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Within-population variation in localized and integrated responses of *Trifolium repens* to biotically patchy environments

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Summary. Genets of *Trifolium repens* (white clover) were collected from three patches of old permanent pasture dominated by *Agrostis capillaris*, *Holcus lanatus* or *Lolium perenne*. Plants derived from the genets were grown with plants of one grass species present on one side of each *T. repens*, and a different grass species on the other side, in all combinations of two of the three grasses. Different modules (a node with its associated internode, leaf, and axillary bud) on the same clover plant responded independently to the microenvironment provided by their own neighbouring grasses. In contrast, all apical meristems on the plant reacted similarly, showing a unified response and integrating the effects of the different microenvironments experienced by the whole clover plant. This is consistent with what is known both physiologically about the nutrition of meristems and modules, and ecologically about the exploratory growth habit of the species. Averaged over all associated grasses, there was no significant variation in the final dry weight of the different clover genets but these differed in their growth habit response to different grasses. In response to *Agrostis* as a neighbour, each meristem of *T. repens* rapidly produced many small modules. New modules were produced more slowly and were larger when *Holcus* or *Lolium* was the neighbour. The same pattern of differences occurred among clovers sampled from different backgrounds. Either genetic differences paralleled plastic responses, or plastic changes in phenotype that developed in response to different neighbours in the field persisted in the greenhouse. Plants taken from backgrounds of different grass species showed different responses to growing with those grass species. The differences were manifest primarily in a "positive leading diagonal" effect of *Holcus* or not-*Holcus*. They were the result primarily of differences in the dry weight per module and the probability of development of the axillary bud into a branch. This confirms earlier results, and implicates the central importance of branching as a means of local response to the microenvironment.

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A major aim of any population-biological study of plant or animal communities is to describe the effects of the environment on the organisms living in that community. Darwin (1859) suggested that the most important part of an individual's environment is its immediate neighbours. This suggestion certainly appears to be valid for communities of the temperate zones and has formed the basis of many studies of plant communities (e.g. Turkington and Harper 1979).

In permanent pastures in Britain and other temperate regions, the dominant dicotyledon is often white clover, *Trifolium repens*, which grows in association, and in competition, with various grass species. Each horizontal shoot axis (=stolon) of *T. repens* growing in such conditions may be 6–25 cm long, comprises 10–50 modules (a node with its associated internode and, where present, leaf, petiole and axillary bud), and moves through the pasture by approximately its own length annually (Sackville Hamilton and Harper 1989; Sackville Hamilton 1990). The whole axis therefore samples a variety of soil microenvironments (Snaydon 1962, 1985) and of neighbours (Burdon 1980), both spatially and temporally. Each rooted module of the *T. repens* genet gathers resources, so the pattern of module placement in relation to the pattern of resource availability determines the efficiency with which resources are captured (Bülow-Olsen et al. 1984; Solangaarachchi 1985). Thus the architecture of a *T. repens* genet may play a major role in determining its fitness and survival in a pasture. This architecture is determined by characteristics such as the proportion of nodes which branch, and internode length (Sackville Hamilton and Harper 1989), and provides a framework to identify the characteristics that should be measured and analysed in the type of study we are reporting. The way in which *T. repens* experiences a grass-dominated system will also depend on its variation for such morphological characteristics as leaf size, petiole

length, leaf angle, internode length, and branch angle (Burdon 1983; Turkington and Burdon 1983). From the "genet's-eye-view" the environment is fine-grained. In contrast, the individual module spends its entire life in one microenvironment (or a few in a temporally variable environment).

How does the individual genet translate the different environmental pressures that act on its constituent modules into a "whole organism" response? – as one plant in a fine-grained environment, or as a population of modules each responding autonomously to its own coarse-grained environment? The pattern of branching in modular organisms is responsive to their immediate environment (Noble et al. 1979; Chapman 1983; Newton 1986). The growth habit of *T. repens* enables it to exploit locally favourable microenvironments as it wanders through a patchy environment. It might be expected that ideally each module of one genet should be able to respond immediately to its own microenvironment. This study was designed to assess the extent to which genets of *T. repens*, chosen with the prior expectation of having different growth forms, responded to different environmental conditions defined by growing the clover with different companion grass species. To test this, we grew one half of each clover plant with one grass neighbour and the other half with a different grass neighbour. The design of the experiment was such that it allowed the global response of a particular clover plant to be estimated (i.e. its response viewed as an integrated set of modules) as well as the local response of its constituent modules.

Methods

In June 1985 three genets of *Trifolium repens* were sampled from each of three areas in an old pasture (described in Turkington 1989) at Henfaes, Abergwyngregin, North Wales. The three areas were dominated by the grasses *Agrostis capillaris*, *Holcus lanatus* and *Lolium perenne* respectively. The nine genets were multiplied in a greenhouse.

