Identifying alkaloids in Alaska *Lupinus* spp. with reference to crooked calf disease

by:

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Introduction

The western rangelands of the United States have a high incidence of skeletal deformities in calves known as crooked calf disease. Crooked calf disease may develop if a cow eats certain species of the genus *Lupinus* between day 40 and 70 of gestation (Keeler, 1984). There are approximately 100 species of *Lupinus* distributed throughout the temperate regions, and it is particularly numerous in western North America (Davis & Stout, 1986). In Alaska, there are four perennial species of lupine: arctic lupine (*Lupinus arcticus* subsp. *arcticus*), Nootka lupine (*L. nootkatensis* var. *nootkatensis*), large–leaved lupine (*L. polyphyllus* var. *polyphyllus*), and Yukon lupine (*L. kuschei*). Because of hybridization it is difficult to separate the perennial species in northwestern North America (Fig. 1).

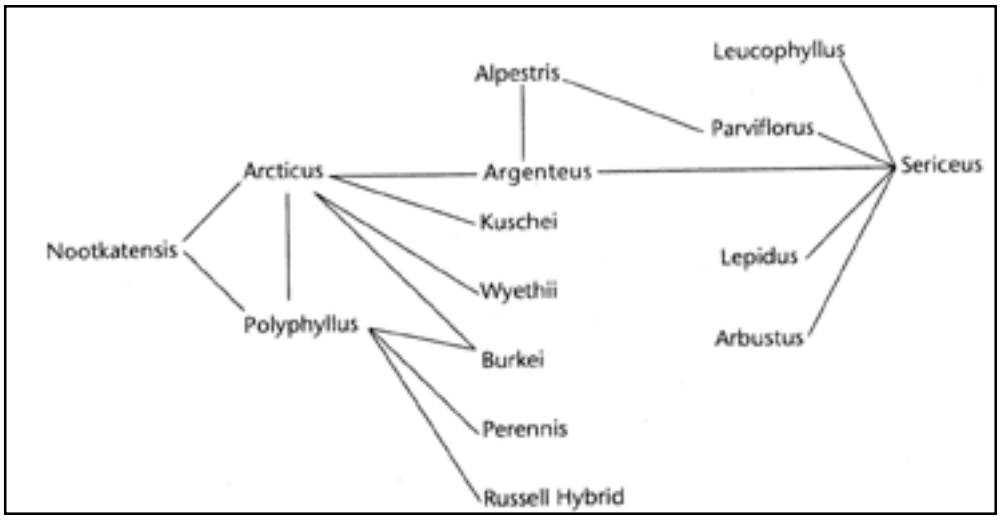

Fig 1. Hybridization of Lupinus species in Canada and Alaska (Dunn and Gillett, 1966).

Teratogenic lupines, those that induce congenital defects, contain quinolizidine and/or piperidine alkaloids. There are three major quinolizidine alkaloids: sparteine, lupanine, and anagyrine. The piperidine alkaloid is ammodendrine (Fig. 2) while anagyrine is the principal quinolizidine alkaloid responsible for crooked calf disease in cattle (Keeler. 1976), ammodendrine has also been implicated (Keeler & Panter, 1989). Crooked calf disease is characterized by arthrogryposis (twisted and ridged limbs, forelimbs are more affected than hind limbs), torticollis (twisted back and/or neck), scoliosis (lateral curvature of the spine), and occasionally palatoschisis (cleft palates), or any combination of these (Fig 3). In one experiment, anagyrine was not detected in either arctic or Nootka lupine (Majak & Ogilvie, 1994). However, in a more recent study, Majak et al. (1994) found significant amounts of anagyrine and ammodendrine in arctic and large–leaved lupine in central British Columbia, Canada. Anagyrine and ammodendrine were present in arctic lupine only at the transition zone where hybridization with large–leaved lupine (containing high levels of anagyrine and ammodendrine) apparently occurred.

