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# Intergenotypic interactions in plant mixtures

Roy Turkington
*Botany Department, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada*

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## Summary

Pastures provide a good model system to study intergenotypic plant interactions because most pastures are mixtures of several sown genotypes and species and the conditions necessary for genetic change are present: (i) an extremely variable biotic and abiotic environment at the local scale, (ii) wide genetic variation, (iii) intense competition so that strong selection is likely, and (iv) long term continuity enables selection to be continuous and cumulative. These together provide a system in which (v) the theoretical outcome of intergenotypic interactions may be readily tested and maximizes the probability of detecting patterns. Interactions between the species will have both ecological and evolutionary consequences, both of which may be of interest to plant breeders. These patterns are detectable at different scales but particular attention will be focused at the level of the individual genotype and especially with mixtures of the grass *Lolium perenne* and the legume *Trifolium repens*. Competition experiments between *L. perenne* and *T. repens* are plentiful, but where specific hypotheses about adaptation at the genotype level have been tested, in all cases *T. repens* grows best when planted with its natural *L. perenne* neighbor – a reciprocal effect in the *L. perenne* has only been reported once. This specific *T. repens* – *L. perenne* neighbor recognition may be mediated through soil microorganisms, particularly *Rhizobium leguminosarum* biovar *trifolii* and *Bacillus polymyxa*.

## Introduction

Some of the most important influences impacting an organism arise from its relationship with other organisms, at the same, or at different, trophic levels. This is especially applicable to plants which, for the most part, are relatively immobile and are forced to deal with their neighbors which are often situated in close promixity and at high density. Ever since Charles Darwin, agronomists, ecologists, and evolutionary biologists have been interested in the ways by which species interact and the consequences of these interactions. The Darwinian tradition has focused on biotic interactions, indeed, he wrote that there is a '. . .deeply-seated error of considering the physical conditions of a country as the most important (for its inhabitants); whereas it cannot be disputed that the nature of the other species with which it has to compete, is as least as important, and generally a far more important element of success.' Agronomists have been primarily concerned with plant competition, and grazing, and have focused research effort on maximizing biomass production and on breeding or selecting for competitive ability. Ecologists have been more interested in how interactions cause organisms to specialize, or adapt, to one another and to the physical environment, and how this impacts abundance, distribution and the organization and structure of communities. Evolutionary biologists have focused attention on how the interactions themselves evolve, the nature and extent of speciation, and whether perhaps the interactions have coevolved. Perhaps not enough effort has been made to cross-fertilize these disciplines, and this of course is not necessarily easy, because the disciplines do have different, although somewhat overlapping, goals. The topic I was specifically assigned to address is intergenotypic interactions in plant mixtures. Taking a little liberty with the title, I will attempt to address some of the areas of common interest to the agronomist, the ecologist and the evolutionary biologist. I will do this by focusing my attention on managed (grazed) temperate pasture systems where grasses and legumes are the major plant association.

## Some ecological principles

John Hill (1990) has well articulated the problem before us when he says that 'coexistence is crucial to the success of any breeding programme designed to raise the productivity of grass/legume pastures...' Various basic ecological assumptions are implicit in this statement. First, one of the preconditions for coexistence is that factors are present which limit the expression of dominance. In pastures this limitation will usually operate through grazing which will act to debilitate potential dominants (Turkington et al., 1993). Second that neighboring species are competing. Competition is one of the conceptual backbones of ecological thinking but it is still poorly understood. It is notoriously difficult to demonstrate, probably because avoidance of it, in nature, is advantageous in most cases. However, in the crowded conditions typical of pastures it is difficult to believe that competition is not occurring. Third, is the assumption that if species have persisted together for a prolonged period it can be taken as axiomatic that some ecological distinction must exist between them. These differences may have always been present, or as is most commonly argued, there has been some degree of population differentiation by one or more of the species, a differentiation driven by competition. Fourth, that this differentiation permits coexistence, and continued interaction may result in coevolution, although Connell (1980) has argued why coevolution between competitors should not be expected to happen frequently. Finally, that coevolved populations will have a higher biomass production than non-coevolved populations. To varying degrees these assumptions have been tested experimentally in ecological studies, and as expected, there is not always a consistent unambiguous result, although a fairly clear consensus and theoretical framework is emerging (Thompson, 1994).

## Intergenotypic interactions – their context in the pasture

Pastures provide us with a good model system to study intergenotypic interactions because most pastures are mixtures of several sown genotypes and species and the conditions necessary for genetic change are present: (i) an extremely variable biotic and abiotic environment at the local scale, (ii) wide genetic variation, (iii) intense competition so that strong selection is likely, and (iv) long term continuity enables selection to be continuous and cumulative. These together provide a system in which (v) the theoretical outcome of intergenotypic interactions may be readily tested and maximizes the probability of detecting patterns. I will consider each of these briefly.

### *Variable biotic and abiotic environment*

The visual impression of pastures being quite uniform is misleading. The only meaningful measure of environmental variation is that which is seen from what I (Turkington, 1975) and Harper (1978) have called the 'plant's-eye-view'. Species with differing growth forms will experience the local environment in entirely different ways. For example, the tillering habit of *Lolium perenne* ensures that genets stay relatively fixed in position and ensures that the individual experiences a relatively constant spatial environment where it is dependent on a strictly local supply of resources. *Trifolium repens* in contrast, has an exploring phenotype that can spread through a grassland by up to 25 cm annually. The whole plant axis therefore samples a diversity of soil microenvironments (Snaydon, 1962) and of neighbors (Burdon, 1980), both spatially and temporally. It is already well established that *T. repens* varies in its aggressiveness to different species of grass (Chestnutt & Lowe, 1970; Turkington & Harper, 1979b; Frame & Newbould, 1986; Harris, 1987), and presumably also in its response to them. MacArthur & Levins (1964) distinguished between environmental variation in which the period of fluctuation is less than the lifespan of the plant and that in which the period is greater. They called this fine-grained and coarse-grained respectively. Thus most tillering pasture grasses will 'see' a coarse-grained environment and rhizomatous or stoloniferous species will 'see' a fine-grained environment.

But the mosaic of vegetation patches that make up a pasture also has a temporal dynamic (Thorhallsdottir, 1990a). Populations of grasses are distributed as a patchwork, corresponding to patterns of past disturbances, colonization, heterogeneity in the physical environment and interspecific interactions (Parish & Turkington, 1990a, 1990b). As plants grow, and patches expand, they inevitably meet and interact with other patches which they may invade, or by which they may be invaded. Plants will die and the gaps produced will be invaded by the same, or a different species (Grubb, 1977). In temperate grasslands dominated by perennials, the dominant species mostly colonize small disturbances by vegetative spread. Growth and death of plants, disturbance, and colonization, all contribute to the dynamics of the pasture community. These dynamics then impose their own structure on the community. As patches meet and interact, their structure will change as they expand or are invaded (Thorhallsdottir, 1990b; Turkington, 1993). Patches may change in age and size distributions, densities, relative abundances, and relative proportions of genotypes, which may in turn alter the local resource levels. These changes in patches and resources will in turn affect the outcomes of interactions. Consequently, there will be a patch dynamics of interactions and a patch dynamics of species distributions, abundances, and composition (Thompson, 1985, 1988). So what is an apparently uniform system to the human observer, is a dynamic and variable system when seen from a plant's-eye-view. This level of variation can clearly impose an array of local directional selection forces which are likely to dominate the fitness of individuals and promote microevolution.

