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BEAVERS AS MOLECULAR GENETICISTS: A GENETIC BASIS TO THE FORAGING OF AN ECOSYSTEM ENGINEER

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Abstract. Ecological genetics is increasingly recognized as critical to understanding interactions among organisms and ecosystem processes. Using a common garden with pure and hybrid cottonwood trees of known genotype, two years of field surveys, and a cafeteria feeding experiment, we link introgression of Fremont genetic markers, condensed tannins (a genetically based plant trait), and foraging by beavers. These data support two major arguments. First, hybridization is an important mechanism for the transmission of ecologically functional traits. Second, links between a genetically based plant trait in a dominant riparian-forest tree species and the foraging behavior of beavers, an ecosystem engineer, emphasize that genetically based plant traits can directly and indirectly link population, community, and ecosystem processes.

Key words: *beavers; chemistry; community genetics; cottonwoods; ecological genetics; genetic markers; herbivory, selective; hybridization; phytochemistry; Populus; tannins; terrestrial-aquatic linkages.*

INTRODUCTION

Linking molecular biology to population, community, and ecosystem processes is fundamental to the burgeoning field of ecological genetics (Jackson et al. 2002). Although one might think that the effects of genetic variation or specific genetically based plant traits would become more diffuse across higher levels of organization (i.e., population, community, and ecosystems), recent studies have shown that specific genetically based plant traits can have direct and indirect effects that extend beyond the individual or population to affect the dependent community, and have ecosystem consequences (i.e., have extended phenotypes; Whitham et al. 2003).

Naturally occurring plant hybridization is useful for quantifying the genetic basis of specific plant traits and their population-, community-, and ecosystem-level effects (Stebbins 1950, Stace 1987, Floate and Whitham 1993, Fritz 1999, Whitham et al. 1999, Driebe and Whitham 2000, Graham et al. 2001). Throughout North America cottonwoods (i.e., *Populus* spp.) are a dominant component of riparian ecosystems. Where the distributions of two or more species of cottonwoods over-

lap, they naturally hybridize (see Plate 1). Cottonwood hybrid zones occur naturally in almost every river system throughout the Rocky Mountains (Eckenwalder 1984*a, b, c*), and range in size from a few to nearly 1000 river kilometers.

Beavers (*Castor canadensis*) are also distributed throughout North America and have a long ecological and evolutionary history of association with cottonwoods and other species in the genus *Populus*, and commonly encounter the genetic variation within this hybridizing complex. Beavers are considered keystone species and the epitome of an ecosystem engineer through multiple pathways of habitat modification. Prominent among these is selective herbivory (Basey et al. 1988, 1990, Fryxell 1997), which can alter plant community composition (Johnston and Naiman 1990), stand age structure (Basey et al. 1988), and the spatial distribution of habitat resources (i.e., landscapes; Turner 1989). As ecosystem engineers, their presence can determine whether habitats develop as a wetland or dry meadow (Chadde and Kay 1991). Thus, changes in the distribution of habitat and resources that result from the foraging behavior of beavers affect many community interactions and ecosystem processes (Jones et al. 1997, Wright et al. 2002).

Along the Weber River, near Ogden, Utah, USA, the ranges of Fremont cottonwood (*Populus fremontii*) and narrowleaf cottonwood (*P. angustifolia*) overlap in a

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PLATE 1. (Top) Representative cottonwood hybrid zone and associated beaver pond along the Snake river in Grand Teton National Park. Photo credit: Joe Bailey. (Lower left) Beaver–cottonwood interaction at Legoland, California. Photo credit: Steve Hart. (Lower right) Hybrid cottonwood riparian forest along the Snake river in Grand Teton National Park. Photo credit: Tom Whitham.

13-km zone where the two species readily hybridize and introgress unidirectionally (Keim et al. 1989). In the field, F_1 hybrids cross only with narrowleaf to form backcross hybrids. Cottonwoods are the dominants in this riparian vegetation type and beavers are found throughout the entire drainage (Rood and Mahoney 1990). We tested the hypothesis that genetic differences among pure and hybrid cottonwoods would result in different phytochemistry (*sensu* Orians 2000) and affect the foraging preferences of beavers.