Another poisonous plant found in Alaska is false hellebore (*Veratrum eschscholtzii*). False hellebore is in the lily family (*Liliaceae*), and is known to be poisonous to livestock (Shupe & James, 1983). Western false hellebore (*Veratrum califomicum*) when maternally ingested by ewes on the 14th day of gestation has caused cyclopian and related craniofacial deformities in lambs (Fig. 4). Limb and tracheal defects occur when Veratrum is ingested during later periods of

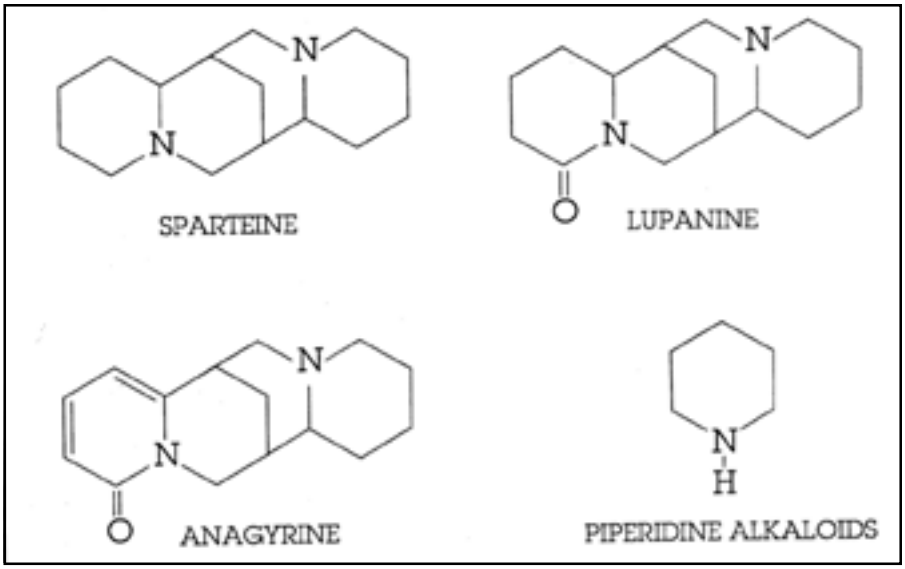

Fig 2. Chemical Structures of Alkaloids in lupine.

gestation (Keeler et al., 1985; Keeler & Stuart, 1987). Congenital defects reported by Leipold et al. (1977), on Kodiak Island, concur with false hellebore ingestion reported from other studies. Also, torticollis, contracted limbs, and cleft palate have been observed on Kodiak Island and are associated with the maternal ingestion of *Lupinus* species. Nootka lupine is found on Kodiak Island.

Spinal dysraphia has been observed, however, it is not associated with crooked calf disease. The cause of spinal dysraphia on Kodiak Island is not known. Cattle have been observed grazing lupine and false hellebore on Kodiak Island during the critical period of gestation (F. Husby, personal communication. Feb. 15, 1994). We believe the congenital defects observed here in cattle may be due to the consumption of either/or both lupine and false hellebore, and may be different from those seen in the western United States. These defects therefore are referred to as Kodiak Island crooked calf disease (E Husby, personal communication, Feb. 15, 1994). The objective of this pilot study was to determine if the alkaloids responsible for crooked calf disease in cattle are present in lupine species in Alaska.

Teratology

Congenital malformations were once thought to be entirely of genetic origin. Today, research has isolated other factors that may cause malformations in animals. Commonly cited factors include: viruses, radiation, deficiency or excess of certain nutrients, chemicals and/or drugs, hereditary defects, and plants that contain natural teratogens.

