The evolution of thermoregulatory nesting behaviour in *Peromyscus* mice

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ABSTRACT

Structures built by animals, such as nests, are often extended phenotypes that can facilitate the study of animal behaviour. For rodents, nest building is both an important form of behavioural thermoregulation and a critical component of parental care. Changes in nest structure or the prioritization of nesting behaviour are therefore likely to have consequences for survival and reproduction, and both biotic and abiotic environmental factors are likely to influence the adaptive value of such differences. Here we use a novel assay to investigate interspecific variation in the thermoregulatory nesting behaviour of deer mice (genus *Peromyscus*). We find that, while there is some variation in the complexity of the nests built by *Peromyscus* mice, differences in the latency to begin nest construction are more striking. Four of the seven taxa examined here build nests within an hour of being given nesting material, but this latency to nest is not related to ultimate differences in nest structure, suggesting that the ability to nest is relatively conserved within the genus, but species differ in their prioritization of nesting behaviour. Latency to nest is not correlated with body size, climate, or the construction of burrows that create microclimates. However, the four taxa with short nesting latencies all have monogamous mating systems, suggesting that evolved differences in nesting latency may be related to social environment. This detailed characterization of nesting behaviour within the genus provides an important foundation for future studies of the genetic and neurobiological mechanisms that contribute to the evolution of behaviour.

Key words: behavioural evolution, comparative method, deer mice, extended phenotype

Running head: Evolution of nesting behaviour
INTRODUCTION

Animal architectures - from the webs spun by spiders to the dams built by beavers - are useful to the study of how and why behaviours evolve. This is largely because these structures can be considered “extended phenotypes,” or traits influenced by genetics but extended outside the body of the individual organism (Dawkins, 1982), which both facilitate the study of behaviour and can provide insight into the selective forces at work (Hansell, 1984, 2005). Building behaviours are often innate and species-specific; for example, the resulting structures have been used historically for classification purposes in insects and some birds (Hansell, 1984; Knerer et al., 2012; Schmidt, 1964; Winkler et al., 1993). These structures reflect stereotyped patterns of behaviour and the neural circuits that generate these motor patterns, allowing us to study behaviour and the nervous system by proxy. Moreover, the structures themselves serve important functions and can confer readily quantifiable fitness benefits on the animals that construct them (Hayward, 1965; Mainwaring et al., 2014; Sealander, 1952).

A widespread and important type of building behaviour is the collection and processing of environmental materials to produce a nest. Nests serve a wide variety of purposes for the animals that construct them. For small-bodied animals, such as rodents, a nest can be used for thermoregulation, providing insulation and reducing the energy expended on the maintenance of body temperature (O. P. Pearson, 1960; Sealander, 1952; Vogt et al., 1982). In animals with altricial young, like many birds and rodents, nests are especially critical to protect offspring from heat loss and predation (Bult et al., 1997; Collias, 1964; Lynch et al., 1978; Southwick, 1955). Structures such as the arboreal nests built by chimpanzees may also ensure a safe sleeping site for adult animals (van Lawick-Goodall, 1968). The nest can even serve as a catalyst for complex social behaviour — nest and bower construction can be integral to courtship in birds (Mainwaring et al., 2014), and investment in elaborate nests likely has been instrumental in the evolution of eusociality in insects (Hansell, 2005). Depending on the species in question and the environment in which they live, nests may be built in trees, in pre-existing cavities, or
in burrow systems that are also constructed by the animal (Collias, 1964; Dooley et al., 1990; Weber et al., 2009). While the excavation of burrows is itself a type of animal architecture, nests are often separate structures, made by collecting and processing vegetation and other material from an animal’s environment.

Both the structure of a completed nest and the timing of nest-building may be relevant traits for natural selection, and each has distinct implications for the proximate and ultimate factors that contribute to behavioural differences among taxa. Variation in nest structure, as is observed in birds, suggests that animals may differ either in their ability to construct nests or in the desired properties of their nests (Mainwaring et al., 2014). At the level of proximate mechanism, variation could result from morphological differences in the animals, fundamental changes in their stereotyped motor patterns, or changes in a more abstract encoding of the animal’s target structure, for example. Moreover, evolved variation in nest structures can suggest that the characteristics of the nest itself have different fitness consequences in different environments. Prime examples of such relationships between fitness and structural variation in bird nests include the pendulous entrances of some weaverbird nests, which are protective against snake predation (Collias, 1964; Crook, 1963), or the increased size and weight of robin, warbler, and finch nests built at colder northern versus southern latitudes (Crossman et al., 2011).

Variation in the timing of nesting behaviour, on the other hand, implies that animals differ in their motivation to engage in otherwise conserved behavioural patterns, and suggests that the prioritization of nesting relative to other elements of the animal’s behavioural repertoire is relevant for selection. Prioritization can occur at different scales, from time invested over the course of a single night to relative time spent on the behaviour during different seasons. As the collection of nesting material can be energetically costly and expose the animal to predation (Collias, 1964; Mainwaring et al., 2014), it may be beneficial for an animal to prioritize other behaviours in environmental conditions where heat loss, for example, is not a pressing concern.
For rodents, environmental conditions can alter nesting behaviour within a species, both by inducing acute changes in the behaviour of individuals and via selection for heritable differences in behaviour among populations. For example, cold temperatures (Lynch et al., 1973) and short photoperiods (Lynch, 1973) increase the amount of nesting material that animals use to build a single nest. On longer timescales, access to nesting material has consequences for survival at low temperatures (Sealander, 1952), and environmental temperature differences have led to heritable differences in nesting material use in *Mus* (Lynch, 1992). In addition, pregnancy and the hormonal changes associated with pregnancy elicit increases in nesting behaviour in female mice (Lisk, 1971; Lisk et al., 1969) and rats (Fleming et al., 1974). While nesting is an important component of parental care in species with altricial offspring, and parental nesting has been studied in a variety of rodents (e.g. Bendesky et al., 2017; Lynch et al., 1978; Stewart et al., 2017), interspecific variation in nesting performed for the purpose of individual thermoregulation is less well understood.