### *Genetic variation*

*Trifolium repens* is a highly variable species commonly showing considerable differences within and between populations in a wide range of morphological and physiological characters (Turkington & Burdon, 1983; Burdon, 1983; Frame & Newbould, 1986; Harris, 1987). *Trifolium repens* is polymorphic for petiole length (Kerner von Marilaun, 1895), date of first flowering (Davies & Young, 1967), nitrogen fixing ability (Connolly et al., 1969), susceptibility for infection by *Rhizobium* (Mytton, 1975), leaf marks and leaf size (Cahn & Harper, 1976a, 1976b; Evans & Turkington, 1988), relative growth rate (Burdon & Harper, 1980; Trathan, 1983), cyanogenic glycosides (Dirzo & Harper, 1982) and their consequent palatability to some herbivores (Burgess & Ennos, 1987), and stolon length and flower production (Aarssen & Turkington, 1985c). Various studies of intraspecific diversity have been conducted in populations of *T. repens* from an old (approx. 100 years) pasture in North Wales. Burdon (1980) examined 50 plants and showed that they usually differed from each other in several characters and on average differed significantly in 3.3 characters. Cahn & Harper (1976a) identified an average of between 3 and 4 clones per 10 cm × 10 cm square, while Trathan (1985) using isoenzyme analysis, identified 48–50 genotypes per square meter. Gliddon & Trathan (1985) estimated the genetic variation within seven sub populations of *T. repens* and *L. perenne*. All 15 polymorphic loci studied in *T. repens* and 21 out of 23 in *L. perenne* showed significant heterogeneity of gene frequency, indicating that there was genetic differentiation between samples. The level of diversity found within this single population is of a magnitude more commonly encountered in comparisons between populations taken from distinctly different environments. Unfortunately, such detailed data are not available for any of the companion grasses but Aarssen & Turkington (1985c) showed extensive variation for some characters in *L. perenne* and *Holcus lanatus* from pastures in British Columbia. In addition, Breeze et al. (1965) showed that the rate of tillering in *L. perenne* can be significantly altered by selection within clones; the response depends on the age of the clone and is genotype-dependent. This is presumably due to some type of somatic mutation. Kelley & Clay (1987) used two grasses, *Danthonia spicata* and *Anthoxanthum odoratum*, from a 40-year-old field in North Carolina to show that naturally co-occurring genotypes of these species differed in interspecific competitive ability. The competitive performance of a given genotype often depended on the genotypic identity of the competing species, especially in *D. spicata*.

### *Competition and selection*

There seems to be little doubt that competition occurs in pastures. Donald (1963) claims 'intense competition' and 'acute competitive stress' and Snaydon (1978) states that 'intense competition usually occurs within the stand (pasture)...' Almost invariably, when species are experimentally removed, there is a positive growth response in remaining species. However, Fowler (1981, 1982) only detected generally diffuse competition and weak competitive relationships among species in a 30-year-old pasture in North Carolina. The problem generally seems not to be one of demonstrating that competition is happening, but to demonstrate that it is having a structuring effect on the community (Turkington & Mehrhoff, 1990).

There is abundant evidence that genetic changes occur rapidly in both sown and natural populations and it is mostly assumed that these changes are due to competition-driven mortality. Brougham & Harris (1967) have shown that the genetic composition of a sward of *L. perenne* may change by as much as 40%

within 4 months of sowing. The most comprehensive series of studies was that undertaken by Charles and coworkers from 1961–1973 (e.g. Charles, 1961, 1972) and perhaps as few as 10% survive the establishment year. They demonstrated that the genetic composition of a number of species, including *L. perenne* and *T. repens*, changes substantially within a few years. This shift was mostly caused by high mortality in the establishment year but the data from older swards showed further marked changes in populations in established grasslands. Changes were reported in biomass production, flowering date, plant height and tillering. Ennos (1981) demonstrated a significant change in the frequency of linamarase-containing phenotypes of *T. repens* during the transition from the seed to established plants in populations exposed to selection at three different sites.

In older pastures, although the level of variation is still apparently quite high, it is nevertheless declining. McNeilly & Roose (1984) documented a decline from about 40 different genotypes of *L. perenne* per 0.25 $m^2$ in a 10-year-old pasture to 5 in a 40-year-old pasture. The reason for the death of these individuals and the loss of variation may be related to many factors such as grazing, competition, pathogens, and variable abiotic factors, and it is not clear what role competition plays in this sorting process. These cautions also apply to the survey by Aarssen & Turkington (1985c) who collected 100 clones each of *H. lanatus*, *L. perenne* and *T. repens* from four pastures ranging from 4 to 67-years old. Although it did not occur for all characters, many characters, especially for *L. perenne* and *T. repens*, showed a decline in mean value and variance with increasing pasture age.

### *Long-term persistence and stability*

There is little evidence for seedling establishment in pastures except on molehills (Jalloq, 1975), gopher mounds (Peart, 1989), decayed dung pats (Parish & Turkington, 1990b), and on ant hills (King, 1977). However, this small amount of new recruitment may be important in maintaining genetic diversity within populations (Soane & Watkinson, 1979). Pastures are therefore primarily perennial systems and it is this that ensures that selection will be continuous and cumulative, and provides the opportunity to assess the long-term consequences to community structure.

It is very difficult to get long term data on any one system even though White (1985) reports the census (not repeated) of plants from about a dozen European grasslands from the last century. Few as they are, such data have become available through a number of recent papers (Silvertown, 1980; Silvertown et al., 1994; Dodd et al., 1994, 1995). These studies were based on the Park Grass Experiment at Rothamsted, in England, on plots established in 1856, and provide a superb opportunity to assess stability or equilibrium in plant communities. Initial changes in botanical composition, in response to different fertilizer treatments, was rapid, but within 50 years no further significant changes were detectable in the proportions by weight of grasses, legumes and other species in seven of nine plots. Dodd et al. (1995) call this a dynamic equilibrium because there was year-to-year variation caused by the vagaries of yearly weather conditions, nevertheless there was a range of long-term equilibria in community composition. A similar stabilizing of floristic composition has been demonstrated by Aarssen & Turkington (1985a) using pastures of different age and stringing them together as a time sequence (also see Turkington & Mehrhoff, 1990).

### *The theoretical outcome of intergenotypic interactions*

When a pasture is established with two or more species that are genetically variable, their relationship may change in at least two ways. First, the different populations may adapt separately and independently to the local environment and the response will be intensified by intrapopulation competition. Secondly, the populations may adapt to each other or coevolve and the response here will be intensified by interpopulation, or interspecific, competition (Antonovics, 1978). Various consequences of interspecific competition have been proposed such as character displacement and character convergence. But the most noteworthy feature of such studies is the variability of the results. Many fit the expectation of improved performance through divergence. Just as frequently, competitive performance declines or remains unchanged (Antonovics, 1978). The conclusion of most theoretical studies (e.g. MacArthur & Levins, 1964, 1967; Lawlor & Maynard Smith, 1976) is that populations will tend to diverge in their ecological requirements so that they increasingly use different resources and so reduce or avoid competition. In the ecological literature this theme has become nearly axiomatic even though most of the evidence is circumstantial with little empirical support, but compelling (Grant, 1972; Arthur, 1982; Schoener, 1983; Benton, 1987). Clear evidence in support of the hypothesis is provided by Allard & Adams (1969) with barley and by Joy & Laitinen (1980) with *Trifolium pratense* and *Phleum pratense*. Alternative outcomes of competition have been proposed, such as the evolution of interference mechanisms (Aarssen, 1983; Hairston et al., 1987), and convergence in resource use (Ghilarov, 1984). Nevertheless, the expectation of interspecific, or intergenotypic, competition, is divergence.

## Intergenotypic interactions – consequences in the pasture

There are two major methods by which to increase biomass productivity (outside of fertilizing). First, is a straightforward systematic search for good combinations of species or cultivars that are high yielding. Often this has lead to attempts to identify characteristics, and to their subsequent inclusion in breeding programs, of the components of the high-yielding mixtures (Hill & Michaelson-Yeates, 1987; Hill, 1990; Collins & Rhodes, 1990; Annicchiarico & Piano, 1994). Conventional variety improvement programs endeavor to identify superior i.e. higher yielding, genotypes on the basis of pure stand performance. The general conclusion from these studies was stated in Donald's (1963) landmark paper and have been restated elsewhere (Trenbath, 1974; Haynes, 1980; Zannone et al., 1983, 1986) and have not really needed upgrading, that is, that the yield of a mixture (legumes excluded) will usually be less than the higher yielding pure component and higher than the lower yielding pure component, and the total yield may be either greater than, or less than, the mean yield of the two pure components. In terms of yield there seems to be little benefit from mixing grass forage species. Trenbath (1974) describes a few exceptions (Rhodes, 1968, 1970). Such yield advantages of genotypes in binary mixtures may occur when the components are of contrasting growth habit (Rhodes, 1970) or have different seasonal growth patterns (Zannone et al., 1983). Most of these studies have been short-term (usually single generation) and made with random pairs of cultivars or genotypes. There is some evidence that coadaptation can occur among genotypes of cereals (Allard & Hansche, 1964; Allard & Adams, 1969) but these studies ran for up to 18 generations of selection and the Allard and Adams composite cross was derived from 31 varieties of barley.