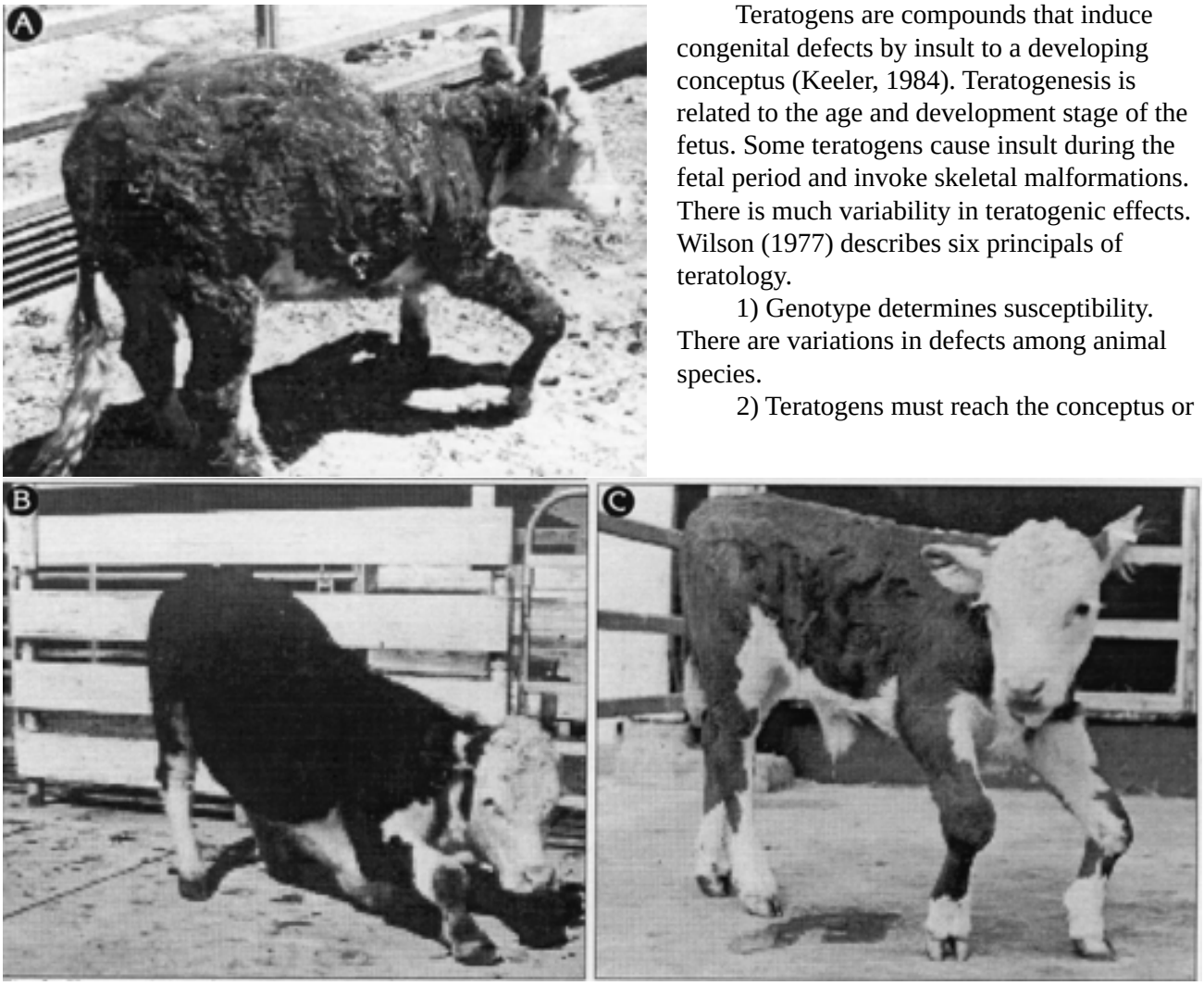

Fig. 3 Characteristics of typical crooked calf disease. A. This calf exhibits signs of arthrogryposis and scoliosis. B & C. These calves exhibit signs of arthrogryposis and torticollis. Photos courtesy of Dr. R. Keeler, USDA–ARS, Poisonous Plant Research Laboratory and Dr. J. Shupe, Veterinary Science Dept..,Utah State University.

Teratogens are compounds that induce congenital defects by insult to a developing conceptus (Keeler, 1984). Teratogenesis is related to the age and development stage of the fetus. Some teratogens cause insult during the fetal period and invoke skeletal malformations. There is much variability in teratogenic effects. Wilson (1977) describes six principals of teratology.

1) Genotype determines susceptibility. There are variations in defects among animal species.

