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The influence of plant defensive chemicals, diet composition, and winter severity on the nutritional condition of a free-ranging, generalist herbivore

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When consuming plants, herbivores must deal with both low nutritional quality from cell wall constituents and potentially toxic plant secondary metabolites, which are often inversely related. Herbivores that consume a highly nutritious, but chemically defended plant, may consume high levels of toxins that require energy for detoxification. Alternatively, herbivores may avoid consuming high levels of toxins by consuming a diverse diet that may be lower in overall nutritional quality. In this study, we assessed the relationship among nutritional restriction, detoxification and diet diversity in a free-ranging wild herbivore. We collected urine deposited in the snow (hereafter, snow-urine) and feces by free-ranging moose *Alces americanus*, a generalist browser, during winter. We used the ratio of urinary urea nitrogen to creatinine (UN:C), measured in snow-urine samples, as an indicator of nutritional restriction, and the ratio of glucuronic acid to creatinine (GA:C), as an indicator of investment in detoxification. We used microhistology to determine diet composition from fecal pellets. GA:C and UN:C were positively associated, suggesting that nutritional condition tends to be worse for individuals investing more in detoxification. We found, after accounting for the influence of winter severity, diet diversity and UN:C to be negatively related, suggesting that increasingly diverse diets were associated with improved nutritional condition. Overall, the most important predictor of UN:C was winter severity and proportion of diet comprised of balsam fir *Abies balsamea*. Physiological indicators of nutritional restriction tended to be worse during severe winters and among individuals that had consumed more balsam fir. These results highlight complex relationships among environmental conditions, foraging decisions, and costs of detoxification that can influence nutritional condition of herbivores.

Many plant secondary metabolites (PSMs) deter herbivores by being toxic in one way or another (Freeland and Janzen 1974, Provenza et al. 2003). For example, PSMs can inhibit activity of enzymes (Forbey et al. 2011, Kohl et al. 2015) and reduce nutrient uptake, energy budgets and reproductive output (Sorensen et al. 2005, DeGabriel et al. 2009, Au et al. 2013). In response, herbivores have developed various physiological mechanisms to detoxify and excrete ingested PSMs (McLean and Duncan 2006, Sorensen et al. 2006). For that reason, one might expect increased investment in detoxification of PSMs would lead to improved nutritional condition of an herbivore. However, detoxification is energetically costly (McLean et al. 2001, Mangione et al. 2004, Sorensen et al. 2005). If sufficiently costly, then increased investment in detoxification of PSMs would lead to worse nutritional condition. We are unaware of prior research to distinguish these two possibilities in a free-ranging mammalian herbivore and provide such an assessment here.

To assess the relationship between nutritional condition and detoxification in free-ranging wild herbivores requires methods that are non-invasive, practical, and assess both attributes in a similar way. One method that has been used to measure nutritional condition of wild herbivores is the ratio of urinary urea nitrogen to creatinine (UN:C) in samples of snow that contain urine (DelGiudice et al. 1997). That ratio is useful for comparing the condition of individuals of the same population that consume similar diets (DelGiudice 1995). Increased excretion of urinary urea nitrogen during mid and late winter indicates increased catabolism of endogenous protein as a result of prolonged nutritional restriction. Because the analyzed sample is a mixture of snow and urine, the concentration of nitrogen in a sample is influenced by the amount of snow (and the degree to which an individual is dehydrated). To account for those sources of variation, the measured concentration of UN is standardized by dividing by the concentration of C, which is excreted at a constant rate over time (DelGiudice et al. 1988, 1996, 1997, Servello and Schneider 2000). Finally, it is important to note that UN:C can be high both when animals are in poor condition and using their body stores for protein and when in animals

are eating high protein food. Because this is a winter study, the former is likely the case. Also, because UN:C is not a linear index inferences drawn from UN:C results could be misleading.