Secondly, and of more direct interest to me are the ecological and evolutionary consequences of species interactions. Although it is a false premise that competitive ability of individuals and productivity of the community are synonymous, it may not be so errant to argue that success (fitness of individuals) and their productivity are often synonymous; this may be less true in pastures where grazing is a dominant force of selection. Nature selects for success, and if this can be translated as productivity, perhaps nature is already doing the systematic selection which we try to mimic in a simplified fashion in field trials, and natural selection is probably more effective than artificial selection. In our field and pot experiments the measures of performance used most often are dry weight and tiller production, measures of interest to the agronomist.

It would seem likely that coexisting genotypes, if they continue to coexist, should coevolve so that they increase their yield in each other's presence i.e. have ecological combining ability (Harper, 1967). It is unlikely that random selections of genotypes grown in mixture for one, or very few, generations, will generate any type of synergistic effect resulting in increased yield. This requires a shift to using multigeneration selection trials, and genotypes grown in mixture, or, deliberately selecting target combinations of coexisting individuals from natural situations where coevolution is assumed to have already happened. This way, both components of the mixture are exposed to the combined effects of both intra- and inter-genotypic competition (Hill & Michaelson-Yeates, 1987; Hill et al., 1987). It is odd that *T. repens* and grasses are usually bred separately in spite of the fact that *T. repens* is almost universally grown in mixture with grasses. Besides, a coevolved mixture will probably form a more stable combination of genotypes. If, however, the components of a mixture complement each other nutritionally then over-yielding will not be unexpected (de Wit et al., 1966) and it is not surprising that mixtures of grasses and legumes provide the most striking and repeatable examples.

Phenotypic plasticity will act to buffer some of the effects of natural selection and thus act to minimize genetic variation. Within any population there is an interplay of genetic diversity and phenotypic plasticity. Both are a means by which a population may adjust to its environment. Plasticity is particularly crucial in allowing fairly rapid and reversible adjustment to environmental change. Turkington (1989b) has argued that in more 'uniform' pastures we may expect to find more evidence of microevolution, or multiple adaptive optima, than in more 'variable' pastures where more of the variation detected will be phenotypic. However, the consequences of a patchy environment may be increased phenotypic plasticity and/or population differentiation (presumably having a genetic basis) among habitats. In the pasture context where environmental heterogeneity is high, where genetic variability within populations is high, and where competition is intense, there are a number of mechanisms by which a population can apparently become locally specialized. Local differentiation may have occurred by natural selection of genotypes leaving most progeny. This is unlikely because of the low rate of recruitment of seedlings into pastures. If this were the case, given the extremely fine scale at which this differentiation occurs (Aarssen & Turkington, 1985b), the intensity of natural selection would need to be extremely high. Alternatively, and much more likely, the differentiation may have occurred by spatial reassortment of the mobile population of genotypes, such that each genotype is most likely to be found in microenvironments that are most favourable to it. This will be especially so in rhizomatous and stoloniferous species. A third hypothesis is that there is no genetic basis to the apparent differentiation and the local specialization is a result physiological carryover of a plastic response (e.g. Evans & Turkington, 1988; Turkington, 1989b). Whatever the mechanism, the result would be that genotypes of one species found with genotypes of another particular species are specifically 'adapted' and have the optimal growth habits for coexisting. Arguably this should result in an increased yield of the mixture. Genetic change by non-random differential mortality among genotypes is potentially a very important process of adaptation in plant populations.

## Ecological and evolutionary consequences

The range of responses by plants to the challenges of their ever-changing environment is quite remarkable. Once we have figured out why the plant population does this or that, it confounds us with yet another ecological or evolutionary solution to a problem that nature has thrown at it. We can detect responses at different scales and some of these may be of a purely academic rather than agronomic interest. I will first of all consider the larger scale (between species) patterns, then within-species patterns, and finally at the individual genotype level.

### *Between-species patterns*

In most of the studies considered here, the specific hypothesis being tested was that the presence or absence of coexistence in the history of a population would influence its fitness in interspecific competition. Evans et al. (1985, 1989) sampled coexisting individuals of *L. perenne* and *T. repens* and from four long-established pastures in Switzerland, France, and Italy, and a 7-year-old pasture in southern England. *Trifolium repens* was grown in two-species mixtures either with its coexisting *L. perenne* or with one of two other *L. perenne* companions with which it had not previously coexisted. By the end of the second harvest year, those mixtures based on coexisting populations yielded 22% more on average than the other mixtures. After the fourth harvest year, overall, *T. repens* yields were significantly higher in the coexisting mixture. In addition, for all five populations the average annual yield of *T. repens* was highest when they were grown in mixture with their coexisting grass. While they detected no significant differences between the grasses, a pattern seems to be emerging (Table 1) where there is a benefit to the grass to be grown with its coexisting clover. On average, coexisting mixtures outyielded the remaining mixtures. Menchaca & Connolly (1990) also examined species interference in *L. perenne* and *T. repens* and their results are based on five sequential harvests after about 3 months. Initial growth of *L. perenne* was vigorous and its influence on *T. repens* was great but it declined dramatically with time. The influence of *T. repens* on *L. perenne* was initially small but it had an enhancing effect in later harvests, presumably due to the increased soil fertility caused by nitrogen fixation. Using the relative resource total (RRT) they showed that there was initially no benefit from mixing but there was a large benefit by the final harvest; this resulted because *T. repens* developed from being a competitor to a net facilitator over time, while *L. perenne* remained a competitor of *T. repens* throughout. In the final two harvests, *L. perenne* individuals performed better in mixture with *T. repens* than in pure stand at the same density. Turkington & Jolliffe (1996) followed the dynamics, over one growing season, of *L. perenne* and *T. repens* in mixtures varying in density and frequency, and using plants from different ages of pastures. Interactions between *Lolium* and *Trifolium* are complex, and change dramatically with pasture age, harvest date, and density. Over successive harvests, the two species' view of each other, and of themselves, changed in a dynamic way. In addition, individuals of

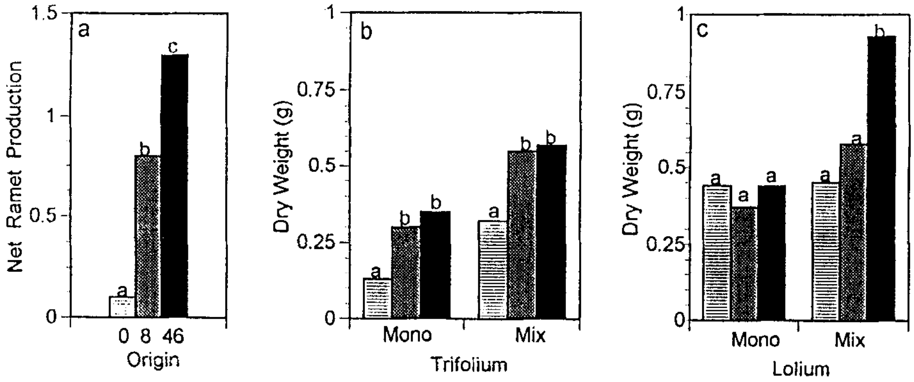

*Figure 1.* Average growth of *Trifolium repens* (Net ramet production, NRP (A) and dry weight (B) and *Lolium perenne* (dry weight (C)) collected from three pastures of age 0 (seed) (▨), 8 (▩) and 46 (■) years and (a) planted into undisturbed swards in 8- and 46-year-old pastures, (b, c) planted as mixtures and as monocultures into grazed patches in the two pastures. Within a graph, bars sharing a common letter are not significantly different ($p < 0.05$). (From Mehrhoff & Turkington, 1990).

