

Diversity peaks at intermediate productivity in a laboratory microcosm

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The species diversity of natural communities is often strongly related to their productivity. The pattern of this relationship seems to vary: diversity is known to increase monotonically with productivity, to decrease monotonically with productivity, and to be unimodally related to productivity, with maximum diversity occurring at intermediate levels of productivity^{1–3}. The mechanism underlying these patterns remains obscure, although many possibilities have been suggested^{3–6}. Here we outline a simple mechanism—involving selection in a heterogeneous environment—to explain these patterns, and test it using laboratory cultures of the bacterium *Pseudomonas fluorescens*. We grew diverse cultures over a wide range of nutrient concentrations, and found a strongly unimodal relationship between diversity and productivity in heterogeneous, but not in homogeneous, environments. Our result provides experimental evidence that the unimodal relationship often observed in natural communities can be caused by selection for specialized types in a heterogeneous environment.

Productivity is the rate of production of organic matter by a community⁷, and is in general determined by the rate of energy supply. At continental scales, the diversity of plants and animals usually increases monotonically with productivity, or some suitable surrogate measure^{3,8}. At the smaller scale of regions within continents, the patterns are more complex. The prevailing view has been that diversity and productivity are unimodally related^{9–13}, such that diversity at first increases with productivity or production, reaches a maximum for intermediate values, and then declines to lower levels in highly productive systems. Support for this view comes from nutrient addition experiments in terrestrial plant communities and aquatic systems^{14–19}, although the generality of the unimodal pattern has been questioned and the theory criticized^{3,6}.

The mechanism underlying this pattern remains unclear, although it is likely to involve competition in heterogeneous environments. Diversity can be maintained if relative competitive ability, or fitness, varies among niches within an environment²⁰, where ‘niches’ is usually interpreted as a collection of sites offering different conditions of growth. This will create antagonistic selection that preserves local adaptation, provided that each niche is capable of supporting similar numbers of individuals in the community²¹. If niches are unable to support approximately equal numbers of individuals, then the type that is best-adapted to the most productive niche will come to dominate the community^{22,23} and diversity will be low.

The effect of varying productivity can be described by a simple modification of Levene’s²⁰ model for the maintenance of diversity in a heterogeneous environment. In the classic version of the model two (or more) types, which may be species or asexual genotypes, are distributed at random into two (or more) niches. Selection occurs within each niche, followed by reproduction. Each niche then contributes a fixed proportion of offspring c_j , such that $\sum c_j = 1$, to

a common pool, and the sequence is repeated. With just two types, the frequency of type 1 at equilibrium is

$$p_1^* = \frac{[c_1(w_{11}w_{22} - w_{12}w_{21}) + w_{21}(w_{12} - w_{22})]}{(w_{21} - w_{11})(w_{12} - w_{22})} \quad (1)$$

where w_{ij} is the fitness of the i th type in the j th niche, and both types coexist if $0 < p_1^* < 1$ (for a full derivation, see Box 1). Note that two conditions must be met to ensure coexistence of both types. The first is the relative fitness of the types within each niche: each type must be superior in one niche but not the other, giving rise to antagonistic selection. This will be the case when the term describing genotype-by-environment interaction for fitness, $(w_{11}w_{22} - w_{12}w_{21})$, is non-zero. The second is the fraction of the total population contributed by each niche, c_j . Each niche must contribute approximately equal proportions to the total population. When both conditions hold, the equilibrium point is stable and diversity is maintained through negative frequency-dependent selection; either type has greater fitness when rare because it is able to invade the niche to which it is better adapted.

