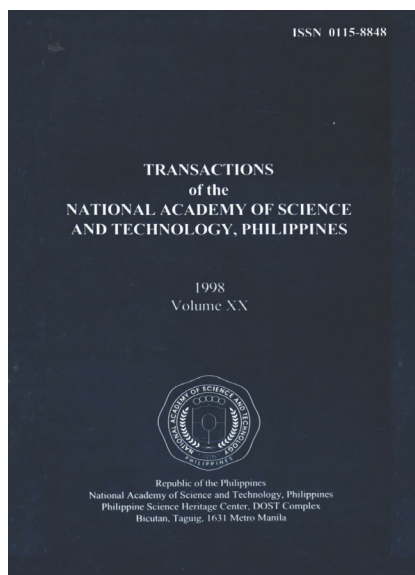


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## Patterns and Processes in Population Divergence of *Microlaena stipoides* (Labill.) R. Br.

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**PATTERNS AND PROCESSES IN POPULATION  
DIVERGENCE OF *Microlaena stipoides*  
(LABILL.) R. BR.**

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**ABSTRACT**

Morphological, behavioral, and genotypic patterns of variation among the four populations of *M. stipoides* found growing in association with *L. perenne* [M (Lpe)], *P. pratensis* [M (Ppr)], *D. glomerata* [M (Dgl)] and *P. aquatica* [M (Paq)] were examined. *Microlaena stipoides* (Ppr) had narrower leaves than the other populations. Seeds of M (Ppr) weighed significantly less and had a faster rate of germination than the other three populations. *Microlaena stipoides* (Ppr) exhibited greater shade tolerance, while M (Dgl) showed greater tolerance to full light. *Microlaena stipoides* (Dgl) and M (Paq) exhibited a greater tolerance to water stress than M (Ppr) and M (Lpe). Random amplified polymorphic DNA banding patterns of the four populations showed greater base sequence divergence of in M (Ppr) compared with the other three populations. It is suggested that the greater divergence of M (Ppr) from the three other populations resulted from interspecific competition with the associated naturalized perennial species, *P. pratensis*. Coexistence between *M. stipoides* populations and introduced and naturalized perennial grass species in permanent pastures could be due to the balancing of competitive abilities between natural neighboring pairs.

**Key words:** plant competition, population divergence, microevolution, genotypic variation, light intensity, water stress tolerance, DNA fingerprinting, natural selection, plant association, coexistence, competitive abilities

**INTRODUCTION**

Genetic variation within populations is a prerequisite for adaptation and evolutionary change (Ennos 1983). High intraspecific variability has been observed in many species having populations that diverge spatially into various ecotypes, each uniquely adapted to a local microenvironment (Baker 1974; Linhart and Grant 1996). Heterogeneity in the abiotic and biotic environment generate different selection pressures leading to genetic heterogeneity (Antonovics 1971; Hedrick *et al.* 1976).

Pasture communities are assemblages of coexisting species that have become adapted to local abiotic and biotic environmental conditions. These pasture communities exhibit patterns in time and space caused by variations in the physical attributes of the environment which interact with the species present (Pemadasa et al. 1974) and by interactions among neighboring species (Van Valen 1973). Thorhallsdottir (1990a) found that patterns in a permanent grassland community were generated and maintained by plants themselves through species-specific interactions. Each species imposes its own rate and scale of change and patterns in plant communities cannot be viewed on a single spatial or temporal scale (Thorhallsdottir 1990b).

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 (1989) 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 neighbor-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 are reduced or eliminated resulting from selection for niche differentiation (Turkington and Aarssen 1984) and balancing of competitive abilities (Aarssen 1983, 1985, 1989). 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). 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 (Aarssen 1989).

*Microlaena stipoides* (Labill.) R. Br. is a slender, tufted, year-long green perennial grass native to Australia, New Zealand, Indonesia, Hawaii, Papua New Guinea, and some Pacific Islands (Connor and Matthews 1977; Wheeler et al. 1990). It is an important component of native, natural, and improved pastures on the Northern Tablelands of New South Wales providing forage during critical winter-early spring period (Taylor and Hedges 1984; Robinson and Archer 1988). When grown with *Trifolium* spp., the productivity of the resultant pasture approaches that of an improved pasture (Lodge and Whalley 1989; Munich et al. 1991). *Microlaena stipoides* grows well in association with introduced exotic pasture species including *Lolium perenne* L., *Dactylis glomerata* L., and *Phalaris aquatica* L., and naturalized grasses such as *Poa pratensis* L. in permanent pastures on the Northern and Southern Tablelands of New South Wales.

The aim of this study was to determine morphological, ecological, and genotypic patterns of variation among the four populations of *M. stipoides*, each

found growing in association with a different species of another grass, *L. perenne* L., *P. pratensis* L., *D. glomerata* L., and *P. aquatica* L., in permanent pastures. A divergence experiment was conducted in the field to determine the occurrence of microevolution among *M. stipoides* populations. A plant competition experiment was designed to determine the growth performance and competitive abilities of four *M. stipoides* populations growing in association with their natural and non-natural neighboring perennial grasses under glasshouse conditions.

It was hoped that the results of this research would add significantly to knowledge of the competitive relationship of *M. stipoides* with four naturalized or sown pasture grasses in permanent pastures. More importantly, the research aimed to expand understanding of the divergence among the populations of one grass species arising from association with neighboring different species of grasses.

## METHODS

### Collection Sites of Four *M. stipoides* Populations

Samples of three *M. stipoides* populations growing in association with *L. perenne*, termed as [M (Lpe)]; *P. pratensis*, [M (Ppr)]; and *D. glomerata*, [M (Dgl)]; respectively, were collected from a permanent pasture at 'Karuah', a commercial grazing property about 50 km north-east of Armidale. The three collection sites at 'Karuah' were located within a 10.2 ha paddock that had uniform pasture management strategies such as phosphate fertilization, pasture improvement, minimal soil disturbance, stocking rate, spell period, and grazing intensity. Samples of a fourth population, growing in association with *P. aquatica* [M (Paq)], were collected from another permanent pasture grazed by cattle and sheep at 'Powalgarh', about 40 km north of Armidale. At each site measuring 3 to 4 m in diameter, four closely spaced bulk plants of *M. stipoides* and the associated grass species were dug 10-cm deep together with the soil underneath to keep the root system intact. The plants were placed inside plastic bags and the leaves and culms were clipped (Whalley and Brown 1973) before adding a small amount of water to keep the soil moist.

Samples of M (Lpe) were collected from the flat, south-western corner of the paddock at 'Karuah' near a gravel road, where the soil is clayey and becomes sticky when wet. *Lolium perenne* has a strong clonal growth pattern with a balance between growth at the apex and death of the old basal stem (Brock and Fletcher 1993). It is most suitable for high fertility conditions (Levy 1970) and showed considerable increase in yield with added nitrogen (Wedderburn et al. 1993). However, the yield of *L. perenne* is limited by its poor adaptation to summer heat and drought (Charmet et al. 1993).

Samples of M (Ppr) were collected from the lower south-eastern part of the paddock about 100 m away from the collection site of M (Lpe), where the soil is also clayey and since it is a low-lying area, the soil is wetter most of the time

compared with the other two collection sites. The collection site was shaded by a big tree and M (Ppr) was growing in association with *P. pratensis*. *Poa pratensis* naturally occurs more often in hollows than on ridges because the higher soil moisture content and fertility levels in the hollows favor its increased tillering (Skinner and Noll 1919; Hartwig 1938; Bennett et al. 1972; Reader and Bonser 1993).

Samples of M (Dgl) were collected from the upper slope on the north-eastern part of the paddock about 200 m away from the other two collection sites. Soil in this site is stoney where M (Dgl) was growing in association with *D. glomerata*, a tufted perennial suitable for moderate fertility and dry regions (Levy 1970). *Dactylis glomerata* was found to grow more commonly on the ridges where soil fertility levels and soil moisture content were probably lower than in the hollows (Reader and Bonser 1993).

Samples of M (Paq) were collected from an open north-facing site with a stony soil growing in association with *P. aquatica*. *Phalaris aquatica* has a rhizomatous growth habit (Rumball 1980), survives under hard grazing (Hutchinson 1970), and shows high pest tolerance (Stevens et al. 1993). In addition, *P. aquatica* has high persistence during drought (Robinson 1952).

'Karuah' and 'Powalgarh' have an elevation of about 1200 to 1330 m above sea level with gentle slopes varying from 1 to 8%. Median rainfall is 75-150 mm in January, 50-125 mm in April, 50 mm in July, and 75 mm in October (Lea et al. 1977a). Mean daily minimum and maximum temperatures are 12-16°C and 28°C in January, 6-10°C and 20-22°C in April, 0-2°C and 12-14°C in July, and 6-10°C and 22-24°C in October (Lea et al. 1977a). The soil at 'Karuah' is a red podzol derived from paleozoic metamorphic shales and sandstones while that at 'Powalgarh' is a chocolate soil and both soil types have moderate organic matter and nutrient contents (Lea et al. 1977b).

