to range from 14 to 19, depending on local resource abundance. This lies well in the range of what previous studies found in comparable landscapes (Danner et al., 2017; Requier et al., 2015). There was no general effect of diversity of available flowers on diversity of pollen samples on genus level, regardless of communication treatment. Even without being able to communicate resource locations, honey bee colonies seemed to exploit a variety of available resources. This is in line with earlier studies on effects of landscapes on diversity of honey bee pollen diets which found no effect of local resource diversity on diversity of foraged pollen in temperate landscapes (Colwell et al., 2017; Danner et al., 2017). In low-diversity environments a diverse pollen diet could be maintained by increasing foraging ranges (Danner et al., 2017) and increasing the scouting activity if few pollen sources are advertised (Beekman, Gilchrist, Duncan, & Sumpter, 2007). Contrary to the findings in temperate environments, local species richness in a species-poor non-temperate desert scrub and grassland was found to be positively correlated with species richness of foraged pollen, regardless of dance communication (Donaldson-Matasci & Dornhaus, 2014). It remains to be tested if this is also the case in particularly species-poor temperate landscapes or if this is an effect of a different resource distribution in non-temperate and less intensively managed landscapes (Dornhaus & Chittka, 2004).

Our study illustrates that directional communication changes the way mass-flowering crops affect pollen foraging. In landscapes with relatively little amounts of mass-flowering crops in the local resource environment, colonies with intact communication foraged on fewer pollen genera than colonies with disrupted directional communication. Communication could have enabled colonies to concentrate on the most profitable plant genera while still maintain a relative high pollen diversity. This is in line with previous findings that communication enables honey bee colonies to forage for pollen more consistently on the presumably most valuable plant species (Donaldson-Matasci & Dornhaus, 2014). In intensively managed landscapes, where resources were predominantly mass-flowering crops and floral resource diversity was low, colonies with intact dance communication foraged on an increasing proportion of available resource genera. This led to a higher total number of pollen genera found on colony level, when compared to colonies with disrupted dance communication. Colonies might have increased the number of plant genera they used for pollen forage in mass-flowering crop dominated resource environments in order to gather considerable amounts of alternative pollen species, even when individual species may only be present at very low abundances and quickly depleted. Colonies with disrupted directional communication seemed to have failed to do so. They foraged on a smaller proportion of available pollen resources in landscapes with higher proportions of easy to find mass-flowering crops, leading to a decreased generic richness in pollen samples on colony level when compared to colonies with intact communication. This implies that colonies with intact communication might better escape malnutrition based on pollen from few mass-flowering crop species than colonies with disrupted dance communication. We could not show an effect of communication treatment on the abundance of *Helianthus* (sunflower) in pollen diets. Sunflower was the most dominant mass-flowering crop that was detected in pollen samples and seemed to be a highly attractive resource for honey bees. However, our data did show that colonies with intact communication foraged less pollen from *Trifolium* (red and white clover), another dominant mass-flowering crop genus present in the studied landscapes. *Medicago* (represented by alfalfa), which was also a common mass-flowering crop in the studied landscapes, was never foraged in considerable amounts, regardless of dance communication treatment. Alfalfa is known to be avoided by honey bee foragers (Gary, Witherell, & Marston, 1973) as they seem to be irritated by the spring-like mechanism within its flowers (Westerkamp, 1991). Earlier studies in comparable landscapes revealed that honey bee colonies foraged less pollen on mass-flowering crops (mostly oilseed rape, which was not present in our study period) than expected regarding their abundance in the landscapes (Danner et al., 2016; Requier et al., 2015). Instead colonies were shown to forage over-proportionally much on alternative resources, e.g. crop weeds and flowers found in semi-natural habitats, even if a restricted availability requires to increase flight distances significantly (Couvillon, Schürch, & Ratnieks, 2014; Danner et al., 2016). The ability to collect pollen from diverse flowers in semi-natural habitats was shown to benefit honey bee health (C. Alaux et al., 2017) and the waggle dance communication seems to be crucial for an efficient exploitation of these relatively rare and hard to find resources. It is remarkable that the overall effect of dominance of mass-flowering crops on diversity of pollen diets was actually small and, according to our results, colonies that were not able to communicate resource locations to nest mates still foraged on 15 different plant genera on average, even in landscapes in which resources consisted predominantly of mass-flowering crops (i.e. over 95% of flower cover). A field study on bumble bees also showed that pollen nutritional value in terms of pollen-lipid ratio did not differ between colonies in different landscapes (Vaudo, Farrell, Patch, Grozinger, & Tooker, 2018), presumably because foragers discriminate for pollen with favourable nutrition properties (Vaudo et al., 2016). However, the resource landscape did affect total nutrient uptake of bumble bee colonies, which had significant consequences for colony fitness (Vaudo et al., 2018). Contradicting findings of I'Anson Price et al. (2019), in our experimental setting colonies with disrupted directional dance communication gathered smaller amounts of pollen than unimpaired colonies (Nürnberger et al., 2017). As diversity of flowering resources had no effect on pollen diversity and dominance of mass-flowering crops only had a relatively small effect, this suggests that dance communication mainly helps to increase the efficiency of a foraging strategy which already ensures that a diverse and healthy pollen diet is maintained on colony level.