Ninety flats, each 36 cm × 21 cm and 5 cm deep, were filled with John Innes No. 3 compost. Seeds of *A. capillaris*, *H. lanatus*, and *L. perenne* were broadcast at high density into 30 each of the flats and placed on a bench in a heated greenhouse. Over a period of 11 weeks, the grasses were periodically clipped to encourage tillering. On three occasions the grasses were sprayed with Daconil to control fungal infection, particularly by *Fusarium*.

The grass flats were arranged into 15 pairs each of *Agrostis* and *Holcus*, *Agrostis* and *Lolium*, and *Holcus* and *Lolium*. Between each pair of grass flats was placed a plastic slot, 36 cm long, 1.5 cm wide and 5 cm deep, filled with John Innes No. 1 compost. For each set ($n=15$) of each grass pair ($n=3$), three shoot apex cuttings from each of the nine genets of *T. repens* were transplanted into the slots, and the main shoot axis directed to grow along the slot. In most cases there were two cuttings per slot, one at each end (Fig. 1).

Successive primary branches of the main shoot axis of *T. repens* are produced on alternate sides and therefore invade the two different grass flats alternately (Fig. 2). The plants were allowed to grow, without defoliation, for 15 weeks until early April. The above-ground parts of individual plants of *T. repens* were then removed, intact, from the grass swards and placed on white card. A "map" was drawn of each plant by tracing the main shoot axis and all branches and marking the position of each node.

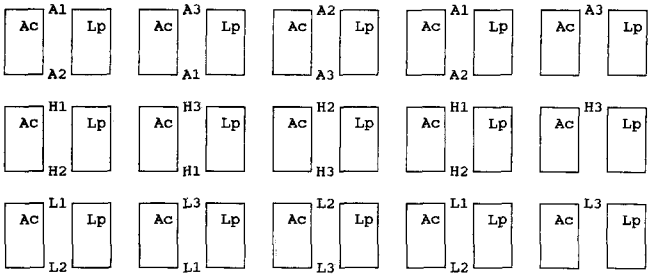

Fig. 1. An example of the experimental layout showing the fifteen pairs (= one set) of *Agrostis capillaris (Ac)* and *Lolium perenne (Lp)* flats. Between each pair of flats are either 1 or 2 shoot apex cuttings of *Trifolium repens* collected from either an *A. capillaris (A)*, *Holcus lanatus (H)* or *Lolium perenne (L)* dominated area of an old pasture; these were allowed to grow as shown in Fig. 2. There were three genets *(1–3)* of *T. repens* from each origin. The experimental design also included *A. capillaris* – *H. lanatus* pairs and *H. lanatus* – *L. perenne* pairs

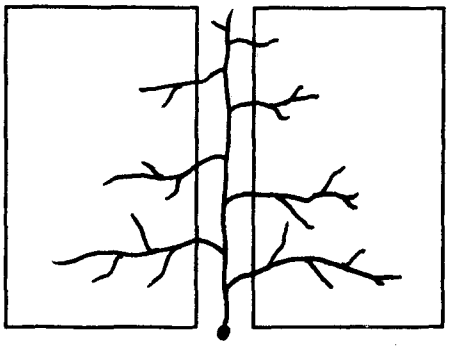

Fig. 2. The growth pattern of stolons of *Trifolium repens* showing how successive primary branches are produced on alternate sides of the main shoot axis. This permits an experimental design where one side of the *T. repens* plant grows into one grass environment and the other half of the same plant grows into a different grass environment

The growth of Trifolium repens

The total dry weight attained by one plant of *T. repens* is the product of the number and mean dry weight of the modules it produces, less those that die. Each axillary bud may develop into a branch, with a new apex iterating more modules.

The rate of production of modules at any one time is the product of the number of apices producing modules and the rate at which each apex produces new modules. The number of apices producing modules is the product of the number of existing modules and the proportion of those modules whose axillary buds have developed into new branches. This in turn is determined by the age at which an axillary bud can develop into a new branch and the probability that, once old enough, it does so.

Data collection

So far as possible, within the constraints of an experiment with a single harvest and no repeated observations, data were collected and analysed to extract the components of growth described above. Specifically, we measured:

1. Dry weight. After the maps were drawn, each plant was divided into two parts – one on either side of the main shoot axis – each part having grown with a different grass neighbour. The main shoot axis was discarded. The above-ground plant material was oven-dried at

70° C for 96 h, and weighed. These data were $\log_{10}$ transformed for analysis.

2. Number of modules. The total number of modules, on all branches of all orders on each side of the plant. These data were $\log_{10}$ transformed for analysis.

3. Dry weight per module. Total plant dry weight was divided by the number of modules.

4. Age at branching. The number of modules distal to the youngest branched module on a shoot axis is the approximate number of plastochrons between the appearance of that module and the appearance of the branch in its leaf axil – i.e. the age of the module, in units of plastochrons, when its branch appeared. A mean age at branching for each side of each clover plant was calculated as the mean number of distal unbranched modules on all its primary branches.

5. Probability of branching. The probability that an axillary bud will develop into a branch is estimated by:

$$NB/(NP-ND)$$

where:

NB = number of branched nodes on primary branches of one side of a plant;
NP = number of nodes on primary branches of one side of a plant;
ND = number of young nodes distal to the youngest branched node on a primary branch, summed over all primary branches of one side of a plant (see 4 above). Data were converted to percentages and arcsine transformed for analysis.