On the other hand, methods for assessing detoxification in mammalian herbivores have been more difficult to obtain. For example, most such research involves longitudinal sampling of blood (McLean et al. 2008), which is typically not possible to do for free-ranging, wild animals. However, glucuronic acid (GA), a metabolite produced from Phase II detoxification, or conjugation, can be found in urine. Among mammalian herbivores, GA excreted in urine is a useful indicator of an organism's investment to detoxify PSMs (Marsh et al. 2006). Conjugation of PSMs with GA is one detoxification pathway that converts PSMs into more water-soluble compounds, which is a prerequisite for being excreted through urine (Villalba et al. 2005). The excretion of GA is also positively correlated with increased intake of individual PSMs (Guglielmo et al. 1996), extracts of plants (Mangione et al. 2004) and whole plants (Sorensen et al. 2005). GA conjugation also incurs a metabolic cost involving the loss of endogenous glucose (Sorensen et al. 2005, Villalba et al. 2005). To account for the influence of snow and dehydration on the concentration of GA, we standardized its concentration by dividing by the concentration of C. We take GA:C to be an indicator of the investment in detoxification.

If the investment in detoxification is positively associated with nutritional condition, it may indicate a net benefit, whereby the nutritional benefits of detoxification outweigh the metabolic cost. However, the concentration of GA could more simply be indicative of an herbivore that has been eating a particularly toxic diet; and although the investment in detoxifying PSMs is necessary, it is also associated with poor nutritional condition resulting from a poor diet. In this paper, we assessed the relationship between GA:C excretion and nutritional restriction for a population of free-ranging moose *Alces americanus* during the winter.

During winter, forage is low in energy and high in potentially toxic PSMs (Shipley et al. 1998, Servello and Schneider 2000). We conducted this assessment in two different winters, one of which was average and the other was severe (i.e. deep snow). During severe winters, the energetic cost of locomotion is greater, which is likely to influence foraging decisions (Parker et al. 1984) and may compete with energy allocated to the detoxification of PSMs (Sorensen et al. 2005). Moose foraging is further impacted by severe winters because deep snow covers forage that is closer to the ground.

In addition to detoxifying PSMs, an herbivore can also manage PSMs by consuming a diverse diet. A diverse diet may minimize the rate at which any particular kind of toxic PSMs is ingested. Additionally, because many detoxification pathways are rate limited (i.e. Phase I pathways, Casarett et al. 2008) and PSMs are detoxified by different enzymes, diversifying intake of PSMs reduces the risk of saturating any one detoxification pathway (Freeland and Janzen 1974, Marsh et al. 2006). That diverse diets are higher quality diets is supported by both theoretical (Westoby 1974, Marsh et al. 2006) and empirical evidence (Bernays et al. 1994, Marsh et al. 2006, Coltrane and Barboza 2010). Diverse diets also provide the best composition of carbohydrates, protein and micronutrients for a variety of herbivore species (Westoby 1974, Seccombe-Hett and Turkington 2008, Wang et al. 2010), including moose (Oldemeyer et al. 1977). During the summer, captive moose also consume less preferred species, even when most preferred species are provided ad libitum (Miquelle and Jordan 1979). Free-ranging moose, foraging during the summer, also feed on multiple species when it seems possible for them to acquire all of their energetic needs from a single species (Miquelle and Jordan 1979). Those ideas suggest that diet diversity would improve nutritional condition. We hypothesize diet diversity to be associated with improved nutritional condition in moose.

However, diet diversity is likely to be advantageous only up to a point. For example, too much diversity may lead to a diet with suboptimal proportions of energy and protein (Wang et al. 2010). Additionally, ingesting a diverse diet may lead to increased search costs and longer foraging time to seek out rare items while passing up common forage items. That increased foraging time could result in a reduction of overall intake rate or intake rate per unit effort spent foraging. Either case could result in reduced nutritional condition. Most of what is known about the relationship between diet diversity and nutrition has been derived from organisms raised under relatively benign conditions, i.e. captive-raised and/or raised on summer forage (Seccombe-Hett and Turkington 2008, Wang et al. 2010; but see Coltrane and Barboza 2010).

In this study, we tested three hypotheses. First, animals may eat more toxins in attempt to get more nutritious food (more digestible nitrogen). Often plants defend the most nutritious parts of plants, such as new growth. If so, UN:C would be directly correlated with GA:C. Second, animals that eat more toxic plants might have lower body condition because of the energetic cost of detoxification, thus UN:C would be inversely correlated with GA:C. Third, we test the hypothesis that UN:C is correlated with diet diversity. To test these hypotheses, we measured UN:C and GA:C in samples of snow-urine, where the urine was deposited from moose on Isle Royale, UN:C was taken as an indicator of nutritional restriction in late winter, and GA:C was taken as an indicator of the investment in detoxification.

