Canadian Journal of Zoology
Revue canadienne de zoologie

Wild Peromyscus adjust maternal nest-building behaviour in response to ambient temperature

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2016-0236.R1
Manuscript Type:	Article
Date Submitted by the Author:	31-Jan-2017
Complete List of Authors:	Stewart, Frances; University of Victoria, McAdam, Andrew; University of Guelph, Department of Integrative Biology
Keyword:	BEHAVIOUR < Discipline, heat dissipation limit hypothesis, nest-building, Peromyscus maniculatus, deer mouse, THERMOREGULATION < Discipline, TEMPERATURE < Discipline

Wild *Peromyscus* adjust maternal nest-building behaviour in response to ambient temperature

F.E.C. Stewart and A.G. McAdam

F.E.C. Stewart and A.G. McAdam. University of Guelph, Department of Integrative Biology, 50 Stone Road East, Guelph, Ontario, Canada, N1G 2W1 (emails: fecstewart@gmail.com and amcadam@uoguelph.ca)

Corresponding author:
Frances E.C. Stewart
University of Victoria
School of Environmental Studies
3800 Finnerty Rd.
Victoria, BC, Canada
V8W 2L7
fecstewart@gmail.com
Ph: 250-818-0796
Fax: 250-721-8985

F.E.C. Stewart. Present address: University of Victoria, School of Environmental Studies, 3800 Finnerty Rd., Victoria, BC, Canada, V8W 2L7 (email: fecstewart@gmail.com)

Wild *Peromyscus* adjust maternal nest-building behaviour in response to ambient temperature

F.E.C. Stewart and A.G. McAdam

Abstract: The heat dissipation limit hypothesis (HDL) suggests that energy output during lactation in mammals might be constrained by their ability to dissipate heat. This hypothesis predicts that wild mammals ought to adjust nest insulation in response to heat load, but these predictions have rarely been tested in wild mammals. Here we developed a simple score of nest-building for wild deer mice (*Peromyscus maniculatus*, Wagner 1845) on an ordinal scale from 0 to 4, based on three qualitative and easy to observe aspects of nest-building behaviour: bedding quality, nest shape, and mouse visibility. We used this measure to track 472 nest-building observations across 14 wild deer mice that were brought into captivity, and housed under pseudo-ambient temperatures across one reproductive event. Our observations of *Peromyscus* nest-building behaviour provide varying support for the HDL hypothesis; there is a negative effect of ambient temperature on nest-building behavior and lactating females became more sensitive to temperature as days post-partum increased, however, females generally build more elaborate nests in lactation than other reproductive states and there are no effects of litter size, total pup mass, or days post-partum on nest scores during lactation. Our observations have broad implications for quantifying behaviours in nest-building species and metabolic relationships in wild mammals.

Keywords: behaviour, heat dissipation limit hypothesis, nest-building, *Peromyscus maniculatus*, deer mouse, temperature, thermoregulation

Introduction

The heat dissipation limit hypothesis (HDL) proposes that the ability of mammals to export energy during lactation can be limited by their ability to dissipate heat generated by high rates of metabolism (Król and Speakman 2003). Laboratory experiments manipulating thermal conductance in rodents have identified that heat load can have important effects on milk production across species (Król and Speakman 2003; Król et al. 2007; Wu et al. 2009; Valencak and Ruf 2009; 2010; Speakman and Król 2010; Yang et al. 2013; Sadowska et al. 2016), but the degree to which lactation in wild rodents is affected by heat load is not yet known. Unlike laboratory rodents, wild animals have greater capacity to adjust heat load during lactation through behaviour, by changing space use or the thermal properties of nests. The heat dissipation limit hypothesis would, therefore, predict that wild rodents ought to change behaviours that affect heat dissipation in response to changes in heat load during lactation. Alternatively, the nest may have no thermoregulatory function and instead act as a resting place. In a rare test of this hypothesis, Guillemette et al. (2009) found that red squirrels raised offspring in more insulated nests when heat load was low, but switched to less insulated nests when heat load associated with lactation was high. In addition to switching between nests with different insulative capacities, wild rodents might also adjust the insulative properties of nests in response to heat load, but this has not yet been investigated.

