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Lactating red squirrels experiencing high heat load occupy less insulated nests

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The heat dissipation limit hypothesis suggests that the capacity for lactating mammals to transfer energy to their offspring through milk may be constrained by limits on heat dissipation, particularly in species that raise offspring in well-insulated nests. We tested a prediction of this hypothesis by evaluating whether lactating free-ranging red squirrels (*Tamiasciurus hudsonicus*) occupy less insulated nests when experiencing conditions that increase heat load. In support of the hypothesis, when climate normal ambient temperatures were warm, squirrels supporting large litter masses of furred offspring occupied nests of lower insulative value. These results support the heat dissipation limit hypothesis and suggest that free-ranging mammals may select nests based on their insulative value, not only to reduce heat loss in cold conditions but also to dissipate heat during periods of heat stress.

Keywords: endothermy; heat stress; heat dissipation limit hypothesis; parental care

1. INTRODUCTION

The parental care hypothesis for the evolution of endothermy proposes that endothermy evolved to facilitate the increased aerobic capacity and warm temperatures required for rapid juvenile provisioning and growth (Farmer 2000; Koteja 2000). Considerable research has focused on what limits energy export during lactation (Hammond & Diamond 1997; Bacigalupe & Bozinovic 2002), because higher energy export should allow females to raise more, faster-growing offspring and thereby increase reproductive success (Clutton-Brock 1991; Thompson 1992). The recently proposed heat dissipation limit hypothesis suggests that energy export during lactation is limited by the ability of mothers to dissipate heat associated with milk production (Król & Speakman 2003). Experimental manipulations of the conductance of laboratory animals demonstrates that heat stress reduces the quantity and quality of milk exported (Król & Speakman 2003; Król *et al.* 2007). These findings are intriguing because, although endothermy may have evolved to enhance parental care (Farmer 2000; Koteja 2000), heat stress may presently be limiting energy export during lactation in some mammals.

The implications of the heat dissipation limit hypothesis to free-ranging mammals have never been examined. Heat load experienced by lactating females is a function of heat production, determined by the combined mass and metabolic rate of mothers and offspring occupying the nest, and heat dissipation, determined by the thermal conductance of the mother, nest insulation and environmental conditions. Thus, negative consequences of heat production during lactation are likely to be experienced by mammals raising litters of large mass, in well-insulated nests, during periods of warm ambient temperature. To prevent excessive insulation from becoming a liability to parental care, free-ranging mammals may avoid heat stress by selecting nests with differing insulative values (Puchalski *et al.* 1988). Thus, a key prediction arising from the heat dissipation limit hypothesis is that the insulation of nests occupied by lactating females should be affected by the conditions that influence heat production and dissipation. We tested this prediction on a high latitude population of free-ranging red squirrels (*Tamiasciurus hudsonicus*) that nurse pups across a wide range of ambient conditions, by evaluating whether the insulation of nests occupied by lactating females is less under conditions when heat production within the nest is high and ambient conditions limit heat dissipation.

2. MATERIAL AND METHODS

Adult and pre-weaned juveniles on five study areas in southwestern Yukon, Canada (61° N, 138° W) were enumerated using standardized methodology (McAdam *et al.* 2007). Females give birth to litter sizes averaging 3.1 naked offspring (1–7 pups) that are weaned at approximately 70 days (McAdam *et al.* 2007). Squirrels occupy multiple nests within their territories (S. Boutin 1987–2008, unpublished data). Nests are constructed of grass balls situated in the branches of white spruce (*Picea glauca*) trees, within witches' brooms (*Chrysomyxa arctostaphyli*) infecting spruce trees, spruce or aspen (*Populus tremuloides*) cavities or underground.

We entered 45 nests and recorded the number and mass of pups when they were furless (0–10 days old; furless nest entry) and when they had fur (18–28 days old; furred nest entry). We examined 17 furless and 28 furred nest entries (range of entries 27 May 2007 to 14 August 2007). Nest types included 22 grass, 11 witches' brooms, nine cavities and three underground nests.

