INVERTEBRATE COLONIZATION AND DEPOSITION RATES OF GUANO IN A MAN-MADE BAT CAVE, THE CHIROPTORIUM, TEXAS USA

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A man-made bat cave, the Chiroptorium, was built on *Selah*: the Bamberger Ranch Preserve near Johnson City, Texas, USA, by Margaret and J. David Bamberger. The Chiroptorium was built in 1998 on the principle of "if we build it, they will come". It took a few years, but the Chiroptorium was colonized by *Tadarida brasiliensis* bats in summer 2003. We began monitoring the bat guano in the winter of 2004-2005 to see when the full community of guano invertebrates (excluding mites) would develop in what amounts to a primary succession in a virgin environment. In Year 1, average guano depths in both domes was about 5.5 cm, and the invertebrates very limited in number and diversity, with none of the characteristic beetles. A pseudoscorpion, probably *Hesperochernes mirabilis*, was common in the guano and on walls from the beginning of our study. Spiders *Spermophora senoculata* Duges and *Tidarren sisyphoides* Walckenaer, some with egg cases, were found on the walls. By the winter of 2005–06, guano depth had roughly doubled to about 10.5 cm, and a diverse community of invertebrates was described, including the dermestid beetles *Metoponium* sp. and *Dermestes* sp. In the third winter (2006–07), all structure of the guano deposits had been reduced to dust, probably by the action of a large population of beetles and aided by cattle. Several spiders, *Oecobius annulipes* Lucas, were found on the walls. The Bambergers built it, and the bats and the invertebrates did come, and very quickly.

1. Introduction

The Bambergers, J. David and Margaret, own *Selah* the Bamberger Ranch Preserve, in Johnson City, Texas, USA. Avid naturalists and conservationists, they wanted to add a bat colony to the ranch property and began planning in consultation with Bat Conservation International to build a man-made bat roost they described as the Chiroptorium: Chiropt- for bats and -torium from auditorium, a place to gather. The Chiorptorium was designed to house one million bats in two domes, a large outer dome 12.2 m D x 6.1 m H (40 ft D x 20 ft H) and a second, smaller inner dome that is 6.1 m D x 6 m H (20 ft D x 18 ft H), with a connecting tunnel, an entrance tunnel, and an observation area behind glass. Figure 1 shows the rebar stage of construction of the Chiroptorium with the entrance tunnel to the right and the small tunnel leading to the observation level at the top of the picture. The rebar was then sprayed with gunite, a liquid concrete. To maintain more bat-friendly temperatures, the structure was covered with tar and earth (BATS, 1997). The structure was completed in 1998, but did not attract significant numbers of bats until the summer of 2003. Gary McCracken confirmed a maternity colony of Mexican free-tailed bats (*Tadarida brasiliensis*) in 2003. Bat biologist Thomas Kunz estimated a population of 27,000 free-tailed

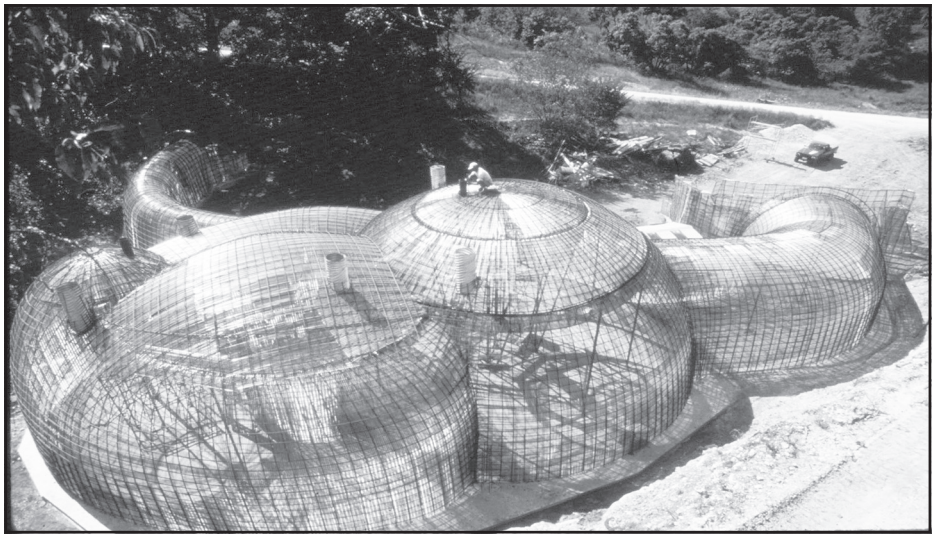

Figure 1: The Chiroptorium under construction. Note person on top of the large outer dome for scale. Photo by James Smith and Brian Keeley.

