Tree phylogenetic diversity promotes host-parasitoid interactions

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Electronic supplementary material

Supplementary methods

(a) Sampling

Naturally, cavity-nesting Hymenoptera construct their nests in a wide variety of materials, as long as species-specific requirements for length and diameter are met (Krombein 1967). Most species take any suitable cavity, from abandoned galleries of wood-dwelling beetles and crevices under bark, to hollow sticks and twigs, the latter which are mimicked by the trap nests used here (Tscharntke *et al.* 1998, Staab *et al.* 2014).

Trap nests consisted of PVC sewer tubes (length: 22 cm, diameter: 12.5 cm) evenly filled with dry *Arundo donax* L. (Poaceae) internodes of varying diameters (2-20 mm) to offer nesting possibilities for a broad size range of Hymenoptera. In every plot, four trap nests each were attached to two wooden posts so all trap nests were situated approximately 1.5 m above the ground (see figure 1*a* in Staab *et al.* 2014). The two posts were positioned approximately 15 m from each other at two opposite corners of the central 10 x10 m area of each plot. As natural nesting possibilities for cavity-nesting Hymenoptera are often clumped (O'Neill 2001), the four directly adjacent trap nests on a post represent a single nesting possibility in the same local environment. Thus, the data from the four trap nests per post were pooled before analysis and treated as statistical replicates nested in the same plot (see main text).

A fungicide (Folicur®, Bayer CropScience, Monheim, Germany) was applied regularly to prohibit mould, which commonly infests trap nests in warm and humid climates. Collected internodes were carefully opened, placed in individual test tubes and reared at

ambient conditions until hatching (see figure 1*c* in Staab *et al.* 2014). All species were identified to species or morphospecies level by the authors and the taxonomic experts listed in the acknowledgements. Voucher specimens have been deposited at the University of Freiburg (Department for Nature Conservation and Landscape Ecology) and the collections curated by the respective taxonomists.

Our sampling was restricted to the understory, which possibly has different habitat properties than the canopy and for some insects (e.g. ants: Floren *et al.* 2014) markedly dissimilar faunas. However, the only trap-nest study comparing forest strata in subtropical forests found no differences in host-parasitoid interactions (Morris *et al.* 2015) and we are confident that our study represents general patterns of how those interactions are influenced by the environment.

(b) Calculation of mean phylogenetic distance

A phylogeny of all 147 tree species growing on the 27 study plots was built with sequences from the marker genes *mat*K, *rbc*L, and the ITS region including the *5.8s* gene as previously described in detail by Baruffol *et al.* (2013). Sequences were either extracted from GenBank (http://www.ncbi.nlm.nih.gov/genbank/) or, for a few species, created with standard barcoding protocols (GenBank accession numbers: KF569888-KF569899). Maximum likelihood tree interference was calculated with PHYML (Guindon & Gascuel 2003) using the GTR+I+G model. An ultrametric tree was created using non-parametric rate smoothing in R8S (Sanderson 1997) and 27 fossil calibration points (see electronic supplementary material of Baruffol *et al.* 2013 and references therein). An illustration of the complete tree can be found in the electronic supplementary material of Schuldt *et al.* (2014). Based on the ultrametric tree, mean phylogenetic distance (MPD) per plot was calculated as the abundance weighted phylogenetic distance among all angiosperm tree species in a plot (see Kembel *et al.* 2010).

(c) Network analyses

Network analyses were done with species-level host-parasitoid interaction data based on single parasitized host brood cells. If in the same nest a host species was parasitized by two different parasitoid species it was counted as two different interactions. Of the manifold postulated indices for quantifying network properties, we selected 'linkage density' and 'H2'. Linkage density (LD) measures the weighted mean number of interaction links per species and is an index for network stability (Bersier *et al.* 2002). The index obtains values ≥1, with larger values referring to more stable networks. H2 measures network specialization between 0 and 1, with higher values referring to higher specialization (Blüthgen *et al.* 2006). Both indices are based on weighted, quantitative links and relatively robust against variations in network size.

A common problem in network analysis is that meaningful network indices can only be calculated with a sufficient number of interactions. Thus, indices were only calculated for the full, pooled network and for plots with at least ten parasitized host brood cells (14 plots), reducing the statistical power of the plot-level network analyses that should hence be interpreted with caution. The biotic and abiotic environmental variables for this subset did not differ from the complete dataset (*t*-test, $p>0.05$ for all variables) and had the same variances (*F*-test, $p>0.05$ for all variables). This indicates that the habitat heterogeneity of all plots is well represented in the subset and that results are unlikely to be compromised.

