Subterranean transport and deposition of quartz by ants in sandy sites relevant to age overestimation in optical luminescence dating

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ABSTRACT

An artificial layered sandy site was created using a combination of native sand and colored sand (3 parts native uncolored quartz, 1 part colored quartz) in Apalachicola National Forest near Tallahassee, Florida. Twelve layers of sand, each 1 by 1 m in horizontal extent by 10 cm in thickness were emplaced to a depth of 2 m followed by implantation at the surface of a Florida harvester ant (*Pogonomyrmex badius*) colony (the lower two layers were 50 cm thick). The colony excavated a nest, and after 7 months, the sand layers were excavated to the base to test the hypothesis that sand grains were moved upward within the ant nest without reaching the surface. The ants penetrated 11 of the 12 colored layers reaching a depth of 130 cm. Thirty nine sticky-acetate peels of ant chamber floors were collected and colored sand grains were counted under a microscope. More than 16,000 grains were identified in layers that did not originally host them. Of these, more than 80% were unambiguously moved upward. This means that possibly as many as 54,000 upwardly mobile grains were present (ratio of 3:1 uncolored to colored). In relation to optical luminescence (OSL) dating, this means that grains that would not have been optically zeroed by transport to the surface (defined here as subterranean-transported) were present in abundance, and that if the site was ancient, there would have been found many grains that were older than the layers they presently reside in, even if only one colony of harvester ants had disturbed the layers. This is in addition to the fact that backfilling of chambers and tunnels may contribute even more significantly to the presence of a subterranean-transported component of an OSL sample. We conclude that ants can significantly affect the age distributions in sandy archaeological sites. Multiple examples of such disturbances have been documented in the literature. Most relevant to our results are recent studies of the OSL chronology of Pre-Clovis-age and Palaeoindian age archaeological sites in sandy environments in North America that may have been compromised by ant bioturbation of quartz sand grains. Here we have examined in detail the potential effects of one episode of ant nest-building on the age overestimation of affected sediments. From this we found that as few as 12 episodes of bioturbation involving backfilling of chambers in the same volume of sand could lead to the presence of 1 contaminant grain per 50 grains of sample.

1. Introduction

1.1. Nature of optical luminescence dating

Unlike radiocarbon dating of archaeological and geological sites, which has been employed since the late 1940's (Arnold and Libby, 1949), OSL dating is a relatively recent methodology (Huntley et al., 1985), which is in increasingly common use. OSL has several advantages, the most important of which is the ability to obtain dates from strata that lack associated, datable carbon. Like the development of radiocarbon dating, as OSL use becomes more widespread, the factors that can affect the association of ages and the strata of interest need to be fully explored.

Optical luminescence dating of quartz grains is based upon the response of light-exposed quartz grains to the cumulative effects of

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natural radiation on the grains during dark burial. Upon light exposure, quartz grain defects lose previously stored charge (in the form of trapped electrons at the defects) thereby zeroing the amount of luminescence that can be detected in a laboratory experiment. At the moment of burial the luminescence clock is therefore set to zero time. During burial in dark conditions, natural radioactivity in the form of alpha, beta, and gamma radiation associated with the decay of natural uranium, thorium and potassium will cause electrons to again be trapped in the defects. In the laboratory, the electrons will be untrapped, emitting ultraviolet-wavelength photons that are counted as the luminescence whose intensity is proportional to the radiation dose acquired during burial in dark conditions. The time since burial is the ratio of the total dose divided by the annual radiation dose experienced at the burial position.

The effects of bioturbation on sequences should be understood in relation to how OSL dating works. Ideally, all grains that are buried with artifacts are zeroed at the time of burial. If this occurs, the OSL age will provide the age of burial of the artifacts. This age is determined using the central age model (CAM; Galbraith et al., 1999), which is the weighted mean value determined from all of the grain ages. In some cases it is possible for grains to move downward, carrying grains that were recently buried downward into older layers, making OSL ages too young when calculated with the CAM (Forrest et al., 2003). Upward movement of grains to the surface (exhumation) is also problematic (Bateman et al., 2003) because these grains are set to zero by light, and then if mixed by downward movement into a layer with grains that have not been reset since burial, their presence can lead to age underestimation using the CAM.

The other main problem for OSL that arises with grain movement is when more deeply buried grains are moved upward into shallower levels without reaching the surface. These grains carry larger doses due to their longer period of burial. If such a layer is analyzed, one will find a distribution of doses associated with multiple burial events, because the upwardly mobile grains were not zeroed again at the time they attained their position in relation to the artifacts. Finding the grains that carry the smallest non-zero doses can provide a best estimate of the burial age, and OSL technology has some ability to do this (by using a minimum age model [MAM] (Galbraith et al., 1999) to constrain that portion of the burial doses).

Another reason that higher dosed grains in a layer can be found mixed with lower dosed grains is related to incomplete zeroing before burial. It is possible that some grains do not receive sufficient light at burial to erase the previous charge in the defects, while some do. It is not possible at this time to demonstrate whether incomplete zeroing at burial or later upward mixing of unzeroed grains can lead to the observations of age overestimation described herein. However, most demonstrated scenarios of incomplete zeroing are associated with fluvial deposition, e.g. Rodnight et al. (2005). The purpose of this article is to give actual experimental results that quantify grain movement by ants and thus to contribute to a better understanding of how these displaced grains might affect OSL ages.

