# The impact of nest tube dimensions on reproduction parameters in a cavity nesting solitary bee, *Osmia bicornis* (Hymenoptera: Megachilidae)

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**Supplement:** Weather conditions, calculation and statistical analysis of the covariate "normalized season date", impact of tube length on progeny sex ratio, analysis of brood cell length and volume, and impact of tube inner diameter on parasitization of brood cells

#### Characterization of the weather conditions

The cleaned cocoons were exposed to outdoor conditions without a previous incubation on 7. April 2010, 15. April 2011, and 5. April 2012.

Table S1: Weather conditions during the *O. bicornis* nesting seasons of the study years. Temperature and humidity were averaged for daylight hours (800 h to 2100 h) only.

Year	Nesting Period* (Duration [d])	Mean Daylight Temp. [°C]	Mean Daylight Rel. Humidity [%]	Mean Daily Activity Hours	
2010	25 Apr – 04 Jun (41)	$14.2 \pm 3.7$	76.9 ± 17.3	$2.43 \pm 2.89$	
2011	21 Apr – 16 Jun (26)	$17.2 \pm 3.6$	$52.8 \pm 12.6$	$5.10 \pm 3.22$	
2012	19 Mai – 10 Jul (53)	$19.7\pm3.6$	$68.5\pm14.0$	$3.67\pm3.34$	

\* given as date of first female observed nesting and date of last nest sealed.

#### Calculation and statistics of the covariate 'normalized season date'

The normalized season date was used to take mother bees' age related shifts of reproduction parameters into statistical account. The mean construction date of the nest was chosen as parameter describing the age of the female. As only the date of the completion of the nest plug was directly accessible, the occupation of the tube for nesting was estimated on the basis of the number of provisioned cells and the activity hours of the previous days. The time bees need to provision a cell depends on the weather conditions that trigger bee flight activity. Based on personal unpublished data on temperature-dependent bee activity (light barrier method, monitoring of 3 – 298 nesting bees simultaneously, 4282 data points from 5 years) and of individually observed nesting bees (996 nests of 3 years) the mean temperature-hours to produce one brood cell in dependence of female's age was calculated. This function was used to estimate the unknown start date for each nest using the climate curves of the respective year. The mean of start and closure date of a given nest was normalized to a total nesting period of 50 days to make the different years statistically comparable.

**Table S2:** Results of the GLM univariate ANOVA for the covariate 'normalized season date' describing the impact of female bees' age on various reproduction parameters. To analyse the direction of the effect of females' age, partial correlations were calculated.

Depending Variable	GLM univariate ANOVA			Partial correlation*		
Depending variable	df	F	р	df	R	р
Brood cell number	1, 658	64.595	< 0.001	653	-0.410	< 0.001
Progeny sex ratio <sup>1</sup>	1, 658	4.687	0.031	653	-0.237	< 0.001
Progeny body mass	1, 4608	49.151	< 0.001	4602	-0.212	< 0.001
Usage of tube length	1, 651	31.961	< 0.001	646	-0.420	< 0.001
Room efficiency	1, 4575	24.439	< 0.001	4570	-0.053	< 0.001
Partition mass <sup>2</sup>	1, 1125	9.343	0.002	1120	-0.023	0.450
Parasitism rate	1, 658	39.579	0.001	653	0.238	< 0.001
Praeimaginal mortality	1, 658	7.314	0.007	653	0.224	< 0.001

\* Control variables: 'diameter', 'length', and 'year'

<sup>1</sup> shifted toward sons in nests constructed by aged females

<sup>2</sup> Maximum in 3th decade (univ. ANOVA:  $F_{4, 1125} = 22.51, p < 0.001$ ).

#### **Impact of Tube Length on Progeny Sex Ratio**

Progeny sex ratio did not depend on nest tube length in general ( $F_{4,658} = 3.20$ , p = 0.074, see results). However, an analysis of the separate years revealed that sex ratio increased with length in each individual year (Fig. S1; 2010:  $F_{2,212} = 5.84$ , p < 0.001; 2011:  $F_{2,244} = 5.40$ , p < 0.001; 2012:  $F_{2,202} = 3.07$ , p = 0.018). The general trend of increasing sex ratios with lengthened tubes was masked by an outlier found at 190 mm nests and at 130 mm nests in 2010 and 2011, respectively.