To determine how and why these features of thermoregulatory nesting behaviour evolve among closely related, yet ecologically and behaviourally divergent, species of rodents, we focused on deer mice (genus *Peromyscus*). Deer mice have adapted to a wide range of habitats and microhabitats across North America, and include animals as diverse as semi-fossorial burrowing beach mice and arboreal forest-dwelling species (Bedford et al., 2015; Blair, 1950; Dewey et al., 2001). They live in a broad array of climates with pronounced differences in winter temperatures (King et al., 1964), vary in body size, a trait associated with adaptation to cold in other rodents (Lynch, 1992), and have distinct social behaviour and parental care (Jašarević et al., 2013; Turner et al., 2010), all of which may affect nest-building behaviour. Importantly, while these species have evolved in different environments, we also can capitalize on animals that have been bred in a laboratory setting, allowing us to perform behavioural experiments under carefully controlled conditions using animals that share a common environment.
(Bedford et al., 2015). These mice therefore provide an opportunity to explore the evolutionary consequences of different environmental parameters on heritable variation in nest-building behaviour.

Here we use a novel behavioural assay that allows us to evaluate natural variation in both ability and motivation to nest in seven species and subspecies of *Peromyscus* mice. This detailed characterization of thermoregulatory nesting behaviour then provides a foundation to understand the evolution of this behaviour in natural populations.

**METHODS**

*Ethical Note*

All experimental procedures were approved by the Harvard University IACUC. The animal housing facility in which these tests were performed maintains full AAALAC accreditation.

*Experimental Cohort*

We selected adult animals from seven laboratory colonies of *Peromyscus*, representing five species, with well-characterized ecology and social systems (Table 1). While these colonies were isolated from natural populations (brought in from the wild between 2 and 71 years ago, depending on strain; Table 1), all animals in this study were born in captivity and tested as adult virgins.

*Animal Husbandry*

All animals were bred and maintained under the same controlled conditions. We kept the animal housing rooms on a 16:8 LD cycle at 22°C. We housed animals in ventilated polysulfone ventilated mouse cages (Allentown, NJ) of standard size (7.75” wide x 12” long x 6.5” high), with the exception of the *P. californicus* animals, which were housed in rat cages (11.25” wide x 15.5” long x 7.6” high) due to their large body size (Allentown, NJ). For ordinary housing, we provided all cages with
<table>
<thead>
<tr>
<th>Species (common name)</th>
<th>County Isolated</th>
<th>Year in Captivity*</th>
<th>Sample Size</th>
<th>Avg. Weight, Males (g ± sd)</th>
<th>Avg. Weight, Females (g ± sd)</th>
<th>Avg. Age (days ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. maniculatus nubiterrae</em> (cloudland deer mouse)</td>
<td>Westmoreland County, PA</td>
<td>2010</td>
<td>47 (31,16)</td>
<td>18.7 ± 2.3</td>
<td>15.8 ± 2.8</td>
<td>164 ± 184</td>
</tr>
<tr>
<td><em>P. maniculatus bairdii</em> (deer mouse)</td>
<td>Washtenaw County, MI</td>
<td>1946-1948</td>
<td>95 (62,33)</td>
<td>20.3 ± 3.5</td>
<td>16.9 ± 1.6</td>
<td>106 ± 50</td>
</tr>
<tr>
<td><em>P. polionotus subgriseus</em> (oldfield mouse)</td>
<td>Marion County, FL</td>
<td>1952</td>
<td>130 (80,50)</td>
<td>14.3 ± 1.9</td>
<td>15.5 ± 1.7</td>
<td>107 ± 57</td>
</tr>
<tr>
<td><em>P. polionotus leucocephalus</em> (Santa Rosa Island beach mouse)</td>
<td>Okaloosa County, FL</td>
<td>2015</td>
<td>37 (23,14)</td>
<td>14.2 ± 1.1</td>
<td>14.4 ± 2.7</td>
<td>71 ± 17</td>
</tr>
<tr>
<td><em>P. leucopus</em> (white-footed mouse)</td>
<td>Avery County, NC</td>
<td>1982-1985</td>
<td>35 (22,13)</td>
<td>21.7 ± 4.1</td>
<td>20.1 ± 2.7</td>
<td>66 ± 7</td>
</tr>
<tr>
<td><em>P. gossypinus</em> (cotton mouse)</td>
<td>Jackson County, FL</td>
<td>2009</td>
<td>27 (19,8)</td>
<td>25.2 ± 7.0</td>
<td>21.9 ± 3.3</td>
<td>72 ± 9</td>
</tr>
<tr>
<td><em>P. californicus</em> (California mouse)</td>
<td>Ventura County, CA</td>
<td>1979-1987</td>
<td>48 (25,23)</td>
<td>42.1 ± 5.2</td>
<td>41.5 ± 7.0</td>
<td>126 ± 30</td>
</tr>
</tbody>
</table>

*Some species were brought into captivity multiple times over several years, see (Bedford et al., 2015).*
2.5g of compressed cotton “Nestlet” (Ancare, Bellmore, NY), 8-10g folded paper “Enviro-Dri” nesting material (Shepherd Specialty Papers, Watertown, TN), a ¼" layer of Anderson’s Bed-o-cob (The Andersons, Inc., Maumee, OH), and enrichment consisting of a red polycarbonate (3 ¾" x 1 ⅞" x 3") mouse hut (BioServ, Flemington, NJ) or a 6" x 3" inside diameter rat tunnel for the large *P. californicus* animals (BioServ, Flemington, NJ). All animals had *ad libitum* access to water and irradiated LabDiet Prolab Isopro RMH 3000 SP75 (LabDiet, St. Louis, MO). We socially housed animals in groups of 2-5 by species and sex after weaning (23 days for most species, 30 days for *P. californicus*), then tested them as adult virgins, averaging 2-6 months old (Table 1).

**Behavioural Paradigm**

**Standard Behavioural Assay:** Nesting behaviour in rodents is often assessed by measuring the weight of nesting material an animal uses over 24 hours (Hartung et al., 1979; King et al., 1964; Layne, 1969; Lynch et al., 1973), which is readily quantifiable but can obscure variation in the timing of the behaviour or the structure of the nests the animals are able to construct. To measure these aspects of nesting behaviour, we designed a novel assay that consists of an overnight habituation period followed by three consecutive days of testing. On the day before a trial began, we weighed and singly housed adult virgin animals in new mouse cages (including *P. californicus*) with 5g of compressed cotton nesting material (or two “nestlets”, see above), ¼ in layer of Anderson’s Bed-o-cob, and a red polycarbonate mouse hut. On the morning following habituation to the novel cage, we took photos of the nest from up to three angles (top and two side views), then removed the mouse hut and replaced all cotton nesting material with 5g of fresh compressed cotton nestlet. The replacement of nesting material during these trials always occurred between 4.5 and 6.5 hours after the lights came on. At one hour after the replacement of nesting material, we again took photographs of the nest from multiple angles and added the mouse hut back to the cage. We repeated this process on the following two mornings for a total of
three sets of photographs (day 1, day 2, and day 3) at each of the two time points (1h and overnight). Research assistants, blinded to the species and sex of the animal, later scored these nest photographs according to a standardized scale (Fig. 1; Supplemental Table S1). Scores ranged from 0 (no visible shredding) to 4 (a full “dome” nest with overhead coverage) with only full and half scores given.