6. Number of modules per primary branch. The number of modules on a primary branch varies with position of the branch on the main shoot axis, mainly because branches tend to be initiated in sequence along a shoot axis as the axis grows. The analysis was therefore based on a linear regression of the number of modules per primary branch on position of the branch. Data for the four most basal branches were omitted because, having been initiated while the original transplant was becoming established as a rooted plant, their early growth was retarded.

7. Photosynthetic area per module. The photosynthetic area of each side of each plant was measured using a Delta-T Area Meter. This value was divided by the number of modules for analysis.

8a. Internode length: young internodes. On each primary branch that was long enough, the length of the three *youngest* fully elongated internodes was measured. On shorter branches, zero, one or two internodes were measured. The three basal internodes on each branch were excluded from consideration, partly because they had not entered the grass environment, and partly because of the ontogenetic drift in module morphology associated with the early development of a branch. Data were square-root transformed for analysis. The analysis was based on the means for all primary branches on each side of each plant.

8b. Internode length: old internodes. As for (8a), except that the three *oldest* internodes were measured, excluding the three basal internodes.

Data analysis

All data, except those on number of modules per branch, were analysed by ANOVAR (GENSTAT 1987). The treatment design was hierarchical – 3 genets taken from each of 3 original sites – and 2-way factorial – the 9 genets × 3 grass neighbours. The three sites of origin and the three grass neighbours in each instance were *Agrostis*, *Holcus*, or *Lolium*. The block design was an incomplete-split-plot, randomized block: 2 sides of each of 27 clover plants with 3 replicates. Site of origin, and genet within site of origin, were assigned to main plots, i.e. clover plants, and grass neighbours to sub-plots, i.e. sides. Since each clover plant experienced only two of the three grasses, the effects of grass neighbour were estimated partly within plants – by comparisons between the two sides of a plant – and partly between plants.

If a plant responds as an integrated whole to its two neighbouring grasses, both sides will have the same phenotype (the within-plant estimates of the effects of grass neighbour will be zero). Conversely, if each side of a plant responds independently to its own grass neighbour, the phenotype of a side under one neighbour is not affected by the grass species associated with the opposite side of the same clover plant (the within- and between-plant estimates of the effects of grass neighbour will be the same). The ANOVAR therefore provides the means of assessing the extent to which connected parts of the same plant can respond independently, each to its own particular microenvironment.

For the origin and origin × grass neighbour interaction terms, two significance levels may be given, with genet treated respectively as a fixed or random-effect factor. The first tests only for differences among origins for the particular genets used in the present study. The second tests whether any random sample of genets from the same three origins would give the same results.

The number of nodes per primary branch was analysed with a General Linear Model (GLM: GENSTAT 1987). The maximal model contained the same block and treatment terms as the ANOVARS, together with a regression on branch position and its interaction with each of the other terms. A Parallel Curve Analysis was applied to obtain one ANOVAR of the number of nodes per primary branch at the mean branch position, and one ANOVAR of the slope of the regression of number of nodes per branch on branch position.

The terms in the above GLM are not orthogonal, so the analysis is affected by the order in which terms are added to the model. In the present case, the nature of the model itself determined the order of adding almost all terms. The only exception was that the effects of genet (both within and between origins) could be added before or after the between-plant effects of grass neighbour. Both orders were used, the order genet-grass neighbour to estimate variance associated with grass neighbour, and grass neighbour-genet to estimate that associated with genet.

Results and interpretation

1. Main effects of origin of T. repens *(Tables 1 and 2)*

Averaged over all genets and all grass neighbours, there were no differences in dry weight of *T. repens* attributable to the background from which it was sampled. There were, however, differences in the morphology of the genets. The three genets from a background of *Agrostis* produced more, but smaller modules with lower dry weight, photosynthetic area and internode length than the genets from *Holcus*.

There were also differences in the pattern of production of modules. Modules on genets from a background of *Holcus* branched earlier. Despite this, these genets had fewer modules on their primary branches, whereas the genets from a background of *Agrostis* had more. This implies that meristems on *T. repens* from an *Agrostis* background produced new modules more rapidly than did those from a *Holcus* background.

Linear regressions of (number of nodes on a primary branch) on (position of the primary branch on the main shoot axis) show slopes shallower than -1 only for genets from the *Agrostis* background ($b = 0.869$). The most likely interpretation is ontogenetic drift in these genets, by which successive modules on the main shoot axis started to branch progressively sooner after their birth.