Study system

We assessed the relationships among nutritional restriction (UN:C), investment in detoxification (GA:C), and diet diversity for moose in Isle Royale National Park during the winters of 2013 and 2014. Isle Royale National Park is a remote island (544 km^2) located in the northwest portion of Lake Superior, North America (47°50′N, 89°00′W). The island is inhabited by a population of moose known to be influenced by predation, climate and forage quantity (Vucetich et al. 2002, Wilmers et al. 2004, Vucetich and Peterson 2014). During the study period, moose density was between 1.7 km^{-2} and 2.1 km^{-2} (Vucetich and Peterson 2014). Those densities are high compared to many North American sites, but near the long-term average for this particular site.

The climate is characterized by warm summers and cold, snowy winters. For the two winters of this study, one was

typical and the other was severe with respect to snow depth. In particular, the mean snow depth during the winter of 2012–2013 was 29.6 cm, which represents the 13th percentile of snow depths for the period 1971–2014. During the winter of 2013–2014, mean snow depth was 72.4 cm (98th percentile). Both winters were associated with lower than average predation risk as indicated by predation rate having been much lower than average (Vucetich and Peterson 2014), where predation rate was estimated using aerial survey methods described in Vucetich et al. (2011). Both winters were associated with greater food quantity (i.e. larger bite sizes as a result of increased current annual growth) than has been typical of recent years (Vucetich and Peterson 2014).

The dominant components of winter diet for this moose population is (in order of prevalence) balsam fir *Abies balsamea*, a variety of deciduous trees and shrubs, especially American mountain ash *Sorbus americana*, red osier dogwood *Cornus stolonifera*, paper birch *Betula papyrifera* and cedar *Thuja occidentalis* (Risenhoover 1987). White pine *Pinus strobus*, which is rare on Isle Royale, represents a very small portion of moose diet.

The eastern and western regions of Isle Royale are distinguished by important differences in vegetative composition and herbivory (Brandner et al. 1990). Although moose density is similar across regions, the western region has relatively more cedar (Sanders and Grochowski 2012), greater browsing damage to balsam fir (Brandner et al. 1990), and smaller bite sizes of balsam fir (Fig. 19 in Vucetich and Peterson 2014), than the eastern region. The western region also has more abundant and more diverse woody browse than the eastern region (DelGiudice et al. 1991). These differences are less likely attributable to differences in moose density, which is similar in both regions (Montgomery et al. 2013); and more likely the result of differences in soil (De Jager et al. 2009) and glacial history (Brandner et al. 1990). DelGiudice et al. (1997) also found that UN:C tended to be greater among moose living in the eastern portion of Isle Royale.

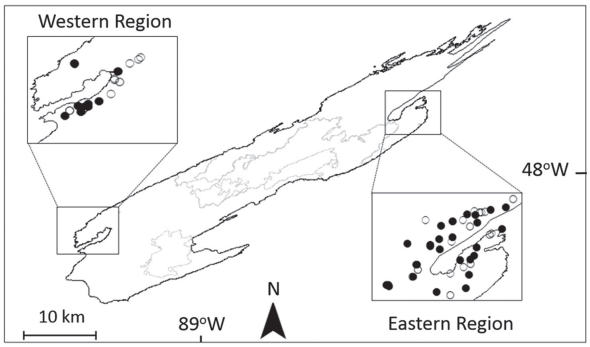

Figure 1. The distribution of sites in Isle Royale National Park where feces and snow-urine samples from free-ranging moose were collected in 2013 (n = 34; open circles) and 2014 (n = 34; filled circles). The eastern and western regions of Isle Royale, which differ with respect to forest composition, are separated by the boundaries of a historic forest fire in the central portion of the island.

Methods

Sample collection

During January–February of 2013 and 2014, we found and followed the tracks of moose in the snow. We sampled from two different geographic regions of Isle Royale (Fig. 1). We collected matched samples of pellets and urine deposited in the snow (hereafter, snow-urine) from the tracks of individual moose (DelGiudice et al. 1991). Every sample was collected within six days of deposition, and 88% of the samples were collected with 72 h of deposition. We collected 34 samples during each of the two field seasons. Urine was collected as a handful of yellow snow (~6 cm^3) into a re-sealable plastic bag. Tracks from which we collected samples were sufficiently spaced such that most samples represent different individuals (Supplementary material Appendix 1).