Disruption of dance communication could have been confounded with hive rotation effects, but this was unlikely to affect the results. Pollen was stripped off before foragers entered the hive, so that any effects of comb position on pollen storing would not have affected the experiment. Pollen foraging is closely linked to brood rearing activity, but no effects of hive rotation on brood rearing activity were detected and the random reassignment of treatments after short time periods would have minimised any bias caused by effects of hive rotation on brood rearing (Nürnberger et al., 2017). Still, effects of hive rotation on foraging cannot completely be ruled out. Further, our experimental setting allowed only to detect short-term effects of waggle dance communication on pollen foraging. Impacts of dance communication on foraging success can change in the long term, depending on quality of available resource patches and foraging strategy (I'Anson Price et al., 2019). Hence, long-term effects of dance communication on pollen foraging should be considered in future studies.

Conclusions

We conclude that honey bee waggle dance communication plays an important role in pollen foraging as it facilitates a non-random exploitation of available resources. Especially in intensively managed agricultural landscapes waggle dance communication could support a diverse pollen forage by allowing honey bees to preferentially forage on a number of rarer plant species with high efficiency and potential positive effects for honey bee colony health (C. Alaux et al., 2017) and wild plant pollination.

Acknowledgements

We would like to thank Stefan Berg from the Bayerische Landesanstalt für Weinbau und Gartenbau, Veitshöchheim, for his expert support and provision of honey bee colonies for the experiment, Susanne Schiele for her excellent assistance during the fieldwork, and Beate Krischke and Gudrun Grimmer who did all the lab work for meta-barcoding the pollen samples. We thank three anonymous reviewers for valuable comments on an earlier version of the manuscript. The Bundesamt für Kartographie und Geodäsie kindly provided access to land use data on the study site. This study was funded by the German Research Foundation (DFG) within the CRC 1047: Insect timing, Project C2.

References

Alaux, C., Allier, F., Decourtye, A., Odoux, J. F., Tamic, T., Chabirand, M., . . . Henry, M. (2017). A 'Landscape physiology' approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats. *Scientific Reports, 7*, 10. doi:10.1038/srep40568

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters, 6*, 562-565. doi:10.1098/rsbl.2009.0986

Ankenbrand, M. J., Keller, A., Wolf, M., Schultz, J., & Förster, F. (2015). ITS2 database V: Twice as much. *Molecular Biology and Evolution, 32*(11), 3030-3032. doi:10.1093/molbev/msv174

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7.

Beekman, M., Gilchrist, A. L., Duncan, M., & Sumpter, D. J. T. (2007). What makes a honeybee scout? *Behavioral Ecology and Sociobiology, 61*(7), 985-995. doi:10.1007/s00265-006-0331-9

Benjamini, Y., & Yekutieli, D. (2001). The Control of the False Discovery Rate in Multiple Testing under Dependency. *The Annals of Statistics, 29*(4), 1165-1188.

Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., & Wheeler, D. L. (2008). GenBank. *Nucleic Acids Research, 36*(Database issue), D25-D30. doi:10.1093/nar/gkm929

Colwell, M. J., Williams, G. R., Evans, R. C., & Shutler, D. (2017). Honey bee□collected pollen in agro□ecosystems reveals diet diversity, diet quality, and pesticide exposure. *Ecology and Evolution, 7*(18), 7243-7253. doi:10.1002/ece3.3178

Couvillon, M. J., Schürch, R., & Ratnieks, F. L. W. (2014). Waggle Dance Distances as Integrative Indicators of Seasonal Foraging Challenges. *PLOS ONE, 9*(4), e93495. doi:10.1371/journal.pone.0093495

Danner, N., Härtel, S., & Steffan-Dewenter, I. (2014). Maize pollen foraging by honey bees in relation to crop area and landscape context. *Basic and Applied Ecology, 15*(8), 677-684. doi:10.1016/j.baae.2014.08.010

Danner, N., Keller, A., Härtel, S., & Steffan-Dewenter, I. (2017). Honey bee foraging ecology: Season but not landscape diversity shapes the amount and diversity of collected pollen. *PLOS ONE, 12*(8), e0183716. doi:10.1371/journal.pone.0183716

Danner, N., Molitor, A. M., Schiele, S., Härtel, S., & Steffan-Dewenter, I. (2016). Season and landscape composition affect pollen foraging distances and habitat use of honey bees. *Ecological Applications, 26*(6), 1920-1929. doi:10.1890/15-1840.1

Di Pasquale, G., Alaux, C., Le Conte, Y., Odoux, J.-F., Pioz, M., Vaissière, B. E., . . . Decourtye, A. (2016). Variations in the Availability of Pollen Resources Affect Honey Bee Health. *PLOS ONE, 11*(9), e0162818. doi:10.1371/journal.pone.0162818

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., . . . Alaux, C. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLOS ONE, 8*(8), e72016. doi:10.1371/journal.pone.0072016

Donaldson-Matasci, M. C., & Dornhaus, A. (2012). How habitat affects the benefits of communication in collectively foraging honey bees. *Behavioral Ecology and Sociobiology, 66*(4), 583-592. doi:10.1007/s00265-011-1306-z

Donaldson-Matasci, M. C., & Dornhaus, A. (2014). Dance Communication Affects Consistency, but Not Breadth, of Resource Use in Pollen-Foraging Honey Bees. *PLOS ONE, 9*(10). doi:10.1371/journal.pone.0107527

Dornhaus, A., & Chittka, L. (2004). Why do honey bees dance? *Behavioral Ecology and Sociobiology, 55*(4), 395-401. doi:10.1007/s00265-003-0726-9

Garbuzov, M., Couvillon, M. J., Schürch, R., & Ratnieks, F. L. W. (2015). Honey bee dance decoding and pollen-load analysis show limited foraging on spring-flowering oilseed rape, a potential source of neonicotinoid contamination. *Agriculture, Ecosystems & Environment, 203*, 62-68. doi:10.1016/j.agee.2014.12.009

Gary, N. E., Witherell, P. C., & Marston, J. M. (1973). Distribution of Foraging Bees Used to Pollinate Alfalfa. *Environmental Entomology, 2*(4), 573-578. doi:10.1093/ee/2.4.573

Haydak, M. H. (1970). Honey bee nutrition. *Annual Review of Entomology, 15*(1), 143-156. doi:10.1146/annurev.en.15.010170.001043

Hendriksma, H. P., & Shafir, S. (2016). Honey bee foragers balance colony nutritional deficiencies. *Behavioral Ecology and Sociobiology, 70*(4), 509-517. doi:10.1007/s00265-016-2067-5

Holzschuh, A., Dainese, M., González‐Varo, J. P., Mudri‐Stojnić, S., Riedinger, V., Rundlöf, M., . . . Bommarco, R. (2016). Mass‐flowering crops dilute pollinator abundance in agricultural landscapes across Europe. *Ecology Letters, 19*(10), 1228-1236. doi:10.1111/ele.12657

I'Anson Price, R., Dulex, N., Vial, N., Vincent, C., & Grüter, C. (2019). Honeybees forage more successfully without the "dance language" in challenging environments. *Science Advances, 5*(2), eaat0450. doi:10.1126/sciadv.aat0450

Kaluza, B. F., Wallace, H., Keller, A., Heard, T. A., Jeffers, B., Drescher, N., . . . Leonhardt, S. D. (2017). Generalist social bees maximize diversity intake in plant species-rich and resource-abundant environments. *Ecosphere, 8*(3), e01758. doi:10.1002/ecs2.1758

Keller, A., Danner, N., Grimmer, G., Ankenbrand, M., Ohe, K., Ohe, W., . . . Steffan-Dewenter, I. (2015). Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. *Plant Biology, 17*(2), 558-566. doi:10.1111/plb.12251