*Table 1.* Dry matter yield (kg ha$^{-1}$) of *Lolium perenne* and *Trifolium repens* when grown in mixtures. Mixtures based on *T. repens* growing with its coexisting *L. perenne* and with other *L. perenne* companions. Data are from 1984 and from a mean annual yield from 1981–1984. (From Evans et al., 1989)

| Origin of | *L. perenne* | 1984 | | | Mean 1981–1984 | | |
|---|---|---|---|---|---|---|---|
| *T. repens* | companion | *T. repens* | *L. perenne* | Mixture | *T. repens* | *L. perenne* | Mixture |
| Switzerland | 1 | 1093 | 1531 | 2624 | 1673 | 2640 | 4313 |
| | 2 | 682 | 1596 | 2278 | 1880 | 3139 | 5019 |
| | Coexisting pop | 1176 | 2176 | 3352 | 2680 | 3069 | 5749 |
| Switzerland | 1 | 1600 | 1380 | 2980 | 3853 | 2578 | 6431 |
| | 2 | 1171 | 1742 | 2913 | 4016 | 2958 | 6974 |
| | Coexisting pop | 1616 | 2823 | 4439 | 4312 | 3237 | 7549 |
| Italy | 1 | 1674 | 1618 | 3292 | 3820 | 2605 | 6425 |
| | 2 | 1110 | 1956 | 3066 | 3626 | 3038 | 6664 |
| | Coexisting pop | 1803 | 1625 | 3428 | 4110 | 2791 | 6901 |
| France | 1 | 1371 | 2245 | 3616 | 3156 | 3334 | 6490 |
| | 2 | 1392 | 2384 | 3776 | 3275 | 3711 | 6986 |
| | Coexisting pop | 1409 | 2698 | 4107 | 3615 | 3278 | 6893 |
| England | 1 | 1835 | 1564 | 3399 | 3825 | 2709 | 6534 |
| | 2 | 1796 | 2257 | 4053 | 4316 | 2832 | 7148 |
| | Coexisting pop | 1648 | 1405 | 3053 | 4368 | 2761 | 7129 |
| Overall means | 1 | 1515 | 1668 | 3183 | 3265 | 2773 | 6038 |
| | 2 | 1230 | 1987 | 3217 | 3423 | 3136 | 6559 |
| | Coexisting pop | 1531 | 2145 | 3676 | 3817 | 3027 | 6844 |

species collected from different ages of pasture perceived each other in different ways, and never in a reciprocal manner. An analysis of some component of final yield would conceal these dynamic interactions and would lead to erroneous conclusions concerning the behaviour of the components in the mixture. Generally however, *L. perenne–T. repens* mixtures used resources more effectively and were more productive than their corresponding monocultures. Chirwa (1985) collected coexisting pairs of *L. perenne* and *T. repens* from a pasture in Surrey and from a Swiss Alpine pasture, and grew them in all four combinations of *L. perenne* and *T. repens*. He did not detect a leading diagonal effect where the productivity of mixtures based on coexisting components is higher than others, but in three of the four mixtures (including both coexisting pairs) he detected a reduction in the interspecific pressures exerted by both components. He suggests that they may have developed 'tolerance' to one another. Martin & Harding (1981) posed a similar question using *Erodium cicutarium* and *Erodium obtusiplicatum*, common plant species in Californian annual grasslands. They also showed that total seed output and total reproductive rates of coexisting mixtures were higher than those of non-coexisting mixtures. McNeilly (1981) showed evidence of ecotypic differentiation of *Poa annua* with respect to competitive ability against *L. perenne*.

Mehrhoff & Turkington (1990, 1996) propagated individuals of *L. perenne* and *T. repens* from seed and from two different-aged (8 and 46-year old) pastures. The material was then transplanted back into the two pastures as monocultures and as *L. perenne-T. repens* mixtures. The experiments were planted in spring, grazed during summer and fall, allowed to grow until May, then harvested. *T. repens* showed a significant age-related pattern in growth where plant material from older pastures outyielded that from younger pastures both when planted into cleared plots without competitors and into the dense sward (Figure 1a). This indicates that microevolution can apparently occur quite rapidly. In addition, yield per plant for both species was higher in mixture than in monoculture, and the highest mixture yield came from the plant material collected from the oldest pasture (Figure 1b, 1c). It is of interest that the age-related increases in *L. perenne* growth were evident only when grown in mixture with *T. repens*, not in monoculture. In another series of studies, two-species mixtures of *L. perenne* grown with *D. glomerata, H. lanatus*, and *T. repens* were planted in a common garden. Genets of each species were collected from different pastures ranging in age from 0 (freshly sown) to 46 years. For each of the three species combinations, total ramet production was significantly greater in mixtures from older pastures than from younger pastures.

### *Within-species patterns*

Turkington & Harper (1979b) collected ramets of *T. repens* from within patches dominated by each of four different grasses. The *T. repens* populations were grown in all possible combinations with the four species of grass in greenhouse flats containing a standard potting compost. Each clover population tended to grow best with the grass from which it had originally been sampled, i.e., a principal diagonal effect (see Luescher & Jacquard, 1991). A similar result was reported when the same design was done in the field although a local soil influence was also noted. It is evident that the different grasses impose different constraints on the growth of *T. repens* and that the *T. repens* populations has differentiated into subpopulations defined by the identity of the grass neighbor. This experimental design could not rigorously test (i) if the shift was genetic, or merely a plastic response to changing conditions, or (ii) if competition really was the driving force. Neither claim was made in the paper (Turkington & Harper, 1979b) although strongly implied (see Snaydon, 1985), and a subsequent study by Evans & Turkington (1988) highlights the problem.

From an old pasture in British Columbia, Evans & Turkington (1988) collected 100 ramets of *T. repens* from each of four neighborhoods dominated by *D. glomerata, H. lanatus, L. perenne* and *Poa compressa*. These were transplanted into a common garden, without competitors, and later scored for twelve morphological characters. For ten of the characters, a significant proportion of the variation between sampled ramets was accounted for by the identity of the neighboring grass species with which the *T. repens* ramet had been growing in the pasture (Table 2). These same clovers were grown for two more years in the common garden and were then scored for morphological characters as before. In no case was a significant proportion of the variation in characters now found to be due to the previous grass neighbors (Table 2). The clear implication of this study is that divergence patterns in *T. repens* morphology have a plastic rather than a genetic basis.

As a consequence of these apparently conflicting results, Turkington (1989a) constructed a much more complicated and rigorous form of this design, following the protocol of Connell (1980). Specifically he was asking (i) has population divergence happened, (ii) does it have a genetic basis, and (iii) was it caused by competition. Again, a home-site advantage was demonstrated for *T. repens* i.e. divergence had occurred. This was the first to actually demonstrate a genetic shift in the *T. repens* populations in response to competition from their grass neighbors.

### *Between-genotype patterns*

Given Darwin's emphasis on the fate of individuals as the cornerstone of natural selection, it is surprising that we know so little about how the events occurring at this scale are relevant to influencing community structure.

Aarssen & Turkington (1985b) reduced the questions of the previous section from investigating species to investigating genotypes. They collected neighboring pairs of genotypes of *L. perenne* and *T. repens* from four different locations in a 40-year-old pasture

*Table 2.* Summary of analyses of variance for measured morphological characters of *Trifolium repens* from neighborhoods dominated by *Dactylis glomerata* (D), *Holcus lanatus* (H), *Lolium perenne* (L), and *Poa compressa* (P). The 1982 measurements were made shortly after the original collections, and the 1984 measurements were made on the same plant material four generations (2 years) later. The multiple range tests are on the means for measured characters classified by neighborhood type from which the ramets of *T. repens* were collected; means are ranked from smallest to largest, and underlined sets of means are not significantly different (p > 0.05). (From Evans & Turkington, 1988)

| | 1982 | | Multiple range test | 1984 | |
|---|---|---|---|---|---|
| | Percent variation accounted for | Significance | | Percent variation accounted for | Significance |
| Root weight | 4.8 | ** | LD HP | 0.0 | NS |
| Shoot weight | 4.8 | ** | LD HP | 0.0 | NS |
| Total weight | 5.3 | ** | LD HP | 0.0 | NS |
| Primary stolon number | 5.7 | ** | DL HP | | Not measured |
| Total stolon number | 2.7 | * | DL HP | 0.0 | NS |
| Internode number | 0.7 | NS | | | Not measured |
| Primary stolon length | 0.7 | NS | | 0.17 | NS |
| Secondary stolen length | 2.0 | * | LD PH | | Not measured |
| Total stolen length | | Not measured | – | 0.0 | NS |
| Internode length | 2.4 | * | LD HP | 0.0 | NS |
| Petiole length | 20.2 | ** | LD HP | 0.0 | NS |
| Leaf weight | 11.8 | ** | – | 0.0 | NS |
| Leaf length | 19.6 | ** | – | 0.0 | NS |

in British Columbia. All 16 possible interspecific combinations of *L. perenne* and *T. repens* genotypes were planted together in pots, the mixture clipped regularly for 1 year, and the cumulative yield of each of the two species recorded. For each pair, the yield of *T. repens* was generally highest when grown in mixture with its natural *L. perenne* neighbor, although a similar pattern was not detected for the grass (cf. Evans et al., 1989). The natural neighbor mixtures did not differ significantly in total yield from pairs of nonnatural neighbors (Figure 2). These results were more-or-less mimicked by Lüscher et al. (1992) using five genotypes each of the same two species. For each of the five natural combinations the percentage of clover was higher than the overall non-neighboring mean. Averaged over six harvests, the diagonal yield of *T. repens* was about 10% higher than that of the average off-diagonal yield (Figure 3). The diagonal yield of *L. perenne* was about 10% lower and total yield about the same as their respective off-diagonal averages.