Table 1. Analyses of variance (degrees of freedom, mean square, significance) for nine genets of *Trifolium repens* taken from three different sites of origin within one pasture, and grown with two of three grasses. The characteristics and analysis are explained in Methods. Significance levels in parentheses were calculated assuming that genet is a random-effect factor

Characteristic	Total dry weight (log_{10})	Number of modules (log_{10})	Dry weight (mg module^{-1})	Branching		Number of nodes per branch		Pthoto-synthetic area (cm^2 module^{-1})	Internode length (sqrt cm)	
				Age (sqrt plastochrons)	% (arcsin)	At mean position	Regression on position		Apical	Basal
Source of variation										
A) Variation among plants										
Origin	2 0.055NS	0.166*(*)	467.35***(***)	0.249*(*)	226NS	133.8**(NS)	32.12**(NS)	1.722***(*)	0.577***(NS)	0.857***(NS)
Genet within origin	6 0.035NS	0.105NS	63.28**	0.076NS	715***	66.72*	21.59***	0.311**	0.315***	0.292***
Neighbouring grass	2 0.661***	0.742***	21.41NS	0.355**	167NS	197.1***	7.37NS	0.847***	0.033NS	0.030NS
Origin × neighbouring grass	4 0.148*(*)	0.078NS	26.82NS	0.059NS	447*(*)	37.36NS	6.7NS	0.190**(NS)	0.073**(NS)	0.086**(NS)
Genet within origin × neighbouring grass	12 0.054NS	0.088NS	44.23**	0.077NS	181NS	26.4NS	4.43NS	0.158*	0.048**	0.053**
Residual	52 0.056	0.054	16.94	0.066	150	23.34	4.19	0.073	0.016	0.017
B) Variation within plants										
Neighbouring grass	2 0.049*	0.012NS	43.48*	0.019NS	2NS	0.070NS	1.49NS	0.086NS	0.034*	0.020*
Origin × neighbouring grass	4 0.101***(***)	0.074***(***)	11.77NS	0.046NS	177*(*)	6.00NS	7.06**(NS)	0.034NS	0.009NS	0.005NS
Genet within origin × neighbouring grass	12 0.015NS	0.010NS	8.52NS	0.053NS	38NS	2.29NS	5.39**	0.022NS	0.017NS	0.005NS
Residual	63 0.013	0.012	10.25	0.044	58	3.48	1.87	0.032	0.011	0.006

*** $P<0.001$, ** $P<0.01$, * $P<0.05$, NS not significant

Table 2. Mean phenotypes of *Trifolium repens* taken from three sites dominated by grass species (origins) in one pasture

	Original neighbour			S.E.D.
	Agrostis	*Holcus*	*Lolium*	
Characteristic				
1. Total dry weight ($\log_{10}$ g)	0.230	0.294	0.256	0.046
2. Total number of modules ($\log_{10}$)	2.179	2.069	2.134	0.045
3. Dry weight (mg module^{-1})	11.85	17.58	13.54	0.79
4. Age at branching (sqrt plastochrons)	1.814	1.721	1.854	0.050
5. Percent branching (arcsin)	42.67	46.50	45.83	2.36
6a. Number of nodes per branch at mean branch position	8.32	6.88	7.45	0.42
6b. Regression of node number per branch on branch position	− 0.869	− 1.054	− 0.979	0.051
7. Photosynthetic area (cm^2 module^{-1})	0.711	1.063	0.838	0.052
8a. Length of apical internodes (sqrt cm)	1.385	1.567	1.391	0.025
8b. Length of basal internodes (sqrt cm)	1.304	1.539	1.345	0.025

There was no significant variation in the probability of an axillary bud developing into a branch. However, genets from the *Agrostis* background appeared to be more branched than those from the *Holcus* background, because of the greater rate of production of modules by each meristem.

In conclusion, genets of *T. repens* from an *Agrostis* background tended to produce many small modules, because each meristem produced modules rapidly, and each module had a short internode. Genets from the *Holcus* background, in contrast, tended to produce few, large modules with long internodes and with axillary buds that develop rapidly into new branches. Genets from the *Lolium* background produced new modules at an intermediate rate, each module having short internodes but of intermediate dry weight, and with axillary buds that are slow to develop. The *T. repens* from the three different origins represent different ways of achieving the same overall dry weight, averaged over all grass neighbours.

2. Differences among genets within origins (Tables 1 and 3)

Formal genetic analyses were not made and real genetic differences were not established. For the purposes of this report we will use the term "genetic" variation to describe phenotypic variation among genets grown in the same environment. There was no significant "genetic" variation in total dry weight among genets taken from the same origin, averaged over all neighbouring grasses, nor in the total number of modules. There was, however, highly significant "genetic" variation within origins both in the morphology and in the pattern of production of modules. There was variation in the size of modules (dry weight, photosynthetic area, and length of internodes), in the probability of an axillary bud developing into a branch, and in the rate of production of modules by individual meristems. The variation among genets of different origins in internode length and in the rate of module production, described in (*1*), was not significantly greater than the variation among genets within origins, although the significance tests indicated that these effects were weak.

3. Mean response of T. repens *to grass neighbours (Tables 1 and 4)*

Averaged over all genets, *T. repens* developed the highest dry weight when growing with *Holcus* or *Lolium*, provided that *Agrostis* was competing with the other side of the same plant. In contrast, it developed the lowest dry weight when *Holcus* and *Lolium* were the neighbours.

