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Acknowledgements

European Space Agency (ESA) remote-sensing satellite (ERS) synthetic aperture radar (SAR) imagery for this study was acquired as a Category 1 research project from the ESA. We thank A. Linde for a critical review, L. Rivera and R. Lohman for modelling software, S. de Silva for an electronic version of his volcano database, and H. Zebker, Y. Fialko, P. Segall, E. Brodsky and M. Battaglia for useful discussions. This material is based upon work partially supported by the National Science Foundation under a grant to M.S. M.E.P. was partly supported by NASA and NSF fellowships.

Competing interests statement

The authors declare that they have no competing financial interests.

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Local dispersal promotes biodiversity in a real-life game of rock–paper–scissors

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One of the central aims of ecology is to identify mechanisms that maintain biodiversity[1,2]. Numerous theoretical models have shown that competing species can coexist if ecological processes such as dispersal, movement, and interaction occur over small spatial scales[1–10]. In particular, this may be the case for non-transitive communities, that is, those without strict competitive hierarchies[3,6,8,11]. The classic non-transitive system involves a community of three competing species satisfying a relationship similar to the children's game rock–paper–scissors, where rock crushes scissors, scissors cuts paper, and paper covers rock. Such relationships have been demonstrated in several natural systems[12–14]. Some models predict that local interaction and dispersal are sufficient to ensure coexistence of all three species in such a community, whereas diversity is lost when ecological processes occur over larger scales[6,8]. Here, we test these predictions empirically using a non-transitive model community containing three populations of *Escherichia coli*. We find that diversity is rapidly lost in our experimental community when dispersal and interaction occur over relatively large spatial scales, whereas all populations coexist when ecological processes are localized.

Microbial laboratory communities have proved useful for studying the generation and maintenance of biodiversity[15–17]. In particular, communities containing toxin-producing (or colicinogenic) *E. coli* have been the centre of much attention from both theoretical ecologists[3,6,8,18–20] and microbiologists[21–27]. Colicinogenic bacteria possess a 'col' plasmid, containing genes that encode the colicin (the toxin), a colicin-specific immunity protein (which renders the cell immune to the colicin) and a lysis protein (which is expressed when the cell is under stress, causing partial cell lysis and the subsequent release of the colicin)[26,27]. In general, only a small fraction of a population of colicinogenic cells will lyse and release the colicin[27]. Colicin-sensitive bacteria are killed by the colicin but may occasionally experience mutations that render them resistant to the colicin. The most common mutations alter cell membrane proteins that bind or translocate the colicin[23,24,26,27]. In some cases, the growth rate of resistant cells (R) will exceed that of colicinogenic cells (C), but will be less than the growth rate of sensitive cells (S). This occurs because resistant cells avoid the competitive cost of carrying the col plasmid[21,22,26,27] but suffer because colicin receptor and translocation proteins are also involved in crucial cell functions such as nutrient uptake[21,23,24,26,27]. In such cases, S can displace R (because S has a growth-rate advantage), R can displace C (because R has a growth-rate advantage) and C can displace S (because C kills S). That is, the C–S–R community satisfies a rock–paper–scissors relationship.

Using a modification of the lattice-based simulation of Durrett and Levin[6], we theoretically explored the role of the spatial scale of

