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A plant toxin mediated mechanism for the lag in snowshoe hare population recovery following cyclic declines

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A necessary condition for a snowshoe hare population to cycle is reduced reproduction after the population declines. But the cause of a cyclic snowshoe hare population's reduced reproduction during the low phase of the cycle, when predator density collapses, is not completely understood. We propose that moderate-severe browsing by snowshoe hares upon preferred winter-foods could increase the toxicity of some of the hare's best winter-foods during the following hare low, with the result being a decline in hare nutrition that could reduce hare reproduction. We used a combination of modeling and experiments to explore this hypothesis. Using the shrub birch *Betula glandulosa* as the plant of interest, the model predicted that browsing by hares during a hare cycle peak, by increasing the toxicity *B. glandulosa* twigs during the following hare low, could cause a hare population to cycle. The model's assumptions were verified with assays of dammarane triterpenes in segments of *B. glandulosa* twigs and captive hare feeding experiments conducted in Alaska during February and March 1986. The model's predictions were tested with estimates of hare density and measurements of *B. glandulosa* twig growth made at Kluane, Yukon from 1988–2008. The empirical tests supported the model's predictions. Thus, we have concluded that a browsing-caused increase in twig toxicity that occurs during the hare cycle's low phase could reduce hare reproduction during the low phase of the hare cycle.

The snowshoe hare *Lepus americanus* cycle is central to the food web of the North American boreal forest (see Fig. 1b in Krebs 2011). Thus, understanding the cycle's mechanism has long been a goal of ecologists interested in boreal forest ecosystem dynamics (Elton and Nicholson 1942, Keith 1963, Bryant 1981, Boonstra et al. 1998, Krebs et al. 2001, Krebs 2011, Sheriff et al. 2011). A large-scale experiment done in the Kluane Lake Region of Canada's Yukon Territory has provided strong evidence that the decline phase of the cycle is caused by a tritrophic interaction involving the hare's winter-food, the hare and the hare's predators (Krebs et al. 1995). But the cause of the hare's reduced reproduction during the hare cycle's low phase, which provides the time delay in hare population growth that is required to generate a hare cycle (May 1974, Boonstra et al. 1998) is not completely understood. The objective of the research described in this paper was to explore the possibility that browsing by hares on their preferred winter foods during a hare peak may, by increasing the toxicity of this food, result in a reduction in hare reproduction during the following hare low. This objective was achieved by using a combination of modeling, laboratory feeding experiments and a long-term field monitoring experiment. The conclusion that we reached was that it is likely that an increase in browse toxicity caused by hare browsing during the winter of a hare peak could reduce hare reproduction during the following hare low.

The toxicity of some of the hare's most preferred winter-foods is related to the process of aging by woody plants. An excellent review of woody plant aging can be found in chapter 4 of Kozlowski (1971), which is titled Aging. Aging is not related to the chronological age of the plant: it can occur in chronologically younger plants as well as chronologically older plants; it can occur either during the juvenile phase of development or the mature phase of development (Kozlowski 1971, chapter 4 titled Maturation or phase change).

Aging is caused by the increased competition for mineral nutrients among shoots that occurs within the crown of a woody plant as the crown grows in size and increases in architectural complexity (Moorby and Waring 1963, Kozlowski 1971). Aging reduces both the lengths and the diameters of a twig's current-annual-shoots (Kozlowski 1971). This decrease is necessarily retained during following growing seasons. This progressive annual reduction in segment size can be seen in the left stick drawing presented in Fig. 1, which is an adaptation of the stick drawings presented in Fig. 1 of Moorby and Waring (1963).

