Survival strategies of stingless bees (*Melipona subnitida*) in an unpredictable environment, the Brazilian tropical dry forest

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Electronic Supplemental material

1. DATA ANALYSIS

1.1. Best Subset Regression Model

The potential relationship between the monthly number of available pollen sources (P-FLO) and abiotic environmental factors (T_{MAX}, maximum temperature; T_{MIN}, minimum temperature; RH_{AVG}, average relative humidity; RAIN, total precipitation; SUN, average time of sunrise) was assessed using Multiple Linear Regression Analysis (Best Subset Regression Model; Zar 1999). Mallow's *Cp*, calculated by the statistical software (SigmaPlot 10.0/SigmaStat 3.5; Systat Software Inc., U.S.A.), was used as measure for the goodness of fit of the Regression Models. Being less dependent on the number of effects in the model than is R^2, Mallow's *Cp* tends to find the *best subset* that includes only the important predictors of the respective dependent variable (Gorman and Toman 1966). The adjusted coefficient of determination R^2_{ADJ} (R^2 adjusted for the number of independent variables in the model) indicated the proportion of variability in the data explained by the respective model (Zar 1999).

1.2. Canonical Correlation Analysis

To assess the potential interaction between environmental factors and colony activity, we used Canonical Correlation Analysis (CCA). This form of data analysis is most appropriate when evaluating the relationship between two sets of variables (Hotelling 1936; Schneider and McNally 1992; Sherry and Henson 2005), in our case, between environmental variables (EV: T_{MAX}, maximum temperature; T_{MIN}, minimum temperature; RH_{AVG}, average relative humidity; RAIN, total precipitation; SUN, average time of sunrise; P-FLO, number of available pollen sources) and colony variables (CV: F_{ON}, average time of foraging onset; F_{PEAK}, average time of peak activity; F_{END}, average end of pollen foraging activity; F_{MAX}, average maximum number of forages; P-COL, total number of plant species collected; CONST, average brood cell construction rate). CCA calculates the maximum (canonical) correlation between two synthetic variables, one for each variable set. These synthetic variables are established through a linear combination among the variables within each set. There are as many orthogonal canonical functions as there are variables in the smaller of the two variable sets, each successive function less correlated than the first canonical function. Following values are relevant for the interpretation of CCA: the *canonical correlation coefficient* (R_C) is the Pearson r-relationship between to synthetic variables. The *squared canonical correlation coefficient* (R_C^2) represents the proportion of variance shared between the variable sets. The *standardized canonical function coefficients* (*SCC*) are the coefficients of the linear combination among the variables in each set. The *structure coefficient* (r_S) is the correlation between a given variable within a variable set and the synthetic variable calculated for this set. The *squared structure coefficient* (r_S^2) indicates the proportion of variance a given variable shares with the synthetic variable.

1.3. REFERENCES

Gorman, J. W., Toman, R. J. (1966) Selection of variables for fitting equations to data. Technometrics **8**, 27-51

Hotelling, H. (1936) Relations between two sets of variables. Biometrika **28**, 321-377

Schneider, S. S., McNally, L. C. (1992b) Factors influencing seasonal absconding in colonies of the African honey bee, *Apis mellifera scutellata*. Insect. Soc. **39**, 403-423

Sherry, A., Henson, R. K. (2005) Conducting and interpreting Canonical Correlation Analysis in personality research: a user-freindly primer. J. Pers. Assess. **84**, 37-48

Zar, J.H. (1999) Biostatistical analysis. Prentice Hall Inc., Upper Saddle River

2. VARIATIONS OF ENVIRONMENTAL PARAMETERS

Variation of environmental variables (EV) in the course of our study. Given are the respective values for each month: T_{AVG}, average temperature; T_{MAX}, maximum temperature; T_{MIN}, minimum temperature; RH_{AVG}, average relative humidity; RAIN, total precipitation; SUN, average time of sunrise; P-FLO, number of plant species in bloom