Deer mice (*Peromyscus*) are a widely studied genus of rodents amenable to both field and laboratory investigation (Wolff 2003; Bedford and Hoekstra 2015). This species has a 21-day gestation length and experiences post-partum estrus, during which weaning takes approximately the same duration as the next gestation. They typically inhabit deciduous forests and build both arboreal and sub-terrestrial nests (King 1968). Nest-building behaviour was first described for

Peromyscus in Eisenberg (1968) and Layne (1969) and refers to searching, assembling, and arranging insulating materials that compose an individual's nest (Eisenberg 1968). It has previously been measured as the mass (g) of cotton used to build the nest (Wolfe 1970; Lynch 1974; Glaser and Lustick 1975). Deacon (2006) developed a protocol for scoring nest-building in laboratory mice on a 5-point scale, but a consistent protocol for quantifying nest-building behaviour in wild rodents has yet to be developed. Previous mouse studies have associated nest-building behaviour with measures of fitness (lifetime reproductive success; Bult and Lynch 1997), stereotypies (Wolmarans et al. 2016), and thermoregulatory behaviours (Lynch and Possidente 1978). In *Mus*, nest-building behaviour is heritable (Bult and Lynch 1996; 1997) and strongly selected for when associated with thermoregulation (Bult and Lynch 1996; 1997). Lynch (1974) found that *P. leucopus* built larger nests under simulated winter conditions compared to summer conditions, but it is not clear whether reproductive mice adjust nest-building in response to changes in summer ambient temperatures that might reflect heat load during lactation.

Here we quantified nest-building behaviour by *Peromyscus maniculatus* (Wagner 1845) on an ordinal scale, and used this to test the hypothesis that mice would adjust nest-building behaviour in response to heat load during lactation. In particular, we brought wild *P. maniculatus* into captivity but allowed ambient temperatures in captivity to fluctuate with natural temperatures during lactation. Following the heat dissipation limit hypothesis, we predicted 1) that wild mice would make larger and more covered nests when ambient temperatures were lower but smaller and more open nests when ambient temperatures were higher, and 2) that reproductive females would demonstrate a greater response to temperature than non-reproductive

females, with lactating rather than pregnant females demonstrating the lowest nest-insulation values.

Materials and methods

Study area

Wild *P. maniculatus* from Algonquin Provincial Park, Ontario, Canada (45°37'N, 78°21'W) were brought into captivity for the month of June and July 2011 as part of a study of maternal nursing behaviour (Stewart and McAdam 2014; Stewart et al. 2014). Briefly, female deer mice were captured within 25 km of the Algonquin Wildlife Research Station using Longworth live traps (13.8 x 6.4 x 8.4 cm). All traps were baited with polyester bedding and soaked sunflower seeds (*Helianthis* sp.) at dusk, and then checked at dawn (*sensu* Fryxell et al. 1998; Falls et al. 2007).

Laboratory protocol and nest-building behaviours

Fourteen female *P. maniculatus* captured from the field were transported to a field laboratory at the Algonquin Wildlife Research Station for maternal observation. Females were housed individually in transparent plexiglass cages (35 x 15 x 17 cm) and were provided with *ad libitum* access to water and food (2014 Rodent Chow 14% protein). Importantly, a condensed square of cotton bedding common to laboratory rodents (Ancare Nestlets; 2.5 g) was provided in addition to Corncob (Harlan Corn Cob bedding No, 7092) and paper bedding (Andersons CN Crink-I' nest^{TM}), as well as a toilet paper roll for environmental enrichment (Macri and Würbel 2006). Photoperiod in captivity was consistent with seasonal changes in photoperiod and unlike most laboratory studies, natural ventilation allowed ambient temperatures in the laboratory to

track natural variation in ambient temperatures in the surrounding forest. Temperature recordings were averaged from two ThermoChron iButton® data loggers (model # DS1990A; San Jose, CA) placed on observation desks at $\frac{1}{4}$ and $\frac{3}{4}$ length of the room.

Females were allowed a 24-hour acclimation period after being brought into the laboratory before nest-building behaviours were observed. All laboratory animals were checked twice daily, once at sunrise and once at sunset; at these points the condition of each nest was scored on an ordinal scale from 0 to 4 based on qualitative characteristics of the cotton bedding Nestlet, nest shape, and visibility of the mouse (Table 1). This protocol was similar to Deacon (2006), but some levels of the scoring system differed in important ways because of our personal observations of nest-building by wild *Peromyscus*. First, wild *P. maniculatus* very rarely ignored the Nestlet or did not build a nest at all, so Deacon (2006) levels 2 and 3 were combined. Second, there appears to be much variation in the form of the nest once all the Nestlet has been shredded and a complete nest has been formed. As a result we added an additional level that identified nests that formed a complete dome covering the mouse (Table 1). At each sampling point the ambient temperature in the lab was also recorded. Thus, diurnal and nocturnal nest-building activity was scored for 14 wild *P. maniculatus* females across 48 days (June 2, 2011 through July 12, 2011), resulting in 472 nest scoring observations.