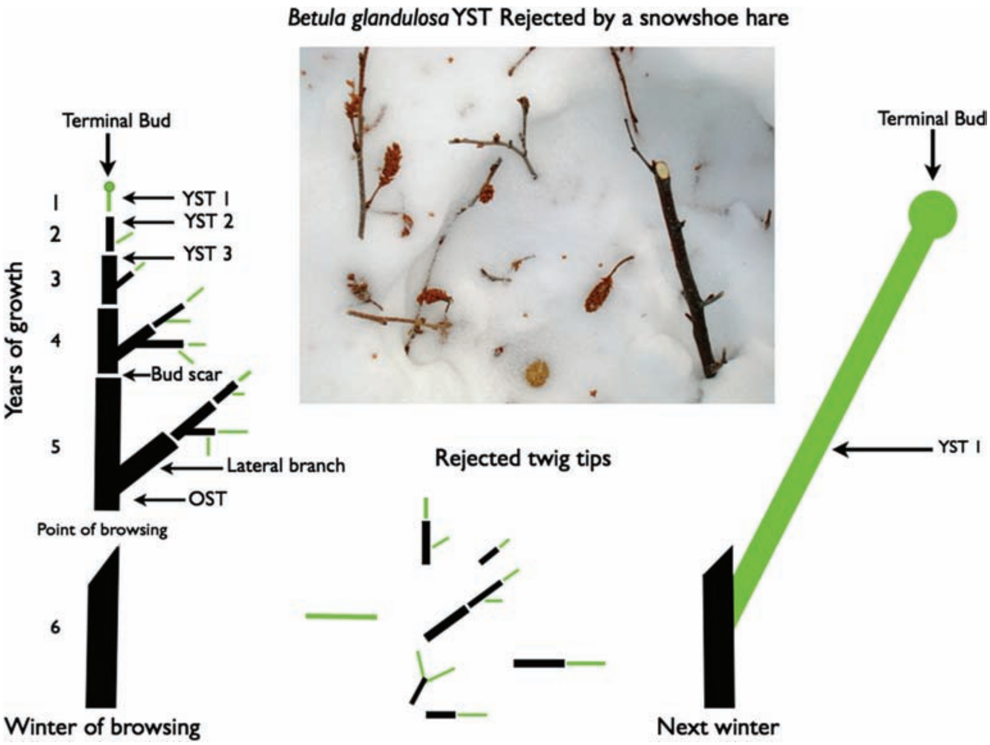

Figure 1. Stick figure drawings of a twig of an 'Aged' woody plant (left drawing) and the same twig after browsing in winter by snowshoe hares has reversed aging (right drawing). The green segments are the current-annual-shoot segments (YST 1 segments). In the left stick drawing, the terminal bud scars that delineate twig segments that differ in chronological age are indicated by breaks in the twigs. The middle stick drawing represent comparatively toxic younger segments of twigs (YST segments) that have been rejected by snowshoe hares. The photograph above the stick drawings of rejected YST segments shows the YST segments of a *B. glandulosa* that have been rejected by real free-ranging snowshoe hares residing at Wiseman, Alaska. Wiseman (67°41′56″N, 150°13′11″W), which is at the northern limit of the snowshoe hare's range in Alaska, is located on the south slope of the Brooks Range: for Wiseman's location see 'Brooks Range' in the map that is in the Supplementary material Appendix 1 PowerPoint slide show named 'Places'. Donna L. DiFolco (Cartographic Technician, US National Park Service Gates of the Arctic National Park & Preserve and Yukon-Charley Rivers National Park) took the photograph in March 2009.

Pruning (here browsing by hares in winter), by removing shoots competing for nutrients, reverses aging (Moorby and Waring 1963). An aging reversal caused by hare browsing should, therefore, result in increases in both the lengths and the diameters of the current-annual-shoots produced in the summer following the browsing event. The right stick drawing in Fig. 1 depicts this increase.

The twigs of some of the woody deciduous angiosperm species that the snowshoe hares prefer to eat in winter produce and store toxins in surface tissues such as the epidermis. This allocation of toxins to surface tissues prevents the toxins from killing the deeper living tissues such as the phloem and cambium that are necessary for further growth (McKey 1979). A consequence of this allocation of anti-herbivore defense toxins to surface tissues is a reduction in their concentration in a twig segment during the secondary growth that increases the twig's diameter. In most species, after the first year of shoot growth, outer tissues such as the epidermis cannot keep up with the growth in area of the twig itself (Kozlowski 1971). Hence, sometime after the first year of secondary growth, during the formation of bark, these outer tissues die, split and disintegrate, thus destroying any of the toxins that they contain. Moreover, since toxins are in surface tissues, increasing diameter also reduces their concentration by dilution: toxin concentration is diluted by wood production. Thus, from a snowshoe hare's perspective, the smaller diameter younger segments of an angiosperm twig (acronym YST) that are nearest the twig's terminal bud can be more toxic than the larger diameter older segments of the twig (acronym OST) that support the YST (Reichardt et al. 1990a, b): in Fig. 1 the toxicity of YST 1 > YST 2 > toxicity of YST 3 > toxicity of all OST, where YST1, 2, and 3 are, respectively, the current year's twig segment, last year's segment and the segment of the year before. In the left stick drawing in Fig. 1 the terminal bud scars that mark the demarcation line between annual increments of twig growth are depicted as breaks in twigs.

From a snowshoe hare's perspective, the secondary growth driven change in twig toxicity puts the hare between a proverbial 'rock and a hard spot'. The rock is the comparatively high toxicity of the small diameter, nutrient rich and highly digestible YST segments. The hard spot is the diameter at which OST segments contain so much wood that simple indigestibility and nutrient dilution renders them very poor winter-food: as depicted in Fig. 2, the larger diameter OST twig segments ultimately become starvation food. The snowshoe hare must feed in the 'goldilocks zone' that contains twig segments that are both comparatively nontoxic and

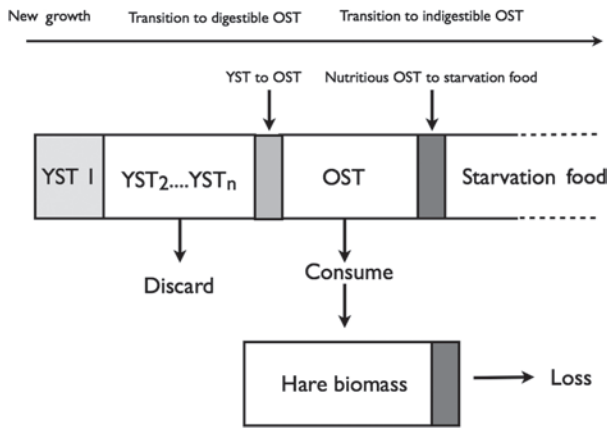

Figure 2. Conceptual diagram of how biomass is transferred among compartments in discrete time steps. On a particular time step, new growth biomass (YST 1 biomass) (light gray increment), is added to the YST compartment, biomass (medium gray increment) is transferred from YST to OST, and biomass (dark gray increment) is transferred from OST compartment to starvation food, which is twig segments that contain so much wood that they are highly indigestible and also deficient in nutrients. In addition, there is a transfer of OST biomass to hare biomass through consumption, and a loss of YST through hares discarding the comparatively high toxicity YST segments found near the a twig's tip (in Fig. 1 see middle stick figures and photograph).

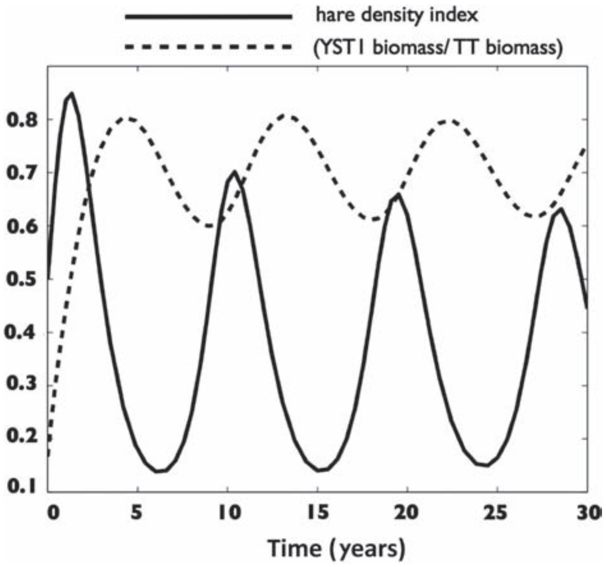

Figure 3. Results of the twig segment model (TSM) simulation. Note that the maximum magnitude of the ratio (YST biomass/ TT biomass), which stands for (YST biomass/total twig biomass), occurs during the hare decline and that this ratio remains comparatively high during the subsequent low of the simulated hare cycle. The parameter values and their sources can be found in Supplementary material Appendix 1. The units of the hare density index and the ratio (YST biomass/TT biomass) have been normalized to range from 0–1. Thus, these units are dimensionless.

comparatively digestible. The snowshoe hare uses foraging behavior to solve this problem.