	Month												
EV	05/11	06/11	07/11	08/11	09/11	10/11	11/11	12/11	01/12	02/12	03/12	04/12	05/12
T_{AVG} (°C)	26.0	25.8	24.4	25.4	27.4	28.2	28.7	28.7	28.6	27.2	27.3	28.1	27.9
T_{MAX} (°C)	31.1	31.9	31.1	36.1	38.3	38.3	38.3	39.2	39.2	38.3	35.3	35.7	36.6
T_{MIN} (°C)	21.3	20.2	19.4	17.9	18.3	19.0	19.4	20.2	20.6	21.3	20.6	19.8	19.4
RH_{AVG} (%)	96.2	91.5	80.1	73.4	60.9	64.1	63.3	64.2	66.5	73.8	75.5	71.2	67.5
RAIN (mm)	163	15	27	0	0	11	0	49	5	59	40	28	3
SUN (hours)	05:30	05:35	05:39	05:38	05:27	05:12	05:05	05:14	05:32	05:37	05:33	05:30	05:30
P-FLO	37	20	11	15	7	6	9	5	3	11	18	11	6

3. THERMAL WINDOW FOR POLLEN FORAGING

We evaluated the foraging activity of the colonies by counting the number of foragers returning to the nests with pollen loads between 0500 h and 1200 h. As established in preliminary observations, all pollen foraging, with few exceptions, occurred in this time frame. During peak foraging activity (usually between 0500 h and 0900 h), the number of pollen collectors/colony was registered for 5 minutes with intervals of 10 minutes (one observation per colony every 15 minutes). When the foragers' flight activity decreased, bee counts were made for 5 minutes with intervals of 25 minutes (one observation per colony every 30 minutes). To assess the preferred temperature range for pollen collection (thermal window of pollen collection), we evaluated the number of foragers returning to their nest at a given ambient temperature (to the nearest °C). This method slightly overestimated the actual foraging temperatures of the individuals because ambient temperatures steadily increased in the course of our observations and, thus, incoming pollen collectors, which forage for several minutes, were registered at the maximum temperature of their foraging trip. Nonlinear Regression Analysis (Gaussian Peak Model) with ambient temperature as predictor and the percentage of returning foragers as dependent variable was used to evaluate the preferred temperature range for pollen collection. We found that foraging occurred mainly at temperatures between 22 and 28°C (Figure ES1). Nonlinear Regression Analysis calculated a peak foraging activity of *M. subnitida* at an ambient temperature of 25.5°C (Nonlinear Regression Analysis, Gaussian Peak Model: $R^2_{ADJ} = 0.94$, $P < 0.001$).

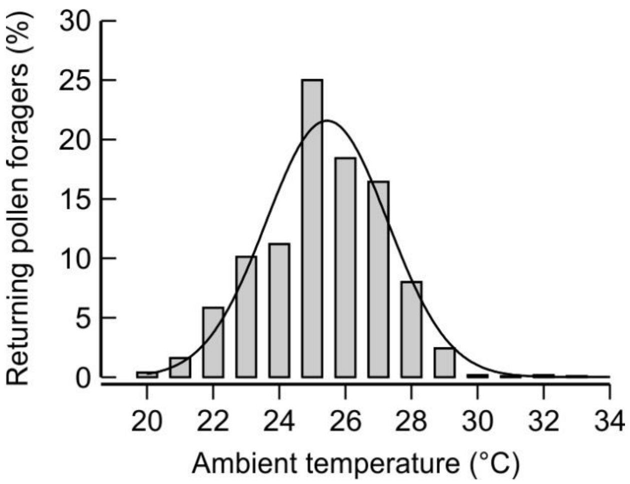


Figure ES1. Thermal window for pollen foraging of *Melipona subnitida*. Frequency distribution of pollen foragers returning to the nest at a given ambient temperature (to the nearest °C) along our study ($N = 2{,}311$ foragers). Line indicates the Gaussian Peak Regression Model ($R^2_{ADJ} = 0.94$, $P < 0.001$) with a calculated maximum pollen foraging activity of *M. subnitida* at an ambient temperature of 25.5°C.

4. POLLEN RESOURCES

4.1. Plant species in the study area

Phenology of plant species (tree, shrubs, herb and lianas) sampled in the study area (Brazilian tropical dry forest) during the observation period (May 2011 to May 2012). The potential resources of the plants were: P = pollen, N = nectar, *n.o.*= not observed.