**Increased Nesting Material:** To examine whether the amount of nesting material had an impact on nest scores in the largest species (*P. californicus*; approximately 42g, on average), we modified the nesting experiment in two ways. First, we singly housed an independent cohort of 21 adult *P. californicus* animals as above, but provided them with an increasing amount of cotton nesting material on four consecutive days: 5g on day 1, 10g on day 2, 15g on day 3, and 20g on day 4. We photographed nests and exchanged cotton nesting material once every 24 hours, and a research assistant blind to experimental conditions scored these photographs as above to establish whether this increase was sufficient to alter overnight nest scores. Based on the results of these experiments, we then assayed an independent group of 23 adult *P. californicus* animals to evaluate their overnight nesting behaviour using 20g of cotton nesting material in an otherwise standard nesting assay (see “Standard Behavioural Assay” above).

**Climate Data**

We drew average winter (December/January/February) temperature data from National Oceanic and Atmospheric Administration (NOAA) 30-year climate normals (Arguez et al., 2010), and averaged these data by state or county of origin for each colony (Table 2).

**Data Analysis**
Figure 1: Nest scoring scale. Nests are scored on a scale from 0 (no manipulation of the nesting material) to 4 (a full cotton “dome”) in increments of 0.5. Representative nests for each of the five integer scores are shown from both the top and side view, and a brief descriptor is provided for each. Classification is according to criteria provided in Supplementary Table S1.
### Table 2: Environmental Context

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Latitude (°N)</th>
<th>Avg. Winter Temp. (°C)</th>
<th>Habitat</th>
<th>Habitat Use</th>
<th>Habitat Ref.</th>
<th>Burrows^C</th>
<th>Nest Location</th>
<th>Mating System^D</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. m. nubiterrae</em></td>
<td>40.2</td>
<td>-1.2</td>
<td>forest</td>
<td>semi-arboreal</td>
<td>(Blair, 1950)</td>
<td>simple/short</td>
<td>arboreal; tree cavities (Wolff et al., 1986; Wolff et al., 1982)</td>
<td>M</td>
</tr>
<tr>
<td><em>P. m. bairdii</em></td>
<td>42.3</td>
<td>-3.4</td>
<td>prairie/grassland</td>
<td>terrestrial</td>
<td>(Blair, 1950)</td>
<td>simple/short</td>
<td>in burrows (Morris et al., 1981)</td>
<td>P</td>
</tr>
<tr>
<td><em>P. p. subgriseus</em></td>
<td>29.2</td>
<td>14.4</td>
<td>sandy soil/grassland</td>
<td>semi-fossorial</td>
<td>(Blair, 1950)</td>
<td>complex/long</td>
<td>in burrows (Dawson et al., 1988)</td>
<td>M</td>
</tr>
<tr>
<td><em>P. p. leucocephalus</em></td>
<td>30.4</td>
<td>11.0</td>
<td>white sand beach</td>
<td>semi-fossorial</td>
<td>(Blair, 1950; Sumner, 1926)</td>
<td>complex/long</td>
<td>in burrows (Blair, 1951)</td>
<td>M</td>
</tr>
<tr>
<td><em>P. leucopus</em></td>
<td>36.1</td>
<td>0.9</td>
<td>deciduous forest</td>
<td>semi-arboreal</td>
<td>(Blair, 1950; Lackey et al., 1985)</td>
<td>simple/short</td>
<td>seasonally dependent; arboreal or ground nests (Wolff et al., 1986; Wolff et al., 1982)</td>
<td>P</td>
</tr>
<tr>
<td><em>P. gossypinus</em></td>
<td>30.7</td>
<td>11.3</td>
<td>hardwood forest, mesic hammocks, swamps</td>
<td>semi-arboreal</td>
<td>(Wolfe et al., 1977)</td>
<td>no laboratory data</td>
<td>diverse but arboreal preferred; in or under logs and stumps, in tree cavities (Ivey, 1949; Klein et al., 1978; Wolfe et al., 1977)</td>
<td>P</td>
</tr>
<tr>
<td><em>P. californicus</em></td>
<td>34.1</td>
<td>12.3</td>
<td>scrub/chaparral</td>
<td>semi-arboreal</td>
<td>(Clark, 1936; M’Closkey, 1976; Merritt, 1978; Meserve, 1977)</td>
<td>none</td>
<td>under logs, in woodrat (Neotoma) dens (Merritt, 1974, 1978)</td>
<td>M</td>
</tr>
</tbody>
</table>

A. Latitude of county of origin (Table 1).
B. 30-year NOAA climate normals for average Dec/Jan/Feb temperatures, pooled by county of origin for each colony (Arguez et al., 2010).
C. Burrows produced in a laboratory assay from (Weber et al., 2009), with the exception of *P. m. nubiterrae* (Hu et al., 2017).
D. Mating system (Monogamous or Promiscuous) according to (Turner et al., 2010), with the exception of *P. m. nubiterrae* (Wolff et al., 1991) and *P. gossypinus* (Dewsbury et al., 1980; McCarley, 1959; Pearson, 1953).
We performed statistical analyses in R using non-parametric methods for the ordinal nest scores. We summarized an animal’s behaviour across the three trial days by its median score (to reflect central tendency) or its maximum score (to represent best effort) at each time point after the replacement of nesting material. To identify differences between groups, we used Kruskal-Wallis rank-sum tests, and then used Bonferroni-corrected Wilcoxon rank-sum tests for subsequent pairwise comparisons between species or sexes. For the experiment that tests the effects of increased access to nesting material in a cohort of *P. californicus* animals, we used a Friedman rank-sum test.

