CHAPTER SIX

MICROEVOLUTION IN *Microlaena stipoides*

6.1 GENERAL INTRODUCTION

Evolution, the net change in gene frequencies of a population, is a result of natural selection acting on genetic variation within species. Darwin (1859) stated that extensive genetic variation within species is the crucial factor in species evolution by natural selection and it has been reported that every species studied in the literature is genetically variable at about 30% of its loci (Antonovics 1976). Genetic variation and selective effects of the environment are the two crucial factors determining the occurrence and rate of evolution which could be rapid and local (Bradshaw and Mortimer 1986). Community-generated environmental factors are important components influencing the evolutionary process (Bradshaw and Mortimer 1986).

The hierarchy of evolutionary processes in a community include the abiotic and biotic environmental effects and the evolutionary changes in genetic constitution of populations (Bradshaw and Mortimer 1986). There is much evidence for genetic variation and differentiation which allows individuals to coexist in communities by differences in resource utilisation (Bradshaw and Mortimer 1986) and improvement in competitive abilities of component species (Aarssen 1983, 1985, 1989).

It is generally accepted among ecologists that competition is a major force in natural selection (Benton 1987) and that improved ecological combining ability (Pianka 1981) and evolutionary equilibration in competitive abilities (Aarssen 1983, 1985, Aarssen and Turkington 1985a) are the main consequences of interspecific competition. Turkington and Harper (1979c) interpreted the observed 'principal diagonal effect' in *T. repens* as a microevolutionary response of *T. repens* to diversifying selection imposed by neighbouring grass species.

The aims of this chapter were to test the hypotheses that competition is the driving force in the divergence of *M. stipoides* populations and that divergence among *M. stipoides* populations allows coexistence with neighbouring perennial grass species.

6.2   COMPETITION AND DIVERGENCE OF *Microlaena stipoides* WITH ASSOCIATED PERENNIAL GRASS SPECIES

6.2.1   Introduction

Plant-plant interactions play an important role in the structuring of communities and in maintaining genetic diversity in populations (Turkington and Aarssen 1984). Evans and Turkington (1988) and Turkington (1989a) found that a mosaic of patches of species of perennial grasses that dominate pastures leads to diversification among *Trifolium repens* genotypes on the basis of neighbour-specific compatibilities. The close association between grasses and *T. repens* imparts a selection on *T. repens* individuals for persistence, growth and reproductive compatibilities with individual grass species (Evans and Turkington 1988).

Martin and Harding (1981) reported that adaptation of populations to each other may result from interspecific competition. It is possible that competitive interactions among coexisting species is reduced or eliminated resulting from selection for niche differentiation (Turkington and Aarssen 1984) and balancing of competitive abilities (Aarssen 1983, 1985, 1989).

The reciprocal transplant technique is a powerful method of testing fitness of populations (Bradshaw 1984). Different populations of a species have very different adaptations to different environments and it is common that fitness of populations in an alien environment would be only half that of the native population (Bradshaw 1984). Connell (1980) suggested appropriate field experiments to demonstrate coevolutionary divergence of competitors which must satisfy these three criteria: (1) divergence of populations, (2) competition by the neighbouring species as the driving force for divergence, and (3) divergence has a 'genetic basis'. To demonstrate divergence of a population, niche breadth and position along the niche axis of an allopatric population (X_A) transplanted into a sympatric location (Treatment 1, Connell 1980) was compared with the niche breadth and position along the niche axis of a sympatric population (X_S) transplanted into a sympatric location where the native sympatric population (X_S) was removed but not the competitor species (Y) (Treatment 4, Connell 1980). Divergence was also assessed by comparing the niche breadth and position along the niche axis of a sympatric population (X_S) transplanted in sympatric (Treatment 4) and allopatric locations (Treatment 6, Connell 1980) where the native sympatric populations were removed.

According to Connell's (1980) design, a test for competitive effects of the associated species (Y) on the divergence of species X can be done by comparing the niche breadth and position along the niche axis of allopatric populations (X_A) transplanted into a sympatric location where both the native sympatric population (X_S) and the competitor species (Y) were removed (Treatment 2) and into a sympatric location where only the native sympatric population (X_S) was removed (Treatment 1). This is in effect comparing the performance of allopatric populations in sympatric locations in the presence (Treatment 1) or absence (Treatment 2) of competitor species (Y). Competitive effects were also tested by comparing the niche breadth and position along the niche axis of sympatric (X_S) (Treatment 5) and allopatric (X_A) populations (Treatment 2) transplanted into a sympatric location where both the native sympatric population (X_S) and the competitor species (Y) had been removed. If the niche of the transplanted allopatric population (X_A) changed significantly compared with the niche of the sympatric population (X_S) in the presence but not in the absence of a competitor species (Y), then there is strong evidence that competition with species Y was the major factor influencing the divergence of the sympatric population (X_S) (Connell 1980, 1985).

Davic (1985) criticised Connell's (1980) proposed experimental design as biased towards commitment of a Type II experimental error, i.e. that competition had not occurred during coevolution when in fact intense competition resulted in non-overlapping use of resources and genetic segregation in allopatric populations (X_A) and competitor species (Y). According to Connell (1980), to prove that competition has occurred during coevolution of species, one must get all affirmative results in the comparisons mentioned above. Davic (1985) modified and presented Grant's (1972) ten models of selection leading to divergent and convergent character displacement. He argued that it is possible that coevolution will result in character displacement without concomittant divergence or convergence in resource use. Davic (1985) proposed a set of experimental treatments involving addition and removal perturbations of allopatric populations of both species which is necessary if Connell's (1980) treatments 1 and 2 infer lack of competition.

Connell (1985) defended his experimental design stating that all of Davic's ten models can be answered by his design. He reasoned that if no changes were observed in the niche of allopatric populations transplanted into a sympatric location where the competitor species (Y) was either removed or left in place, this was due to the following reasons: (a) that competition was not involved, (b) the experiment was not carried out long enough or in sufficient detail to detect change, or (c) changes occurred in the environment or the genetic structure of the allopatric populations since the original coevolution.

Roughgarden (1983) criticised Connell's (1980) presentation of his proposed experiment to determine that coevolution of competing species has led to niche separation: [it] "lacks in clear justification, misleadingly suggests that there is an element of certainty obliging the acceptance of a conclusion based on his protocol and that no other protocol is necessary". Roughgarden (1983) reported that Connell's proposed experiment is neither workable, convincing nor on the right track for a number of reasons: (a) divergence is only one of the possible outcomes of coevolution of competing species, (b) there is an unspecified relationship between observations on niche compression and expansion to shifts in niche position, (c) simple transplant/removal experiments could hardly detect the genetic basis for the difference between populations of the same species that are sympatric and allopatric with a presumed competitor, and (d) it is an unworkable protocol for detecting residual competition after divergence.

Turkington (1989a) adapted Connell's (1980) proposed field experimental design to study coevolution between *Trifolium repens* and associated grass species. Turkington (1989a) collected ramets of *T. repens* from sites dominated by *Agrostis capillaris*, *Holcus lanatus* and *Lolium perenne* within a permanent pasture and multiplied them in a glasshouse. He then transplanted the allopatric and sympatric populations into sympatric and allopatric sites with either the removal or non-removal of competitor species following Connell's experimental design. He monitored transplant survival and did a series of above ground biomass harvests in each plot. His results showed that populations of *T. repens* had diverged into a number of subpopulations resulting from interspecific competition with neighbouring grass species and strongly inferred that this divergence had a 'genetic basis'. Each *T. repens* showed home site advantage due to both local soil conditions and neighbouring grass species.

The present study followed Connell's (1980) proposed experimental design with the aim of determining the occurrence of microevolution among *M. stipoides* populations with four associated grass species in permanent pastures, namely *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata* and *Phalaris aquatica*. The three criteria specified by Connell (1980) were tested: (a) divergence in *M. stipoides* populations, (b) competition as the driving force for population divergence, and (c) that divergence had a 'genetic basis'.

6.2.2 Materials and methods

6.2.2.1 Study sites

The study was conducted in permanent pastures grazed by cattle and sheep on two

properties, 'Karuah' and 'Powalgarh', on the Northern Tablelands of NSW. Descriptions of the paddocks used in this study are presented in Chp. 4.2.1 ('Karuah') and Chp. 5.2.1 ('Powalgarh'). Four sites, three at 'Karuah' and one at 'Powalgarh', each 160 cm x 100 cm were chosen for the study and surveyed for percentage basal cover of perennial herbaceous species (Table 6.2.1) before setting-up the experiment. A 2-m long board, 10-cm wide with ten 30-cm wires 20 cm apart was positioned and the perennial herbaceous species whose base was touched by each point was recorded. Where a point rested on bare ground or litter, the species of nearest plant was recorded. The procedure was repeated 9 times 20 cm apart to make a total of 100 points. Percentage composition by basal cover of species was computed for each site. The three chosen sites at 'Karuah' were dominated by each of *Lolium perenne*, *Poa pratensis* and *Dactylis glomerata* while the one site at 'Powalgarh' was dominated by *Phalaris aquatica* with other species of grasses infrequent or absent. It was not possible to choose a site at 'Karuah' dominated by *P. aquatica* and *M. stipoides* as the former species was not abundant on this property. *Microlaena stipoides* was abundant at all sites.

Table 6.2.1. Percentage composition by basal cover of perennial species in the four experimental sites at 'Karuah' and 'Powalgarh' on the Northern Tablelands.

| Species | Site | | | |
	Lpe	Ppr	Dgl	Paq
Microlaena stipoides	18	24	32	41
Lolium perenne	61	18	12	4
Poa pratensis	2	42	0	0
Dactylis glomerata	1	0	50	0
Phalaris aquatica	0	0	0	33
Trifolium repens	18	16	5	8
Eleusine tristachya	0	0	1	7
Danthonia sp.	0	0	0	7

6.2.2.2   Experimental design

The experimental design proposed by Connell (1980) to demonstrate coevolution of competitors and adopted by Turkington (1989a) in his study of coevolution of *T. repens* with associated grasses *A. capillaris*, *H. lanatus* and *L. perenne* was implemented in this study. Ramets of populations of *M. stipoides* growing in association with each of the

grasses *L. perenne*, *P. pratensis*, *D. glomerata* and *P. aquatica* were collected from the field and propagated in the glasshouse as described in Chp. 5.2.2.

For purposes of consistency, the terminologies used by Connell (1980) and Turkington (1989a) were adopted. Sympatric was used to refer to native *M. stipoides* populations growing in association with a particular competitor grass species at the site dominated by both the *M. stipoides* population and the grass species. Allopatric referred to *M. stipoides* populations growing in association with another competitor grass species at alien sites dominated by other *M. stipoides* populations and other competitor grass species.

There were six treatments at each site. Native *M. stipoides* was removed in the first three treatments and both native *M. stipoides* and the competitor grass species were removed in the other three treatments. Experimental treatments in one site, *L. perenne* (Table 6.2.2), will be elaborated here and the same treatments were imposed at the other sites. Ramets of allopatric *M. stipoides* populations collected from the *P. pratensis*, *D. glomerata* and *P. aquatica* sites were transplanted into the sympatric *L. perenne* site where native *M. stipoides* was removed and the grass competitor, *L. perenne*, was either present (Treatment 1) or absent (Treatment 2). In Treatment 3, ramets of the allopatric *M. stipoides* populations collected from *P. pratensis*, *D. glomerata* and *P. aquatica* sites were transplanted back in their original allopatric sites, i.e. *P. pratensis*, *D. glomerata* and *P. aquatica* sites respectively, where only the native *M. stipoides* population was removed. Ramets of the sympatric *M. stipoides* population collected from the *L. perenne* site were transplanted back in the *L. perenne* site where the native *M. stipoides* population was removed and *L. perenne* was either present (Treatment 4) or absent (Treatment 5). In Treatment 6, ramets of the sympatric *M. stipoides* population were transplanted in the allopatric sites (*P. pratensis*, *D. glomerata* and *P. aquatica*) where the native *M. stipoides* population was removed but not the grass competitor species.

6.2.2.3 Experimental methods

At each site a 2 m x 2 m square exclosure was erected. An inner area measuring 1 m x 1.6 m was marked with pegs and was divided into five rows and eight columns, resulting in 40 plots with each plot measuring 20 cm x 20 cm. Native *M. stipoides* was removed from twenty randomly pre-selected plots and both native *M. stipoides* and competitor grass species were removed from the remaining twenty randomly pre-selected plots. Each row represented one replication. A systemic herbicide, glyphosate, was used to paint the leaves of *M. stipoides* and associated grasses in designated plots according to treatments. Dead plant materials were removed from the plots after two weeks. Painting of herbicide was

repeated in plots where live *M. stipoides* and competitor grasses were still present after two weeks. Other minor species were also removed, either by herbicide painting or carefully digging out the root system.

Table 6.2.2. Experimental treatments used to test for the coevolution of *M. stipoides* and competitor grass species, *L. perenne*. In the sympatric site, *M. stipoides* and *L. perenne* occur together naturally, and in the allopatric site *M. stipoides* is present but *L. perenne* is naturally absent or at relatively low abundance. Treatments 1-6 are consistent with Connell (1980) and Turkington (1989a).

Treatment Number (T)	Origin of *M. stipoides* population	Trans-planting site	Removal or no removal of competitor species	Removal of native *M. stipoides* population
T1	allopatric	sympatric	not removed	removed
	M (Ppr)	Lpe		
	M (Dgl)	Lpe		
	M (Paq)	Lpe		
T2	allopatric	sympatric	removed	removed
	M (Ppr)	Lpe		
	M (Dgl)	Lpe		
	M(Paq)	Lpe		
T3	allopatric	allopatric	not removed	removed
	M (Ppr)	Ppr		
	M (Dgl)	Dgl		
	M (Paq)	Paq		
T4	sympatric	sympatric	not removed	removed
	M (Lpe)	Lpe		
T5	sympatric	sympatric	removed	removed
	M (Lpe)	Lpe		
T6	sympatric	allopatric	not removed	removed
	M (Lpe)	Ppr		
	M (Lpe)	Dgl		
	M (Lpe)	Paq		

Eighty new ramets of each of the four populations of *M. stipoides* were rooted in 5.5 cm diameter pots (Chp. 5.2.2). All the ramets were clipped to a height of 5 cm above the ground and were transplanted into appropriate plots in the field sites. Two ramets of each population were planted in each plot by digging holes and transferring the ramets with the soil left intact so as not to disturb the rooting system. Care was also taken to avoid

destroying plants of the competitor species in the plots where these occurred. Each row in the sympatric site contained four treatments, T1 (3 allopatric populations), T2 (3 allopatric populations), T4 (1 sympatric population) and T5 (1 sympatric population). All the four treatments were assigned randomly in each row and were replicated five times at each site. Treatments 3 and 6 are included in the corresponding treatments in the allopatric sites. Transplants were monitored weekly during the first two months and dead transplants were replaced immediately. Plots were cleared of any germinating weeds by pulling them out.

6.2.2.4   Measurements

Ramets were transplanted in the field late in spring 1992, and the number of panicles produced by each ramet was counted every four weeks during the following summer and autumn months of 1993. Total above-ground material of two ramets of *M. stipoides* in each plot were harvested by clipping to 5 cm above the ground in January 1993, March 1993, May 1993 and January 1994. A census of transplants was also taken at these times. Oven dry weight (70° C for 48 h) of above ground herbage was determined.

6.2.2.5   Data analysis

Data screening using histograms and normal probability plots of total plot *M. stipoides* dry weight, total panicle number and number of survivors were done using the SYSTAT statistical package. Logarithmic transformation was done on the dry weight and number of panicles data. Analysis of variance of the means of log total dry weight and log total panicle number was done using the STATVIEW statistical package. Significant differences among site, population, and site x population interaction means were determined using Scheffé's test at the 5% significance level. Pairwise comparisons of treatment means for log total dry weight and log total panicle number were conducted using Bonferroni t-tests (BMDP 7D). The Mann-Whitney t-test (BMDP 3S, Nonparametric statistics) was used for nonparametric pairwise comparison of the number of survivors.

6.2.3 Results

6.2.3.1 Survival

In general, there was high percentage survival in almost all the four sites. At the last harvest time (8 January 1994), the *P. pratensis* and *P. aquatica* sites had 100% survival, while the *L. perenne* site had 95% and *D. glomerata* had 85% survival. *Microlaena stipoides* (Lpe), M (Ppr), M (Dgl) and M (Paq) had 95%, 90%, 95% and 100% survival at the *L. perenne* site and 75%, 80%, 95% and 90% survival at the *D. glomerata* site, respectively.

6.2.3.2 Total dry weight

Averaged over all transplanting sites, there were no significant differences in the total dry weights of the four *M. stipoides* populations (Table 6.2.3) when only the native *M. stipoides* population was removed. When both native *M. stipoides* and grass competitor species were removed, M (Ppr) had a significantly higher mean total dry weight than the other three *M. stipoides* populations (Table 6.2.4).

Averaged over all *M. stipoides* populations, there were significant differences in the total dry weight of the populations among the four transplanting sites in the presence of the competitor grass species (Table 6.2.3). The trend in total dry weight was: *P. pratensis* site > *L. perenne* site > *P. aquatica* site > *D. glomerata* site. In the absence of both *M. stipoides* and the competitor grass species, the total dry weights in the *P. pratensis* and *L. perenne* sites did not differ significantly and were significantly higher than the yields in the *D. glomerata* and *P. aquatica* sites, which likewise did not differ significantly (Table 6.2.4).