1.2. Previous experimental studies on bioturbation in relation to optical luminescence dating

The most extensive studies on bioturbation effects in OSL dating have been conducted by Bateman et al. (2003, 2007a,b), Wilder et al. (2007). These studies show that mismatches in OSL age and artifact age (constrained by radiocarbon) may in large part be either due to bioturbation by burrowing creatures or pedoturbation. Bateman et al. (2003) provide a variety of model scenarios of upward and downward bioturbation with respect to their effect on OSL results. They convincingly demonstrate through two experiments that gopher activity in modern and old gopher mounds, and in infilled krotovinas, yield equivalent dose values higher than the nearby undisturbed sediments. In their models, both upward and downward movement of grains have detrimental effects on OSL age estimation and lead to age estimate errors that depend upon the rate and depth of bioturbation (pedoturbation), and the geological sedimentation rate (addition of grains at the surface due to non-biological processes). By comparing single aliquot measurements to single grain measurements they showed that even larger departures from the true dose were found in the single grain measurements. Additionally, some grains carried zero doses as a result of zeroing at the surface during bioturbation. In our own study here, we are able to provide data on upward and downward grain movement that support their findings, however our data are for ants, while theirs are for gophers and creatures that produce large krotovinas.

Studies on OSL dating of archaeological sites in Florida led by Bateman also show discordance between radiocarbon ages and OSL ages attributable to bioturbation. Wilder et al. (2007) show overestimates of OSL ages in upper layers of the sites at Avon Park, Florida in relation to radiocarbon age estimates for the same layers. Though they do not identify ants as the bioturbators, they clearly show that the upper layers were contaminated by older grains that apparently were moved upward by biological processes without being zeroed (that is they were moved upward without reaching the surface).

Bateman et al. (2007a,b) showed that bioturbation in sites in Texas and Florida had the ability to rejuvenate ancient weathered sand bedrock and/or to alter depositional stratigraphies through exhumation and sub-surface mixing of sediment. In both the Texas and Florida sites radiocarbon dates are consistently older than OSL dates. In addition to the discovery that fully zeroed grains were found within the bioturbated layers, there were clear signatures of upward mixing of older grains into the sediment column, specifically ones that did not reach the surface during bioturbation.

1.3. Study location and behavior of harvester ants

Our research was conducted in the Apalachicola National Forest at a study area located 15 km southwest of Tallahassee, Florida. The study area is fairly open with excessively drained deep sands supporting a scattering of turkey oaks and a thin groundcover of grasses and herbs, along with longleaf pines planted in the mid-1970s. The study area harbors a dense population of the Florida harvester ant, *Pogonomyrmex badius* on which one of us (WRT) has carried out behavior and natural history studies for 30 years. These studies have resulted in a detailed understanding of the development of colonies from founding to maturity, the distribution of workers within the nest in relation to their work-duties, the seasonal cycle and its relation to colony reproduction and other aspects of the colony life cycle (Tschinkel, 1998, 1999; Smith and Tschinkel, 2005). Most relevant here is a detailed description of the geometry and size of subterranean nest architecture, and how the ants and seeds are distributed within it (Tschinkel, 2004). Harvester ant colonies move about once a year, excavating up to 4–6 l of sand in 4–7 days to create nests up to 2.5 m in depth (Tschinkel, unpublished; Carlson and Gentry, 1973). Altogether, this detailed knowledge of the biology and nest architecture of the Florida harvester ant made this site ideal for the experiment we report here.

The Florida harvester ant, *P. badius*, is a large ant of the longleaf pine forests of Georgia, South Carolina and Florida (Harrison and Gentry, 1981; Gentry and Stiritz, 1972; Nickle and Neal, 1972). Colonies consist of up to 10,000 workers that excavate deep nests in

the sandy soils they prefer. The architecture of these subterranean nests is known in detail through making plaster or aluminum casts of them (Tschinkel, 2004, 2011a,b). Fig. 1 shows some typical harvester ant nests of a range of sizes. Nests are composed of one to four vertical helical shafts connecting horizontal chambers that at maturity are between 2 and 3 m deep (Tschinkel, 2004).

Colonies are founded by newly mated queens, that dig the initial nest and rear the first brood of workers from reserves stored in their bodies. As the colony grows, workers enlarge the nest by deepening it, adding chambers and enlarging chambers. Moreover, colonies move about once a year, constructing an entirely new nest nearby and moving into it (unpublished data, WRT). During excavation, workers form the damp sand into pellets which they hold between their mandibles during transport. Dry sand is often moved by propelling it backwards between the legs, like a dog, or it may be packed into the psammophore on the ventral side of the head, but dry sand is found only within the top few cm of soil, assuring that almost all excavated sand is moved in the form of pellets.

The requirement that ants transport significant volumes of sand upward to create their nests, combined with the series–parallel nature of ant work (Oster and Wilson, 1978), led to our idea that this process may not be 100% efficient, and that some grains might only be transported upward within the nest and not reach the surface (defined here as subterranean transport).

2. Materials and methods

2.1. Study site

A pit measuring 1 × 2 m and 2 m deep was dug and a plywood box measuring 1 × 1 × 2 m was constructed in one side of the pit (Fig. 2). Beginning at 2 m depth, layers of 12 different colors of sand were deposited, flattened and tamped. The bottom two layers were each 50 cm thick, but all layers shallower than 100 cm were 10 cm thick. As each layer was installed, the open side of the plywood box was fitted with a plank to contain the sand. The final disposition of the colored sand layers with depth is shown in Table 1.