For comparative analyses, we first generated an ultrametric tree using Grafen’s method (Grafen, 1989; Symonds et al., 2014) and the known topology of the species relationships (Fig. 2A; Bedford et al., 2015; Bradley et al., 2007; Weber et al., 2009). To test for a relationship between species-level median 1h nest scores and species-level average weights, latitudes of origin, and winter temperatures at sites of origin, we performed phylogenetic generalized least squares (PGLS) analysis using the ape and nlme packages in R (Paradis E., 2004; Pinheiro et al., 2017; Symonds et al., 2014). Covariance due to relatedness was modelled by Brownian motion using the corBrownian function in ape. The covariance was then included as a correlation parameter in the generalized least squares analyses in nlme. The effect of each environmental variable on 1h nest scores was tested independently. To test whether short nesting latency is dependent on other discrete traits (complex burrowing or mating system, as indicated in Table 2), we performed Pagel’s binary character correlation test using the fitPagel function in the phytools package in R (Pagel, 1994; Revell, 2012). For this test, we utilized the fitMk method, allowed all rates of change to be different between states (model=”ARD”), and set nesting latency (short vs. intermediate/long) to be dependent on the state of either mating system (monogamous vs. promiscuous) or burrow complexity (complex vs. simple/absent). As there are no laboratory data on burrowing behaviour in *P. gossypinus*, this species was excluded from the latter analysis.
RESULTS

Interspecific variation in nesting latency

To measure an animal’s motivation to nest, we assayed individuals from seven *Peromyscus* taxa with known evolutionary relationships (Fig. 2A). First, we analysed the median of the three scores an animal received one hour after the replacement of nesting material, which reflects the tendency of the animal to begin nesting shortly after their nest is disturbed. Scores at 1h were significantly correlated across the three days in the full dataset (Spearman rank correlations: day 1 vs. day 2 $r_s = 0.75$, day 1 vs. day 3 $r_s = 0.68$, day 2 vs. day 3 $r_s = 0.78$, $N=419$, $P<2.2\times10^{-16}$ for each), and species comparisons were largely the same whether three-day medians or maxima were used (see below). The median nest scores at 1h following the initiation of the trial varied dramatically among the taxa we assayed (Fig. 2B; Kruskal-Wallis test: $H_6=216.85$, $P<2.2\times10^{-16}$). Four taxa (*P. m. nubiterrae*, *P. p. subgriseus*, *P. p. leucocephalus*, and *P. californicus*) had high, statistically indistinguishable scores at the 1h time point (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected $P>0.05$ for each pairwise comparison), suggesting that they began to construct their nests relatively quickly. In addition, these four taxa differed significantly from the other three taxa we assayed (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected $P<0.05$ for each pairwise comparison). *P. leucopus* animals received intermediate scores that reflect a tendency to shred the material, but not arrange it into a nest, at the 1hr time point. These scores were significantly different from those received by all other species (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected $P<0.05$ for each pairwise comparison). Finally, *P. m. bairdii* and *P. gossypinus* animals had equivalently low scores (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected $P=0.29$), which indicate that they did not manipulate the nesting material in the first hour and suggest that they are relatively slow to initiate nest construction. Ranking of each taxon’s performance largely followed the same pattern whether maximum or median nest scores were used (Supplemental Figure S1). The only exception was one
Figure 2: Interspecific differences in nesting behaviour. (A) Phylogenetic relationships among the Peromyscus taxa included in this study; modified from (Bedford & Hoekstra, 2015; Weber et al., 2009). (B) Median nest scores 1h after receiving new nesting material and (C) maximum overnight nest scores for each animal over the 3 trial days. Letters indicate species groups that do not significantly differ from one another, while all other pairwise comparisons are significant (Wilcoxon rank-sum test, Bonferroni-corrected P<0.05). (D) P. californicus animals given 20g of nesting material do not differ in maximum overnight scores from those given 5g of nesting material (Wilcoxon rank-sum test, P=0.47). Sample sizes are provided below.
species difference: while the median nest scores of *P. gossypinus* and *P. m. bairdii* animals at 1h were indistinguishable, *P. gossypinus* were slightly more likely to shred the nesting material on at least one of the trial days and therefore had slightly, but significantly, higher maximum scores (Wilcoxon rank-sum test: \( W=839, N_1=27, N_2=95, \) Bonferroni-corrected \( P = 0.049 \)). Based on our analysis of 1h median scores, we identified three main groups of nest builders in our assay: those with short, intermediate or long latencies to nest.

**Interspecific differences in nesting ability**

We next asked whether these taxa differed in their overall ability to construct a three-dimensional nest. To establish the highest-scoring nest that an animal was capable of producing, we used the maximum score achieved over the individual’s three overnight time points, which represents the animal’s best effort during the longest interval of the trial. Maximum overnight scores varied significantly among taxa (Fig. 2C; Kruskal-Wallis test: \( H_6=127.21, P<2.2x10^{-16} \)), although most animals built full or partial domes. The highest scoring nests were consistently constructed by *P. m. nubiterrae*, *P. p. leucocephalus*, *P. leucopus*, and *P. gossypinus* animals, which tended to build statistically indistinguishable full domes (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected \( P>0.05 \) for each pairwise comparison). Three taxa – *P. m. bairdii*, *P. p. subgriseus*, and *P. leucopus* – had equivalently high maximum scores (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected \( P>0.05 \) for each pairwise comparison), and *P. m. bairdii* and *P. p. subgriseus*, which tended to build domes with only partial cover, were significantly different from all but *P. leucopus* animals (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected \( P<0.05 \) for each pairwise comparison). Finally, *P. californicus* animals tended to build nests with walls but without overhead cover, and had significantly lower maximum nest scores than all other species tested (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected \( P<0.05 \) for each pairwise comparison). Notably,
we found that all species had at least one individual who constructed a domed nest with full cover (maximum nest score, “4”) during the assay, suggesting that all species are capable of building a “complete” nest if given enough time. However, some species showed a large variance in nest scores, and *P. californicus* tended to have lower maximum scores than the other species.