				Months												
				2011								**2012**				
Family	**Species**	**Stratum**	**Resources**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**
Amaranthaceae	*Froelichia humboldtiana* (Roem. & Schult.) Seub.	herb	N											■		
Amaranthaceae	*Alternanthera tenella* Collad	herb	N	■	■	■	■	■								
Anacardiaceae	*Myracrodruon urundeuva* Allemão	tree	P/N						■							
Anacardiaceae	*Spondias* sp.	tree	P/N						■	■	■	■	■			
Anacardiaceae	*Spondias tuberosa* Arruda	tree	P/N						■	■	■	■				
Arecaceae	*Copernicia prunifera* (Mill.) H.E. Moore	tree	P						■			■				
Asteraceae	*Stilpnopappus pratensis* Mart. ex DC.	herb	*n.o.*	■												
Bignoniaceae	Indeterminate (1)	liana	N		■			■								
Bignoniaceae	Indeterminate (2)	liana	N						■	■						
Bixaceae	*Cochlospermum vitifolium* (Willd.) Spreng.	tree	P					■								
Boraginaceae	*Cordia oncocalix* Allemão	tree	P/N	■	■											
Burseraceae	*Commiphora leptophloeos* (Mart.) J.B.Gillett	tree	N									■				
Cactaceae	*Cereus jamacaru* P. DC	tree	P/N							■						
Capparaceae	*Cynophalla flexuosa* (L.) J.Presl	tree	N								■					
Combretaceae	*Combretum leprosum* Mart.	tree	P/N	■												
Commelinaceae	*Commelina erecta* L.	herb	N											■		
Convolvulaceae	*Evolvulus cordatus* Moric	herb	P/N	■												
Convolvulaceae	*Ipomoea nil* (L.) Roth.	liana	P/N	■												
Convolvulaceae	*Ipomoea* sp. (1)	liana	P/N		■											
Convolvulaceae	*Ipomoea* sp. (2)	liana	P/N			■										
Convolvulaceae	*Ipomoea asarifolia* (Desr) Roem. & Schult.	liana	P/N	■	■	■	■	■	■	■					■	■
Convolvulaceae	*Ipomoea bahiensis* Willd. ex Roem. Schult.	liana	P/N		■	■										