Microlaena stipoides (Ppr) produced significantly higher dry weights when transplanted into the *P. pratensis* site than the *D. glomerata* site, and was intermediate when transplanted into the *L. perenne* and *P. aquatica* sites in the presence of competitor species (Table 6.2.3). On the other hand, total dry weights produced by M (Lpe), M (Dgl) and M (Paq) did not differ significantly when transplanted into the four sites in the presence of competitor species (Table 6.2.3). With the removal of competitor species, the dry matter yield of M (Lpe) transplanted into the *P. pratensis* site was significantly higher (P < 0.05) than when transplanted into the *P. aquatica* site, and was intermediate in the *D. glomerata* and *L. perenne* sites (Table 6.2.4). In the absence of competitor species, M (Ppr)

Table 6.2.3. Log total dry weight (sum of four harvests) and log panicle number (in brackets) per plot (sum of two ramets) of four *M. stipoides* populations collected from four sites dominated by different perennial grasses and transplanted into all four sites where the native *M. stipoides* and other minor species were removed but not the associated grass species. Values are means of five replicates. Values which share the same letter(s) are not significantly different (P > 0.05).

Transplanting site	*Microlaena* M(Lpe)	*stipoides* M(Ppr)	population M(Dgl)	M(Paq)	Mean
L. perenne	0.81^a	0.81^{ab}	1.14^{ab}	1.06^{ab}	0.95^b
	(1.05^{ab})	(1.26^{ab})	(0.83^b)	(1.10^{ab})	(1.06^{ab})
P. pratensis	1.18^{ab}	1.45^a	1.07^{ab}	1.20^{ab}	1.23^a
	(1.15^{ab})	(1.96^a)	(0.56^b)	(0.98^{ab})	(1.16^a)
D. glomerata	0.56^b	0.77^b	0.66^b	0.55^b	0.64^c
	(0.69^b)	(1.21^{ab})	(0.76^b)	(0.77^b)	(0.86^b)
P. aquatica	0.77^b	0.87^{ab}	0.87^{ab}	0.76^b	0.82^{bc}
	(0.89^{ab})	(1.36^{ab})	(1.13^{ab})	(1.06^{ab})	(1.11^{ab})
Mean	0.83	0.97	0.94	0.89	
	(0.94^b)	(1.45^a)	(0.82^b)	(0.98^b)	

transplanted into the sympatric *P. pratensis* site had a significantly higher total dry weight than when transplanted into the *P. aquatica* site (Table 6.2.4), while yield was intermediate when transplanted into the *L. perenne* and *D. glomerata* sites. *Microlaena stipoides* (Dgl) and M (Paq) had significantly lower yields when transplanted into the *D. glomerata* site than when transplanted into the *L. perenne* site (Table 6.2.4).

6.2.3.3 Total panicle number

The mean total number of panicles exserted by *M. stipoides* (Ppr) was significantly larger than that of the other three populations both in the presence and absence of the competitor grass species, averaged over all planting sites (Tables 6.2.3 and 6.2.4). The mean total number of panicles exserted by all four *M. stipoides* populations was significantly higher when transplanted in the *L. perenne*, *P. pratensis* and *P. aquatica* sites than in the *D. glomerata* site in the presence of the competitor species (Table 6.2.3). In the absence of the competitor species, the mean total number of panicles exserted was

significantly higher in the *P. aquatica* site than in the *D. glomerata* site, while it was intermediate in the *L. perenne* and *P. pratensis* sites (Table 6.2.4).

Table 6.2.4. Log total dry weight (sum of four harvests) and log panicle number (in brackets) per plot (sum of two ramets) of four *M. stipoides* populations collected from four sites dominated by different perennial grasses and transplanted into all four sites where the native *M. stipoides*, associated grass species and other minor species were removed. Values are means of five replicates. Values which share the same letter(s) are not significantly different (P > 0.05).

Transplanting site	*Microlaena* M(Lpe)	*stipoides* M(Ppr)	population M(Dgl)	M(Paq)	Mean
L. perenne	1.07bcd	1.17abc	1.22ab	1.18abc	1.16a
	(1.21abcd)	(1.70abcd)	(0.84d)	(1.11abcd)	(1.22ab)
P. pratensis	1.30ab	1.56a	1.08bcd	1.02bcd	1.24a
	(1.30abcd)	(2.02a)	(0.77d)	(0.97bcd)	(1.26ab)
D. glomerata	0.78bcd	1.14abcd	0.68d	0.68d	0.82b
	(0.90cd)	(0.88cd)	(0.69d)	(1.82abc)	(1.04b)
P. aquatica	0.72cd	1.08bcd	0.83bcd	0.74cd	0.84b
	(1.22abcd)	(1.89ab)	(1.28abcd)	(1.16abcd)	(1.39a)
Mean	0.97b	1.24a	0.95b	0.91b	
	(1.16b)	(1.62a)	(0.86c)	(1.27b)	

There were no significant differences in the total number of panicles exserted by all four *M. stipoides* populations when transplanted into the four sites in the presence of competitor species (Table 6.2.3). In the absence of competitor species, M (Ppr) had a significantly larger number of exserted panicles when transplanted into the *P. pratensis* site than in the *D. glomerata* site, while it was intermediate in the *L. perenne* and *P. aquatica* sites. There were no significant differences in the number of panicles exserted by M (Lpe), M (Dgl) and M (Paq) when transplanted into the four sites in the absence of competitor species (Table 6.2.4).

6.2.3.4 Divergence of *Microlaena stipoides* populations

Divergence in *M. stipoides* populations was determined by comparing the performance of sympatric *M. stipoides* populations (T4) with allopatric *M. stipoides* populations (T1) when transplanted into sympatric sites in the presence of the grass competitor. If the performance of sympatric *M. stipoides* populations is higher than allopatric populations in

the presence of the grass competitor, then divergence of *M. stipoides* populations has occurred. Results of Bonferroni pairwise comparisons (T4 v T1) for total dry weight showed that no significant divergence occurred among the four populations (Fig. 6.2.1). In terms of the total number of panicles exserted, divergence was exhibited by sympatric M (Ppr) against all the three allopatric populations transplanted into the sympatric *P. pratensis* site (Fig. 6.2.2).

Divergence was also assessed by comparing the performance of sympatric *M. stipoides* populations transplanted into sympatric sites (T4) and allopatric sites (T6) in the presence of the grass competitor. If the performance of a sympatric *M. stipoides* population is higher in a sympatric site (T4) in the presence of its competitor species (Y) than when transplanted into an allopatric site (T6) in the presence of a different competitor species, then divergence of the original *M. stipoides* population has occurred. Pairwise comparisons (T4 v T6) for total dry weight showed that divergence occurred in M (Ppr), which had a significantly higher total dry weight when transplanted into its sympatric site compared with its yield when transplanted into the other three allopatric sites (Fig. 6.2.1). Significant differences between the growth of sympatric M (Dgl) in the sympatric *D. glomerata* site and allopatric *L. perenne* site did not indicate divergence as the growth of M (Dgl) was significantly higher in the allopatric site than in the sympatric site (Fig. 6.2.1). Likewise, the growth of sympatric M (Paq) in the allopatric *P. pratensis* site was also significantly higher than in the sympatric *P. aquatica* site (Fig. 6.2.1), thus it did not show divergence of M (Paq).

There were no significant differences among the pairwise comparisons (T4 v T6) for panicle production except for M (Ppr) transplanted into the allopatric *D. glomerata* site (Fig. 6.2.2). *Microlaena stipoides* (Ppr) produced a significantly larger number of panicles when transplanted into its sympatric site compared with its panicle production when transplanted into the allopatric *D. glomerata* site (Fig. 6.2.2).

6.2.3.5 Divergence in response to competition with the associated grass species

Divergence in response to current competition with the associated grass species is assessed by comparing the performance of allopatric populations transplanted into a sympatric site where the native *M. stipoides* population was removed and the grass competitor was either not removed (T1) or removed (T2). In this way, divergence in response to interference competition with the associated grass was being tested. If the performance of the allopatric *M. stipoides* population was greatly reduced with the presence
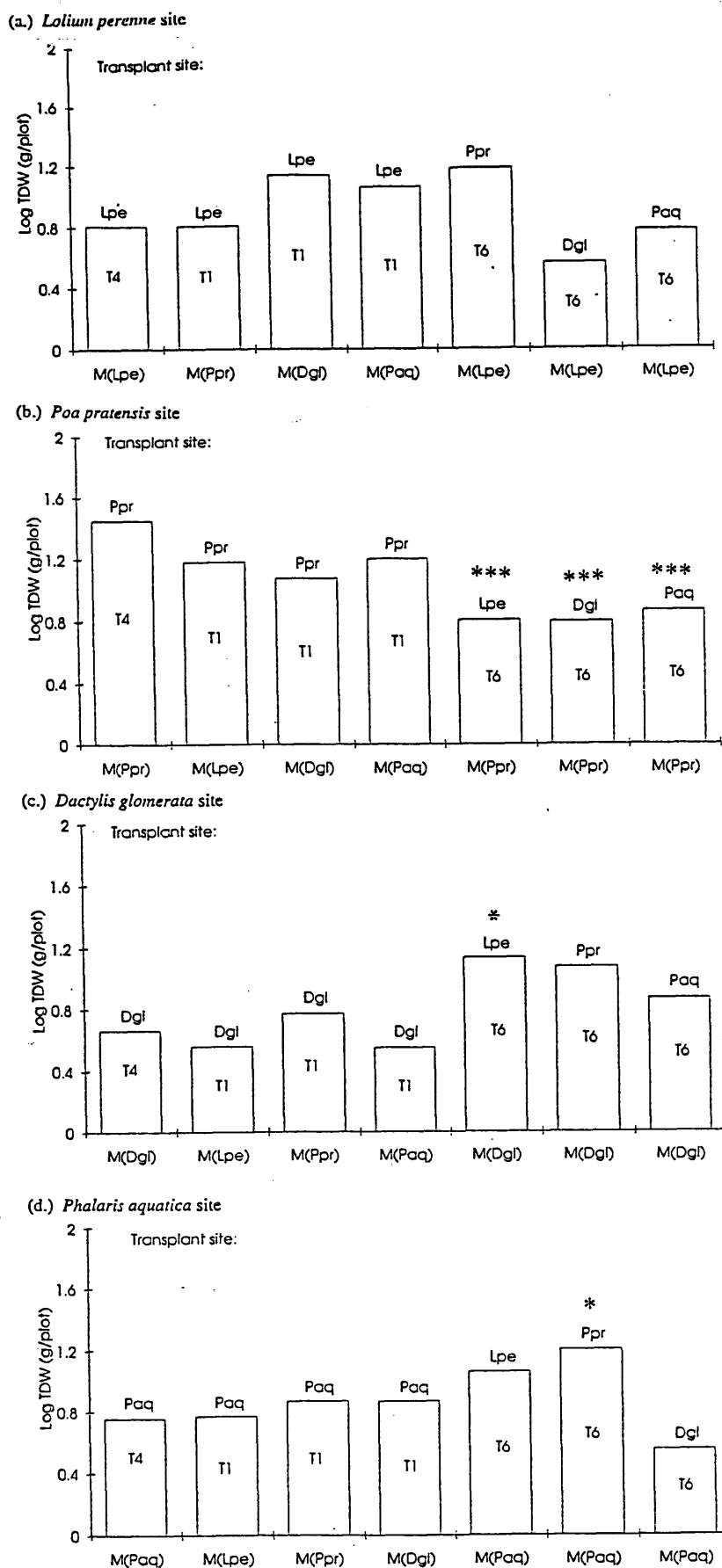
(a.) *Lolium perenne* site

(b.) *Poa pratensis* site

(c.) *Dactylis glomerata* site

(d.) *Phalaris aquatica* site

Fig. 6.2.1. Total plot dry weight (TDW) of *M. stipoides* populations collected from and transplanted into four different sites where only the native *M. stipoides* population was removed. (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments (T4 *v* T1 and T4 *v* T6) are necessary to demonstrate divergence of *M. stipoides* populations. Asterisks refer to the results of Bonferroni pairwise comparisons (***, 0.1%; **, 1%; *, 5% level of significance).
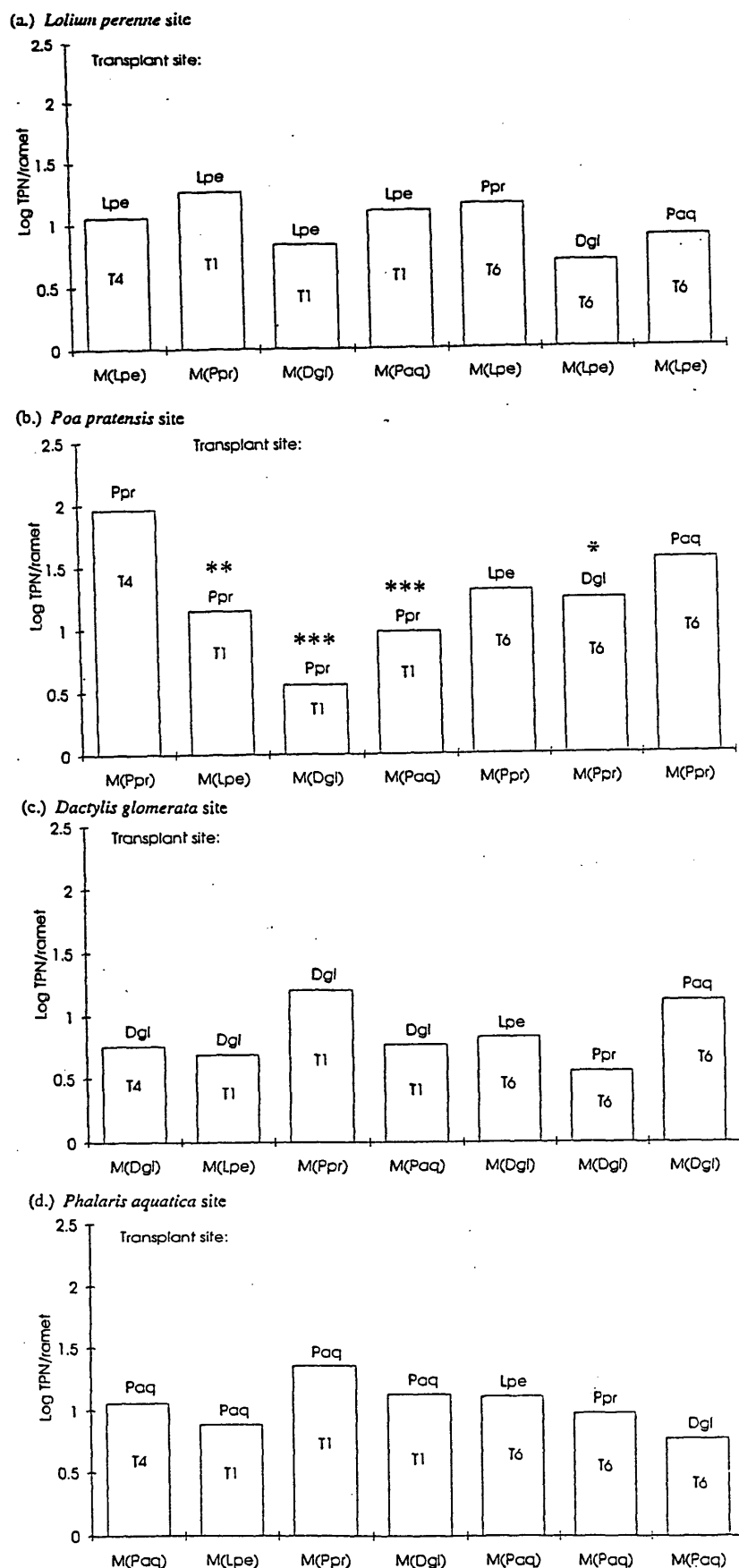
Fig. 6.2.2. Total panicle number (TPN) per ramet of *M. stipoides* populations collected from and transplanted into four different sites where only the native *M. stipoides* population was removed. (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments (T4 *v* T1 and T4 *v* T6) are necessary to demonstrate divergence of *M. stipoides* populations. Asterisks refer to the results of Bonferroni pairwise comparisons (***, 0.1%; **, 1%; *, 5% level of significance).

of the grass competitor (T1) compared with when the grass competitor was absent (T2), then current competition with the associated grass was the likely mechanism for divergence of the population.

Results of pairwise comparisons (T1 v T2) for the total dry weight and panicle number of allopatric populations transplanted into a sympatric site showed no significant differences in their yields (Figures 6.2.3 and 6.2.4). All three allopatric populations gave higher, although not statistically significant, total dry weights upon removal of the competitor species when transplanted into *L. perenne* and *D. glomerata* sites (Figure 6.2.3). When transplanted into *D. glomerata* and *P. aquatica* sites, all three populations produced a larger number of panicles upon removal of competitor species, although the differences were not statistically significant (Fig. 6.2.4).

Divergence due to past competition could also be determined by comparing the performance of a sympatric population (T5) with the allopatric populations (T2) when transplanted into sympatric sites and both the native *M. stipoides* and grass competitors were removed in the sympatric site. If the performance of allopatric populations were greatly reduced compared with performance of the sympatric population, then divergence due to past competition may have occurred.