These pastures were mixtures of perennial and annual grasses, sedges, and herbaceous dicots including *Trifolium repens* L. At 'Karuah', 0.34 kg/ha of *T. repens* (white clover cv. 'NZ') was seeded in the paddock in July 1961, while in the autumn of 1972, 0.84 kg/ha each of *L. perenne* (hybrid rye and perennial rye) and *D. glomerata* (Danish cocksfoot and Curry cocksfoot) were aurally seeded. Superphosphate was applied annually at a rate of 125 kg/ha from 1961 until 1990. At 'Powalgarh', *P. aquatica* (3.37 kg/ha), *L. perenne* (1.12 kg/ha), and *T. repens* (1.12 kg/ha) were broadcast in 1960. Superphosphate fertilizer was applied annually in the pasture (125 kg/ha) from 1960 to 1980.

### Propagation of Planting Materials

Each plant of *M. stipoides* and *L. perenne* was divided and its ramets planted individually in 9.5 cm diameter plastic pots using 1:1:1 sand:soil:peat moss mixture. The ramets were propagated under glasshouse conditions, fertilized with 0.05% Aquasol solution (23% N, 4% P, 18% K, 0.05% Zn, 0.06% Cu, 0.0013% Mo,

0.04% S, 0.15% Mn) weekly and watered regularly. Repeated subdivision and cutting of the plants were done until enough ramets were generated for the different experiments.

### **Response to Light Intensity Experiment**

Potted plants of the four populations were grown in the field under four light treatments; full sunlight, one layer, two layers, and three layers of Sarlon shade cloth used to cover all four sides and top of 750 mm x 950 mm x 550 mm steel frames. The weekly accumulated intensity of transmitted light under the Sarlon cloth-covered steel frames as well as in the open were measured using light integrators (Magcale-Macandog 1994) positioned in the centre of each cage at a 60° angle from the horizontal facing magnetic north using 300 mm high wooden pole. The weekly accumulated light intensity under each of the frames was expressed as a percentage of the light intensity in the open.

A ramet of each of the four samples of each population was transplanted in 15 cm pots filled with a 1:1:1 sand:soil:peatmoss mixture on 26 September 1990. There was thus a total of sixteen pots under each light treatment and the pots were arranged in a completely randomized design under each light treatment. Slow-release fertilizer (1.3 g Osmocote/pot) was applied every three months and the plants were watered as necessary.

Biomass was harvested four times by cutting the shoots 5 cm from the surface of the soil on the following dates: July 1992, November 1992, February 1993, and May 1993. Oven-dry weights (80°C for at least 48 h of the harvested shoot biomass were determined and the total shoot dry matter was summed across all four sampling dates. Numbers of panicles exerted between November 1991 and April 1992 were counted weekly and the total number of panicles produced during this period was computed. In July 1991, ten full grown leaves per ramet were selected randomly to measure leaf length and leaf width.

In November 1991, full grown leaves were sampled randomly from the ramets for chlorophyll analysis. Chlorophyll content was determined by cutting a 0.1 g sample of fresh leaf material into small pieces and homogenizing for 1 min with 5 mL of 1:4 water:acetone solution (Arnon 1949). An additional 10 mL of the water:acetone solution was added and mixed. The tubes were stoppered, covered with aluminum foil, and left in the dark for 20 h to allow extraction of chlorophyll from the leaf tissue. After chlorophyll extraction, the tubes were centrifuged at 3,000 rpm for 5 min, decanted and the absorbance at 652 nm was read against a water:acetone blank using a spectrophotometer. The amount of chlorophyll (mg chlorophyll/g of leaf fresh weight) was calculated following Arnon (1949).

Analyses of variance of all the data on harvested shoot dry mass, leaf length, leaf width, total number of panicles exerted in one flowering season, and chlorophyll content were done using the STATVIEW statistical package. Significant differences among means were determined using Scheffe's test at 5% significance level.

## Response to Water Stress

The plants were grown in pots in a 4:1 mixture of sandy loam and river sand. The air-dry water content of the mixture was determined gravimetrically by weighing and then drying for 24 and 48 h at 110°C. No further loss of water was recorded after 24 h. The field capacity of the soil mixture was determined by allowing a volume of water equivalent to 7.5% of the fresh weight of the soil to drain through a column of the soil mixture for 72 h. The moisture content of the middle portion of the soil column was determined by the gravimetric method as described above.

Heavy-duty plastic bags were used to line the inside of 100 (small), 125 (medium), and 150 (large) mm diameter plastic pots. Small pots were filled with 500 g, medium pots with, 1,000 g, and large pots with 1,800 g of the soil mixture.

From ramets of the four samples of each population of *M. stipoides*, cleistogamous grains were collected and pooled. Cleistogamous grains of the four *M. stipoides* populations and grains of *Lolium perenne* L. were germinated on moist germination pads in petri dishes inside a 25°C incubator. The germinated grains were transplanted individually into pots when the radicle length was about 1 cm. There were ten replicates for each population at each pot size, making 150 pots altogether. The soil mixture in the pots was brought to field capacity with a 0.05% Aquasol solution prior to transplanting. The soil surface of each pot was covered with a known mass of white polythene granules to minimize water loss from the soil surface. Pots were arranged randomly in the glasshouse. The water content of the soil mixtures was brought up to field capacity daily with appropriate volumes of 0.05% Aquasol solution for 40 days before the first stress cycle commenced.

The first stress cycle consisted of leaving each pot unwatered for one day. Aquasol solution (0.05%) was then added to bring each pot back up to field capacity. Increasing stress periods of 1, 2, 4, 5, 7, 9, 11, 11, 13, 13, 16, 20, and 25 days were used. A plant was considered dead when all the leaves had totally lost green color or no new shoots emerged after watering.

At the death of each plant, the number of drying days at which 100% of the leaves had died was computed. Analysis of variance of the data on 100% leaf mortality was done using the STATVIEW statistical package and significant differences among means were determined using Scheffe's test at the 5% level of significance.

## Grain Weight Measurement and Grain Germination Tests

Grains produced by cleistogamous inflorescences in the glasshouse were collected from several plants of each of the four samples of each *M. stipoides* population. The grains were air-dried and 4 lots of 100 mature, plump grains (enclosed in their ancillary structures) weighed and mean grain mass calculated. Four lots of twenty-five grains of each *M. stipoides* population were arranged on a

wet germination pad in square plastic petri plates. The grains were incubated at 25°C and the number of germinated seeds were counted daily. A seed was considered germinated if 0.5 cm of the radicle had emerged.

### DNA Extraction

Leaves from several plants of each of the four *M. stipoides* populations and of *L. perenne* were harvested, pooled for each population, washed with distilled water, and stored in a freezer at -70°C. Plant genomic DNA was extracted following a procedure modified from Guidet et al. (1991). Leaves were cut into small pieces and ground in liquid nitrogen using a mortar and pestle. The powdered leaves were transferred to 10-mL plastic centrifuge tubes and suspended in 4 volumes (e.g., 4 mL/g powdered leaf) of extraction buffer (0.1 M Tris-HCl, pH 8, 10 mol m<sup>-3</sup> EDTA, 4% sarkosyl), then an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added and the mixture was shaken end over end (20 rpm) for 1 h at 4°C. The tubes were centrifuged for 5 min at 7,000 rpm and 4°C, then the organic layer was discarded and the aqueous layer and interphase containing DNA were re-extracted as described above. Following centrifugation for 5 min at 7,000 rpm and 4°C, the aqueous layer was transferred to another tube and extracted by shaking end to end with an equal volume of chloroform-isoamyl alcohol (24:1) for 1 h at 4°C. After centrifugation (7,000 rpm, 4°C for 5 min), the aqueous layer was transferred into another centrifuge tube and genomic DNA was precipitated with 0.1 volume of 3 M Na acetate (pH 4.8), and then 2.5 volumes of 99% ethanol was layered on top of the solution. The mixture was carefully hand-shaken end over end for 1 min and then centrifuged for 15 min at 15,000 rpm and 4°C. The ethanol was carefully removed using a Pasteur pipette and the precipitated DNA was washed three times with 2 mL of 70% ethanol and dried under vacuum for 5 min. DNA was redissolved in 300 µL of TE buffer (10 mol m<sup>-3</sup> Tris-HCl, 1 mol m<sup>-3</sup> EDTA, pH 8.0).

