Density Triggers Maternal Hormones That Increase Adaptive Offspring Growth in a Wild Mammal

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In fluctuating environments, mothers may enhance the fitness of their offspring by adjusting offspring phenotypes to match the environment they will experience at independence. In free-ranging red squirrels, natural selection on offspring postnatal growth rates varies according to population density, with selection favoring faster-growing offspring under high-density conditions. We show that exposing mothers to high-density cues, accomplished via playbacks of territorial vocalizations, led to increased offspring growth rates in the absence of additional food resources. Experimental elevation of actual and perceived density induced higher maternal glucocorticoid levels, and females with naturally or experimentally increased glucocorticoids produced offspring that grew faster than controls. Therefore, social cues reflecting population density were sufficient to elicit increased offspring growth through an adaptive hormone-mediated maternal effect.

Fluctuations in food availability and the resultant changes in the population density of consumers are thought to be important ecological agents of natural selection in many animal populations (1, 2). Temporal variation in natural selection characterized by recurrent pulses in food or density can favor the evolution of adaptive phenotypic plasticity when there are reliable cues that predict the direction or the magnitude of these agents of selection (3, 4). Phenotypic plasticity is beneficial in such changing environments because it enables individuals to track fluctuating fitness optima (5, 6). Similarly, if the parental environment or phenotype provides reliable cues of the conditions that offspring will experience, parents may induce adaptive changes in offspring that increase both parental and offspring fitness [adaptive parental effects (7, 8)].

The role of parental effects in the adaptation of offspring to changing environments is intriguing, but little is known about their importance in free-living animals. Not only do the agents of natural selection on offspring phenotype need to be identified, but the cues parents use to predict changes in the agent of selection, and the mechanism that mediates the parental effect, also need to be known. This is further complicated when considering population density as a cue because it is often confounded with food availability, which might also relieve resource constraints and cause resource-mediated or permissive parental effects (7). Identification and experimental manipulation of the mechanisms that mediate parental effects requires a combination of field physiology, experimental ecology, and longitudinal studies of natural selection that have not been achieved to date. We identified and experimentally manipulated the social density cues and stress hormones responsible for an adaptive maternal effect in a natural population of North American red squirrels (*Tamiasciurus hudsonicus*).

Individual male and female red squirrels defend exclusive territories around a central midden (9, 10) containing cached white spruce cones [*Picea glauca* (11, 12)], and juveniles that fail to acquire a territory before their first winter do not survive (13). Red squirrels experience recurrent fluctuations in population density because of pronounced episodic fluctuations in the availability of white spruce seeds (Fig. 1A) (11, 12). Increased autumn spruce cone production is associated with increased squirrel density in the following spring (Fig. 1B) (14). In our 23-year study in the Yukon, Canada, we found that these changes in density have notable effects on red squirrels because we documented density-dependent selection on offspring postnatal growth rates. In years when spring density was high, females that produced faster-growing offspring had more offspring survive their first winter and recruit into the adult population, whereas when density was low there was no benefit to producing faster-growing offspring [$n = 463$ females, offspring growth × density, $t_{726} = 2.15$, $P = 0.016$ (table S1)].

In such variable environments, the evolution of adaptive maternal effects may be favored, but this requires the presence of reliable cues that enable an accurate prediction of natural selection on offspring (3, 4). Therefore, cues of population density in red squirrels might induce adaptive increases in offspring growth when density is high. Red squirrels emit territorial vocalizations called rattles to defend their territories, and the

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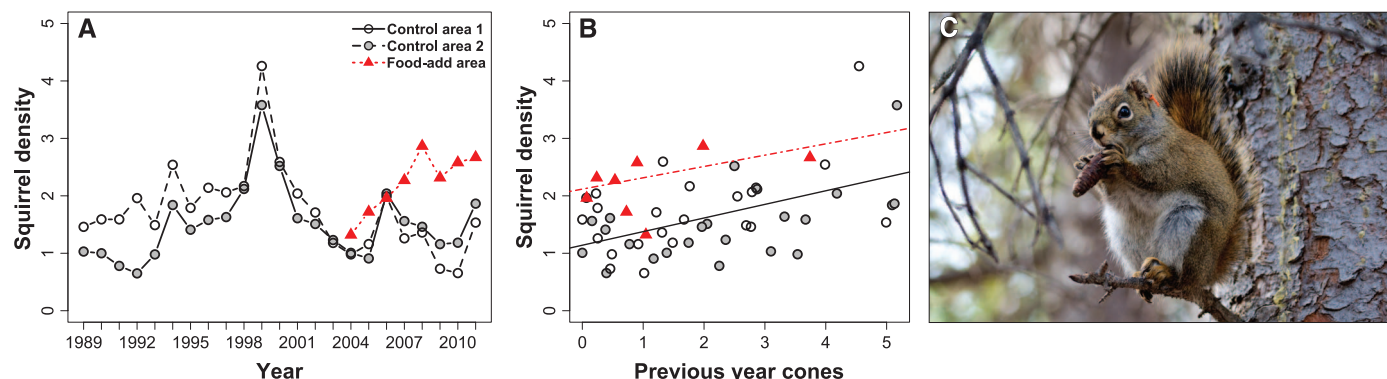†Present address: Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK.

