Productivity and genotypic diversity of *Trifolium ambiguum* in the Central Caucasian Mountains, Georgia.

R.G. Patterson[1], P.R. Espie[2]
1 'Longslip Station, Private Bag, Omarama, New Zealand
2 'Highview', 36 Patterson Road, Wingatui, New Zealand

ABSTRACT
Trifolium ambiguum occurred in12 diverse grassland communities, ranging from 1700 m to 2850 m, in the Kasbegi region, Central Caucasus. Total herbage mass in mid summer ranged from 310 to 763 g/m^2 with *T. ambiguum* composition ranging from 0.5 to 51%. Maximum *T. ambiguum* production occurred in fertile, regularly mown hay meadows, but was reduced in severely grazed pastures. It has persisted in these communities after centuries of grazing or cutting, without fertiliser input. *T. ambiguum* ploidy levels were predominantly diploid (82% frequency of occurrence), often in conjunction with triploid (18%) or tetraploid (27%) genotypes. The triploid *T. ambiguum* genotype (2n = 24) has not been previously reported. Further evaluation for use in temperate montane pasture systems is warranted.

KEYWORDS
Caucasian clover, Kura clover, ploidy, forage legume.

INTRODUCTION
In many humid temperate regions symbiotically-fixed leguminous nitrogen (N) is the basis for high producing quality pastures (Scott and Maunsell, 1974). In New Zealand hill and high country pastures, the legume content is often low (Suckling, 1966) or widely used species, such as white clover (*Trifolium repens* L.), persist poorly. There is therefore considerable interest in evaluating other perennial legumes for special purpose pastures or for particular environments (Hill et al. 1993, Scott et al. 1995).

Caucasian or Kura clover (*Trifolium ambiguum* M. Bieb.) is a perennial legume that is attracting attention for its persistence under drought and intensive grazing, and its winter-hardy and N-fixing attributes (Lane 1985, Dear & Zorin 1985, Yates 1993, Peterson et al. 1994, Virgona & Dear 1996). It naturally occurs throughout the greater Caucasus geographical region, extending from the Black Sea, to NE Asia minor and NW provinces of Iran, with a wide ecological distribution, ranging from steppe communities to the subnival zone (3000 m a.s.l.). Little is known regarding its agronomic performance in these environments in the English scientific literature.

A major problem with evaluating the persistence of *Trifolium ambiguum* is the relatively short timeframes (<20 yr) available with existing experiments and also that many studies have either been well fertilised or had nil or infrequent grazing. Furthermore there is wide morphological diversity within the species and further potential for genetic selection for desired traits such as N-fixation (Spencer and Hely, 1982), seedling vigour/ inoculation requirements (Hay et al. 1993, Patrick et al. 1994) or soil acidity tolerance (Barnard and Folscher, 1988). Therefore we systematically evaluated *T. ambiguum* production in its natural environments where it shows long-term persistence (> 100 yr) in unfertilised grazed grasslands and to identify superior genotypes suitable for immediate pastoral use or plant breeding. Bioclimatically zones in the greater Caucasus region are similar to those in the New Zealand high country.

METHODS
We assessed *T. ambiguum* mid-summer productivity (July 1995) in 13 different communities arrayed along an altidudinal gradient from 1700 to 2850 m., in the Mt. Kazbegi region, central Greater Caucasus (42^0 48' N, 44^0 39' E). Here a suite of siliceous shales, schists, granites, andesites and basalts rise to 5000 m. Winters are cold with cool summers, mean summer temperatures ranging between 14 to 18 ^{0}C with maximum temperatures of around 30 ^{0}C. The mean annual precipitation in the subalpine belt is 700 to 800 mm with a spring maxima and fohn winds often cause late summer drought. Timberline occurs at around 2,400 m a.s.l. Grasslands have traditionally been used for centuries as hay meadows or pasturage for both sheep and cattle, with composition and use remaining similar within living memory (ca 100 yr). Soils in the study area (ratings according to Blakemore et al.1981) are usually moderately acidic but varied to nearly neutral pH. Cation exchange capacity varies from low to medium with very high base saturation (81-100%). Olsen extractable phosphates were low (<10) except for hay meadows on talus fans where they exceeded 10 me/ 100g. C: N ratios were generally high, indicative of N deficiencies.

In each community herbage was removed in three replicate 20 x 20 cm plots, separated by species, and dried to constant weight. *T. ambiguum* chromosome numbers were counted in root meristem cells from fresh samples collected between 7-13 July 1995, after 1-1.5 hr pre treatment in 0.05% colchicine, fixing in Carnoy's solution and staining with Aceto-Carmine solution.

