

Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection

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Abstract The global ecological impacts of introduced and exotic species can be dramatic, leading to losses in biodiversity and ecosystem “melt-down”, however, the evolutionary impacts of introduced species are much less understood. Further, very few studies have examined whether mammalian herbivores can act as agents of natural selection for plant traits. We examined the hypothesis that variation in aspen phytochemistry resulted in selective herbivory by *Cervus elaphus* (elk), an introduced mammalian herbivore. With the experimental removal of a large elk enclosure, elk selectively eliminated 60% of

an aspen population previously protected from herbivory resulting in a dramatic shift in the phytochemical composition of the aspen forest. Selection gradients (β) varied from 0.52 to 0.66, well above average relative to other studies of selection. These results indicate that introduced herbivores can have rapid evolutionary consequences even on long lived native species. Because there are fundamental links between phytochemistry, biodiversity and ecosystem processes, the effects of an introduced herbivore are likely to have cascading impacts on the services ecosystems provide.

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Introduction

Natural selection can profoundly influence the direction and pace of evolutionary change within populations (e.g., Arnold 1983; Lande and Arnold 1983; Endler 1986; Brodie et al. 1995; Kingsolver et al. 2001). Natural selection within animal and plant populations can be extremely rapid, resulting in resistance to pesticides or herbicides within human lifespans, among other examples (reviewed in Brown 1996; Thompson 1998; Thompson et al. 2001; Hairston et al. 1999; Richter and Ronald 2000). Indeed, intense selection and potentially rapid evolution may be a defining trait of evolutionary interactions that are relatively recent, such as when plants or herbivores are introduced. While much research has shown that mammalian herbivores respond to variation in defensive phytochemistry in plants (Bryant 1981; Basey et al. 1988, 1990; Verheyden-Tixier and Duncan 2000; Pusenius et al. 2002; O'Reilly-Wapstra et al. 2002, 2004; Bailey et al. 2004), relatively few studies indicate that herbivores can act as agents of natural selection on plant traits in general and defensive phytochemistry in particular (Mauricio and Rausher 1997, but see Stinchcombe and Rausher 2001, 2002; Bailey et al. 2004).

Aspen (*Populus tremuloides*) has the largest distribution of any North American deciduous tree species, although throughout the intermountain West aspen is in decline (Romme et al. 1995; Baker et al. 1997; Bailey and Whitham 2002). Browsing by large ungulates (e.g., elk) is considered to be one of the principle causes of the change in populations of aspen (Romme et al. 1995; Baker et al. 1997; Bailey and Whitham 2002). Because of aspen's substantial economic and environmental value, this decline is of considerable concern (Romme et al. 1995; Baker et al. 1997; Bailey and Whitham 2002). In northern Arizona, elk (*Cervus elaphus*) were introduced early in the 20th century (Hoffmeister 1986) and are thought to have very different

ecological roles than the dwarf native elk that was native to AZ, but extirpated by 1914 (Rolf 2001). Studies in Arizona have shown that elk herbivory in these forests can reduce aspen abundance by up to 90% in just 2 years after their emergence following fire (Bailey and Whitham 2002, 2003). If this herbivory is selective, such high mortality may result in rapid shifts of mean values of many plant population traits and alter genetic interactions with other associated species.

We investigated if elk herbivory imposed selective pressures on both survivorship and chemical content of aspen due to their lack of evolutionary history together. We note that, at this early stage, the interaction is decidedly one-sided, in that elk in large herds clearly have negative effects on aspen. We provide evidence for dramatic change in the chemical composition of aspen populations after herbivory by elk. Our findings hold importance for invasion biology, because they show that introduced herbivores can dramatically alter the chemical composition of populations of foundation species (Ellison et al. 2005), and that selection can be rapid even for long-lived species.

Methods

Selection experiment

To examine whether elk are a selective force shaping the chemical composition of aspen populations in northern Arizona, we utilized the planned removal of a large-scale (~100 ha) experimental exclosure. In 2001, we randomly selected and tagged 60 winter-dormant aspen saplings that had been protected from elk since their emergence after a crown fire in 1996. These aspen were all 5–6 years old and 3–5 m in height. Dormant twig samples from these saplings were collected for phytochemical analysis (see below) to examine potential correlations between phytochemistry and herbivory. Because aspen are very clonal and sexual reproduction is rare, we tried to minimize the possibility of collecting replicate genets from the same parent and thus saplings were randomly collected from the entire 100 ha area.