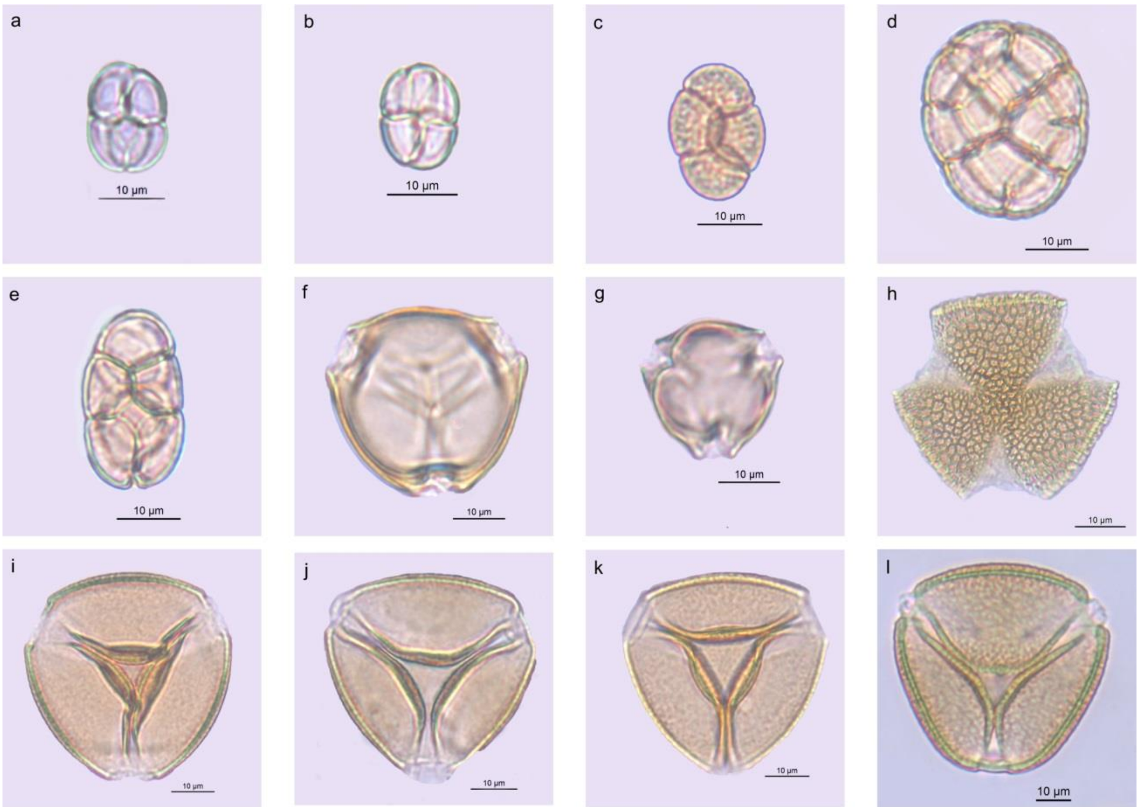
				Months												
				2011								**2012**				
Family	**Species**	**Stratum**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**
Convolvulaceae	*Jacquemontia multiflora* (Choisy) Hallier f.	liana	P/N	■	■	■	■									
Convolvulaceae	*Merremia aegyptia* (L.) Urb.	liana	P/N		■		■									
Convolvulaceae	*Operculina macrocarpa* (L.) Urb.	liana	P/N		■											
Cucurbitaceae	*Cucumis anguria* L.	liana	*n.o.*/P	■												
Cucurbitaceae	*Momordica charantia* L.	liana	*n.o.*/P		■											
Euphorbiaceae	*Cnidoscolus urens* L. Arthur	shrub	P/N	■	■		■						■	■		
Euphorbiaceae	*Dalechampia pernambucensis* Baill.	liana	P/Resin										■			
Euphorbiaceae	*Jatropha mollissima* (Pohl) Baill.	tree	P/N								■		■			
Euphorbiaceae	*Manihot glaziovii* Muell. Arg.	tree	P/N										■	■		
Euphorbiaceae	*Croton hirtus* L'Hér.	herb	P/N											■		
Euphorbiaceae	*Croton sonderianus* Müll. Arg.	tree	P/N										■	■		
Fabaceae, Caesalpinioidae	*Bauhinia cheilantha* (Bong.) Steud.	tree	P/N	■												
Fabaceae, Caesalpinioidae	*Chamaecrista calycioides* (DC. ex Collad.) Greene	herb	P	■	■		■							■	■	
Fabaceae, Caesalpinioidae	*Chamaecrista duckeana* (P.Bezerra & Afr.Fern.) H.S.Irwin & Barneby	shrub	P	■	■		■									
Fabaceae, Caesalpinioidae	*Chamaecrista pilosa* var *luxurians* (Benth.) H.S. Irwin & Barneby	herb	P	■												
Fabaceae, Caesalpinioidae	*Chamaecrista supplex* (Mart. ex Benth.) Britton & Rose ex Britton & Killip	herb	P	■												
Fabaceae, Caesalpinioidae	*Libdibia ferrea* (Mart. ex Tul.) L.P.Queiroz	tree	P/N	■	■										■	
Fabaceae, Caesalpinioidae	*Poincianella bracteosa* (Tul.) L.P.Queiroz	tree	P/N	■	■					■	■					
Fabaceae, Caesalpinioidae	*Senna macranthera* (DC. ex Collad.) Irwin & Barneby	tree	P	■	■		■	■								
Fabaceae, Caesalpinioidae	*Senna obtusifolia* (L.) H.S. Irwin & Barneby	shrub	P	■										■	■	
Fabaceae, Caesalpinioidae	*Senna occidentalis* (L.) Link	shrub	P							■						
Fabaceae, Caesalpinioidae	*Senna uniflora* (Mill.) H. S. Irwin & Barneby	shrub	P	■										■	■	
Fabaceae, Mimosoidae	*Anadenanthera colubrina* (Vell.) Brenam	tree	P/N							■			■			
Fabaceae, Mimosoidae	*Mimosa arenosa* (Willd.) Poir.	tree	P/N	■	■	■	■						■	■	■	■
Fabaceae, Mimosoidae	*Mimosa caesalpiniifolia* Benth	arboreal	P/N	■	■								■	■	■	■