RESULTS
Total herbage mass and *T. ambiguum* production differed significantly between communities (Table 1, $P < 0.001$) with the highest yields in the *Hordeum violaceum* association. Here *T. ambiguum* comprised 51% and white clover only 10% of the 6,900 kg/ha total yield. *T. ambiguum* productivity and leaf area index, excluding this community and a *Bromopis variegata* association infested with *Hieracium pannoniciforme* as special cases, remained remarkably constant across the 1700 - 2850 m range in altitude, as shown by a low regression coefficient of determination (DM (g/m^2) = 0.008 Alt.(m) + 15.8; R^2= 0.01). In the *Bromopis* association, *T. ambiguum* yield decreased by 53% in plots where it occurred with *Hieracium pannoniciforme*, attributed to competition, though how generally this may apply is not known.

Three different ploidy levels were found in *T. ambiguum* in the study area, diploids being the most frequent, occurring at all altitudes (Table 2). Triploids and tetraploids were less frequent, sometimes co-occurring with diploids, though the small sample size restricts generalisations regarding their ecological differentiation. The hexaploid genotype was apparently absent.

DISCUSSION
There is a striking contrast between the performance of *T. ambiguum* in its natural habitat in the Caucasian mountains and *T. repens* in New Zealand under similar rainfall, mean annual temperatures and soils (pH 4.9 to 8.1). In the Caucasus, *T. ambiguum*, was present, sometimes dominant, in nearly all of the plant communities studied to altitudes approaching 3000 m, while white clover was increasingly rare above 2300 metres a.s.l.. The high production on the *Hordeum* association hay meadow, in comparison to the other sites is almost certainly partly due to the greater P availability from the younger talus soils and the and low Olsen phosphate on the other soils strongly suggests P deficiency. If so, it is reasonable to expect similar P responses would occur in the Caucuses to those demonstrated in New Zealand (Strachen et al. 1994). The ninefold reduction in *T. ambiguum* production in a severely grazed *Hordeum* association compared with that under hay management, supports recent results showing that grazing over long rotations maximises production (Petersen et al. 1994). *T. ambiguum* production in this association in the Caucuses compares favourably with that of Australian cultivars (Yates 1993, Virgona & Dear 1996). Similarly, its productivity (200 kg/ha) at 2850m suggests considerable potential for high country pastoralism in areas with similar soils and climate.

The major result from this study is that *T. ambiguum* has successfully persisted in montane to sub-alpine pastures under centuries of traditional grazing without applied fertiliser. This corroborates results from other studies regarding its long term persistence (Yates 1993) under low fertiliser inputs (Virgona & Dear 1996), its ability to survive summer drought (Spencer et al. 1975) and severe grazing (Allan & Keoghan 1995), and makes it an increasingly attractive perennial legume with the increasing requirements for sustainable pastoral farming in upland areas. The local ecotypes, particularly the triploid which has not been recorded previously, warrant further evaluation.

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Table 1
Community diversity, total herbage mass and *T. ambiguum* percentage yield and Leaf Area Index

Community Dominants	Altitude	Aspect	Slope (^{0})	DM (g/m^2)	*T. amb.* (g/m^2)	*T. amb.* (%)	*T. amb.* (LAI m^2/m^2)
Poa depressa-Trifolium repens-Trifolium ambiguum	1750	nil	0	333	12	4	0.3
Hordeum violaceum-Ranunculus elegans-Trifolium ambiguum	1850	nil	0	697	357	51	6.0
Hordeum assn. Plus severe grazing	1850	nil	0	365	40	11	0.8
Bromopsis variagata-Agrostis tenuis-Trifolium ambiguum	1900	S	3-5	310	54	17	1.0
Bromopsis assn. Plus Hieracium pannoniciforme	1900	S	3-5	339	1	0.4	0.03
Bromopsis variagata-Agrostis tenuis-Trifolium ambiguum	2050	nil	3-5	611	35	6	0.9
Festuca varia-Carex meinshauseniana	2000	NW	40-45		20	5	
Festuca rupicola-Carex buschiorum-Pulsatilla violacea	2000	S	0-12	315	5	2	0.1
Cobresia capilliformis-C. persicae-Carum caucasicum	2150	NW	2-3	348	48	14	1.2
Cobresia capilliformis-C. persicae-Carum caucasicum	2450	nil	0	425	31	7	
Nardus stricta-Anthoxanthum alpinum-Leontodon caucasicum	2300	S	3	763	13	2	0.3
Sibbaldia semiglabra-Alchemilla retinervis	2500	N	10-12	688	74	11	1.3
Festuca supina-Carex tristis-Cobresia capilliformis	2850	SW	10-12	576	20	3	0.4

Table 2
T. ambiguum ploidy level and vegetation association, Mt Kasbegi, Georgia.

Community Dominant	Altitude	Chromosome Number			
Hordeum violaceum	1750	16			
Poa depressa	1750		16		
			32		
Nardus stricta	1800	16			
Bromopsis variegata	1900		16		
			32		
Nardus stricta	1900	16			
Bromopsis variegata	2000	16			
Festuca rupicola	2000	16	24		
Sibbaldia semiglabra	2100	16			
Cobresia capilliformis	2150			32	
Festuca supina	2450		24		
Sibbaldia semiglabra	2470	16			
Frequency (%)		82	18	27	