The difference between the two sides of a clover plant was attributable solely to differences in the size (dry weight, photosynthetic area and internode length) of individual modules, there being no differences in the rate of module production by individual meristems, nor in the probability of branching, nor in the age-at-branching of modules. The side growing with *Agrostis* had small modules with short internodes. The side growing with *Holcus* had heavy modules with long internodes. The side growing with *Lolium* had heavy modules but short internodes.

The size of individual modules developed with one species of grass was not affected by the grass species on the other side of the same plant. These results are consistent with the hypothesis of a local response: modules respond solely to their immediate microenvironment.

In contrast, the number of modules was the same on both sides of each plant, but differed on plants growing with different pairs of neighbours. It was highest when *Agrostis* was present, and lowest when it was absent. These differences in number were determined primarily by the rate at which individual meristems produced new modules. This is consistent with the hypothesis of a global response: all apices on one plant are integrated so that they produce modules at the same rate, even if they occupy different microenvironments.

The differing patterns of response of modules and apices explains the complex response in dry weight as follows: *Trifolium repens* responds to *Agrostis* by producing more, smaller modules whereas with *Holcus* and with *Lolium* it produces fewer, heavier modules.

Table 3. Mean phenotypes of nine genets of *Trifolium repens* taken from three sites dominated by different grass species in one pasture

Original neighbour	Agrostis			Holcus			Lolium			S.E.D.
Genet	1	2	3	1	2	3	1	2	3	
Characteristic										
1. Total dry weight ($\log_{10}$ g)	0.253	0.178	0.258	0.333	0.247	0.301	0.230	0.308	0.231	0.079
2. Total number of modules ($\log_{10}$)	2.162	2.189	2.185	2.191	2.008	2.007	2.098	2.222	2.082	0.078
3. Dry weight (mg module^{-1})	12.89	9.93	12.74	14.68	18.17	19.88	13.78	12.58	14.26	1.37
4. Age at branching (sqrt plastochrons)	1.841	1.768	1.832	1.670	1.766	1.717	1.950	1.764	1.846	0.086
5. Percent branching (arcsin)	43.73	43.89	40.38	52.98	39.24	47.27	40.43	55.29	41.76	4.08
6a. No of nodes per branch at mean branch position	7.22	8.53	9.29	7.79	6.64	6.13	7.72	7.14	7.46	0.72
6b. Regression of node no. per branch on branch position	−0.903	−1.040	−0.664	−0.964	−0.998	−1.198	−0.895	−0.952	−1.091	0.088
7. Photosynthetic area (cm^2 module^{-1})	0.751	0.617	0.764	0.867	1.059	1.264	0.916	0.767	0.832	0.090
8a. Length of apical internodes (sqrt cm)	1.414	1.438	1.302	1.576	1.525	1.599	1.225	1.632	1.315	0.043
8b. Length of basal internodes (sqrt cm)	1.311	1.368	1.232	1.548	1.476	1.594	1.187	1.572	1.275	0.044

Table 4. Half-plant phenotypes of *Trifolium repens* as affected directly and indirectly by three different grass species. The two halves of each clover plant were grown in separate containers. Direct effects come from the grass neighbours growing in the same container as the half of the clover plant measured, and the indirect effects from the grass in the same container as the other half. The S.E.D. for within-plant comparisons is for comparing the two halves of one plant (i.e. two columns which have the direct and indirect grass neighbours reversed); the other S.E.D. is for all other comparisons

Direct neighbour	*Agrostis*		*Holcus*		*Lolium*		S.E.D.	
Indirect neighbour	*Holcus*	*Lolium*	*Agrostis*	*Lolium*	*Agrostis*	*Holcus*	within-plant	between-comparisons
Characteristic								
1. Total dry weight ($\log_{10}$ g)	0.296	0.291	0.370	0.121	0.337	0.144	0.031	0.065
2. Total number of modules ($\log_{10}$)	2.191	2.172	2.243	1.996	2.169	1.993	0.030	0.063
3. Dry weight (mg module^{-1})	13.23	13.66	14.01	14.16	16.01	14.89	0.87	1.12
4. Age at branching (sqrt plastochrons)	1.831	1.881	1.768	1.722	1.869	1.708	0.057	0.070
5. Percent branching (arcsin)	46.62	44.88	46.85	43.44	45.21	42.99	2.07	3.33
6a. Number of nodes per branch at mean branch position	7.78	8.45	7.73	6.88	8.75	6.70	0.23	0.59
6b. Regression of node number per branch on branch position	−1.034	−0.966	−1.059	−0.921	−0.982	−0.842	0.048	0.072
7. Photosynthetic area (cm^2 module^{-1})	0.724	0.819	0.762	0.992	0.934	0.994	0.048	0.074
8a. Length of apical internodes (sqrt cm)	1.435	1.421	1.484	1.489	1.417	1.439	0.028	0.035
8b. Length of basal internodes (sqrt cm)	1.373	1.379	1.405	1.444	1.373	1.400	0.020	0.036