2) Teratogens must reach the conceptus or

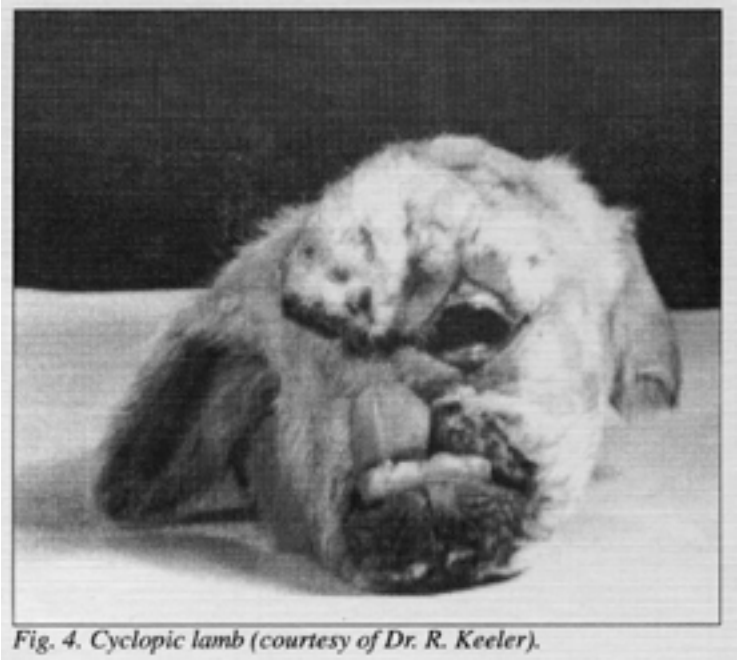

Fig. 4. Cyclopic lamb (courtesy of Dr. R. Keeler).

produce an influence that does.

3) Induced deformities by teratogens are dose dependent. The amount of plant consumed, metabolized, passing the placental barrier, and finally reaching the site of insult at the vulnerable period of gestation.

4) Teratogens can produce death instead of deformities. High doses may kill the conceptus and/or the dam. Abortions or reabsorptions, seen as a poor calf crop, may signal a problem with a teratogenic plant.

5) The conceptus (fetus) must be exposed at the susceptible developmental period. In cattle, the calf is susceptible to insult over a wide window during gestation. Through feeding trials, Keeler (1976) has shown that between day 40 and 70 of gestation is the most susceptible period.

6) Teratogens exert their effects by specific mechanisms. Structurally different teratogens may cause similar deformities.

Poisonous Plants

Plant poisoning may occur when cattle or other herbivores are overstocked and the range is overgrazed. Poisoning may also occur due to mismanagement or inability to provide adequate controls as occasionally occurs on large rangelands with unrestricted grazing as seen in the southwest and western continental United States. The large size of the ranges adds difficulty in the detection of animal poisoning and the necessary management to avoid potential hazardous areas. Eliminating poisonous plants is often not an economical option in this situation. The presence of poisonous plants on rangelands is no assurance that poisoning will occur. For example, lupine–which may be indigenous to certain ranges—may not be a problem every year. Toxicity in lupine varies among sites, stage of growth, and plant parts (James & Ralphs. 1987). It is dependent on the season of the year (some plants are toxic in the spring and others in the autumn), availability of non-toxic forage, and the amount of toxic plant eaten (Klebesadel, 1972).

Livestock poisoning is a serious economic problem in range management. The rancher should be familiar with the different toxic plants on the range. If animals are stressed from overstocking, the range is overgrazed, or the animal has a nutritional deficiency, then foraging patterns may shift to consumption of vegetation that would normally be avoided. As agriculture expands in Alaska to include more diversified livestock such as reindeer (*Rangifer tarandus*), wapiti (*Cervus elaphus*), and bison (*Bison bison*), there will be an increasing need for more information on poisonous range plants and their effects on these species.

Methods and Materials

A botanical collection of arctic, Nootka, and large–leaved lupine was made in July 1994 from Eagle Summit, Delta Junction, Homer, and Kodiak Island (Fig. 5). Ten entire plants of each species, including roots, were randomly collected from each site for botanical identification and chemical analysis. The samples were immediately put in Ziploc® plastic bags to reduce desiccation, placed in a cooler with ice, and then transported to the laboratory where they were stored at –70 °C. Individual plants were separated by plant parts: flower, stem, upper leaves, lower leaves, root, and seed pods.

Lower leaves were classified as those between 12 cm and 24 cm, depending on the plant size above the root. Those leaves above 12 cm and 24 cm were considered upper leaves. The samples were freeze–dried using a Labconco® freeze dry system and ground in a Wylie® mill. Arctic and Nootka lupine were shipped to