Among the four *M. stipoides* populations, M (Ppr) showed significant divergence due to past competition with *P. pratensis*. Total dry weight and panicle number of sympatric M (Ppr) (T5) was significantly higher compared with the total dry weights and panicle numbers of allopatric populations (T2) of M (Dgl) and M (Paq) when transplanted in the sympatric *P. pratensis* site (Figs. 6.2.3b and 6.2.4b). The significant difference between the total dry weight and panicle number of sympatric M (Dgl) and allopatric M (Ppr) transplanted into the sympatric *D. glomerata* site did not indicate divergence due to past competition (Fig. 6.2.3c). This is because sympatric M (Dgl) had significantly less total dry weight and panicles produced compared with allopatric M (Ppr) when transplanted in the sympatric *D. glomerata* site (Fig.6.2.3c). The significant difference in total number of panicles produced by sympatric M (Paq) and allopatric M (Ppr) when transplanted into the sympatric *P. aquatica* site did not mean divergence due to past competition either since the total number of panicles produced by the allopatric M (Ppr) was significantly higher than the panicles produced by the sympatric M (Dgl) (Figure 6.2.4d).
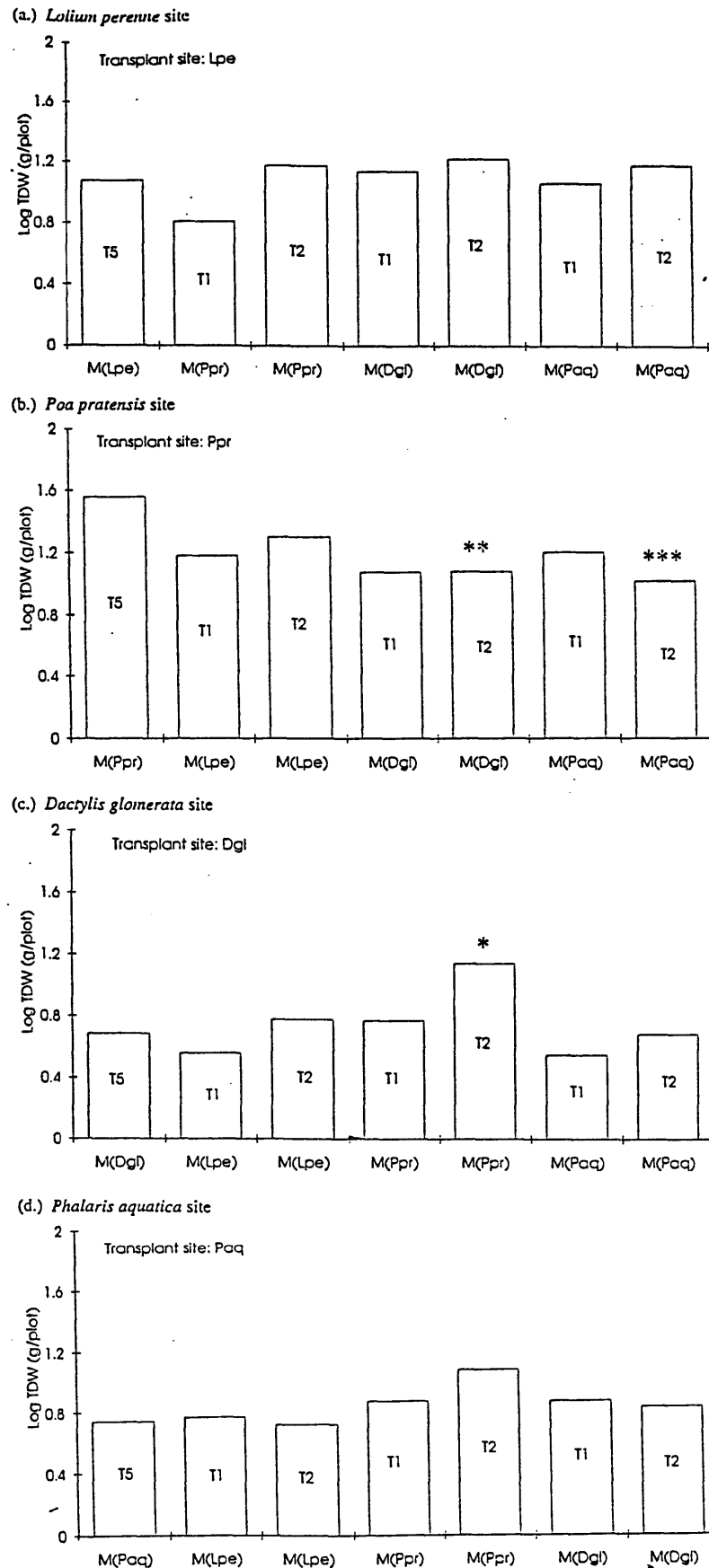
Fig. 6.2.3. Total plot dry weight (TDW) of *M. stipoides* populations collected from and transplanted into four different sites where the native *M. stipoides* population and associated grass species were removed. (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments (T2 *v* T1 and T5 *v* T2) are necessary to demonstrate competition with the associated grass species as the driving force in the divergence of *M. stipoides* populations. Results of all pairwise T2 *v* T1 comparisons were non-significant. Asterisks refer to the results of T5 *v* T2 pairwise comparisons (***, 0.1%; **, 1%; *, 5% level of significance).
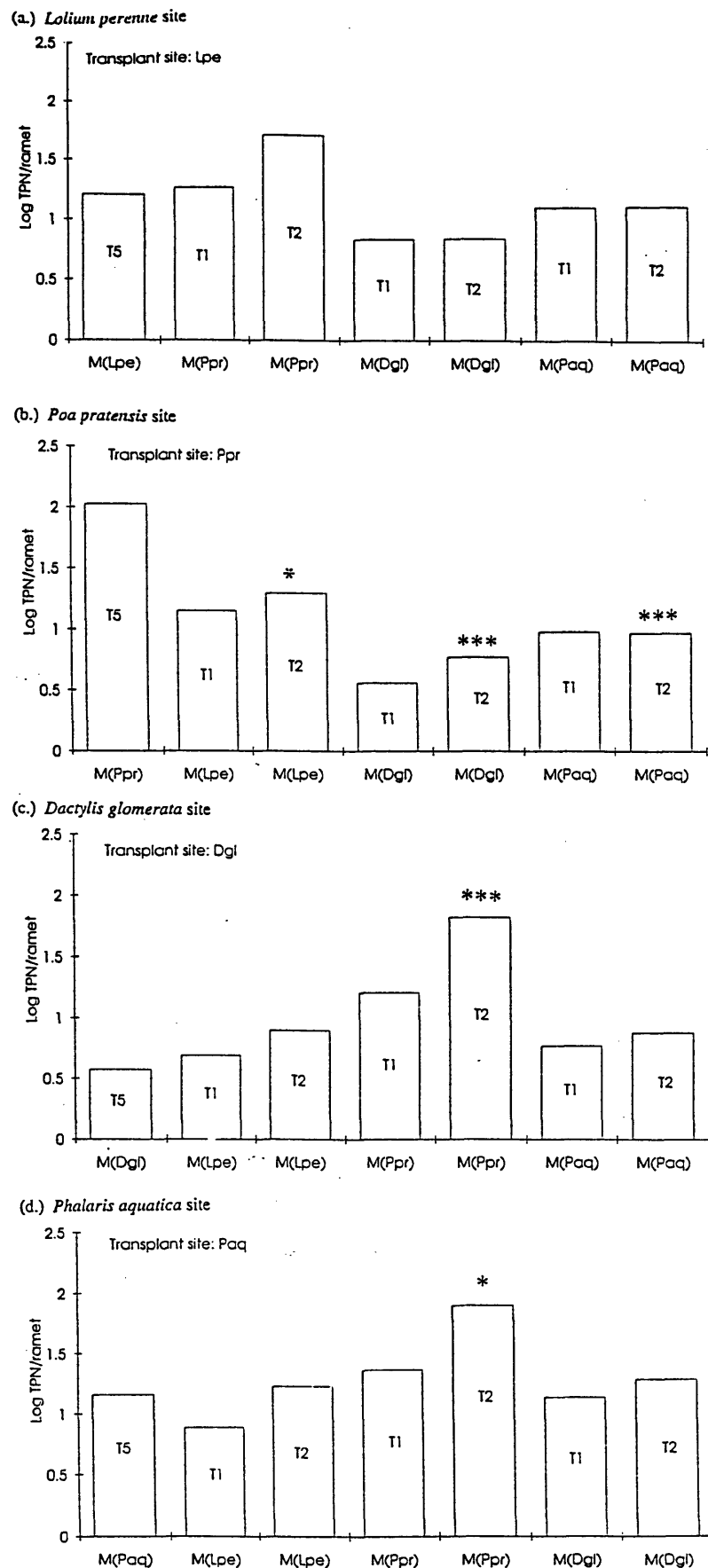
(a.) *Lolium perenne* site

Transplant site: Lpe

Log TPN/ramet

T5 T1 T2 T1 T2 T1 T2

M(Lpe) M(Ppr) M(Ppr) M(Dgl) M(Dgl) M(Paq) M(Paq)

(b.) *Poa pratensis* site

Transplant site: Ppr

Log TPN/ramet

T5 T1 * T2 T1 *** T2 T1 *** T2

M(Ppr) M(Lpe) M(Lpe) M(Dgl) M(Dgl) M(Paq) M(Paq)

(c.) *Dactylis glomerata* site

Transplant site: Dgl

Log TPN/ramet

T5 T1 T2 T1 *** T2 T1 T2

M(Dgl) M(Lpe) M(Lpe) M(Ppr) M(Ppr) M(Paq) M(Paq)

(d.) *Phalaris aquatica* site

Transplant site: Paq

Log TPN/ramet

T5 T1 T2 T1 * T2 T1 T2

M(Paq) M(Lpe) M(Lpe) M(Ppr) M(Ppr) M(Dgl) M(Dgl)

Fig. 6.2.4. Total panicle number (TPN) per ramet of *M. stipoides* populations collected from and transplanted into four different sites where the native *M. stipoides* population and associated grass species were removed. Pairwise comparison of treatments (T2 *v* T1 and T5 *v* T2) are necessary to demonstrate competition with the associated grass species as the driving force in the divergence of *M. stipoides* populations. Results of all pairwise T2 *v* T1 comparisons were non-significant. Asterisks refer to the results of T5 *v* T2 pairwise comparisons (***, 0.1%; **, 1%; *, 5% level of significance).

6.2.3.6 Genetic basis of population divergence

The genetic basis of the observed divergence among the four populations may be assessed by comparing the performance of sympatric populations transplanted into sympatric sites in the presence (T4) or absence (T5) of a grass competitor. If the performance of the sympatric population did not increase significantly when the competitor was removed, then the observed differences in the sympatric and allopatric populations have a genetic basis. Results of all pairwise comparisons of total dry weights and total panicle numbers produced by all *M. stipoides* populations transplanted in sympatric sites with (T4) or without (T5) the grass competitor yielded non-significant differences (Fig. 6.2.5).

Genetic basis could also be assessed by comparing the performance of sympatric populations (T6) with the performance of allopatric populations (T3) transplanted into allopatric sites. If the performance of the sympatric population (T6) transplanted into an allopatric site did not change or if it increased, the performance should not be greater than the performance of a natural allopatric population in the allopatric site (T3), then divergence of populations has a genetic basis. Results of pairwise comparisons showed no significant differences in the total dry weights of all sympatric *M. stipoides* populations compared with allopatric populations transplanted into allopatric sites (Fig. 6.2.5). In terms of panicle production, it was only the allopatric M (Ppr) which produced a significantly higher total number of panicles compared with the sympatric populations transplanted into the three allopatric sites (Fig. 6.2.6). There were no significant differences in the panicle production of the other three allopatric populations when transplanted into allopatric sites. The results seem to indicate that, except for the panicle production of M (Ppr) in allopatric sites, in terms of dry mass production of all populations and panicle production of M (Lpe), M (Dgl) and M (Paq), the observed divergence of the populations had a genetic basis.

6.2.3.7 Influence of local site conditions

Removal of *M. stipoides*, associated grass species and other minor species within the plots in Treatments 2 and 5 left the plots bare of plant cover. Comparison of the performance of a sympatric *M. stipoides* population transplanted in sympatric and all other allopatric sites would yield an assessment of the effect of local soil conditions. Total dry weight of sympatric M (Lpe) was significantly higher when transplanted into its sympatric site compared with its total dry weight when transplanted into its allopatric *P. aquatica* site (Table 6.2.5). *Microlaena stipoides* (Lpe), however, produced the largest total dry weight and panicle number when transplanted into the *P. pratensis* site, although not statistically
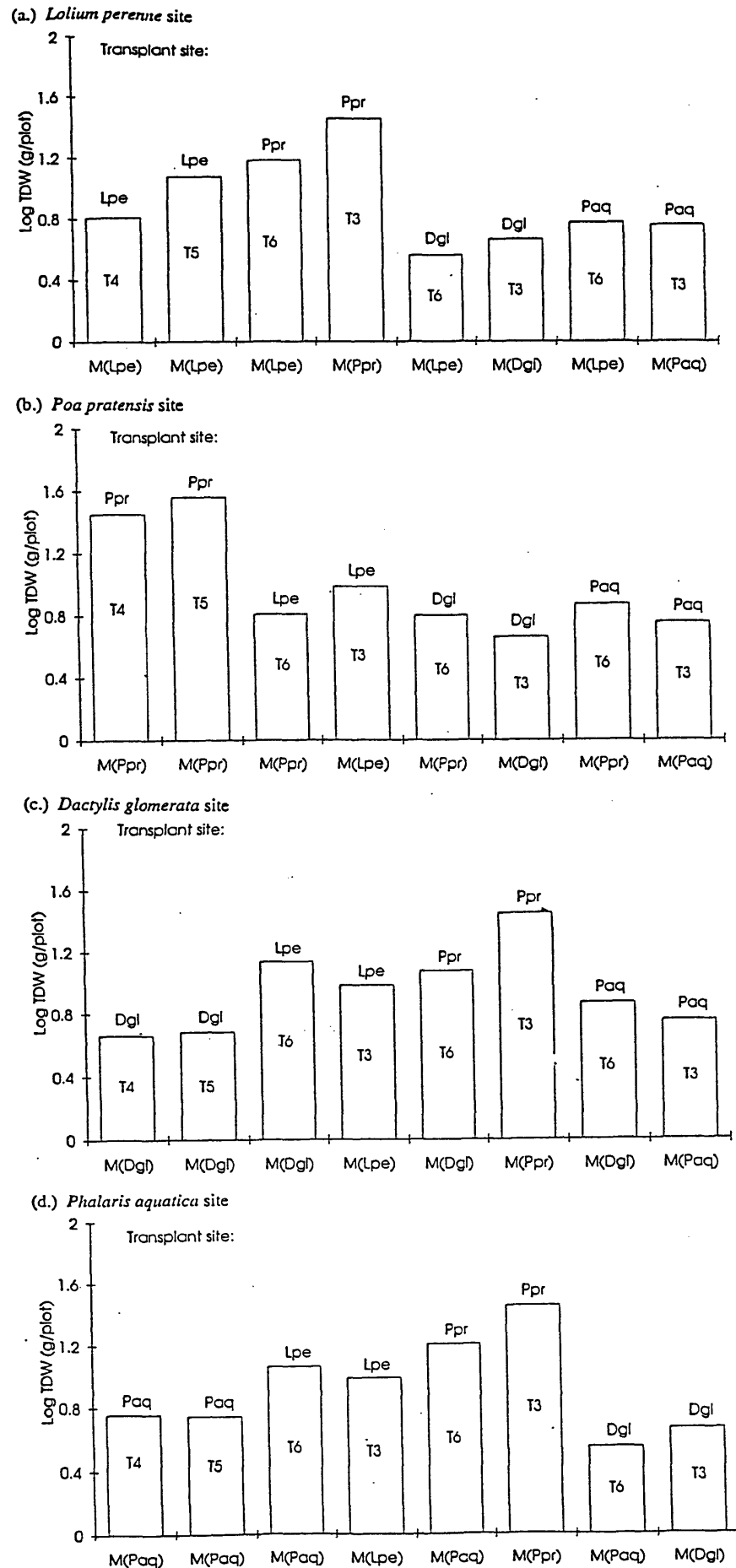
(a.) *Lolium perenne* site

Transplant site:

Log TDW (g/plot)

Lpe — T4 — M(Lpe)
Lpe — T5 — M(Lpe)
Ppr — T6 — M(Lpe)
Ppr — T3 — M(Ppr)
Dgl — T6 — M(Lpe)
Dgl — T3 — M(Dgl)
Paq — T6 — M(Lpe)
Paq — T3 — M(Paq)

(b.) *Poa pratensis* site

Transplant site:

Log TDW (g/plot)

Ppr — T4 — M(Ppr)
Ppr — T5 — M(Ppr)
Lpe — T6 — M(Ppr)
Lpe — T3 — M(Lpe)
Dgl — T6 — M(Ppr)
Dgl — T3 — M(Dgl)
Paq — T6 — M(Ppr)
Paq — T3 — M(Paq)

(c.) *Dactylis glomerata* site

Transplant site:

Log TDW (g/plot)

Dgl — T4 — M(Dgl)
Dgl — T5 — M(Dgl)
Lpe — T6 — M(Dgl)
Lpe — T3 — M(Lpe)
Ppr — T6 — M(Dgl)
Ppr — T3 — M(Ppr)
Paq — T6 — M(Dgl)
Paq — T3 — M(Paq)

(d.) *Phalaris aquatica* site

Transplant site:

Log TDW (g/plot)

Paq — T4 — M(Paq)
Paq — T5 — M(Paq)
Lpe — T6 — M(Paq)
Lpe — T3 — M(Lpe)
Ppr — T6 — M(Paq)
Ppr — T3 — M(Ppr)
Dgl — T6 — M(Paq)
Dgl — T3 — M(Dgl)

Fig. 6.2.5. Total plot dry weight (TDW) of *M. stipoides* populations collected from and transplanted into four different sites. (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments (T4 v T5 and T6 v T3) are necessary to demonstrate a genetic basis of *M. stipoides* population divergence. Asterisks refer to the results of Bonferroni pairwise comparisons (***, 0.1%; **, 1%; *, 5% level of significance).