Microhistological analysis of fecal pellets

To assess the relationship between diet diversity, nutritional condition and detoxification, we first determined diet composition. To do so, we conducted microhistological analyses of pellet samples (Holechek and Gross 1982). Specifically, samples were dried, then ground in a food processor. Each sample was then passed through two sieves (1 mm and 0.2 mm), rinsed with tap water and then drained. Afterwards, we bleached the sample by incubating it for 5 min with 5 ml nitric acid. The sample was agitated three times during the incubation period. Next, we poured the sample into a flask with 45 ml distilled water and rinsed the incubation tube with 45 ml distilled water. Then we brought the mixture of 90 ml distilled water, nitric acid, and sample to a boil for five minutes. After allowing the sample to cool, we decanted it and placed the processed sample in a vial. Using forceps and a probe, we spread a small amount of processed sample on a microscope slide and allowed it to dry for 24 h. Afterwards, we applied three to four drops of Permount, covered the sample with a 18 × 18 mm coverslip, and allowed it to dry for 24 h.

We viewed the samples at 40 × magnification, using polarized light. We identified the plant fragments located closest to the center of the field of view for 100 stations per slide. These stations were arranged in a grid, 10 columns and 10 rows, across the slide. Diet composition and diversity was calculated directly from the identification of these 100 fragments.

We identified each plant fragment on the basis of the structure of stomata and other distinguishing cells. Identifying structures were determined from a reference collection that we prepared, representing the plant species that Isle Royale moose are known to eat (Risenhoover 1987). These reference samples were ground and processed in the same manner as moose pellet samples. Because many deciduous species are indistinguishable under a microscope, we pooled all deciduous species into one category.

We used the diet composition data to calculate *Evenness* (an index of diet diversity) as $E = H/\ln(S)$, where S is species richness, $H = -(\sum p_i \times \ln(p_i))$, and p_i is the proportion of diet comprised of one of the four food types, i (Keylock 2005).

Measurement of UN:C and GA:C

To determine the relationship between nutritional condition and investment in detoxification, we first measured the

concentration of UN, C and GA in snow-urine samples. We melted snow-urine sample, poured into a 15 ml tube, and stored it at –20°C. Concentrations of UN and C was determined by Wolff Laboratories (Minneapolis, MN), using protocols described in DelGiudice et al. (1987). UN and C were both measured for diluted samples of snow-urine with spectrophotometry. Concentrations of GA in snow-urine was determined using a colorimetric assay following techniques adapted from Blumenkrantz and Asboe-Hansen (1973). GA was used as the standard. Quantification of GA was done in duplicate for each sample and values were averaged.

Statistical analysis

We evaluated whether diet composition differed between the two regions of Isle Royale and between the two years of the study by using a two-sample equality of proportions test. We evaluated whether diet diversity differed between the two regions of Isle Royale using a two-sample t-test.

To understand the factors that might influence UN:C, we implemented the backward option in the stepAIC() command of R (<www.r-project.org>), which selects models on the basis of Akaike's information criterion (AIC). We implemented that procedure using a full model that included GA:C; *Fir*, *Evenness*, *Region*, *Year* and each of the pairwise interactions that can be constructed from those main effects. *Fir* is the proportion of diet that is balsam fir (balsam fir is both the most dominant and most variable component of the diet). *Evenness* is the Shannon evenness index of diet diversity. In addition to those predictors, we also considered *Year* (2013 and 2014) and *Region* (east and west) as candidate predictors. *Year* is an important candidate predictor because the two years differed greatly with respect to winter severity. Although we do not know the precise mechanisms, previous research indicates that *Region* may also be important. We also compared the best model to result from the stepAIC() function with a null model that included only an intercept.

We also implemented the stepAIC() function with the backward option to understand potential predictors of GA:C, starting with a full model that included *Fir*, *Evenness*, *Region*, *Year*, and every and each of the pairwise interactions that can be constructed from those main effects.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.8f973> (Parihk et al. 2016).