When feeding upon a twig a hare bites the twig off in an OST segment (point of browsing in Fig. 1). Then the hare consumes the twig from the point of browsing toward the tip where the twig's terminal bud is located. Initially the hare eats some toxic biomass (buds and woody internodes of YST segments). But after the hare reaches its tolerance level for the twig's toxin (or toxins) it rejects the more toxic sections of the YST and eats only the less toxic OST. The stick drawings in the middle of Fig. 1 depict this rejected YST. An example of this foraging behavior can be seen in the photographs in Fig. 3 of Reichardt et al. (1984).

In the case of the shrub birch *B. glandulosa* (Dugle 1966), which is the plant of interest in this study, rejection of the more toxic YST appears to be widespread throughout northwestern North America, having been seen at several locations in interior Alaska (J. P. Bryant – e.g. Denali National Park, Goldstream Valley, Nelchina Basin; D. DeFolco – Brooks Range; J. Whitman – Upper Kuskokwim River Valley), at Kluane, Yukon (D. Hik) and in the Mackenzie River Valley in Canada's Northwest Territories (D. Allaire). The locations of these observations are shown in the PowerPoint slide show named "Places" that is in the Supplementary material Appendix 1. An example of this rejection of *B. glandulosa* YST is shown in the photograph that is in Fig. 1. Furthermore, laboratory experiments at the Inst. of Arctic Biology (IAB) have shown that in the case of previously unbrowsed mature phase *B. glandulosa*, the palatability of twig segments to Alaskan hares increases with increasing diameter up to a diameter of 4 mm, with the greatest rate of increase occurring between a diameter of 2 mm and a diameter of 3 mm (Fox and Bryant 1984).

The effects of browsing on *B. glandulosa* YST toxicity and the resultant effects on hare browsing are incorporated in a model, with which the consequences for the hare's dynamics, particularly during the low phase of the cycle, can be studied. Because the plant unit of interest is a segment of a twig, the model's acronym is TSM, which stands for twig segment model. In the Methods section the TSM model is described, along with the laboratory and field studies used to test it. A conceptual diagram of the TSM is presented in Fig. 2. We emphasize that the TSM does not describe either an induced defense (sensu Karban and Baldwin 1997) or a pruning caused reversion to the juvenile developmental phase (sensu Kozlowski 1971, chapter 3), both of which can also increase anti-herbivore defense.

Methods

Model

The TSM model, which was developed in Liu et al. (2012), attempts to simulate the effects that woody plant chemical defenses may have on boreal snowshoe hare populations, which, in winter, feed almost entirely on twigs. We focus particularly on the fact that twig segment toxicity often changes chronologically. The woody internodes of the chronologically youngest segments of the twigs (YST) of the deciduous angiosperm species that these hares prefer to eat can be more defended by toxins than the woody internodes of the chronologically older segments (OST) that subtend and support the younger segments (Reichardt et al. 1990a, b). Thus, the per capita daily intake of the biomass of the

OST segments of a twig by boreal snowshoe hares can be much higher than their intake of the biomass of the twig's YST segments. This chronological age-dependent toxicity of twig segments is modeled using age-structured equations which are reduced to a system of delay differential equations by aggregating twig segments into three discrete classes that vary in chronological age: YST twig segments; edible and comparatively digestible OST twig segments; and older, comparatively indigestible OST twig segments that are starvation food (Fig. 2). Transitions between these twig segment age classes involve multiple delays, which have consequences for the woody plant–hare dynamics.

A novel aspect of the modeling was that it had to account for mortality of non-consumed YST biomass when OST biomass is bitten off and consumed. The rejected, and therefore, non-consumed YST biomass is depicted by the twig segment stick drawings in the middle of Fig. 1. *Betula glandulosa* YST biomass that has been rejected by real free-ranging snowshoe hares is shown in the photograph of Fig. 1.

Basic mathematical properties of the model have been established (Liu et al. 2012) together with upper and lower bounds on the solutions. Also, necessary and sufficient conditions were found for the linear stability of the equilibrium in which the hare is extinct, and sufficient conditions were found for the global stability of this equilibrium. Numerical simulations confirmed the analytical results and demonstrated the existence of limit cycles over ranges of parameters reasonable for hares browsing on woody vegetation in boreal ecosystems. This showed that age dependence in plant chemical defenses has the capacity to cause hare–plant population cycles. The TSM's mathematical formulation is described in detail in the Supplementary material Appendix 1, as are the values and sources of the parameters used.

The flow chart presented in Fig. 2 shows the model's dynamics. This figure shows the flow of increments (in different shades of gray) of biomass in the twig in one time step, starting from YST 1, transfer from YST to more digestible OST, and transfer from OST to the less digestible OST biomass that is starvation food. At the same time, the more digestible OST biomass is removed and ingested by the hare, while the more toxic YST biomass is discarded (see middle of Fig. 1). In the simplified version of the model used here, no YST biomass is consumed; all is discarded.

Application of model to plant of interest – the shrub birch Betula glandulosa

We selected *B. glandulosa* for use in TSM simulations for these reasons. 1) The *B. glandulosa* twig growth data collected at Kluane, Yukon, from 1988 to 2008 (see 'Shrub growth' worksheet in Kluane Monitoring Data Excel workbook, <www.zoology.ubc.ca/~krebs/kluane.html>) is sufficient to test the predictions made by the TSM simulations (Fig. 3). 2) The twigs of *B. glandulosa*'s mature developmental phase (sensu Kozlowski 1971, chapter 3, titled Maturation or phase change) are the Kluane hare's favorite winter-food (Krebs 2011). However, at Kluane the twigs of juvenile *B. glandulosa* are rarely browsed in winter by free-ranging snowshoe hares (Williams et al. 1992). Moreover, the last two years of growth of these twigs produced a resin that strongly deterred feeding by snowshoe hares captured at Kluane: in Fig. 1 these 2 segments are the YST 1 segment and the YST 2 segment. 3) This resin contained two dammarane triterpenes (papyriferic acid, acronym PA, Reichardt 1981 and 3-*0*-malyonylebetulafolientriol oxide I, acronym 3*0*I, Reichardt et al. 1987). PA deters snowshoe hare feeding (Reichardt et al. 1984) and is also toxic to Alaskan snowshoe hares (Sorensen-Forbey et al. 2011).