				Months												
				2011								2012				
Family	**Species**	**Stratum**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**
Fabaceae, Mimosoidae	*Mimosa modesta* Mart.	herb	*n.o.*/P	■												
Fabaceae, Mimosoidae	*Mimosa quadrivalvis* L.	herb	*n.o.*/P	■												
Fabaceae, Mimosoidae	*Mimosa tenuiflora* (Willd.) Poir.	tree	P/N	■		■	■									■
Fabaceae, Mimosoidae	*Pityrocarpa moniliformis* (Benth.) Luckow & R.W.Jobson	tree	P/N											■		
Fabaceae, Mimosoidae	*Senegalia polyphylla* (DC.) Britton & Rose	tree	P/N											■	■	
Fabaceae, Papilionoidae	*Canavalia brasiliensis* Mart. ex Benth.	liana	N	■	■	■		■								
Fabaceae, Papilionoidae	*Centrosema* sp.	liana	N	■	■	■	■							■		
Fabaceae, Papilionoidae	*Macroptilium* sp.	liana	N			■	■									
Lamiaceae	*Marsypianthes chamaedrys* (Vahl) Kuntze	herb	N	■												
Malpighiaceae	*Heteropterys* sp.	liana	P						■	■						
Malvaceae	*Herissantia tiubae* (K. Schum.) Brizicky	shrub	P/N	■	■	■	■						■	■		
Malvaceae	*Pavonia cancellata* (L) Cav.	herb	P/N	■												
Malvaceae	*Sida ciliares* L.	herb	P/N	■			■							■		
Malvaceae	*Sida cordifolia* L.	shrub	P/N	■										■	■	■
Malvaceae	*Triumphetta rhomboidea* Jacq.	shrub	P/N	■												
Malvaceae	*Waltheria americana* L.	shrub	P/N							■				■	■	■
Malvaceae	*Waltheria rotundifolia* Schrank	shrub	P/N				■	■								
Nictaginaceae	*Guapira tomentosa* (Casar) Lundell.	tree	N							■						
Nyctaginaceae	*Boerhavia diffusa* L.	herb	P/N	■										■		
Onagraceae	*Ludwigia* sp.	herb	P			■										
Oxalideae	*Oxalis divaricata* Mart. ex Zucc.	herb	P/N										■			
Plantaginaceae	*Scoparia dulce* L.	herb	P/N											■		
Rhamnaceae	*Ziziphus joazeiro* Mart.	tree	P/N								■					
Rubiaceae	*Borreria scabiosoides* Cham. & Schltdl.	herb	P/N	■												
Rubiaceae	*Borreria verticillata* (L.) G.Mey.	herb	P/N	■	■	■	■	■								