Results

On average, balsam fir was the most abundant food item and cedar was the least abundant of the common food items (Fig. 2). White pine represented only a trace of the diet. We observed considerable variation in diet composition (Fig. 3A). A significant portion of that variation was attributable to region of the island (Fig. 2, $p < 10^{-5}$). Diet diversity was significantly higher in the western portion of the island ($p < 10^{-3}$). Mean composition of the diet did not differ significantly between the two years ($p = 0.82$).

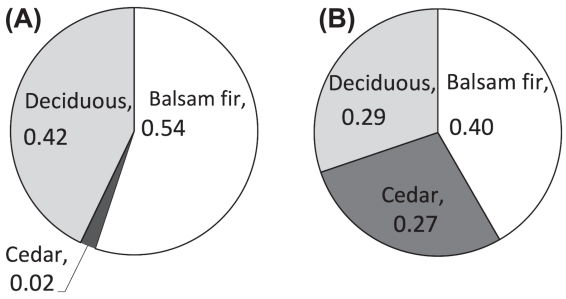

Figure 2. Composition of winter diet for moose living in the eastern (A) and western (B) regions of Isle Royale National Park. White pine is not depicted because it represents less than one half of one percent of diet. Sample sizes are 47 (eastern region) and 21 (western region).

The result of the stepAIC() algorithm was a model that explained 56% of the variation in UN:C and included *Fir*, *GA:C*, *Evenness* and *Year* as predictors. None of the interaction terms were included. The coefficients and standard errors for that model (with p-values in parentheses) were, $Fir = 2.22 \pm 0.75$ ($p = 4.5 \times 10^{-3}$), $GA{:}C = 0.05 \pm 0.02$ ($p = 0.03$), $Evenness = -1.00 \pm 0.63$ ($p = 0.11$), and $Year = 1.26 \pm 0.21$ ($p < 10^{-5}$). Variance inflation factors were low (< 1.9) indicating that multicollinearity was not a concern. The null model had a ΔAIC of 47.8 in comparison to the model resulting from the stepAIC() algorithm. The relationships between UN:C and each of the predictors is also depicted in Fig. 3.

These relationships could conceivably be statistical artifacts if estimates of diet composition are overly affected by differential digestibility of the different food items. In vitro digestibility trials indicate that balsam fir is 36% digestible, cedar is 42% digestible, and the deciduous species in this diet are 26% digestible (Risenhoover 1987; see also Fig. 4). To mitigate this concern, we repeated the analyses on UN:C except that diet composition was adjusted to account for different digestibilities. The results of that analysis are qualitatively identical and quantitatively very similar (Supplementary material Appendix 2).

The stepAIC() algorithm produced a model that explained 15% of the variation in GA:C and included *Region*, *Year* and the interaction between *Region* and *Year*. The coefficients and standard errors for that model (with p-values in parentheses) were, $Region = 2.07 \pm 1.6$ ($p = 0.20$), $Year = 3.77 \pm 1.3$ ($p = 0.01$), and $Year{:}Region = -6.96 \pm 1.3$ ($p = 0.01$). The null model had a ΔAIC of 4.9 in comparison to the model resulting from the stepAIC() algorithm.

Discussion

Nutritional restriction for moose was associated with greater investment in detoxification during both the average winter and the severe winter (Fig. 3A). That association is consistent with the idea that detoxifying larger quantities of toxic PSMs is energetically costly (Sorensen et al. 2005) and can impair the nutritional condition of an individual (Villalba et al. 2005). Moreover, this association does not support the idea that increased investment in detoxification is associated