METHODS

Observational and experimental surveys

Throughout the hybrid zone along the Weber River (Utah, USA) we quantified beaver preference for the different cottonwood cross types every month for a 26-month period beginning in September 1998 and ending in November 2000. We surveyed 625 cottonwood trees that were felled by beaver as well as the four nearest cottonwoods that remained standing to quantify for-

aging preferences and the abundance of different cross types in this riparian habitat. Because beaver herbivory often occurs in patches, in estimating abundance, we counted trees that were present in multiple samples only once. The cross type of felled trees was determined by examining the foliage of resprout growth that had emerged from stumps. Narrowleaf and backcross hybrids are morphologically indistinguishable in the field and were therefore considered as a single category for analysis.

To experimentally test the potential genetic components of this apparent selective foraging, we presented beavers with branches of trees derived from a 10-year-old common garden in which the genetic composition of the trees had been quantified using RFLP (restriction fragment length polymorphism) molecular techniques (Keim et al. 1989). Throughout September and October of 2000 we ran 12 feeding trials in pure and hybrid zones. To ensure that different beavers were involved with each trial, trials were conducted over a

65-km reach of the Weber River. Overall, we found eight lodges associated with our sites and estimate that there were ~30 beavers included in the study (four beavers per lodge is the average family size; Nowak 1999). We used a randomized grid design with all four cross types at equal abundance among the grids. There were 16 branches in each grid with four branches from four different genotypes (i.e., four rows with four branches in each row). We attempted to keep genotype constant, but our heavy use of branches from garden trees left some genotypes bereft of suitable branches for the cafeteria experiment. Overall, there were four Fremont, four F_1 hybrids, five backcross hybrids, and six narrowleaf cottonwood genotypes used in the study, and different genotypes from each cross type were used to make the four replicates of that cross type in the grid. Although the number of branches per cross type was held constant, cross types with more genotypes had fewer branches per genotype. Because there was an unequal chance of beavers removing each genotype, we standardized our data by calculating the percentage of each genotype selected by beavers. Branches were flagged, labeled, mapped, and placed along the stream margin and were surveyed consecutively for three nights following the initial placement. Among all grids, branches that beavers had selected were ultimately determined by their absence from the grid. In the majority of cases, we recovered tagged branches in which beavers had characteristically fed on the inner bark. Twenty-nine branches were taken and data from all grids were pooled for analysis.

Chemical analysis

To determine the potential mechanism behind the selection preferences of beavers, we examined total nitrogen, phenolic glycosides, and condensed tannin concentrations, which are the principal secondary metabolites in cottonwoods that are known to affect herbivores (Palo 1984, Lindroth and Hwang 1996). Chemical analysis was done on the same genotypes of cottonwoods from the common garden that were used in the feeding trials. Bark was collected on dry ice, lyophilized, and ground to pass a number 40 (425- μm) mesh screen in a Wiley mill. Nitrogen levels were determined using a LECO nitrogen analyzer (LECO, Saint Joseph, Missouri, USA) with glycine *p*-toluene sulfonate as nitrogen standard. Salicortin was quantified using high-performance thin-layer chromatography (Lindroth et al. 1993), using purified salicortin as a standard (Still et al. 1978). Condensed tannin levels were measured on 70% acetone extracts of leaf samples using the butanol-HCl method of Porter et al. (1986). Narrowleaf cottonwood condensed tannins purified by the method of Hagerman and Butler (1980) were used as the reference standard.