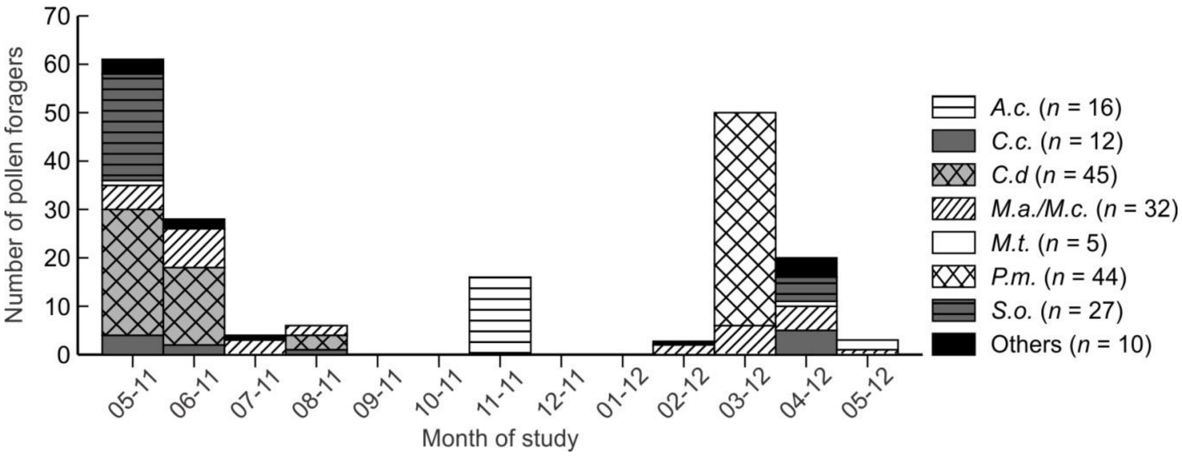
				Months												
				2011								**2012**				
Family	**Species**	**Stratum**		**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**1**	**2**	**3**	**4**	**5**
Rubiaceae	*Diodella teres* (Walter) Small	herb	P/N	■												
Rubiaceae	*Richardia grandiflora* (Cham. & Schlecht.) Steudel	herb	P/N	■	■	■	■	■								
Sapindaceae	*Cardiospermum corindum* L.	liana	P/N	■												
Solanaceae	*Solanum* sp.	shrub	P	■												
Turneraceae	*Turnera subulata* SM	herb	P/N	■	■	■	■	■						■	■	
Turneraceae	*Piriqueta* sp.	herb	P/N	■												
Indeterminate	Indeterminate 3	herb	*n.o.*		■											
Indeterminate	Indeterminate 4	herb	*n.o.*										■			
Indeterminate	Indeterminate 5	herb	*n.o.*											■		

4.2. Main pollen types identified

Main pollen types identified in the pollen samples of *Melipona subnitida* foragers. (**a**) *Mimosa arenosa*. (**b**) *Mimosa caesalpiniifolia*. (**c**) *Mimosa tenuiflora*. (**d**) *Anadenanthera colubrina*. (**e**) *Pityrocarpa moniliformis*. (**f**) *Chamaecrista calycioides*. (**g**) *Chamaecrista duckeana*. (**h**) *Turnera subulata*. (**i**) *Senna uniflora*. (**j**) *Senna trachypus*. (**k**) *Senna obtusifolia*. (**l**) *Desmantus* (type). Bar indicated 10µm. Photos C.M.S. Given that flowering of the tree species *Mimosa arenosa* and *M. caesalpiniifolia* occurred simultaneously and due to the high similarity between the pollen types (compare **a** and **b**), it was impossible to separate these two species in the samples. We, therefore, referred to these pollen samples as *M. arenosa/M. caesalpiniifolia*.



4.3. Variation of pollen types collected by *Melipona subnitida*



Total number of foragers of the four studied colonies returning to the nest with the following pollen types (different shading of bars). *A.c.*, *Anadenanthera colubrina*; *C.c.*, *Chamaecrista calycioides*; *C.d.*, *Chamaecrista duckeana*; *M.a.*/*M.c.*, *Mimosa arenosa/ M. caesalpiniifolia*; *M.t.*, *Mimosa tenuiflora*; *P.m.*, *Pityrocarpa moniliformis*; *S.o.*, *Senna obtusifolia*; Others: *Desmantus* (type), n = 3; *Eucalyptus* sp., n = 1; *Psidium guajava*, n = 1; *Senna* sp., n = 2; *Senna trapchpus*, n = 1; *Senna uniflora*, n = 1; *Turnera subulata*, n = 1. Different shadings on white background indicate mass-flowering plants; different shadings on grey background indicate poricidal flowers.

4.4. Mass-flowering trees visited

Plant species visited by *Melipona subnitida* in the Brazilian tropical dry forest. (**a**) *Mimosa tenuiflora*. (**b**) *Anadenanthera colubrina.* (**c**) *Mimosa arenosa.* (**d**) Comparison of pollen collected from mass flowering tree species and pollen collected from other plants species, mostly shrubs, and herb species. Photos: M.H.