The sand colors were purchased from Sandblast Entertainment in Pensacola, Florida. Using a gasoline-powered, portable cement mixer, each color was mixed in a ratio of 25% colored sand, 75% native sand. The native sand was excavated from the pit. This mixture was layered in the column within the plywood box in the pit.

2.2. Planting the ant colony

In November 2010, a queenright colony of about 9000 *P. badius* workers was excavated nearby and released on the surface of the layered sand column. A few starter holes encouraged the ants to dig their new nest in the layered column. Small groups initiating nests elsewhere were dug up and reunited with the main nest. Once established in the layered column, the colony remained there until it was excavated in May 2011.

2.3. Excavation and mapping

In May 2011, the colony was excavated as described in Tschinkel (1998, 1999). A pit was dug adjacent to the colored sand column, and the wooden planks making up the side of the box were removed as needed, exposing the side of the colored sand column. Sharpened brick trowels and shovels were used to lift off the layers of sand to expose the chambers, and a portable shop vacuum was used to remove caved-in chamber roofs. In this way, clean chamber floors were exposed one by one as the dig proceeded. All chambers found were numbered and mapped on large sheets of acetate, each of which recorded the chamber positions found over specific depth intervals. Decisions to collect chambers were not based on whether we believed contamination by exogenous grains had or had not occurred. The chamber floors and shafts often contained sand that was different in color than the host sand layer. Chamber floors were captured by pressing down sticky, transparent acetate sheets and drawing the chamber outlines on the sheet. Most of the chambers in the native and chocolate layer were captured, along with all chambers below 10 cm depth. The exposed layer of sand stuck to these sheets, which were stored for later counting of the different colored grains under a microscope. One acetate is shown in Fig. 3, which shows lime green and orange grains among the purple of the host color, the former having been moved down and the latter up.

Detailed photographs of chambers and any features of interest were also made during the excavation.
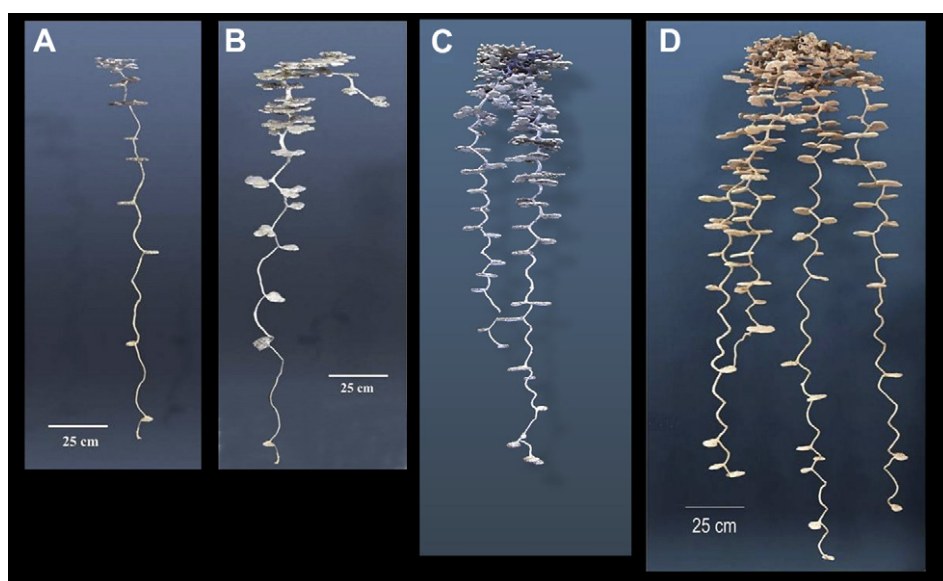

Fig. 1. Metal or plaster casts of harvester ants of increasing size, showing the basic architectural features and how they increase with nest and colony growth.

Fig. 2. Construction of the layered sand column within the plywood box. Beginning at 2 m depth, different colors of sand were layered and packed. The open side of the box was closed with boards as the level of sand rose. The yellow sand layer is shown in place here. Once complete, all sides around the box, and the work pit were refilled with sand. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.4. Assessment of grain movement and deposition

The basic data consist of the depth and outline tracings of each chamber, and the number of grains of each color that did not belong to the host color. Grains on acetate sheets were counted using variable magnification ($10\times$–$30\times$) by means of a second acetate overlay that was ruled into lanes 15 mm wide to keep track of position under the microscope. Colors of grains not belonging to the host layer color were counted sequentially and tallied.

2.5. Data analysis

Quantitative movement of grains was assessed for all layers. The raw numbers of colored grains moved upward or downward (Tables 1–3) formed the basis of subsequent calculations. Because

the chambers from which this sand was excavated varied in size, the grain counts were adjusted for chamber volume (chamber area × assumed 1 cm height). These adjusted counts were proportional to the probability of deposition in a host layer of a different color. Normalization of these adjusted counts yielded the fraction of grains of each color deposited in each layer, and summing these fractions yielded a number that was proportional to the rate of deposition of all sand colors together (shown in Fig. 4).

3. Results

Table 1 shows the disposition of the colored sand layers, the area of each chamber and details of the total numbers of upward and downward grains in each colored layer (referred to as host colors). Tables 2 and 3 show the total number of colored grains moved

Table 1
Depth distribution of colored layers and recovered grain totals.