(a.) *Lolium perenne* site

(b.) *Poa pratensis* site

(c.) *Dactylis glomerata* site

(d.) *Phalaris aquatica* site
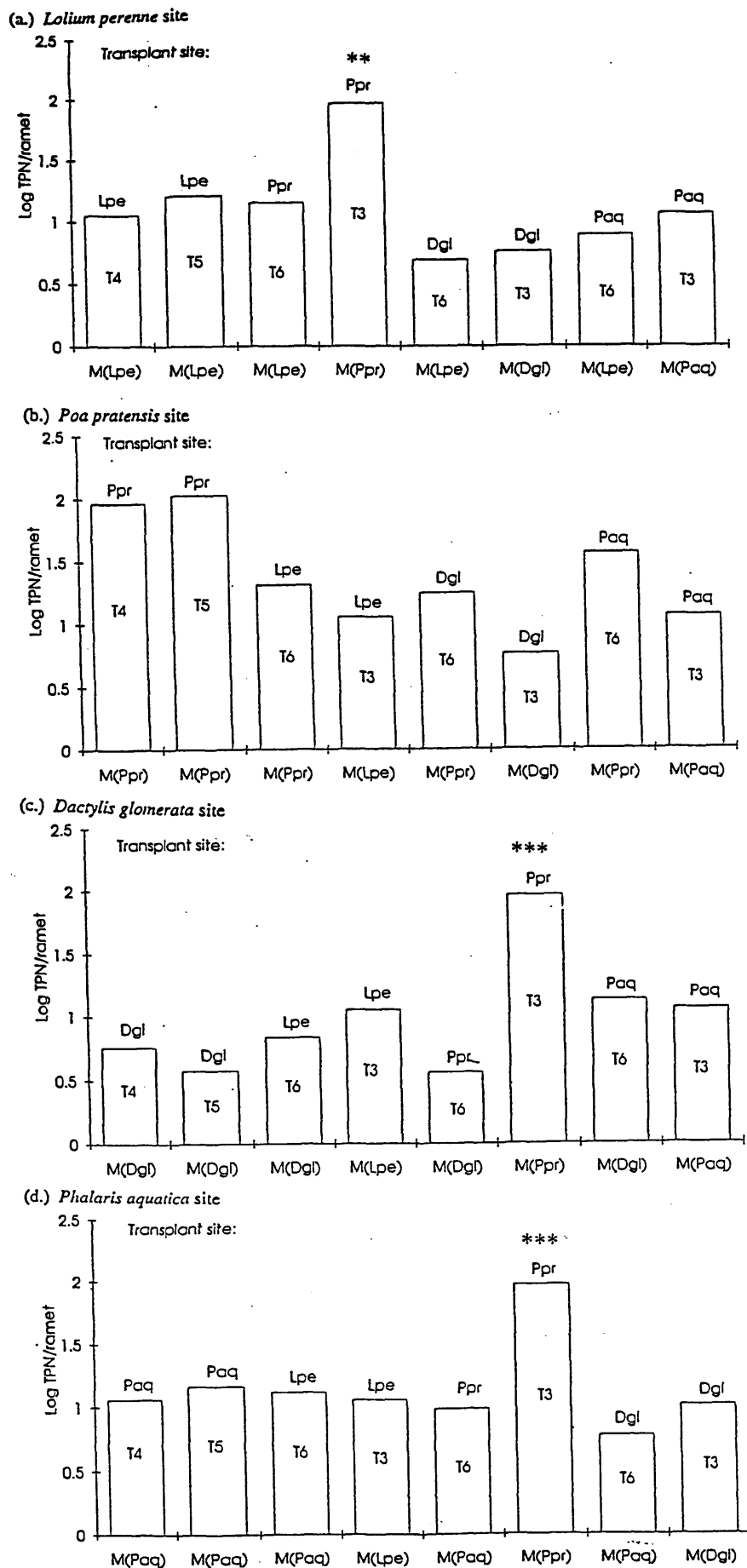
Fig. 6.2.6. Total panicle number (TPN) per ramet of *M. stipoides* populations collected from and transplanted into four different sites. (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments (T4 *v* T5 and T6 *v* T3) are necessary to demonstrate a genetic basis of *M. stipoides* population divergence. Asterisks refer to the results of Bonferroni pairwise comparisons (***, 0.1%; **, 1%; *, 5% level of significance).

Table 6.2.5. The effect of local soil conditions on the mean log total dry weights, log total panicle numbers and number of survivors of the four *M. stipoides* populations transplanted in sympatric and allopatric sites where both native *M. stipoides* and competitor species were removed. Significant results from pairwise t-tests comparing the performance of *M. stipoides* populations transplanted in sympatric and allopatric sites where both native *M. stipoides* and competitor species were removed to determine the effect of local soil conditions are denoted by asterisks.

	Log total dry weight	Log total panicle number	Number of survivors/plot
Sympatric site/popn (Allopatric site)			
L. perenne	1.07	1.21	2
(P. pratensis)	1.30	1.30	2
(D. glomerata)	0.78	0.90	1.6
(P. aquatica)	0.72 *	1.22	2
P. pratensis	1.56	2.03	2
(L. perenne)	1.17 ***	1.70 *	2
(D. glomerata)	1.14 ***	1.82	2
(P. aquatica)	1.08 ***	1.89	2
D. glomerata	0.68	0.69	1.6
(L. perenne)	1.22 ***	0.84	2
(P. pratensis)	1.08 ***	0.77	2
(P. aquatica)	0.82	1.08	2
P. aquatica	0.74	1.16	2
(L. perenne)	1.18 **	1.11	2
(P. pratensis)	1.02	0.97	2
(D. glomerata)	0.68	0.88	2

Notation for Bonferroni significance levels:
A single comparison must have a P value less than 0.008333 to be significant at the 0.05 level when comparing 6 pairs of means.
***   0.1% significance
**   1% significance
*   5% significance

significant compared with its yield when transplanted into its sympatric *L. perenne* site. Total dry weight of M (Ppr) transplanted into its sympatric site was significantly higher compared with total dry weights of M (Ppr) transplanted into all other allopatric sites (Table

6.2.5). Total dry weights of M (Dgl) transplanted into allopatric *L. perenne* and *P. pratensis* sites were significantly higher compared with its total dry weight when transplanted into its sympatric site (Tables 6.2.5). When M (Paq) was transplanted into its allopatric *L. perenne* site, its total dry weight was significantly greater compared with its total dry weight when transplanted in its sympatric site (Table 6.2.5). With regards to total panicle number, only M (Ppr) transplanted into its sympatric site had significantly higher total panicle number compared with its panicle production when transplanted into its allopatric *L. perenne* site (Tables 6.2.5). There were no significant differences in the survivorship of the four *M. stipoides* populations transplanted in both sympatric and allopatric sites (Table 6.2.5).

6.2.4 Discussion

Coexistence of species in the same habitat is a function of several factors (Harper *et al.* 1961): (a) ancestral populations had been subjected to different opportunities for divergence into several subpopulations; (b) migration had occurred after divergence, (c) populations have acquired different reproductive strategies before meeting, (d) tolerance to hazardous components of the environment has developed after divergence, and (e) evolutionary divergence has resulted in species having different controlling factors affecting site requirements for seedling establishment, susceptibility to parasites and predators, germination time or requirements for breaking dormancy.

This study addressed the first factor and was conducted to obtain evidence for the divergence of the original *M. stipoides* population into subpopulations resulting from competition with associated grass species in a permanent pasture. It is hypothesised that prior to the introduction of the exotic perennial grass species, *L. perenne* and *D. glomerata* in 1961, there was one original population of *M. stipoides* at 'Karuah'. It is assumed that the present subpopulations of *M. stipoides* coexisting with *P. pratensis*, *L. perenne* and *D. glomerata* at 'Karuah' have a common ancestor. *Poa pratensis* was not sown in the paddock but is assumed to have invaded the pasture some time after European settlement so it has been present for a longer time than *L. perenne* and *D. glomerata*. *Microlaena stipoides* coexisting with *P. aquatica* was included in the study because *P. aquatica* was another exotic species widely introduced on the Northern Tablelands and *M. stipoides* can grow well in association with *P. aquatica* in permanent pastures.

The general performance of M (Ppr) in terms of biomass and panicle production was significantly greater than the other three *M. stipoides* populations, in the absence of grass competitor species. In the presence of grass competitor species, M (Ppr) had relatively higher dry weight ($P > 0.05$) and produced significantly higher panicle numbers than the

other three populations. Results of the pot experiment on the effect of light intensity (Chp. 5.3) have shown that, averaged over all light intensity levels, M (Ppr) had significantly larger dry mass, number of leaves and panicle number and longer leaves than the other three *M. stipoides* populations. Fingerprints of the amplified DNA segments of the four *M. stipoides* populations showed greater variation in the banding pattern of M (Ppr) (Chp. 5.5), clearly indicating divergence of M (Ppr) from the other three populations.

Population divergence can be determined by comparing the performance of populations before and after contact if we assume that before and after contact are represented by allopatric and sympatric populations (Connell 1980). Divergence of *M. stipoides* populations was assessed by comparing the performance of sympatric (T4) and allopatric (T1) *M. stipoides* populations transplanted into sympatric sites in the presence of competitor species. Divergence was also assessed by comparing the performance of sympatric populations transplanted into sympatric (T4) and allopatric sites (T6) in the presence of different competitor species at each site.

Performance of the different *M. stipoides* populations was measured in terms of vegetative (or biomass) and reproductive (or panicle) production. Among the four *M. stipoides* populations, it was M (Ppr) that exhibited significant divergence against all three allopatric populations transplanted into the sympatric *P. pratensis* site in the presence of competitor species in terms of the total number of panicles exserted. It was also M (Ppr) that exhibited significant divergence in terms of dry mass production when transplanted into its sympatric site compared with when transplanted into all three allopatric sites in the presence of competitor grass species.

To determine if competition with the associated grass species was the driving force in the observed divergence among the *M. stipoides* populations, Connell (1980) and Turkington (1989a) proposed two criteria. The first one is significant reduction in the performance of allopatric populations transplanted into a sympatric site in the presence of competitor species (T1) compared with its performance in the absence of competitor species (T2). The second criterion is that removal of competitor species did not cause a significant increase in the performance of a sympatric population (T5) compared with its performance in the presence of a competitor species (T4) when transplanted into a sympatric site. This means that the sympatric population had diverged with the competitor species such that the presence of a competitor species did not reduce the performance of the sympatric population.

The results of this study indicated that competition may be an important factor in the observed pattern of population divergence in the *P. pratensis* site. Among the four *M. stipoides* populations, M (Ppr) showed large increases in performance, though not

statistically significant, both in terms of dry matter production and panicle production with the removal of competitor species when transplanted in all three allopatric sites. Removal of the competitor species (T5) did not cause a significant increase in performance of M (Ppr) compared with its performance in the presence of a competitor species when transplanted in its original site, the sympatric site. There was no significant evidence to show that population divergence due to competition occurred at the *L. perenne*, *D. glomerata* and *P. aquatica* sites.

Among the four *M. stipoides* populations studied, it was only M (Ppr) that showed divergence resulting from inter-specific competition with the associated grass species. The divergence exhibited by M (Ppr) had a genetic basis since there was no significant difference in the total dry weights and panicles exserted by M (Ppr) transplanted into its sympatric site in the presence (T4) or absence (T5) of the competitor species, *P. pratensis*, and in the dry weights of M (Ppr) (T6) and allopatric populations (T3) transplanted into the allopatric site. It was only in terms of panicle production of sympatric M (Ppr) (T6) compared with panicle production of allopatric populations (T3) transplanted into an allopatric site that did not satisfy the criteria for genetic divergence.

Turkington (1989a) found strong evidence of divergence among the populations of *T. repens* growing in association with different perennial grasses in a 100-year old permanent pasture. The species in this pasture had been co-occurring for 100 years and had more opportunity for genetic-based microevolutionary changes (Turkington 1989a). Results from the present study showed that population divergence was evident only between M (Ppr) and *P. pratensis*. This could be because both *M. stipoides* and *P. pratensis* could have co-occurred for a long time before the introduction of the exotic species, *L. perenne* and *D. glomerata* in 1961 and *P. aquatica* in 1960.

Evans and Turkington (1988) and Turkington (1989a) found that a mosaic of patches of several species of perennial grasses that dominate the pastures provides a major environmental factor that leads to diversification among *T. repens* genotypes on the basis of neighbour-specific compatibilities. This may be true with the *P. pratensis* patch where the close association between *P. pratensis* and M (Ppr) provided selection pressures on *M. stipoides* individuals for persistence, growth, reproduction (Evans and Turkington 1988) and mortality (Connell 1980). It is possible that coexistence between M (Ppr) and *P. pratensis* may have resulted from natural selection under pressure of competition resulting in species divergence such that each species occupies a different niche (MacArthur 1972) or a balancing of competitive abilities (Aarssen 1983). Divergence was evident in M (Ppr) but not in other *M. stipoides* populations, probably because thirty years of coexistence between *M. stipoides* and the introduced species is not long enough for competition pressures to

result in significant population divergence. *Poa pratensis* is an invader in the pasture and possibly had been coexisting with *M. stipoides* in the area for more than thirty years. It would be interesting to find out species associations in the same pasture in seventy years time.

Local site conditions also had significant effects on the biomass production of the different *M. stipoides* populations. All four *M. stipoides* populations had better performance when transplanted into *P. pratensis* and *L. perenne* sites. *M. stipoides* (Ppr) showed a home site advantage, yielding significantly higher dry matter and panicle number than the other three allopatric populations transplanted into the sympatric *P. pratensis* site. The results indicate that it is possible that local-scale variation in edaphic factors may also influence the micro-distribution of *M. stipoides* in this paddock. Snaydon (1962) reported that small-scale variations in edaphic factors influenced the micro-distribution of *Trifolium repens*. The better performance of all populations at the *P. pratensis* site could be related to the location and soil properties of the site in the paddock. The *P. pratensis* site is situated at the lower slope of the paddock and has a loamy soil texture which retains more moisture than the other sites. The *D. glomerata* and *P. aquatica* sites were stony and situated at the upper slope of the paddock, while the *L. perenne* site was situated in a flat area and characterised by clayey soil. It is highly possible that soil pH and soil fertility levels could vary among the sites and may influence the micro-distribution of *M. stipoides*. Magcale-Macandog and Whalley (1994) have found that *M. stipoides* abundance and distribution on the Northern Tablelands is correlated with soil pH.

6.3 COMPETITIVE ABILITIES OF FOUR *Microlaena stipoides* POPULATIONS AND THEIR NATURAL NEIGHBOURING PERENNIAL GRASSES IN THE GLASSHOUSE

6.3.1 Introduction

Aarssen (1983) presented two evolutionary mechanisms that permit the coexistence of species which interact competitively. The first mechanism stipulates that coexistence is a consequence of selection which results in avoidance of competitive interactions through niche differentiation. It may also refer to the ability of a species pair to produce a higher mixture yield than their yields in monoculture. This is termed ecological combining ability in plant interactions (Aarssen 1983). The second mechanism, termed competitive combining ability, stipulates that coexistence is a consequence of selection wherein species coexist because there are numerous possible permutations and combinations of biological attributes in plants leading to balanced competitive abilities. These attribute complexes are under constant fine-tuning by coevolution that alters the way members respond to one another. A species can maintain the potential to generate and propagate new genetic variants with increased competitive power under the prevailing competitive pressures.

Competitive ability is a pooled measure of the capacity of an individual plant to decrease the availability of contested resources to another plant and the capacity to sustain a decrease in the contested resource availability produced by another plant (Aarssen 1983). Competitive ability may involve both resource removal and hindrance of access to resources as both mechanisms affect resource availability (Aarssen 1983). Aarssen (1989) listed the primary attributes of competitive abilities, including greater depletion rate of water, nutrients and light; greater ability to attract pollinators and dispersal agents; greater interference with recruitment of neighbours and with pollination of neighbours; release of more toxic allelopathic chemicals to the soil; higher tolerance of depletion of water, minerals and light by neighbours; resistance to allelopathic chemicals released by neighbours and greater ability to maximise fecundity using resources denied to neighbours.

Variability in competitive performance among different populations of many plant species is so high that it is unrealistic to deduce 'species-wide' performance from one or two representative populations (Merhoff and Turkington 1990). Merhoff and Turkington (1990) collected clones of three sets of species pairs; *Holcus lanatus* and *Lolium perenne*, *Dactylis glomerata* and *L. perenne*, and *Trifolium repens* and *L. perenne* from a series of differently aged pasture populations. After multiplying the clones under glasshouse conditions, they

grew the clones from each population in monoculture at two levels (full density and half density) and in mixtures with their naturally occurring competitor species under different conditions; garden, three year old pasture and glasshouse. They found large variations in growth performance of the different populations of each species, making it difficult to make generalisations about species performance.

McNeilly (1981, 1984) collected *Poa annua* plants from three 'closed' habitats (pastures, old lawns) and three 'open' habitats (car park, shrubbery, churchyard foot path), grew them in the glasshouse and, as *P. annua* is an inbreeding species, collected seeds to grow plants for competition and cutting experiments with *L. perenne*. Populations of *P. annua* from 'closed' habitats gave higher yields under cutting and competitive situations than those from 'open' habitats. When left uncut, *P. annua* populations collected from 'open' habitats out-yielded *L. perenne*. Based upon these findings he concluded that genetically-based inter-population differences in the competitive ability of *P. annua* had arisen in response to competition, grazing and mowing.