Productivity can be incorporated into the classic Levene model by allowing absolute fitness, w_{ij} , to be a function of resource supply rate, R . The Michaelis–Menton relation provides a suitable functional form:

$$w_{ij} = \frac{w_{\max,ij}(R - R_{\min,ij})}{K_{m,ij} + (R - R_{\min,ij})} \quad (2)$$

where, for each type i in niche j , R_{\min} is the minimal resource supply rate required to maintain viability, w_{\max} is the maximum number of individuals that could be produced at maximal resource supply rates, and K_m , the resource supply rate at $w_{\max}/2$, is a measure of the ability to convert resources into individuals at half-maximal resource supply rates. These parameters thus determine the number of individuals produced by a given type within a niche across the entire range of resource supply rates. We assume, as in the original model, that relative fitness within a niche remains constant across the entire range of resource supply rates. Given antagonistic selection, the outcome of selection is then governed solely by the contribution of each niche to the total population, which responds to resource supply rate as specified in equation (2).

To see this more clearly, consider Figs 1a and 2a, which depict two sorts of relationship between fitness and resource supply rate for two types, each specialized to a different niche (and therefore satisfying the necessary condition that selection be antagonistic). In both cases, the community is dominated at low resource supply rates by the type that is fittest in the niche where growth requires the lowest minimum resource supply rate (Figs 1a and 2a). As resource supply rate increases, the production of the two niches becomes more nearly equal and diversity can be maintained. At high resource supply rates, however, the production of the niches diverge, one contributing a larger fraction of the total population than the other. The type that is best adapted to the more productive niche then prevails, and diversity is lost. This generates a unimodal relationship between diversity and resource supply rates (Figs 1b and 2b). Note that the type dominating the community at high productivity depends on how fitness varies with resource supply rate (Figs 1c and 2c).

We note that the results of our model do not seem to be specific to the set of parameter values that we have chosen or to the use of the Michaelis–Menton function. Any set of curves that cross once within the range of resource supply rates and that share the same qualitative form as the Michaelis–Menton function will probably lead to similar results. We have found that it is possible to obtain more complex patterns, such as a bimodal relationship between diversity and productivity, if the curves for each niche cross more than once (data not shown). Nevertheless, such complex patterns have not been observed in nature³ and so we do not consider them further.

Our theory thus represents a simple and general explanation for unimodal diversity–productivity curves at a single trophic level. Note that it does not require a shift from resource limitation to light limitation (or any other single limiting factor) along a productivity gradient^{1,5,6} or predation^{24,25} to generate the unimodal relationship. Its key features are simply niche specialization, leading to changes in the ranking of relative fitness across niches, and the relative rates of production of different niches, both of which are readily amenable to experimental manipulation using microbial systems. Microbes are well-suited for studying community ecology and evolution; their small size and rapid generation time allow large populations to be maintained under defined conditions. Bacterial populations have the further advantage of being asexual, meaning that each genotypic lineage corresponds to a species in a sexual community, and we can thus test theories of species diversity by the response of genotypic diversity to experimental manipulations.

We used cultures of *Pseudomonas fluorescens* which undergo rapid adaptive radiation producing a variety of genotypes specialized to different ecological niches (for example, the surface, liquid phase or walls of the culture vessel) that are easily identified by their colony morphology when grown on agar plates²⁶. Diversity is maintained through negative frequency-dependent selection in

spatially structured microcosms²⁶. When the spatial structure is removed by shaking, diversity is rapidly lost from previously diverse cultures and fails to become established in genetically uniform cultures. To create a gradient in productivity we used serial dilutions of a standard medium to which the base population is well-adapted, creating environments differing by up to three orders of magnitude in nutrient concentration. In this way, we can contrast the effects of productivity on diversity in homogeneous and heterogeneous environments.

Our model predicts a unimodal relationship between diversity and productivity in heterogeneous environments. In homogeneous environments, the model predicts little or no relationship between diversity and nutrient concentration. Further, the model predicts that the community should be dominated by a single type at both very low and very high nutrient concentrations, implying that diversity at these extremes should be similar in heterogeneous and homogeneous environments.