Gliddon & Trathan (1985) did a rather elegant field experiment using *T. repens* and *L. perenne* transplants from four sites in the old (100 years) pasture in North Wales. They clearly showed that both *T. repens* (especially for number of survivors) and *L. perenne* (for dry weight) are highly locally specialized (Figure 4). A final competition experiment using plant material from two of the sites showed a significant leading diagonal effect.

A final example concerns the work of Collins & Rhodes (1990). They grew six *T. repens* varieties with three *L. perenne* companions: (a) the coexisting *L. perenne* collected from the same location as the *T. repens*, (b) the variety Ba 9462 bred specifically to be compatible with *T. repens*, and (c) S23, a variety of *L. perenne* that has been used to evaluate clover varieties during breeding programs. For all the *T. repens* the greatest *T. repens* yield, and the total mixture yield, was highest in mixtures with the coexisting grass than with Ba 9462 (Table 3). It is notable that all *T. repens*

*Table 3.* Annual dry matter yield (t.ha$^{-1}$) of *Trifolium repens* populations in mixture with *Lolium perenne*. (From Collins & Rhodes, 1990)

| | | Yield (t.ha$^{-1}$) | | |
|---|---|---|---|---|
| *T. repens* population | *L. perenne* companion | *T. repens* | *L. perenne* | Total |
| Ac 3441 | Coexisting | 4.6 | 3.5 | 8.1 |
| | S. 23 | 1.2 | 2.1 | 3.3 |
| | Ba 9462 | 3.4 | 2.8 | 6.2 |
| Ac 3160 | Coexisting | 8.4 | 3.1 | 11.5 |
| | S. 23 | 6.4 | 2.2 | 8.6 |
| | Ba 9462 | 7.9 | 3.2 | 11.1 |
| Ac 4512-13-14 | Coexisting | 7.5 | 3.2 | 10.7 |
| | S. 23 | 6.0 | 2.5 | 8.5 |
| | Ba 9462 | 6.8 | 2.7 | 9.5 |
| Ac3449-53 | Coexisting | 7.0 | 3.4 | 10.4 |
| | S. 23 | 5.5 | 2.7 | 8.2 |
| | Ba 9462 | 6.4 | 3.4 | 9.8 |
| Menna | Coexisting | 7.9 | 2.8 | 10.7 |
| | S. 23 | 6.4 | 2.3 | 8.4 |
| | Ba 9462 | 7.5 | 2.9 | 10.3 |

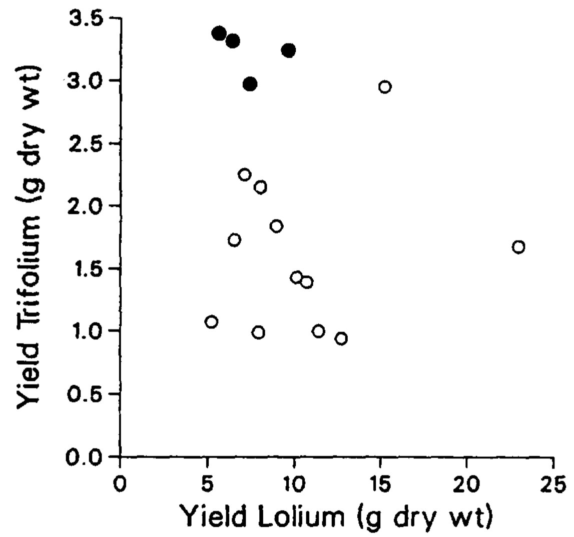

*Figure 2.* Yield of *Trifolium repens* and *Lolium perenne* when genotypes of the two species were grown in mixture. Mixtures that were collected as natural neighboring combinations in the field are designated by (●) and mismatched combinations by (○) (Data from Aarssen & Turkington, 1985b).

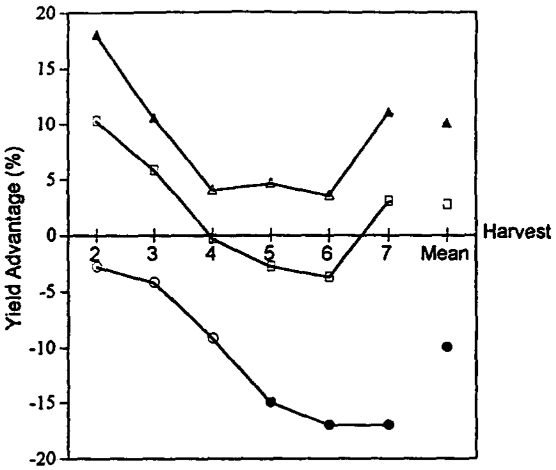

*Figure 3.* Comparative yield of *Trifolium repens* (Δ), *Lolium perenne* (○), and their mixture (●), over six successive harvests. The yield advantage is a comparative measure of the percentage increase or decrease in yield of natural neighboring combinations compared with the mean yield of all mismatched combinations. Filled symbols indicate a significant neighbour effect ($p < 0.05$) (From Lüscher et al., 1992).

yielded more with Ba 9462 than with S23 suggesting that it may be possible to breed for more general compatibility in grasses. It must be noted however, that some studies have reported that not all *T. repens* grow best with their coexisting grass neighbors (e.g. Collins & Rhodes, 1989).

Competition experiments between *L. perenne* and *T. repens* (at different scales) are plentiful, but where

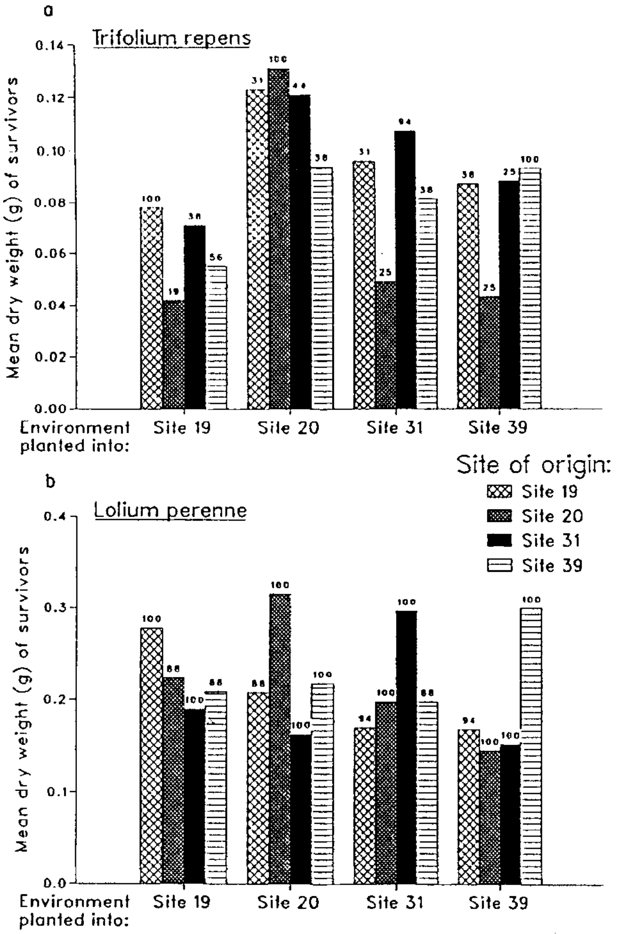

*Figure 4.* Mean dry weight of survivors of (a) *Trifolium repens* and (b) *Lolium perenne* collected from four sites and replanted into their site of origin and also transplanted into the other three sites. Numbers on top of bars are percentage survival values. (Data from Gliddon & Trathan, 1985).

a specific hypothesis about adaptation has been tested (Turkington & Harper, 1979b; Aarssen & Turkington, 1985b; Evans et al., 1985, 1989; Gliddon & Trathan, 1985; Chanway et al., 1989; Turkington, 1989a), in all cases *T. repens* grew best when planted with its natural neighbor yet a reciprocal effect in the *L. perenne* has only been reported once (Gliddon & Trathan, 1985).