**Nest-building behaviour in the large *P. californicus* mice**

*P. californicus* animals are much larger than the other taxa included in this study (Table 1), and therefore might require more material to construct a dome nest with overhead cover. To test the possibility that these animals built lower-scoring nests because 5g of nestlet was an insufficient amount of nesting material, we conducted two additional experiments. First, we gave a group of *P. californicus* animals increasing amounts of nesting material on four consecutive days and evaluated the nests they produced in each 24-hour interval. We found that increasing nesting material from 5g to 20g could increase overnight nesting scores (Supplemental Fig. S2; Friedman test: $X^2_3=13.468, p=0.004$). However, when we provided an independent group of *P. californicus* animals with 20g of nesting material during a three-day trial (Fig. 2D), there was no difference in overnight maximum scores between those *P. californicus* given 5g of nestlet and those given 20g (Wilcoxon rank-sum test: $W= 609, N_1=23, N_2=48, P=0.47$). Moreover, the maximum overnight nest scores for *P. californicus* given 20g of nestlet remained significantly lower than the maximum nest scores for all other species (Supplemental Table S2, Wilcoxon rank-sum test, Bonferroni-corrected $P<0.05$ for each pairwise comparison). Thus, the poor nest construction of *P. californicus* in this assay cannot be attributed simply to insufficient nesting material relative to its large body size.

**Sex differences in nesting**
Figure 3: Sex differences in nesting latency. Sex-specific median 1h nest scores for each *Peromyscus* taxa tested. Significant sex differences in median nest score occurred in only two groups: *P. maniculatus nubiterrae* (Wilcoxon rank-sum test, Bonferroni-corrected \( P = 0.03 \)) and *P. polionotus subgriseus* (\( P = 0.002 \)). Sample sizes are provided below. NS = non-significant.
We next investigated whether there were any sex differences in the nest scores produced by each species and subspecies. Only two taxa showed evidence of sexual dimorphism in nesting (Fig. 3). Surprisingly, both male *P. m. nubiterrae* and male *P. p. subgriseus* animals built higher scoring nests than their female counterparts one hour after the start of the assay (Supplemental Table S3, Wilcoxon rank-sum test, Bonferroni-corrected \( P = 0.03 \) and 0.002, respectively), and *P. p. subgriseus* males also built higher scoring nests at the overnight time point (Supplemental Table S3, Supplemental Fig. S3; Wilcoxon rank-sum test, Bonferroni-corrected \( P = 0.008 \)). No other species showed evidence of sex differences in nest scores at either time point (Supplemental Table S3, Wilcoxon rank-sum test, Bonferroni-corrected \( P > 0.05 \) for each pairwise comparison). Therefore, while there was no sexual dimorphism in nesting behaviour for most taxa, in both instances when sex differences were observed, males constructed higher-scoring nests than the females.

**Association between body size and nest building**

To determine whether body size had an effect on nest-building behaviour, we tested for correlations between weight and performance in the nesting assay. We found that weight significantly varied by species, sex, and species-by-sex interactions in our experimental cohort (two-way ANOVA, main effect of species: \( F_{6,352} = 365.9, P < 2 \times 10^{-16} \); main effect of sex: \( F_{1,352} = 5.3, P = 0.02 \); interaction: \( F_{6,352} = 4.1, P = 0.0005 \)). However, there was no evidence that species-level average weights alter median 1hr nest scores (Fig. 4A, phylogenetic generalized least squares, median 1hr nesting score by average weight: coefficient = -0.01, SE = 0.06, \( t = -0.15, P = 0.89 \)). Likewise, when we divided the animals by species and sex, we found no correlation between weight and median nest score at 1h (Supplemental Table S4, Spearman’s rank correlations, Bonferroni-corrected \( P > 0.05 \)) or maximum overnight nest score (Supplemental Table S4, Spearman’s rank correlations, Bonferroni-corrected \( P > 0.05 \)) within any of the
Figure 4: Environmental Factors and Nesting Behaviour
**Figure 4: Environmental factors and nesting behaviour.** (A) Species median 1h nest scores and average weight are plotted with bars indicating interquartile range (nest score) and standard deviation (weight). (B) Sites of colony origin (on US map), burrow shape (by symbol), and nesting latency (by colour) are indicated for each taxon (following legend). Map colours represent average winter temperatures by state (Arguez et al., 2010). Nesting latency category (short, intermediate, long) was determined by the significant species groups depicted in Fig. 2B. (C) Association between a taxon’s mating system and nesting latency.
species-sex groups. Thus, while average weight varied almost three-fold among species, weight was not associated with nesting behaviour in our assay.

**Association between environment and nest construction**

We next asked whether there was an association between performance in the nesting assay and several additional environmental covariates, including latitude and average winter temperature of origin, burrow construction, and mating system. Neither latitude nor average winter temperatures were significantly associated with median 1hr scores in these species (Table 2, Fig. 4B; phylogenetic generalized least squares, median 1hr nest score by latitude: coefficient = -0.05, SE= 0.08, t=-0.71, P=0.51; median 1hr nest score by average winter temperature: coefficient = 0.03, SE= 0.05, t=0.69, p=0.52). Moreover, nesting latency does not appear to be influenced by burrowing behaviour: a model in which short nesting latency is dependent on building complex burrows does not fit the data significantly better than a model where the two traits are independent (Table 2, Fig. 4B; Pagel’s binary character correlation test: AIC (independent model) = 21.97, AIC (dependent model) = 23.88, likelihood ratio=2.09, P=0.35). However, a model in which short nesting latency depends on mating system fits the observed data significantly better than a model assuming the two traits are independent (Table 2, Fig. 4C; Pagel’s binary character correlation test: AIC (independent model)= 26.42, AIC (dependent model)=21.21, likelihood ratio = 9.21, P=0.01). With the caveat that the sample size for comparisons among taxa is small, these data suggest that mating system influences nesting latency but the other abiotic environmental factors we examined do not.

**DISCUSSION**

Nesting is important for survival in rodents, but it is not clear how this behaviour varies among species or which evolutionary pressures may drive these changes. Here we find that closely related deer
mice generally are able to construct dome-shaped nests, but exhibit considerable variation in their latency to do so. When we tested for correlations between several abiotic and biotic variables and latency to nest, we found that only mating system is predictive of nesting behaviour, with monogamous species beginning to build nests more quickly than promiscuous species.

Nesting has been well studied in laboratory models, where researchers have historically investigated the proximate genetic and physiological mechanisms that contribute to the behaviour in rodents (e.g. Lisk et al., 1969; Lynch, 1980; Lynch et al., 1978). However, the majority of nesting experiments in laboratory settings, including some studies in Peromyscus, measure the amount of nesting material that an animal pulls into its cage or the final nest structure achieved over a 24-hour period (Hartung et al., 1979; King et al., 1964; Layne, 1969; Lynch et al., 1973). By contrast, we focus on the timing of the behaviour. By evaluating nests just one hour after the replacement of nesting material, we are able to assess whether the animals differ in their latency to begin nest construction—what might be considered a baseline motivation to nest. This is complemented by a second measurement at the more permissive overnight time point, which allows us to evaluate whether animals vary in their overall ability to build three-dimensional structures. This novel phenotyping paradigm therefore allows us to distinguish between animals that differ in their motivation to construct nests of similar shape from those that differ in their ability to construct nests.