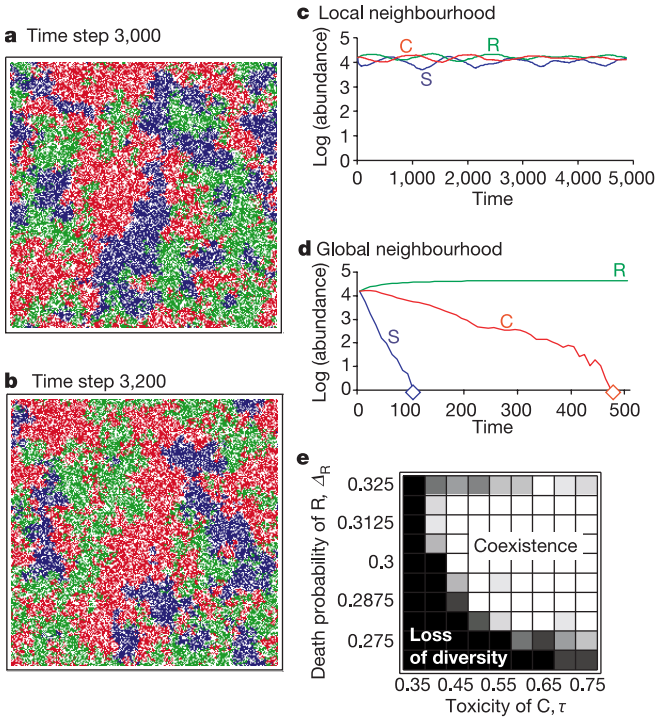

Figure 1 Predictions of the lattice-based simulation (see Box 1). **a**, **b**, Snapshots of the lattice in a simulation with a local neighbourhood at times 3,000 (**a**) and 3,200 (**b**). The unit of time is an 'epoch', equal to 62,500 lattice point updates (an epoch is the average turnover of any given lattice point in the 250 × 250 grid). The strains are colour-coded as follows: C is red, S is blue and R is green; empty lattice points are white. **c**, The complete community dynamics for the same simulation run. **d**, Community dynamics for a simulation with a global neighbourhood. The abundances in **c** and **d** are log transformed. When the abundance of a strain goes to zero, we represent this event with a diamond on the abscissa of the relevant graph at the relevant time. For **a**–**d** we used the following parameters: $\Delta_C = 1/3$, $\Delta_{S,0} = 1/4$, $\Delta_R = 10/32$, and $\tau = 3/4$ (see Box 1). **e**, Sensitivity of qualitative dynamics to changes in a subset of parameter values. For the (τ, Δ_R) values plotted, the greyscale indicates the number of 'local' simulated runs (out of 10) in which coexistence occurred for at least 10,000 epochs, with the lighter area indicating a higher probability of coexistence. For all simulations, we require $\Delta_{S,0} < \Delta_R < \Delta_C < \frac{\Delta_{S,0}+\tau}{1+\tau}$, which (at least for the mixed system) ensures that S displaces R, R displaces C, and (if C has sufficient density) C displaces S.

ecological processes in our C–S–R community (see Box 1). When dispersal and interaction were local, we observed that 'clumps' of types formed (Fig. 1a). These patches chased one another over the lattice—C patches encroached on S patches, S patches displaced R patches and R patches invaded C patches (Fig. 1a, b). Within this fluid mosaic of patches, the local gains made by any one type were soon enjoyed by another type. The result of this balanced chase was the maintenance of diversity (Fig. 1c). However, this balance was lost when dispersal and interaction were no longer exclusively local (that is, in the 'well-mixed' system—see Box 1). In the mixed system, continual redistribution of C rapidly drove S extinct, and then R outcompeted C (Fig. 1d). Durrett and Levin[6] describe a qualitatively similar effect of spatial scale in their model of colicinogenic, sensitive, and 'cheater' strains (where a cheater was defined as a strain producing less colicin at a lower competitive cost).

When ecological processes were local in the simulation, coexistence occurred over a substantial range of model parameter values (Fig. 1e), suggesting that the result was not very sensitive to the specific choice of parameter values. In the case of the mixed system, coexistence never occurred for the region of parameter space shown in Fig. 1e. In agreement with Durrett and Levin[6], our simulation results suggested that three strains with the abovementioned non-hierarchical relationship could coexist when dispersal and interaction are local, whereas one strain excludes the others when the community is well mixed.

To test this conclusion, we used three strains of the bacterium *E. coli*: a colicin-producing strain (C), a sensitive strain (S), and a resistant strain (R), which satisfied a rock–paper–scissors competitive relationship (see Methods). We placed the C–S–R community in the following three environments: (1) 'Flask' (a well-mixed environment in which dispersal and interaction are not exclusively local); (2) 'Static Plate' (an environment in which dispersal and interaction are primarily local); and (3) 'Mixed Plate' (an environment intermediate between these two extremes).

For the Flask environment, the bacteria were grown in shaken flasks containing liquid media. We transferred an aliquot of the community to fresh media every 24 h. In the Static Plate environment, the bacteria were grown on the surface of solid media in Petri plates. Every 24 h, we pressed each plate onto a platform covered with a sterile velveteen cloth and then placed a fresh plate on the velvet. This method transferred a small sample of the community and allowed the transferred sample to retain the spatial pattern that developed on the previous plate. The Mixed Plate environment was identical to the Static Plate environment, except that at each transfer the fully-grown community plate was pressed on the velvet several times, each time rotated at a different angle (see Methods).