Statistical analyses

We assessed the effect of ambient temperature, Julian date, and reproductive status on nest-building score in two sets of cumulative link mixed models; 1) across all females, and 2) focusing on breeding females. In these models, female identity was fitted as a random effect to

account for the repeated measures collected for each female and date was fitted as a random effect to account for observations of multiple females within a single day. Ambient temperature was confounded with the time of day when regressed against all nest values ($\beta = 3.77 \pm 0.25$, $Z = 14.63$, $p < 0.0004$), but there is still a lot of variation within morning ($18.0\text{ }^{\circ}\text{C} \pm 0.4\text{ }^{\circ}\text{C}$), and evening ($19.9\text{ }^{\circ}\text{C} \pm 0.22\text{ }^{\circ}\text{C}$) temperatures.

Across all females, ambient temperature, Julian date, and reproductive status (nonbreeding, pregnant, or lactating) were fitted as fixed effects. A likelihood ratio test between models with and without female identity investigates the variation of nest-building behavior among females. To assess if nest-building scores are being driven by the time of day a separate model assessed the effect of ambient temperature on nest-building by reproductive status. This model involved an interaction between ambient temperature and reproductive status, with Julian date and reproductive status as fixed effects.

Across breeding mice we tested for an effect temperature and days post-partum on nest-building behavior by incorporating morning ambient temperature, time of day, and an interaction between days post-partum and morning ambient temperature as fixed effects. Effects of litter size, days post-partum, total pup mass, and their interactions with temperature on building behavior of lactating females were investigated by adding each as a fixed effect to a cumulative link model. These models always involved morning laboratory temperature as a fixed effect, with female identity and Julian date as random effects.

All analyses were conducted in the *ordinal* package in R version 3.2.5 (R Core Team 2015), where a linear mixed effects regression framework incorporating a log link function is used, the response variable is treated as ordered, and parameters are reported on a log-odds

scale (Christensen 2015). All model assumptions were met and values represent means $\pm$ *SE* unless otherwise stated.

Ethical note

All research was conducted within Canadian ethical standards under the Canadian Council on Animal Care and conforms to published guidelines for the use of wild animals in research. This research was approved by the University of Guelph Animal Care Committee under the Animal Use Permit No. 08R063.

Results

Female mice conceived their litters between June 22 and July 28 2011. Nine of 14 females gave birth, with 11.7 ± 1.3 days in the laboratory prior to parturition. Litters contained 5.7 ± 0.3 offspring and weighed a total of 9.4 ± 0.4 g.

Nest-building latency was less than 24 hours, as all but one of the first nest scores for each female was greater than zero (median = 2, interquartile range = 1). Morning temperatures in the housing facility averaged 18.0°C ($\pm 0.4^{\circ}\text{C}$), but ranged from 10.0 to 24.5°C . In our cumulative link mixed model, ambient temperature, Julian date, and reproductive status were significantly associated with nest score across all 14 females. Females build more extensive nests when temperatures were low ($\beta = -0.16 \pm 0.04$, $Z = -4.5$, $p < 0.001$), built more extensive nests as the season progressed ($\beta = 0.08 \pm 0.02$, $Z = 3.5$, $p < 0.0004$), and non-breeding females build smaller nests than breeding females ($\beta = -1.4 \pm 0.5$, $Z = -2.6$, $p = 0.009$). A likelihood ratio test between models with and without a random effect of female identify demonstrates that there is significant variation in nest-building behavior among females (likelihood ratio statistic = 18.87, p

< 0.001). Ambient temperature does not vary significantly by reproductive status ($\beta = 0.07 \pm 0.08$, $Z = 0.9$, $p = 0.36$).