bats in summer 2006 (T. Kunz, personal communication). The plan is to ultimately use the guano as fertilizer at the Ranch. (For a panorama of the large outer dome, go to http://chriswjohnson.blogspot.com/2007/12/bamberger-ranch-chiroptorium-main.html)

Our interest in the Chiroptorium is that is a virgin environment, never before colonized by animals and invertebrates. We wanted to follow the rate of guano deposition and the process of primary succession to determine how long it would take for a full guano invertebrate community to develop, especially Dermestid beetles, and to inventory the invertebrates. We did not include mites in our study, which can represent 95% of the invertebrates in guano. Thomas Kunz from Boston University and his students are following the changes in bat populations over the same period of time.

2. Material and Methods

We began our study in January of 2005, well after the majority of bats had left for the winter. We set up an octagonal grid in the large dome, and a hexagonal grid in the second smaller dome. Both domes had wall to wall transects crossing through one central point and an inner and an outer ring 1/3 and 2/3 of the way from the center point to the walls. The points where the rings intersected the transects resulted in n=8 and n=6 sampling sites for the rings in the large and small domes, respectively. At each sampling site we measured guano depth using a meter stick. We did a visual census of invertebrates at each point for 10 cm around the intersection and below the surface. Each dome also contained a large bat box that was particularly heavily colonized by the bats. We measured the piles and made observations of invertebrates. We did a visual survey of the walls of the domes and tunnels, sampling as we encountered something of interest. Year 1 was in January of 2005, Year 2 in November 2005, and Year 3 in January of 2007.

Samples of bat guano were taken for a molecular, culture-independent analysis to detect changes in the guano from year to year (January 2005: CH050106-1 (Dome 2, big pile), 2 (Dome 1, big pile) and 3 (Dome 1, central room); November 2005: CH051112-1 (Dome 2, fresh guano), 2 (Dome 2, older guano), 3 (Dome 1, bat ring), 4 (Dome 1, big pile), 5 (Dome 1, outer ring); CH070115-3 (fresh guano), 4 (fresh guano).

DNA was extracted and purified using the MoBio PowerSoil™ DNA Isolation Kit using the manufacturer's protocol (MoBio, Carlsbad, CA). Extracted DNA was amplified with universal bacterial primers 46 forward (5'-GCYTAAYACATGCAAGTCG-3') and 1409 reverse (5'-GTGACGGGCRGTGTGTRCAA-3')(Vesbach, personal communication). Amplicons were cleaned and purified using the Qiagen PCR cleanup kit (Qiagen, Germantown, Maryland) and were cloned using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) and sent to Washington University Genome Sequencing Facility for sequencing with primers M13F and M13R. Once received, sequences were edited and contiged with Sequencher 4.8. (Gene Codes, Ann Arbor, Michigan). To check the orientation of our sequences and to convert from antisense to sense, OrientationChecker (www.cardiff.ac.uk/biosi/research/biosoft/) was used. Sequences were classified using the Ribosomal Database Program Classifier software (http://rdp.cme.msu.edu/). Denaturing Gradient Gel Electrophoresis (DGGE) was carried out on guano samples. The 16S rRNA gene was amplified with universal primers 338 forward with GC clamp(5'CGC_3 $GCCGCGC_4$ $GCGC_3GTC_3GCCGC_5GC_3TCCTACG_3$ AGGCAGCAG-3'; and 907 reverse (5'-CCGTCAATTC CT_3 RAGT3-3'). DGGE was conducted using a DGGE-2001 System (C.B.S.*Scientific Company, Inc.). PCR products were run on 6% (w/v) acrylamide gels with a denaturing gradient of 30–60%.