Statistical analysis

We used chi-square analysis for observational data and simple linear regressions for analysis of relation-

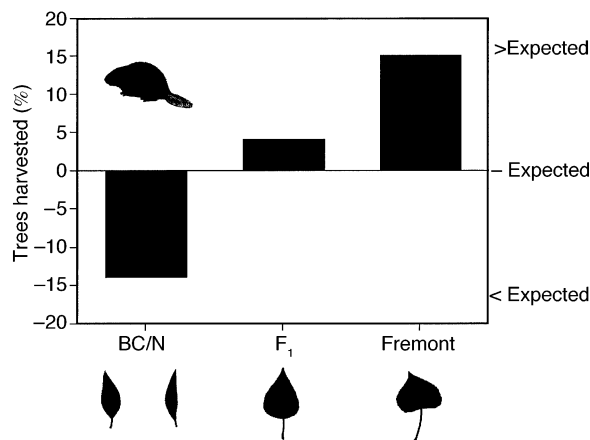


FIG. 1. Beaver preference among cottonwoods in the hybrid zone (Weber River, Utah, USA). In the wild, beaver selectively discriminated among related cottonwood species and their naturally occurring hybrids ($X^2 = 13.94$, $df = 2$, $P < 0.001$; $n = 676$ trees). Beaver felling of trees was monitored for 26 months along a 13-km reach of the Weber River. Overall, beaver selectively felled more Fremont and fewer backcross/narrowleaf (BC/N) cottonwoods than expected. Beavers selected 43% of the BC/N cottonwoods, 52% of the F_1 hybrids, and 57% of the Fremont cottonwoods that were available.

ships among molecular, chemical, and preference data. All data used in the statistical analysis were transformed such that there was no significant lack of fit, no autocorrelation among residuals (Durbin-Watson test), and no inequality of variances based on the distribution of residuals (regression models are provided in the figure legends). In all cases, the relationships were linear. The figures show actual, nontransformed data.

RESULTS

After two years of observations along the 13-km stretch of the cottonwood hybrid zone, within-patch preferences showed that beavers selected 29% more Fremont, and 18% more F_1 hybrids than expected, relative to the availability of backcross hybrid and pure narrowleaf cottonwoods (Fig. 1). These observational data show a preference by beavers for Fremont cottonwoods and suggested that plant traits related to cottonwood hybridization might influence patterns of beaver herbivory.

The observational patterns were confirmed in the field using the cafeteria choice experiment. The patterns of branch removal from these tests showed a significant positive relationship in beaver selectivity and the proportion of Fremont markers per tree type (Fig. 2a). On average, beavers selected $13.6\times$ and $2.4\times$ more Fremont than narrowleaf and backcross hybrids, respectively. These data show that beavers discriminated at the level of closely related cottonwood species and their natural hybrids.