For breeding females, there is a significant effect of morning ambient temperature ($\beta = -0.12 \pm 0.06$, $Z = -2.1$, $p = 0.03$) and days post-partum ($\beta = 0.41 \pm 0.12$, $Z = 3.5$, $p = 0.0004$) on nest-building behavior. Lactating females also became more sensitive to temperature as days post-partum progressed (interaction between morning ambient temperature and days post-partum: $\beta = -0.01 \pm 0.005$, $Z = -2.5$, $p = 0.01$). There is no effect of days post-partum ($\beta = -0.02 \pm 0.08$, $Z = -0.20$, $p = 0.77$), litter size ($\beta = 0.37 \pm 0.59$, $Z = 0.62$, $p = 0.53$), total pup mass ($\beta = -0.29 \pm 0.39$, $Z = -0.74$, $p = 0.46$), or their interaction with morning laboratory temperature (days post-partum: $\beta = -0.01 \pm 0.02$, $Z = -0.51$, $p = 0.61$; litter size: $\beta = 0.03 \pm 0.08$, $Z = 0.43$, $p = 0.66$; total pup mass: $\beta = -0.01 \pm 0.06$, $Z = -0.30$, $p = 0.76$) on nest-building behavior of lactating females.

Discussion

Our observations and results demonstrate varied support for the heat dissipation limit hypothesis (HDL). Consistent with predictions of HDL (Król and Speakman 2003), there is a negative effect of ambient temperature on nest-building behavior across females. Within breeding females this negative correlation persists, and the sensitivity of lactating females to temperature increases with the days post-partum. Inconsistent with HDL, females build more elaborate nests in lactation than other reproductive states (Figure 1). There were no effects of litter size, total pup mass, or days post-partum on nest-building behavior, however, sample size is limited. Significant variation among females suggest that maternal nest-building behavior may be repeatable.

Confounds between time of day and ambient temperature may be affecting our results. Across all females, these two effects are collinear, and we therefore did not consider effects of time of day when modeling all 14 females. For nest-building behavior of lactating females, the parameter for temperature across morning and evening ($\beta = -0.21 \pm 0.07$, $Z = -3.1$, $p = 0.002$) was stronger than the parameter for temperature within either morning ($\beta = -0.14 \pm 0.13$, $Z = -1.1$, $p = 0.28$) or evening ($\beta = -0.13 \pm 0.14$, $Z = -0.88$, $p = 0.37$) readings. The effects of ambient temperature and time of day on nest-building behavior are very hard to separate and our current results must be interpreted with caution. Discrepancies in this confound may be larger if we had kept females and their offspring in the laboratory for longer. To follow our Animal Use Protocol we released females and offspring back to the wild on post-natal day seven, and therefore only have nest-building scores from early lactation. However, investigating the interaction between days post-partum and temperature demonstrates that females became more sensitive to temperature through lactation.

An alternative interpretation of these data is that mice build nests that are sufficient to minimize thermoregulation costs, rather than maximize heat dissipation (Additive heat load hypothesis; Hammond et al. 1994). This interpretation could explain why females build more elaborate nests when it gets colder and why there is no effect of litter size, but it would not explain why they subsequently reduce the size of their nests when temperatures become warmer, as observed here. Experimental manipulations of temperature, litter size, and nesting material would aid in teasing apart these two interpretations, however, within our current study we do not show support for the additive heat load hypothesis. It is possible that nonbreeding females simply use the nest as a resting structure, but we reject this proposed alternative hypothesis

because of the general negative relationship between nest-building and ambient temperature across females.

Changes in nest-building have the potential to affect investigations of maternal behaviour. *Peromyscus* have been a common laboratory and field model for many observational and experimental studies (Sadlier 1965; Gubernick and Nelson 1989; Wolff 2003; Bedford and Hoekstra 2015; Borniger and Nelson 2016). However, behaviours located within the nest, such as maternal care, nursing, resting, sleeping, or potentially communication, may be visually obstructed if the nest score is high. Stewart and McAdam (2014) were not able to tease apart different types of maternal care in wild *Peromyscus* as accurately as laboratory *Mus musculus* studies (Curley et al. 2008; Champagne 2011), partly because high-scoring nests obscured maternal behaviours. This problem may also be present for other important aspects of *Peromyscus* life history, such as maternal infanticide and ano-genital licking, which are associated with important endocrine effects (Zehr et al. 2000; Kerin et al. 2003; Ophir and delBarco-Trillo 2007; Manno 2008). As such, environmentally driven adjustments or individual variation in nest-building behaviour have the potential to pattern missing data in a nonrandom way (Hadfield 2008).

Acknowledgements

Funding for this project was received from the Natural Sciences and Engineering Research Council of Canada, as well as the Ontario Ministry of Research and Innovation. We are grateful to R. Do, D. Pond, P. LeSommer and S. Boyle for field assistance, Algonquin Wildlife Research Station staff for technical assistance, and Algonquin Provincial Park for research and logistical support.