Keller, I., Fluri, P., & Imdorf, A. (2005). Pollen nutrition and colony development in honey bees—Part II. *Bee World, 86*(2), 27-34. doi:10.1080/0005772X.2005.11099650

Krebs, C. (1999). *Ecological Methodology*. Menlo Park, California: Addison Welsey Educational Publishers.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software, 82*(13), 26. doi:10.18637/jss.v082.i13

Menzel, R., Kirbach, A., Haass, W.-D., Fischer, B., Fuchs, J., Koblofsky, M., . . . Greggers, U. (2011). A Common Frame of Reference for Learned and Communicated Vectors in Honeybee Navigation. *Current Biology, 21*(8), 645-650. doi:10.1016/j.cub.2011.02.039

Nürnberger, F., Steffan-Dewenter, I., & Härtel, S. (2017). Combined effects of waggle dance communication and landscape heterogeneity on nectar and pollen uptake in honey bee colonies. *Peerj, 5*, e3441. doi:10.7717/peerj.3441

Okada, R., Akamatsu, T., Iwata, K., Ikeno, H., Kimura, T., Ohashi, M., . . . Ito, E. (2012). Waggle dance effect: dancing in autumn reduces the mass loss of a honeybee colony. *Journal of Experimental Biology, 215*(10), 1633-1641. doi:10.1242/jeb.068650

R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., & Bretagnolle, V. (2015). Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. *Ecological Applications, 25*(4), 881-890. doi:10.1890/14-1011.1

Richardson, R. T., Lin, C.-H., Sponsler, D. B., Quijia, J. O., Goodell, K., & Johnson, R. M. (2015). Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. *Applications in Plant Sciences, 3*(1), 1400066. doi:10.3732/apps.1400066

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: a versatile open source tool for metagenomics. *Peerj, 4*, e2584. doi:10.7717/peerj.2584

Roulston, T. H., & Cane, J. H. (2000). Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution, 222*(1), 187-209. doi:10.1007/bf00984102

Scheper, J., Bommarco, R., Holzschuh, A., Potts, S. G., Riedinger, V., Roberts, S. P., . . . Wickens, J. B. (2015). Local and landscape□level floral resources explain effects of wildflower strips on wild bees across four European countries. *Journal of Applied Ecology, 52*(5), 1165-1175. doi:10.1111/1365-2664.12479

Schürch, R., & Grüter, C. (2014). Dancing Bees Improve Colony Foraging Success as Long-Term Benefits Outweigh Short-Term Costs. *PLOS ONE, 9*(8). doi:10.1371/journal.pone.0104660

Seeley, T. D. (1986). Social Foraging by Honeybees - How Colonies allocate Foragers among Patches of Flowers. *Behavioral Ecology and Sociobiology, 19*(5), 343-354. doi:10.1007/bf00295707

Sherman, G., & Visscher, P. K. (2002). Honeybee colonies achieve fitness through dancing. *Nature, 419*(6910), 920-922. doi:10.1038/nature01127

Sickel, W., Ankenbrand, M. J., Grimmer, G., Holzschuh, A., Härtel, S., Lanzen, J., . . . Keller, A. (2015). Increased efficiency in identifying mixed pollen samples by meta-barcoding with a dual-indexing approach. *BMC Ecology, 15*(1), 20. doi:10.1186/s12898-015-0051-y

Vaudo, A. D., Farrell, L. M., Patch, H. M., Grozinger, C. M., & Tooker, J. F. (2018). Consistent pollen nutritional intake drives bumble bee (Bombus impatiens) colony growth and reproduction across different habitats. *Ecology and Evolution, 8*(11), 5765-5776. doi:10.1002/ece3.4115

Vaudo, A. D., Patch, H. M., Mortensen, D. A., Tooker, J. F., & Grozinger, C. M. (2016). Macronutrient ratios in pollen shape bumble bee (*Bombus impatiens*) foraging strategies and floral preferences. *Proceedings of the National Academy of Sciences, 113*(28), E4035-E4042. doi:10.1073/pnas.1606101113

von Frisch, K. (1967). *The dance language and orientation of bees*. Cambridge, MA: Harvard University Press.