Fig. 1. Population density of North American red squirrels in the Yukon, Canada, fluctuates annually in response to the availability of spruce cones. (**A**) Yukon red squirrels experience recurrent fluctuations in population density (squirrels/ha) because of interannual variation in white spruce cone abundance (11, 12). (**B**) Spruce cone production in the previous autumn is associated with increased spring population density in two control study areas ($b = 0.24 \pm 0.05$, $t_{53} = 4.3$, $P < 0.0001$) and one study area (Food-add) where squirrels have been provided with supplemental food since autumn 2004 ($b = 0.20 \pm 0.19$, $t_{53} = -0.22$, $P > 0.5$). Autumn spruce cone production is an index on a ln scale (11). Regression lines from a linear mixed-effects model. (**C**) Red squirrel extracting seeds from a white spruce cone. [Photo credit: R. W. Taylor]

frequency with which they hear rattles in their neighborhood accurately predicts density (*10*). We hypothesized that territorial vocalizations provide a cue of density that allows females to adaptively adjust offspring growth in anticipation of the density-dependent selection that they will experience. We tested this hypothesis by simulating high-density conditions using audio playbacks of red squirrel rattles (*9*, *10*). This corresponded to a perceived density of 4.92 squirrels/ha, which was sixfold higher than the perceived density of females exposed to a control stimulus (bird vocalizations, 0.81 squirrel/ha) and similar to the maximum historical density (Fig. 1A) (*10*). Such a high-density environment would typically be associated with a strong positive relationship between offspring growth and fitness (table S1), whereas offspring growth does not affect fitness in the low-density control environment.

As predicted, offspring produced by females experiencing experimentally heightened perceived density grew significantly faster than those produced by control females (Fig. 2). Consistent with life-history theory (*15*), the growth rates of offspring produced by control females declined significantly as litter size increased, but this effect was attenuated by 67% in females exposed to playbacks of territorial vocalizations [playback × litter size, $t_{186} = 1.98$, $P = 0.024$ (table S2 and Fig. 2)]. In fact, the trade-off between litter size and growth rate in females exposed to playbacks of territorial vocalizations was greatly reduced ($r = -0.12$, $t_{66} = -1.57$, $P = 0.06$) compared with that in control females ($r = -0.37$, $t_{64} = -4.43$, $P < 0.0001$). Female red squirrels, therefore, increase offspring growth in response to conspecific density because of the fitness benefits of doing so in high-density years. These growth-enhancing maternal effects in high-density years are adaptive for mothers and offspring by increasing the probability that their offspring will survive their first winter (*16*), which is a major component of their lifetime fitness (*17*). However, faster offspring growth rates are not favored under low-density conditions [≤1 squirrel/ha (table S1)], and in some years there is significant negative selection on offspring growth (*16*). Increased reproductive effort does not appear to incur a survival cost to mothers (*18*, *19*). However, offspring born in high-density years have a reduced adult life span (*20*), suggesting that faster offspring growth, which enhances recruitment when density is high, might incur a cost to offspring later in life. Such conditions will promote the evolution of plasticity in maternal effects, whereby increased offspring growth coincides with the high-density conditions under which it enhances fitness.

These adaptive maternal effects on offspring were mediated by the physiological stress responses of females experiencing heightened population density. Across 6 years (2006 to 2011), we found a positive relationship between local density and concentrations of fecal cortisol metabolites [FCM; $t_{155} = 3.63$, $P = 0.0002$ (table