Even closely-related Peromyscus species vary dramatically in their latency to begin nesting, but variation in final nest structure is much more modest. This is in contrast to studies of nesting in birds and insects, where the structures of complete, species-typical nests are highly variable (Collias, 1964; Healy et al., 2008; Knerer et al., 2012; Price et al., 2017; Schmidt, 1964), or even burrowing behaviour in Peromyscus, where species excavate cavities that significantly differ in size and shape (Hu et al., 2017; Weber et al., 2009). Most Peromyscus species in this study demonstrated a capacity to build dome nests with some degree of overhead cover. An exception to this result was the behaviour of the much larger...
P. californicus, which could not be explained by a simple limitation of nesting material, and therefore might reflect a true difference in nesting ability or desired nest structure. Importantly, latency to nest appears to be decoupled from the slight variation in final nest shape — P. gossypinus animals, which have some of the lowest scores at one hour, build the highest scoring nests overnight, and P. californicus, which have some of the highest scores at one hour, build the lowest scoring nests at this longer time point. Long latencies to nest are therefore not simply explained by reduced ability to nest. Together, these results indicate that ability to nest is relatively conserved within the genus, but latency to nest is quite variable. This, in turn, implies that being able to produce dome-shaped nests is important for most animals, but that the prioritization of the behaviour may vary among species. Thus, variation in nesting in the genus is likely due to altered motivation rather than changes in stereotyped motor patterns, morphology, or target nest structure.

While nesting is largely a reproductive behaviour in birds (Collias, 1964) and is sometimes viewed as primarily the provenance of female mammals (Kleiman, 1977), we show that Peromyscus mice are willing and able to nest in a purely thermoregulatory context. We also find only limited support for sex differences in nesting latency or nest complexity within the genus in adult, reproductively inexperienced animals. In fact, in most species, no sex differences exist. Moreover, in the two species with evidence of sexual dimorphism, males produce slightly higher scoring nests than females in the first hour. Intriguingly, in these two species there is evidence for a monogamous mating system (Foltz, 1981; Turner et al., 2010; Wolff et al., 1991), and males therefore are likely to contribute to parental care of their young, which could include paternal nest building (Bendesky et al., 2017; Kleiman, 1977). It is important to note, however, that these sex differences are relatively subtle and are dwarfed by the interspecific variation in nest scores. Furthermore, as these results reflect propensity to nest in adult virgin animals, they may change when animals are tested at other life-history stages.
Based on data from latitudinal clines in *Mus* (Lynch, 1992) and *Peromyscus* (King et al., 1964), one might expect to see a positive relationship between propensity to nest and body size in animals found in different climates. This expectation is also aligned with Bergmann’s Rule, which posits that there should be selection for increased body size and other thermoregulatory adaptations in cold climates (King et al., 1964; Lynch, 1992). It is worth noting that the relationship between body weight and weight of nesting material used that has been observed in these studies of rodent nesting (King et al., 1964; Lynch, 1992; Wolfe, 1970) might be at least partially explained by larger animals requiring more material to build equivalently shaped structures. By focusing on the structure of the nest rather than the weight of nesting material used to construct it, we minimize this confounding factor. Alternatively, others have argued that mammals with smaller bodies, and hence higher relative surface areas, are more prone to heat loss and therefore may build larger nests or be more motivated to begin nesting at the same temperature (Hartung et al., 1979). As body weight varies significantly within this experimental cohort of *Peromyscus* species, we can test both hypotheses. When we divide the cohort by species and sex we find no evidence for a correlation between weight and nesting scores at either time point. Moreover, there is no evidence for a relationship between average weight by species and median nesting scores at one hour. This suggests that there is not a simple (or strong) mechanistic link between body size and nesting behaviour in *Peromyscus* mice — differences in motivation to nest are not simply a result of differences in body size and corresponding changes in thermal inertia, even though minor changes in weight can impact physiological forms of thermoregulation in rodents (McNab, 1974).

Body size aside, it is reasonable to hypothesize that climate could alter this thermoregulatory behaviour. Other studies have suggested that climate (King et al., 1964) or microclimate (Wolfe, 1970) is associated with use of nesting material by animals in the genus. We find no evidence for a relationship between nesting latency and average winter temperatures or latitude of origin, which is frequently used as a proxy for temperature. Of course, these species might use habitats in ways that mitigate the effects
of ambient winter temperatures. For example, both subspecies of \textit{P. polionotus} construct elaborate burrows, which function as microclimates and buffer the animals from dramatic changes in ambient temperature (Hayward, 1965; Sealander, 1952; Weber et al., 2009). Thus, we tested for a specific association between burrowing behaviour and nesting, which may arise from a trade-off between time invested in the two behaviours (for a negative relationship), or alternatively, reflect some common home-building impulse (in the case of a positive relationship). However, we found no evidence for a relationship between nesting latency and burrowing behaviour in the genus – the short nesting latency observed in these burrowing species is also shared by a species that does not burrow at all, \textit{P. californicus} (Weber et al., 2009), and one that builds only short, simple burrows, \textit{P. m. nubiterrae} (Hu et al., 2017). Short nesting latency, therefore, does not appear to be merely a by-product of burrowing behaviour. In fact, none of these broader abiotic environmental factors appear to have a simple relationship with nesting behaviour in our assay.