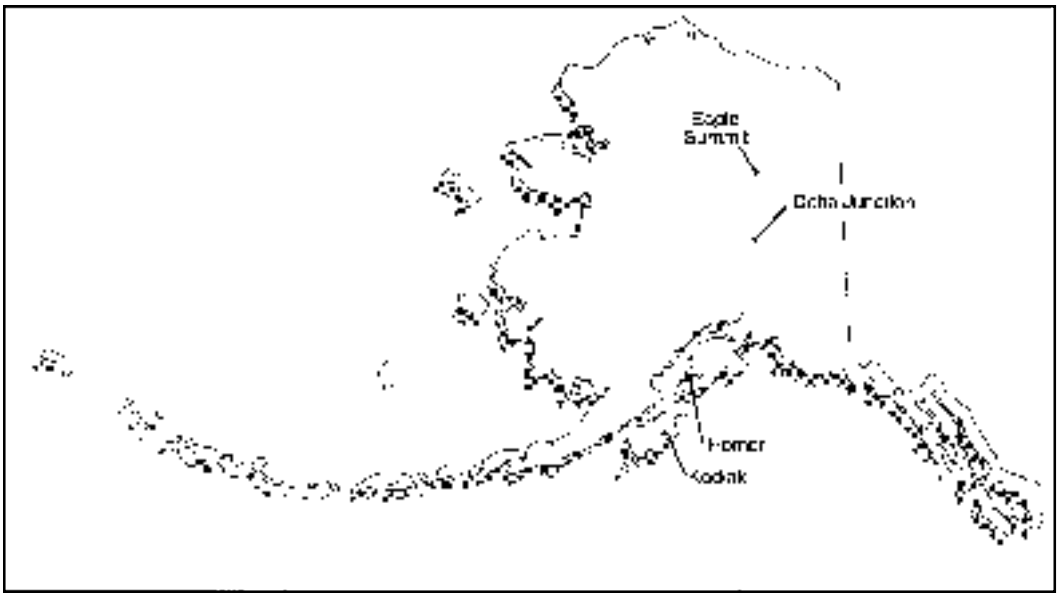

Fig. 5. The 1994 collection sites.

the USDA Poisonous Plant Research Lab in Logan, Utah for analysis. An attempt was made to collect lupine on the Seward Peninsula, Alaska, but no species were found. However, according to Hultèn (1968), lupine is present in that geographic area. The discrepancy between Hultèn and our study may have resulted from Hultèn's collection made at a specific site on the Seward Peninsula and the lines extrapolated to cover the entire area.

Chemical Analysis

Total alkaloid level and the percent of individual alkaloids were measured for stem, pods, and root parts of arctic and Nootka lupine by gas chromatography (GC) using a slightly modified procedure previously reported (Gardner & Panter, 1994). Dried plant material (approximately 100 mg) was finely ground and extracted with a mixture of lN hydrochloric acid (HCL) (5 ml) and 4 ml chloroform ($CHCL_3$) with mechanical shaking for 15 minutes. The sample was centrifuged at 1000 g for five minutes, the acidic aqueous layer removed and pH adjusted to 9.0–9.5 with concentrated ammonium hydroxide (NH_4OH). The basic solution was then extracted twice with $CHCL_3$, first with 4 ml and then with 2 ml. The combination $CHCL_3$ solutions were filtered through sodium sulfate (Na_2SO_4) and the solvent evaporated under N_2 at 60 °C. Ethanol (EtOH) (1 ml) was added to this final extract and mixed. One L of this final solution was injected into an HP 5890 GC equipped with a split/splitless injector, FID detector, and a J&W DB–l (30m x 0.33 mm i.d.) capillary column. Injector temperature was 250 °C and operated in the splitless mode. Split vent flow rate was 60 ml/min. and purged after 1 min. Detector temperature was programmed: 100 °C for 1 min; 100–200 °C at 50 °C/min; 200–260°C at 5 °C/min.; 260–320 °C at 50 °C/min.; and held at 320 °C for the final 8.8 min. to clean off the column. Alkaloid peaks, retention times and peak areas were compared with two previously analyzed standard plant samples—*L. caudatus* known to have quinolizidine alkaloids and *L. formosus* known to have piperidine alkaloids.