We obtained two highly diverse populations, each constituting the base of a replicate experiment, by allowing cultures to proliferate in unshaken microcosms containing standard KB medium for 4 days. These were used to inoculate tubes containing medium that varied in concentration over the range of factors between 1/128 and 8 times that of standard. After two days of growth the experimental cultures were vortexed and plated to enable colony

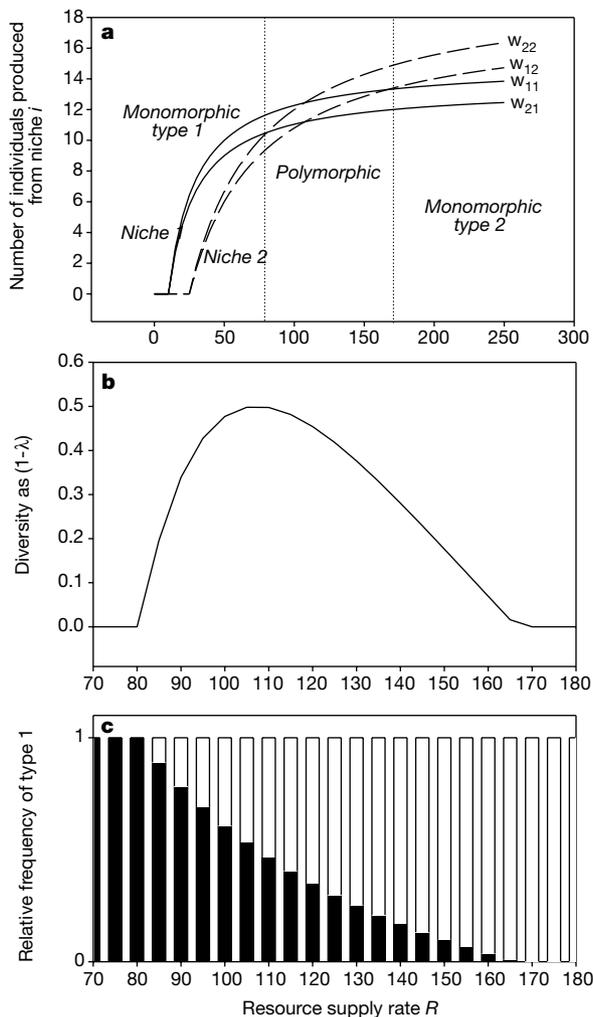


Figure 1 Maintenance of two types in two ecological niches over a range of productivities. **a**, Theoretical curves, explained in text. Parameter values are type 1 in niche 1, $R_{\min} = 10$, $K_m = 20$, $w_{\max} = 15$; type 2 in niche 2, $R_{\min} = 25$, $K_m = 50$, $w_{\max} = 20$. The less-fit type in each niche has a fitness equal to 90% of that of the more-fit type at all resource supply rates. **b**, The behaviour of the diversity index $1 - \lambda$, where $\lambda = \sum p_i^2$ is Simpson's index. **c**, Relative frequencies of the two types.