In 2002, after the fence had been removed for 1 year, we re-surveyed the 60 tagged aspen

saplings for elk herbivory to determine if elk were selectively foraging. From the 60 tagged saplings in 2001 we found 51 tagged saplings in 2002. Rather than assuming the nine missing samples were dead, they were removed from the analyses. Herbivory and mortality were characterized as present/absent (e.g., based on whether shoots showed signs of characteristic browsing) or alive/dead. The mortality survey was repeated in 2005.

Phytochemistry

Although elk browse aspen year-round, much of their damage to aspen in this region occurs in the winter when other food resources are scarce. Winter dormant twigs were collected once (March 2001, immediately prior to fence removal) for phytochemical analyses. To determine the potential mechanism underlying the selective foraging of elk, we examined total nitrogen (an index of protein), phenolic glycosides (i.e., salicortin and tremulacin) and condensed tannins, the chemical constituents in aspen likely to affect herbivores (Palo 1984; Lindroth and Hwang 1996; Lindroth et al. 1993). Chemical analysis was done on terminal twigs, representing the most recent year's growth. Whole twigs were flash-frozen on dry ice in the field, lyophilized and ground to pass a 40-mesh screen in a Wiley Mill. Nitrogen levels were determined using a LECO nitrogen analyzer (LECO, St. Joseph, MI) with glycine *p*-toluene-sulfonate as a nitrogen standard. The two most abundant, biologically active phenolic glycosides, salicortin and tremulacin, were quantified using high performance thin layer chromatography (Lindroth et al. 1993). Aspen salicortin and tremulacin purified via flash chromatography (Still et al. 1978) served as standards. Condensed tannin levels were measured on 70% acetone extracts of samples using the butanol-HCl method (Porter et al. 1986). Aspen tannin purified by the method of Hagerman and Butler (1980) was used for reference standard.

Statistics

We used a Chi-square analysis to determine if herbivory of aspen by elk and mortality of aspen due to herbivory were related. As previously

mentioned herbivory and mortality were characterized as categorical data. We then examined the relationship between phytochemistry and mortality. Simple logistic regression was used for relating the continuous variables (i.e., phytochemistry) to categorical data (i.e., mortality) (Sokal and Rohlf 1995; Janzen and Stern 1998) in order to identify which foliage chemical characteristics of the plant samples related to mortality of aspen by elk. A Bonferroni adjustment was used to take into account the number of individual tests ($\alpha = 0.0125$). From these analyses we found that aspen mortality was only related to a single phytochemical compound and thus did not proceed to more complex multiple regression models.

Selection (S) was measured as the slope of the linear regression of relative fitness (w) on the standardized phytochemical traits (z) following methods described in Kingsolver et al. (2001), Conner and Hartl (2004) and Stinchcombe (2005). Relative fitness was measured by subtracting the population mean survival from each individual survival score (See Stinchcombe (2005) for details on relativizing categorical data). Standardized traits were measured similarly, i.e., subtracting the population mean trait score from each individual trait score and then dividing by the population standard deviation. While the above calculations estimate overall selection they do not account for correlations among traits, such as the phenolic glycosides tremulacin and salicortin. Using multiple regression to control for correlations among phytochemical traits, we reanalyzed the selection gradients (β) between relative fitness and tremulacin and salicortin (Conner and Hartl 2004; Stinchcombe 2005) to examine the direct and indirect actions of selection on specific phytochemical targets. Partial regression coefficients (i.e., β) can be obtained directly from the regression output. We used the program SAS-JMPN 5.1 for all analyses.