DNA concentrations and purity were determined using a UV spectrophotometer and electrophoresis through 1.5% agarose gel. A 1 µL aliquot of DNA suspension was diluted to 100 µL with sterile water and UV absorbance was determined using a UV spectrophotometer. One µL of DNA suspension was diluted to 10 µL with 8 µL sterile water and 1 µL loading buffer and was electrophoresed through 1.5% agarose gel using TBE (Tris-borate-EDTA) buffer. The DNA was further purified of RNA impurities by RNase digestion. Ten µL of RNase (10 ng/µL) was added to the DNA solution and incubated at 35°C for at least 1 h. The DNA was re-precipitated by adding 0.1 volume of 3 M Na acetate, mixing well and adding 2.5 volumes of 99% ethanol. After centrifugation for 10 min at 13,000 rpm in a microfuge, ethanol and Na acetate were removed using a Pasteur pipette. The DNA was rinsed three times with 70% ethanol, dried under vacuum for 5 min, resuspended in 50 µL of TE buffer, and diluted with 100 µL of sterile water.

### DNA Amplification Fingerprinting (Random Amplified Polymorphic DNA)

Amplification of DNA fragments was performed in a polymerase chain reaction (PCR) mixture consisting of 2  $\mu\text{L}$  10 X PCR reaction buffer, 2  $\mu\text{L}$  25 mol  $\text{m}^{-3}$   $\text{MgCl}_2$ , 0.25  $\mu\text{L}$  16 mol  $\text{m}^{-3}$  each of dATP, dCTP, dTTP, and dGTP (or 1  $\mu\text{L}$  16 mol  $\text{m}^{-3}$  dNTPs), 2  $\mu\text{L}$  20 mol  $\text{m}^{-3}$  primer, 1  $\mu\text{L}$  *Thermus aquaticus* DNA polymerase (1 unit/ $\mu\text{L}$ ), 2  $\mu\text{L}$  template DNA and 10  $\mu\text{L}$  sterile water. The reaction was overlaid with a drop of mineral oil to prevent evaporation of the reaction mixture when heated at 94°C and the mixture was incubated in a Gene Machine-thermocycler (Bartelt Instruments, Melbourne, Australia). The DNA amplification cycles were: denaturation at 94°C for 5 min, annealing at 37°C for 30 sec, and polymerization at 72°C for 60 sec in the first cycle followed by 44 cycles of denaturation at 94°C for 20 sec, annealing at 37°C for 30 sec, and polymerization at 72°C for 30 sec. PCR products were separated on a 1.5% agarose gel electrophoresis at 80 V using TBE (Tris-borate-EDTA) buffer at 80 V for 1.5 h.

### Divergence Experiment

The study was conducted in permanent pastures grazed on 'Karuah' and 'Powalgarh'. Four sites, three at 'Karuah' and one at 'Powalgarh', each 160 cm x 100 cm were chosen for the study. 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.

The experimental design proposed by Connell (1980) to demonstrate coevolution of competitors and adopted by Turkington (1989) 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.

For purposes of consistency, the terminologies used by Connell (1980) and Turkington (1989) 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 4), 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.

At each site a 2 m x 2 m square enclosure 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 systematic 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. Other minor species were also removed, either by herbicide painting or carefully digging out the root system.

Eighty new ramets of each of the four populations of *M. stipoides* were rooted in 5.5 cm diameter pots. 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 were 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.

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 aboveground parts 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 aboveground herbage was determined.

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 Scheffe'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.

### Plant Competition Experiment

The four *M. stipoides* populations and the four associated grass species were paired in all possible ways resulting in sixteen combinations. 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 each other. The pots were arranged in a completely randomized 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 fertilizer (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 fertilizer (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.

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.

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 analyzed using the STATVIEW statistical package. Significant differences among population means were determined using Scheffe's test at the 5% significance level.

## RESULTS

### Response to Varying Light Intensity Levels

One layer of shade cloth reduced the amount of transmitted light to 47% of the incident radiation, two layers to 16%, and three layers of shade cloth to 6%.

There were clear differences among the four *M. stipoides* populations in total aboveground dry mass produced, length and width of leaves, chlorophyll content, and production of panicles across a range of light intensity levels.

#### *Total above ground dry mass*

Total aboveground dry mass of plants varied greatly across the four light intensity treatments. The plants yielded their greatest biomass when grown under 47% transmitted light, followed by 16% transmitted light, and full sunlight (Fig. 1). Dry mass was lowest in plants grown under severe shading (6% transmitted light).

The light intensity x population interaction was significant ( $P < 0.05$ ). The biomass production of plants of M (Lpe) was highest when grown under 47% transmitted light, was reduced by 38% (NS) when grown under 16% transmitted light, by 40% (NS) when grown under full light, and was reduced significantly by 78% ( $P < 0.05$ ) when grown under severe shading (6% transmitted light) (Fig. 1). Plants of M (Ppr) exhibited shade tolerance, having only a 16% yield reduction (NS) when grown under 16% transmitted light, 42% reduction (NS) when grown under full light, and 72% reduction ( $P < 0.05$ ) under severe shading compared with its dry mass yield under 47% transmitted light (Fig. 1). Those of M (Dgl) demonstrated light tolerance, having the least reduction in yield, though non-significant, when grown under full light in proportion to its highest yield (when grown under 47% transmitted light) among the four populations (Fig. 1). The dry mass yield of plants of M (Dgl) was highest when grown under 47% transmitted light, was reduced by 18% (NS) when grown under full light, 30% reduced (NS) under 16% transmitted light, and 76% reduced ( $P < 0.05$ ) when grown under severe shading (Fig. 1). It was only plants of M (Dgl) which exhibited the following trend in dry mass production: 47% transmitted light > full sunlight > 16% transmitted light > 6% transmitted light. The trend of dry mass yields in plants of the other three populations under the different light intensities can be summarized as follows: 47% transmitted light > 16% transmitted light > full sunlight > 6% transmitted light.

#### *Chlorophyll content*

The average leaf chlorophyll content (mg/g fresh weight) of plants of the four populations grown under shade were significantly higher than those grown under full light (Table 1). There were no significant differences in the mean chlorophyll contents of leaves of plants of the four *M. stipoides* populations grown under the three shade intensities (Table 1). Mean leaf chlorophyll contents of plants of the four *M. stipoides* populations averaged over all light intensity levels