### *Is there a mechanism?*

Watkin Williams (1970), in his opening address to a White Clover Research symposium, sponsored by the British Grassland Society, stated that 'Another major gap in our understanding. . . is the precise relationship between the clover plant *together with its symbiont* (emphasis added), and co-habiting grass species in a mixed sward.' In spite of the plea, and

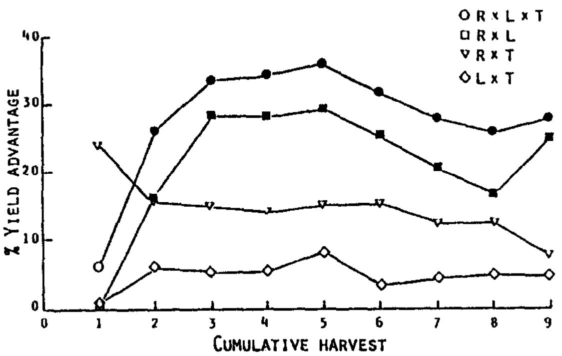

*Figure 5.* The influence of *Lolium perenne* genotype and *Rhizobium leguminosarum* biovar *trifolii* strain on the dry weight yield of *Trifolium repens*. The yield advantage is a comparative measure of the percentage increase or decrease in yield of natural neighboring combinations compared with the mean yield of all mismatched combinations. Filled symbols indicate a significant yield advantage ($p < 0.05$). (From Chanway et al., 1989).

considering that one entire section of the symposium (almost 80 published pages) was devoted to the white clover/Rhizobium symbiosis, there have been few attempts to include the associated microorganisms in these investigations, and almost nothing that I know of, to do it in the field. A number of more recent studies has attempted to partially remedy this situation (Chanway et al., 1988, 1989, 1991; Holl et al., 1988; Turkington et al., 1988; Thompson et al., 1990). Specifically Chanway et al. (1989) hypothesized that if individual genotypes of *L. perenne* and *T. repens* had become specifically adapted to one another, then the identity of the *Rhizobium* genotypes should be matched to the *L. perenne*-*T. repens* association. The objectives of the work were to (i) assess the effect of *Rhizobium* on the genotype specificity between individual *L. perenne* and *T. repens* plants collected from a permanent pasture, and (ii) to determine the effectiveness of *Rhizobium* strains when tested with coexistent or unrelated *T. repens* host genotypes grown in species mixture with *L. perenne*. We used the term 'homologous' to describe cuttings of *L. perenne* and of *T. repens*, and *Rhizobium* isolated from *T. repens* root nodules, all collected from the same point in the pasture. The experiment consisted of growing these three components (n = 3 for each factor) in a factorial design. To corroborate the patterns observed by Aarssen & Turkington (1985b), we would have predicted a yield advantage to ramets of *T. repens* when growing with matched *L. perenne* tillers; this effect was detected but it was not significant (Figure 5) i.e. specificity between *L. perenne* and *T. repens*

*Table 4.* Effect of *Bacillus polymyxa* inoculation on plant dry weight (mg) in a 50:50 (by numbers) mixture of *Lolium perenne* and *Trifolium repens* as genotypic homology is increased. (From Chanway et al., 1988)

| Degree of | *Lolium perenne* | | | *Trifolium repens* | | |
|---|---|---|---|---|---|---|
| homology | Inoculation | | % change | Inoculation | | % change |
| | - | + | | - | + | |
| Partial[b] | 1631 | 1502 | - 9 | 688 | 845 | + 23[a] |
| Substantial[c] | 1856 | 1820 | - 2 | 721 | 1025 | + 43[a] |
| Total[d] | 1624 | 1876 | + 16 | 619 | 985 | + 59 |

[a] Significant at $p < 0.05$.

[b] Plant populations comprised mixtures of three genotypes each of the two species, one of which was homologous to the *Bacillus* inoculum strain.

[c] The yield species was homologous to *Bacillus*; the neighboring plant population comprised a mixture of three genotypes, one of which was homologous to the *Bacillus* inoculum strain.

[d] The yield species was homologous to both the *Bacillus* and the neighboring plant population.

is lost if the coexisting *Rhizobium* is not present, a result not corroborated by Lüscher et al. (1992). When *Rhizobium* from homologous sites is used to inoculate the mixture (Figure 5), the effect becomes very pronounced, with homologous groups outyielding nonhomologous groups by up to 35%. However, a *T. repens* yield advantage of up to 30% can also be achieved when only homologous *Rhizobium-L. perenne* combinations are used, regardless of the identity of the *T. repens*. This indicates that it is the specific associations of *Rhizobium-L. perenne* genotypes that have the greatest impact on *T. repens* yield, even though the *Rhizobium* is symbiotic with *T. repens*.

The work also considered a grass-associated bacterium, *Bacillus polymyxa* (Chanway et al., 1988). It is clear from the data that the genotypic match between plant species and bacterial inoculant is critical to the direction and magnitude of the growth response. As the level of homology was increased between participating species, yield of *L. perenne* increased from slightly below those of the uninoculated controls to 16% ($p > 0.5$) greater under conditions of total homology (Table 4). However, data for the white clover clones reflected a significant yield increase in response to inoculation and increasing homology from 23% with partial homology, to 42% with substantial homology and finally 59% under total homology. These effects appear to be truly novel in an agricultural context and may represent the discovery of a significant component of the variation observed in plant growth promotion by rhizosphere bacteria. Exploitation of these relationships could contribute to the development of inoculants which consistently and effectively promote crop growth.

## A final comment

A number of the studies I have described in this paper were done in a pasture in North Wales when it was about 100 years old (Peters, 1980). The pasture has large areas dominated by single grasses; the *L. perenne*-dominated site is about 150 $m^2$ (Turkington & Harper, 1979a; Thorhallsdottir, 1990a). Here, during its lifetime, the individual *T. repens* will not extend its stolons through many different environments, but will sample its environment in a coarse-grained manner, and may spend many generations coexisting with one species of grass. In contrast, many of the other studies I described were done in an old pasture in British Columbia which was 35–45 years-old during the studies. This pasture has a similar botanical composition to the Welsh pasture but has very different patterns of species distribution. The pasture is a complex mosaic of grass patches where most patches are probably less than 1 $m^2$ and closer to 0.25 $m^2$ (Evans & Turkington, 1988). Here, an individual *T. repens* will extend its stolons through many different neighborhoods and sample the different biotic environments in a fine-grained way. Thus, one might expect to detect genetically based microevolutionary changes in the *T. repens* population in the Welsh pasture in response to different coarse-grained patches. In contrast, the British Columbia pasture population of *T. repens* might be expected to respond in a plastic manner to the fine-grained mosaic environment.

## References

Aarssen, L.W., 1983. Ecological combining ability and competitive combining ability in plants: Toward a general evolutionary theory of coexistence in systems of competition. Am Nat 122: 707–731.

Aarssen, L.W. & R. Turkington, 1985a. Vegetation dynamics and neighbour associations in pasture-community evolution. J Ecol 73: 585–603.

Aarssen, L.W. & R. Turkington, 1985b. Biotic specialization between neighbouring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. J Ecol 73: 605–614.

Aarssen, L.W. & R. Turkington, 1985c. Within-species diversity in natural populations of *Holcus lanatus, Lolium perenne*, and *Trifolium repens* from four different-aged pastures. J Ecol 73: 869–886.

Allard, R.W. & J. Adams, 1969. Population studies in predominantly self-pollinating species. XIII. Intergenotypic competition and population structure in barley and wheat. Am Nat 163: 621–644.

Allard, R.W. & P.E. Hansche, 1964. Some parameters of population variability and their implications in plant breeding. Adv Agron 16: 281–325.

Annicchiarico, P. & E. Piano, 1994. Interference effects in white clover genotypes grown as pure stands and binary mixtures with different grass species and varieties. Theor Appl Gen 88: 153–158.

Antonovics, J., 1978. The population genetics of mixtures. In: J.R. Wilson (Ed.), Plant Relations in Pastures, pp 233–252. CSIRO, Melbourne.