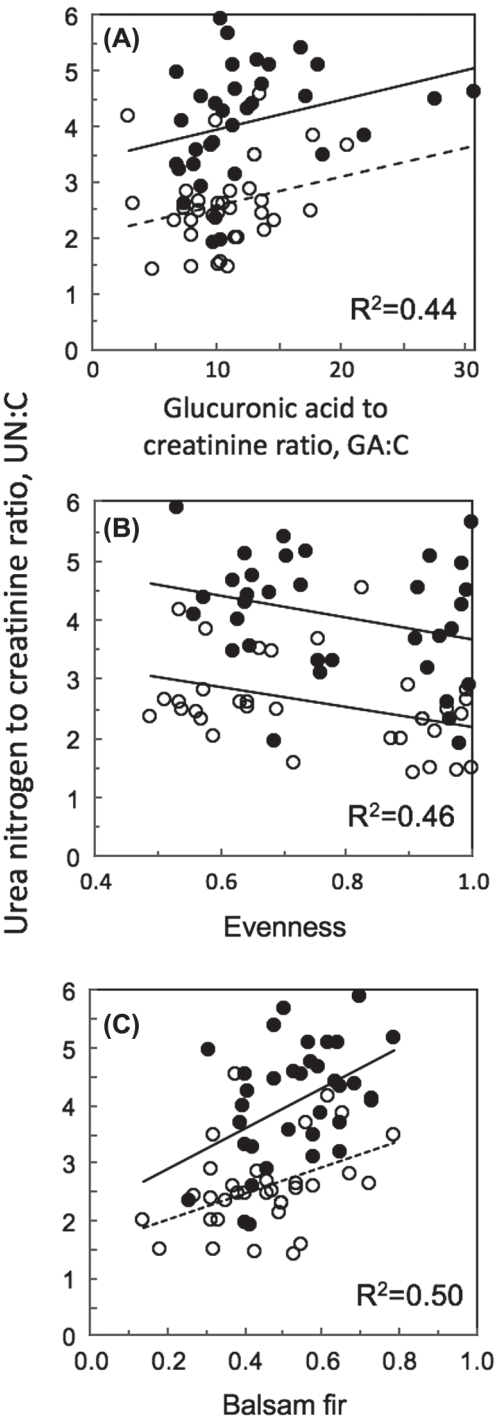

Figure 3. The ratio of urea nitrogen to creatinine (UN:C) in samples of urine that had been deposited in the snow by moose in Isle Royale National Park during the winters of 2013 (open symbols) and 2014 (closed symbols) and its associations with ratio of urinary glucuronic acid to creatinine, GA:C (A), Shannon evenness index of diet diversity (B), and proportion of balsam fir in the diet (C). The differences between years are significant in each case ($p < 10^{-3}$). For context, UN:C > 3.5 indicates severe nutritional restriction (DelGiudice et al. 1997). The lines depict best fit regressions. The R^2-values represent the proportion of variance explained by year and the covariate depicted in each panel. For context, year, by itself explains 39% of the variance in UN:C.

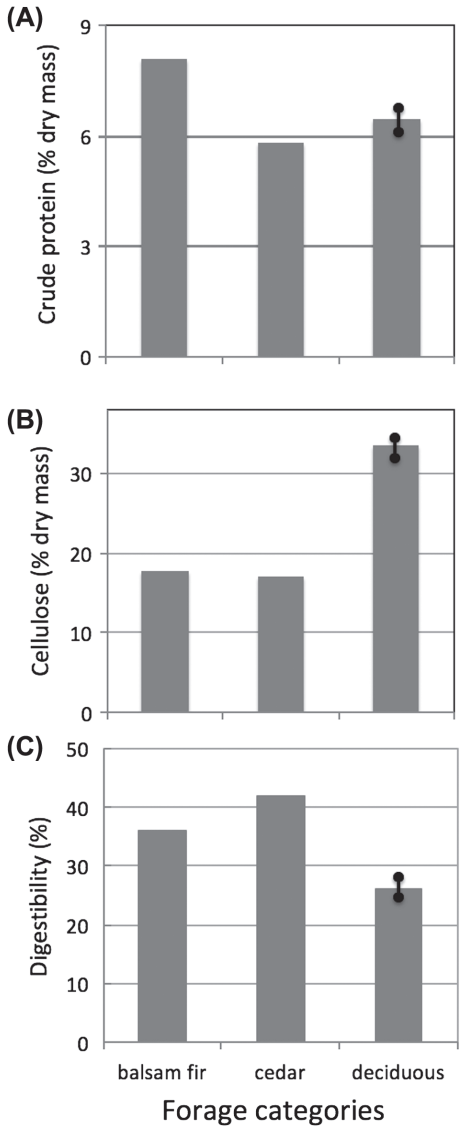

Figure 4. Protein content (A), cellulose content (B), and digestibility (C) for the common categories of forage for moose on Isle Royale National Park during the winter. The deciduous category is an average of 15 different species that are common in the diet. The vertical bars represent standard errors. The data were taken from Appendix III of Risenhoover (1987).

with improved nutritional condition. From this association (i.e. Fig. 3A) it may be reasonable to infer that high energy intake, which could in principle allow for greater investment in detoxification, does not seem to offset the cost of detoxifying associated PSMs (Reid et al. 2011).