Finally, to test if observed network indices were different from chance, random networks and the corresponding indices were simulated with 10000 runs of Patefield null models (Dormann *et al.* 2009).

Supplementary results

(a) General community patterns

Of the totally 2933 host brood cells, 79% had been constructed by 19 wasp species and 21% by 6 bee species (table S3, electronic supplementary material). All bee species were members of the family Megachilidae while the wasp community contained Pompilidae (323 brood cells / 7 species), Sphecidae (204 / 2) and Vespidae (1424 / 10). The five most abundant species accounted for 85% of all brood cells and were *Anterhynchium flavomarginatum curvimaculatum* (Cameron, 1903) (1042 brood cells, Vespidae) (figure S2*a*), *A. f. flavomarginatum* (Smith, 1852) (324 brood cells, Vespidae), *Osmia taurus* Smith, 1873 (275 brood cells, Megachilidae), *Deuteragenia ossarium* Ohl, 2014 (213 brood cells, Pompilidae) and *Hoplammophila aemulans* (Kohl, 1901) (199 brood cells, Sphecidae) (figure S2*b*). Six species (24%) were only found in one internode and eight species (32%) were only found in one of the 27 plots. None of the species is considered to be exotic to China.

Similarly to the host community, a few parasitoid species accounted for the majority of parasitized brood cells. The five most abundant species were Sarcophagidae sp. CN02 (81 brood cells, Diptera: Sarcophagidae), *Chrysis principalis* Smith, 1874 (62 brood cells, Hymenoptera: Chrysididae) (figure S2*c*), *Apanteles* sp. CN01 (43 brood cells, Hymenoptera: Braconidae), *Lycogaster violaceipennis* Chen, 1949 (38 brood cells, Hymenoptera: Trigonalidae), and Sarcophagidae sp. CN01 (19 brood cells, Diptera: Sarcophagidae). Those species parasitized 74% of all brood cells attacked by parasitoids. Eight species (30%), such as *Leucospis japonica* Walker, 1871 (Hymenoptera: Leucospididae) (figure S2*d*), parasitized only a single host nest while 11 species (41%) did only so in a single plot. From six parasitized host brood cells no specimens hatched. Those brood cells were included in the calculation of parasitism rates but excluded from network analyses.

(b) Species-richness estimation

Species richness estimation using first order jackknife estimators and species accumulation curves indicated that host and parasitoid (figure S3, electronic supplementary material)

communities were sampled equally well and to a similar extent. Of the expected 32 ± 3 (SE) host species, 78% were collected. The expected species richness of parasitoids was 38 ± 4 species and slightly larger, and the observed sampling efficiency of 71% slightly smaller when compared to hosts.

(c) Network analyses

In the subset of plots with calculable network indices, H2 was high (mean ± SD: 0.86 ± 0.20), suggesting consistently specialized host-parasitoid interactions. Linkage density in the subset was 2.15 ± 0.53 suggesting about two links per species. The best-performing linear models for H2 and LD retained no environmental variable, revealing that network properties were unrelated to the environment. There was no sign of spatial autocorrelation.

Null models showed that the observed network were consistently more specialized ($H2_{obs} > H2_{null}$) and less linked ($LD_{obs} < LD_{null}$) than expected by chance. For the total pooled network and for H2 in the subsets, the differences between observed and null indices were large and the associated *p*-values always <0.05. The same was true for LD, with the exception of two plots with non-significant LD-null model comparisons. Thus, in total species interactions in 28 out of 30 index-null model comparisons were significantly different from chance.

Supplementary references

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Table S1. Environmental variables characterizing the 27 study plots. Shown are values ranges, medians and means (± SD). Variables marked by # were log-transformed prior to analyses to improve normality and homoscedasticity. See table S2 for pairwise correlations of all variables.

Environmental variable	Range	Median	Mean ± SD
Aspect East	-1.00 - 1.00	-0.06	-0.08 ± 0.72
Aspect North	-1.00 - 1.00	-0.07	-0.18 ± 0.69
Canopy layer cover (%)#	5 - 50	20	21 ± 12
Elevation (m)	251 - 903	569	547 ± 168
Herb layer cover (%)#	1 - 80	5	18 ± 22
Leaf functional diversity	0.27 - 0.42	0.34	0.34 ± 0.03
Mean phylogenetic distance	154 - 209	192	190 ± 14
Shrub layer cover (%)#	50 - 80	10	22 ± 19
Successional age (years)	22 - 116	72	67 ± 26
Tree abundance#	207 - 1233	513	597 ± 290
Tree species richness#	25 - 69	39	42 ± 10

Table S2. Spearman correlation coefficients (r_s; above the diagonal) and *p*-values (below the diagonal) for all pairwise comparisons of all environmental variables. Bold numbers indicated when two variables were correlated with $r_s > 0.70$ and hence one of the variables (marked by *) was excluded from all following analyses.