**Figure S1:** Estimated means of progeny sex ratio per nest depending on nest tube length for individual study years (GLM univariate ANOVA, tube dimensions as fix factors, normalized season day as cofactor). The solid line refers to the analysis of the whole data set with 'year' included as a random factor. Whiskers indicate standard errors.

#### **Brood Cell Length and Volume**

Females partially compensated the increase in space due to thicker tube inner diameters by a reduction of the brood cell length (Fig. S2;  $F_{4,5362} = 34.75$ , p < 0.001). Nevertheless, the cell volume increased substantially (Fig. S3;  $F_{4,5362} = 88.66$ , p < 0.001). Remarkably, also the tube length had an impact on brood cell length ( $F_{4,5362} = 12.21$ , p = 0.001) and volume ( $F_{4,5362} = 17.95$ , p < 0.001). The length of brood cells ( $F_{1,5362} = 4.09$ , p = 0.160) and the brood cell volume ( $F_{1,5362} = 3.63$ , p = 0.178) were independent of the size-dimorphism-adjusted sex.



**Figure S2**: *Osmia bicornis* brood cell length for sons (open symbols, solid lines) and daughters (grey symbols, dashed lines) depending on tube inner diameter (circles, bottom axis, sum of all length classes) and tube length (squares, top axis, sum of all diameter classes). Symbols refer to mean  $\pm$  sd.



**Figure S3**: *Osmia bicornis* brood cell volume for sons (open symbols, solid lines) and daughters (grey symbols, dashed lines) depending on tube inner diameter (circles, bottom axis, sum of all length classes) and tube length (squares, top axis, sum of all diameter classes). Symbols refer to mean  $\pm$  sd.

### **Impact of the Inner Tube Diameter on the Parasitization of Brood Cells**

Nests of *O. bicornis* were attacked by two different types of parasites: species that infest cells under provisioning (open-cell parasites like *Chaetodactylus osmiae*, *Cacoxenus indagator*, or *Anthrax anthrax*) and species that parasitize the nest after completion of the nest (e.g. *Monodontomerus obscurus*). The success of both types of parasites may depend directly or indirectly on the nest tube diameter. Direct effects may arise from the distance between tube wall and cocoon or the stability of partitions, while indirect effects result from larger provisions stored in tubes of wider inner diameter. To control for indirect effects of diameter-depending provision masses, the weighted mean of the

cocoon masses of the nest was used as an additional covariate in the statistical analysis of species-specific parasitism rates by GLM univariate ANOVA.

The parasitism rate increased with inner tube diameter ( $F_{4, 658} = 4.28$ , p = 0.030) while indirect effects from larger provisions were not present in general ( $F_{1, 658} = 1.87$ , p = 0.172). The cardinal parasites *C. indagator*, *Ch. osmiae*, and *M. obscurus* infested sufficient cells to allow a detailed analysis of this effect. Only the parasitism by the drosophilid fly *C. indagator* depended on the tube diameter while the infestation rates of the mite *Ch. osmiae* and the chalcidoid wasp *M. obscurus* did not (Table S3). Due to the experimental design, the bees were released without parasites. The mites were picked up from contaminated flowers (Chmielewski 1993) and therefore the risk of an infestation increased with the provision mass stored. Corresponding, only parasitism of the mite depended on the provision mass (Table S3). Females of the drosohilid fly, however, enter open brood cells under provisioning to lay eggs (Coutin and de Chenon 1983). The chance to escape returning bees when caught parasitizing a brood cell might be higher in wider tubes than in narrow ones. This could result in a preference of the parasite for nests in large diameter tubes.

**Table S3:** Results of the GLM univariate ANOVA for the impact of the fixed factor 'tube inner diameter' and the covariate 'mean of cocoon masses' on the parasitism rate by various parasite species. The direction of an effect was analysed by partial correlations between 'diameter' and 'parasitism rate'.

	ANOVA				Doutial completion*			
Parasite Species		'diameter'		'cocoon mass'		Parual correlation*		
	df	F	р	F	р	df	R	р
Chaetodactylus osmiae	1, 658	1.638	0.246	4.647	0.032	653	0.127	0.001
Cacoxenus indagator	1, 456	4.356	0.042	0.055	0.815	451	0.152	0.001
Monodonto- merus obscurus	1, 202	0.380	0.823	0.351	0.554	198	0.073	0.306

\* Control variables: 'length', 'normalized season date', 'mean cocoon mass' and 'year'

## References

- Chmielewski, W. (1993) Biology of *Chaetodactylus osmiae* (Duf. 1866) (Acarida,
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