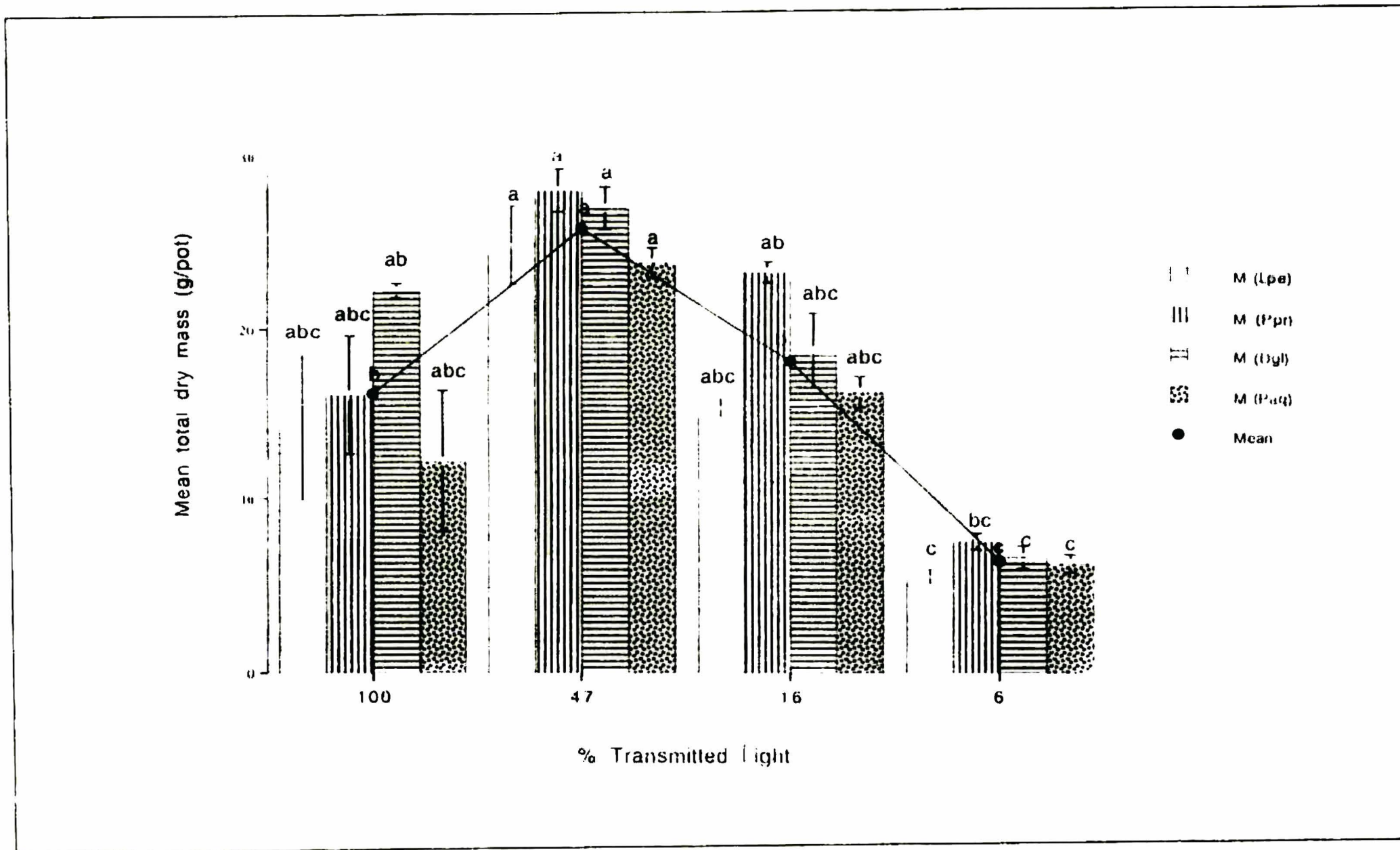


Figure 1. Mean harvested dry mass of shoots of plants from four *M. stipoides* populations grown under varying light intensities. Columns which share the same letter(s) are not significantly different at  $P > 0.05$ . Vertical bars indicate  $\pm$  SE.

Table 1. Mean leaf chlorophyll (mg/g fresh weight) contents of four *M. stipoides* populations grown under varying light intensity levels. Within each group, means which share the same letter are not significantly different at  $P > 0.05$ .

<i>M. stipoides</i> population					
Transmitted light (%)	M (Lpe)	M (Ppr)	M (Dgl)	M (Paq)	Mean
Mean chlorophyll content (mg/g fresh weight)					
100	1.88	2.33	2.03	2.01	2.06 <sup>b</sup>
47	2.52	3.35	2.83	2.73	2.86 <sup>a</sup>
16	2.60	2.92	2.95	2.93	2.85 <sup>a</sup>
6	2.62	3.13	2.77	2.55	2.77 <sup>a</sup>
Mean	2.40 <sup>b</sup>	2.93 <sup>a</sup>	2.64 <sup>ab</sup>	2.56 <sup>ab</sup>	

significantly. The average chlorophyll content of plants of M (Ppr) was significantly higher than those of M (Lpe), while plants of M (Dgl) and M (Paq) were intermediate (Table 1). There was no significant light treatment x population interaction in leaf chlorophyll content.

#### *Leaf length*

The mean leaf (mm) in plants of the four populations was significantly higher when grown under 47% (87.7<sup>a</sup> mm) and 16% (95.1<sup>a</sup> mm) transmitted light than when grown under full light (50.2<sup>b</sup> mm) and severe shading (59<sup>b</sup> mm). The interaction between light intensity treatments and population was not significant ( $P > 0.05$ ) and there were no significant differences in mean leaf lengths between plants of the four *M. stipoides* populations averaged over all light intensity treatments.

#### *Leaf width*

Shading had no significant effect on the average width (mm) of the leaves in plants of the four *M. stipoides* populations. However, significant differences resulted when average leaf width of each population was computed over all light intensity levels. Plants of M (Lpe) (4.42<sup>a</sup>), M (Dgl) (3.94<sup>a</sup>), and M (Paq) (4.06<sup>a</sup>) had significantly ( $P < 0.05$ ) wider leaves than those of M (Ppr) (3.15<sup>b</sup>).

*Panicle production*

Mean total number of panicles exerted for one flowering season by the ramets of the four populations was significantly ( $P < 0.05$ ) higher when the ramets were grown under 47% transmitted light than when grown under 16% transmitted light (Table 2). The mean total number of panicles exerted by the ramets when grown under 16% transmitted light was, however significantly greater than when the ramets were grown under severe shading, while those grown under full light were intermediate (Table 2). The average number of panicles exerted by ramets of M (Ppr) was significantly higher than by those of M (Lpe) and M (Dgl), while that of M (Paq) was intermediate (Table 2). Light intensity treatment and population had no significant interacting effects on panicle production.

**Water Stress Tolerance**

Generally, plants grown in large pots took longer to reach 100% leaf mortality than plants grown in medium and small pots and there was a significant population x pot size interaction ( $P < 0.05$ ) (Table 3). When the total numbers of drying days to 100% leaf mortality for each population were compared across all three pots sizes, seedlings of M (Dgl) and M (Paq) had live leaves significantly longer when grown in the large pots than when grown in the small and medium pots (Table 3). Leaves of seedlings of M (Lpe) and *L. perenne* survived significantly longer when

Table 2. Mean total number of panicles/pots produced by four *M. stipoides* populations grown under varying light intensity levels. Within each group, means which share the same letter(s) are not significantly different  $P > 0.05$ .

<i>M. stipoides</i> population					
	M (Lpe)	M (Ppr)	M (Dgl)	M (Paq)	Mean
Transmitted light (%)	Panicles/pots				
100	9.33	38	13.5	19.25	20.02 <sup>bc</sup>
47	30.25	108	59	57.25	63.62 <sup>a</sup>
16	19.75	49.67	24	41.75	33.79 <sup>b</sup>
6	4.75	20.67	3.75	15.5	11.17 <sup>c</sup>
Mean	16.02 <sup>b</sup>	54.08 <sup>a</sup>	25.06 <sup>b</sup>	33.44 <sup>ab</sup>	

Table 3. Cumulative number of drying days required to 100% leaf mortality of plants of four *M. stipoides* populations and *L. perenne* grown in three pot sizes. Means which share the same letter(s) are not significantly different at  $P > 0.05$ . Comparisons are made in both directions as there was a significant population x pot size interaction ( $P < 0.05$ ).

Pot size	Population					Mean
	M (Lpe)	M (Ppr)	M (Dgl)	M (Paq)	<i>L. perenne</i>	
Small (100 mm D)	30.2 <sup>bc</sup>	37.9 <sup>bc</sup>	43.5 <sup>bc</sup>	42.1 <sup>bc</sup>	25.5 <sup>c</sup>	36.8
Medium (125 mm D)	40.2 <sup>bc</sup>	52.6 <sup>bc</sup>	42.4 <sup>bc</sup>	46.6 <sup>bc</sup>	34.2 <sup>bc</sup>	44.0
Large (150 mm D)	78.4 <sup>ab</sup>	64.8 <sup>abc</sup>	97.8 <sup>a</sup>	94.5 <sup>a</sup>	53.3 <sup>bc</sup>	77.1
Mean	49.6	51.8	61.2	61.1	37.6	

grown in large pots than when grown in small pots (Table 3). There were no significant differences in the total number of drying days to 100% leaf mortality in seedlings of M (Ppr) when grown in all three pot sizes (Table 3). In the total number of drying days to 50% leaf mortality, there was no significant pot size x population interaction.

### Grain Weight Measurement and Seed Germination Tests

Mean grain weight from ramets of M (Ppr) was significantly smaller (3.55<sup>b</sup> mg/grain) than the mean grain weights from ramets of the other three *M. stipoides* populations growing in association with *L. perenne* (M (Lpe), 5.14<sup>a</sup> mg/grain), *D. glomerata* (M (Dgl), 5.3<sup>a</sup> g/grain), and *P. aquatica* (M (Paq), 5.58<sup>a</sup> mg/grain).

Grains from ramets of M (Ppr) germinated faster than bigger grains from ramets of the other three populations (Fig.2). Grains from ramets of M (Lpe) had the lowest rate of germination, while those from ramets of M (Dgl) and M (Paq) were intermediate (Fig. 2).