Westerkamp, C. (1991). Honeybees are poor pollinators — why? *Plant Systematics and Evolution, 177*(1), 71-75. doi:10.1007/bf00937827

Zarchin, S., Dag, A., Salomon, M., Hendriksma, H. P., & Shafir, S. (2017). Honey bees dance faster for pollen that complements colony essential fatty acid deficiency. *Behavioral Ecology and Sociobiology, 71*(12), 172. doi:10.1007/s00265-017-2394-1

[dataset] Nürnberger, F., Keller, A. Härtel, S. & Steffan-Dewenter, I. (2019). DNA sequencing raw data: Honey bee waggle dance communication increases diversity of pollen diets in intensively managed agricultural landscapes. *European Nucleotide Archive (ENA)*, http://www.ebi.ac.uk/ena/data/view/PRJEB32797

Data accessibility

All source data analysed is accessible: field data can be found in the supplemental material and raw data from DNA sequencing is available at the EBI-ENA (www.ebi.ac.uk/ena) public repository under accession number PRJEB32797.

Author contributions

FN, SH, and ISD designed the research. FN conducted the research. AK performed the DNA metabarcoding of pollen samples. FN and AK analysed the data. FN visualized the data and wrote the first draft. FN, AK, SH, and ISD edited the manuscript. SH and ISD acquired the funding for the research.

Tab. 1 Resource environment parameters of the eight selected landscapes for a 2000 m radius buffer around experimental colonies. Parameters are given for the two distinct mapping periods. Means ± standard errors and ranges

Landscape parameter	**Mean ± *SE***	**Range**
Flower cover - Period A [ha]	23.9 ± 2.9	11.1 - 33.3
Flower cover - Period B [ha]	12.5 ± 3.2	3.4 - 26.0
% Mass-flowering crops – Period A	69.7 ± 3.4	17.1 – 95.7
% Mass-flowering crops – Period B	35.4 ± 4.3	1.5 – 91.0
Generic richness – Period A	24.6 ± 0.1	23 – 24
Generic richness – Period B	34.8 ± 0.1	34 – 35

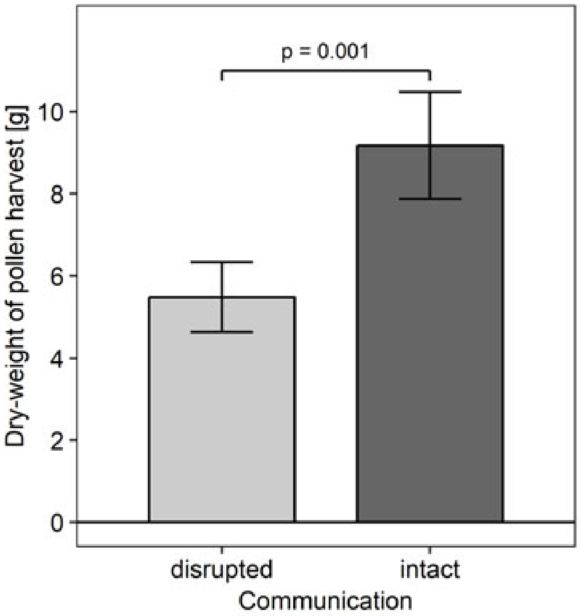

Fig. 1 Disrupting dance communication affected the total dry-weight of pollen collected by honey bee colonies. Figure adapted from Nürnberger et al. (2017), which was based on the same field experiment

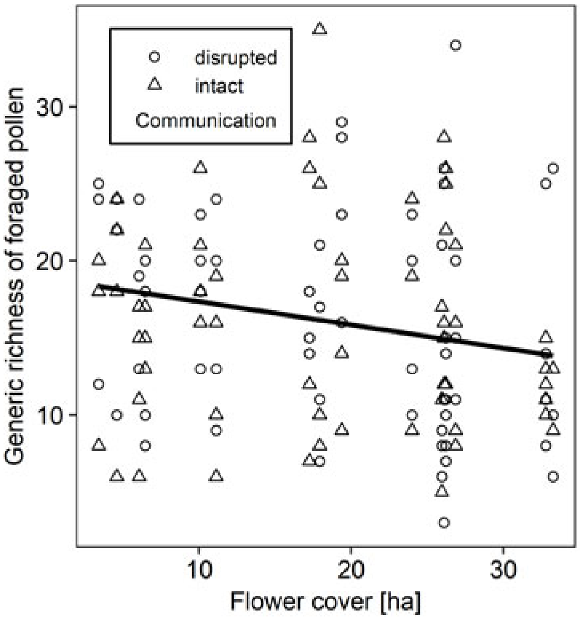

Fig. 2 The total flower cover affected the richness of foraged pollen on genus level, regardless of communication treatment. Data points represent individual pollen samples. Regression line based on negative binomial generalized linear mixed-effects model with identity link