	Tree species richness	Tree abundance*	Successional age (yrs)	Shrub layer cover (%)	Mean phylogenetic distance	Leaf functional diversity	Herb layer cover (%)	Elevation (m)	Canopy layer cover (%)*	Aspect North	Aspect East
Aspect East	0.22	0.14	-0.06	0.1	0.11	0.44	-0.02	0.34	-0.06	0.04	
Aspect North	0.04	0.25	-0.14	0	-0.21	-0.04	0.46	-0.18	-0.18		0.86
Canopy layer cover (%)*	0.15	-0.61	**0.76**	-0.39	0.4	0.09	-0.19	0.25		0.37	0.78
Elevation (m)	0.05	0.13	0.22	0.25	-0.27	0.45	-0.37		0.2	0.37	0.09
Herb layer cover (%)	0.14	0.00	-0.08	0.06	0.23	-0.05		0.05	0.34	0.02	0.93
Leaf functional diversity	0.41	0.04	0.12	0.45	0.18		0.81	0.02	0.64	0.83	0.02
Mean phylogenetic distance	0.47	-0.64	0.56	-0.34		0.38	0.25	0.17	0.04	0.29	0.6
Shrub layer cover (%)	0.03	0.6	-0.33		0.09	0.02	0.76	0.2	0.04	0.99	0.62
Successional age (yrs)	0.25	**-0.74**		0.09	<0.01	0.55	0.69	0.28	**<0.01**	0.47	0.76
Tree abundance*	0.05		**<0.01**	<0.01	<0.01	0.84	1	0.52	0	0.21	0.48
Tree species richness		0.81	0.21	0.88	0.01	0.04	0.47	0.82	0.45	0.84	0.27

Table S3. Host and parasitoid brood cells collected with trap nests in subtropical South-East China. Values in brackets for host species are species specific parasitism rates in %. The numbers in the first column refer to the species codes in figure S2 and figure 4.

Number in figures	Species	Family	Brood cell number
Hosts			
	Allorhynchium chinense (de Saussure, 1862)	Vespidae	1 (0)
	Ancistrocerus nigricornis (Curtis, 1826)	Vespidae	2 (0)
	Ancistrocerus trifasciatus shibuyai (Müller, 1776)	Vespidae	14 (0)
1	*Anterhynchium flavomarginatum curvimaculatum* (Cameron, 1903)	Vespidae	1042 (24.4)
2	*Anterhynchium flavomarginatum flavomarginatum* (Smith, 1852)	Vespidae	324 (11.1)
	Anterhynchium sp. CN01	Vespidae	1 (0)
	Auplopus sp. CN02	Pompilidae	8 (0)
	Auplopus sp. CN03	Pompilidae	4 (0)
3	*Auplopus* sp. CN04	Pompilidae	75 (2.7)
	Auplopus sp. CN05	Pompilidae	4 (0)
4	*Chalybion japonicum* (Gribodo, 1883)	Sphecidae	5 (20.0)
5	*Deuteragenia ossarium* Ohl, 2014	Pompilidae	213 (3.3)
	Deuteragenia sp. CN01	Pompilidae	11 (0)
	Deuteragenia sp. CN02	Pompilidae	8 (0)
	Discoelius nigriclypeus Zhou & Li, 2013	Vespidae	1 (0)
6	*Epsilon fujianensis* Lee, 1981	Vespidae	24 (16.7)
	Eumenes quadratus quadratus Smith, 1852	Vespidae	2 (0)
7	*Hoplammophila aemulans* (Kohl, 1901)	Sphecidae	199 (0.5)
	Hoplitis carinotarsa. Wu, 1987	Megachilidae	29 (0)
8	*Megachile sculpturalis* Smith, 1853	Megachilidae	87 (15.0)
9	*Megachile abluta* Cockerell, 1911	Megachilidae	33 (12.1)
10	*Megachile monticola* Smith, 1853	Megachilidae	17 (23.5)
11	*Orancistrocerus drewseni* (de Saussure, 1857)	Vespidae	13 (23.1)
	Osmia taurus Smith, 1873	Megachilidae	275 (0)