Aarssen (1989) collected seeds of *Senecio vulgaris* from different geographical localities and conducted competition experiments in growth chambers under controlled conditions involving monoculture at two densities and mixtures with *Phleum pratense*. He collected seeds for three generations from the experiment and found that the third generation *S. vulgaris* seeds germinated more rapidly than seeds from earlier generations in all three treatments. Intense selective forces, however, were observed in *S. vulgaris* growing in competition with *P. pratense*. He concluded that competition in the mixed culture provided a strong selective force for improved competitive combining ability. Selection for competitive combining ability is an evolutionary mechanism of coexistence as it implicates genetic changes resulting from selection from competition and depends upon genotypic variation within species.

Seeds of previously chosen populations of *Erodium cicutarium* and *E. obtusiplicatum* were collected from isolated and sympatric sites in the field, germinated in the glasshouse, grown and fertilised, and a replacement series competition experiment (de Wit 1960) was conducted (Martin and Harding 1981). Seed yields of sympatric cultures were higher than those of allopatric cultures and two populations of *E. obtusiplicatum* displayed specific responses to the genetic composition of the population of *E. cicutarium* competitors which showed that evolution in response to competition occurs at the population level (Martin and Harding 1981).

Evans *et al.* (1985, 1989) collected five pairs of *T. repens* and *L. perenne* populations growing together in the field from different countries in Europe and grew the population

pairs in the field. They also grew the five populations of *T. repens* in mixtures with two other commercial populations of *L. perenne*. The yield of each *T. repens* population was highest when grown with its natural neighbouring *L. perenne* population. The yield of coexisting *T. repens* - *L. perenne* mixtures was greater than other mixtures, indicating increased ecological combining ability. It was also observed that as the yield of the associated *L. perenne* increased, the yield of *T. repens* decreased and vice versa. Component species of the coexisting mixtures had undergone selective pressures to reduce aggression towards each other either through arrangement of spatial parts or complementary growth patterns (Rhodes 1981).

The component yield quotient (CYQ) is a ratio of the yield of component species in a species combination and is a measure of competitive relationships among component species. It gives an indication of the probability of coexistence of two species or the capacity to avoid competitive exclusion (Aarssen and Turkington 1985a). CYQ values close to 1.0 indicate more similar competitive abilities of component species, while CYQ values close to zero indicate dissimilar competitive abilities. An increase in CYQ values may result from either an increase in performance of the inferior competitor with or without a decrease in performance of the superior competitor. Decreases in CYQ values result from increases in performance of the superior competitor but not the inferior competitor. Aarssen and Turkington (1985a) reported four possible trends in the relationship between total yield and CYQ: (1) increase in total yield without any decrease in CYQ indicating niche differentiation, (2) decrease in CYQ indicating dissimilar competitive abilities reflecting a process of competitive exclusion, (3) increase in CYQ without any increase in total yield indicating more similar competitive abilities, and (4) no significant change in total yield but CYQ changes significantly, in which case a change in yield for one component is accompanied by an opposite change in yield for the other component.

Aarssen and Turkington (1985a) collected four different genet pairs of *L. perenne* and *T. repens* growing together as immediate neighbours in the field (natural neighbours) from four widely separated locations in a 40-year old pasture. After propagation of each genet type in a glasshouse, they conducted a competition experiment consisting of 16 possible paired-genotype combinations. *Trifolium repens* populations gave their highest yields when grown with their natural neighbours. Genet pairs of *L. perenne* and *T. repens* which were sampled as natural neighbours in the field had a higher component yield quotient (CYQ) than those pairs in which the two components came from different neighbourhoods. These results indicated that natural selection, through competition resulted in more balanced competitive abilities for contested resources of natural neighbouring species. Members of natural pairs contributed more equitably to the total yield instead of niche differentiation,

which may have been a result of continuous selection of genetically-based differences in competitive abilities with local neighbours (Aarssen 1985, 1989).

Turkington and Harper (1979c) compared the growth of ramets of *T. repens* from four different sites dominated by four different grass species: *Agrostis tenuis*, *Cynosurus cristatus*, *Holcus lanatus* and *L. perenne*. The ramets were mutiplied in the glasshouse and were transplanted back into the swards in the field in all possible *T. repens* 'type' and site of origin combinations. A concurrent experiment was done in the glasshouse where seeds of *A. tenuis*, *C. cristatus*, *H. lanatus* and *L. perenne* were sown separately in boxes filled with soil to form grass swards. Phytometers of *T. repens* were sown in the grass swards in all possible combinations of *T. repens* 'type' and grass species. They found that *T. repens* ramets had higher biomass production when transplanted back into their site of origin and when grown in a mixture with the grass species that dominated the area from where the *T. repens* 'type' was collected. They interpreted these results as the results of microevolution of *T. repens* in response to selection pressures exerted by neighbouring grass species (Turkington and Harper 1979c, Turkington 1989a). It is possible that similar divergence in populations of *M. stipoides* in response to interspecific competition with neighbouring introduced perennial grasses in a permanent pasture has occurred.

The study was designed to determine the growth performance and competitive abilities of four *M. stipoides* populations when grown in association with their natural and non-natural neighbouring perennial grass species under glasshouse conditions.

6.3.2 Materials and methods

6.3.2.1 Plant propagation

Ramets of the four *M. stipoides* populations and of the neighbouring perennial grasses *Lolium perenne*, *Poa pratensis*, *Dactylis glomerata* and *Phalaris aquatica* were propagated in the glasshouse as described earlier (Chp. 5.2).

6.3.2.2 Competition experiment

The four *M. stipoides* populations and the four associated grass species were paired in all possible ways resulting in sixteen combinations in March 1990. Four replicates were conducted for each pair, totalling 64 pots. Four equidistant holes were dug in the 12.5 cm diameter plastic pots containing a sand:soil:peatmoss (1:1:1) mixture and two ramets of an

M. stipoides population were planted opposite each other while the other two ramets of the associated grass species were planted in the adjacent holes to the *M. stipoides* ramets opposite to each other. The pots were arranged in a completely randomised block design. The plants were maintained inside the glasshouse and watered regularly. Forty ml of 0.1% Aquasol® (Hortico (Aust.) Pty. Ltd., Inverton North, Victoria) complete fertiliser (23%N, 4%P, 18%K, 0.05%Zn, 0.06%Cu, 0.0013%Mo, 0.04%S, 0.15%Mn, 0.06%Fe, 0.011%B, 0.165%Mg) was applied to each pot every two weeks during the first 20 weeks and from then on 1.3 g of Osmocote® (Sierra Chemical Co., Castle Hill, NSW), a slow release complete fertiliser (14%N, 6.1%P, 11.6%K, 3%S, 2.2%Ca) was applied every 12 weeks. Malathion® (Chemspray Pty. Ltd., Smithsville, NSW) and Zineb® (Chemspray Pty. Ltd., Marayong, NSW) were applied, as needed, according to the manufacturers' directions to control aphids and rust, respectively.

6.3.2.3 Harvest

Both *M. stipoides* and the associated grass were cut 5 cm above the ground every two weeks after transplanting until 24 weeks and from then on were harvested every four weeks. Oven dry weight (80° C for 48 h) of the harvested material was determined.

6.3.2.4 Data analysis

Accumulated dry biomass of two ramets of each component species was summed after 45 weeks of growth. Mean, standard deviation (SD), coefficient of variation (CV) and variance among means (AOV) were analysed using the STATVIEW statistical package. Significant differences among population means were determined using Scheffé's test at the 5% significance level. Component yield quotient (CYQ), a measure for determining competitive relationships among neighbouring species, was calculated for each *M. stipoides* population and associated grass species combination for every harvest using the following formula (Aarssen and Turkington 1985a, b):

$$CYQ = Y/Y' \qquad (6.3.1)$$

where Y and Y' are the observed dry weights of the lower and higher yielding components of a combination at each harvest.

6.3.3 Results

6.3.3.1 General performance

Microlaena stipoides (Ppr) had a significantly higher mean component yield than M (Dgl), while M (Lpe) and M (Paq) were intermediate (Table 6.3.1). *Microlaena stipoides*

Table 6.3.1. Accumulated component yields (g/pot) of four *M. stipoides* populations grown for 45 weeks in mixtures with four associated grass species. Means which share the same letter are not significantly different (P > 0.05).

Associated grass species	*M. stipoides* population			
	M(Lpe)	M(Ppr)	M(Dgl)	M(Paq)
L. perenne	3.21	5.53	2.23	2.81
P. pratensis	2.03	4.00	2.03	2.18
D. glomerata	2.47	2.62	2.10	1.98
P. aquatica	1.52	3.15	1.30	2.32
Mean	2.31[ab]	3.82[a]	1.92[b]	2.32[ab]
SD	0.72	1.27	0.42	0.35
CV	0.31	0.33	0.22	0.15

(Ppr) had a higher standard deviation than the other three populations, indicating large deviations from the mean yield when grown with different associated grass species. *Microlaena stipoides* (Lpe) and M (Ppr) had higher coefficients of variation than the other two populations, reflecting a high sensitivity to the nature of the associated grass species it is growing with, while M(Paq) had the least coefficient of variation reflecting its low sensitivity to its associates (Table 6.3.1).

Poa pratensis and *P. aquatica* had significantly higher mean component yields than *L. perenne*, while *D. glomerata* was intermediate (Table 6.3.2). Generally, the associated grass species had lower coefficients of variation compared with the *M. stipoides* populations (Tables 6.3.1 and 6.3.2). Among the associated grass species, *P. pratensis* and *D. glomerata* had a lower sensitivity, while *L. perenne* had the highest sensitivity to the associated *M. stipoides* populations.

Table 6.3.2. Accumulated component yields (g/pot) of four associated grass species grown for 45 weeks in mixtures with four *M. stipoides* populations. Means which share the same letter are not significantly different (P > 0.05).

Associated *M. stipoides* population	Grass Species			
	L. perenne	*P. pratensis*	*D. glomerata*	*P. aquatica*
M(Lpe)	4.77	6.94	6.17	7.31
M(Ppr)	3.09	6.6	5.83	5.86
M(Dgl)	4.98	6.88	5.72	6.64
M(Paq)	4.52	5.74	5.02	5.35
Mean	4.34^b	6.54^a	5.68^{ab}	6.29^a
SD	0.85	0.55	0.48	0.86
CV	0.20	0.08	0.08	0.14

6.3.3.2 Component yield

The component yield of M(Lpe) grown with *L. perenne* was relatively higher than when it was grown with the other three non-natural neighbouring grass species, though non significant (P > 0.05) (Fig. 6.3.1a, bar graph). The component yields of the three non-natural neighbouring grass species of M(Lpe), i.e. *P. pratensis, D. glomerata* and *P. aquatica*, were higher than the component yield of *L. perenne* when grown with M(Lpe), though non significant (P > 0.05) (Fig. 6.3.1a). This resulted in a relatively higher total combined yield of M (Lpe) and each of the three non-natural neighbouring associated grass species (Fig. 6.3.1a, line graph) than the combined yield with *L. perenne*, although they did not differ significantly (P > 0.05). The difference in total dry weights of the component species of the M(Lpe) - *L. perenne* mixture was low (P > 0.05) compared with the difference between the total dry weights of the component species of the other M(Lpe) - grass species mixtures (P < 0.05) (Fig. 6.3.1a). The M(Lpe) - *P. aquatica* mixture had the largest discrepancy in the total dry weights of the component species (Fig. 6.3.1a).

Growth of M(Ppr) was relatively higher when grown with *L. perenne* but the growth of *L. perenne* in turn was significantly reduced compared with the growth of *P. pratensis* and the *D. glomerata* and *P. aquatica* intermediates (Fig. 6.3.1b, bar graph). In most cases, the associated grass species was the superior yielding component, but in the M(Ppr) - *L. perenne* mixture, the reverse occurred with M(Ppr) out yielding *L. perenne* to the extent of suppressing the growth of *L. perenne*, although the difference in component yields of the two species was non significant (P > 0.05). Combined total yield of component species was significantly higher for the M(Ppr) - *P. pratensis* mixture than the other mixtures (Fig.
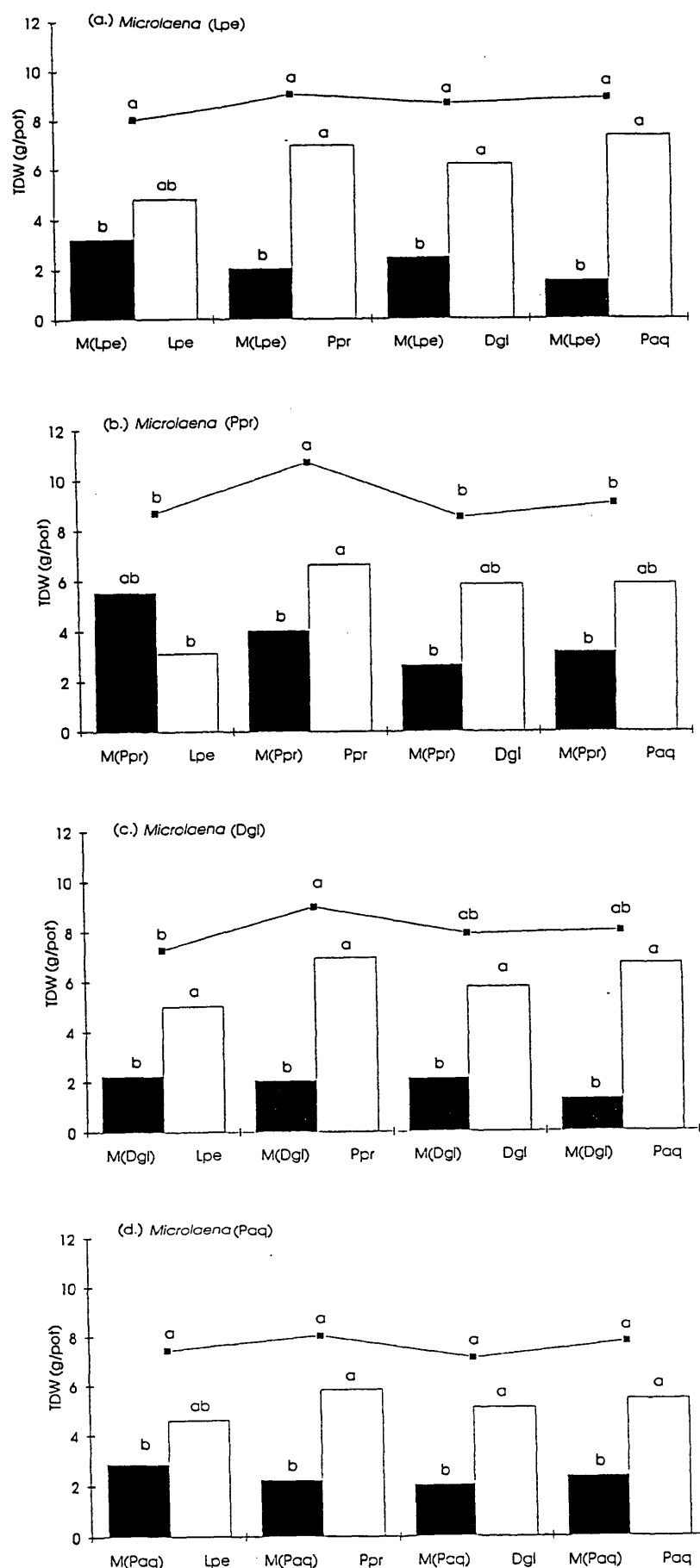
Fig. 6.3.1. Total dry weight (TDW) over 45 weeks of growth of four *M. stipoides* populations grown with four grass species. Filled and open bars indicate mean dry weights of *M. stipoides* populations and associated grass species, respectively. Line graph indicate total yield of mixtures. Points in the line graph and bars in the bar graph which share the same letter(s) are not significantly different (P < 0.05).

6.3.1b, line graph). The differences in total dry weights of the component species of all mixtures of M(Ppr) and the four grass competitor species were generally narrower than all the other mixtures of the other three *M. stipoides* populations and grass competitor species (Fig. 6.3.1).

There were no significant differences in the component yields of the M(Dgl) and M(Paq) populations when grown with the four different grasses (Fig. 6.3.1c and d, bar graphs). The difference in total dry weights of the component species in the M(Dgl) - *D. glomerata* and M(Dgl) - *L. perenne* mixtures were lower than the differences in the other M(Dgl) - grass mixtures (Fig. 6.3.1c). The combined total yield was largest in the M (Dgl) - *P. pratensis* mixture and smallest in the M (Dgl) - *L. perenne* mixture (P < 0.05) with the other species composition intermediate (Fig. 6.3.1c, line graph). There were no significant differences in the combined total yields (line graph) of M(Paq) and the grass mixtures (Fig. 6.3.1d). Total dry weights of the component species in the M(Paq) - *L. perenne* mixture did not differ significantly (Fig. 6.3.1d).