Here we must provide a caveat. Our choice of *B. glandulosa* necessarily limits the specific conclusions that we draw in the discussion to habitats in which *B. glandulosa* is an important component of the snowshoe hare's winter diet. At Kluane, Yukon, *B. glandulosa* is a comparatively rare species that does not occur everywhere (Smith et al. 1988). Thus, *our discussion should only be interpreted as an example of the potential value of the TSM* as a tool to be used when studying the role anti-browsing toxic chemical defense may play in reducing snowshoe hare reproduction during the cycle's low phase.

Experiments

The experimental data required to test the TSM are the previously unpublished results of two earlier experiments. The Alaska experiment was conducted at the Institute of Arctic Biology, Univ. of Alaska, Fairbanks, during February and March 1986. The Kluane experiment provided monitoring data from 1988 to 2008 (<www.zoology.ubc.ca/~krebs/kluane.html>). The location of the Kluane region, along with the locations of all other places in this paper, is shown in the Supplementary material Appendix 1 PowerPoint slide show named 'Places'.

Alaska experiment

Objectives

The Alaska experiment had four objectives. The first objective was to measure the concentrations of 3*0*I and PA in three *B. glandulosa* twig segments: 1) the YST 1 segment of the twigs of mature phase *B. glandulosa* genets that had not been browsed for at least four years (hereafter called unbrowsed genets); 2) the OST segments of the twigs of these unbrowsed genets; and 3) the YST 1 regrowth segment produce by *B. glandulosa* genets that had been severely browsed in the previous winter (hereafter called browsed genets). The second objective was to determine if 3*0*I, when added to an artificial diet (rolled oats) at below the concentration found in the YST 1 segment of unbrowsed *B. glandulosa* genets, deterred feeding by captive snowshoe hares. Reichardt et al. (1984) had previously shown that PA strongly deterred feeding by captive snowshoe hares. The third objective was to measure the per capita daily consumption by captive snowshoe hares of the biomasses of the YST 1 segment and the older segments of unbrowsed genets. The fourth objective was to measure the per capita daily consumption by captive snowshoe hares of the biomass of the regrowth YST 1 segment biomass that was produced by the browsed genets in the summer that followed the a browsing event (summer 1985).

Twig collection sites

The twigs assayed for their concentrations of 3*0*I and PA were collected in January 1986 at each of five collection sites located on a 200 km long north to south transect that originated in Goldstream Valley, Alaska (64°96′32″N, 147°95′08″W) and ended at Denali National Park, Alaska (64°39′32″N, 147°95′59″W). The twigs used to obtain the *30*I that was used in the feeding deterrence bioassay and the *B. glandulosa* twigs used in feeding trials were both collected in the Goldstream Valley site during February and March 1986.

Sampling *B. glandulosa* genets within collection sites

Two sets of *B. glandulosa* genets were sampled within each collection site, unbrowsed genets and browsed genets. The unbrowsed genets were growing at least 100 m from the dense thickets of spruce *Picea* spp. and/or alder *Alnus* spp. that are common near Fairbanks, Alaska. The snowshoe hares in this region retreat into these thickets during a hare low in order to evade their predators (Wolff 1980). Presumably distance from predation refuges explains why these genets were unbrowsed: at the time of their collection (start of a hare increase) fear of predation was probably still keeping hares very near predation refuges (Wolff 1980, Hik 1995). The browsed genets were growing within 5 m of a predation refuge. In the previous winter (winter 1984–1985) hares had browsed all twigs < 5 mm diameter, hares had gnawed off most main stems at a diameter > 10 mm and hares had also girdled (ring-barked) most main stems.

Twig collection protocol

Twigs were collected by clipping at a diameter of 3 mm, because in the case of snowshoe hares residing in interior Alaska, large diameter twig segments are starvation food (Wolff 1980). The twigs of the unbrowsed genets were separated into two parts (YST 1 segment biomass; OST segment biomass) by clipping at the terminal bud scar (diameter ≈ 1.5 mm) that separated the YST 1 segment biomass from the OST segment biomass. Since the twig segments collected from the browsed genets were 100% YST 1 biomass, they were not clipped into two parts. The resulting three samples of twig segments from each of the five collection sites were stored at –40°C until their concentrations of *30*I + PA were assayed in May 1986.

*30*I + PA assays

At each of the five twig collection sites, we randomly selected 10 unbrowsed genets and 10 browsed genets. Five twigs were collected from each unbrowsed genet and five twigs were collected from each browsed genet. After the twigs from the unbrowsed genets had been divided into two biomass types (YST 1 segment biomass, OST segment biomass), the twig segment collections were pooled by twig segment type: unbrowsed genet YST 1 segment biomass; unbrowsed genet OST segment biomass; browsed genet YST 1 regrowth biomass. This procedure resulted in five samples per twig segment type. In the case of the unbrowsed genets, a one-tailed paired t-test with 4 degrees of freedom [$t_{(1,4df)}$] and $\alpha = 0.05$ was used for statistical analysis of the comparison of the concentrations of *30*I + PA in the YST 1 segment biomass versus the older segment biomass. Because free-ranging hares rejected the YST 1 segment biomass, we expected this biomass to have the highest concentration of *30*I + PA. A two-tailed two-sample t-test with 8 degrees of freedom [$t_{(2,8df)}$] and $\alpha = 0.05$ was used to test the statistical significance of the difference in the concentrations of 30I + PA, if any, existing between the YST 1 segment biomass of unbrowsed genets versus the regrowth YST 1 biomass of the browsed genets. The method of triterpene assay can be found in Reichardt et al. (1984). Unfortunately at 60 MHz the spectral signals used for quantifications of *30*I and PA coincide, so we can report only the combined concentration *30*I + PA. Based upon relative spot size on thin layer chromatography plates (visualized by H_2SO_4 followed by heating), we estimate the *30*I:PA ratio to be ca. 10:1. This result is consistent with the results obtained by Williams et al.'s (1992) chemical analysis of Kluane *B. glandulosa* YST 1 segment biomass + YST 2 segment biomass.