3. Results

Figure 2 shows the changes in guano depths from Year 1 to Year 2 at the sampling sites in each ring. Figure 2A is from

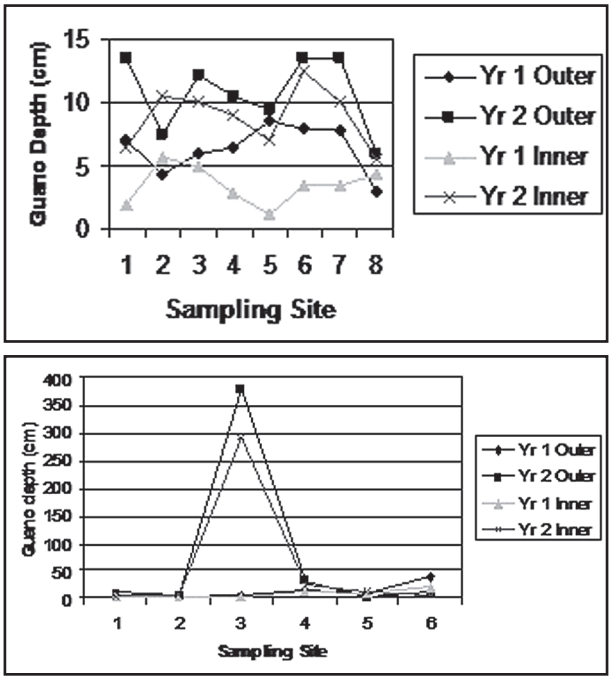

Figure 2: Changes in guano depth from Year 1 to Year 2 by sampling ring in A, the large dome, and B, the small dome.

the large dome, and Figure 2B is from the small dome. Note the difference in scale. The outer ring in the small dome included the large guano pile under the bat box at site 3.

The large guano pile under the bat boxes increased in extent from Year 1 to Year 2 by:

Large Dome. Year 1: 31 cm H x 160 cm L x 101.6 cm W to Year 2: 435 cm H x 167.6 L x 127 cm W

Small Dome. Year 1: 64 cm H x 182.9 cm L x 152.4 cm W to Year 2: 530 cm D x 323 cm L x 198.1 cm W.

Invertebrate numbers from a representative transect from wall to wall in the Large Dome are shown in Table 1 for Year 1 and Year 2.

Sample location	Year 1	Year 2
Outer 0-1	(dead lacewing)	4 dermestids
Inner 0-2	Nothing	1 flea, 1 dermestid
Center	1 ant	Nothing
Inner 5-2	Nothing	2 dermestids
Outer 5-1	Nothing	1 ant, 1 pseudoscorpion
Total	**1**	**9**

Table 1: Invertebrate numbers from a representative transect from wall to wall in the Large Dome.

A DGGE analysis of all nine guano samples (Fig. 3) reveals much similarity among samples within a given sampling time, but major differences between sampling times. Samples from January 2005 and November 2005 showed many bands, indicating the presence of many species, without dominance by any one band/species. The samples from November 2005 appear to be more diverse than those from January 2005, possibly due to sampling earlier in the winter. The samples from January 2007 are dominated by one major band/species and lack diversity.

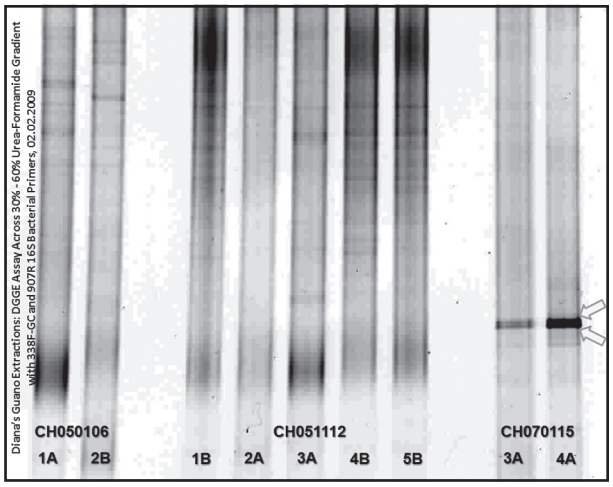

Figure 3: DGGE analysis of all nine guano samples: January 2005, November 2005 and January 2007. Each band represents a separate species.

The samples from January 2007 are dominated by one major band/species and did not have as much diversity as formerly seen. The one dominant sequence is *Lactobacillus lactis*, in the Order *Lactobacillales*.