Because of the unified response of apices over the whole plant, the rate of production of modules will increase on both sides of the plant if *Agrostis* is growing on one side. Because of the local response of modules, the side of the plant with *Holcus* or *Lolium* as neighbours will have larger modules. So, if a side with *Holcus* or *Lolium* as a neighbour has *Agrostis* on the other side, then it will have larger modules because of the local response of modules, and more modules, than a plant without *Agrostis* on the other side, because of the global response of apices. Thus, the highest dry weight of whole plants occurs in plants with *Agrostis* as one neighbour, whereas

Table 5. Interaction between the original neighbour (in the pasture) and the experimental neighbour (in the glasshouse) in their effects on the phenotypes of *Trifolium repens* sampled from a patch dominated by the original neighbour and then grown with a neighbour. In each cell of the table, the upper value is the between-plants estimate, and the lower value the within-plants estimate of the interaction effects. See methods for explanation of the characteristics

Original neighbour (in the pasture)	Agrostis			Holcus			Lolium			S.E.
Experimental neighbour (in the glasshouse)	*Agrostis*	*Holcus*	*Lolium*	*Agrostis*	*Holcus*	*Lolium*	*Agrostis*	*Holcus*	*Lolium*	
Characteristic										
1. Total dry weight (log_{10} g)	0.000	−0.005	0.005	− 0.151	0.203	−0.053	0.151	−0.199	0.048	0.112
	−0.049	−0.061	0.111	0.001	0.054	−0.056	0.048	0.007	−0.055	0.039
2. Total number of modules (log_{10})	−0.066	0.079	−0.012	− 0.051	0.093	−0.042	0.117	−0.171	0.054	0.110
	−0.045	−0.048	0.093	0.015	0.053	−0.067	0.030	−0.005	−0.025	0.030
3. Dry weight (mg module^{-1})	1.77	−1.81	0.04	− 2.97	2.50	0.47	1.20	−0.68	−0.51	1.94
	0.04	−0.39	0.34	− 0.78	0.00	0.79	0.74	0.39	−1.13	0.87
4. Age at branching (sqrt plastochrons)	−0.058	0.126	−0.068	0.103	− 0.105	0.002	−0.044	−0.022	0.066	0.121
	0.043	−0.016	−0.027	0.033	− 0.013	−0.020	−0.076	0.028	0.048	0.057
5. Percentage branching (arcsin)	2.26	−2.23	−0.03	−10.49	11.27	−0.78	8.23	−9.04	0.81	5.77
	−2.49	−1.67	4.16	0.08	2.90	−2.98	2.41	−1.23	−1.18	2.07
6a. Number of nodes per branch at mean branch position	−0.542	0.447	0.094	− 0.907	− 0.115	1.022	1.448	−0.332	−1.116	1.019
	−0.285	−0.05	0.334	0.160	0.146	−0.306	0.125	−0.096	−0.029	0.227
6b. Regression of node number per branch on branch position	0.141	−0.061	−0.080	− 0.245	0.075	0.170	0.104	−0.014	−0.090	0.123
	0.095	−0.02	−0.075	− 0.076	0.006	0.069	−0.020	0.014	0.006	0.047
7. Photosynthetic area (cm^2 module^{-1})	0.182	−0.134	−0.048	− 0.253	0.198	0.055	0.071	−0.064	−0.007	0.128
	−0.017	−0.003	0.020	− 0.035	− 0.007	0.041	0.052	0.010	−0.062	0.048
8. Length of apical (youngest) internodes (sqrt cm)	0.09	−0.106	0.016	− 0.124	0.158	−0.034	0.035	−0.052	0.017	0.061
	−0.023	0.013	0.011	0.010	0.014	−0.024	0.013	−0.026	0.013	0.028
9. Length of basal (oldest) internodes (sqrt cm)	0.074	−0.155	0.081	− 0.103	0.159	−0.056	0.028	−0.003	−0.025	0.066
	0.004	−0.022	0.018	− 0.007	0.014	−0.007	0.003	0.008	−0.011	0.020

the side with the highest dry weight is the one opposite *Agrostis*.

4. Response to grass neighbours by T. repens *from different origins (Tables 1 and 5)*

The between-plant estimates of the origin × grass neighbour interaction effects (Table 5) show the effect on a whole plant from a given origin, of the presence of the grass neighbour on one side of the plant. The corresponding within-plant estimates show the effect, relative to the whole plant mean, of the neighbour on the side of the plant with which it is growing. The between-plants effect is expressed as the deviation from the value predicted under the null hypothesis that whole plants, whatever their origin, show the same response to grass neighbours. The within-plants effect is expressed as the deviation from the difference (between the two sides of the plant) predicted under the null hypothesis that half plants, whatever their origin, show the same local response to grass neighbours.