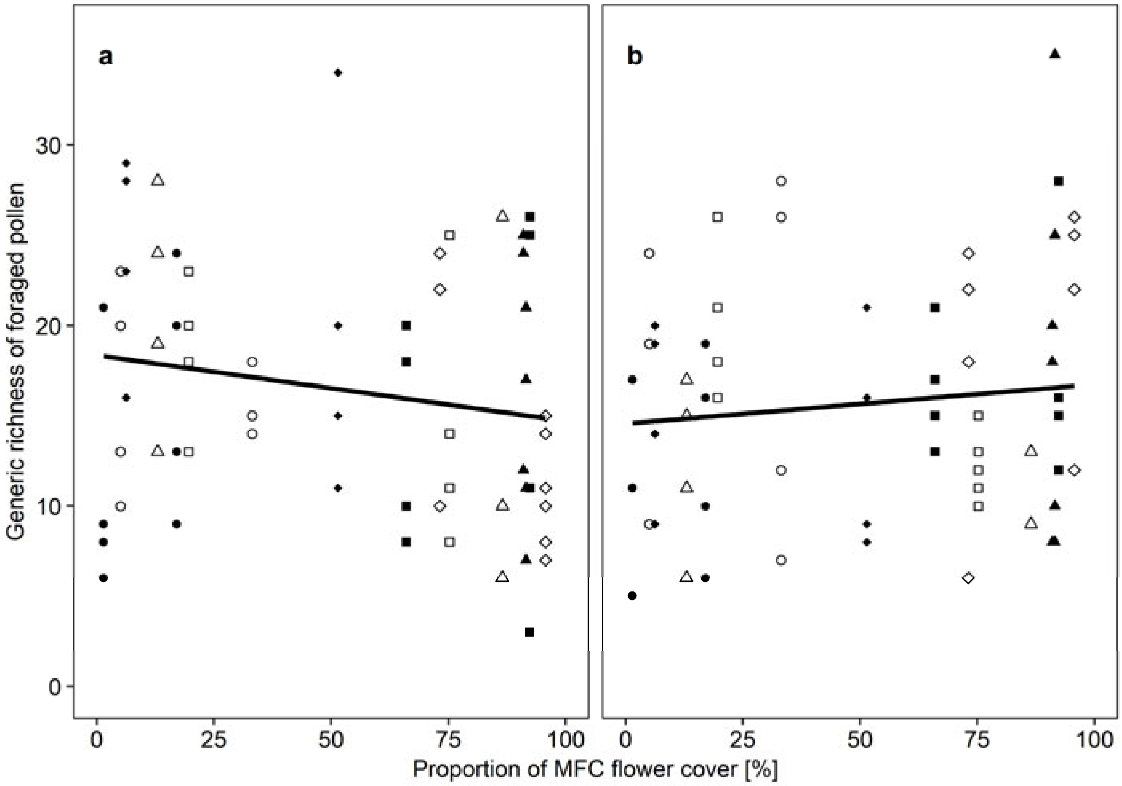

Fig. 3 The effect of the proportion of flower cover that was made up of mass-flowering crops (MFC) on the richness of foraged pollen on genus level depended on whether dance communication was disrupted (a) or intact (b). The different symbols depict individual pollen samples from the eight different landscapes. Regression lines based on negative binomial generalized linear mixed-effects model with identity link