Layer color (Host color)	Depth interval (cm below surface)	Layer color code	Total chambers sampled	Total colored upward grains	Total colored downward grains	Total upward plus downward grains	Upward fraction (%)	Downward fraction (%)	Ratio downward/upward	Total estimated upward grains (4× colored upward grains)
Native	+2 to 0	N	16	7511	Not applicable		100	0		30,044
Chocolate	0 to −10	C								
Emerald Green	−10 to −20	EG	2	3142	854	3996	78.6	21.4	0.27	12,568
Violet	−20 to −30	V	2	991	191	1182	83.8	16.2	0.19	3964
Blue	−30 to −40	B	3	898	96	994	90.3	9.7	0.11	3592
Pink	−40 to −50	PI	2	319	111	430	74.2	25.8	0.35	1360
Black	−50 to −60	BLA	4	124	229	353	35.1	64.9	1.9	412
Lime Green	−60 to −70	LG	3	36	315	351	10.2	89.8	8.8	144
Purple	−70 to −80	P	1	298	316	614	48.5	51.5	1.1	1192
Orange	−80 to −90	O	2	27	637	664	4.1	95.9	23.4	108
Yellow	−90 to −100	Y	2	116	132	248	46.8	53.2	1.1	464
Divot Green	−100 to −150	DG	3	Not applicable	206	206	0	100		
Red Clayey	−150 to −200	RC	0	Not applicable						
Totals			40	13,462	3087	16,549				53,848

Fig. 3. An example of a sticky acetate peel of the floor of a chamber (outlined in green at a depth of 79 cm. The host sand color was purple, but grains of lime green (moved downward and orange (moved upward are visible. The basic data were derived by counting the non-host colored grains in such acetate stick sheet peels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

upward and downward, respectively, for each nest chamber sample. Table 1 clearly shows that upward transport and deposition greatly exceeds downward, and that the likelihood of deposition of all colors, no matter how far transported, increased dramatically toward the surface, that is, less than about 20–30 cm below the surface.

Rather than simply plotting the number of grains moved upward and downward, Fig. 4 depicts a more meaningful analysis, which is based on the actual volume of chambers created in each host sand color. We determined chamber volume only below 35 cm depth, but this still includes seven host colors. The largest volume of sand was removed from the blue host color, and we proportionated all other colors to the same volume. Proportionating the colored grain counts by the same factors gives the counts of each color that would be present if the same volume of chambers had been dug in each host color. In other words, these numbers represent the probability of deposition independent of the volume of sand removed (Fig. 4). As such, they represent the probability of within-nest sand deposition in relation to distance transported, depth and by extension, time of transport.

Fig. 4 clearly shows that deposition is not related to the distance of transport: if it were, sand colors from the deepest layers would have been found in significant quantities at intermediate depths, but were not. Table 1 also provides an estimate of the total upward grain movement including non-colored grains. This value arises from the fact that in building the colored sand layers, the colored sand was mixed with native sand excavated at the site in a ratio of 1:3, so that upward colored sand grain movement 4× yields an estimate of the total upward grain movement. Table 1 shows that 13,462 colored grains were moved upward and 3087 colored grains downward, yielding a total of 16,549 total colored grains moved

Table 2
Upward colored grain counts.

Chamber no.	Chamber area (cm²)	Host color	Emerald green	Violet	Blue	Pink	Black	Lime green	Purple	Orange	Yellow	Divot green	Total
1	26.3	Chocolate	58	75	9	22	143	6	6	1	2	2	324
2	30.6	Chocolate	77	133	35	14	56	7	5	8	5	3	343
3	33.2	Chocolate	21	60	9	9	3	0	0	0	7	0	109
4	31.3	Chocolate	197	111	125	45	64	1	2	1	2	0	548
5	33.7	Chocolate	245	127	22	11	49	2	5	4	1	1	667
6	33.9	Chocolate	44	6	39	12	17	0	0	3	5	0	126
7	26.3	Chocolate	117	96	279	143	64	17	6	9	11	0	742
8	18.8	Chocolate	13	1	27	4	9	1	1	1	0	1	58
9	26.9	Chocolate	19	21	47	16	13	5	1	27	11	36	196
11	49.1	Chocolate	452	255	152	66	107	98	78	294	238	96	1836
12	24.3	Chocolate	209	41	28	18	32	3	2	1	4	1	339
13	71.9	Chocolate	172	132	278	134	85	65	53	86	34	3	1042
14	25.9	Chocolate	77	60	44	12	44	5	0	4	2	0	248
15	33.8	Chocolate	109	92	49	13	19	5	52	11	6	0	306
16	27.3	Chocolate	253	69	62	83	66	16	5	20	33	20	627
17	123.0	Em. Grn.		282	225	92	124	181	386	362	604	0	2256
18	27.0	Em. Grn.		433	81	34	105	51	59	58	61	4	886
19	34.1	Violet			156	12	39	4	5	5	5	0	226
21	67.6	Violet			279	60	143	44	39	61	86	53	765
24	11.8	Blue				202	274	94	1	2	2	0	575
25	50.7	Blue				12	61	4	1	3	0	0	81
27	26.2	Blue					172	58	5	7	0	0	242
28	32.0	Pink					120	46	4	0	2	0	172
29	25.4	Pink					107	12	7	3	2	16	147
30	19.3	Black						13	0	16	2	0	21
31	17.7	Black						7	13	12	4	0	36
32	24.7	Black						32	0	0	1	0	33
33	25.7	Black						20	3	5	4	2	34
34A	11.9	L. Green							3	2	2	0	9
34B	13.3	L. Green							1	3	1	0	5
35	35.4	L. Green						15	7	0	0	22
37	15.9	Orange								215	6	77	298
39	46.8	Orange									5	8	13
40	5.8	Orange									6	8	14
41	23.0	Yellow										16	16
42	22.0	Yellow										100	100

Table 3
Downward colored grain counts.

