BEHAVIORAL ADAPTATION

Phenotype–environment mismatch errors enhance lifetime fitness in wild red squirrels

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Mismatches between an organism's phenotype and its environment can result in short-term fitness costs. Here, we show that some phenotype–environment mismatch errors can be explained by asymmetrical costs of different types of errors in wild red squirrels. Mothers that mistakenly increased reproductive effort when signals of an upcoming food pulse were absent were more likely to correctly increase effort when a food pulse did occur. However, mothers that failed to increase effort when cues of an upcoming food pulse were present suffered lifetime fitness costs that could only be offset through food supplementation. In fluctuating environments, such phenotype–environment mismatches may therefore reflect a bias to overestimate environmental cues and avoid making the costliest error, ultimately enhancing lifetime fitness.

An organism's ability to match its phenotype to environmental conditions is central to hypotheses related to the evolution of adaptive phenotypic plasticity (*1*). Organisms producing phenotypes that are adaptive in a particular environment (i.e., a "match") can enhance their reproductive success and survival. Alternatively, organisms that mismatch their phenotypes to environmental conditions often suffer fitness costs. For example, birds that mistime breeding to a fluctuating food supply experience poorer offspring growth (*2*) and recruitment (*3*). Such mismatches have historically been viewed as a cost of phenotypic plasticity (*4*). Yet, emerging research demonstrates that mismatched phenotypes may also promote long-term population persistence (*5*, *6*). Organisms may therefore aim to manage the costs and potential benefits of phenotype–environment mismatches over their life course to maximize fitness.

Existing theory about how organisms balance the costs of making different types of errors can help explain the occurrence of mismatches (*7–9*). For example, in the presence or absence of information about the future environment, an organism may respond or not respond, creating a minimum of four conditions (two matches, two mismatch errors). Each type of mismatch error is expected to have a distinct fitness cost, and organisms should err toward making the least costly of the two (*7*). For a mismatched phenotype to be adaptive, the costs of not responding to the cue (false negative) should be greater than the cost of responding erroneously in the absence of the cue (false positive) (*7*). Empirical tests of this framework

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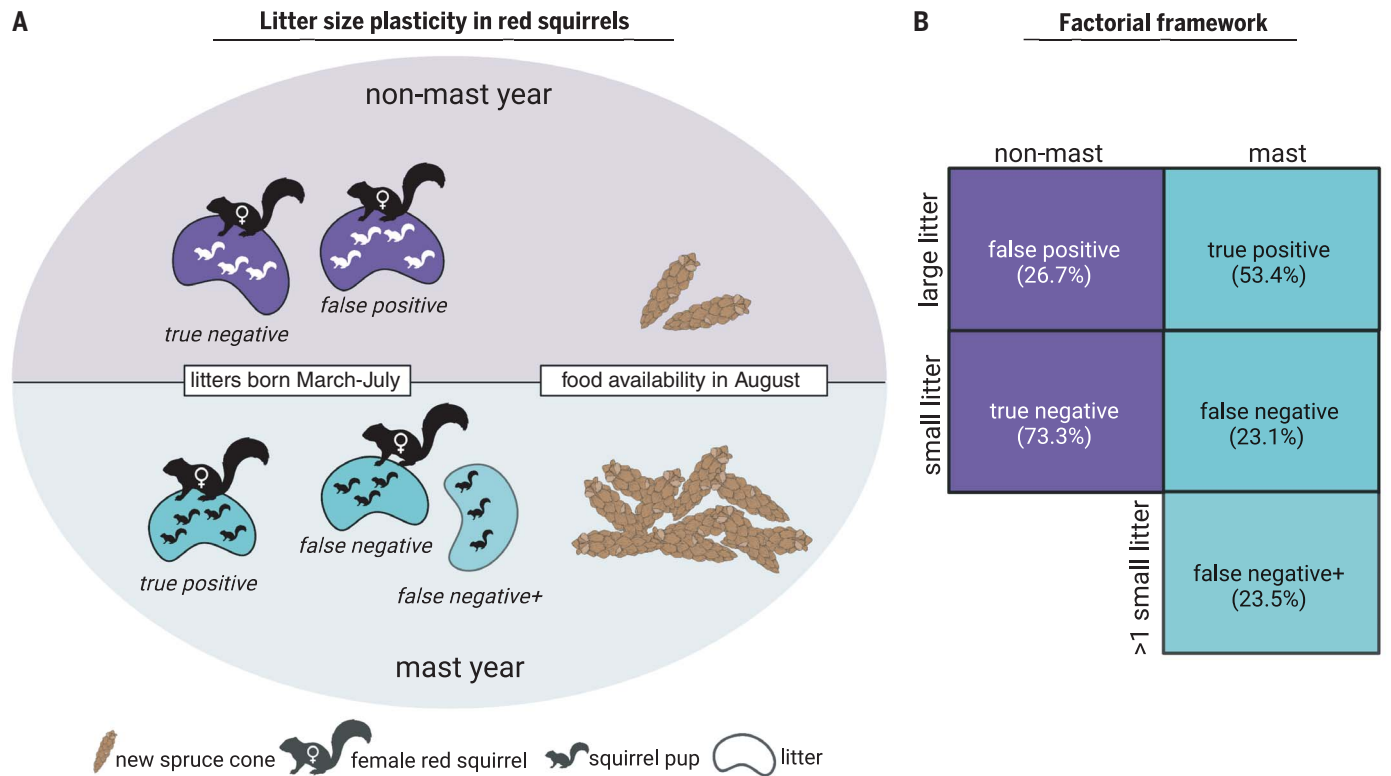*Corresponding author. Email: petrullo@umich.edu (L.P.); dantzer@umich.edu (B.D.)