	Trachusa sp. CN01	Megachilidae	11 (0)
Parasitoids			
1	*Apanteles* sp. CN01	Braconidae	43
2	Braconidae sp. CN01	Braconidae	1
3	*Chrysis principalis* Smith, 1874	Chrysididae	62
4	*Coelioxys brevicaudata* Friese, 1935	Megachilidae	4
5	*Coelioxys ducalis* Smith, 1853	Megachilidae	6
6	Drosophilidae sp. CN01	Drosophilidae	2
7	*Euaspis basalis* (Ritsema, 1874)	Megachilidae	3
8	*Eurytoma* sp. CN01	Eurytomidae	4
9	Ichneumonidae sp. CN01	Ichneumonidae	2
10	Ichneumonidae sp. CN02	Ichneumonidae	1
11	Ichneumonidae sp. CN03	Ichneumonidae	14
12	Ichneumonidae sp. CN04	Ichneumonidae	2
13	Ichneumonidae sp. CN05	Ichneumonidae	3
14	*Irenangelus* sp. CN01	Pompilidae	4
15	*Leucospis japonica* Walker, 1871	Leucospididae	1
16	*Lycogaster flavonigrata* Chen, van Achterberg, He & Xu, 2014	Trigonalidae	12
17	*Lycogaster* sp. CN03	Trigonalidae	1
18	*Lycogaster violaceipennis* Chen, 1949	Trigonalidae	38
19	Mutillidae sp. CN01	Mutillidae	1
20	Phoridae sp. CN01	Phoridae	2
21	Sarcophagidae sp. CN01	Sarcophagidae	19
22	Sarcophagidae sp. CN02	Sarcophagidae	81
23	Tachinidae sp. CN01	Tachinidae	2
24	Tachinidae sp. CN02	Tachinidae	1
25	Tachinidae sp. CN03	Tachinidae	11
26	Tachinidae sp. CN04	Tachinidae	4
27	*Zonitis* sp. CN01	Meloidae	5

Table S4. Complete results of the averaged mixed-effect models (within 2 AICc units of the model with the lowest AICc) for parasitism rate (binomial model) and abundance and species richness (Poisson models) of parasitoids and hosts. Shown are standardized model estimates ± SE allowing a direct comparison of effect sizes, *z*-values, *p*-values of the *z*-statistics and the relative importance of variables in the averaged models. Variables within each averaged model are sorted by their relative importance. Significant *p*-values are indicated in bold.

Environmental variable	Estimate ± SE	*z*	*p*	Relative importance
Elevation	-0.439 ± 0.139	3.073	**0.002**	1.00
Mean phylogenetic distance	0.427 ± 0.149	2.801	**0.005**	1.00
Aspect East	-0.060 ± 0.115	0.514	0.608	0.32
Shrub layer cover	0.042 ± 0.101	0.412	0.680	0.26
Host brood cell number[a]	0.021 ± 0.084	0.248	0.805	0.13
Tree species richness	-0.017 ± 0.068	0.238	0.812	0.13
Parasitoid abundance				
Host brood cell number[a]	1.050 ± 0.182	5.611	**<0.001**	1.00
Elevation	-0.361 ± 0.126	2.868	**0.004**	1.00
Mean phylogenetic distance	0.388 ± 0.134	2.825	**0.005**	1.00
Aspect East	-0.050 ± 0.010	0.494	0.621	0.31
Shrub layer cover	0.025 ± 0.074	0.326	0.744	0.20
Parasitoid species richness				
Host brood cell number[a]	0.808 ± 0.165	4.775	**<0.001**	1.00
Elevation	-0.229 ± 0.111	2.023	**0.043**	1.00
Shrub layer cover	0.162 ± 0.119	1.339	0.181	0.79
Mean phylogenetic distance	0.204 ± 0.150	1.348	0.180	0.77
Successional age	0.056 ± 0.116	0.484	0.629	0.23