To examine how selective herbivory could change specific phytochemicals within a population of aspen, we used one-sample *t*-tests to compare the initial mean values of phytochemical traits in all the unbrowsed aspen trees prior to enclosure removal (i.e., 2001 collection) with the mean values of those initial traits in the subset of

samples representing aspen that remained after exclosure removal and subsequent elk-induced mortality (i.e., 2002 collection). Thus, we examined the chemical composition of initial aspen versus the chemical composition of surviving aspen, but all chemical measures reflect chemical condition prior to elk foraging. We chose not to compare chemistry of aspen samples collected both before and after elk herbivory for several reasons. First, chemical profiles of young trees change substantially with age (Donaldson et al. 2006), so collection of post-herbivory samples would have confounded changes due to selection with changes due to ontogeny. Second, surviving aspen may have induced production of secondary metabolites following browsing, in which case a plastic phenotypic response would have been misinterpreted as a selective, genetic response.

To examine how selective herbivory affected the chemical composition of an aspen population we created a dissimilarity matrix in the statistical package Primer© 5.0. To create our dissimilarity matrix, we used the combined concentrations of salicortin, tremulacin, nitrogen, and condensed tannins found in the winter dormant twigs of our aspen samples. Because phytochemicals occur in measurable concentrations, Euclidian distance was used for determining the phytochemical compositional similarity among samples. As above, a one sample *t*-test was used to determine if the chemical composition of aspen populations differed before and after selective herbivory by elk. This site was reanalyzed after the 2005 survey. We used Non-metric Multidimensional Scaling (NMDS) to represent the chemical composition of the aspen population before and after herbivory by elk. NMDS is a robust multivariate approach that places samples with similar phenotypes close, and dissimilar samples far away in ordination space based on the measure of ecological distance (Minchin 1987).

Results

Just 1 year after the removal of the elk exclosure, we found that trees browsed by elk were significantly more likely to die as a result of herbivory than trees that were unbrowsed (2002: $X^2 = 21.23$, $P < 0.0001$, 2005: $X^2 = 30.36$, $P < 0.0001$).

Sixty-three percent of the aspen that had been protected from elk for the previous 5–6 years had been browsed and forty-one percent of the aspen were dead due to herbivory. Four years after the removal of the exclosure, 73% of the aspen had been browsed and 61% were dead as a result.

Of the four phytochemicals examined, concentration of tremulacin in the dormant twigs was the only phytochemical significantly related to aspen mortality ($X^2_{(4)} = 9.07$, $P = 0.003$). As tremulacin concentrations increased, mortality of aspen related to herbivory by elk declined. Overall, saplings experienced an 80% chance of dying, due to severe herbivory, when tremulacin concentration was 2%, but only a 5% chance of dying when the concentration was 8% (Fig. 1a). After 4 years

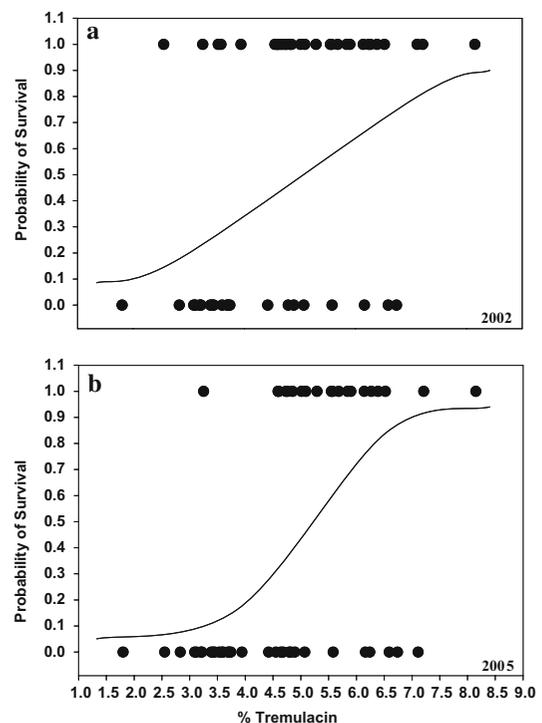


Fig. 1 In contrasting nascent populations of aspen that were protected from elk since their regeneration after fire, with those that survived elk herbivory after the removal of fences, using logistic regression we found that elk herbivory was negatively associated with tremulacin concentration. Panel A shows selection in 2002 and Panel B shows increasing selection in 2005. Aspen with high concentrations had a 95% lower chance of dying relative to aspen with a low concentration. Each point represents an individual aspen sapling, some data points may be stacked on each other

these results became even stronger, again tremulacin concentration was the only factor significantly related to aspen mortality (Fig. 1b, $X^2_{(4)} = 12.35$, $P = 0.0004$).