Increasing diet diversity was associated with less nutritional restriction during both the severe winter and the average winter (Fig. 3B). This result is similar to previous research showing that increased diet diversity was associated with improved nutrient intake and better growth in captive herbivores (Bernays et al. 1994, Dearing et al. 2000, Nersesian et al. 2012). Generalist herbivores also benefit from consuming a variety of forage species because of interspecific variation in nutritional content (Nersesian et al. 2012). Diverse diets

also minimize the intake of any one type of PSM (Freeland and Janzen 1974, Provenza et al. 2003). For these reasons, diet diversity is beneficial for a generalist herbivore. Finally, the observed pattern (Fig. 3B) seems consistent with the idea that a diverse diet may increase search time, but not to the point of worsening an individual's nutritional condition (Wang et al. 2010). The connection between Fig. 3B and search time is that maintaining a diverse diet can entail passing up common forage items and searching more fore rarer items. The assessment presented here on the relationship between diet diversity and nutritional condition is the first to our knowledge for a free-ranging mammalian herbivore living in environmental conditions that are less benign than those found in captive environments (Miquelle and Jordan 1979, Bernays et al. 1994, Marsh et al. 2006, Coltrane and Barboza 2010).

Nutritional restriction also varied with diet composition. In particular, UN:C increased with proportion of fir in the diet (Fig. 3C). This result is, at least superficially, counter-intuitive because of the two dominant food items in moose diets (balsam fir and deciduous species), balsam fir has greater concentration of protein, lower concentration of cellulose, and higher in vitro digestibility than the average deciduous species on which Isle Royale moose feed (Fig. 4). Moose also take larger bite sizes of balsam fir, compared to deciduous forage (Table 9 in Risenhoover 1987). This is relevant because bite size is an important predictor of intake rate for browsing ungulates (Shipley 2007), including moose on Isle Royale (Fig. 165 in Renecker and Schwartz 1997).

The increased nutritional restriction associated with increased proportion of fir in the diet (Fig. 3C) may be the result of high concentrations of particularly toxic PSMs (Terra-Berns 1993, Servello and Schneider 2000). While that inference is inconsistent with the lack of association between GA:C and the proportion of balsam fir in the diet (Fig. 5), the inference remains plausible because the proportion of the diet comprised of fir may not be a good indicator of the absolute rate at which fir is consumed. Moreover, this inference is consistent with the detoxification limitation hypothesis, which predicts that higher concentrations of PSM (which may limit an herbivore's capacity to detoxify and excrete PSMs) will limit food intake (Freeland and Janzen 1974, Marsh et al. 2006). For example, captive white-tailed deer fed large amounts of balsam fir substantially reduced food intake (Ullrey et al. 1968, Servello and Schneider 2000). Also, captive mule deer refuse a single species diet to the point of starving to death (Milchunas et al. 1978). It is plausible that free-ranging moose eating higher proportions of fir experience nutritional restriction because fir contains high concentrations of PSMs that are toxic to moose (DelGiudice et al. 1997).

The GA:C ratio was not associated with diet composition (Fig. 5). Given this result and the expectation that GA:C should be associated with some aspect of diet, one would expect GA:C to be associated with intake rate of balsam fir (and consequently PSMs). For example, GA:C has been shown to be associated with higher intake of species with high concentrations of toxic PSMs (Servello and Schneider 2000). Analyzing intake rate of balsam fir, rather than proportion of the diet, would be a more robust approach to assess the relationship between investment