Fig. 1. Litter size plasticity generates phenotype–environment mismatches.
(**A**) Yukon red squirrels inhabit a resource pulse system where fluctuations in new food production (no pulse = nonmast year, pulse = mast year) combine with variation in litter sizes (large = three or more pups, small = less than three pups) to create matched (true positive, true negative) and mismatched (false positive, false negative) phenotypic responses. Some females that produced small litters in mast years went on to produce a second litter later that year (false negative+), potentially mitigating the costs of an initial reproductive mismatch. (**B**) A factorial framework showing frequency rate of each response category (percentage of litters falling into each category in a given year) in parentheses (N = 2729 litters).

are rare because they require a system where individuals experience within-generation environmental fluctuations [fine-grained environments (10)] and where lifetime fitness can be quantified to determine the net effects of matches and mismatches. Individuals must also receive reliable cues about the future environment (11), and phenotypic responses must vary enough to permit a factorial assessment of relative fitness costs across all conditions.

Assessing the costs and benefits of mismatching in a resource pulse system

Here, we used a long-term study of wild North American red squirrels (*Tamiasciurus hudsonicus*) living in the Yukon, Canada, to probe whether phenotype–environment mismatches can be adaptive. Red squirrels in this region have been monitored continuously since 1987 (12). Using 31 years of life-history and fitness data from over 1000 female squirrels, we tested whether reproductive phenotype–environment mismatch errors allow females to avoid making the costliest type of error and thus enhance lifetime fitness. Yukon red squirrels inhabit a resource pulse ecosystem where they experience profound fluctuations in food availability as a result of a swamp-and-starve strategy by their primary food source, seeds from the cones of masting white spruce (*Picea glauca*) trees (13). Squirrels

store spruce cones containing seeds underground in middens and rely on these resources in lieu of bodily fat reserves (14). In nonmast years, few to no new seeds are produced, intraspecific competition for territories is high, and offspring overwinter survival is at its lowest (15). In these nonmast years, litter sizes experience stabilizing selection around a population mean of 3.0 pups (16) (fig. S1), where the fecundity benefit of producing more pups is balanced by the benefit of producing small litters of fast-growing offspring that can acquire territories and survive winter (16, 17). By contrast, in mast years [approximately every 4 to 7 years (13)], a superabundance of cones is produced in the autumn, leading to higher offspring survival, and directional selection favoring increased litter sizes [optimum litter size = 5.2 pups (16); population mean = 3.5 pups (fig. S1)]. Females use ecological cues to adaptively increase litter sizes in the months before the mast occurs, capitalizing on the future food boom by recruiting more pups into the breeding population (tables S1 and S2) (16). These increases in litter size may be caused by physiological responses to phytohormonal cues of an upcoming mast (18) but not by energetic differences (19) or differences in the number of hoarded spruce cones in an individual's midden (table S3).

Most females responded to mast cues by increasing litter sizes before the food pulse (true positive, 53.4%), but others mismatched by failing to increase litter size (false negative, 23.1%) or by increasing litter size in the absence of mast cues [false positive, 26.7% (Fig. 1)].