### DNA Amplification Fingerprinting (Random Amplified Polymorphic DNA)

The DNA fingerprints of ramets of the four populations showed polymorphism although there were similarities in the location of three major conserved bands (labelled a, b, and c; Fig. 3) and a minor conserved band (labelled ‘;’; Fig. 3) among the four populations. Among the four *M. stipoides* populations,

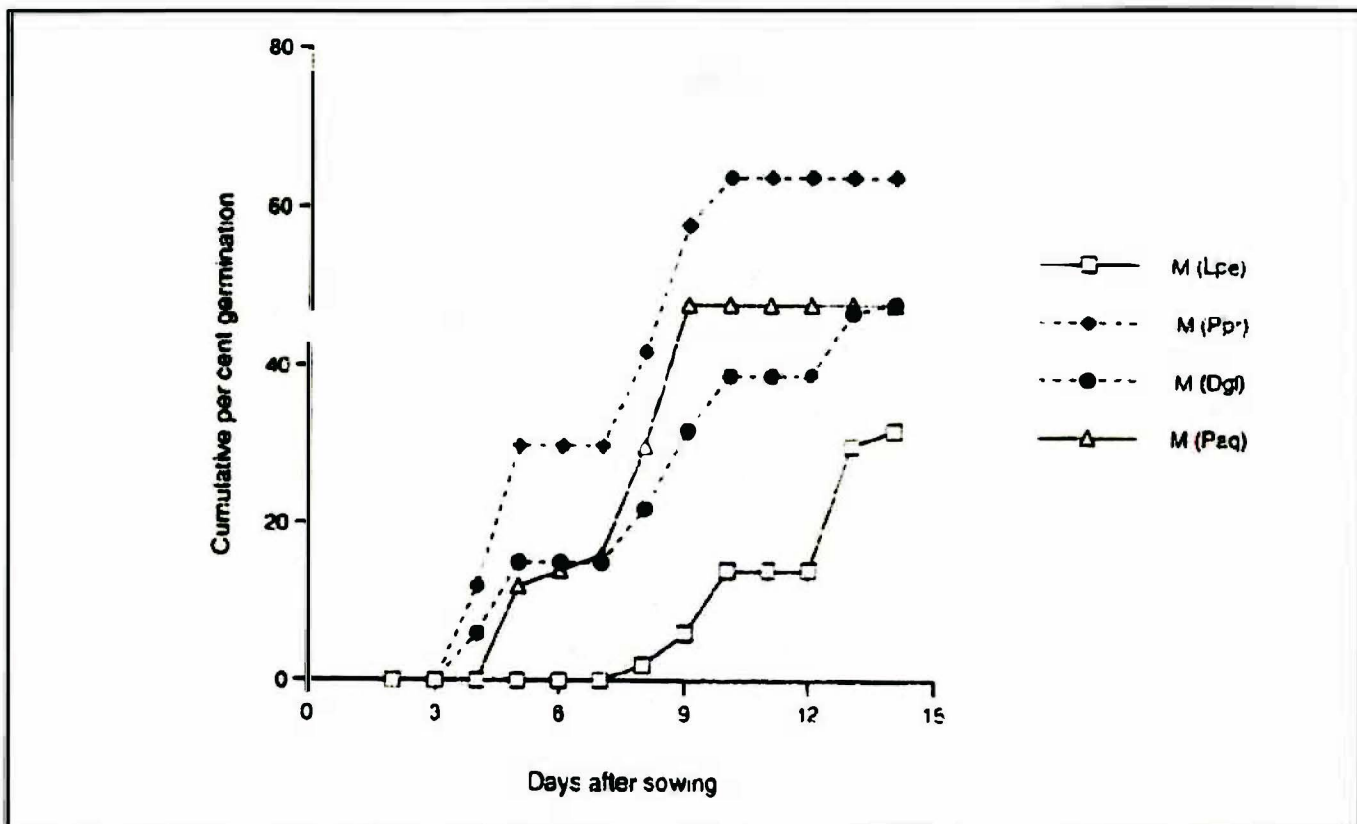


Figure 2. Cumulative seed germination of grains produced in the glasshouse by plants from four *M. stipoides* populations.

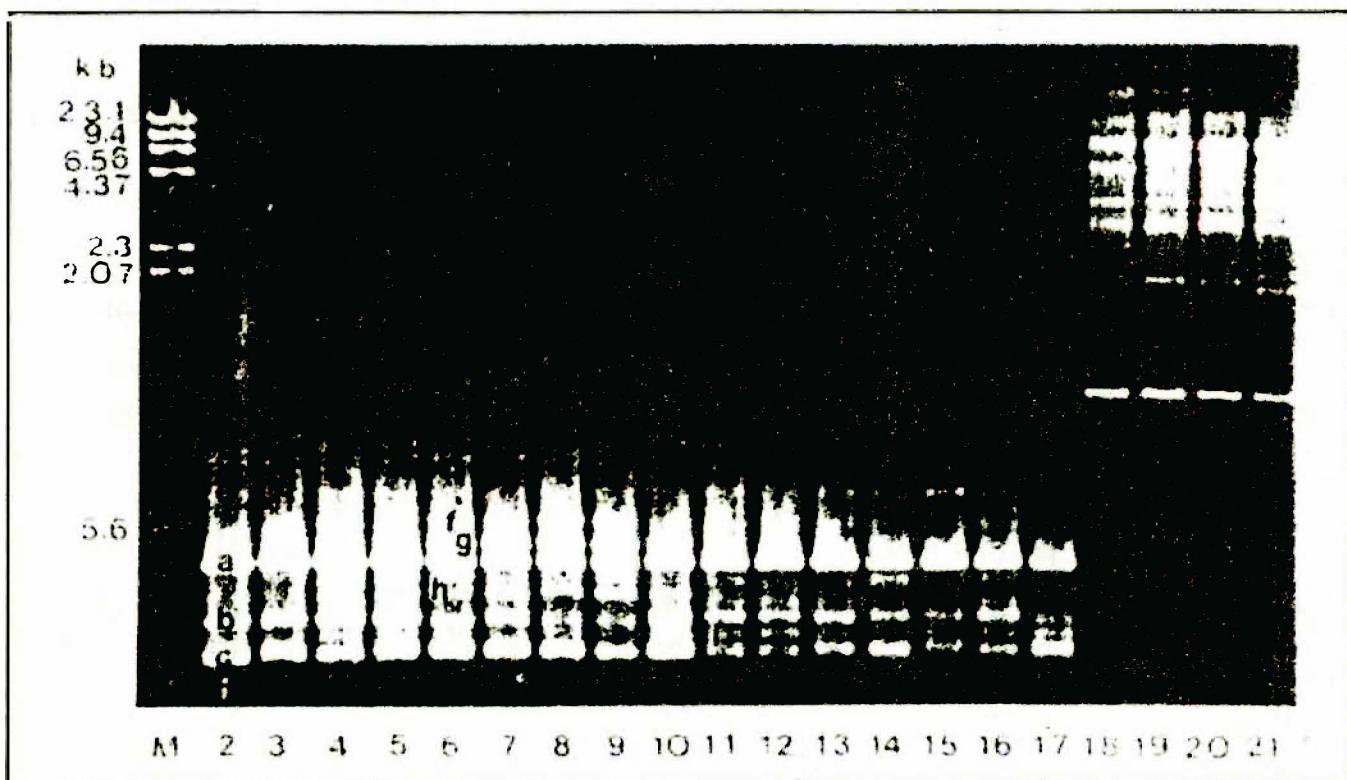


Figure 3. Intraspecific polymorphisms among plants from four *M. stipoides* populations and interspecific polymorphism between *M. stipoides* and *L. perenne* revealed by amplification with primer 12MER10. Each population is represented with four lanes: two replicates at each concentration: 1 and 2 ng/mL. lanes 2-5: M (Lpe), lanes 6-9: M (Ppr) lanes 10-13: M (Dgl), lanes 14-17: M (Paq), and lanes 18-21: *L. perenne*.

M (Ppr) showed the greatest variation in the banding pattern of major and minor bands from the other three populations (Fig. 3). Two major variable bands (labelled f and g; Fig. 3) and a minor variable band (h; Fig. 3) were present in M (Ppr) fingerprints, but not in the other three populations. Two minor bands (d and e; Fig. 3) were present in the three *M. stipoides* populations, (M (Lpe), M (Dgl), and M (Paq)), but absent or very faint in the M (Ppr) banding pattern. A minor band (I; Fig. 3) was distinctly present in the M (Dgl) and M (Paq) banding patterns and faintly present in the M (Lpe) banding pattern, but not in the M (Ppr) banding pattern. Ramets of the other species, *L. perenne*, gave distinctly different banding patterns compared with those of the four *M. stipoides* populations both in the major and minor bands.

Variations in the DNA fingerprints among the ramets within the population were also evident (Fig. 3). Minor bands (d, e, and I) found in M (Lpe), M (Dgl), and M (Paq) varied among the four samples within the populations in terms of width and distinctness. Within the M (Ppr) population, variation in the width and distinctness of bands f, g, and h were also observed. A minor band above the band labelled 'h' is distinct in the first and fourth samples (lanes 6 and 9) of the M (Ppr) population but very faint in the two middle ones.

## Divergence Experiment

### *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 (Table 4). 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. 4).

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 allopatric sites (Fig. 4). 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. 4).

Table 4. 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 (1989).