We next asked whether nesting latency might be influenced by social environment—in particular, mating system, which varies even among these closely related species (Turner et al., 2010). In three of these species, there is strong evidence for a monogamous mating system (Foltz, 1981; Jašarević et al., 2013; Ribble et al., 1990; Turner et al., 2010), and in an additional subspecies, \textit{P. m. nubiterrae}, there is evidence for nest co-occupancy and a degree of paternal care that is generally indicative of monogamy (Wolff et al., 1991). \textit{P. m. bairdii} and \textit{P. leucopus} animals are thought to be promiscuous (Jašarević et al., 2013; Turner et al., 2010) and, while the mating system of \textit{P. gossypinus} animals has not been tested directly, home range overlap, sexual dimorphism in size, and some field observations are all consistent with promiscuity (Dewsbury et al., 1980; McCarley, 1959; P. G. Pearson, 1953). Our results suggest that mating system and nesting latency are not independent, with monogamous species having short latencies to nest. It is possible that the nesting latency phenotype reflects a tendency to invest in a home territory that is more beneficial for monogamous animals than for promiscuous ones. Patterns of
territory use and mating system are often linked in mammals (Kleiman, 1977). In voles, for instance, home range and mating system are correlated, with males of promiscuous species ranging further in search of mating opportunities (Gaulin et al., 1988), and intraspecific variation in territory use is linked to mating success in monogamous prairie voles (Okhovat et al., 2015). If there is a trade-off between investing time and resources in building a nest versus searching for mates, the optimal allocation of time to nesting may be very different for promiscuous species than monogamous ones. It is also possible that selection for increased paternal care, a hallmark of monogamous mating systems that includes reproductive nesting (Kleiman, 1977), might result in increased nesting even in a purely thermoregulatory context. This is particularly interesting given the slightly higher nest scores achieved by males in two of these monogamous species, who would not undergo the physiological changes that improve nesting in pregnant and lactating rodent mothers of other species (Fleming et al., 1974; Lisk et al., 1969). In either case, we find baseline motivation to engage in nesting behaviour is more related to species-typical social environment than to any of the abiotic environmental factors we examined.

CONCLUSION

Measurement of extended phenotypes such as nests allows us to study how behaviours evolve within and between species. Here we showed that, while the ability to nest is relatively conserved in the genus Peromyscus, latency to begin nest construction is highly variable. This result suggests that evolution of nesting behaviour in these animals is characterized by differences in the prioritization of an otherwise conserved behavioural pattern. Intriguingly, while abiotic environment cannot explain these species differences in nesting behaviour, there is a link between latency to nest and mating system, with monogamous species prioritizing nesting. This highlights the importance of considering social environment when investigating the causes of behavioural change between species. Finally, as the differences in nesting behaviour in Peromyscus appear to be largely changes in the motivation to nest,
future studies in this system may elucidate genetic and neurobiological mechanisms that lead to
differences in motivation to engage in particular behaviours, a topic with implications far beyond nesting
behaviour.

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**Supplemental Table S1: Detailed Nest Scoring Criteria**

<table>
<thead>
<tr>
<th>Score</th>
<th>Score Description</th>
<th>Shredding</th>
<th>Nest Site¹</th>
<th>Walls²</th>
<th>Overhead Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No Manipulation</td>
<td>None</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>Minor Shredding</td>
<td>Minor: ≤ top of 1 nestlet</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Extensive Shredding</td>
<td>Extensive: &gt; top of 1 nestlet</td>
<td>No: neither a nor b is true</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>1.5</td>
<td>Ambiguous Nest Site</td>
<td>Extensive</td>
<td>Unclear: either a OR b is true</td>
<td>No</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Platform Nest</td>
<td>-</td>
<td>Yes: both a and b are true</td>
<td>No: &lt;½ sphere height for &lt;½ circumference</td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>Partial Cup Nest</td>
<td>-</td>
<td>Yes</td>
<td>Partial: &lt;½ sphere height for &gt; ½ circumference OR ≥½ sphere height for &lt;½ of circumference</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Cup Nest</td>
<td>-</td>
<td>Yes</td>
<td>Yes: ≥½ sphere height for ≥½ circumference</td>
<td>No overhead cover</td>
</tr>
<tr>
<td>3.5</td>
<td>Partial Dome Nest</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Partial: &lt;50% of the sphere is covered or there are multiple entrance holes</td>
</tr>
<tr>
<td>4</td>
<td>Full Dome Nest</td>
<td>-</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes: ≥50% of the sphere is covered and there is at most one entrance hole</td>
</tr>
</tbody>
</table>

1. A nest site is defined according to two criteria: (a) there is a contiguous concentration of nestlet around a central point consisting of ≥90% of any material the animal has shredded, and (b) the shape of the putative nest site is defined by the shredded material (>50% of the nestlet at the nest site is shredded).

2. To evaluate walls, imagine that the nest cavity is filled by a sphere, sensu (Hess et al., 2008). The walls are compared to the height of the sphere within the cavity, and the proportion of the circumference of the sphere that is surrounded by walls is noted.
### Supplemental Table S2: Pairwise species comparisons of 1hr median and overnight maximum scores

<table>
<thead>
<tr>
<th>Species</th>
<th>man. nub.</th>
<th>man. baird</th>
<th>pol. sub.</th>
<th>pol. leuco</th>
<th>gossy.</th>
<th>calif. (5g)</th>
<th>calif. (20g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N:</td>
<td>47</td>
<td>95</td>
<td>130</td>
<td>37</td>
<td>35</td>
<td>27</td>
<td>48</td>
</tr>
<tr>
<td>man. baird</td>
<td>W=4046, P&lt;2.2x10^{-16}</td>
<td>W=5306.5, P=1</td>
<td>W=1353.5, P=0.0025</td>
<td>W=746.5, P=1</td>
<td>W=362, P=0.59</td>
<td>W=1438.5, P=2.9x10^{-7}</td>
<td>W=666, P=0.00024</td>
</tr>
<tr>
<td>pol. sub.</td>
<td>W=2565, P=1</td>
<td>W=734, P&lt;2.2x10^{-16}</td>
<td>W=1353.5, P=0.0025</td>
<td>W=746.5, P=1</td>
<td>W=362, P=0.59</td>
<td>W=1438.5, P=2.9x10^{-7}</td>
<td>W=666, P=0.00024</td>
</tr>
<tr>
<td>pol. leuc.</td>
<td>W=924.5, P&lt;2.2x10^{-16}</td>
<td>W=256.6, P=1</td>
<td>W=3088.5, P=0.16</td>
<td>W=746.5, P=1</td>
<td>W=362, P=0.59</td>
<td>W=1438.5, P=2.9x10^{-7}</td>
<td>W=666, P=0.00024</td>
</tr>
<tr>
<td>leucop.</td>
<td>W=1159.5, P=0.027</td>
<td>W=375, P=1.7x10^{-15}</td>
<td>W=3589.5, P=2.5x10^{-6}</td>
<td>W=929.5, P=0.023</td>
<td>W=362, P=0.59</td>
<td>W=1438.5, P=2.9x10^{-7}</td>
<td>W=666, P=0.00024</td>
</tr>
<tr>
<td>gossy.</td>
<td>W=1095, P=2.3x10^{-6}</td>
<td>W=1036, P=0.29</td>
<td>W=3176, P=4.6x10^{-10}</td>
<td>W=881.5, P=1.8x10^{-9}</td>
<td>W=763.5, P=0.0002</td>
<td>W=1221, P=1.2x10^{-9}</td>
<td>W=576, P=4.9x10^{-7}</td>
</tr>
<tr>
<td>califor.</td>
<td>W=990.5, P=1</td>
<td>W=103, P&lt;2.2x10^{-16}</td>
<td>W=3492, P=1</td>
<td>W=678.5, P=1</td>
<td>W=281, P=3.1x10^{-6}</td>
<td>W=74.5, P=2.4x10^{-9}</td>
<td>W=745, P=2.4x10^{-9}</td>
</tr>
</tbody>
</table>