Feeding trials with captive hares

The twigs used for the maintenance of captive hares and the twigs used in the feeding trials with captive hares both came from the Goldstream Valley site. All twigs were collected weekly and, before use, were stored in tightly sealed plastic bags in the hare facility described by Reichardt et al. (1984). The adult 10 hares (six females, four males; age undetermined) that were used in these feeding trials were captured November 1985 in Goldstream Valley. The hares were kept in the above mentioned hare facility. One wall of this building was open, so the ambient temperature in the building was approximately the ambient temperature outside the building. The lowest temperature recorded in the building during the period that hares were housed in it was –44°C. The hares were released in April 1986 where they had been captured. When released, the hares had lost no weight.

From capture until two weeks before the feeding experiments began, the hares were fed a pure browse diet that had been previously used by the IAB to maintain captive hares for several months in winter with no weight loss and in a positive nitrogen balance: 350 g wet mass of winter-dormant twig tips (<3 mm diameter) from the upper crown of the mature phase (sensu Kozlowski 1971) of *B. neoalaskana* (200 g), *Salix alaxensis* (100 g), *Populus tremuloides* (30 g) and *Picea glauca* (20 g). Over the 14 days preceding the feeding trials the hares were gradually acclimated to eating *B. glandulosa* twig biomass by gradually replacing 100 g of the *B. neoalaskana* twig biomass with 100 g of *B. glandulosa* twig biomass that was collected from unbrowsed mature phase *B. glandulosa*. Although the *B. glandulosa* twigs contained both YST biomass and OST biomass, their predominant biomass was OST segment biomass. Like the free ranging hares that reside in Goldstream Valley, the hares that were captured in Goldstream Valley primarily ate OST biomass and rejected most of the YST biomass, and especially the most resinous biomass of the YST 1 segment.

The feeding trials were conducted during February and March 1986. During all feeding trials the temperature within the hare facility was below –20°C. The feeding trials began one hour after sunset. After each trial, the hares were returned for a week to the maintenance diet that contained mature phase *B. glandulosa* twig biomass. The first feeding trial used twigs collected from unbrowsed genets. Its objective was to compare the hares' preference for YST 1 biomass versus the biomass of older segments, which contained YST 2 biomass, YST 3 biomass and OST biomass. The locations of these biomass segments within a twig and the segments' chronological ages are shown in the left stick drawing in Fig. 1. The two types of biomass were simultaneously offered to the hares for 24 h in two separate pans: one pan contained 150 g of the YST 1 segment biomass; one pan contained 150 g of the older segment biomass. The second feeding trial measured the hares' per capita daily intake of the YST 1 regrowth biomass produced by genets that had been severely browsed in the previous winter: winter 1984−1985. This feeding trial lasted only 48 h because the hares consumed so little biomass (1.4 ± 0.3 g dry mass kg^{-1} hare day^{-1}) that continuing the trial for a longer period would have killed hares. The third and fourth feeding trials, respectively, tested the feeding deterrent capacity of *B. glandulosa* resin and this resin's primary constituent, 3*O*I. In these feeding trials the hares were simultaneously offered for six h a pan containing 35 g of the control diet (rolled oats treated with the extraction solvent (diethyl ether), but not treated with an extract) and a pan of the treatment diet (rolled oats that had been treated with an extract – resin or 3*O*I but not both). When testing *B. glandulosa* resin for feeding deterrent capacity the treatment diet was 35 g rolled oats with a resin concentration of 17% dry mass. This is the concentration of resin found in the YST 1 segment biomass of unbrowsed *B. glandulosa* genets growing in Goldstream Valley, Alaska. The 3*O*I used to test its feeding deterrent capacity had been purified by flash chromatography. Because of the difficulty in isolating large amounts of pure 3*O*I, the treatment diet was 11 g of 3*O*I treated oats. The 3*O*I concentration of the treatment diet was 2.9% dry mass. This 3*O*I concentration was about 58% of the dry mass concentration of 3*O*I + PA mix found in the YST 1 segment of the unbrowsed *B. glandulosa* genets that was fed to hares in the feeding trial that compared the hares' preference for YST 1 biomass versus the biomass of older segments. We did not test the feeding deterrence capacity of PA because Reichardt et al. (1984) had already established that PA strongly deterred hare feeding. Because all of the four feeding trials used the same 10 hares, the statistical significance of all pairwise comparisons was tested with a one-tailed paired t-test with 9 degrees of freedom [$t_{(1,9df)}$] and $\alpha = 0.05$.

Kluane experiment

The raw data of the Kluane experiment came from the Kluane Monitoring Project Excel workbook (<www.zoology.ubc.ca/~krebs/kluane.html>): three worksheets were used – Shrub growth, Hares, and Lynx tracks. The data in the worksheet sheet 'Shrub growth' were used to estimate aging and its reversal by hare browsing. These data are the percentage-annual-increase in the biomass of a 5 mm basal diameter *B. glandulosa* twig that is YST 1 biomass; that is, the ratio (YST 1 segment biomass/twig total biomass); in Fig. 4 this ratio is labeled (YST 1 segment biomass/TT biomass) where TT biomass means total twig biomass. Aging was expected to reduce the magnitude of this ratio (Kozlowski 1971). A pruning caused reversal of aging was expected to increase the value of this ratio (Moorby and Waring 1963). The sampling units used were individual 5 mm twigs collected from winter-dormant *B. glandulosa* spaced at a minimum of 15 m apart. Five millimeters is the maximum diameter at the point of browsing recorded during the comparatively high-density hare peak that occurred at Kluane in the early 1980s (Smith et al. (1988). During the 21 years from 1988 through 2008 the number of twigs sampled per year has ranged from 129–544 twigs $year^{-1}$ (mean ± 1 SD is 338 ± 110 twigs $year^{-1}$). A more detailed description of this method of measuring twig growth can be found in Melnychuck and Krebs (2005). The density of hares can be found in the Kluane monitoring project in worksheet 'Hares'. Live trapping (86 traps on 20 × 20 grids, 30 m spacing) was used to estimate hare density. DENSITY 5 (<www.otago.ac.nz/density>, Efford et al. 2009) was used to calculate the estimate. Lynx density was estimated by the method of Sheriff et al. (2011) from the number of lynx *Lynx canadensis* tracks counted annually in a 350 km^2 study area.