Host abundance				
Successional age	-0.160 ± 0.168	0.940	0.347	0.61
Herb layer cover	-0.086 ± 0.130	0.655	0.513	0.44
Shrub layer cover	0.093 ± 0.141	0.654	0.513	0.41
Mean phylogenetic distance	-0.097 ± 0.151	0.634	0.526	0.40
Leaf functional diversity	0.022 ± 0.071	0.314	0.754	0.15
Aspect North	0.017 ± 0.064	0.256	0.798	0.11
Elevation	0.012 ± 0.054	0.221	0.825	0.08
Tree species richness	0.003 ± 0.028	0.096	0.924	0.03
Host species richness				
Aspect East	0.180 ± 0.080	2.193	**0.028**	0.91
Successional age	-0.223 ± 0.089	2.458	**0.014**	0.86
Mean phylogenetic distance	-0.057 ± 0.103	0.549	0.583	0.31
Tree species richness	0.035 ± 0.078	0.445	0.656	0.25
Leaf functional diversity	0.026 ± 0.063	0.407	0.684	0.20
Elevation	0.011 ± 0.040	0.270	0.787	0.11
Shrub layer cover	0.007 ± 0.033	0.209	0.834	0.08
Aspect North	0.004 ± 0.025	0.170	0.865	0.07

[a] host brood cell number is our definition of host abundance and consequently not included in models for host abundance and species richness.

Table S5. Complete results of the averaged linear model (within 2 AICc units of the model with the lowest AICc) for Shannon interaction diversity of host-parasitoid interactions. Shown are standardized model estimates ± SE allowing a direct comparison of effect sizes, *t*-values, *p*-values of the *t*-statistics and the relative importance of variables in the averaged model. Variables are sorted by their relative importance. Significant *p*-values are indicated in bold.

Environmental variable	Estimate ± SE	*t*	*p*	Relative importance
Host brood cell number	0.562 ± 0.106	5.058	**<0.001**	1.00
Mean phylogenetic distance	0.205 ± 0.166	1.209	0.227	0.74
Elevation	-0.106 ± 0.127	0.820	0.412	0.52
Shrub layer cover	0.072 ± 0.118	0.602	0.547	0.36
Leaf functional diversity	-0.060 ± 0.108	0.543	0.587	0.30
Tree species richness	-0.029 ± 0.074	0.389	0.697	0.21
Successional age	0.028 ± 0.079	0.353	0.724	0.15

Table S6. Pearson correlation coefficients, explained variance (R^2) and probabilities p (based on 10.000 permutations) for the relationship between the environmental variables (ordered by decreasing R^2) and the plot axes scores of the first two NMDS axes (NMDS 1, NMDS 2) for host and parasitoid ordinations (obtained by the R-command 'envfit'). Significant p-values are indicated in bold.

Environmental variable	NMDS 1	NMDS 2	R^2	p
Host community				
Elevation	-0.852	-0.523	0.393	**0.004**
Mean phylogenetic distance	0.962	-0.272	0.332	**0.006**
Shrub layer cover	-0.988	-0.156	0.292	**0.015**
Leaf functional diversity	-0.960	-0.280	0.284	**0.017**
Aspect East	-0.442	0.897	0.162	0.122
Successional age	0.619	-0.786	0.089	0.325
Herb layer cover	0.743	0.669	0.046	0.565
Aspect North	0.068	0.998	0.030	0.695
Tree species richness	0.086	-0.996	0.004	0.959
Parasitoid community				
Mean phylogenetic distance	-0.474	-0.881	0.335	**0.009**
Aspect East	0.648	-0.762	0.174	0.096
Successional age	-0.800	-0.601	0.121	0.210
Host brood cell number	0.316	0.949	0.110	0.248
Elevation	0.085	0.996	0.102	0.272
Aspect North	-0.004	1.000	0.076	0.398
Leaf functional diversity	-0.412	0.911	0.030	0.690
Tree species richness	-0.427	0.904	0.006	0.929
Herb layer cover	-0.846	0.532	0.001	0.986
Shrub layer cover	-0.367	-0.930	0.001	0.994

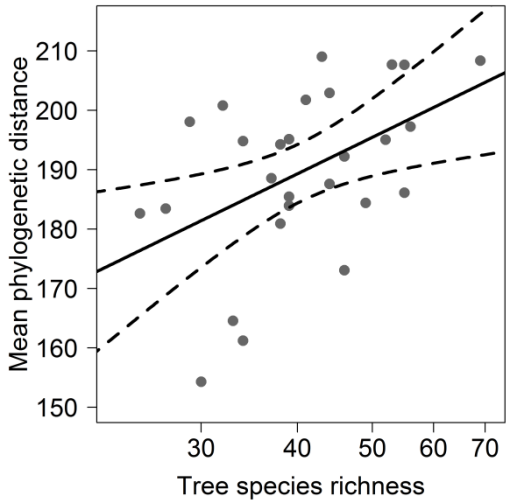

Figure S1. Relationship between tree species richness and mean phylogenetic distance. Shown is the prediction (solid line) of a linear model (t=2.697, p=0.012) and 95% CI (dashed lines). Please note that the x-axis is log-scaled.