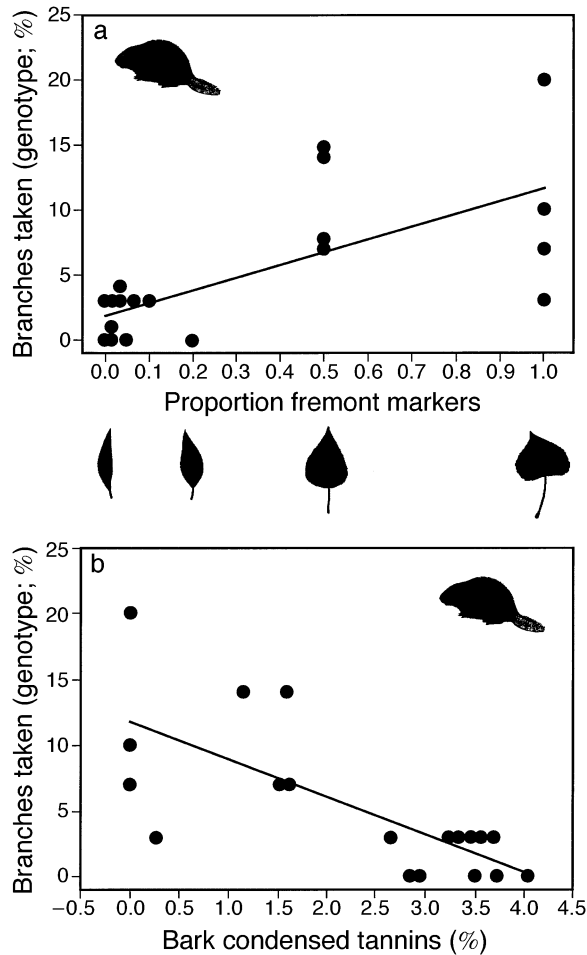


FIG. 2. Beaver preference among cottonwoods in a cafeteria choice branch-removal experiment. (a) In a cafeteria feeding experiment, using trees of known genotype from a common garden, beaver preferences were significantly correlated with the molecular composition of individual tree genotypes (i.e., in effect, beavers are molecular geneticists; $F = 14.52$; $n = 19$ genotypes, $df = 1, 18$, $r^2 = 0.46$). The percentage of each genotype taken by beaver was related positively to the proportion of Fremont markers in each genotype (regression model: $\ln + 1\%$ of each genotype taken = $0.815 + 1.73 \times$ proportion of Fremont markers). Beavers favored individual tree genotypes high in Fremont markers, e.g., they selected Fremont 41%, F_1 hybrids 39%, backcross hybrids 17%, and narrowleaf cottonwoods 3% of the time. (For leaf-shape key, see Fig. 1.) (b) Using the same genotypes from the cafeteria experiment, inner-bark tannin concentration was related negatively to the percentage of branches taken by beavers ($F = 18.72$, $df = 1, 18$, $r^2 = 0.52$; regression model: $\ln + 1\%$ of each genotype taken = $2.56 - 0.51 \times$ bark condensed-tannin concentration).

Populus spp. possess an array of bark chemical constituents likely to influence beaver foraging, including nitrogen (an index of protein), and two dominant classes of secondary metabolites: phenolic glycosides and condensed tannins. We assayed cottonwood bark tissues for these compounds and found that beaver preferences were negatively correlated with the concentra-

tion of condensed tannins (Fig. 2b). On average, beavers were $12\times$ more likely to select a genotype without tannins than a genotype with 4% tannins by dry mass. By holding genotype diversity, size class, and distance to the food resource constant, we specifically addressed the links among plant genotype, secondary chemistry, and foraging behavior of beavers. Overall, these data suggested that foraging patterns of beavers are influenced by condensed-tannin concentration in the bark tissue of cottonwoods, and they indicated that tannins may be the plant trait related to the introgression of Fremont alleles. Neither nitrogen ($r^2 = 0.003$, $P = 0.8$) nor phenolic glycosides ($r^2 = 0.15$, $P = 0.10$) were found to affect beaver choice.

As both the concentration of condensed tannins in the bark and the proportion of Fremont markers were related to the foraging preferences of beavers, we predicted that there would be a significant negative correlation between tannin concentrations of individual genotypes and the proportion of Fremont markers contained in those trees. Using the same genotypes from the common garden that were used in the feeding trials, we regressed the concentration of condensed tannins in the bark to the proportion of Fremont markers and found a highly significant linear relationship between Fremont markers and tannins ($r^2 = 0.93$, Fig. 3). As the proportion of Fremont markers decreased in the

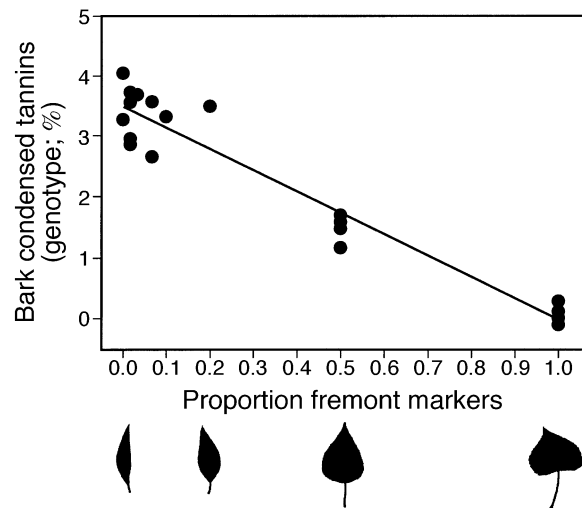


FIG. 3. Concentrations of condensed tannin in cottonwood bark as a function of proportion of Fremont markers in the trees. A strong linear relationship exists between introgression of Fremont cottonwood markers and condensed-tannin concentration in the bark of cottonwood genotypes ($F = 225.11$, $df = 1, 18$, $P \ll 0.001$, $n = 19$ genotypes; $r^2 = 0.93$, percentages bark condensed-tannin concentration = $1.927 - 1.729 \times$ proportion of Fremont markers in an individual tree genotype). This pattern was not driven by the pure genotypes; the relationship was also significant when hybrids were analyzed alone ($F = 47.22$, $df = 1, 12$, $P \ll 0.001$; $r^2 = 0.81$, $n = 13$ genotypes, percentages bark condensed tannins = $3.51 - 3.93 \times$ proportion of Fremont markers). (For leaf-shape key, see Fig. 1.)

genetic composition of individual trees, tannins increased $>300\times$.