The results of pairwise Wilcoxon rank-sum tests for species differences in median 1hr scores (below diagonal) or maximum overnight scores (above diagonal). For each comparison, test statistics (W) and Bonferroni-corrected p-values are reported; significant results (p<0.05) are in bold.
Supplemental Table S3: Sex differences in nest scores

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Males</th>
<th>Females</th>
<th>1hr Median Score</th>
<th>Maximum Overnight Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>maniculatus nubiterrae</em></td>
<td>31</td>
<td>16</td>
<td>( W=119.5, P=0.03 )</td>
<td>( W=266, P=1 )</td>
</tr>
<tr>
<td><em>maniculatus bairdii</em></td>
<td>62</td>
<td>33</td>
<td>( W=858, P=0.11 )</td>
<td>( W=1173, P=1 )</td>
</tr>
<tr>
<td><em>polionotus subgriseus</em></td>
<td>80</td>
<td>50</td>
<td>( W=1262.5, P=0.002 )</td>
<td>( W=1361, P=0.008 )</td>
</tr>
<tr>
<td><em>polionotus leucocephalus</em></td>
<td>23</td>
<td>14</td>
<td>( W=163.5, P=1 )</td>
<td>( W=133, P=1 )</td>
</tr>
<tr>
<td><em>leucopus</em></td>
<td>22</td>
<td>13</td>
<td>( W=125.5, P=1 )</td>
<td>( W=152, P=1 )</td>
</tr>
<tr>
<td><em>gossypinus</em></td>
<td>19</td>
<td>8</td>
<td>( W=87, P=1 )</td>
<td>( W=84, P=1 )</td>
</tr>
<tr>
<td><em>californicus</em></td>
<td>25</td>
<td>23</td>
<td>( W=219.5, P=1 )</td>
<td>( W=238.5, P=1 )</td>
</tr>
</tbody>
</table>

The results of pairwise Wilcoxon rank-sum tests for sex differences in 1hr median nest scores or maximum overnight nest scores. Sample sizes, test statistics (W), and Bonferroni-corrected p-values are reported; significant results (\( P<0.05 \)) are in bold.
**Supplemental Table S4:** Spearman correlations between weight and nest scores within species/sex groups

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Weight vs. 1hr Median Score</th>
<th>Weight vs. Maximum Overnight Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>maniculatus nubiterrae</td>
<td>$r_s=0.24, N=7, P=1$</td>
<td>$r_s=-0.09, N=6, P=1$</td>
</tr>
<tr>
<td>maniculatus bairdii</td>
<td>$r_s=0.22, N=58, P=1$</td>
<td>$N=29$, see below</td>
</tr>
<tr>
<td>polionotus subgriseus</td>
<td>$r_s=0.01, N=76, P=1$</td>
<td>$r_s=0.04, N=48, P=1$</td>
</tr>
<tr>
<td>polionotus leucocephalus</td>
<td>$r_s=0.03, N=23, P=1$</td>
<td>$r_s=-0.10, N=14, P=1$</td>
</tr>
<tr>
<td>leucopus</td>
<td>$r_s=0.41, N=22, P=0.82$</td>
<td>$r_s=0.22, N=13, P=1$</td>
</tr>
<tr>
<td>gossypinus</td>
<td>$r_s=-0.25, N=17, P=1$</td>
<td>$r_s=-0.24, N=7, P=1$</td>
</tr>
<tr>
<td>californicus</td>
<td>$r_s=-0.08, N=23, P=1$</td>
<td>$r_s=0.14, N=23, P=1$</td>
</tr>
</tbody>
</table>

Sample sizes, Spearman correlation coefficient ($r_s$), and Bonferroni-corrected p-values are reported. Sample sizes are smaller than for other tests due to missing weight data. We were unable to perform correlations between 1hr scores and weight within *maniculatus bairdii* females because all 29 animals received a median score of 0 at 1 hour. Similarly, all female *gossypinus* animals produced maximum scores of 4 at the overnight time point.
Supplemental Figure S1: Maximum 1 hour nest scores. Maximum nest score achieved 1h after receiving new nesting material over the 3 trial days. Letters indicate species groups that did not significantly differ from one another, while all other pairwise comparisons were significant (Wilcoxon rank-sum test, Bonferroni-corrected $P<0.05$), largely consistent with median scores (Fig. 2B, Supplemental Table S2), with the exception of a significant difference in maximum scores between $P.\ gossypinus$ and $P.\ m.\ bairdii$ animals (Wilcoxon rank-sum test, Bonferroni-corrected $P=0.049$). Sample sizes provided below.
Supplemental Figure S2: Effect of increasing nesting material in *P. californicus*. Adult animals (*N*=21) were given increasing amounts of nesting material (5g, 10g, 15g and 20g) on 4 sequential days. Higher amounts of nesting material increased overnight nest scores (Friedman repeated measures test, *P*=0.003).
Supplemental Figure S3: Sex differences in maximum overnight nest scores. Evidence for a significant sex difference overnight nest score occurred in only one taxon: in *P. polionotus subgriseus*, males built higher scoring nests at the overnight time point than females (Wilcoxon rank-sum test, Bonferroni-corrected *P*=0.008). Sample sizes are provided below. NS = not significant.
Supplemental Figure S4: Effect of body weight on nesting behaviour by species and sex. (A) Median nest scores at 1h were not correlated with weight within any species-sex group (Spearman correlation, Bonferroni-corrected $P>0.05$). (B) Overnight maximum nest scores were not correlated with weight within any species-sex group (Spearman correlation, Bonferroni-corrected $P>0.05$). Note different x-axis for each species as indicated. Sample sizes are provided by sex and were the same for both time points.