Overall estimates of selection (S) showed that selection intensity on tremulacin and salicortin varied from (0.28–0.45, Table 1). As expected, the strength of selection increased through time. Using the partial regression coefficients from the multiple regression we found that selective herbivory by elk was acting directly on tremulacin ($\beta = 0.52$ –0.66) and indirectly on salicortin. And similar to the estimates of selection, β also indicated that selection tended to increase through time, indicating a sustained rapid selection event (i.e., 61% of a nascent aspen population were dead within 4 years) that is directly related to the concentration of tremulacin.

We evaluated the change in phytochemical concentration in aspen by comparing the chemical profiles of trees alive prior to, and following, elk browsing. We compared the mean values of phytochemical traits in the trees before elk herbivory to the values of those traits in the subpopulation of unbrowsed aspen trees that remained after removal of the exclosure (i.e., one-sample *t*-test). Our data showed that mean population trait values of the phenolic glycosides tremulacin and salicortin in unbrowsed saplings were 5–7% greater after one year (Table 2) and 8–13% greater after 4 years, relative to values in the initial population of saplings. In support of

our hypothesis that selective herbivory by elk could result in significant changes to the chemical composition of aspen populations, we found that after just 1 year the overall chemical composition of aspen saplings that survived elk herbivory was significantly different than the chemical composition of aspen saplings that had died due to herbivory after 1 and 4 years, respectively (Fig. 2: $t = 2.74$, $P = 0.0063$, $t = 2.85$, $P = 0.0039$).

Discussion

Our data show that a large introduced mammalian herbivore can exert a selective force for dramatic change in chemical composition of a dominant forest tree species and impact evolutionary dynamics in this system. This is important as herbivory is a ubiquitous component of most terrestrial ecosystems (Cyr and Pace 1993). Understanding the evolutionary impact of invasive species becomes especially important when considered in the light of their increasing global ecological and economical impacts (Simberloff 2001; Simberloff et al. 2005). In some cases, introduced herbivores can exert devastating effects, such as consuming native plants to extinction (Groombridge 1992; Parker et al. 2006). While we see similar ecological effects in this elk-aspen system (Bailey and Whitham 2002), where nearly 84% of the aspen trees that were

Table 1 Estimates of selection based on simple linear regressions between relative fitness, as measured by aspen mortality, and aspen phytochemistry

Chemical	Year	df	S	F	ρ	β	ρ
Salicortin	2002	1,50	0.28	4.01	0.051	–0.17	0.50
Tremulacin	2002	1,50	0.38	7.88	0.007	0.52	0.04
Con. Tannins	2002	1,50	NS				
Nitrogen	2002	1,50	NS				
NMDS score	2002	1,50	0.32	5.53	0.023		
Salicortin	2005	1,50	0.32	5.35	0.025	–0.22	0.32
Tremulacin	2005	1,50	0.45	12.2	0.001	0.66	0.006
Con. Tannins	2005	1,50	NS				
Nitrogen	2005	1,50	NS				
MDS Score	2005	1,50	0.39	8.58	0.005		

Estimates of selection gradients (β) are based on multiple regression using phytochemical factors that were significant in simple linear regression models

Notes: S = selection intensity, β = selection gradient between the trait and relative fitness, Values in bold represent phytochemical factors upon which selection acted directly and significantly

Table 2 One-sample *t*-test comparing change in aspen phytochemistry before and after selective herbivory by elk (initial = before fence removal, after = after fence removal)