The component yields of the associated grasses grown with its natural neighbouring *M. stipoides* population were generally not higher than when grown with the other non-natural *M. stipoides populations* (Fig. 6.3.2). The total dry biomass of *L. perenne* did not differ significantly (P > 0.05) when grown with the four *M. stipoides* populations (Fig. 6.3.2a, bar graph) although when grown with M(Ppr) it had a slightly lower component yield. The accumulated dry biomass of *P. pratensis* grown with M(Ppr) did not differ significantly from when it was grown with the other three species (Fig. 6.3.2b, bar graph). However, the component yield of M(Ppr) was relatively higher than the component yields of the other *M. stipoides* populations grown with *P. pratensis*, resulting in a significantly higher combined yield of the M(Ppr) - *P. pratensis* mixture (Fig. 6.3.2b, line graph). The component yield of *D. glomerata* did not differ significantly when grown with the four *M. stipoides* populations. *Phalaris aquatica* grown with M(Paq) had a significantly lower component yield than when grown with M(Lpe) (Fig. 6.3.2d, bar graph). The component yield of M(Ppr) was significantly higher when grown with *P. aquatica* than the component yield of M(Dgl), while the component yields of the other *M. stipoides* populations were intermediate (Fig. 6.3.2d, bar graph). The difference in total dry weight of component species in the M (Lpe) - *P. aquatica* mixture was greater than the differences in the other *M. stipoides* - *P. aquatica* mixtures (Fig. 6.3.2d).
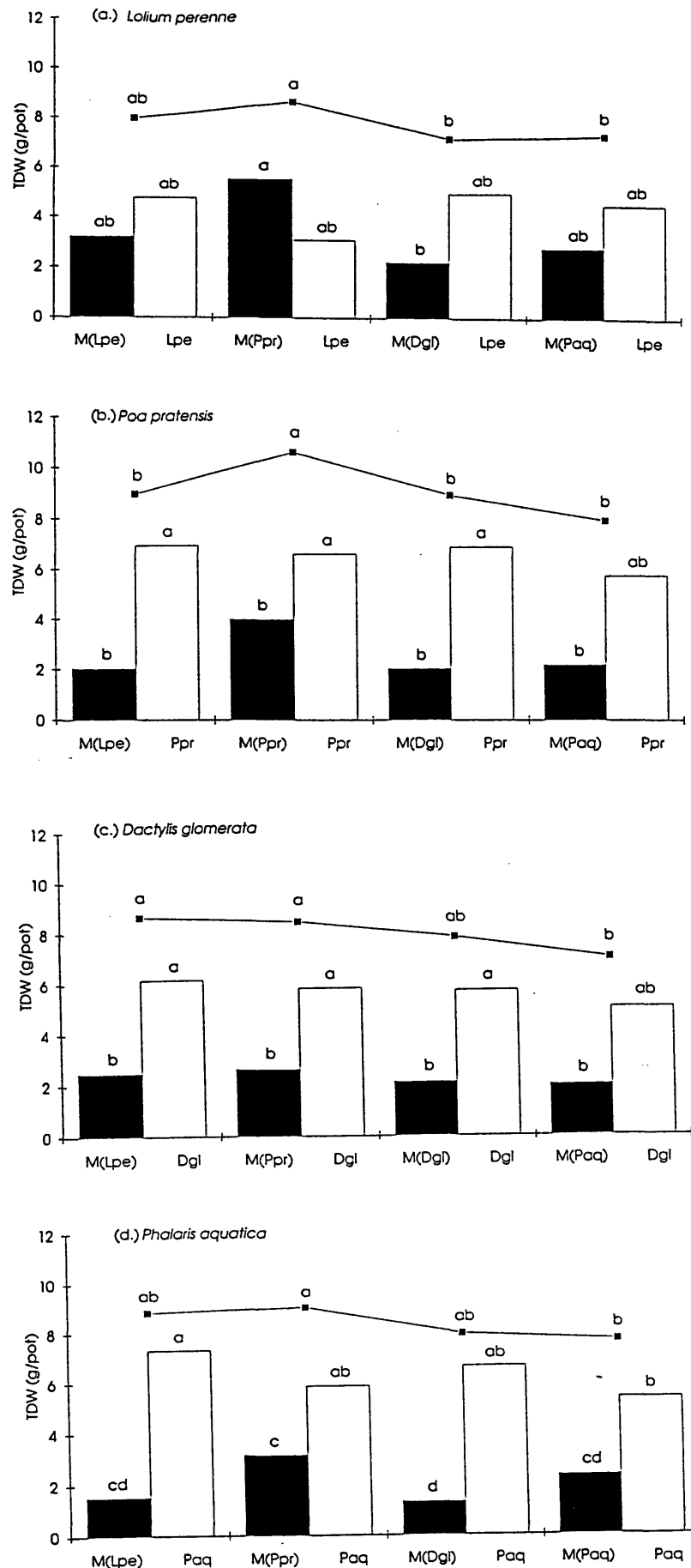
Fig. 6.3.2. Total dry weight (TDW) over 45 weeks of growth of the associated grass species grown with four *M. stipoides* populations. Filled and open bars indicate mean dry weights of *M. stipoides* populations and associated grass species, respectively. Line graph indicate total yield of mixtures. Points in the line graph and bars in the bar graph which share the same letter(s) are not significantly different (P < 0.05).

6.3.3.3 Component yield quotient

The M (Lpe) - *L. perenne* combination (Fig. 6.3.3a) had initially low component yield quotient (CYQ) values (bar graph) which gradually increased during the first 20 weeks with no significant changes in total yield (line graph), indicating similar competitive abilities. Starting at week 20, CYQ values remained high, accompanied by an increase in total yield of the mixture (Fig. 6.3.3a). When M (Lpe) was grown in combination with *P. pratensis* (Fig. 6.3.3b) and *D. glomerata* (Fig. 6.3.3c) there was an increase in CYQ values without much variation in total yield, indicating more similar competitive abilities in the first twenty weeks. After week 20, there was a reduction in CYQ values accompanied with an increase in total yields. The M(Lpe) - *P. aquatica* mixture had lower CYQ values compared with the other three M(Lpe) - grass mixtures (Fig.6.3.3d).

The M (Ppr) and *L. perenne* mixture had high CYQ values with relatively constant total yield during the first 17 weeks (Fig. 6.3.3e). Starting from week 18, there was a decline in CYQ values and total yields, indicating unbalanced competitive abilities. This period was also accompanied by the reversal in the superior yielding component, with M(Ppr) having higher component yields. However, after 30 weeks, the mixture showed a gradual increase in CYQ values and total yields (Fig. 6.3.3e). When M(Ppr) was grown in combination with *P. pratensis* (Fig. 6.3.3f) and *P. aquatica* (Fig. 6.3.3h), CYQ values were lower during the first 12 weeks with constant yield, however, from week 14, there were generally higher CYQ values with increasing total yields. In the M(Ppr) - *D. glomerata* mixture, a decline in CYQ values during the later part of the experiment was observed with a slight increase at the last harvest (Fig. 6.3.3g).

Generally, an increasing trend in CYQ values was observed when *M. stipoides* (Dgl) was grown in combination with *L. perenne*, *P. pratensis* and *D. glomerata* in the first 30 weeks (Fig. 6.3.3 i, j, and k). Substantial decreases in CYQ values were observed in the M(Dgl) - *L. perenne* and M(Dgl) - *P. pratensis* mixtures after 30 weeks (Figs. 6.3.3 i and j). Higher CYQ values were observed when M(Dgl) was grown in combination with *L. perenne*. When grown with *P. aquatica*, CYQ values were generally lower compared with the other M(Dgl) - grass combinations (Fig. 6.3.3 l). The M(Dgl) - *P. aquatica* mixtures exhibited fluctuations in CYQ values with constant combined yields during the first 18 weeks and a decline in CYQ values with increase in total yields after 29 weeks.

The M(Paq) - *L. perenne* mixture had higher CYQ values during the later part of the experiment, accompanied by an increase in total yields (Fig. 6.3.3m). The M(Paq) - *P. pratensis* and M(Paq) - *P. aquatica* mixtures had increasing CYQ values and constant total yields during the first 30 weeks, followed by a decline in CYQ values and an increase in
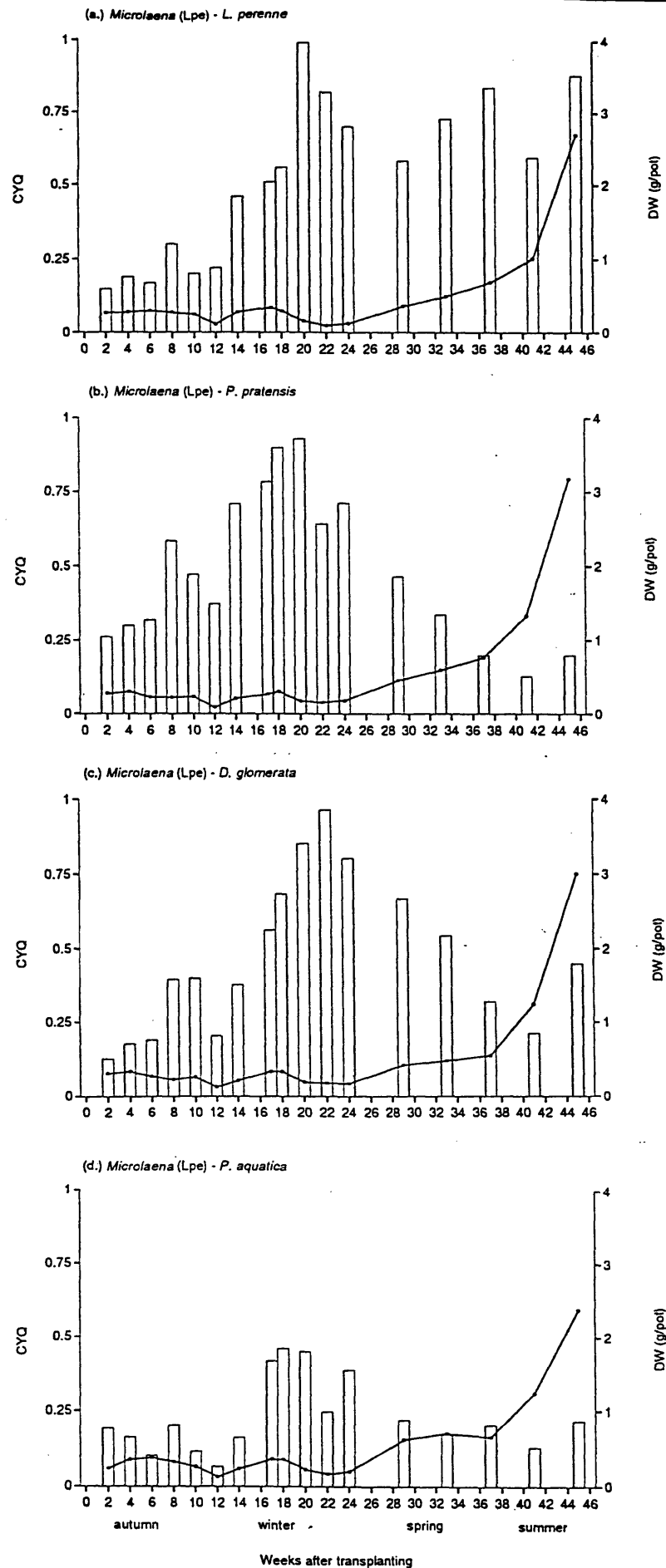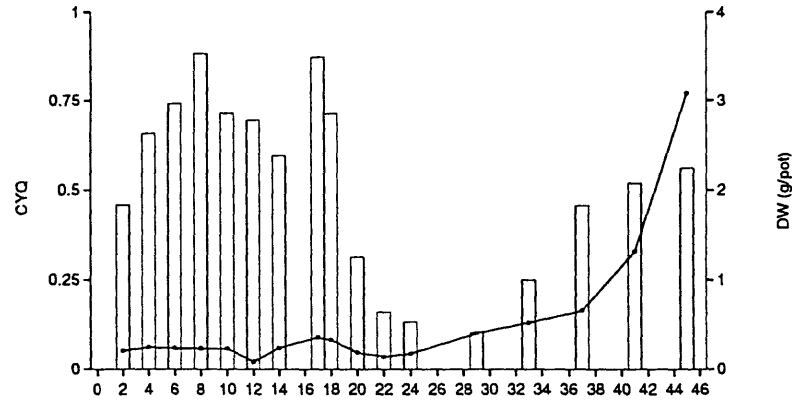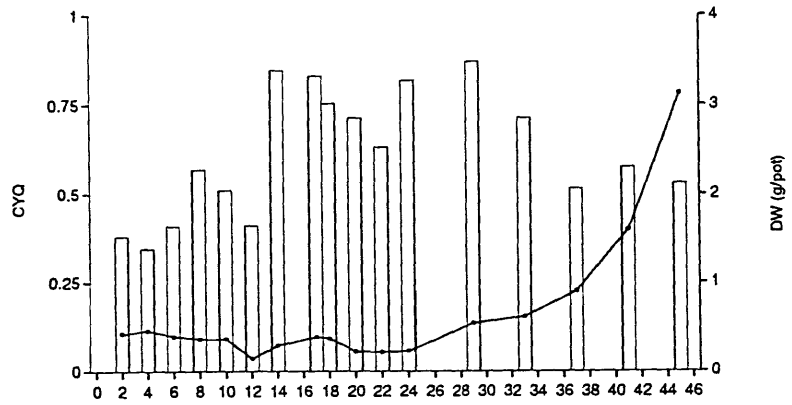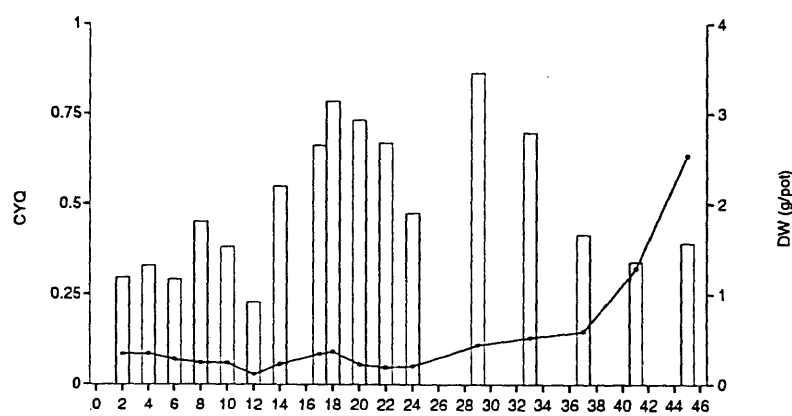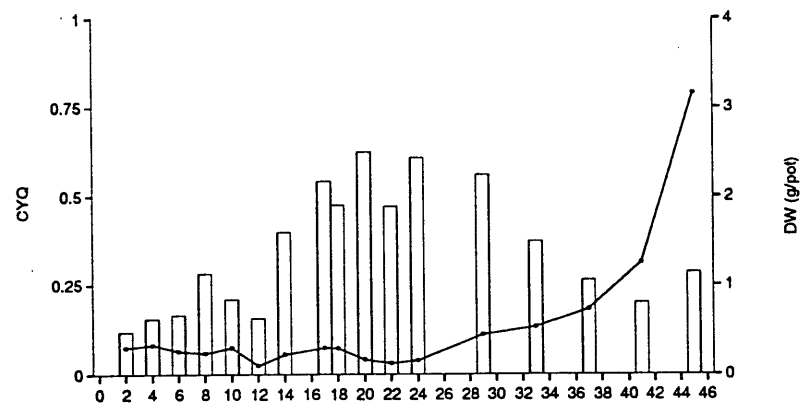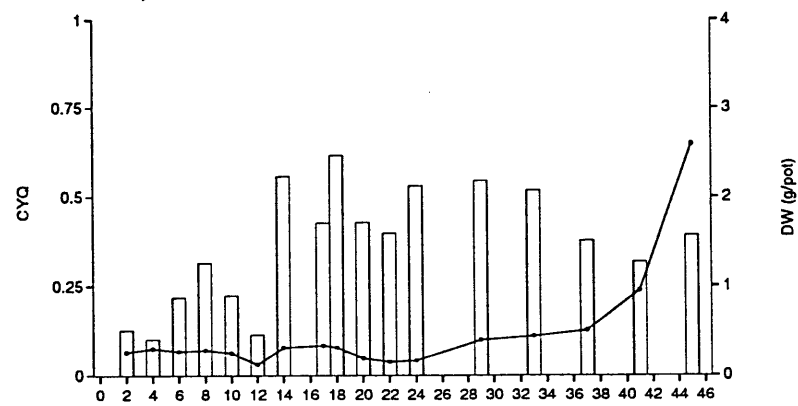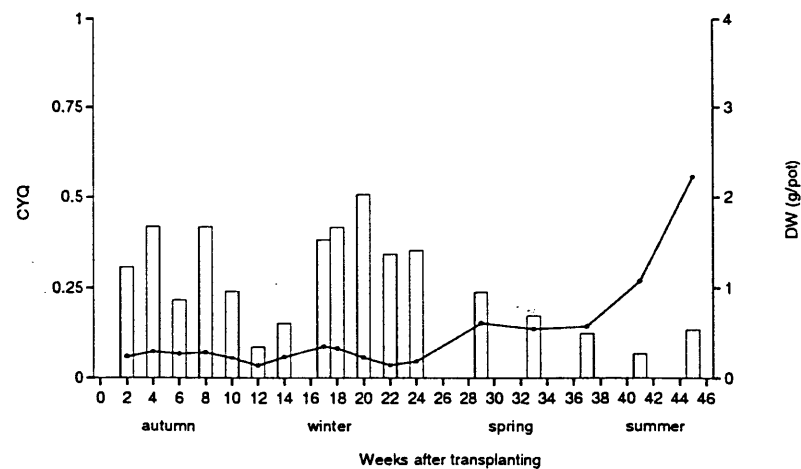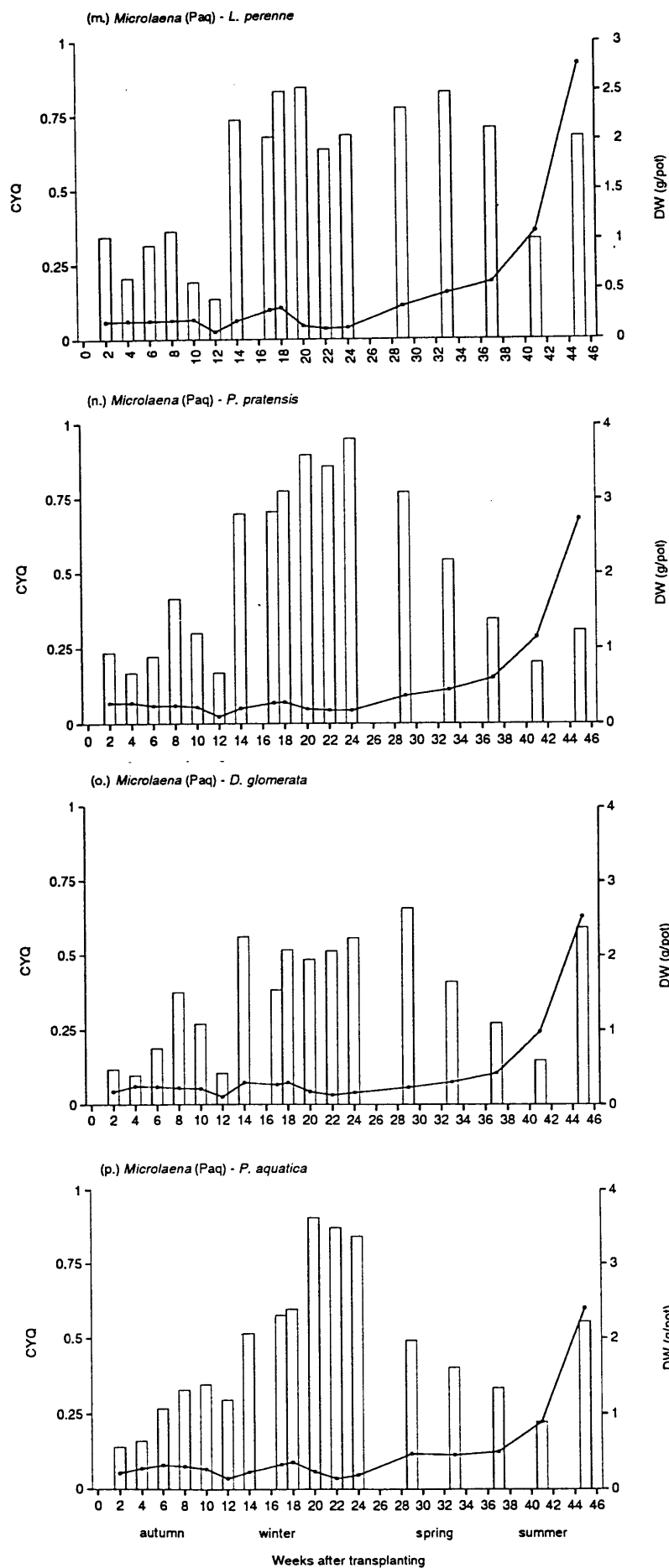
Fig. 6.3.3. Component yield quotient (CYQ) (bar graph) and combined total dry weight (TDW) of each pair (line graph) of *M. stipoides* population and associated grass species at each harvest.