Chamber no.	Host color	Chocolate	Emerald green	Violet	Blue	Pink	Black	Lime green	Purple	Orange	Yellow	Total
17	Em. Grn.	162										162
18	Em. Grn.	692										692
19	Violet	3	84									87
21	Violet	34	70									104
24	Blue	2	1	35								38
25	Blue	14	10	25								49
27	Blue	1	7	1								9
28	Pink	3	3	0	27							31
29	Pink	4	22	4	48							78
30	Black	2	1	1	5							9
31	Black	2	1	0	0	6						9
32	Black	3	1	0	5	160						169
33	Black	4	14	5	13	6						42
34A	L. Green	0	12	4	8	8	49					81
34B	L. Green	2	3	2	7	1	32					47
35	L. Green	13	40	8	33	8	85					189
37	Purple	1	4	1	3	1	37	269				316
39	Orange	6	6	0	2	0	18	3	582			617
40	Orange	2	2	0	1	0	4	0	11			20
41	Yellow	9	7	1	1	1	22	0	7	51		99
42	Yellow	2	1	1	2	0	8	1	8	10		33
43	Divot Grn.	3	0	0	0	1	11	0	1	3	134	153
44	Divot Grn.	5	1	4	0	0	21	0	2	2	1	36
45	Divot Grn.	2	0	1	0	0	14	0	0	0	0	17
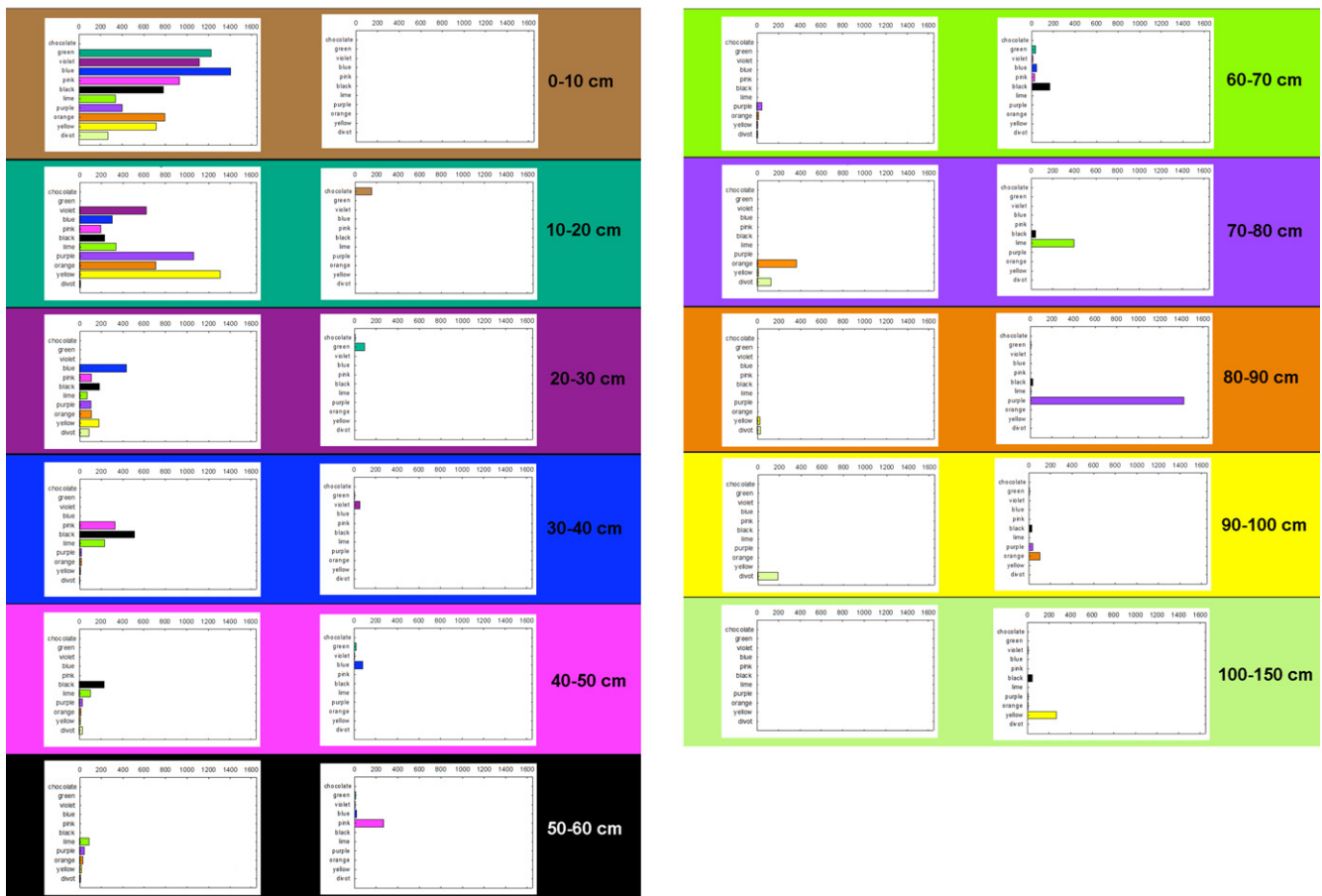
Fig. 4. Chamber volume normalized plots of upward and downward grain movement.

through subterranean transport. Thus the upward colored grain movement is 81.3% of total colored grains moved, whereas only 18.7% were moved downward. An important point here is that the use of sticky sheets to sample chamber floors did not yield the true total of all colored grains deposited on each floor because only the surface layer of sand grains was captured, so the total grain counts (both upward and downward) are minimum values. The total grains counts likely are several times this conservative estimate.