Figure S2. Examples of species from this study illustrating the morphological, taxonomic and life-history diversity of hosts and parasitoids. (*a*) *Anterhynchium flavomarginatum* (Vespidae, host 1), mainly a predator of Noctuidae caterpillars, of which several individuals are provisioned in each brood cell, was the most abundant host species. (*b*) *Hoplammophila aemulans* (Sphecidae, host 7), a conspicuous large-bodied predator of Geometridae caterpillars; each brood cell is provisioned with a single caterpillar only. (*c*) *Chrysis principalis* (Chrysididae, parasitoid 3), a common kleptoparasitoid attacking brood cells of Vespidae such as *A. flavomarginatum*. (*d*) *Leucospis japonica* (Leucospididae, parasitoid 15) an endoparasitoid on the larvae of Megachilidae bees. Numerical codes are identical to figure 4 and refer to table S3 where species authors are given. All photographs by Michael Staab.

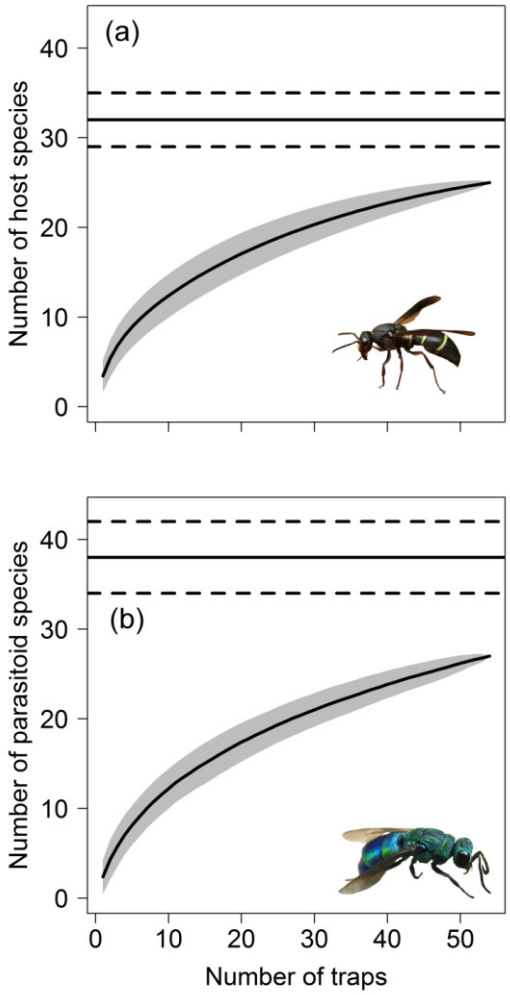

Figure S3. Sample-based species accumulation curves of solitary cavity-nesting Hymenoptera (*a*) and their parasitoids (*b*), based on 10.000 permutations each. Shown are the observed number of species (solid curves), the 95% CI of the accumulation curves (grey shadings), and the expected numbers of species ± SE based on jack1 estimators (solid and dashed vertical lines, respectively). Both communities were sampled approximately equally well with 78% (25 species) of the total expected host species and 71% (27 species) of the expected parasitoid species having been collected.

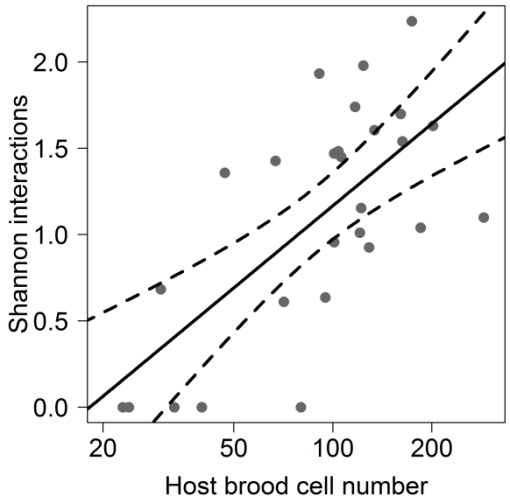

Figure S4. Relationship between Shannon interaction diversity of host-parasitoid interactions and host abundance. Shown is the prediction of a linear model (solid line, significant at $p<0.001$) and 95% CI (dashed lines). Please note that the x-axis is log-scaled. See table S5 for details on model averaging.