(e.) *Microlaena* (Ppr) - *L. perenne*

(f.) *Microlaena* (Ppr) - *P. pratensis*

(g.) *Microlaena* (Ppr) - *D. glomerata*

(h.) *Microlaena* (Ppr) - *P. aquatica*

autumn   winter   spring   summer

Weeks after transplanting
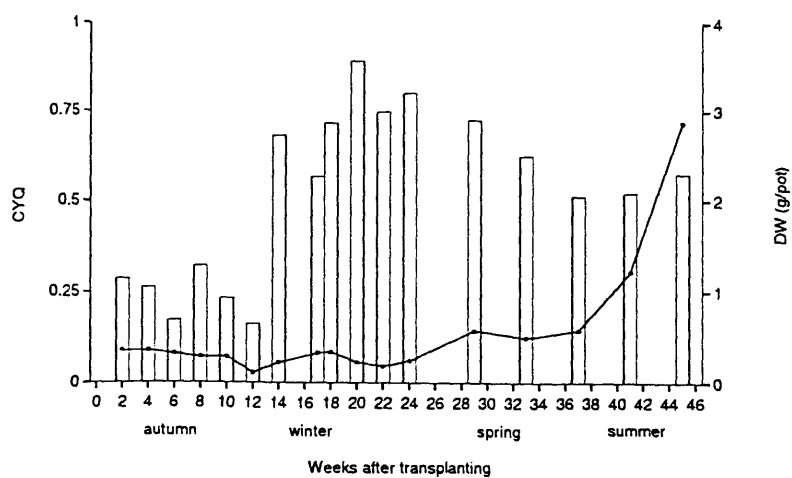
(i.) *Microlaena* (Dgl) - *L. perenne*
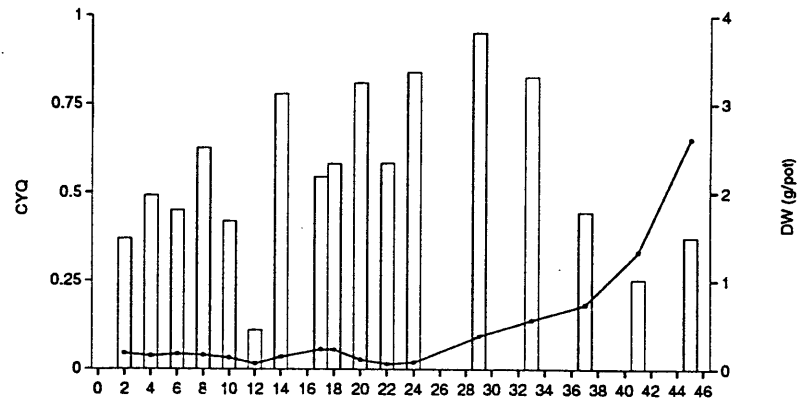
(j.) *Microlaena* (Dgl) - *P. pratensis*

(k.) *Microlaena* (Dgl) - *D. glomerata*

(l.) *Microlaena* (Dgl) - *P. aquatica*

autumn   winter   spring   summer

Weeks after transplanting

(m.) *Microlaena* (Paq) - *L. perenne*

(n.) *Microlaena* (Paq) - *P. pratensis*

(o.) *Microlaena* (Paq) - *D. glomerata*

(p.) *Microlaena* (Paq) - *P. aquatica*

autumn   winter   spring   summer

Weeks after transplanting

total yields (Figs. 6.3.3 n and p). A similar trend was also observed in the M(Paq) - *D. glomerata* mixture, although CYQ values were generally lower (Fig. 6.3.3o).

6.3.4 Discussion

The mean total component yield of M (Ppr), averaged over all mixtures with associated grass species, was significantly higher than the other three *M. stipoides* populations. The results of the pot experiment on the effect of light intensity (Chp. 5.3) have shown that averaged over all light intensity levels, M (Ppr) had significantly higher total dry weight, number of leaves, panicle number and narrower leaves than the other three populations. Among the four *M. stipoides* populations, M (Lpe) and M (Ppr) exhibited high sensitivity to the associated grass species. Growth of M (Lpe) was higher when grown with *L. perenne* and was lower when grown with *P. pratensis* and *P. aquatica*. Growth of M (Ppr) was higher when grown with *L. perenne* and was lower when grown with *D. glomerata*.

Among the associated grass species, *L. perenne* had a significantly lower mean total component yield than *P. pratensis* and *P. aquatica*, while *D. glomerata* was intermediate. *Lolium perenne* and *P. aquatica* had higher sensitivity as shown by large fluctuations in its growth with the different associated grass species compared with the other two grass species. Growth of *L. perenne* was inhibited when grown with M (Ppr) to the extent that the superior yielding component in the M (Ppr) - *L. perenne* mixture was M (Ppr). Generally, in most of the *M. stipoides* - grass mixtures, the associated grass was the superior yielding component, but in the M (Ppr) - *L. perenne* mixture, the reverse occurred.

Two of the four natural neighbouring pairs studied, the M (Lpe) - *L. perenne* and M (Ppr) - *P. pratensis* mixtures, showed lesser differences in total dry weights of component species. This could perhaps indicate evolutionary equilibration in competitive abilities as a consequence of ongoing selection in order to be able to coexist in the same community (Aarssen 1983, 1989, Aarssen and Turkington 1985a).

The reduced biomass produced by M (Lpe) when grown with *P. aquatica*; of M (Ppr) when grown with *D. glomerata* and *P. aquatica*; of M (Dgl) when grown with *P. aquatica* and *P. pratensis*; of *L. perenne* when grown with M (Ppr); and the increased growth of *P. aquatica* and *P. pratensis* when grown with M (Lpe) show the aggressiveness of non-neighbouring pairs. Selective pressures may have reduced the aggression of the neighbouring species populations towards each other through arrangement of plant parts and complementary growth patterns (Rhodes 1981).

The results show changes with time in competitive interactions between the four *M. stipoides* populations and the four associated grass species. A number of sequences of competitive interactions were observed in the data: (1) increase in CYQ values without any increase in total yields, followed by sustained high CYQ values and increasing total yields, e.g. M(Lpe) - *L. perenne,* M(Ppr) - *P. pratensis,* M(Ppr) - *P. aquatica,* M(Dgl) - *D. glomerata,* M(Paq) - *L. perenne*; (2) increase in CYQ values without any increase in total yields followed by a decrease in CYQ values accompanied by an increase in total yields, e.g. M(Lpe) - *P. pratensis,* M(Lpe) - *D. glomerata,* M(Ppr) - *D. glomerata,* M(Dgl) - *L. perenne,* M(Dgl) - *P. pratensis,* M(Paq) - *P. pratensis,* M(Paq) - *D. glomerata* mixtures; (3) fluctuating and generally low CYQ values , e.g. M(Lpe) - *P. aquatica,* M(Dgl) - *P. aquatica*; (4) high or increasing CYQ values without any increase in total yields, followed by a decrease in CYQ values without any change in total yields and then followed by increases in CYQ values and total yields, e.g. M(Ppr) - *L. perenne* and M(Paq) - *P. aquatica.*

Aarssen and Turkington (1985a) discussed three alternative consequences of competition: (a) improved performance of the inferior competitor without reduction of the superior competitor, resulting in an increase in CYQ values and total yields. This may arise from niche differentiation or release from resource competition; (b) improved performance of the inferior competitor along with reduction in the performance of the superior competitor, resulting in an increase in CYQ values but not in total yields. This may arise from balancing of competitive abilities for contested resources; (c) improved performance of the superior competitor relative to the inferior competitor resulting in a decrease in CYQ values. This may arise from asymmetric improvement of competitive abilities and may ultimately result in competitive exclusion of the inferior competitor.

The four *M. stipoides* populations clearly showed different competitive interactions with the four different grass species. Note that from week 24, harvests were done at four-weekly intervals, providing the ramets with a longer growing time, and resulting in higher dry weights compared with the previous harvests. All the ramets showed increased dry weights at the last four harvests which corresponded to the summer months when rising temperatures resulted in increased growth of the grasses. The first trend characterised by an increase in CYQ values without any increase in total yields, followed by sustained high CYQ values and increasing total yields, may indicate balancing of competitive abilities for contested resources as described by Aarssen and Turkington (1985a). The increase in total yields after week 24 could have been attributed to the longer intervals between harvests which allowed more growing time for the plants and the rising temperature during the warmer months favoured a faster growth rate. Generally, more similar competitive abilities were exhibited by natural neighbouring pairs as shown by their lesser differences in total dry weights of component species and higher CYQ values. Non-natural neighbouring pairs

either showed low CYQ values throughout the duration of the experiment or showed improved competitive abilities at the early stages of the experiment, but ended up with decreasing CYQ values, indicating asymmetrical improvement of competitive abilities. This may possibly result in competitive exclusion later on. A few non-natural neighbouring pairs showed the possibility of coexistence, e.g. the M(Ppr) - *P. aquatica*, and M(Paq) - *L. perenne* mixtures. Generally, M(Ppr) showed potential for coexistence with all four associate grass species having higher or increasing CYQ values until the last harvest.

It was only the growth of *P. aquatica* that did not exhibit preference with its associated M (Paq), and their combined dry mass was lower than the other combinations of *P. aquatica* growing with the other three *M. stipoides* populations. This may be explained by the fact that M(Paq) and its associated grass, *P. aquatica*, were collected from a different paddock ('Powalgarh') 60 km north-west of the other 10.2 ha-paddock ('Karuah') where the other three populations of *M. stipoides* and their associated grasses were collected. Natural selection, through competition, may have occurred for more than thirty years since the Karuah paddock was sown with *L. perenne* and *D. glomerata* seeds in 1961. Coexistence among M (Lpe), M (Ppr) and M (Dgl) and their associated grasses were made possible by the numerous possible permutations and combinations of plant biological attributes to maintain a balance of overall competitive abilities (Aarssen 1983). When *P. aquatica* was grown with M(Lpe) and M(Dgl) populations, it exhibited aggressiveness, hence the higher dry mass than when it was grown with its own associated M (Paq). However, when *P. aquatica* was grown with M(Ppr), it did not exhibit aggressiveness and M(Ppr) had a high component yield relative to the other *M. stipoides* populations, indicating the possibility of coexistence.

Turkington and Harper (1979c) indicated that the influence of a plant's neighbours may be great enough to result in microevolution. This may be possible with *M. stipoides*. It was observed that the four *M. stipoides* populations studied have different seed weight (Chp. 5.2), morphology (Chp. 5.3), growth performance under varying light intensity levels (Chp.5.3), responses to water stress (Chp. 5.4), DNA banding patterns (Chp. 5.5) and thermal denaturation profiles (Chp. 5.6). These differences among populations of *M. stipoides* may indicate divergence resulting from the expression of numerous combinations of different biological attributes that define alternative ways of being an effective competitor (Aarssen 1983).

6.4 DISCUSSION

Competition studies employing manipulative field experiments are ideal in that other factors such as soil heterogeneity and climate, which affect species performance in their local microenvironment, are included in the experimental design. A field competition experiment following Connell's (1980) proposed design was excuted to test the hypothesis that *M. stipoides* had diverged into populations resulting from competition with associated grass species in a permanent pasture. It was postulated that the present populations of *M. stipoides* coexisting with *P. pratensis*, *L. perenne* and *D. glomerata* at 'Karuah' and with *P. aquatica* at 'Powalgarh' had a common ancestor.

The results showed that the greatest population divergence occurred in M (Ppr). Among the four *M. stipoides* populations, M (Ppr) exhibited significant divergence from all three allopatric populations transplanted into the sympatric *P. pratensis* site in the presence of competitor species in terms of the total number of panicles exserted. It was also M (Ppr) that exhibited significant divergence in terms of dry matter production when transplanted into its sympatric site compared with production when transplanted into all three allopatric sites in the presence of competitor grass species.

The divergence exhibited by M (Ppr) resulted from inter-specific competition with the associated grass species, *P. pratensis*. *Microlena stipoides* (Ppr) showed large increases in performance, though not statistically significant, both in terms of dry matter production and panicle production when transplanted into all three allopatric sites following removal of the competitor species. When transplanted into its sympatric site, removal of the competitor species did not cause a significant increase in the performance of M (Ppr) compared with its performance in the presence of the competitor species. There was no significant evidence to show that population divergence due to competition occurred at the *L. perenne*, *D. glomerata* and *P. aquatica* sites.

The divergence exhibited by M (Ppr) probably had a genetic basis since there was no significant difference in the total dry weights and number of panicles exserted by M (Ppr) transplanted into its sympatric site in the presence or absence of the competitor species, *P. pratensis*, and in the dry weights of M (Ppr) and allopatric populations transplanted into allopatric sites. This evidence was supported by the results from the amplified polymorphic DNA experiment which showed that M (Ppr) had a more divergent banding pattern compared with the other populations.

The observed divergence in M (Ppr) due to competitive interactions with its neighbouring grass species, *P. pratensis,* may be because both *M. stipoides* and *P. pratensis* co-occurred before the introduction of the sown species, *L. perenne* and *D. glomerata* in 1961, and *P. aquatica* in 1960. The close association between *P. pratensis* and M (Ppr) provided selection pressures on *M. stipoides* individuals for persistence, growth, reproduction (Evans and Turkington 1988) and mortality (Connell 1980). It is possible that coexistence between M (Ppr) and *P. pratensis* may have resulted from natural selection under the pressure of competition, resulting in species divergence such that each species occupies a different niche (MacArthur 1972) or a balancing of competitive abilities (Aarssen 1983). Divergence was evident in M (Ppr) but not in the other *M. stipoides* populations, probably because thirty years of co-existence between *M. stipoides* and the introduced species has not been long enough for competition pressures to result in population divergence. *Poa pratensis* is an invader in the pasture and possibly had been coexisting with *M. stipoides* in the area for more than thirty years. It would be interesting to examine the species associations in the same pasture in another thirty years time.

Local site conditions also had significant effects on the biomass production of the different *M. stipoides* populations. All four *M. stipoides* populations had better performance when transplanted into the *P. pratensis* and *L. perenne* sites. *Microlaena stipoides* (Ppr) showed a home site advantage as it yielded significantly higher dry matter and panicle numbers than the other three allopatric populations transplanted into the sympatric *P. pratensis* site. The results indicate that it is possible that local-scale variation in edaphic factors may also influence the micro-distribution of *M. stipoides* in this paddock. The better performance of all populations at the *P. pratensis* site could be related to the location and soil properties of that particular site in the paddock. The *P. pratensis* site is situated on the lower slope of the paddock and has a loamy soil texture which probably retains more moisture than the other sites. The *D. glomerata* and *P. aquatica* sites were stony and situated on the upper slope of the paddock, while the *L. perenne* site was situated in a flat area and characterised by clayey soil. It is highly possible that soil pH and soil fertility levels could vary among the sites and may influence the micro-distribution of *M. stipoides.* Magcale-Macandog and Whalley (1994) found that *M. stipoides* abundance and distribution on the Northern Tablelands is correlated with soil pH.