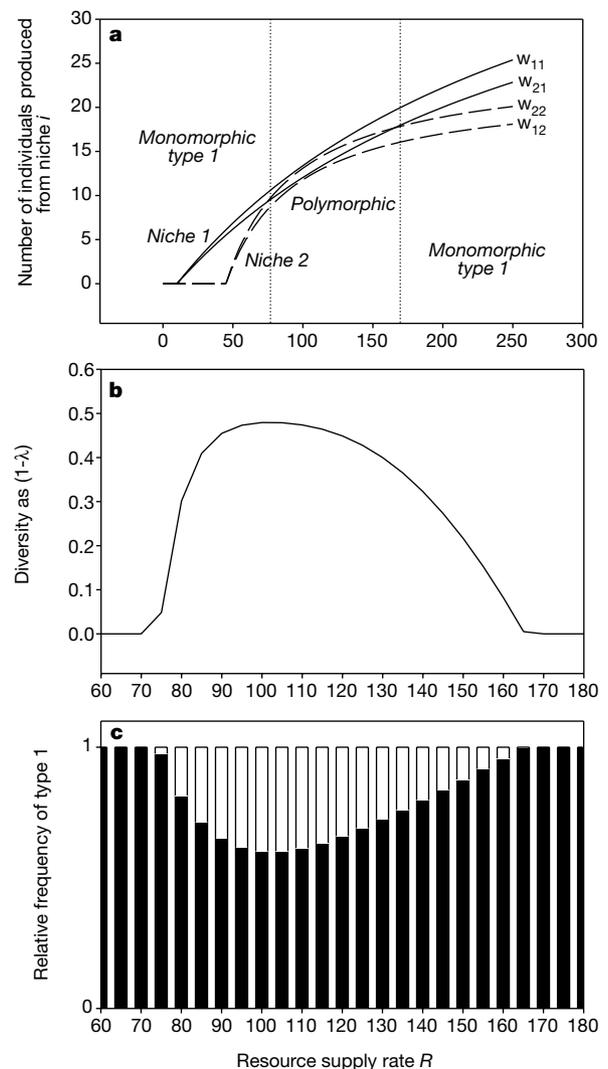


Figure 2 As in Fig. 1 but with different parameter values. Type 1 in niche 1, $R_{\min} = 10$, $K_m = 280$, $w_{\max} = 55$; type 2 in niche 2, $R_{\min} = 45$, $K_m = 50$, $w_{\max} = 25$.

morphotypes to be scored. The three basic types appearing in *P. fluorescens* cultures are 'smooth', the ancestral type which lives chiefly in the broth phase; 'fuzzy-spreader', which occupies the bottom of the vessel; and 'wrinkly-spreader', which colonizes the air-broth interface. There is considerable variation within each of these categories, and we distinguished about 10 types during this experiment.

The results, shown in Figs 3 and 4, are striking: diversity in the heterogeneous (unshaken) environments is clearly unimodally related to productivity, while in the homogeneous (shaken) environments it is not. In shaken cultures there was only a weak response of diversity to increased nutrient concentration (Figs 3 and 4, Table 1). This was significant in one case ($P = 0.032$) although not in the other ($P = 0.12$); combining the two yields $\chi^2 = 11.1$, d.f. = 4, $P \approx 0.025$. The same procedure yields $\chi^2 = 5.79$, d.f. = 4, $P = 0.3$ for the quadratic effect. In short, there was a weak and inconsistent tendency for diversity to increase monotonically with productivity in the homogeneous environments. It is conceivable that such an increase could arise if increasing nutrient concentration is associated with increasing population size, and so a larger sample of the total range variation. However the large population sizes in our experiment (at least $\sim 10^6$ – 10^7 per culture) make this an unlikely explanation. In the unshaken cultures there was again some tendency for an overall increase of diversity with productivity; this was