Red squirrels routinely move their litters to different nests following entries (S. Boutin 1987–2008, unpublished data), allowing us to quantify the insulation of recently vacated nests. Nest insulation was measured in the evening (start times 18.45–23.45) on the same day of the nest entry ($n=34$ nests), the day after ($n=9$) or two days after ($n=2$). The insulation of vacated nests was determined by comparing the cooling rates of one water bottle that was placed inside the nest, and another that was hung above ground less than 1 m away (Redman *et al.* 1999). Water bottles (HDPE plastic 125 ml, Nalge Nunc International) were filled with hot water from a common thermos. Temperature data loggers (iButton; Maxim Integrated Products) were suspended in the water bottles using a nylon stocking to avoid contact with the bottle walls. Bottle water temperature (T_w; 0.5°C increments) was recorded every 5 s while air filled white balls (ovoids 4.5×3.1 cm), hung less than 5 m from the nest and recorded ambient temperature (T_a). The start point of cooling rates was 10 min after the bottle was placed in the nest, to ensure that the bottle inside the nest had warmed the interstitial air space (Lamprecht & Schmolz 2004). T_w readings taken over 2 h were converted to levels of heat ($Q = C_{H_2O}/[T_w - T_a]$; $C_{H_2O}=$ specific heat of H_2O). Nest insulation was measured as the ratio of

the cooling rate slopes (ln[Q] versus ln[time]) of the outside bottle ($b_{outside}$) over the nest bottle (b_{nest}; insulation $= b_{outside}/b_{nest}$; Redman *et al.* 1999); therefore, more insulated nests had higher insulation values. These measurements were conducted in the evening to reduce sunlight and wind on the nest, and outside bottle. Insulative values did not vary according to T_a at the start time ($p = 0.73$) or wind speed (land adapted Beaufort scale; $p = 0.77$), and there was no difference between the insulation of nests quantified on the day of the nest entry and those that were quantified ≥ 1 day(s) later ($F_{1,43} = 0.40$, $p = 0.53$).

We evaluated how insulation varied according to nest type, heat production within the nest, nest stage (furless versus furred) and ambient temperature. The combined body mass (M_B) of all offspring at the time of nest entry (litter mass: M_L) was used as an index of heat production within the nest. Previous estimates of the field metabolic rate (FMR, kJ d^{-1}; Speakman 1997) of pre-weaned juveniles revealed a strong relationship between $\log_{10} M_B$ and FMR ($b = 1.21 \pm 0.12$ s.e.; $r^2 = 0.50$, $F_{1,104} = 104.7$, $p < 0.001$; Q. E. Fletcher, J. R. Speakman & M. M. Humphries 2003–2006, unpublished data). Lactating female M_B was not incorporated into our index of heat production within the nest because there is no overall relationship between $\log_{10} M_B$ and FMR of lactating females ($b = 0.21 \pm 0.39$; $r^2 = 0.002$, $F_{1,141} = 0.29$, $p = 0.59$; Q. E. Fletcher, J. R. Speakman & M. M. Humphries 2003–2006, unpublished data). We compared the furless versus furred nest stages because furless pups were unlikely to be homeothermic (Knight 1987), and thus contributed less to heat load. To evaluate whether nest insulation varied according to prevailing ambient temperatures during the 24 h prior to the nest entry or according to the expected seasonal temperatures, we obtained mean and maximum daily temperature (T_{mean}, T_{max}; Environment Canada: Haines Junction, Yukon, 60° N, 136° W; approx. 30 km from study areas), and climate normal mean temperature (T_{cn}; 1967–2006; Burwash, Yukon, 61° N, 139° W; approx. 70 km from study areas; T_{cn} values were not available from Haines Junction).

We evaluated the predictors of insulation using linear (LM) and linear mixed models (LME; www.r-project.org; v. 2.5.1). Squirrel identification (ID) was included as a random factor because we determined the insulation of both the furless and furred nest entries for eight females. The inclusion of ID did not improve the fit of the full model (likelihood ratio test: $p > 0.43$), so ID was excluded from subsequent models. Model simplification was used to test our hypotheses that factors increasing the heat production and reducing heat dissipation would be associated with lower insulation levels. To simplify our full factorial models, one effect was removed at each step ($p > 0.10$), with higher order interactions considered first and main effects second. Main effects were retained if their interactions were retained.