Fig. 2. Female red squirrels experiencing increased perceived or actual density produced faster-growing offspring than controls. Female red squirrels experiencing experimentally increased perceived population density (rattle playbacks, $n = 19$ females, 67 pups) produced offspring that grew significantly faster than those produced by controls ($n = 19$ females, 65 pups) but similar to those produced by food-supplemented females ($n = 16$ females, 55 pups) experiencing increased actual density. Values on the y axis represent residuals from a linear mixed-effects model (table S2).
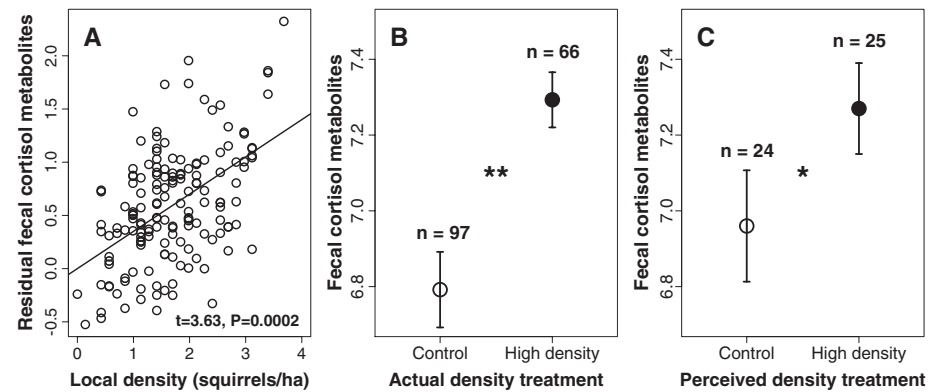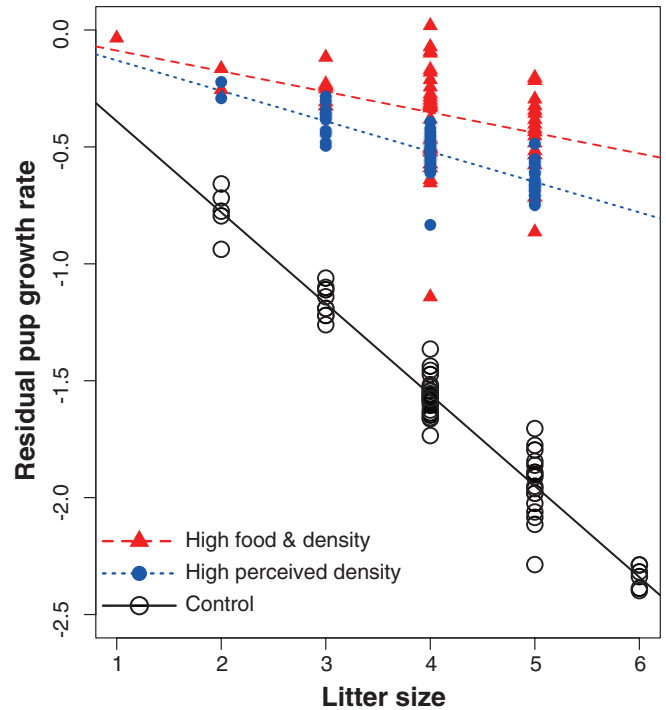

Fig. 3. Female red squirrels experiencing higher population density had higher glucocorticoid levels. (**A**) Female red squirrels living under high-density conditions had higher concentrations of FCM. Squirrels experiencing experimentally increased (**B**) actual density resulting from long-term food supplementation or (**C**) perceived density (rattle playbacks) had significantly higher concentrations of FCM than controls. Values on the y axis represent either (A) residuals from a linear mixed-effects model (table S4) or [(B) and (C)] raw FCM (ln ng/g of dry feces). Sample sizes refer to the number of fecal samples analyzed. **$P < 0.01$ and *$P < 0.05$ (table S4). Error bars indicate ± SE.

S4 and Fig. 3A)]. Females from a study area with experimentally increased density resulting from food supplementation [75% higher density than control study areas (Fig. 1)] had concentrations of FCM that were 49% higher [$t_{162} = 3.82$, $P < 0.0001$ (table S4 and Fig. 3B)] than those of females in control study areas. Females experiencing increased perceived density through the playback experiment had concentrations of FCM that were 30% higher than those of control females [$t_{48} = 2.24$, $P = 0.015$ (table S4 and Fig. 3C)]. These results confirm that increases in concentrations of FCM were driven by perceived density rather than by food abundance (*21*).