Divergent costs of phenotype–environment mismatching

When an upcoming mast was imminent, females that failed to respond (false negative) suffered a 97% decrease in their annual reproductive success (recruiting only 1.07 ± 0.09 pups) compared to females that responded by increasing litter sizes [true positive, recruiting 2.11 ± 0.10 pups (Fig. 2A and tables S4 and S5)]. In nonmast years, females producing large litters (false positive) had annual reproductive success similar to that of females producing small litters [true negative (Fig. 2A and tables S4 and S5)], suggesting a marginal or otherwise undetectable cost of false positive errors (20). A small number of females (6.6%) produced a second successful litter in a mast year. When litter sizes were small but still a part of this multilitter strategy (false negative+), mothers minimized, but did not eliminate, the costs of a false negative error by having an additional litter in that year (Fig. 2A and tables S4 and S5).
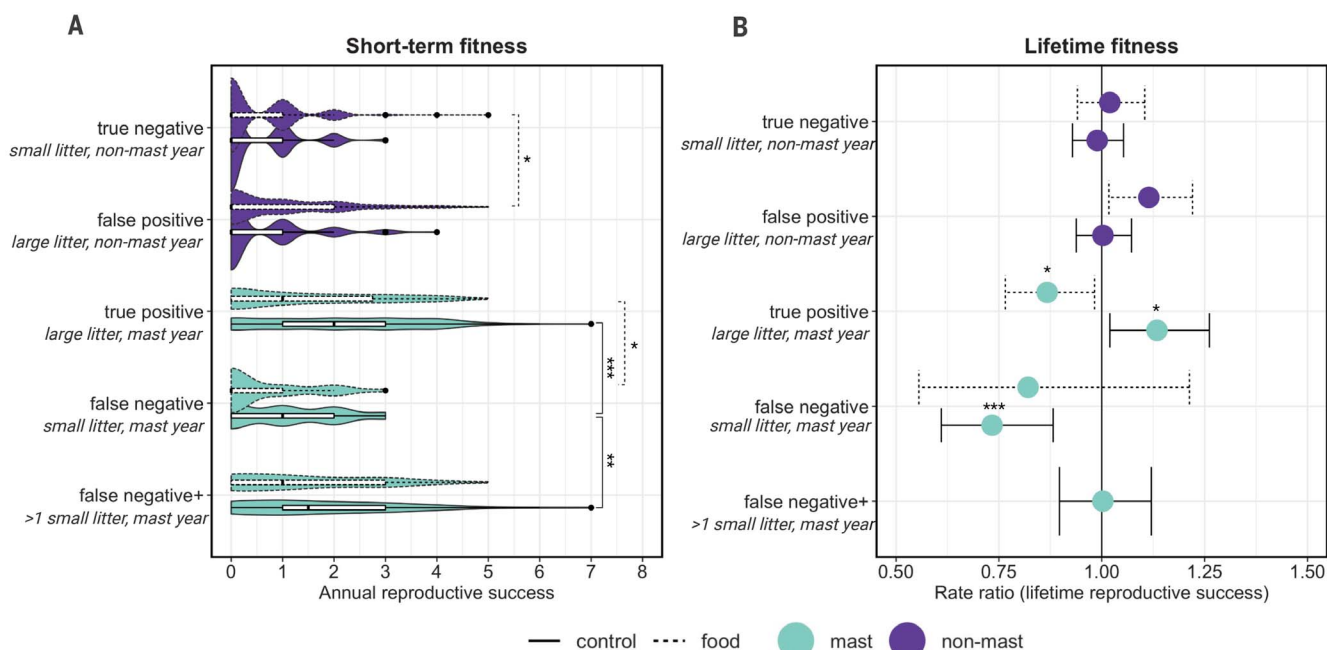

Fig. 2. The fitness costs of mismatch errors are asymmetrical, cumulative, and ameliorated by food supplementation. (**A**) In nonmast years, the difference in annual reproductive success (number of pups recruited into the breeding population that year) between control females that produced large (false positive) and small (true negative) litters was negligible. In mast years, control females that failed to respond to mast cues and produced small litters (false negative) exhibited significantly lower annual reproductive success than those that produced large litters [true positive (tables S4 and S5), N = 2729 litters]. Violin plots show raw data; inset boxplots depict median and outliers. (**B**) There was no cumulative cost to maternal lifetime fitness (number of total pups recruited into the breeding population over the life course) of repeated overestimation of mast cues (false positive), but each missed mast cue (false negative) significantly decreased lifetime fitness among control but not food-supplemented females (N = 1055 females). Filled circles show point estimates of rate ratios (relative difference). Bars denote 95% confidence intervals (CIs). Estimates >1.0 indicate a positive effect on lifetime reproductive success; estimates <1.0 indicate a negative effect. Asterisks denote significance (*P > 0.05, **P > 0.01, ***P < 0.001).