Treatment number (T)	Origin of <i>M. stipoides</i> population	Transplanting site	Removal or no removal of competitor species	Removal of native <i>M. stipoides</i> population
T1	allopatric M (Ppr) M (Dgl) M (Paq)	sympatric Lpe Lpe Lpe	not removed	removed
T2	allopatric M (Ppr) M (Dgl) M (Paq)	sympatric Lpe Lpe Lpe	removed	removed
T3	allopatric M (Ppr) M (Dgl) M (Paq)	allopatric Ppr Dgl Paq	not removed	removed
T4	sympatric M (Lpe)	sympatric Lpe	not removed	removed
T5	sympatric M (Lpe)	sympatric Lpe	removed	removed
T6	sympatric M (Lpe) M (Lpe) M (Lpe)	allopatric Ppr Dgl Paq	not removed	removed

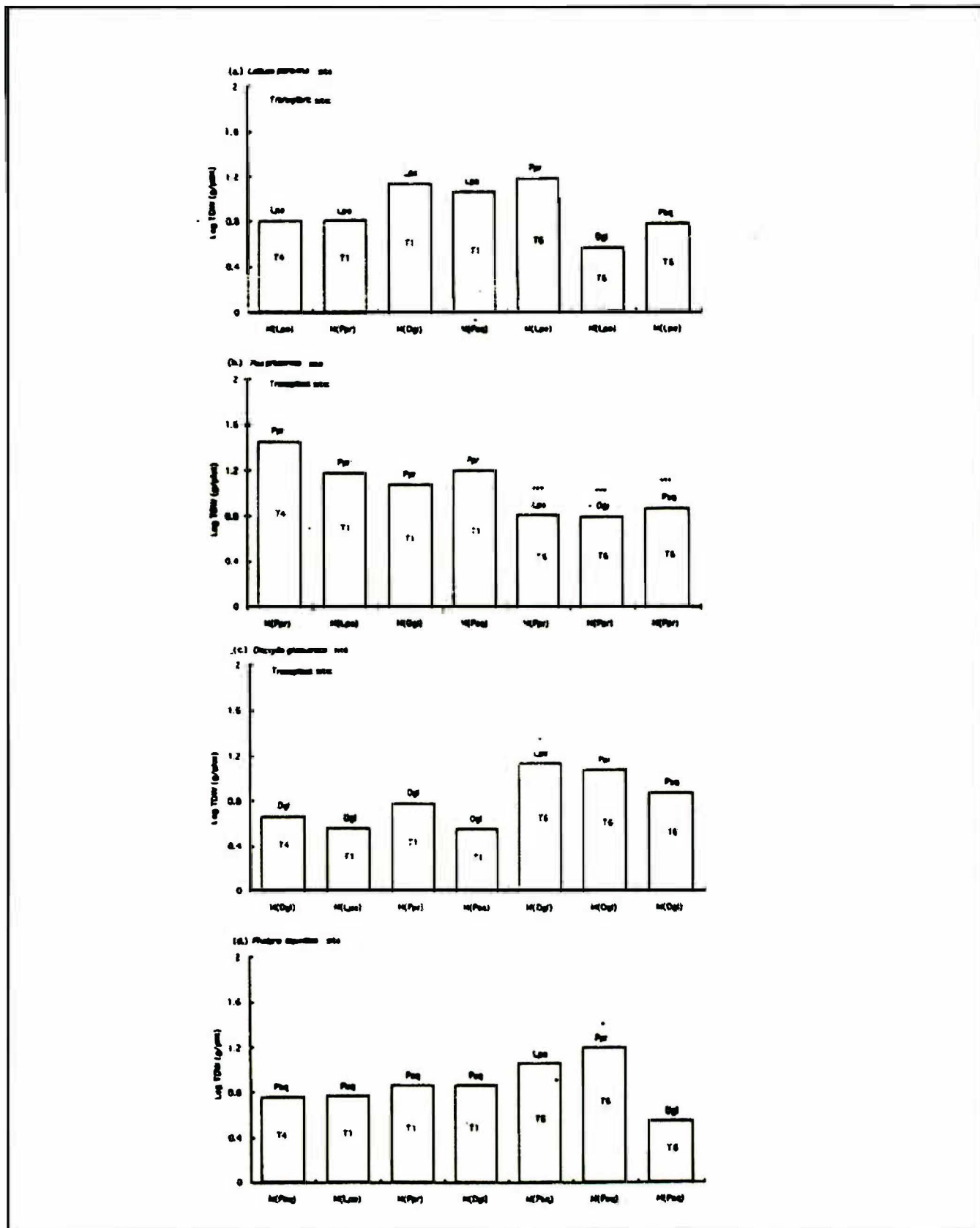


Figure 4. Total plot dry weight (TDW) of *M. stipoides* populations collected from and transplanted into four different sites where only the native *M. stipoides* population was removed (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments (TV v T1 and TV 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).

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. 4); thus it did not show divergence of M (Paq).

#### *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 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 (Fig. 5). 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 (Fig. 5).

Divergence due to the 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 of sympatric M (Ppr) (T5) was significantly higher compared with the total dry weights of allopatric populations (T2) of M (Dgl) and M (Paq) when transplanted in the sympatric *P. pratensis* site (Fig. 5b). The significant difference between the total dry weights of sympatric M (Dgl) and allopatric M (Ppr) transplanted into the sympatric *D. glomerata* site did not indicate divergence due to past competition (Fig. 5c). 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. 5c).

#### *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

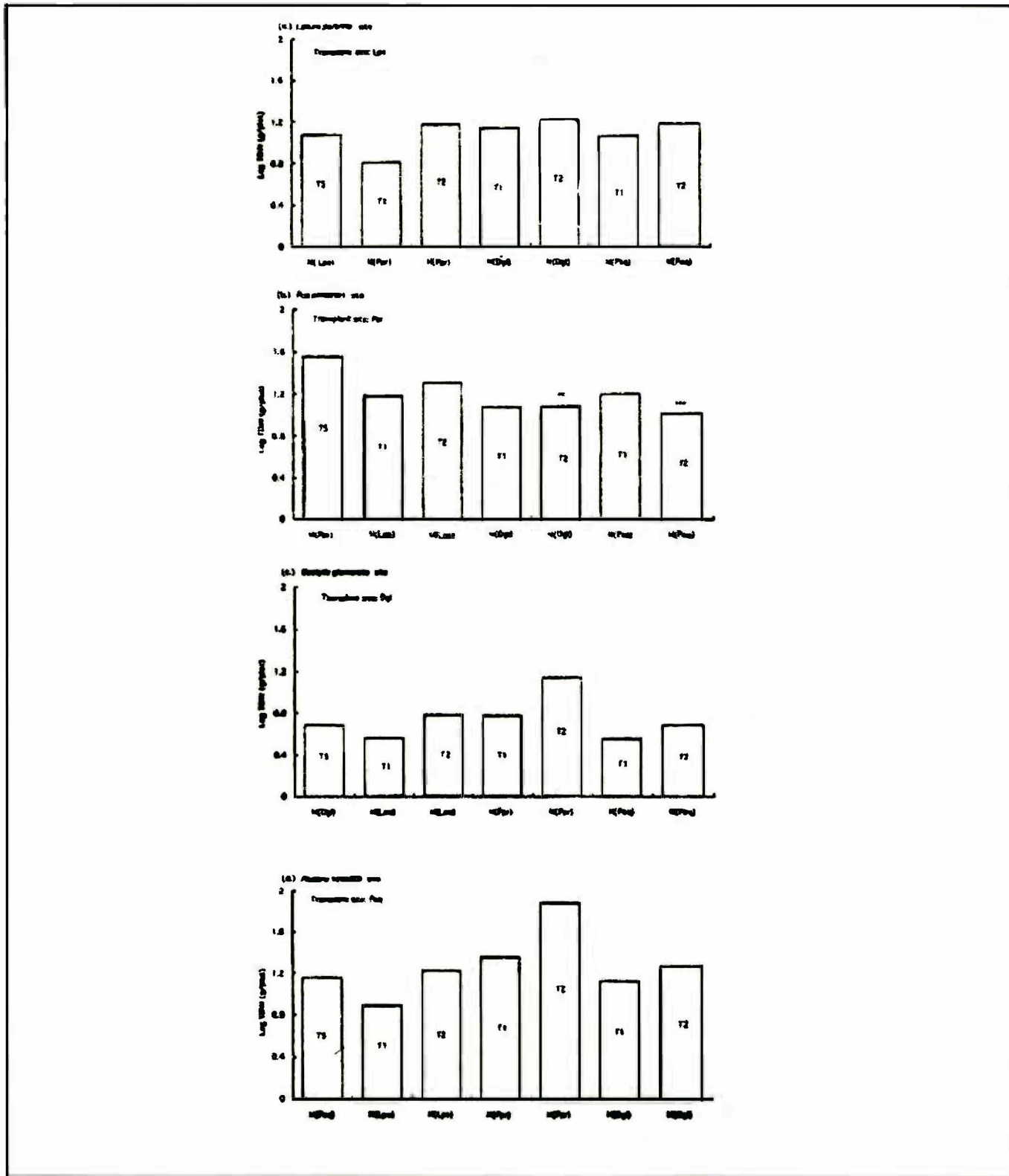


Figure 5. Total plot dry weight (TDW) of *M. stipoides* populations collected from and transplanted into four different sites where the native *M. stipoides* population and associated grass species were removed: (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris 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* population. Results of all pairwise T2 v T1 comparisons were non-significant. Asterisks refer to the results of Bonferroni pairwise comparisons (\*\*\*, 0.1%; \*\*, 1%; \*, 5% level of significance).