Arthur, W., 1982. The evolutionary consequences of interspecific competition. Adv Ecol Res 12: 127–187.

Benton, M.J., 1987. Progress and competition in macroevolution. Biol Rev 62: 305–338.

Breese, E.L., M.D.Haywood & A.C. Thomas, 1965. Somatic selection in perennial ryegrass. Heredity 20: 367–379.

Brougham, R.W. & W. Harris, 1967. Rapidity and extent of changes in genotypic structure induced by grazing in a ryegrass population. NZ J Ag Res 10: 56–65.

Burdon, J.J., 1980. Intra-specific diversity in a natural population of *Trifolium repens*. J Ecol 68: 717–735.

Burdon, J.J., 1983. Biological flora of the British Isles, *Trifolium repens*. J Ecol 71: 307–330.

Burdon, J.J. & J.L. Harper, 1980. Relative growth rates of individual members of a plant population. J Ecol 68: 953–968.

Burgess, R.S.L. & R.A. Ennos, 1986. Selective grazing of acyanogenic white clover: Variation in behaviour among populations of the slug *Deroceras reticulatum*. Oecologia 73: 432–435.

Cahn, M.A. & J.L. Harper, 1976a. The biology of the leaf mark polymorphism in *Trifolium repens* L. I. Distribution of phenotypes at a local scale. Heredity 37: 309–325.

Cahn, M.A. & J.L. Harper, 1976b. The biology of the leaf mark polymorphism in *Trifolium repens* L. II. Evidence for the selection of leaf marks by rumen fistulated sheep. Heredity 37: 327–333.

Chanway, C.P., F.B. Holl & R. Turkington, 1988. Genotypic coadaptation in growth promotion of forage species by *Bacillus polymyxa*. Plant Soil 106: 281–284.

Chanway, C.P., F.B. Holl & R. Turkington, 1989. Effect of *Rhizobium leguminosarum* biovar *trifolii* genotype on specificity between *Trifolium repens* L. and *Lolium perenne* L. J Ecol 77: 1150–1160.

Chanway, C.P., R. Turkington & F.B. Holl, 1991. Ecological implications of specificity between plants and rhizosphere microorganisms. Adv Ecol Res 21: 121–169.

Charles, A.H., 1961. Differential survival of cultivars of *Lolium, Dactylis* and *Phleum*. J Br Grassld Soc 16: 69–75.

Charles, A.H., 1972. Ryegrass populations from intensively managed leys. III. Reaction to management, nitrogen application and *Poa trivialis* L. in field trials. J Agric Sci 79: 205–215.

Chestnut, D.M.B. & J. Lowe, 1970. Agronomy of white clover/grass swards: a review. Occ Sym Br Grassld Soc 6: 191–213.

Chirwa, R.M., 1985. Inter and intra-specific competition in perennial ryegrass-white clover mixtures. MSc Thesis, University of Wales, Aberystwyth, Wales.

Collins, R.M. & I. Rhodes, 1989. Yield of white clover populations in mixture with contrasting perennial ryegrasses. Grass For Sci 44: 111–115.

Collins, R.P. & I. Rhodes, 1990. The basis and significance of compatibility in grass/clover mixtures. Herba 3: 30–32.

Connell, J.H., 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35: 131–138.

Connolly, V., C.L. Masterson & D. Conniffe, 1969. Some genetic aspects of the symbiotic relationship between white clover (*Trifolium repens* L.) and *Rhizobium trifolii*. J Theor Appl Gen 39: 206–213.

Davies, W.E. & N.R. Young, 1967. The characters of European, Mediterranean and other populations of white clover (*Trifolium repens* L.). Euphytica 16: 330–340.

Dirzo, R. & J.L. Harper, 1982. Experimental studies on slug-plant interactions. III. Differences in the acceptability of *Trifolium repens* to slugs and snails. J Ecol 70: 101–118.

Dodd, M., J. Silvertown, K. McConway, J. Potts & M. Crawley, 1994. Stability in the plant communities of the Park Grass Experiment: the relationships between species richness, soil pH and biomass variability. Phil Trans Roy Soc B 346: 185–193.

Dodd, M., J. Silvertown, K. McConway, J. Potts & M. Crawley, 1995. Community stability: a 60-year record of trends and outbreaks in the occurrence of species in the Park Grass Experiment. J Ecol 83: 277–285.

Donald, C.M., 1963. Competition among crop and pasture plants. Adv Agron 15: 1–118.

Ennos, R.A., 1981. Detection of selection in populations of white clover (*Trifolium repens* L.). Biol J Linn Soc 15: 75–82.

Evans, D.R., J. Hill, T.A. Williams & I. Rhodes, 1985. Effects of coexistence in the performances of white clover-perennial ryegrass mixtures. Oecologia 66: 536–539.

Evans, D.R., J. Hill, T.A. Williams & I. Rhodes, 1989. Coexistence and the productivity of white clover-perennial ryegrass mixtures. Theor Appl Gen 77: 65–70.

Evans, R.C. & R. Turkington, 1988. Maintenance of morphological variation in a biotically patchy environment. New Phytol 109: 369–376.

Fowler, N., 1981. Competition and coexistence in a North Carolina grassland. II. The effects of the experimental removal of species. J Ecol 69: 843–852.

Fowler, N., 1982. Competition and coexistence in a North Carolina grassland. III. Mixtures of component species. J Ecol 70: 77–92.

Frame, J. & P. Newbould, 1986. Agronomy of white clover. Adv Agron 40: 1–87.

Ghilarov, A.M., 1984. The paradox of the plankton reconsidered; or, why do species coexist. Oikos 43: 46–52.

Gliddon, C. & P. Trathan, 1985. Interactions between white clover and perennial ryegrass in an old permanent pasture. In: J. Haeck & J.W. Woldendorp (Eds), Structure and Functioning of Plant Populations, 2, pp. 161–169. North-Holland, Amsterdam.

Grant, P., 1975. The classical case of character displacement. Evol Biol 8: 237–337.

Grubb, P.J., 1977. The maintenance of species richness in plant communities: the importance of the regeneration niche. Biol Rev 52: 107–145.

Hairston, N.G., K.C. Nishikawa & S.L. Stenhouse, 1987. The evolution of competing species of terrestrial salamanders: Niche partitioning or interference? Evol Ecol 1: 247–262.

Harper, J.L., 1967. A Darwinian approach to plant ecology. J Ecol 55: 247–270.

Harper, J.L., 1978. Plant relations in pastures. In: J.R. Wilson (Ed.), Plant Relations in Pastures, pp. 3–14. CSIRO, Melbourne.

Harris, W., 1987. Population dynamics and competition. In: M.J. Baker & W.M. Williams (Eds), White Clover, pp. 203–275. Commenwealth Agricultural Bureau, Wallingford, UK.

Haynes, R.J., 1980. Competitive aspects of the grass-legume association. Adv Agron 33: 227–261.

Hill, J., 1990. The three Cs - competition, coexistence and coevolution – and their impact on the breeding of forage crop mixtures. Theor Appl Gen 79: 168–176.

Hill, J., K. Mather & P.D.S. Caligari, 1987. Analysis of competitive ability among genotypes of perennial ryegrass. II. Effect upon dry matter production. Euphytica 36: 109–115.

Hill, J. & T.P.T. Michaelson-Yeates, 1987. Effects of competition upon the productivity of white clover-perennial ryegrass mixtures. Seasonal trends. Plant Breed 99: 251–262.

Holl, F.B., C.P. Chanway, R. Turkington & R. Radley, 1988. Growth response of crested wheatgrass (*Agropyron cristatum* L.), white clover (*Trifolium repens* L.), and perennial ryegrass (*Lolium perenne* L.) to inoculation with *Bacillus polymyxa*. Soil Bio Biochem 20: 19–24.

Jalloq, M.C., 1975. The invasion of molehills by weeds as a possible factor in the degeneration of reseeded pastures. I. The buried viable seed population of molehills from four reseeded pastures in West Wales. J Appl Ecol 12: 643–657.

Joy, P. & A. Laitinen, 1980. Breeding for coadaptation between Red Clover and Timothy. Hankkija's Seed Publ 13. Hankkija Plant Breeding Inst, Finland.

Kelley, S.E. & K. Clay, 1987. Interspecific competitive interactions and the maintenance of genotypic variation within two perennial grasses. Evolution 41: 92–103.