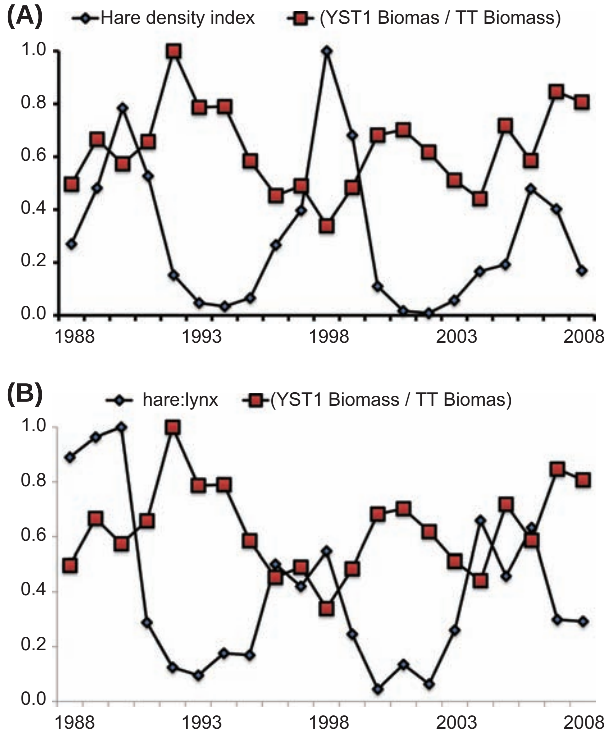

Figure 4. Results of the Kluane experiment. The highest values of the ratio (YST 1 biomass/ TT biomass) occurred during the hare decline and the ratio's value remained comparatively high during the subsequent low phase of the hare cycle (Fig. 4A): TT biomass means total twig biomass. In Fig. 4B, the highest values of the ratio (YST 1 biomass/ TT biomass) are coincident with the lowest values of the ratio of hares:lynx. The units of the ratio hare:lynx and the ratio (YST biomass / TT biomass) have been normalized to range from 0–1. Thus, these units have no dimensions.

Results

Model predictions

Equations 7, 8 and 9 in the Supplementary material Appendix 1, with initial conditions, were simulated, with the assumption of a time delay, $\tau_1 = 3$ years, and other parameter values given in Table 1 of the Supplementary materials Appendix 1. These are reasonable values for Alaskan snowshoe hares and Alaskan *Betula glandulosa* as the browse species. Note that in these simulations we set the limiting diameter of browsing to 5 mm because the data in worksheet 'Shrub growth' that was mentioned above came from twigs collected by clipping at a diameter of 5 mm. The two predictions made by the simulations are represented by the line graphs shown in Fig. 3. The important temporal pattern to note is that the magnitude of the ratio (YST biomass / twig total biomass) peaks during the simulated hare decline and remains comparatively high during the subsequent simulated hare cycle low phase. This result predicts that the toxicity of twigs, which in this case are twigs of winter-dormant *B. glandulosa* genets, will peak shortly after the hare population has peaked, and will remain comparatively high during the subsequent hare low. The increase in toxicity is the expected consequence of a browsing-caused reversal of aging that has greatly increased the percentage of a winter-dormant *B. glandulosa* twig's biomass that is composed of the most toxic YST 1 segment: compare left and right stick drawings in Fig. 1. We again emphasize that this predicted browsing-caused change in twig toxicity is not a result of either an induced defense (sensu Karban and Baldwin 1997) or pruning-caused reversion to the juvenile developmental phase (sensu Kozlowski 1971). It is nothing more than the browsing-caused increase in the size of the YST 1 segment that occurs when aging has been reversed by pruning (Moorby and Waring 1963).

Alaska experiment

The Alaska experiment's results are presented in Table 1. Irrespective of its source (unbrowsed genets, browsed genets), YST 1 segment biomass had about the same dry mass concentration of the potentially toxic dammarane triterpene mix 3*0*I + PA: the concentration difference was only 0.4% dry mass. This statistically insignificant difference ($t_{(2,8df)} = 0.9409$, $p = 0.3743$) strongly suggested that that the primary response to browsing in the previous winter had been a reversal of aging rather than either an induced defense (sensu Karban and Baldwin 1997) or a pruning caused reversion to the juvenile developmental phase (sensu Kozlowski 1971 chapter 3). Either an induced defense or a reversion to the juvenile developmental phase would have caused a greater change in defense chemistry (Bryant et al. 1991b). The concentration of 3*0*I + PA in the YST 1 segment biomass of the twigs from unbrowsed genets (4.5 ± 0.3% dry mass) was about 20 × greater than the concentration of 3*0*I + PA in the biomass of the older segments of twigs from unbrowsed genets (0.23 ± 0.01% dry mass). The lower concentration of 3*0*I + PA found in the biomass of the older segments of twigs from unbrowsed genets was caused by the domination of older segment biomass by the comparatively large diameter of the OST segments. Because of aging, the biomass of the YST segments was small in comparison to the biomass of the OST segments; in chronological time, aging progressively reduces both the lengths and diameters of YST segments (Kozlowski 1971), as depicted in the left stick drawing in Fig. 1. Measurement of the feeding deterrent potencies of *B. glandulosa* crude resin and the purified 3*0*I demonstrated that both the crude resin extract and the purified 3*0*I strongly deterred hare feeding. The feeding trial that compared the consumption of the biomass of the YST 1 segments of the twigs from unbrowsed genets with the biomass of the twig's older segments, which were composed of the predominantly less resinous OST segment biomass, demonstrated that the hares' consumption of the older segment biomass (49.9 ± 7 g dry mass kg^{-1} hare day^{-1}) was about 50 × greater than their consumption of the YST 1 segment biomass (1.0 ± 0.4 g dry mass kg^{-1} hare day^{-1}). When the hares were only offered the YST 1 regrowth segment biomass produced by *B. glandulosa* that had been severely browsed by hares in the previous winter, the hares' per capita daily intake of this biomass was also very low (1.4 ± 0.3 g dry mass kg^{-1} hare day^{-1}), and was comparable to their consumption of the YST 1 segment biomass of the twigs from unbrowsed mature phase genets (1.0 ± 0.4 g dry mass kg^{-1} hare day^{-1}).