In mammalian species, increases in maternal glucocorticoid levels can cause profound changes in offspring phenotype (*22*) and may provide offspring with reliable hormonal cues about their future environment. Three lines of evidence indicate that increases in maternal glucocorticoid levels are responsible for the adaptive increase in offspring growth under high-density conditions. First, females exposed to heightened perceived density had increased concentrations of FCM during pregnancy (Fig. 3C) and also produced faster-growing offspring than controls (Fig. 2). Second, increased maternal FCM concentrations were positively associated with offspring growth in females measured over a 6-year
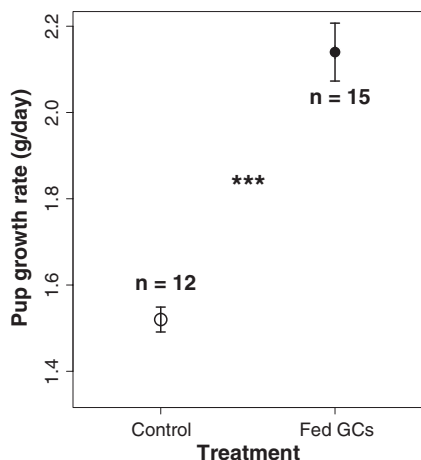
Fig. 4. Offspring produced by female red squirrels provisioned with cortisol grew significantly faster than those from controls. Raw offspring growth rates (mean ± SE) are shown on *y* axis. Sample sizes denote number of pups. Fed GCs corresponds to provisioning with three different cortisol concentrations (fig. S2). ***$P < 0.0001$ (table S6).

period [$t_{98} = 1.94$, $P = 0.028$ (table S5)]. Last, offspring born to females with experimentally increased glucocorticoid levels during pregnancy [fed cortisol (fig. S1)] grew 41% faster than those produced by control females [$t_{26} = 4.98$, $P < 0.0001$ (table S6 and Fig. 4)].

Our results suggest that elevated maternal glucocorticoid levels in response to heightened population density induced an adaptive hormone-mediated maternal effect on offspring growth. In contrast to the widespread assumption that heightened maternal glucocorticoid levels are detrimental to offspring (*22*), our results emphasize that in free-living animals they can instead lead to adaptive adjustments in offspring (*23, 24*). Under high-density conditions, squirrels spend less time feeding and in the nest (*10*), suggesting that increased offspring growth is not a simple outcome of increased maternal care or milk provisioning. Alternatively, elevated exposure to glucocorticoids early in life (*22, 25*) could increase offspring growth by directly influencing offspring physiology or behavior (*22, 26*) and subsequent changes in growth hormone secretion in offspring (*27*).

For nearly 100 years, food availability has been considered to be a universal variable affecting population dynamics and life-history traits (*28*). Increased food availability also increases the population density of consumers, which has made it difficult to distinguish whether the plasticity in life-history traits after periods of high food availability is due to relaxation of food limitation or to adaptive reproductive adjustments to changes in density-mediated selection. Our results provide evidence that female red squirrels can produce faster-growing offspring in the absence of additional resources but only do so when the fitness prospects warrant this increased invest-

ment. In fact, offspring produced by females exposed to high-density cues but with no access to additional food grew as fast as those produced by food-supplemented females that were also experiencing increased density [1.79 ± 0.09 squirrels/ha (Fig. 2 and table S2)]. Therefore, some of the plasticity in female life history traits is due to the expected fitness benefits of producing faster-growing offspring under high-density conditions rather than only reflecting a relaxation of food limitation.

Experimental increases in food resources that result in increased reproductive output are typically interpreted as evidence for resource limitations on reproduction (*29*). However, if animals use food abundance as a cue of upcoming density-mediated selection, then reproductive responses to food supplementation might reflect not only relaxation of food limitation but also an adaptive adjustment to an anticipated change in natural selection resulting from an impending increase in density. Cues of population density may be a general signal that animals use to make adaptive reproductive adjustments in anticipation of density-dependent natural selection on offspring phenotypes.

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The Cross-Bridge Spring: Can Cool Muscles Store Elastic Energy?

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Muscles not only generate force. They may act as springs, providing energy storage to drive locomotion. Although extensible myofilaments are implicated as sites of energy storage, we show that intramuscular temperature gradients may enable molecular motors (cross-bridges) to store elastic strain energy. By using time-resolved small-angle x-ray diffraction paired with in situ measurements of mechanical energy exchange in flight muscles of *Manduca sexta*, we produced high-speed movies of x-ray equatorial reflections, indicating cross-bridge association with myofilaments. A temperature gradient within the flight muscle leads to lower cross-bridge cycling in the cooler regions. Those cross-bridges could elastically return energy at the extrema of muscle lengthening and shortening, helping drive cyclic wing motions. These results suggest that cross-bridges can perform functions other than contraction, acting as molecular links for elastic energy storage.

Elastic energy storage is heralded as a critical design characteristic of animal movement, because it promotes efficient locomotion. Canonical examples of elastic energy-storage sites include tendons of mammals and resilin, the rub-

berlike protein in insect cuticle (*1, 2*). Elastic energy storage is particularly important to flying insects, reducing the otherwise prohibitive inertial power costs of accelerating and decelerating the wings (*3, 4*). Two main sites of elastic