Kerner von Marilaun, A., 1895. The Natural History of Plants. Vol. 2. Blackie, London.

King, T.J., 1977. The plant ecology of ant-hills in calcareous grasslands. II. Succession on the mounds. J Ecol 65: 257–278.

Lawlor, L.R. & J. Maynard Smith, 1976. The coevolution and stability of competing species. Am Nat 110: 79–99.

Luescher, A. & P. Jacquard, 1991. Coevolution between interspecific plant competitors? TREE 6: 355–358.

Lüscher, A., J. Connolly & P. Jacquard, 1992. Neighbour specificity between *Lolium perenne* and *Trifolium repens* from a natural pasture. Oecologia 91: 404–409.

MacArthur, R.H. & R. Levins, 1964. Competition, habitat selection, and character displacement in a patchy environment. Proc Nat Acad Sci USA 51: 1207–1210.

MacArthur, R.H. & R. Levins, 1967. The limiting similarity, convergence and divergence of coexisting species. Amer Nat 101: 377–385.

Martin, M.M. & J. Harding, 1981. Evidence for the evolution of competition between two species of annual plants. Evolution 35: 975–987.

McNeilly, T., 1981. Ecotypic differentiation in *Poa annua*: Intrapopulational differences in response to competition and cutting. New Phytol 88: 539–547.

McNeilly, T. & M.L. Roose, 1984. The distribution of perennial ryegrass genotypes swards. New Phytol 98: 503–513.

Mehrhoff, L.A. & R. Turkington, 1990. Microevolution and site-specific outcomes of competition among pasture plants. J Ecol 78: 745–756.

Mehrhoff, L.A. & R. Turkington, 1996. Growth and survival of white clover (*Trifolium repens* L.) transplanted into patches of different grass species. Can J Bot (in press).

Menchaca, L. & J. Connolly, 1990. Species interference in white clover-ryegrass mixtures. J Ecol 78: 223–232.

Mytton, L.R., 1975. Plant genotype × *Rhizobium* strain interactions in white clover. Ann Appl Biol 80: 103–107.

Parish, R. & R. Turkington, 1990a. The influence of dung pats and molehills on pasture composition. Can J Bot 68: 1698–1705.

Parish, R. & R. Turkington, 1990b. The colonization of dung pats and molehills in permanent pastures. Can J Bot 68: 1706–1711.

Peart, D.R., 1989. Species interactions in a successional grassland. III. Effects of canopy gaps, gopher mounds and grazing on colonization. J Ecol 77: 267–289.

Peters, B., 1980. The demography of leaves in a permanent pasture, Ph.D. thesis, University College of North Wales, Bangor, Wales.

Rhodes, I., 1968. Yield of contrasting ryegrass varieties in monoculture and mixed culture. J Brit Grassl Soc 23: 156–158.

Rhodes, I., 1970. The production of contrasting genotypes of perennial ryegrass (*Lolium perenne* L.) in monocultures and mixed cultures of varying complexity. J Brit Grassl Soc 25: 285–288.

Schoener, T.W., 1983. Field experiments on interspecific competition. Am Nat 122: 240–285.

Seaton, A.P.C. & J. Antonovics, 1967. Population interrelationships. I. Evolution in mixtures of *Drosophila* mutants. Heredity 22: 19–35.

Silvertown, J., 1980. The dynamics of a grassland ecosystem: botanical equilibrium in the Park Grass Experiment. J Appl Ecol 17: 491–504.

Silvertown, J., M. Dodd, K. McConway, J. Potts & M. Crawley, 1994. Rainfall, biomass variation, and community composition in the Park Gras Experiment. Ecology 75: 2430–2437.

Snaydon, R.W., 1962. Microdistribution of *Trifolium repens* and its relation to soil factors. J Ecol 50: 133–143.

Snaydon, R.W., 1978. Genetic changes in pasture populations. In: J.R. Wilson (Ed.), Plant Relations in Pastures, pp. 253–272. CSIRO, Melbourne.

Snaydon, R.W., 1985. Aspects of the ecological genetics of pasture species. In: J. Haeck & J.W. Woldendorp (Eds), Structure and Functioning of Plant Populations, 2, pp. 127–152. North-Holland, Amsterdam.

Soane, I.D. & A.R. Watkinson, 1979. Clonal variation in populations of *Ranunculus repens*. New Phytol 82: 557–573.

Thompson, J.D., R. Turkington & F.B. Holl, 1990. The influence of *Rhizobium leguminosarum* biovar *trifolii* on the growth and neighbour relationships of *Trifolium repens* and three grasses. Can J Bot 68: 296–303.

Thompson, J.N., 1985. Within-patch dynamics of life histories, populations; selection over time in small spaces. In: S.T.A. Pickett & P.S. White (Eds), The Ecology of Natural Disturbance and Patch Dynamics, pp. 253–264. Academic Press, Orlando.

Thompson, J.N., 1988. Variation in interspecific interactions. Ann Rev Ecol Syst 19: 65–87.

Thompson, J.N., 1994. The Coevolutionary Process. University of Chicago Press, Chicago.

Thórhallsdóttir, T.E., 1990a. The dynamics of a grassland community: a simultaneous investigation of spatial and temporal heterogeneity at various scales. J Ecol 78: 884–908.

Thórhallsdóttir, T.E., 1990b. The dynamics of five grasses and white clover in a simulated mosaic sward. J Ecol 78: 909–923.

Trathan, P., 1983. Clonal interactions of *Trifolium repens* and *Lolium perenne*. Ph.D. thesis, University of Wales, Bangor, Wales.

Trenbath, B.R., 1974. Biomass productivity of mixtures. Adv Agron 26: 177–210.

Turkington, R., 1975. Relationships between neighbours among species of permanent grassland (especially *Trifolium repens* L.). Ph.D. thesis. University of Wales, Bangor, Wales.

Turkington, R., 1989a. The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. V. The coevolution of competitors. J Ecol 77: 717–733.

Turkington, R., 1989b. The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. VI. Conditions effects by neighbours. J Ecol 77: 734–746.

Turkington, R., 1993. Effect of propagule source on competitive ability of pasture grasses: spatial dynamics of six grasses in simulated swards. Can J Bot 72: 111–121.

Turkington, R. & J.J. Burdon, 1983. Biology of Canadian weeds. 57. *Trifolium repens*. L. Can J Pl Sci 63: 243–266.

Turkington, R., F.B. Hall, C.P. Chanway & J.D. Thompson, 1988. The influence of microorganisms, particularly *Rhizobium*, on plant competition in grass-legume communities. In: A.J. Davy, M.J. Hutchings & A.R. Watkingson (Eds), Plant Population Ecology, pp. 343–366. Blackwell, Oxford, England.

Turkington, R. & J.L. Harper, 1979a. The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. I. Ordination, pattern and contact. J Ecol 67: 201–218.

Turkington, R. & J.L. Harper, 1979b. The growth, distribution and neighbour relationships of *Trifolium repens* in a permanent pasture. IV. Fine scale biotic differentiation. J Ecol 67: 245–254.

Turkington, R. & P.A. Jolliffe, 1996. Interference in *Trifolium repens* — *Lolium perenne* mixtures: short-term and long-term relationships. J Ecol (in press).

Turkington, R., E. Klein & C.P. Chanway, 1993. Interactive effects of nutrients and disturbance: an experimental test of plant strategy theory. Ecology 74: 863–878.

Turkington, R. & L.A. Mehrhoff, 1990. The role of competition in structuring pasture communities. In: J.B. Grace & D. Tilman (Eds), Perspectives on Plant Competition, pp. 307–340. Academic Press, San Diego.

deWit, C.T., G.P. Tow & G.C. Ennik, 1966. Competition between legumes and grasses. Agric Res Rep 687: 1–30, Wageningen.

White, J., 1985. The census of plants in vegetation. In: J. White (Ed.), The Population Structure of Vegetation, pp. 33–38. Junk Publishers, Dordrecht.

Williams, W., 1970. White clover in British Agriculture. Occ Sym Br Grassld Soc 6: 1–10.

Zannone, L., L. Assemat, P. Rotili & P. Jacquard, 1983. An experimental study of intraspecific competition within several forage crops. Agronomie 3: 451–459.

Zannone, L., P. Rotili, R. Paoletti & C. Scotti, 1986. Experimental studies of grass-legume associations. Agronomie 6: 931–940.