Actual grain counts in Table 1 show there is a strong change in the percentage of upward colored vs. colored downward moved grains at about 50 cm depth. For the interval 10–50 cm, a total of 6602 colored grains were moved, with 5350 upward and 1252 downward, yielding percentages of 81% upward and 19% downward, which are very similar to figures for the total site. However for the interval of 50–100 cm depth, the trend is reversed: a total of 1629 colored grains moved, 601 upward and 1629 downward, yielding percentages of 27% upward and 73% downward. Though percentages remain the same, the numbers of estimated grains (colored plus uncolored) moving were calculated by multiplying the actual numbers by 4.

Table 1 and Fig. 4 demonstrate that workers therefore appear to carry sand continuously upward even from great depth, but as they reach the shallower parts of the nest, there is an increasing probability that they will deposit their loads. The fraction of sand-transporters that travel all the way to the surface is unknown, but it seems likely that loads dropped in the upper chambers are often picked up by other workers and carried to the surface. Indeed, this function may fall primarily to the foragers that are never found deeper than 20 cm (Kwapich and Tschinkel, unpublished data).

The density of the colored sand layers averaged 1.51 g/ml. Counting the grains in weighed samples of sand revealed that each ml contained a mean of about 12,300 grains or 18,600 per g. Collecting individual sand pellets brought to the surface by worker ants showed that the average pellet contained about 160 grains. From the volume of the chambers (determined from the tracings) and the values noted above, combined with the sum of the pellets deposited in chambers or shafts rather than on the surface, it was shown that for every 100,000 sand grains excavated (colored plus uncolored), a mean of 3100 were deposited below the surface. Translated into pellets, about 19 of every 500 pellets (approximately 4%) were deposited below the surface, mostly in the upper 30 cm of the nest. This rate of subterranean deposition, although quite conspicuous during our excavation, does not seem very high. Nevertheless, this is surely an underestimate because it is based on the conservative counts from the sticky sheets and does not include sand deposited in the tunnels and chambers that were actually completely or partly backfilled with sand from other layers. Such backfilling was particularly obvious in the more superficial chambers (<10 cm depth) of the nest, and consisted of relatively large volumes of sand of diverse colors.

4. Discussion

Small but significant fractions of the sand pellets that workers transport upward during nest excavation are not carried to the surface. Most commonly, the pellets are deposited in chambers and shafts, especially in the upper 30 cm of the nest. Our work found that as many as 19 of every 500 sand pellets, about 4%, are thus deposited in chambers beneath the surface. Because the experimental nest was no longer actively growing when it was excavated by us, it seems unlikely that most of these grains were merely being cached before final transport to the surface. With respect to how this species of ants excavate their nests, this suggests that workers bringing sand pellets from deep in the nest are not significantly likely to carry this sand to the surface. Harvester ant workers are

highly stratified within the nest, with younger workers in the lower nest reaches, and foragers (the oldest workers) strictly limited to the upper 20 cm. It is thus likely that workers from deep in the nest cache the excavated sand in the top 20 cm from which it is carried to the surface by foragers. Indeed, caching and repelletizing is suggested by the presence of multiple colors of sand in many pellets (WRT, unpublished data).

With respect to using quartz sand for OSL, it is clear that the major effect observed is that ants move older sand from deeper layers into higher layers without exposing the sand to bleaching by sunlight. These older contaminant grains comprise about 80% of all contaminant grains and would yield age overestimation. The age signal of the sand layers is thus blurred, even within a single harvester ant nest. In light of the fact that the ants move about once a year, excavating a new nest of equal or larger size with each move, it is clear that even this single species of ant can have a significant impact on the utility and simplicity of using sand for archeological and geological dating, especially in deposits that exceed several millennia in age.

Our study did not quantify the amount of sand used to backfill chambers. Such backfilling was particularly common in the upper 10 cm of the nest, but can occasionally be found at almost any level of the soil column (Tschinkel, unpublished observations). Our estimates are thus probably an underestimate of the amount of sand moved from one subterranean level to another.

We also found significant numbers of grains that were moved downward, though they comprise only about 20% of all grains counted. Thus these grains would mix in lower-dosed grains with original unmoved grains and would contribute to an underestimate in OSL ages. This adds a level of complexity to using the minimum age model to identify the true burial age of a level, because these smaller dosed (i.e., younger) grains, if in sufficient quantity, could contribute to the mixed original host (unbioturbated) and upwardly mobile grains and influence the OSL results.

Questions arise regarding how important upward contamination may be in sandy sites for OSL dating. Studies of sand from the area show a bimodal grain-size distribution of 50% of grains in the 100 μm mean grain size range, plus about 50% in the 270 μm mean grain size range (Means, 2012). Though we do not know the relationship between this and the fraction used in OSL dating (normally 90–150 μm), we can make a rough calculation using this grain size range. The aim of this calculation is to better understand how many episodes of nesting might be required to enrich the sample enough to induce age overestimation in OSL ages. For this we assume that we would need the contamination level (referred to here as an enrichment factor) to be 1 upwardly mobile grain in 50 total grains. We assume our OSL tube (4.5 cm in diameter) captures one chamber containing 1900 upwardly mobile grains (similar to the 1836 grains for chamber 11 in Table 2), and they are 100% quartz grains, as are all the in-situ non-mobile grains.