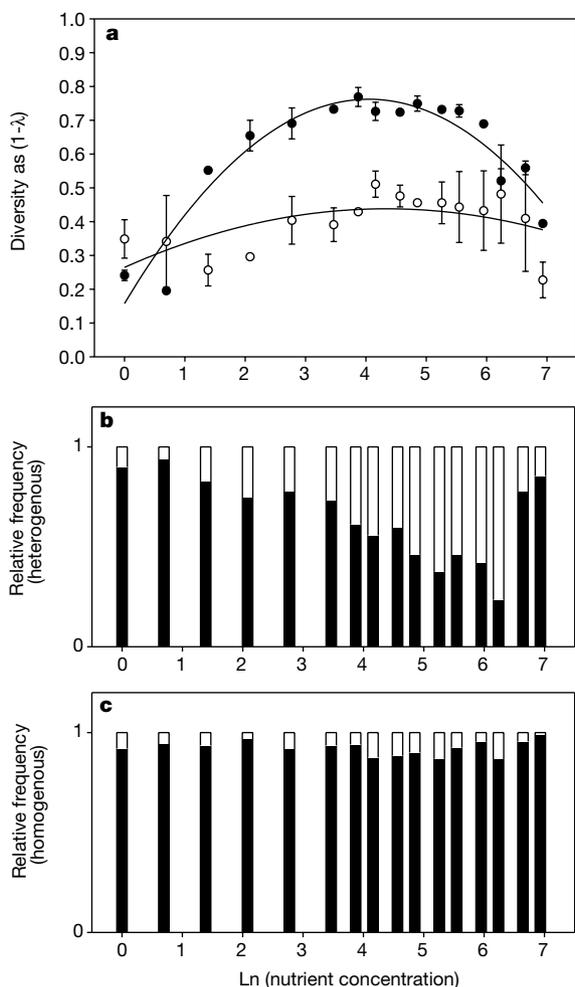


Figure 3 Response of diversity to nutrient concentration in base population 1. **a**, Diversity expressed as $1 - \lambda$. Open circles are homogeneous, and solid circles heterogeneous, cultures, with bars marking ± 1 s.e. of two replicates. **b**, Relative frequency of different colony morphotypes in the heterogeneous environment; 'smooths' (solid bars) versus 'wrinkly-spreaders' and 'fuzzy-spreaders' (open bars). **c**, As in **b**, for the homogeneous environment.

Table 1 Analysis of covariance of the experiments

Structure	Effect	d.f.	MS	F	P	r ²
First base population						
Unshaken	Linear	1	0.088	21.6	<0.0005	0.18
	Quadratic	1	0.357	87.7	<0.0001	0.89
	Error	13	0.004			
Shaken	Linear	1	0.014	2.77	0.12	0.14
	Quadratic	1	0.022	4.41	0.06	0.35
	Error	13	0.005			
Second base population						
Unshaken	Linear	1	0.014	4.34	0.06	0.08
	Quadratic	1	0.122	38.8	<0.0001	0.76
	Error	13	0.003			
Shaken	Linear	1	0.065	5.77	0.03	0.31
	Quadratic	1	0.00	0.00	0.99	0.31
	Error	13	0.011			

The response variable is $1 - \lambda$ as shown in Fig. 1.

somewhat stronger than in homogeneous environments (combined $\chi^2 = 20.9$, d.f. = 4, $P < 0.001$), but diversity in homogeneous and heterogeneous environments was very similar, both at very low and at very high productivity.

The most remarkable feature of the results in the heterogeneous environments, however, was the negative quadratic effect, which was highly significant ($P < 0.0001$) in both replicates (Table 1). This reflects the modal relationship between diversity and productivity, with diversity in the heterogeneous environment exceeding that in the homogeneous environment and peaking at almost the same intermediate concentration in both cases (Figs 3 and 4). Moreover, our model predicts that competition will lead to a sequential