(a) The response of the whole plant. The effects of grass neighbours on the dry weight of whole plants of *T. repens* varied with the origin of the *T. repens*. These differences were mainly in the form of a "positive leading diagonal" effect, principally of *Holcus* or not-*Holcus*. That is, plants sampled from a background of *Holcus* developed a high dry weight with *Holcus* as a neighbour on one side, and a low dry weight in the absence of *Holcus*: and plants growing with *Holcus* on one side developed a low dry weight unless they had come from a site dominated by *Holcus*. There was no leading diagonal effect between *Agrostis* and *Lolium*; plants taken from a site dominated by *Lolium* developed a high dry weight with *Agrostis* as one of the neighbours.

Neither of the components of dry weight – number of modules and mean dry weight per module – showed a significant origin × grass neighbour interaction for whole plants. However, for both variables the estimates of their effects followed the same pattern as for total dry weight. Moreover, similar patterns of effects were significant for one component of module number (probability of branching) and for the components of module size (photosynthetic area per module and internode length). The specificity of *Trifolium* to its original neighbour is therefore mediated through the development of individual modules and their axillary buds; the rate at which apices produce new modules is not involved in the specific response.

The response in terms of internode length represents a reinforcement of the main effects of origin (genets from sites dominated by *Holcus* had longer internodes with all neighbouring grasses) and neighbouring grass (association with *Holcus* increased internode length in all genets). The increase in internode length in response to *Holcus* as a neighbour was greatest in the genets sampled from a site dominated by *Holcus*.

(b) Differences between the two sides of the same plant. The local origin-specific responses of plants to their immediate grass neighbours were broadly similar to those of the whole plant (*4a* above). For dry weight, there was a slight indication of a positive leading diagonal effect for *Holcus* or not-*Holcus*. Differences in dry weight response were attributable to module size and the development of axillary buds, but not in the rate of production of modules by apices.

There was little effect by the grass on the other side on the origin-specific response to the direct grass neighbour. The specific responses of genets to their grass neighbours is thus a local response by each module to its own environment.

This confirms the conclusion reached for the average response of all genets to their grass neighbours, that modules show a local response to their immediate microenvironment. The global response by apices, that was part of the average response of all genets to their grass neighbours, was not involved in the specific response of each genet to its original neighbour.

5. Responses to grass neighbours by genets of T. repens *from the same origin*

(a) The response of the whole plant (Table 1). There were no significant differences among genets of *T. repens* from the same origin in the response of the whole plant dry weight to different grasses. There were, however, differences in internode length, mainly concerning whether a genotype produced shorter or longer internodes in response to growth with *Holcus*.

(b) Differences between the two sides of the same plant (Table 1). There were no significant differences except for variation in the slopes of the regressions of (number of nodes on primary branches) *vs* (position of the primary branch on the main axis), i.e. the mean number of modules adjusted to the same position for all plants.

Discussion

In this study we have examined the performance of individual genets of *T. repens* both in terms of their yield response to a patchy grass environment and the way in which individual phenotypic characters contribute to that yield response. Averaged over all neighbouring grass species, there was no variation among genets of *T. repens* in dry weight production but they did vary in their growth habit. They also varied in their responses to neighbouring grasses, both in dry weight and in growth habit. The differences between genets were partly related to their different sites of origin but there were also differences among genets from the same origin. This suggests that no single architecture is universally advantageous. Averaged over all environments tested here, the different architectures are simply different ways of achieving the same dry weight. Rather, different architectures are specifically advantageous in different environments.

Main effect of neighbours

Two categories of response to neighbours were apparent; a response to the global environment perceived by the entire plant, and a response to the immediately local microenvironment. All apical meristems throughout the whole plant showed a unified response to the combined influence of the two neighbouring grasses. New modules were produced at the same rate on both sides of a plant, despite the presence of different neighbours, but differed for different combinations of neighbouring grasses. In contrast, internode length, probability of development of axillary buds into branches, the dry weight per module and the photosynthetic area per module all showed different responses on the two sides of each plant.

A possible physiological explanation for this difference is that, whereas the development of individual modules is dependent mainly on resources acquired by the module itself (Harvey 1979; Newton 1986), the resources used by an apex in producing new modules are obtained from several other connected modules (Hoshino 1974; Harvey 1979). Therefore, we would expect development of a module to be determined mainly by its rate of acquisition of resources in its local environment. Conversely, we would expect the rate of production of new modules by an apex to be determined by the integrated environment of all modules that supply it.

An ecological explanation invokes the hypothesis that the growth habit of *T. repens* enables it to explore a heterogeneous environment, exploiting locally favourable microenvironments by branching profusely, and passing through locally unfavourable microenvironments by extending the main shoot axis without branching. This hypothesis requires that a branch be initiated in response to the local microenvironment of the parent module of the branch, whereas production of new modules by an apex should continue regardless of the local microenvironment of the apex, exactly as shown by the present results. The present results therefore support what is known both physiologically about the nutrition of meristems and modules, and ecologically about the exploratory growth habit of the species.