3. RESULTS

There was no difference between the insulation of the four nest types ($F_{3,41} = 1.36$, $p = 0.27$). From a full factorial model including nest stage (furless and furred), T_{cn} and M_L (pooling the nest types), model simplification retained the significant or marginally significant two-way interactions of nest stage by T_{cn} ($F_{1,39} = 5.01$, $p = 0.03$) and nest stage by M_L ($F_{1,39} = 3.66$, $p = 0.06$), in addition to the significant main effect of nest stage ($F_{1,39} = 5.26$, $p = 0.03$). Nests were less insulated when pups were furred (3.35 ± 0.13 s.e.) than when pups were furless (3.89 ± 0.21; figure 1*a*). Moreover, the insulation of nests at furred entries, but not furless entries, decreased with increasing M_L and increasing T_{cn} (figure 1*b*). The main effects of T_{cn} ($F_{1,39} = 0.98$, $p = 0.33$) and M_L ($F_{1,39} = 1.78$, $p = 0.19$) were not significant but were retained in the model. In two subsequent analyses that substituted T_{mean} and T_{max} for T_{cn}, neither T_{mean} nor T_{max} were significant ($p > 0.10$) and the same qualitative conclusions for the other parameters remained (analyses not shown). T_{cn} values were left skewed; however, the qualitative conclusions remain with
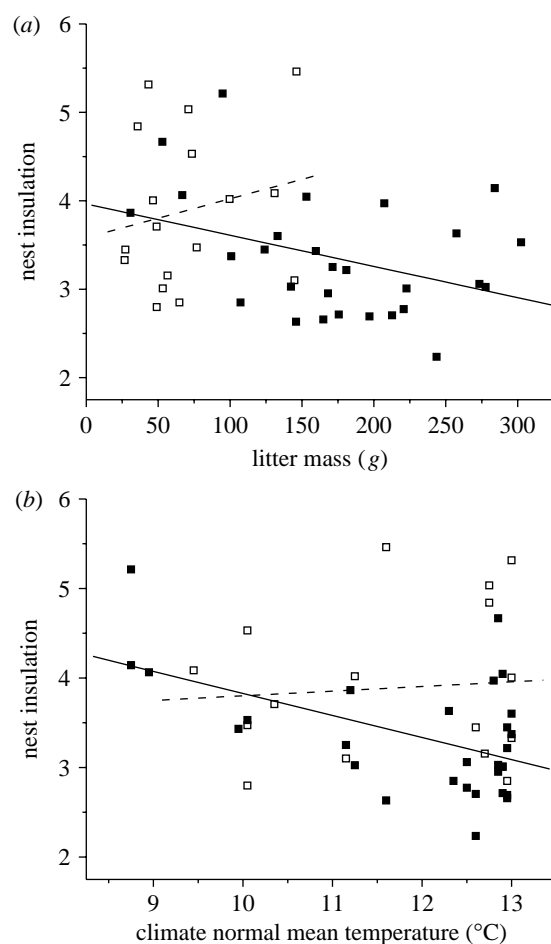

Figure 1. The interactive effects of litter mass (M_L), daily climate normal mean ambient temperature (T_{cn}; 1967–2006) and nest stage on the insulation of nests occupied by lactating squirrels. Nest stages were characterized by juveniles that were furless (open squares and dashed lines) versus furred (solid squares and solid lines). (*a*) The relationship between M_L and insulation was not significant when pups were furless ($r^2 = 0.04$; $F_{16} = 0.59$, $p = 0.46$); however, there was a marginally significant negative relationship when pups were furred ($b = 3.53 \times 10^{-3} \pm 1.72 \times 10^{-3}$ s.e.; $r^2 = 0.14$; $F_{27} = 4.2$, $p = 0.05$). (*b*) The relationship between T_{cn} and insulation was not significant when pups were furless ($r^2 = 0.01$; $F_{16} = 0.09$, $p = 0.76$); however, the relationship was negative when pups were furred ($b = -2.46 \times 10^{-1} \pm 8.17 \times 10^{-2}$, $r^2 = 0.26$; $F_{27} = 9.1$, $p = 0.006$).

reduced significance ($p < 0.076$) when Box–Cox [$\lambda = 8$] transformed T_{cn} values are substituted into the final model.

4. DISCUSSION

Our results suggest that squirrels occupy less insulated nests in response to the conditions that increase heat load. Overall, more insulated nests were occupied when pups were furless and this was not affected by the variation in M_L and T_{cn}. Moreover, females occupied less insulated nests when caring for furred offspring when M_L was larger. Furless pups are unlikely to be homeothermic (Knight 1987); therefore, they are less likely to contribute to excessive heat load and they might also benefit from the warmth and thermal stability offered by well-insulated nests (Newkirk *et al.* 1998).

Weaker relationships suggested that females caring for furred offspring occupied less insulated nests in response to warmer T_{cn}, but were not influenced by T_{mean} or T_{max}. Past research has shown that Djungarian hamsters (*Phodopus sungorus*) alter nest properties in response to both photoperiod and short-term T_a variation (Puchalski *et al.* 1988). Our findings suggest that squirrels may match nest insulation to prevailing climatic norms. Long-term climatic averages may provide a more reliable basis for thermoregulatory decisions than short-term weather patterns, since the latter are less predictable and more prone to rapid reversals (Bronson 1985).