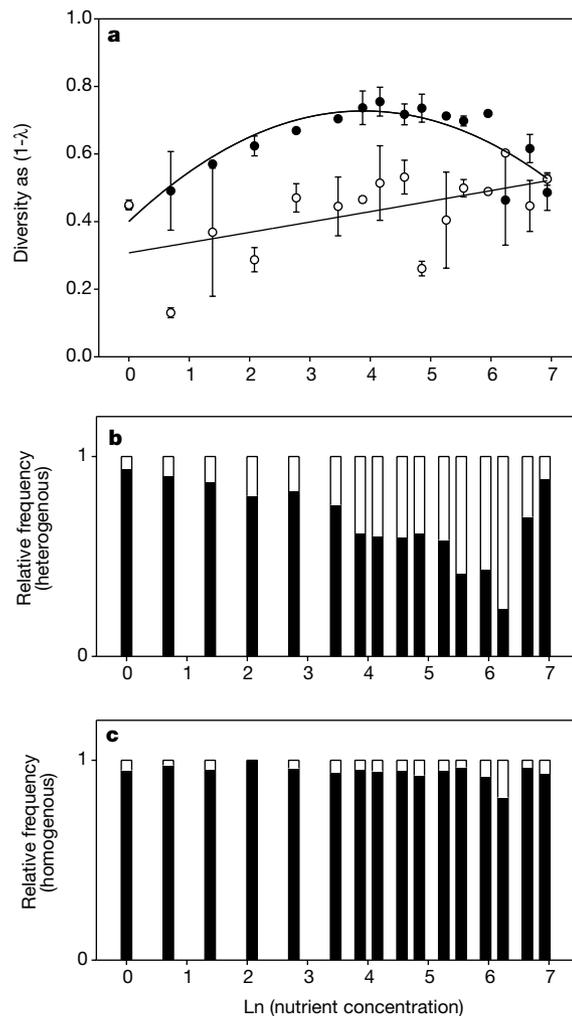


Figure 4 Response of diversity to nutrient concentration in base population 2, as in Fig. 3.

replacement at intermediate productivity of one type by the other (Figs 1c and 2c), which is what we observed in our experiment (Figs 3b and 4b).

These results are broadly consistent with the simple model described in Figs 1 and 2, and mirror the relationships between diversity and productivity or production that have been repeatedly observed at small spatial scales in natural communities. Moreover, the mechanism by which diversity is maintained in this system through negative frequency-dependent selection is well-understood: at standard nutrient concentrations, a rare type will invade when its niche is available. In our experiment, the two most common categories were 'smooth' and 'wrinkly-spreader'. The former occupies the broth phase and was dominant at low nutrient concentration; the latter occupies the surface, and its success depends on the formation of a coherent mat. At low nutrient concentration there are too few cells to make this possible, but at higher concentrations, and thus higher overall population density, 'wrinkly-spreader' can invade and diversity increases. At the highest concentrations, the mat formed by 'wrinkly-spreader' is still visible, but 'smooth' appears to be the superior competitor, a result consistent

with the predictions of the model outlined in Fig. 2. In natural communities, of course, the range of ecological opportunities will be much greater and the degree of specialization much finer than in our simplified laboratory system. Nevertheless, our experiment supports the interpretation of the modal diversity-productivity relationship as arising from selection for niche specialization in a heterogeneous environment.

At larger spatial scales it has been observed that diversity tends to increase linearly with productivity^{3,8}, and our model suggests two reasons for this. First, different kinds of niches will be included as the extent of the environment increases, leading to an overall increase in the environmental variance of fitness²⁷ and likewise an increase in the quantity of genotype-environment interaction for fitness²⁸. Second, immigration will also decrease as the distance between niches becomes larger relative to dispersal distance. The combined effects of stronger selection and lower migration means that local adaptation, and so diversity, can be maintained more readily. Over larger spatial scales, then, the linear relationship between diversity and productivity should be a function of the grain of the environment, relative to the size and dispersal ability of the organisms concerned. □

Box 1

The simplest version of the Levene model

Two asexual types are distributed at random to two niches. All individuals initially present are equally fecund, but the genotypes may survive differently in the two niches. (The same conclusions follow if fecundity rather than survival be the target of selection.) The sequence of events is settlement followed by differential survival, then reproduction, after which the parents die and a fixed number of offspring from each niche form a dispersal pool from which they are distributed at random between the niches. Let w_{ij} signify the fitness (probability of survival) of the i th genotype in the j th niche, which contributes a fraction c_j of the dispersal pool. If the initial frequency of type 1 is p , then its frequency p' after a single round of selection will be

$$p' = \left[\frac{pW_{11}}{pW_{11} + (1-p)W_{21}} \right] c_1 + \left[\frac{pW_{12}}{pW_{12} + (1-p)W_{22}} \right] (1 - c_1)$$