Table 1. Results of the Alaska experiment. Unbrowsed means twigs were collected from *B. glandulosa* genets that had not been browsed for at least four years. Browsed means that twigs were collected from *B. glandulosa* genets that had been severely browsed in the previous winter (winter 1984–1985). The 3*0*I + PA concentrations are % dry mass (% DM). Feeding deterrence (Deterrence) is grams oatmeal eaten per hare in a 6-h period (treated oatmeal versus untreated control oatmeal). Twig segment intake (Intake) is g dry mass (DM) kg^{-1} hare day^{-1}. The data are presented as the mean ± 1 SE of the mean. The statistical significance of the pairwise comparisons was tested with a one-tailed paired t-test. The t-values, their degrees of freedom [$T_{1(df)}$] and the probability of a type I error (p) are shown.

% DM 3B*0*I + PA	YST 1	$T_{1(4df)}$	p	OST
unbrowsed	4.5 ± 0.3	16.0704	$p < 0.0001$	0.23 ± 0.01
browsed YST 1	4.9 ± 0.4			
Deterrence	Treatment	$\mathbf{T}_{1(9df)}$	p	Control
resin	0.5 ± 0.3	10.2287	$p < 0.0001$	23.4 ± 2.5
3*0*I	1.6 ± 0.5	11.4572	$p < 0.0001$	21.3 ± 1.4
Intake	YST 1	$\mathbf{T}_{1(9df)}$	p	OST
unbrowsed	1.0 ± 0.4	6.7604	$p < 0.0001$	49.9 ± 7.3
browsed	1.4 ± 0.3			

Kluane experiment

At Kluane, Yukon, from 1976 through 2013 the hare cycle's period has been 8–9 years (see worksheet Hares in Kluane Monitoring Project Excel workbook, <www.zoology.ubc.ca/~krebs/kluane.html>). At Kluane, from 1988 to 2008 the percentage of a 5 mm *B. glandulosa* twig's biomass that was YST 1 segment biomass peaked in the first year of each hare decline and remained comparatively high during the each following hare low: In Fig. 4 the ratio (YST 1 segment biomass / total twig biomass) biomass peaked in the first year of each hare decline and remained comparatively high during the following hare low. This result indicates that during these 21 years, as predicted by the TSM model, a reversal of aging occurred during the peak of each hare cycle. Thus, during these 21 years the presumed toxicity of twigs of the maximum diameter that Kluane snowshoe hares eat in winter should have been highest during each hare decline and following hare low. During these 21 years the ratio of hares:lynx was lowest during the each hare cycle low exactly when the percentage of a 5 mm twig's biomass that was YST 1 segment biomass was comparatively high (Fig. 4B). Since the risk of predation, and hence the chronic stress caused by fear of predation, is highest when the hare:lynx ratio is lowest (Sheriff et al. 2011), this result indicates that any chronic stress caused by fear of predation was highest exactly when the presumed toxicity of *B. glandulosa* twig biomass to snowshoe hares was highest.

Discussion

In this paper, we explored the possibility that hare browsing in a hare peak, by reversing aging by a woody plant (Moorby and Waring 1963), could cause an increase in the toxicity of the Kluane snowshoe hare's preferred winter-food during the following hare low. This exploration used the twig segment model (TSM model) developed by Liu et al. (2012) to predict how hare browsing should affect twig toxicity during the hare cycle's low phase.

The TSM predicted that the biomass of the proportion of a *Betula glandulosa* twig's total biomass that is younger segment biomass (YST segment biomass) will peak in the winter of the hare decline and remain high throughout the winters of the following hare low (Fig. 3). The implication of this prediction is that, during the winters of the low phase of a hare cycle, hares will be forced by the comparatively high toxicity of YST segment biomass to eat the comparatively less toxic, but larger diameter, indigestible and nutrient deficient older segments of twigs that are starvation food (Fig. 2). The expected result of having to consume starvation food is a case of malnutrition that could reduce hare reproduction during a hare low.

The Alaska experiment demonstrated that severe snowshoe hare browsing in winter of *B. glandulosa*, like that occurring in a hare peak, does in fact, increase the lengths and the diameters of the regrowth YST 1 segment produced in the following summer. This result verified the TSM's assumption that browsing will reverse aging. This experiment further demonstrated that in winter, the YST 1 segments of *B. glandulosa*, irrespective of their diameter, have a high concentration of two feeding deterrent dammarane triterpenes, papyriferic acid (acronym PA) and 3-*0*-malyonylebetulafolientriol oxide I (acronym 3*0*I), one of which, PA, is also toxic to snowshoe hares (Sorensen-Forbey et al. 2011). This increase in toxicity may explain why, in the Alaska experiment, the captive hares' consumption of *B. glandulosa* YST 1 biomass was 50 times lower than their consumption of *B. glandulosa* older segment (OST) biomass. Increased toxicity of *B. glandulosa* YST segment biomass in general presumably explains why free ranging hares residing in interior Alaska (J. P. Bryant – Denali National Park, Goldstream Valley, Nelchina Basin; D. DeFolco – Wiseman; J. Whitman – upper Kuskokwim River Valley), residing at Kluane, Yukon (D. Hik) and residing in the Mackenzie River Valley in Canada's Northwest Territories (D. Allaire) all have selectively consumed the OST segment biomass of winter-dormant *B. glandulosa* twigs and have rejected the presumably more toxic YST biomass segments of the same twigs.