Because squirrels breed annually and fitness accrues with each breeding attempt, we assessed whether mismatched responses had cumulative effects on female lifetime fitness. Although each true positive (responding in presence of cue) produced across the life span increased maternal lifetime reproductive success by 12.5%, each false negative (failure to respond in presence of cue) mismatch decreased lifetime reproductive success by 30% (Fig. 2B and table S6). Females would therefore have to produce three true positive matches to recuperate the lifetime fitness costs of missing a single mast cue, an impossibility given the frequency of mast events [once every ~4 to 6 years (fig. S5)] and the maximum red squirrel life span [8 years, median = 3.5 years (21)].

Avoiding the costliest error benefits mothers but not offspring

The rare nature of spruce mast events provides additional ecological context for the maintenance of false positive mismatches. Females that did not experience a mast event before dying (54.2%) exhibited considerably lower lifetime reproductive success than females that did (table S2). The uncertainty of encountering a mast during a squirrel's lifetime, coupled with the importance of mast years for maximizing lifetime fitness, should favor individuals that are highly sensitive to mast cues even if they mismatch in years when cues are absent (8, 22). Indeed, each false positive error produced over the lifetime increased a female's probability of avoiding the costliest error (false negative) by 67% (Fig. 3A and table S7). Females therefore benefit from an error-avoidance bias (7) or adaptive gullibility (8), such that overestimating cues and responding when cues are absent reduces the likelihood of failing to respond when cues are present.

Using data on 8455 squirrel pups born to these females, we found that this error-avoidance bias, despite enhancing maternal lifetime fitness, came at a cost to offspring. Although females that produced large litters in nonmast years (false positive) reduced the likelihood that they made a mismatch error in a mast year (Fig. 3A), their pups grew slower [$\beta = -0.15 \pm 0.07$, $P = 0.03$ (Fig. 3B and tables S8 and S9)] and were less likely to survive their first winter, thus failing to contribute to the breeding population [$\beta = -1.26 \pm 0.44$, $P = 0.005$ (Fig. 3C and tables S10 and S11)]. Explaining why traits that increase maternal fitness but decrease offspring fitness (i.e., "selfish maternal effects") exist has been contentious (23). Here, we show that traits promoting maternal fitness at the expense of offspring fitness, such as overestimating environmental cues, may be more likely to evolve in systems where mothers must manage asymmetrical costs of reproductive mismatch errors.

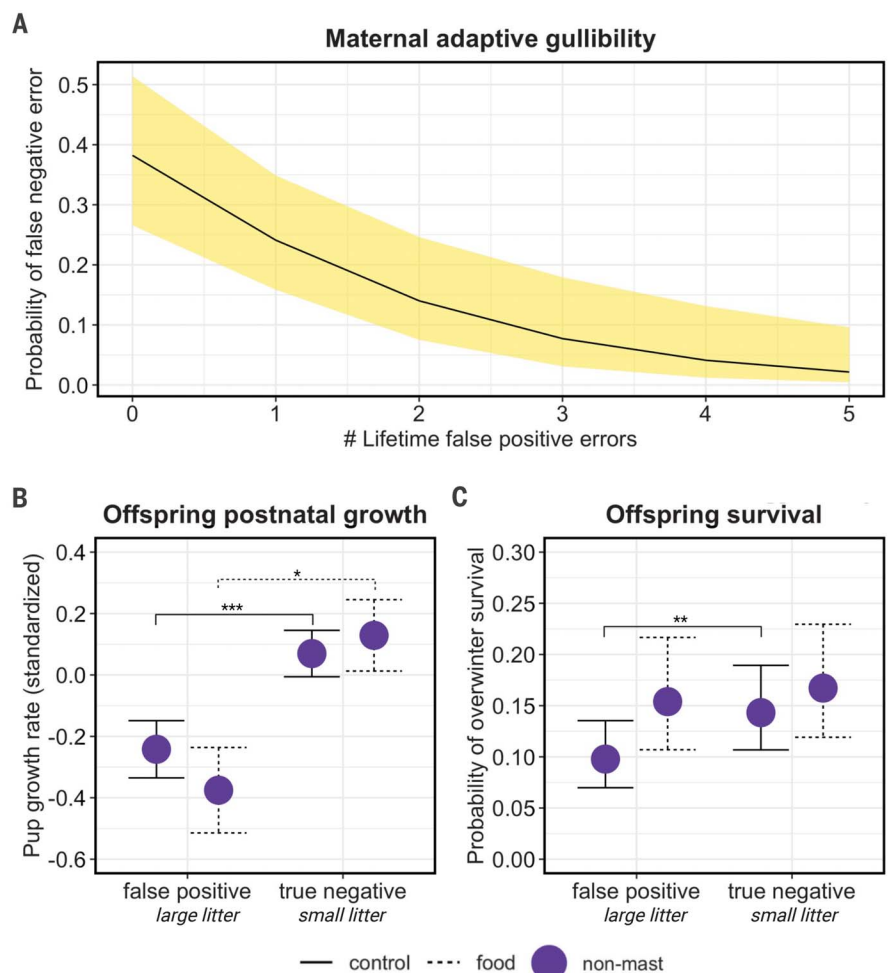It is currently unclear why heterogeneity in female reproductive responses to cues of spruce