Setting $\Delta p = p' - p = 0$, we can solve for the equilibrium frequency of type 1, p^* :

$$p^* = \frac{[c_1(W_{11}W_{22} - W_{12}W_{21}) + W_{21}(W_{12} - W_{22})]}{(W_{21} - W_{11})(W_{12} - W_{22})}$$

which is equation (1) in the text. This expression has three parts. The denominator is a genetic variance, and the right-hand term in the numerator specifies the genotype referred to. The left-hand term in the numerator contains the two crucial elements of the model. The first is the fixed contribution of a niche to the dispersal pool, c_j . This constitutes local density regulation, which creates negative frequency-dependent selection because the probability that a surviving individual of a given type will be included in the dispersal pool will be inversely proportional to the initial frequency of that type in its niche. The second element is genotype-environment interaction. If there is no multiplicative genotype-environment interaction, relative fitness is the same in both niches, so that $W_{11}/W_{21} = W_{12}/W_{22}$, and consequently $W_{11}W_{22} - W_{12}W_{21} = 0$. The term is non-zero only if relative fitness differs between niches. A necessary condition for $0 < p^* < 1$ is that the ranking of relative fitness changes between niches, that is, $w_{11} > w_{21}$ and $w_{22} > w_{12}$ (or the reverse), so that each type is superior in one of the niches and inferior in the other. Sufficient conditions for the maintenance of diversity involve both the intensity of niche-specific selection and the balance of niche-specific production. Setting $w_{12} = w_{21} = 1$ for clarity, these conditions are

$$w_{11} > \frac{[c_1 - (1 - W_{22})]}{c_1 W_{22}} \quad \text{and} \quad w_{22} > \frac{(W_{11} - c_1)}{W_{11}(1 - c_1)}$$

implying that as the share of the dispersal pool contributed by a given niche decreases, the greater the difference in relative fitness in that niche required to maintain diversity.

Methods

Base populations

Each base population was obtained by culturing *Pseudomonas fluorescens* isolate SBW25 in 6 ml of King's B (KB) medium for 4 days.

Experimental procedure

Samples from the two base populations were washed three times in 10 mM phosphate buffer and then starved for two hours. Experimental cultures were then inoculated with about 10^3 cells. Experimental culture media were mixtures of KB nutrients (glycerol and proteose peptone) and M9 mineral salts, made up by serial dilution in M9 salts to concentrations of $8 \times, \dots, 1/128 \times$ standard KB nutrient concentration. After 48 h, cultures had reached 10^8 – 10^9 cells and were stored in 50% glycerol at -80°C . Diversity was estimated by plating the 48-h cultures onto KB agar and scoring the morphology of approximately 100 random colonies.

Statistical procedure

Diversity was expressed as the complement of Simpson's index²⁹, $1 - \lambda = 1 - \sum p_i^2 = \sum p_i(1 - p_i)$ where p_i is the frequency of the i th type. Expressing diversity as richness, or the number of types, gives the same pattern (data not shown).

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Herbivory-induced volatiles elicit defence genes in lima bean leaves

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In response to herbivore damage, several plant species emit volatiles that attract natural predators of the attacking herbivores^{1–5}. Using spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis*)^{1–4}, it has been shown that not only the attacked plant but also neighbouring plants are affected, becoming more attractive to predatory mites^{3,6} and less susceptible to spider mites⁶. The mechanism involved in such interactions, however, remains elusive. Here we show that uninfested lima bean leaves activate five separate defence genes when exposed to volatiles from conspecific leaves infested with *T. urticae*, but not when exposed to volatiles from artificially wounded leaves. The expression pattern of these genes is similar to that produced by exposure to jasmonic acid. At least three terpenoids in the volatiles are responsible for this gene activation; they are released in response to herbivory but not artificial wounding. Expression of these genes requires calcium influx and protein phosphorylation/dephosphorylation.

We placed two lima bean (*Phaseolus lunatus* cv. Sieva) leaves in a plastic cup. Each of the leaves was infested with 100 female *T. urticae*. Within a lidded glass container, four to six lima bean leaves in a vial were kept together with the *T. urticae*-infested leaves in a plastic cup

for 1 or 3 days. The *T. urticae*-infested leaves emitted the ‘*T. urticae*-induced’ volatiles, and the leaves in the vial received the volatiles. We analysed the expression patterns of six defence genes in the leaves emitting the *T. urticae*-induced volatiles and in the leaves receiving the volatiles, and identified the volatile substances that are responsible for eliciting defence genes in the receiver leaves.