References

- Bedford, N.L., and Hoekstra, H.E. 2015. *Peromyscus* mice as a model for studying natural variation. *eLife*, **4**:1–13.
- Borniger, J.C., and Nelson, R.J. 2016. Photoperiodic Regulation of Behaviour: *Peromyscus* as a Model System. *Semin. Cell Dev. Biol.* **61**:82-91. Academic Press.
- Bult, A., and Lynch, C.B. 1996. Multiple selection responses in house mice bidirectionally selected for thermoregulatory nest-building behaviour: Crosses of replicate lines. *Behav. Genet.* **26**(4): 439–446.
- Bult, A., and Lynch, C.B. 1997. Nesting and fitness: lifetime reproductive success in house mice bidirectionally selected for thermoregulatory nest-building behaviour. *Behav. Genet.* **27**(3): 231-240.
- Champagne, F.A. 2011. Maternal imprints and the origins of variation. *Horm. Behav.* **60**(1):4-11.
- Christensen, R. H. B. 2015. ordinal - Regression Models for Ordinal Data. R package version 2015.6-28. <http://www.cran.r-project.org/package=ordinal/>.
- Curley, J.P., Davidson, S., Bateson, P., and Champagne, F.A. 2008. Social enrichment during postnatal development induces transgenerational effects on emotional and reproductive behaviour in mice. *Front. Behav. Neurosci.* **3**: 1–14.
- Deacon, R.M. 2006. Assessing nest building in mice. *Nature Protocols*, **1**(3):1117-1119.
- Falls, J.B., Falls, E.A., and Fryxel, J.M. 2007. Fluctuations of deer mice in ontario in relation to seed crops. *Ecol. Monogr.* **77**(1): 19–32.
- Fryxel, J.M., Falls, J.B., Falls, E.A., and Brooks, R.J. 1998. Long-term dynamics of small-mammal populations in Ontario. *Ecology*, **79**(1):213-225.
- Glaser, H., and Lustick, S. 1975. Energetics and nesting behaviour of the Northern White-Footed

- Mouse, *Peromyscus leucopus noveboracensis*. *Physiol. Zool.* **48**(2): 105–113.
- Gubernick, D.J., and Nelson, R.J. 1989. Prolactin and paternal behaviour in the biparental California mouse, *Peromyscus californicus*. *Horm. Behav.* **23**(2): 203–210.
- Guillemette, C.U., Fletcher, Q.E., Boutin, S., Hodges, R.M., McAdam, A.G., and Humphries, M.M. 2009. Lactating red squirrels experiencing high heat load occupy less insulated nests. *Biol. Lett.* **5**(2): 166–168.
- Hadfield, J.D. 2008. Estimating evolutionary parameters when viability selection is operating. *Proc. Biol. Sci.* **275**(1635): 723–734.
- Hammond, K.A., Konarzewski, M., Torres, R.M. and Diamond, J. 1994. Metabolic ceilings under a combination of peak energy demands. *Physiol. Zool.* **67**(6):1479-1506.
- Kerin, T.K., Volger, G.P., Blizard, D.A., Stout, J.T., McClearn, G.E., and Vandenberg, D.J. 2003. Anogenital distance measured at weaning is correlated with measures of blood chemistry and behaviours in 450-day-old female mice. *Physiol. Behav.* **78**: 697–702.
- King, J.A. 1968. *Biology of Peromyscus (Rodentia)*, second edition. The American Society of Mammalogists Spec. Publ. No. 2.
- Król, E., Murphy, M., and Speakman, J.R. 2007. Limits to sustained energy intake. X. Effects of fur removal on reproductive performance in laboratory mice. *J. Exp. Biol.* **210**(23): 4233–4243.
- Król, E., and Speakman, J. 2003. Limits to sustained energy intake VI. Energetics of lactation in laboratory mice at thermoneutrality. *J. Exp. Biol.* **206**(23): 4255–4266.
- Lynch, C.B. 1974. Environmental modification of nest-building in the white-footed mouse, *Peromyscus leucopus*. *Anim. Behav.* **22**(2): 405–409.
- Lynch, C.B., and Possidente, B.P. 1978. Relationships of maternal nesting to thermoregulatory