The importance of the above results is that, in concert, they experimentally demonstrate that hare browsing, by reversing plant aging, could force snowshoe hares to eat large diameter comparatively indigestible, and nutrient deficient OST biomass during the hare cycle's low phase. We suggest that this reduction in diet quality, by causing malnutrition, could reduce hare reproduction during the hare cycle's low phase.

The Kluane field experiment verified that in the case of *B. glandulosa* that, as predicted by the TSM (Fig. 3), the percentage of a winter-dormant twig's biomass that is YST 1 segment biomass did peak in the winter of each hare decline and did then remain comparatively high throughout each subsequent hare low (Fig. 4A). The importance of this result in concert with the results of the Alaska experiment is that it implies that, at Kluane, during hare declines and subsequent cyclic lows the snowshoe hare's per capita daily intake of their favorite winter-food, twigs of mature *B. glandulosa* (Krebs 2011), may have declined. This possibility has been supported by secondary metabolite assays of *B. glandulosa* twigs collected in winter at Kluane and by laboratory feeding trials using the resin extracted from these twigs and snowshoe hares captured at Kluane (Williams et al. 1992). The last two years of *B. glandulosa* twig growth (YST 1 segment + YST 2 segment) did produce a resin that strongly deterred feeding by Kluane snowshoe hares. Furthermore, this resin's primary constituent was the dammarane triterpene 3*0*I, which strongly deterred feeding by the Alaskan captive hares that were used in this study. The Kluane feeding deterrent *B. glandulosa* resin also contained the dammarane triterpene PA (Williams et al. 1992) that, in interior Alaska, strongly deters snowshoe hare feeding (Reichardt et al. 1984) and is also toxic to snowshoe hares (Sorensen-Forbey et al. 2011).

We have, therefore, concluded that at Kluane, Yukon, browsing by snowshoe hares in the winter of a hare peak could decrease their intake of at least one preferred winter food, the twigs of the shrub birch *B. glandulosa*, during the winters of the following hare low. When the basis for the low palatability of the twigs of other winter-dormant browse species to Alaskan snowshoe hares has been chemically determined, it has always been found to be caused by lipophilic secondary metabolites (Bryant et al. 1983, Reichardt et al. 1984, 1990a, b, Clausen et al. 1986) that are either potentially toxic to mammals or have been experimentally demonstrated to be toxic to mammals (Bryant et al. 1991a). Thus, the TSM model may apply to more browse species than *B. glandulosa*. In short, a browsing-caused reversal of aging, which increases the proportion of the twig biomass of the diameter preferred by snowshoe hares in winter that is toxic to hares, may be widespread. If this hypothesis is true, then browsing in a hare peak, by increasing the toxicity of the snowshoe hare's preferred winter food during the following hare low, could be a cause of the low phase reduction in reproduction that is required to cause a hare population to cycle.

The other hypothesis that shows promise in explaining reduced reproduction in the hare cycle's low phase is the predator-induced chronic stress hypothesis (Boonstra et al. 1998, Sheriff et al. 2011). This hypothesis predicts that during the hare decline, when the hare:lynx ratio is low, fear of predation chronically stresses hares, and this chronic stress reduces reproduction during the hare decline. Furthermore, this stress is then propagated into the following hare low by maternal inheritance of high levels free cortisol.

In Fig. 4B we have graphically compared these two hypotheses by comparing the change in the ratio (YST 1

Table 2. Peak snowshoe hare densities estimated in autumn: LT = live trapping; PC = fecal Pellet Count. Data sources: Northwest Territories (Suzanne Carrier); Alberta (Keith and Windberg 1978); British Columbia (Wildlife Dynamics Consulting); Kluane Ecosystem Monitoring Project (< www.zoology.ubc.ca/~krebs/kluane.html >); Yukon Tanana Uplands (Wolff 1980); Bonanza Creek Long-Term Ecological Research Program; Tetlin National Wildlife Refuge; Kenai National Wildlife Refuge; Wrangle-Saint Elias National Park. The locations of these places can be found in the map in the Supplementary material Appendix 1 PowerPoint named 'Places'.

Region	Hares ha^{-1}
Northwest Territories^{PC}	
1990	2.50
1999	2.38
2009	1.35
Rochester, Alberta^{LT}	
1971	9.16
Skeena British Columbia^{PC}	
2003	1.51
Kluane, Yukon^{LT}	
1981	4.43
1990	1.68
1998	2.73
2006	1.15
Alaska	
Yukon-Tanana Uplands^{LT}	
1971	5.88
Tetlin National Wildlife Refuge^{PC}	
2008	0.95
Bonanza Creek Long-term Ecological Research Site^{LT}	
1990	6.53
1999	6.00
Wrangle-Saint Elias National Park^{PC}	
1991	2.85
2001	3.93
2009	2.68
Kenai National Wildlife Refuge ^{PC}	
1983	3.55
1999	1.23
2011	1.45

biomass/total twig biomass) of Kluane *B. glandulosa* with the change in the hare:lynx ratio at Kluane. The comparison shows that from 1988–2008, when the value of the hare:lynx ratio was low (risk of predation was high) the value of *B. glandulosa*'s (YST 1 biomass/total twig biomass) ratio was high (twig toxicity was high). This comparison therefore suggests that at Kluane the comparative importance of a predator-induced chronic stress versus a browsing-caused increase in twig toxicity in reducing hare reproduction during the cyclic needs to be determined.

Suggested future research

In winter, the snowshoe hare generally requires a multi-species diet (Bookhout 1965, Rodgers and Sinclair 1997), and mature *B. glandulosa* is only one of the snowshoe hare's preferred winter-foods. We therefore suggest that the generality of the TSM model requires testing with a variety of the snowshoe hare's preferred winter-foods. Moreover, the density of hares during the hare cycle's peak has varied both in space and in time (Table 2). This variation implies that the intensity of browsing by hares on their preferred browse species has also varied among hare cycles. We suggest that the TSM model needs testing in several locations that are characterized by a strong hare cycle, and furthermore that this testing will require the use of a long-term monitoring program such as the one at Kluane, Yukon.

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Supplementary material (available online as Appendix oik.01671 at <www.oikosjournal.org/readers/appendix>). Appendix 1.