In the *T. urticae*-infested leaves, we detected the transcripts of six defence genes: genes for the basic type of pathogen-related (PR) proteins (PR-2 (β -1,3-glucanase) and PR-3 (chitinase)); an acidic type of PR-4 (chitinase); lipoxygenase (LOX) in the octadecanoid pathway; phenylalanine ammonia-lyase (PAL) in the phenylpropanoid pathway; and farnesyl pyrophosphate synthetase (FPS) related to isoprene biosynthesis (Fig. 1a). Artificial wounding of lima bean leaves elicited four of the defence genes (the acidic PR-4 and FPS genes were not elicited). We have proposed a signalling mechanism such that infestation by *T. urticae* activates both jasmonic acid (JA)- and salicylic acid (SA)-signalling pathways in lima bean leaves⁷. These compounds are known as active components of wound^{8–10} or pathogen^{10,11} response in higher plants. Exogenous JA or methyl salicylate (MeSA) reproduced the expression patterns of six defence genes observed in the *T. urticae*-infested leaves (Fig. 1a). The present results constitute further evidence supporting the proposed signalling mechanism.

In lima bean leaves exposed to the *T. urticae*-infested leaf volatiles for one day in the same glass container, we also detected the transcripts of five out of the six defence genes (Fig. 1a). Transcripts of the basic PR-3 and PAL genes decreased after three days. In a control experiment, lima bean leaves received the ‘wound-induced’ volatiles that lima bean leaves emit in response to artificial wounding. We detected only the transcript of a basic PR-2 gene in the receiver leaves. Neighbouring lima bean plants can respond to the *T. urticae*-induced and wound-induced volatiles respectively by eliciting different combinations of defence genes.

LOX in the octadecanoid pathway is a key enzyme in the biosynthesis of JA¹². Enzymatic activity of LOX in lima bean leaves that received the *T. urticae*-induced volatiles increased as high as the activity in the *T. urticae*-infested leaves (Table 1). Previous treatment with a LOX inhibitor, SHAM, completely blocked the expression of basic PR-2, basic PR-3, LOX, PAL and FPS genes in the receiver leaves (Fig. 1b). This inhibitory effect was overcome by simultaneous treatment of exogenous JA with the inhibitor. In the receiver leaves, the *T. urticae*-induced volatiles might induce *de novo* synthesis of LOX followed by biosynthesis of JA, which subsequently activates a subset of defence genes.

Treatment with a chelator of extracellular Ca²⁺ ion, BAPTA, or an inhibitor of serine/threonine protein kinases, staurosporine, completely suppressed the expression of the five defence genes in lima bean leaves that received the *T. urticae*-induced volatiles (Fig. 1b). An inhibitor of type 1 and/or type 2A protein phosphatases, calyculin A, suppressed the expression of basic PR-3, PAL and FPS genes completely, but that of basic PR-2 and LOX genes only slightly. Therefore, the signalling pathway(s) mediating expression of defence genes in the receiver leaves would include the calcium influx into cells, protein phosphorylation, and dephosphorylation steps.

Table 1 Inductions of LOX activity in lima bean leaves

Duration (days)	Enzyme activity (relative activity*)		
	Control	Emitter leaves infested by <i>T. urticae</i>	Receiver leaves
0	1.43 ± 0.24 (1.00)	–	–
1	–	2.18 ± 0.33 (1.52)	6.95 ± 1.55 (4.87)
3	1.29 ± 0.19 (0.94)	5.08 ± 1.41 (3.57)	3.80 ± 0.35 (2.66)

*Relative to the activity of the control experiment at day 0. Lima bean leaves were either infested with *T. urticae* or receiving the *T. urticae*-induced volatiles. Values are means ± standard deviation of three experiments (ΔA_{234} per mg protein per min).