- nesting in house mice (*Mus musculus*) at warm and cold temperatures. *Anim. Behav.* **26**: 1136–1143.
- Macrì, S., and Würbel, H. 2006. Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. *Horm. Behav.* **50**(5): 667–680.
- Manno, F.A. 2008. Measurements of the digits lengths and the anogenital distance in mice. *Physiol. Behav.* **93**: 364–368.
- Valencak, T.G. and Ruf, T. 2009. Energy turnover in European hares is centrally limited during early, but not during peak lactation. *J. Comp. Physiol. B.* **179**(8):933–943.
- Sadlier, R.M.F.S. 1965. The Relationship between Agonistic Behaviour and Population Changes in the Deermouse, *Peromyscus maniculatus* (Wagner). *J. Anim. Ecol.* **34**(2): 331–352.
- Sadowska, E.T., Król, E., Chrzascik, K.M., Rudolf, A.M., Speakman, J.R. and Koteja, P. 2016. Limits to sustained energy intake. XXIII. Does heat dissipation capacity limit the energy budget of lactating bank voles? *J. Exp. Biol.* **219**(6): 805–815.
- Speakman, J.R., and Król, E. 2010. Maximal heat dissipation capacity and hyperthermia risk: neglected key factors in the ecology of endotherms. *J. Anim. Ecol.* **79**(4): 726–746.
- Stewart, F.E.C., and McAdam, A.G. 2014. Seasonal plasticity of maternal behaviour in *Peromyscus maniculatus*. *Behaviour* **151**(11): 1641–1662.
- Stewart, F.E.C., Brooks, R.J., and McAdam, A.G. 2014. Seasonal adjustment of sex ratio and offspring masculinity by female deer mice is inconsistent with local resource competition. *Evol. Ecol. Res.* **16**: 153–164.
- Wickham, H. 2009. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Wolfe, J.L. 1970. Experiments on Nest Building Behaviour in *Peromyscus* Rodentia Cricetinae.

- Anim. Behav. **18**(4): 613–615.
- Wolff, J.O. 2003. Laboratory studies with rodents: facts or artifacts? Bioscience **53**(4): 421–427.
- Wolmarans, D.W., Stein, D.J., and Harvey, B.H. 2016. Excessive nest building is a unique behavioural phenotype in the deer mouse model of obsessive-compulsive disorder. J. Psychopharmacol. **30**(9):867-874.
- Wu, S.H., Zhang, L.N., Speakman, J.R. and Wang, D.H. 2009. Limits to sustained energy intake. XI. A test of the heat dissipation limitation hypothesis in lactating Brandt's voles (*Lasiopodomys brandtii*). J. Exp. Biol. **212**(21):3455-3465.
- Yang, D.B., Li, L., Wang, L.P., Chi, Q.S., Hambly, C., Wang, D.H. and Speakman, J.R. 2013. Limits to sustained energy intake. XIX. A test of the heat dissipation limitation hypothesis in Mongolian gerbils (*Meriones unguiculatus*). J. Exp. Biol. **216**(17): 3358-3368.
- Zehr, J.A., Gans, S.E., and McClintock, M.K. 2000. Variation in reproductive traits is associated with short anogenital distance in female rats. Dev. Psychobiol. **38**: 229–238.

Tables

Table 1. – Maternal nest-building behaviour by *Peromyscus maniculatus* was scored based on three qualitative categories. This scoring system differed from Deacon (2006) because *P. maniculatus* almost always had some form of nest (i.e. Deacon scores 2, 3 combined) and there appeared to be much variation in the structure of the nest once all of the Nestlet was shredded and a full nest was formed (i.e. Deacon score 5 was split into two levels).

Score	Cotton bedding quality*	Nest shape	Mouse visibility	Deacon (2006) score
0	Cotton not touched	No nest	Dorsal, ventral, lateral	1
1	Slight tearing	No nest	Dorsal, ventral, lateral	2, 3
2	Mostly torn	Cup-shaped	Dorsal and lateral	4
3	Entirely torn	Bowl-shaped	Only dorsal	5
4	Entirely torn	Complete dome	Could not see mouse	NA

*Ancare Nestlets were used as cotton bedding (approx. 2.5 g). Scores may change if a different product is used.

Figures

Fig. 1. – Nest scores varied with reproductive status across 14 deer mice (*Peromyscus maniculatus* (Wagner 1845)). Inconsistent with the heat dissipation limit hypothesis (HDL) lactating (L) females build more elaborate nests than either pregnant (P) or nonbreeding (NB) females, but a negative effect of temperature supported the HDL. Violin plots, generated from the R package *ggplot2* (Wickham 2009), of raw data (jittered for visibility) are shown. Statistical models also considered effects of Julian date and accounted for repeated observations of individual females within each day.

Draft