Fig. 3. Overestimating mast cues enhances maternal lifetime fitness despite costs to offspring.
(**A**) Each false positive error (erroneously producing a large litter in a nonmast year) made across a control female's lifetime significantly decreased her probability of making the costliest error [false negative (Fig. 2B), $N = 361$ squirrels]. Plot shows predicted probabilities from a binomial model (table S7); ribbon indicates 95% CIs. (**B**) In nonmast years, when new food is low and territory competition is high, pups born into large litters (false positive) had poorer postnatal growth [true negative (tables S8 and S9), $N = 5048$ pups] and (**C**) were less likely to survive their first winter than pups born into small litters [true negative (tables S10 and S11), $N = 8455$ pups]. Pups of supplemented females that made false positive errors did not suffer a survival cost. Asterisks denote significance (*$P > 0.05$, **$P > 0.01$, ***$P < 0.001$).

masting persists in this population, but it may reflect variation in the physiological integration of mast cues. Being born in a mast year did not affect a female's ability to respond to mast cues in the future (table S12). However, first-time (primiparous) mothers were ~1.5 times less likely to make a litter-size mismatch error than were multiparous mothers [$\beta = -0.42 \pm 0.17$, $P = 0.01$ (fig. S3 and table S12)]. This may reflect parity-dependent differences in neuroendocrine sensitivity to phytohormonal (estrogenic) mast cues (18); it may also indicate that, because most females breed only once in their lifetime (fig. S2A), first-time mothers have more to lose by mismatching what may be their only attempt at contributing to the gene pool.

Food supplementation modifies patterns of error avoidance

In addition, environmental fluctuations that are episodic but predictable can create well-adapted systems in which the manipulation of resources is necessary to uncover mismatch costs. We tested the hypothesis that providing squirrels with additional food would disrupt selection for an error-avoidance bias by reducing the fitness costs of mismatching. To do so, we provided ad libitum peanut butter to females living on a nearby but distinct experimental study area for 14 consecutive years. In mast years, food supplementation relaxed selection for larger litters, and directional selection on litter size was negative rather than positive [$\beta = -0.26 \pm 0.12$, $P = 0.03$ (table S13)].

Providing an additional food source diminished and in some cases reversed the costs of mismatch errors. Producing a large litter in the absence of a cue (false positive) was associated with an increase in maternal annual reproductive success (Fig. 2A and tables S4 and S5) and cumulatively positive effects on lifetime reproductive success (Fig. 2B and table S6). Supplemented females who failed to respond to mast cues (false negative) incurred short-term fitness costs similar to those incurred by control females, but without any cumulative cost over the life span (Fig. 2B and table S6). An amelioration of the trade-off between offspring quality and quantity appears to be the mechanism that mitigated these costs. In nonmast years, pups born in large litters (false positive) on the food-supplemented study area did not exhibit poorer survival as compared to pups born into small litters [true negative (Fig. 3C and tables S10 and S11)].

In environments where selective pressures are unpredictable, producing a mix of matched and mismatched phenotypes (i.e., bet-hedging) can maximize fitness (24). By contrast, in environments where selective pressures are predictable, adaptive plasticity is expected to evolve in a manner dependent on the spatiotemporal grain of the environment (25). Organisms inhabiting predictable coarse-grained environments (across-generation fluctuations) should exhibit polymorphisms that are environmentally induced (26) or genetically determined (27). Organisms inhabiting predictable fine-grained environments (within-generation fluctuations), such as Yukon red squirrels, are instead expected to exhibit adaptive phenotypic plasticity (10).

What has remained unclear is why phenotypes that are mismatched to environmental conditions persist. Mismatch errors can occur because the cues that organisms receive about their future environment are imprecise or unreliable (11), a growing phenomenon in the wake of global anthropogenic climate change (28). Or as our results indicate, mismatches can persist because organisms must balance asymmetrical costs of different types of errors to avoid making the costliest error over their lifetimes.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Supplementary Text
Figs. S1 to S5
Tables S1 to S17
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MDAR Reproducibility Checklist

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