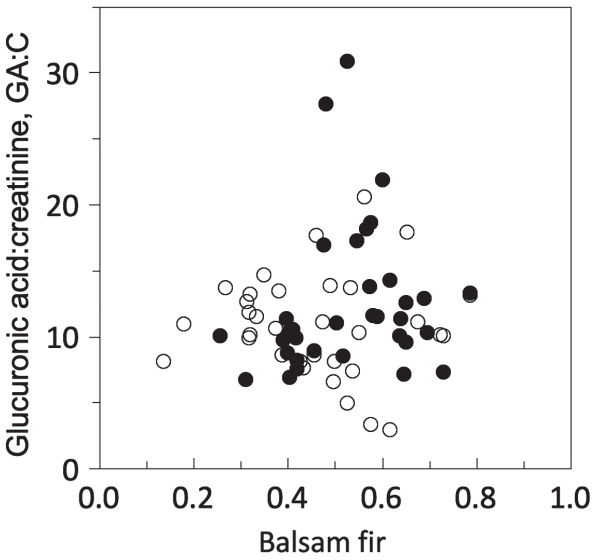

Figure 5. The ratio of glucuronic acid to creatinine (GA:C) in samples of urine that had been deposited in the snow by moose in Isle Royale National Park during the winters of 2013 (open symbols) and 2014 (closed symbols) and its associations with proportion of balsam fir in the diet. There is no significant trend ($p = 0.19$). While the multivariate model suggests that GA:C differed between years ($p = 0.01$) that result depends on the presence of *Region* in the model, which had a p-value of 0.20. Otherwise, there appears to be no significant difference between years ($p = 0.14$, t-test on log-transformed data).

in detoxification, GA:C, and diet mixing. However, this analysis was not possible with the data available. The concentration of potentially toxic PSMs can also vary considerably among plants of the same species (Frye et al. 2013, Ulappa et al. 2014). For example, among balsam fir trees sampled on Isle Royale, the total concentration of 15 different kinds of terpenes varied by nearly a factor of three (range = [2.3×10^3 ppm, 6.5×10^3 ppm]; Terra-Berns 1993). Intraspecific variation in PSM concentration could be an important element of the dynamics associated with detoxification. If so, then an herbivore's capacity to prefer some individual plants and avoid others of the same species could be an important aspect of herbivory.

For emphasis, the results presented here are the results of regression analysis applied to observational (non-experimental) data. Consequently, it is important to interpret the results as indicating associations that are not necessarily reflective of causal relationships. For example, GA:C and UN:C may covary with some other aspect of feeding or food choices, thus if they are directly or inversely correlated, it may not be a cause and effect relationship. It is also important to note that UN:C can be high both when animals are in poor condition and using their body stores for protein and when in animals are eating high protein food. Because this is a winter study, the former is likely the case. Also, UN:C is not a linear index and could lead to misrepresentation of findings.

While our original motivation was to assess how UN:C was associated with investment in detoxification, diet diversity and diet composition, the most important influence on UN:C was winter severity (Fig. 3). Interannual differences in

UN:C account for a substantial portion (39%) of variation in UN:C; that circumstance is a necessary prerequisite for concluding that temporal variation in nutritional condition has an important influence on population dynamics.

The patterns described here are the result, not only of ecological relationships, but also of co-evolutionary relationships between plant and herbivore. As such, our results should also be interpreted while knowing that balsam fir and northern-white cedar evolved in eastern North America, but moose evolved in Eurasia and did not contact either of these species in North America until about fourteen thousand years ago (Hundertmark et al. 2003). Additionally, white-tailed deer which did evolve in eastern North America do not forage on balsam fir except in extreme circumstances, but readily forage on cedar (Sauvé and Cŏté 2007). Those patterns highlight that while many physiological pathways for detoxifying PSMs, including GA conjugation, are relatively generic (i.e. operate on a wide class of PSMs), other important aspects of the chemical ecology of herbivory are species-specific and not well understood.

Investment in detoxification (as indicated by GA:C) is associated with nutritional restriction, which is consistent with the idea that detoxification is energetically expensive and likely associated with a diet high in toxic PSMs. Our results are also consistent with the inference that moose consuming high proportions of balsam fir (which likely possesses high concentrations of toxic PSM) restrict their intake and are consistent with the detoxification limitation hypothesis (Freeland and Janzen 1974, Marsh et al. 2006). The patterns documented here highlight the complex relationships among three processes, i.e. detoxifying PSMs, foraging behavior, and net energy gain.

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Supplementary material (available online as Appendix oik-03359 at <www.oikosjournal.org/appendix/oik-03359>). Appendix 1–2.