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).

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).

### Plant Competition Experiment

The component yield of M (Lpe) grown with *L. perenne* was relatively higher than when it was grown with the other three non-natural neighboring grass species, though non-significant ( $P > 0.05$ ) (Fig. 7a, bar graph). The component yields of the three non-natural neighboring 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. 7). This resulted in a relatively higher total combined yield of M (Lpe) and each of the three non-natural neighboring associated grass species (Fig. 7a, 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. 7a). The M (Lpe) - *P. aquatica* mixture had the largest discrepancy in the total dry weights of the component species (Fig. 7a).

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. 7b, 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) outyielding *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. 7b, line graph). The differences in total dry weights of the component species of all mixtures of M (Ppr) and the four

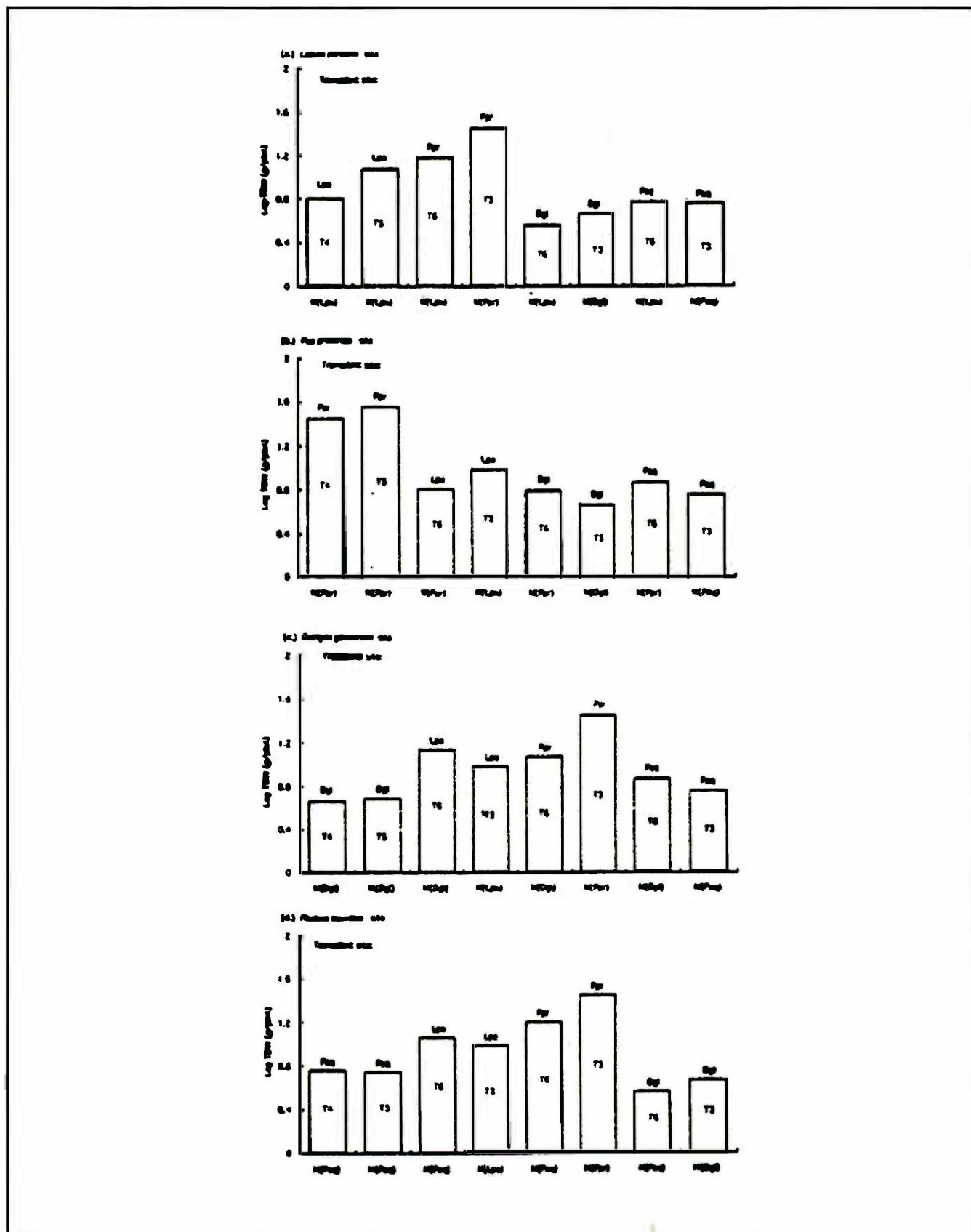


Figure 6. Total plot dry weight (TDW) of *M. stipoides* populations collected from and transplanted into four different sites. (Lpe, *Lolium perenne*; Ppr, *Poa pratensis*; Dgl, *Dactylis glomerata*; and Paq, *Phalaris aquatica*). Pairwise comparison of treatments T4 v T5 and T6 v T3) are necessary to demonstrate a genetic basis of *M. stipoides* population divergence. Asterisks refer to the results of Bonferroni pairwise comparisons (\*\*\*, 0.1%; \*\*, 1%; \*, 5% level significance).

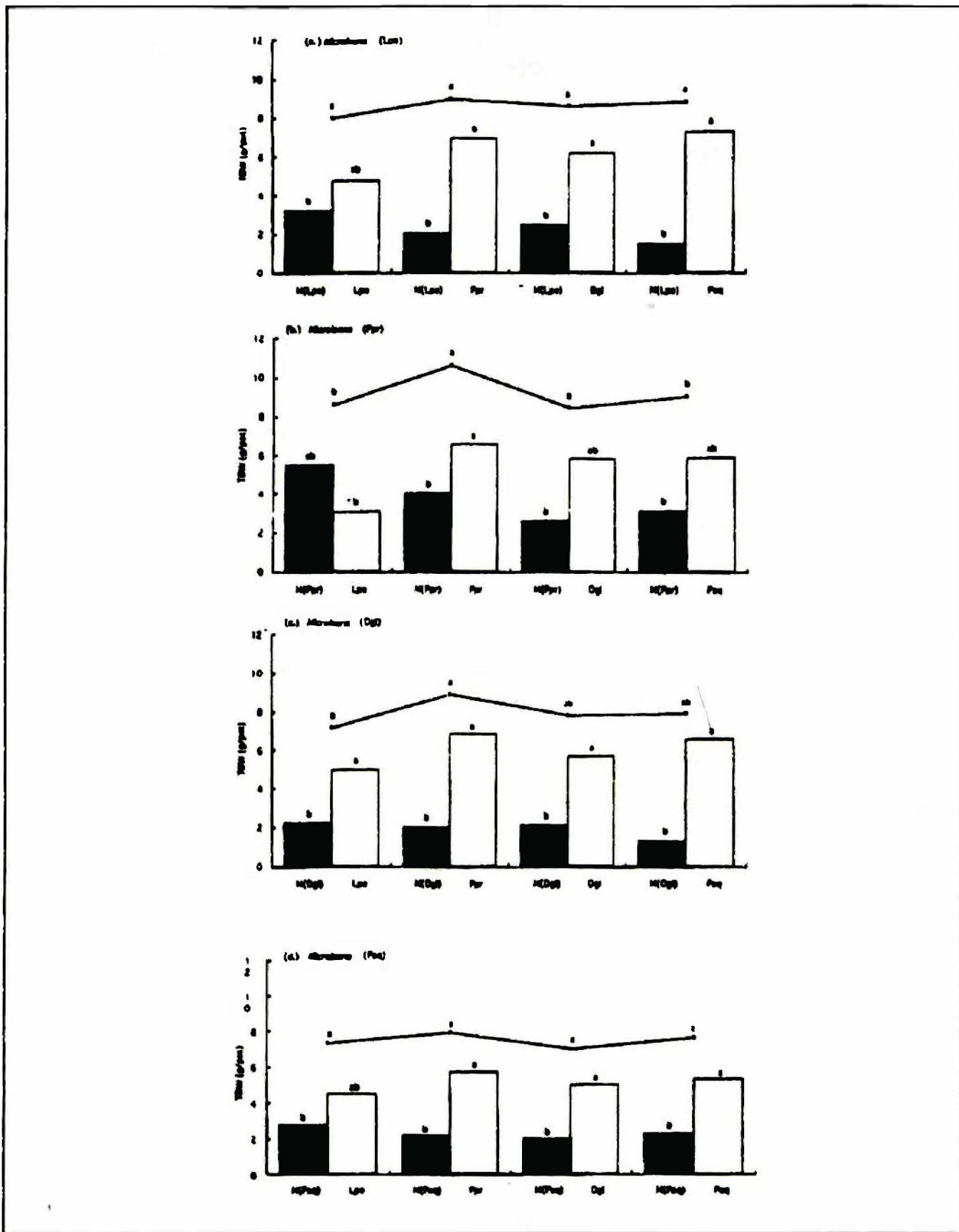


Figure 7. Total plot dry weight (TDW) over 45 weeks of growth of four *M. stipoides* populations grown with four grass species. Filled and open bars indicate mean dry weights of *M. stipoides* populations and associated grass species, respectively. Line graphs indicate total yield of mixtures. Points in the line graph and bars in the bar graph which share the same letter(s) are not significantly different ( $P < 0.05$ ).

grass competitor species were generally narrower than all the other mixtures of the other three *M. stipoides* populations and grass competitor species (Fig. 7).