Chemical	Year	df	Mean _{initial}	Mean _{after}	<i>t</i>	<i>p</i>
Salicortin	2002	29	9.08 ± 0.31	9.52 ± 0.39	2.25	<0.05
Tremulacin	2002	29	4.78 ± 0.19	5.12 ± 0.23	2.32	<0.05
Con. Tannins	2002	29	5.44 ± 0.15	5.43 ± 0.21	0.19	0.43
Nitrogen	2002	29	0.87 ± 0.02	0.87 ± 0.03	0.16	0.44
NMDS score	2002	29	0.00 ± 0.13	0.20 ± 0.14	2.21	<0.05
Salicortin	2005	19	9.08 ± 0.31	9.78 ± 0.39	1.94	<0.05
Tremulacin	2005	19	4.78 ± 0.19	5.39 ± 0.28	2.10	<0.05
Con. Tannins	2005	19	5.44 ± 0.15	5.46 ± 0.21	0.09	0.46
Nitrogen	2005	19	0.87 ± 0.02	0.87 ± 0.04	0.11	0.45
MDS score	2005	19	0.00 ± 0.13	0.35 ± 0.19	2.11	<0.05

Notes: Phytochemistry is measured as % dry wt

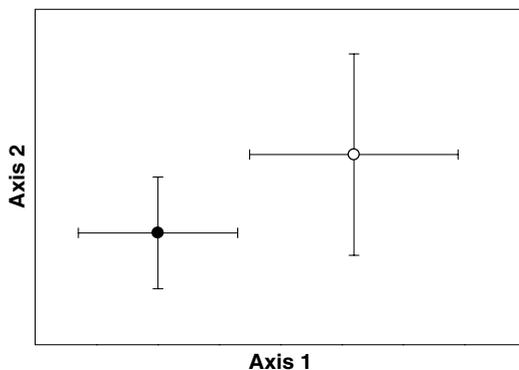


Fig. 2 Selective herbivory results in changes to aspen forest chemical composition. Non-metric multidimensional scaling ordination shows that selective herbivory and the subsequent mortality of aspen results in a significant shift in the chemical composition of the aspen forest. Each point in the figure represents the mean dissimilarity of chemical constituents ± standard error. The combination of both points in the figure represents the natural variation in phytochemistry prior to the removal of the elk enclosure. The black dot represents the aspen ramets that were consumed by elk and those that have persisted after the fence was removed are shown with an open circle (2005 data shown)

browsed by elk died as a result of that herbivory and elk enclosures are necessary for successful aspen regeneration (Rolf 2001; Bailey and Whitham 2002), whether the evolutionary effects of introduced herbivores are different than that of native herbivores is still not well understood.

Few studies examining the relationship between mammalian herbivory and phytochemistry have actually quantified how strongly herbivores may act as agents of selection for phytochemical

defenses, if there are fitness consequences as a result of selection, or examined the impact of selection on the composition of traits within the plant population (Bryant 1981; Basey et al. 1988, 1990; Verheyden-Tixier and Duncan 2000; Pusenius et al. 2002; O'Reilly-Wapstra et al. 2002, 2004; Bailey et al. 2004). Our results indicate that elk do exert a selective force that is above average relative to other published studies that average 0.40 and range from 0 to 1.95 (Roff 1997; Kingsolver et al. 2001; Hereford et al. 2004), suggesting that introduced herbivores may have stronger evolutionary consequences for native plants than average. Previous research in other western forests also indicates that aspen cannot easily respond to the selective pressure by elk and could be eliminated without protection (Bailey and Whitham 2002; Hessl 2002). Elk may play especially important roles in shaping the genetic structure of developing aspen populations following rare periods of successful sexual reproduction in the intermountain West. Romme et al. (2005) reported widespread establishment of seed-derived aspen in Yellowstone National Park following the extensive fires of 1988. Their subsequent censuses were consistent with the perspective that following bouts of sexual reproduction, aspen populations experience a shift from many genets with few ramets to few genets with many ramets. This shift is due to selection by a complex of abiotic and biotic factors, including elk herbivory. Our research with aspen ramets that produced asexually via root sprouts revealed similar selective pressure

from elk herbivory. We suggest that as a consequence of the profound effects of elk herbivory, aspen must respond to this intense selection to persist in this relatively recent evolutionary dynamic. We note that in forest ecosystems that have evolved without the selection pressures of dominant herbivores, introduced species may impose strong selective forces and result in rapid evolutionary processes in plants potentially impacting associated biodiversity and ecosystem processes through changes to genetic interactions among associated species (Parker et al. 2006; Whitham et al. 2006).

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