Our 4.5 cm diameter tube (30 cm long) inserted horizontally in stratum of interest has a volume of 480 cm^3, which at a porosity of 40% (as determined in measurements at our site), yields a volume of 288 g/cm^3 of quartz grains. The total grain volume of upwardly mobile grains (UMG) after summing the six size classes (e.g. 90–100, 100–110, etc., each including 1900/6 = 317 grains) is 0.00182 g/cm^3, yielding a ratio of UMG volume/total grain volume of 6.316×10^{-6}. To increase this ratio to a value of 0.02 (about 1 grain in 50), would require almost 3200 episodes. This suggests that bioturbation would have to be very intense over several thousand years to obtain this level of contamination. However, we note here that our sticky acetate sampling procedure collected only a minimum number of contaminant grains present because it did not sample all grains on a chamber floor, nor any grains which were

just below the chamber floor. More importantly it is clear that backfilled chambers and backfilled tunnels may be much more important in concentrating UMG than contamination of chamber floors. Fig. 5 shows examples of these phenomena. Though we did not collect sticky acetates from these features, the following simple calculation shows how important they could be.

Here we assume that only 1.6 cm^3 of upwardly mobile grains is captured from a chamber containing 50% UGM, which is easy to visualize as possible with reference to the scales in Fig. 5. At 40% porosity this would yield 0.5 cm^3 of UGM. This yields a ratio UGM volume/total grain volume in OSL tube of 0.0017 (0.5/288). In this case only 12 episodes would be required to obtain a ratio of 0.02 (1 UGM in 50 grains).

Fig. 4, based solely on chamber floor data, suggests that any effect associated with downwardly mobile grains (DMG) would be most important below 50 cm depth. Although we acknowledge that this adds more complexity to the overall problem, further modeling and experiments are necessary to estimate the effect of the DMG. These experiments have already been conducted and are in the data analysis stage. Backfilling was more predominant in the zones above 30 cm, and it is expected that backfilling contamination will be dominated by UMG.

In OSL studies containing Palaeoindian artifactual material in sandy sediments at the Wakulla Springs Lodge Site (Florida Master

Fig. 5. Upper Panel: Backfilled ant chamber found within the 0–10 cm depth interval (chocolate layer); Lower Panel: three backfilled ant tunnels found within 20–30 cm depth interval (violet layer).

Site File # 8WA 329), near Tallahassee Florida, Rink et al. (2012a) showed that OSL age estimates of the Palaeoindian artifactual layers were well beyond the expected age of colonization of North America. Only the search for the youngest grains, and the use of the minimum age model for equivalent dose estimation in the layers yielded ages in agreement with other Palaeoindian sites in the area. Similarly Rink et al. (2012b) showed that at the Helen Blazes Site (Florida Master Site File # 8BR 27) near Melbourne, Florida, both Archaic age and deeper levels commensurate with previous Palaeoindian artifact finds showed large OSL age over-estimates without search for the youngest grains in those layers (using the minimum age model for age estimation). The youngest-grain ages yielded good agreement with expectation. In both sites, upward mixing of older grains was proposed as the explanation of the age discrepancies.

At Cactus Hill, where significant Palaeoindian artifacts are present, Feathers et al. (2006) found that mixing of sediments was an issue, and only single-grain dating procedures to identify the youngest grains provided ages in agreement with radiocarbon age estimates. Mean OSL ages without this analysis consistently yielded age overestimation in relation to radiocarbon ages in the same levels. Mixing was not suggested as the cause of this effect, but the possibility of incomplete zeroing at burial was raised.

The pattern of age overestimation (without seeking OSL ages of the youngest grains) is consistent among the sandy sites mentioned here. One possible source of this overestimation is the upward movement of older grains by ants that do not reach the surface (subterranean transported). Our experiments have demonstrated that the major subterranean transport movement is upward for this species of ant. This strengthens the case for the use of single-grain or small single aliquot, employing the minimum age model for OSL age determinations in sites frequented by ants. We believe this is the best available method to compensate for mixed age populations associated with upward bioturbation of sand grains. We acknowl-edge that more complex burial scenarios could occur, that would interfere with the MAM-derived age, such as significant downward movement, but most of the evidence so far from sandy sites is that age overestimation is the most commonly observed problem, and that is best dealt with using MAM.

It is important to consider the degree to which harvester ants disrupt areas which they occupy. The cumulative effect of harvester ant soil movement can be roughly estimated as follows (unpub-lished data, WRT). The study area is 22 ha in extent and contains 320 active harvester ant colonies. The discs of excavated soil on the surface roughly outline the column of soil underneath in which the ants dig their chambers (although the column narrows with depth). The surface discs of all 320 colonies combined cover a total of about 44 m^2. Every year, colonies move an average of 4 m and disturb a fresh 44 m^2 of soil. In 1000 years, colonies will have worked 44,000 m^2 (4.4 ha), or 20% of the study site. In 5000 years, assuming no reworking of previously occupied areas, they will have worked the entire study site.