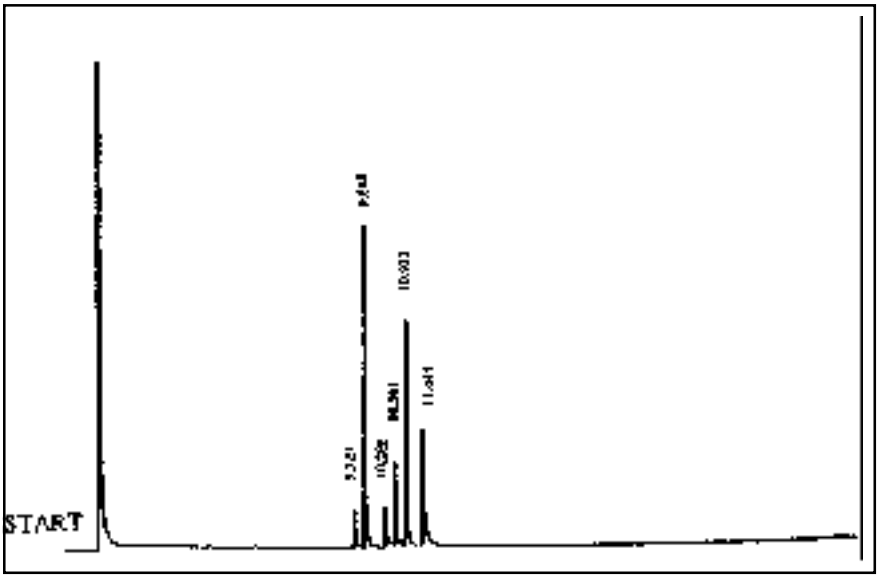

Fig. 6. Gas Chromatographic seperation pattern from Nootka lupin.

Table 1. Total alkaloid level and percent of individual alkaloids in arctic and Nootka lupine.

Plant parts	Material amount (mg)	Alkaloid amount (mg)	lupanine (%)	α isolupanine (%)	5,6 dehydro lupanine (%)	N methyl albine (%)	Winks #9 (%)	Multi-florine (%)	Unknown alkaloids (%)
Arctic									
stem	302	0.4	35.43	9.46	13.58	3.33	5.32	8.96	23.92
pods	1081	1.7	40.89	14.05	21.69	4.51	5.58	5.59	7.75
roots	101	0.6	21.99	—	20.47	16.66	40.6		0.82
Nootka									
stem	99	0.9	38.28	—	1.88	4.75	13.4	20.00	18.69
pods	104	1.0	34.67	—	4.35	9.67	26.2	19.92	5.19
roots	103	0.5	26.57	—	10.06	33.36	13.3	16.72	0

Results

The alkaloids of six GC peaks were identified as the following: peak one, a–isolupanine (trace amount); peak two, 5,6–dehydrolupanine; peak three, lupanine; peak four, N–methylalbine; peak five, Winks #9; and peak six, multiflorine (Fig. 6). Anagyrine, the only quinolizidine alkaloid known to cause crooked calf disease in experimental feeding trails was not present in either arctic or the Nootka lupine collected in Alaska.

The total alkaloid level and percent of individual alkaloids in arctic and Nootka lupine collected in July 1994 are presented in Table 1. Arctic lupine had trace amounts of a–isolupanine, which was not present in Nootka lupine. The highest concentration of alkaloids from this study appeared in the stem and pods, with the lowest concentration in the root. The highest percent of alkaloid in both plants was lupanine. In arctic lupine, Winks #9 was highest in the root compared to Nootka lupine where it was highest in the pod. The alkaloid multiflorine was not present in the root of arctic lupine, but was present in all three samples of Nootka lupine. N–methylalbine appears to be the alkaloid in lowest concentration, that is present in all three samples of arctic lupine. The alkaloid 5,6–dehydrolupanine is in lowest concentration in Nootka lupine. Of the alkaloids observed in this elution, only two have been observed in teratogenic lupines: lupanine and 5,6–dehydrolupanine.