The response of *T. repens* to *Agrostis* was that each meristem rapidly produced many small modules with short internodes. In response to *Holcus*, meristems produced larger modules at a slow rate with long internodes. In response to *Lolium*, meristems produced "chunky" modules (i.e. heavy but with short internodes) at a slow rate. The main effects of the grass species on the numbers of modules and branches were mediated mainly through effects on the rate of production of modules by apical meristems, there being little overall effect on the probability of branching or on the age of modules at branch initiation. That is, in contrast with the specific effects summarized above, module numbers were controlled by the global responses of meristems, not by the local responses of axillary buds.

These responses were seen both as short-term plastic responses to growth with the grasses, and as long-term persistent characteristics of *T. repens* found in association with the grasses. A "panglossian" interpretation, of the correspondence between the effects of original neighbour and those of the experimental neighbour, would be that it is an illustration of the efficacy of natural selection in changing both mean phenotypic expression and phenotypic plasticity in response to the environment.

However, an alternative explanation is equally possible from the results. The differences among the genets may not be truly genetic, but rather may be the remnants of plastic responses that persist at least for several months after removal to another environment. Support for this hypothesis is provided by Evans and Turkington (1988) who demonstrated that genets of *T. repens*, collected from different grass patches in an old pasture, had different morphologies and these differences were retained for at least 1 year in the absence of competition under common garden conditions. However, after 2 years the differences had disappeared and all genets had similar growth forms irrespective of their origin.

Genet origin × neighbouring grass interaction

The differences in response to competitors were manifest primarily in a "positive leading diagonal" effect of *Holcus* or not-*Holcus*, by which the *Holcus*-genets had the greatest, and those from non-*Holcus* backgrounds the lowest, dry weight in competition with *Holcus*. This is broadly in agreement with earlier results demonstrating variation in response to grasses (Chestnutt and Lowe 1970; Hill 1977), and on local specialization, within a single population of *T. repens*, to local variation in the identity of the grass neighbours (Turkington and Harper 1979). The present results show in addition that the specificity of the dry weight response resulted from specificity in the response of modules, primarily the dry weight per module and the probability of development of the axillary bud into a branch. As observed above, these are characteristics that do respond to the immediate microenvironment of the module. The rate of production of modules by each meristem, a globally-determined characteristic of the plant, did not contribute to the specificity. This is in keeping with the probable role of the specificity in enabling each plant to exploit locally favourable microenvironments within the pasture.

There are a number of mechanisms by which the population can apparently become locally specialized. Local differentiation may have occurred by natural selection of genotypes leaving most progeny. If this were the case, given the extremely fine spatial scale at which this substructuring occurs, the intensity of natural selection would need to be extremely high to overcome the effects of gene flow via both pollen and seeds. Alternatively, the genetic substructuring may have occurred by spatial reassortment of the mobile population of genets, such that each genet is most likely to be found in microenvironments that are most favourable to it. A third, equally plausible hypothesis is that there is no genetic substructuring, but that the local specialization is a result of physiological carryover of the plastic response. Whatever the mechanism, the result would be that genets found with one particular species of grass are specifically "ad-

apted" to that grass, and have the optimal growth habit for competing with that grass.

Conclusion

This one experiment has given many insights into the behaviour of *T. repens* and has shown that a single plant responds in a complex manner when growing in a heterogeneous environment. For example, *T. repens* grew best with *Holcus* or *Lolium* so long as *Agrostis* was present nearby, but grew worst with *Holcus* and *Lolium* together in the absence of *Agrostis*. So at first sight *T. repens* appeared to respond to more distant neighbours than to its immediate neighbour. The reason for this apparent anomaly was that some components of growth, growth of modules, responded independently to the different microenvironments, while other components of growth, differentiation of new modules by apical meristems, showed a unified response and integrated the effects of the different microenvironments experienced by the whole plant. Because different components of growth of one plant responded differently to a patchy environment, clover growth is difficult to predict except in relatively homogeneous habitats.

Genets of *T. repens* sampled from backgrounds of different grass species and grown in a common garden show the same patterns of differences as a variety of clover genets grown in mixture with the same range of grasses in a glasshouse. This demonstrates that the grasses have a strong developmental influence on the clover, that these responses are easily altered, and that the grasses produce a consistent and predictable morphological response. No growth form of *T. repens* is universally advantageous, each is specifically advantageous in a particular grass neighbourhood. However, as the clover grows through a patchy grass neighbourhood the phenotype is sensitive and will respond in a predictable morphological manner dependent upon the identity of the current grass neighbour.

Many of our previous studies of *T. repens* have demonstrated within-population specificity to neighbouring grasses. It is apparent from this study (Tables 1 and 5) that this specificity is mediated through the localized responses of individual modules and their axillary buds, and not through the rate at which new modules are produced.

The results presented above have substantial implications for the interpretation of experiments involving *T. repens* and companion grass species. In particular, the observation that responses of particular phenotypic characteristics can be local (occurring at the scale of the module) or global (occurring as an integrated response at the level of the entire plant) can place severe constraints on the design and analysis of such experiments. Further experiments designed to examine the underlying mechanisms of such behaviour, together with studies to discriminate between a genetic and a residual "physiological carry-over" determination of the differences between genets would clearly help in the understanding of the dynamics of clover-grass interactions in long-lived pastures.

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