However, harvester ants are only one species among many that occupy sandhill sites like ours, and many of these other species also excavate nests deep enough to be significant to bioturbation (Tschinkel, 2003). King (2007) collected 40 species of ants in pine upland habitats, while King and Tschinkel (2008) trapped 75 species in pine flatwoods 6–7 km southwest of our site. Of these, perhaps 20–30 species are fairly common to very common.

The following species of ants were common at our site. Among the most common was the fungus gardening ant, *Trachymyrmex septentrionalis* which excavates egg-shaped 50–100 cm^3 cham-bers for its fungus gardens. Not only can these chambers be deeper than 1 m, but the depth and number of chambers change seasonally (Seal and Tschinkel, 2006), suggesting that chambers

are filled and reconstructed at another depth. The source of this sediment is currently uncertain, but is likely to derive from the new chamber under construction. Nest densities as high as 1000 per ha have been reported, depositing as much as 1 metric ton per ha of deeper soil on the surface each year, displacing the top 5 cm of soil in about 800 yr (Seal and Tschinkel, 2006, 2010). Nest densities at our site varied with canopy and groundcover, but averaged about 8.2 (s.d. 4.9) per 100 m^2 sample or about 800 per ha. In May, tumuli averaged 320 (s.d. 170) cm^3, totaling about 0.25 m^3 or 375 kg per ha.

Another common sandhills ant was *Camponotus socius,* the largest ant of the coastal plains forest. Each colony excavates and abandons several up to 70–80 cm deep nests per year (Tschinkel, 2005). Also common were *Formica pallidefulva* and *Formica dolosa,* both of which create nests 50–75 cm deep with total volumes up to 2–3 l (Mikheyev and Tschinkel, 2004; Tschinkel, unpublished data). Another abundant sandhills ant, *Aphaenogaster floridanus,* constructs nests up to 80 cm deep. *Aphaenogaster ashmeadi* and *Aphaenogaster treatae* make shallower nests that are less likely to be important to deep bioturbation (Tschinkel, 2011a,b), but both were uncommon at our site. In contrast, *Dorymyrmex bureni* and *Dorymyrmex bossuta* were both abundant, and excavate slender but often very deep nests (>3 m), while the clustered and locally abundant nests of *Nylanderia arenivaga* are rarely deeper than 1 m (WRT, unpublished data). The trap-jaw ant, *Odontomachus brunneus* occurred mostly in the more densely wooded parts of our site. It commonly makes 1.5 m deep nests of considerable caliber (Cerquera and Tschinkel, 2009). These nests are shallower in summer than winter, suggesting subterranean remodeling (Hart and Tschinkel, 2012). The native fire ant, *Solenopsis geminata* was widely distributed in our site (as opposed to invasive fire ant species). Its large colonies excavate very deep nests in little disturbed sandhills sites such as ours, while in highly disturbed site, the very large colonies of the exotic *Solenopsis invicta* dig high-volume nests up to 2 m deep, bringing 5–20 l of soil to the surface to build its mound (Tschinkel, 2003; WRT, unpublished data). *Pheidole morrisi* is a common flatwoods ant, but also occurred in our site at low densities, excavating nests up to 1 m or more in depth (0.5–2 l volume) and topped with a large mound of excavated soil (Tschinkel, 2003). Probably the deepest nests of all are made by *Prenolepis imparis*, an ant that is active only in the winter, aestivating in nests up to 4 m deep (Tschinkel, 1987), with total chamber volumes of 0.1–2 l.

5. Conclusion

This study investigated how a single species of ant could rearrange the distribution of individual sand grains in a sandy environment in north Florida during one episode of nest-building. We believe this kind of controlled experiment has not been done before, and it provides a detailed look into the mechanics and rates of the redistribution of sediments. Ant bioturbation is shown here to have two possible adverse effects on OSL age estimation: underestimation of grain ages due to subterranean transport of younger grains downward and overestimation due to transport of older grains upward. Our results show that the majority of subterranean transport moves older grains upward, showing the potential for age overestimation in OSL dating. This observation is in accord with OSL age estimations in a number of sandy sites in Florida and elsewhere in North America. Our results also show a relatively smaller flux of downwardly transported (grains sourced from higher levels in the site), though we suggest that it is likely that the effects of this component of transport by ants on OSL age distributions might be masked by the larger effect of upward transport of older grains.

Although narrowly focused, the research has broad implications and importance, both for our specific focus on OSL and more general effects of bioturbation for geological and archaeological research. Next steps in this research include understanding whether the behavior of *P. badius* in subterranean deposition of excavated sand grains is shared with other ant species, how multiple episodes of ant nest-building on the same spot will affect sand redistribution, and evaluating methods to detect and account for the effects of this kind of bioturbation. Our work shows that in appropriate contexts the use of a minimum age model for interpreting results best accounts for the subterranean transport and deposition of older sand grains. In addition, our work implies that the effects of bioturbation by ants, especially over extended time periods, can be a significant contributor to overdispersion of equivalent dose distributions, beyond those that could be associated with incomplete zeroing at burial. Actual OSL dating studies of recent *P. badius* nests and adjacent undisturbed areas are underway to further understand how age profiles developed from equivalent dose distributions might be affected.

Further, the methodology developed here would be useful in testing assumptions that are not directly related to OSL. Controlled experiments using constructed environments in natural settings could evaluate how ants or other important bioturbators, such as gophers and other mammals, can displace charcoal through archaeological strata, or whether ants are significant agents in biomantling (Balek, 2002).

Acknowledgments

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