The heat dissipation limit hypothesis proposes that energy export during lactation is limited by the ability of mothers to dissipate body heat (Król & Speakman 2003). Our results suggest that heat dissipation is an important issue for lactating squirrels, even in high latitude environments, but also show that free-ranging squirrels can mitigate potential negative impacts of heat stress by reducing nest insulation. Squirrels may match nest insulation to heat load conditions by altering the insulative properties of a given nest (e.g. by adding or removing grass) or by switching nests based on thermal properties. Resolving the energetic effectiveness and parental care importance of these insulative adjustments requires expanding our research beyond quantifying nest insulation to examine how factors affecting heat load (e.g. litter mass, ambient temperature) influence the temperature profiles of occupied nests, maternal nest attendance and ultimately reproductive performance.

Bacigalupe, L. D. & Bozinovic, F. 2002 Design, limitations and sustained metabolic rate: lessons from small mammals. *J. Exp. Biol.* **205**, 2963–2970.

Bronson, F. H. 1985 Mammalian reproduction: an ecological perspective. *Biol. Reprod.* **32**, 1–26. (doi:10.1095/biolreprod32.1.1)

Clutton-Brock, T. H. 1991 *The evolution of parental care*. Princeton, NJ: Princeton University Press.

Farmer, C. G. 2000 Parental care: the key to understanding endothermy and other convergent features in birds and mammals. *Am. Nat.* **155**, 326–334. (doi:10.1086/303323)

Hammond, K. A. & Diamond, J. 1997 Maximal sustained energy budgets in humans and animals. *Nature* **386**, 457–462. (doi:10.1038/386457a0)

Knight, F. M. 1987 The development of pelage insulation and its relationship to homeothermic ability in an altricial rodent *Peromyscus leucopus*. *Physiol. Zool.* **60**, 181–190.

Koteja, P. 2000 Energy assimilation, parental care and the evolution of endothermy. *Proc. R. Soc. B* **267**, 479–484. (doi:10.1098/rspb.2000.1025)

Król, E. & Speakman, J. R. 2003 Limits to sustained energy intake. VI. Energetics of lactation in laboratory mice at thermoneutrality. *J. Exp. Biol.* **206**, 4255–4266. (doi:10.1242/jeb.00674)

Król, E., Murphy, M. & Speakman, J. R. 2007 Limits to sustained energy intake. X. Effects of fur removal on reproductive performance in laboratory mice. *J. Exp. Biol.* **210**, 4233–4243. (doi:10.1242/jeb.009779)

Lamprecht, I. & Schmolz, E. 2004 Thermal investigations of some bird nests. *Thermochim. Acta* **415**, 141–148. (doi:10.1016/j.tca.2003.08.029)

McAdam, A. G., Boutin, S., Sykes, A. K. & Humphries, M. M. 2007 Life histories of female red squirrels and their contributions to population growth and lifetime fitness. *Écoscience* **14**, 362–369. (doi:10.2980/1195-6860(2007)14[362:LHOFRS]2.0.CO;2)

Newkirk, K. D., Cheung, B. L. W., Scribner, S. J. & Wynne-Edwards, K. E. 1998 Earlier thermoregulation and consequences for pup growth in the Siberian versus Djungarian dwarf hamster (*Phodopus*). *Physiol. Behav.* **63**, 435–443. (doi:10.1016/S0031-9384(97)00465-4)

Puchalski, W., Bulova, S. J., Lynch, C. B. & Lynch, G. R. 1988 Photoperiod, temperature and melatonin effects on thermoregulatory behavior in Djungarian hamsters. *Physiol. Behav.* **42**, 173–177. (doi:10.1016/0031-9384(88)90294-6)

Redman, P., Selman, C. & Speakman, J. R. 1999 Male short-tailed field voles (*Microtus agrestis*) build better insulated nests than females. *J. Comp. Physiol. B* **169**, 581–587. (doi:10.1007/s003600050258)

Speakman, J. R. 1997 *Doubly-labelled water: theory and practice*. Dordrecht, The Netherlands: Kluwer Academic.

Thompson, S. D. 1992 Gestation and lactation in small mammals: basal metabolic rate and the limits of energy use. In *Mammalian energetics: interdisciplinary views of metabolism and reproduction* (eds T. E. Tomasi & T. H. Horton), pp. 213–259. New York, NY: Cornell University Press.