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. 7c 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. 7c). 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. 7c, line graph). There were no significant differences in the combined total yields (line graph) of M (Paq) and the grass mixtures (Fig. 7d). Total dry weights of the component species in the M (Paq) – *L. perenne* mixture did not differ significantly (Fig. 7d).

## DISCUSSION

The four populations of *M. stipoides* exhibited genotypic differences in their growth performance under varying light intensity levels, survival under varying moisture regimens, grain size, seed germination rate, leaf width, and amplified DNA fingerprints on the basis of the four representative ramets collected from each population. Generally, all the populations produced the highest total dry mass when grown under 47% transmitted light. *Microlaena stipoides* (Ppr) showed greater shade tolerance than the other populations. When grown under 16% transmitted light, M (Ppr) gave the smallest reduction in total dry mass compared with other three populations. *Microlaena stipoides* (Ppr) had a higher chlorophyll content than the other populations when grown under 47% transmitted light. *Microlaena stipoides* (Dgl) showed a significant tolerance to high light intensity, producing greater dry mass when grown under full light compared with the other *M. stipoides* populations.

The different grasses associated with each *M. stipoides* population perhaps alter the transmitted radiation below their canopies because they have different growth habits. Although *P. pratensis* and *D. glomerata* are both tufted perennials, *P. pratensis* produces a 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). 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, with M (Ppr) more adapted to lower light intensity levels compared with M (Dgl), which was more tolerant of higher light intensity levels. Shade provided by neighboring plants and the consequent reduction of available light is a biotic effect which acts as a selective force for evolutionary divergence of plants (Linhart and Grant 1996). Teramura and Strain (1979) reported that the substantial differences in the photosynthetic response of closely-growing *Plantago lanceolata* populations to irradiance and temperature involve

genetic differences in the internal control of photosynthesis and photosynthetic enzyme activity.

*Microlaena stipoides* (Ppr) produced a larger number of panicles at all light intensity levels compared with the other four populations. Because M (Ppr) produced more panicles, perhaps it has higher grain production than the other populations, assuming the same number of grains per panicle. Grains produced by M (Ppr) generally weighed significantly less (3.55 mg/grain) and had a faster rate of germination than grains of the other populations. Production of larger quantities of smaller or lighter grains could be an adaptive strategy of M (Ppr) by allocating less energy resources per seed but producing larger quantities of seed.

Generally, the number of drying days to 100% leaf mortality increased with an increase in pot size for all the four *M. stipoides* populations. *Microlaena stipoides* (Dgl) and M (Paq) showed a relatively longer period to 100% leaf mortality when grown in large pots compared with when grown in small and medium pots. The other two populations, M (Ppr) and M (Lpe) did not exhibit a significant increase in the total number of drying days to 100% leaf mortality when pot size was increased from medium to large. Averaged over all pot sizes, M (Dgl) and M (Paq) took a longer time to reach 100% leaf mortality compared with M (Ppr) and M (Lpe), although this was not statistically significant.

Samples of *M. stipoides* (Dgl) were collected from the upper slope on the north-eastern part of the paddock at 'Karuah', growing in association with *D. glomerata*. *Dactylis glomerata* has been reported to be suitable for dry regions (Levy 1970), with more successful establishment than *L. perenne* on dry hills (White et al. 1972; Barker et al. 1993), and had greater yields than *L. perenne* after moisture stress (Wedderburn et al. 1993). Samples of *M. stipoides* (Paq) were collected from an open north-facing site with a stony soil at 'Powalgarh', growing in association with *P. aquatica*. *Phalaris aquatica* has high persistence during drought (Robinson 1952) and can avoid drought by becoming dormant (McWilliam and Kramer 1968).

The significant variations in the increase of total number of drying days to 100% leaf mortality among the two of the four *M. stipoides* populations indicate variations in physiological adjustment to water stress. Among the four populations, M (Dgl), and M (Paq) exhibited a greater tolerance to water stress compared with M (Lpe) and M (Ppr). These two *M. stipoides* populations which exhibited greater tolerance to water stress were growing in association with neighboring grass species that have been noted for water stress tolerance and were situated in the upper slopes of the paddock where soil moisture is likely to be more limiting compared with the lower slopes or flat areas where the other two populations were situated. It is possible that the neighboring grass species as well as the abiotic environmental conditions such as soil moisture availability and soil physical properties exerted natural selection forces on the *M. stipoides* populations for water stress tolerance. This indicates that sufficient genetic variation in plant water relations existed among *M. stipoides* populations to allow an evolutionary response to the selection pressures on drought resistance (Farris 1987).

DNA fingerprints of the four *M. stipoides* populations exhibited amplified fragments common to all populations, which may indicate the phylogenetically conserved regions as well as individual-specific bands. Variations in the DNA banding patterns among samples within *M. stipoides* populations show that genotypic variation occurs among individuals within each population. Likewise, significant variations among the DNA banding patterns between *M. stipoides* populations show that greater genotypic differentiation exists among the four *M. stipoides* populations. Amplified polymorphic DNA segments of M (Ppr) showed the greatest divergence in banding patterns compared with the other three populations, which parallels the divergent growth performance of M (Ppr) when grown under different light treatments and water stress in terms of varied leaf width, seed size, and seed germination rate.

*Microlaena stipoides* (Ppr) was collected from the lower south-eastern part of the paddock at 'Karuah' growing in association with *P. pratensis*. *Poa pratensis* is a naturalized species in these permanent pastures and might have become established in the area soon after the arrival of Europeans in the early 19<sup>th</sup> century, while *L. perenne*, *D. glomerata*, and *P. aquatica* were introduced in the early 1960s. Association between *P. pratensis* and M (Ppr) populations may have taken place at a much longer time frame compared with the association between the other three *M. stipoides* populations and the other introduced pasture grass species. Exposures to selection pressures exerted by *P. pratensis* over a longer time frame may have accumulated and led to greater evolutionary divergence of M (Ppr) populations compared with the other three *M. stipoides* populations.

These findings show that phenotypic and genotypic variations occur among *M. stipoides* populations growing in association with different neighboring perennial grasses in a permanent pasture. The four populations exhibited morphological and ecological differences which may have evolved through natural selection resulting from competitive interactions with their associated perennial grasses or from selection pressures associated with differences in light and water availability and perhaps, other abiotic microenvironmental factors, at the locations from which they were collected. The complex breeding system of *M. stipoides* (Connor and Matthews 1977) enables it to control or permit rapid genetic changes so as to be able to adjust to changing local biotic and abiotic microenvironments. The process of divergence in the *M. stipoides* populations is likely to involve both biotic interactions with neighboring 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. Competitive interactions between *M. stipoides* and the associated grasses may be influencing genetic differentiation among the four *M. stipoides* populations.

The divergence field experiment 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

hypothesized that prior to the introduction in 1961 of the exotic perennial grass species, *L. perenne* and *D. glomerata*, 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 in 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 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 ( $P > 0.05$ ) than the other three populations. Results of the pot experiment on the effect of light intensity have shown that, averaged over all light intensity levels, M (Ppr) had significantly larger dry mass 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), clearly indicating divergence of M (Ppr) from the other three populations.

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) 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. It was also M (Ppr) that showed large increases in performance, though not statistically significant, in terms of dry matter production with the removal of competitor species when transplanted in all three allopatric sites. 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 of 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.

Turkington (1989) 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 1989). 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 (1989) 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 neighbor-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 (Aarsen 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.

Results of the plant competition experiment have shown that 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. 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*.

Two of the four natural neighboring 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 1985).

The reduced biomass produced by M (Lpe) when grown with *P. aquatica*; of M (Ppr) when grown with *D. glomera* 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-neighboring pairs. Selective pressures may have reduced the aggression of the neighboring species populations towards each other through arrangement of plant parts and complementary growth patterns (Rhodes 1981).

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 was 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 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 (1979) indicated that the influence of a plant's neighbors 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, morphological characters, growth performances under varying light intensity levels, responses to water stress, and DNA banding patterns. 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).

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