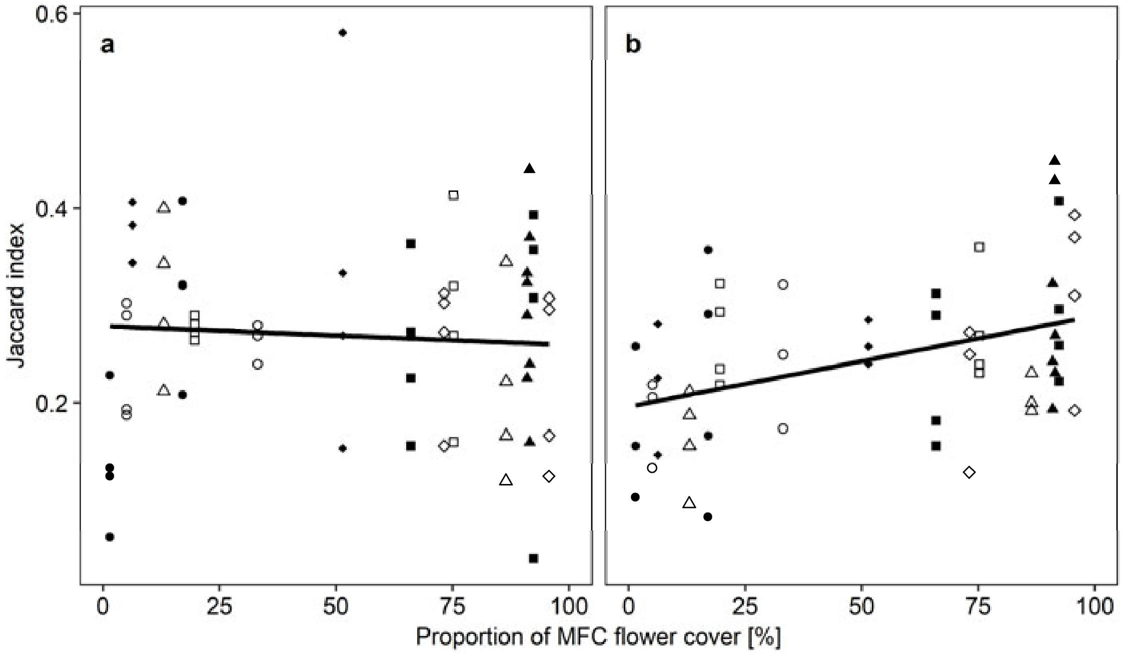

Fig. 4 The effect of the proportion of flower cover that was made up of mass-flowering crops (MFC) on the Jaccard index, i.e. the similarity between available resource genera composition and the genera composition of foraged pollen, depended on whether dance communication was disrupted (a) or intact (b). The different symbols depict individual pollen samples from the eight different landscapes. Regression lines based on linear mixed-effects model

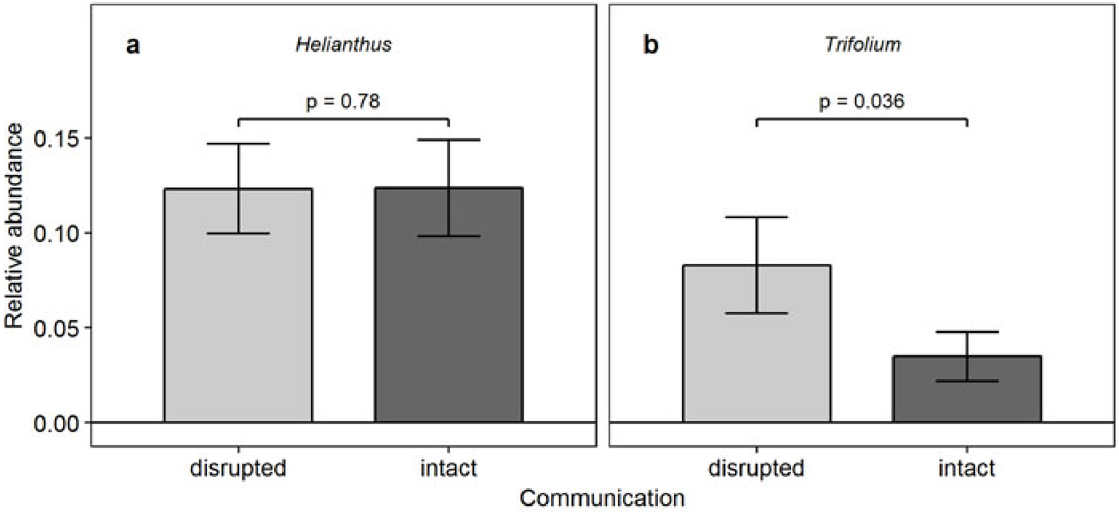

Fig. 5 The relative abundance of the mass-flowering crop genera a) *Helianthus* and b) *Trifolium* in pollen samples (based on numbers of sequence reads) of honey bee colonies in which directional dance communication was either disrupted or intact. Means ± *SE*. P-values based on linear mixed-effects models