Two samples form the November 2005 collection from Dome 1 were sequenced and closest relatives were identified. The two samples showed overlap within the *Firmicutes* phylum, in particular within the *Staphylococcus* and *Sporosarcina* genera. The bat ring in Dome 1 contained *Alphaproteobacteria* and *Gammaproteobacteria*, which were not found in the big pile sample from Dome 1.

4. Discussion

In our Year 1, the winter of 2004–05, average guano depths in both domes was about 5.5 cm, and the invertebrates

Sample	**αProteo** *Bartonella*	**γProteo** *Enterobacteriaceae*	**Firmicutes** *Enterococcus* *Weissella* *Atopostipes* *Lactobacillales*	**Firmicutes** *Staphylcoccus* *Sporosarcina* *Bacillaceae 1* *Bacilliaceae 2* *Bacillales*	**Firmicutes** Unclass Bacilli
Dome 1 Bat Ring 051112-3	1	2	3 + 1 + 0 + 1	4 + 4 + 1 + 15 + 5	1
Dome 1 Big Pile 051112-4	0	0	0 + 0 + 24 + 0	3 + 2 + 0 + 3 + 0	1

Table 2: Taxonomic groups found in clone libraries from two of the guano samples examined with DGGE. Closest relatives by genus or family are given under each phyla (in bold) and the number of clones within each of these classifications is given by sample.

very limited in number and diversity, with none of the characteristic dermestid beetles. A pseudoscorpion, probably *Hesperochernes mirabilis*, was common in the guano and on walls from the beginning of our study. Spiders *Spermophora senoculata* Duges and *Tidarren sisyphoides* Walckenaer, some with egg cases, were found on the walls. Mud dauber wasps and a phoebe nest were already well-established in the entrance tunnel by 2005.

By Year 2, guano depth had roughly doubled to about 10.5 cm, and a diverse community of invertebrates was described, including the beetles *Metoponium* sp. and *Dermestes* sp. (Fig. 4).

Figure 4: Dermestid beetle on the wall of the Chiroptorium (Photo by Kenneth Ingham).

In Year 3 (2006–07), we were surprised to find all structure of the guano deposits and of individual droppings had been reduced to dust, probably by the action of a large population of beetles (Kunz, personal communication) and aided by cattle. Several spiders, *Oecobius annulipes* Lucas, were found on the walls. Sampling was complicated by a major ice storm that made staying in the Hill Country unfeasible.

Major changes in the guano bacterial community composition occurred over the three sampling periods, with the last sampling period showing a change from a diverse community, with relatively even abundances, to a community dominated by one organism. The reworking of the guano by dermestid beetles and cows may have contributed to the major change in the bacterial makeup of the guano in 2007.

A possible complication of our study is the spreading of about 100 pounds of guano from Bracken Bat Cave prior to 2002 (J. Bamberger, personal communication). The guano was placed in the Chiroptorium in the hopes that the smell would attack bats to the structure. We doubt that this action had any significant effects on the succession, since any invertebrates transferred with the guano would have had to go through several lean years without inputs of guano, and that a guano community had not fully developed by the time we began our study, despite an abundance of raw material already in place. However, we are conducting a comparison of the Chirpotorium invertebrate community with Bracken Bat Cave, and we plan to investigate other nearby guano deposits.

5. Conclusions

A robust guano invertebrate community had developed within three years of reliable colonization of the Chiroptorium by bats. The Bambergers built it, and the bats and the invertebrates did come, and very quickly. The study is still on-going, and comparison of the invertebrate guano community in the Chiroptorium with other bat guano caves will provide additional information.

Acknowledgements

We thank the Bambergers for their interest in our project and all things natural, and for building the Chiorptorium. *Selah* Educational Programs Manager Colleen Gardner is our regular contact, and has been a gracious host. We thank James Smith and Brian Keeley for Chiroptorium construction pictures provided by the Bambergers. Tom Kunz gave us insights into both bat and Dermestid beetle behavior. Thanks to Jim Kennedy and BCI for providing guano from Bracken and Dr. Sandy Brantley for identification of the spiders. Jerry Lewis made a presumptive identification of the pseudoscorpion. Monica Moya and Amaka Nwagbologu were very helpful with sequence editing and Dr. Armand Dichosa and Jenny Hathaway helpfully provided the DGGE analysis of the guano samples. We thank Jim Lavoie and Kenneth Ingham for field assistance and photography. Funding was provided through Kenneth Ingham Consulting, L.L.C. and the Research Foundation of SUNY.

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