The aim of the glasshouse experiment was to determine the relative performance of the four *M. stipoides* populations when grown in association with natural and non-natural neighbouring species. The mean total component yield of M (Ppr), averaged over all mixtures with associated grass species, was significantly higher than that of the other three *M. stipoides* populations.

The component yields of species in mixtures involving non-natural neighbouring pairs show large differences, reflecting asymmetrical competitive abilities and aggressiveness (Rhodes 1981) of one component species relative to the other. Conversely, two of the four natural neighbouring pairs studied, the M (Lpe) - *L. perenne* and M (Ppr) - *P. pratensis* mixtures, showed smaller differences in total dry weights of the component species.

The three natural neighbouring pairs collected from the same paddock at 'Karuah', i.e. M (Lpe) - *L. perenne*, M (Ppr) - *P. pratensis* and M (Dgl) - *D. glomerata*, and two non natural neighbouring pairs {M (Ppr) - *P. aquatica* and M (Paq) - *L. perenne*} showed increased and sustained high CYQ values. All the other combinations ultimately exhibited a decrease in CYQ values with time.

The four *M. stipoides* populations clearly showed different competitive interactions with the four different grass species. Generally, more similar competitive abilities were exhibited by natural neighbouring pairs, as shown by their lesser differences in total dry weights of component species and higher CYQ values. This could perhaps indicate evolutionary equilibration in competitive abilities as a consequence of ongoing selection in order to be able to coexist in the same community (Aarssen 1983, 1989, Aarssen and Turkington 1985a). Natural selection, through competition, may have occurred for more than thirty years since the paddock at 'Karuah' was sown with *L. perenne* and *D. glomerata* seeds in 1961. Coexistence among M (Lpe), M (Ppr) and M (Dgl) and their associated grasses were feasible because of the numerous possible permutations and combinations of plant biological attributes resulting in a balance of overall competitive abilities (Aarssen 1983).

Non-natural neighbouring pairs either showed low CYQ values throughout the duration of the experiment or improved competitive abilities at the early stages of the experiment, but ended up with decreasing CYQ values, indicating asymmetrical improvement of competitive abilities. This may ultimately result in either niche differentiation (Aarssen 1983) or competitive exclusion.

Turkington and Harper (1979c) indicated that the influence of a plant's neighbours may be great enough to result in microevolution. This may be possible with *M. stipoides*. It was observed that the four *M. stipoides* populations studied have different seed weights (Chp. 5.2), morphologies (Chp. 5.3), growth performances under varying light intensity levels (Chp.5.3), responses to water stress (Chp. 5.4), DNA banding patterns (Chp. 5.5) and thermal denaturation profiles (Chp. 5.6). A field experiment (Chp. 6.2) showed that intersecific competition between M (Ppr) and *P. pratensis* caused the divergence

in M (Ppr). Natural neighbouring pairs (Chp. 6.3) had more similar component yields in mixture and higher CYQ values, indicating more balanced competitive abilities. These results indicate that divergence among populations of *M. stipoides* may have resulted from the expression of numerous combinations of different biological attributes that define alternative ways of being an effective competitor (Aarssen 1983) or being able to coexist with neighbouring perennial grass species in permanent pastures (Aarssen and Turkington 1985a). Precisely defined biotic specialisation in these natural neighbouring pairs may have occurred, resulting in a more even distribution of yield among the combinations (Aarssen and Turkington 1985a).

CHAPTER SEVEN

INTEGRATING DISCUSSION AND CONCLUSION

One of the primary aims of this thesis was to determine the patterns and processes of divergence in *M. stipoides* populations that permit stable coexistence with introduced exotic and naturalised pasture species. Patterns exhibited by a species in a community are influenced by its growth morphology, variations in physical environmental conditions and by biotic interactions with neighbouring species.

The distribution and abundance of *M. stipoides* on the Northern Tablelands were strongly associated with environmental and pasture management correlates, and pasture type. *Microlaena stipoides* was more common at higher elevations on the eastern side and southern half of the Northern Tablelands and its frequency increased with an increase in soil acidity. It was distributed over a wide range of soil types. Pasture management correlates that were significantly associated with abundant *M. stipoides* were high tree density, minimum soil disturbance and long period since last cultivation. Cultivation either for cropping or for sowing improved pastures results in the destruction of *M. stipoides*.

The abundance of *M. stipoides* was unrelated to the type of grazing animal or whether set stocked or rotationally grazed. However, *M. stipoides* was more abundant in pastures which were continually grazed than those spelled for 2-3 months, indicating that it is tolerant of continuous grazing. The total amount of phosphate fertiliser applied had no significant association with the abundance of *M. stipoides* and it was found in both phosphate-fertilised and phosphate-poor soils. However, it is very responsive to added nitrogen (Vieira 1980) which may be the reason why it is more common along the margins of sheep camps and grows well in association with *Trifolium repens*.

The abundance of *M. stipoides* was associated with the pasture type in individual paddocks. *Microlaena stipoides* was more abundant in paddocks containing other year-long green perennials, cool season annuals and exotic sown species in addition to warm season perennials and annuals compared with pastures composed mainly of warm season perennials and annuals. In terms of the sown exotic species, more *M. stipoides* was observed in the cluster of paddocks having a higher frequency of *Lolium perenne* than in clusters of paddocks having higher frequencies of either *Phalaris aquatica* or *Dactylis*

glomerata. The distribution patterns of these three sown species are probably influenced by the same environmental variables which affect the distribution of *M. stipoides*.

The results of the survey clearly indicated that environmental and pasture management attributes are important correlates of the distribution and abundance of *M. stipoides* on the Northern Tablelands of New South Wales. Biotic interactions between *M. stipoides* and neighbouring pasture species may also be important in determining the dynamic distribution pattern of *M. stipoides* in a permanent pasture.

Vegetation surveys conducted at 'Karuah' in 1990 and 1993 illustrated the dynamic nature of the species associations in this permanent pasture. All the species studied showed variations in their pattern of distribution and species associations within three years.

Microlaena stipoides and *L. perenne* were the dominant perennial grasses in the paddock in both surveys. Although they were both abundant throughout the paddock, *M. stipoides* preferred shaded areas, while *L. perenne* was more abundant in open areas. During the first survey, locations with a high cover of either *M. stipoides* or *L. perenne* in many cases had a lower cover of the other species. The complementary nature of these two species is further illustrated by the separation of the sites dominated by each species in the discriminant analysis territorial map and the separation of them on axis two of the species ordination map. Three years later, close associations between *M. stipoides*, *L. perenne*, *T. repens*, *D. glomerata* and *P. pratensis* were clearly shown by the discriminant analysis territorial map and species ordination.

Although *D. glomerata* is well separated from all other species in the species ordination, it was obvious that at least some genotypes of *M. stipoides* can co-exist with *D. glomerata*. This association was supported by the results of the 1993 survey, where two groups of sampling locations had a high abundance of *D. glomerata* and *M. stipoides*.

In both surveys, it was generally observed that the presence of *L. perenne* was greatly reduced in sampling locations where *D. glomerata* comprised more than 40% of the total basal cover. These sampling locations were confined to the upper slopes on the upper north-eastern part of the paddock above the gravel road. Likewise, *D. glomerata* was rarely observed in sampling locations dominated by *L. perenne*, which was more common on the lower parts of slopes. *Lolium perenne* and *D. glomerata* have the same growth forms: both are tufted perennials with erect or spreading culms so that they form patches in the field. Both the hierarchical fusion and discriminant function analyses using the 20 groups formed by the fusion of the 1990 survey results, clearly separated the locations dominated by *L. perenne* and by *D. glomerata* from the rest. Isonome maps in the 1990 survey showed that

each of these species occupies distinct areas in the paddock, *D. glomerata* being more frequent in the upper slopes and *L. perenne* in the flat areas and lower parts of the slopes.

Poa pratensis had an interesting species association. It was well separated from all the other major species in the species ordination but was closely associated with *L. perenne* at one location and with *M. stipoides* at two others in the 1990 survey. Three years later, territorial maps and the dendrogram showed that *P. pratensis* had improved associations with *M. stipoides*, *L. perenne* and *T. repens*. In both surveys, *P. pratensis* was confined to flat areas and hollows on the lower slopes of the paddock where soil moisture was possibly higher. Again, perhaps the genotypes of *M. stipoides* associated with *P. pratensis* are different from those associated with other species in this paddock.

The increase in species diversity in the 1993 survey could be attributed to several environmental and biotic factors. The decline in the soil phosphorus level due to the cessation of annual superphosphate application since 1990 may have favoured minor species which were able to compete and become dominant in scattered patches in the paddock. The seasonal distribution of rainfall during the period 1990 to 1993 also may have favoured particular species. The decline in the abundance of *T. repens* may have favoured species that do not particularly benefit from the nitrogen fixed by the *T. repens - Rhizobium* sp. association.

The high percentage basal cover of *M. stipoides* in sampling locations in association with the other dominant perennial grasses such as *L. perenne*, *D. glomerata* and *P. pratensis* may indicate that *M. stipoides* can become adapted to growing in close proximity with the other species in the area. Results of the species ordination for the 1993 survey showing *M. stipoides* in close proximity with most of the other dominant grasses may imply a permanency of these species associations. The aggregation of sampling locations dominated by mixed *M. stipoides*, *T. repens* and *L. perenne* in the discriminant function analysis using the 20 groups determined by Bray-Curtis hierarchical classification also suggests stability in this species combination (Turkington and Harper 1979a).

Microlaena stipoides was widespread throughout the paddock and was associated with different species at different locations. It could be possible that there are substantial morphological, behavioural and genetic differences among populations of *M. stipoides* growing in association with the different dominant perennial grasses (Turkington and Harper 1979b).

Patterns of divergence among the four *M. stipoides* populations growing in association with three dominant perennial grasses at 'Karuah' (M (Lpe), M (Ppr) and M

(Dgl)} and with *P. aquatica* {M (Paq)} at 'Powalgarh' were examined. The four populations exhibited differences in leaf morphology, tillering, seed size and rate of seed germination. *Microlaena stipoides* (Lpe) and M (Paq) have shorter but broader leaves and heavier seeds. *Microlaena stipoides* (Ppr), on the other hand, had narrower leaves, larger tiller numbers, small seed size and a faster rate of seed germination. Furthermore, M (Paq) seeds have the slowest germination rate.

All the four *M. stipoides* populations exhibited differences in their growth responses under varying light intensity levels. *Microlaena stipoides* (Ppr) showed a significantly higher vegetative and reproductive performance under low light intensity levels. *Microlaena stipoides* (Dgl) showed tolerance to full light, having a lower reduction in total dry mass when grown under full light compared with the other three populations. The different associated grasses could alter the transmitted radiation below their canopies because they have different growth habits. Although *P. pratensis* and *D. glomerata* were both tufted perennials, *P. pratensis* produces dense foliage and forms large compact tufts growing up to 0.9 m, high while *D. glomerata* grows up to 1.4 m high with erect culms (Harden 1993). It could be possible that the varying transmitted radiation intercepted by *M. stipoides* when growing with these associated grass species had exerted selection pressures on *M. stipoides* populations to become better competitors under varying light intensity levels. The results seem to indicate that the transmitted radiation under or adjacent to *P. pratensis* is lower compared with the transmitted radiation under the *D. glomerata* canopy, thus M (Ppr) was more adapted to lower light intensity levels compared with M (Dgl), which was more tolerant of higher light intensity levels.

The four populations also exhibited differences in their water stress tolerance levels. *Microlaena stipoides* (Paq) and M (Dgl) showed a higher tolerance to water stress than M (Lpe) and M (Ppr), exhibiting a significant increase in the total number of drying days to 100% leaf mortality when grown in large pots than when grown in medium and small pots. Averaged over all pot sizes, M (Dgl) and M (Paq) took a longer time to reach 100% leaf mortality compared with M (Lpe) and M (Ppr), although it was not statistically significant. *Microlaena stipoides* (Dgl) and M (Paq) grow in association with *D. glomerata* and *P. aquatica* respectively, which are water stress tolerant compared with *L. perenne* and *P. pratensis*. In the paddock, *D. glomerata* and *P. aquatica* were more common on the ridges or upper parts of the slope, while *L. perenne* was more abundant in the flat areas and *P. pratensis* was confined to the lower parts of the slope or hollows where soil moisture is much higher. This seems to indicate that natural neighbouring species exerted selection pressures on *M. stipoides* populations for greater water stress tolerance.

Amplified polymorphic DNA fingerprints have shown that among the four populations of *M. stipoides*, M (Ppr) showed greater divergence in the DNA banding patterns compared with the other three populations. This was in agreement with the more divergent growth responses of M (Ppr) on differing light intensity levels, smaller seed size and higher panicle production compared with the other three populations. The thermal denaturation curves from the DNA-DNA hybridisation techniques seemed to single out M (Paq), which was geographically isolated from the other three populations collected from within the same paddock. Results of the amplified polymorphic DNA experiment seem to be correlated with the growth performance of the four populations both in the glasshouse and field experiments while the results of the DNA-DNA hybridisation experiment, seem to be more correlated with the geographical location of the four populations. The DNA-DNA hybridisation technique, however, needs refinement and further replication.

Local site conditions were found to significantly affect the performance and abundance of *M. stipoides* in the paddock. All the four populations showed greater performance when transplanted into the *L. perenne* and *P. pratensis* sites which were located in the flat areas and hollows on the lower slopes of the paddock. These sites were observed to have clayey and loamy soils, respectively. It is possible that the soil moisture content in these sites was less limiting (Reader and Bonser 1993) than the *D. glomerata* and *P. aquatica* sites which were located on the upper slopes and had stony soil. Soil depth and other edaphic factors like soil pH, nutrient status, soil microorganisms and soil physical conditions may contribute to the differences in local microenvironments of the four transplanting sites.

It is postulated that the main driving force for the divergence of *M. stipoides* populations, characterised by a fine-scale patchy distribution in a small paddock, was interspecific competition with neighbouring perennial grass species. It has been shown that association with *P. pratensis* caused genetic divergence in the *M. stipoides* populations. It could be possible that both *M. stipoides* and *P. pratensis* have been co-occurring for a long time to cause such genetic divergence. *Microlaena stipoides* is a native species while *P. pratensis* is a naturalised species and might have established in the area soon after the arrival of Europeans in the 19th century. The results of the manipulative field experiment did not yield any evidence to show that the other *M. stipoides* populations had diverged with the other three exotic grass species introduced in the 1960's.

The results of the controlled glasshouse experiment, however, showed that the three natural neighbouring pairs at 'Karuah' (*L. perenne* - M (Lpe), *P. pratensis* - M (Ppr) and *D. glomerata* - M (Dgl)) exhibited equilibration of competitive abilities. This may indicate some

fine scale tuning process that resulted in the balancing of competitive abilities between natural neighbouring pairs (Aarssen 1983).

Species coexistence occurs if selection pressures resulting from competition lead to evolution, which results in the avoidance of competitive interaction (niche differentiation) or in the reciprocal balancing of competitive abilities for contested resources (competitive combining ability). Selection for niche differentiation and selection for competitive combining ability takes place concurrently in nature (Aarssen 1983). This could be the reason why CYQ values fluctuated throughout the duration of the experiment. It may indicate continuous fine-tuning processes of adjustment between component species in a mixture. Natural neighbouring pairs had high CYQ values while non-natural neighbouring pairs had lower CYQ values most of the time.

There is a vast number of genes which determines the various characters that govern competitive ability (Aarssen 1983, 1989). The competitive ability of M (Ppr)was characterised by a combination of several attributes, including a faster rate of seed germination, abundant seed production, greater shade tolerance by being able to produce a greater number of tillers at lower light intensity levels (16% transmitted light), high leaf production and high leaf chlorophyll content at 47% transmitted light. The competitive ability of M (Dgl), on the other hand, was characterised by a higher tolerance of full light conditions and greater water stress tolerance.

The distribution of *M. stipoides* on the Northern Tablelands is influenced by correlates such as altitude, soil pH and minimal soil disturbance (long period since last cultivated). It is suggested that the neighbouring species growing in association with *M. stipoides* have exerted selection forces which have driven divergence among the *M. stipoides* populations. The complex breeding system of *M. stipoides* enables it to control or permit rapid genetic changes so as to be able to adjust to changing local abiotic and biotic microenvironments.

The process of divergence in the *M. stipoides* populations would involve both biotic interactions with neighbouring grass species and adaptation to local abiotic microenvironments. However, it was not determined from these results what percentage of divergence in *M. stipoides* populations was due to biotic interactions and what was due to abiotic local microenvironments.

The results of the manipulative field competition experiment clearly showed that divergence occurred only in one population, M (Ppr). The glasshouse competition experiment, however, showed a balancing of competitive abilities between the three natural

neighbouring pairs at 'Karuah'. This shows the advantage of conducting manipulative field experiments as all other extraneous factors affecting the growth performance of the plants in nature were inherent in the experimental design.

Stable coexistence between *M. stipoides* and introduced and naturalised perennial grasses in permanent pastures is therefore a consequence of selection resulting from interactions at the genotype level within local neighbourhoods (Aarssen 1989). It is likely that evolutionary changes are reciprocal, such that the associated grass species had been undergoing divergence resulting from selection forces exerted by *M. stipoides* populations. Studies on coevolution between *M. stipoides* populations and neighbouring grass species would be an interesting endeavor for future research.