DISCUSSION

These results link a genetically based plant trait in a dominant plant (i.e., secondary chemistry) and the foraging behavior of an ecosystem engineer. Genetic differences among plants that are related to the introgression of Fremont cottonwood markers can result in large differences ($>300\times$; Fig. 3) in the phenotypic expression of plant secondary compounds that can affect a plant's resistance to herbivores. Such large differences in tannin concentrations provide a genetic basis for beavers to discriminate among different genotypes, and argue for the importance of hybridization and introgression to the transmission of ecologically functional traits.

Selective herbivory and thus the ability to discriminate among plant genotypes that are most easily digested and nutritious should be adaptive (Fryxell 1997). A growing body of literature indicates that many mammalian herbivores including beavers, opossum, hares, voles, and roe deer discriminate among plants that vary in secondary chemical composition (Edwards 1978, Bryant 1981, Basey et al. 1988, 1990, Pusenius et al. 2002, Verheyden-Tixier and Duncan 2000). Specifically, studies have shown that beaver preference for food resources can be influenced by plant secondary metabolites (Basey et al. 1988, 1990). Like Basey et al. (1988), we found that phenolic glycosides did not influence the patterns of foraging by beavers. In contrast, Basey et al. (1988) did not analyze for condensed tannins, which we found to be important. Although our data showed that condensed tannins were related to beaver herbivory, we did not measure lignin concentration, which may be correlated with tannins and could also be correlated to beaver preference.

Basey et al. (1988) argued that animals that exhibit "central place foraging," such as beavers, have multiple strategies related to selective herbivory. Herbivores may try to avoid toxic compounds in their food and select the best available food resource, or, when food resources are not well defended by secondary metabolites, herbivores select food to maximize energy intake. Based on these hypotheses, the relative impact of the interaction of plant genotype and the patterns of foraging of this ecosystem engineer depend upon the composition of cottonwood stands and the level of herbivory by beavers. For example, in stands of pure Fremont cottonwood, which have much lower concentrations of some defensive compounds found in narrowleaf cottonwoods and their hybrids, beavers may forage to maximize net rates of energy intake. In stands where Fremont cottonwoods are rare and trees with many defensive compounds are present, beavers are likely to forage to satisfy nutritional needs while minimizing the ingestion of toxic secondary compounds.

This study suggests that beavers may indirectly influence cottonwood-stand genotype composition, stand age structure, and the spatial distribution of cottonwood habitat through selective herbivory. However, the relative strength of these interactions depends upon the magnitude of beaver herbivory within a site (i.e., minimal herbivory vs. heavy herbivory) and the response of plants to that herbivory (i.e., survival, asexual reproduction, chemical induction, ontogenetic shifts). For example, the cross types of cottonwoods selected by beavers have different abilities to regenerate asexually; Fremont cottonwood does not clone, whereas backcross hybrids and narrowleaf cottonwoods clone prolifically (Schweitzer et al. 2002). Thus, in addition to the direct effects of felling, the indirect effects on regeneration from resprout growth among cross type could result in a change in the genotypic composition of a dominant species and hybridizing complex (i.e., beavers are agents of natural selection in poplars). Their selective foraging can also affect the age structure of cottonwood stands, converting stands that are predominantly composed of mature individuals into mixed-age or juvenile stands, similar to the effects of beavers in aspen forests (Basey et al. 1988, 1990). Furthermore, beavers may affect a diverse community of arthropods and birds that are differentially associated with each cross type (Whitham et al. 1999, 2003). These points emphasize that genetically based plant traits can directly and indirectly link population-, community-, and ecosystem-level processes.

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