Discussion

Although the plants were collected in July, if anagyrine was present it would have been detected by this procedure. Changes in concentration within the plant may vary over the season, but alkaloid composition overall would remain relatively stable. In other studies, Keeler (1972) showed that the elution patterns of lupine containing anagyrine to have four major alkaloid peaks: peak one, 5,6–dehydrolupanine; peak two, lupanine; peak three, possibly 1,3–epimethoxylupanine; and peak four identified as anagyrine (Keeler. 1972). Patterns of the type reported by Keeler (1972) are seen from lupines in areas with a history of crooked calf disease. There is a high incidence of crooked calf disease on Kodiak Island, where Nootka lupine was collected, but this elution pattern was not observed. This may indicate that another quinolizidine alkaloid in arctic or Nootka, or perhaps large–leaved lupine may be responsible for crooked calf disease there. It is difficult to separate the perennial lupine species in northwestern North America due to hybridization. Majak et al. (1994) found significant amounts of anagyrine and ammodendrine in arctic lupine in central British Columbia, Canada, but only at the transition zone where large–leaved lupine grows. The large–leaved lupine contained large amounts of anagyrine and ammodendrine (Majak et al., 1994). This would suggest a hybridization of these two species and also a “hybridization” of the alkaloid composition. Toxicity is caused by minor changes in the structure of active alkaloids (Kingsbury, 1964). If hybridization occurred, it possibly influenced the production of anagyrine and ammodendrine. Sparteine and lupanine are similar to anagyrine in chemical structure (Fig. 2), but have not shown teratogenic activity.

The highest concentration of alkaloids from this study appeared in the pods (Table 1), with the lowest concentration in the root and stem. Finstad and Renecker (1991) reported that on Umnak Island, Alaska, where lichen is not present, reindeer dig and consume the roots of cow parsnip (Heracleum lanatum) and lupine (personal communication). If the low concentration of alkaloids in the roots of lupine collected in Interior Alaska and on Kodiak Island holds true for those on Umnak Island, this may help explain the reindeer's preference for lupine and cow parsnip roots.

In light of this information, we should consider the theory of coevolution of poisonous plants and herbivores. Laycock (1978) suggested that secondary compounds in plants, once described as waste products, may defend against herbivores and insects. In heavily grazed areas, the more palatable plants are grazed first, reducing competition, and allowing poisonous plants to increase (Harris, 1972). In some species, herbicides may increase alkaloid concentration. Two plants that have shown this trend are tall larkspur (*Delphinium barbeyi*) (Williams & Cronin, 1966) and false hellebore (*Veratrum californicum*) (Cronin & Nielsen, 1972). Although the effectiveness of secondary compounds in producing teratogenic effects is clear, we cannot prove they evolved as a anti–herbivore defense. However, it is possible that secondary compounds in lupine are at an earlier stage of evolution on Kodiak Island because grazing has been relatively recent. This may be a genetically isolated population and therefore evolved without herbivore pressure as in other regions.

The presence of poisonous plants on rangelands is no assurance that poisoning will occur (Klebesadel. 1972). Nearly all malformations produced by an environmental teratogen can be matched by one produced by a mutant gene. The variety of congenital malformations , makes it difficult to determine with certainty that any observed malformation has arisen from a particular etiologic agent. Also, hereditary defects should not be overlooked. Only certain lupines produce crooked calf disease. Kellogg's spurred lupine (*L. caudatus*), silky lupine (*L. sericeus*) (Shupe et al., 1967) and Lunara lupine (*L. formosus*) (Keeler & Panter, 1989) are three whose teratogenic properties were established by feeding trials. Crooked calf disease is of high economic importance to ranchers that graze herbivores on ranges where these lupine are present. Severe forms of crooked calf disease can effect from 2 to 25 % of all calves born (Keeler et al., 1976). Most of these calves die or are killed by predators. The simplest way to avoid crooked calf disease is to prevent pregnant cows from grazing teratogenic lupines between the 40th and 70th day of gestation. This management is not always possible, however slight changes in breeding times or grazing strategies in relationship to lupine growth stage (alkaloid levels) can significantly reduce risk. The variability in incidence of the disease from range to range and year to year apparently stems from the amount of anagyrine consumed and gestational timing. Weather conditions and other unknown factors can influence the growth stages of the plant and, hence, anagyrine content of the plant (Keeler et al., 1976). Also, seeds high in anagyrine may or may not be present depending on the insect infestation that year. This may explain the lack of anagyrine in lupine from Kodiak Island, but not the previous high incidence of crooked calf disease in cattle.

This pilot study has shown that anagyrine is not present in two species of lupine found in Alaska, however, not all lupine are teratogenic. Nevertheless, the high incidence of crooked calf disease on Kodiak Island may indicate that another quinolizidine alkaloid–other than anagyrine—in these lupines may be responsible for the disease. Kodiak Island crooked calf disease may also be related to the consumption of both lupine and false hellebore. Further chemical analysis of these and related species needs to be done. It appears that more research is needed to fully understand the incidence of crooked calf disease in Alaska.

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