Figure 2a shows that C, S and R strains were maintained at high densities in the Static Plate environment throughout the experiment. Photographs of the plates show the spatial pattern that developed over the experiment (Fig. 3a). The pink and yellow inter-strain boundaries in Fig. 3b show clearly that R chased C, and C

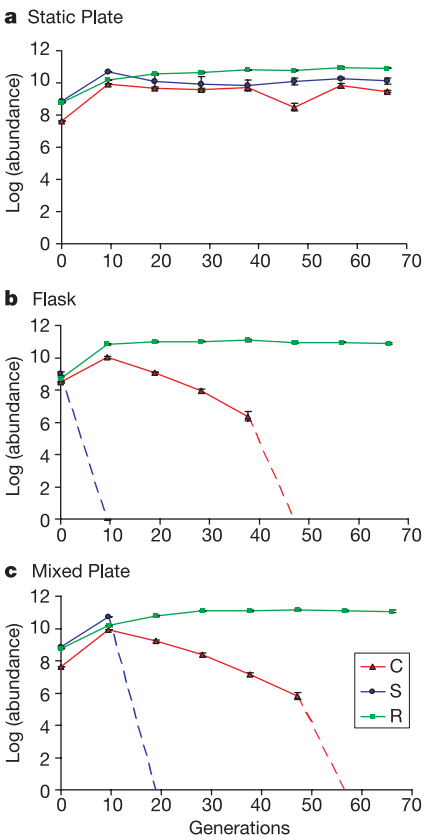

Figure 2 Community dynamics in the experimental treatments: **a**, Static Plate; **b**, Flask; and **c**, Mixed Plate. Dashed lines indicate that the abundance of the relevant strain has decreased below its detection limit. Data points are the mean of three replicates, and bars depict standard errors of the mean. Consecutive data points are separated by 24 h, approximately 10 bacterial generations.

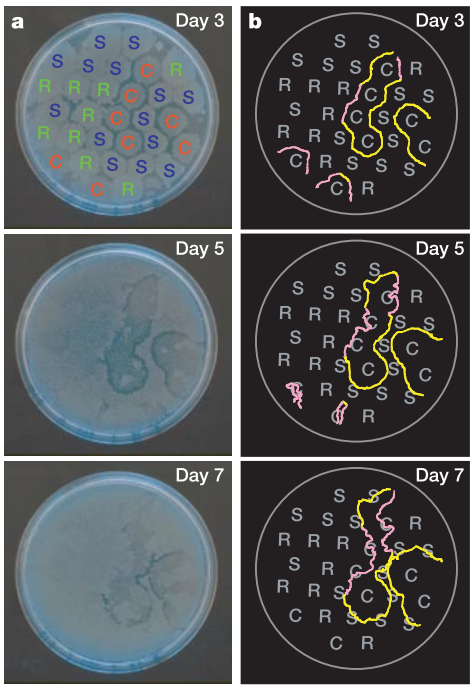

Figure 3 Time series photographs of a representative run of the Static Plate environment. We initiated the plate environments by depositing small droplets from pure cultures in a hexagonal lattice pattern, where the strain at each point was assigned at random. **a**, The changing spatial configuration of the experimental community is shown in this first panel of photographs. Patches inhabited by C cells were less dense and consequently easily distinguished from S and R patches. The dense growing 'spots' that appear inside the C clumps were determined to be resistant cells generated *de novo* from S cells. An empty layer existed between C clumps and S clumps, where diffused colicin had prevented the growth of S cells, but where C cells had not yet colonized. The border between C and R lacked this empty layer. **b**, 'Chasing' between clumps is highlighted in this second panel. The letters giving the initial spatial distribution of the strains are preserved for reference. The borders between C and S are coloured in yellow and the borders between C and R in pink.

chased S, respectively. It was not possible to see S chasing R, because these two strains grew to comparable densities. However, the S density did not consistently decrease over time (Fig. 2a), suggesting that S did indeed chase R. Thus, coexistence did not result from absolute spatial isolation of the three strains. As can be seen in Fig. 3, interaction (for example, competition and killing) occurred at the boundaries between the strains. These results support the prediction that balanced chasing in a spatially structured, non-hierarchical community may result in the maintenance of diversity.

The results for the Flask environment are shown in Fig. 2b. In contrast to the Static Plate environment, both S and C dropped below their detection limits before the study was completed. The difference in dynamics between the Flask and Static Plate environments did not merely reflect differences between liquid culture and surface growing conditions. Simply mixing the surface growing community at each transfer event (that is, our Mixed Plate environment) produced dynamics very similar to those in the Flask environment (Fig. 2c). Thus, it appears that dispersal and interactions must be local for coexistence to occur in this community.

To explore the robustness of our results, we repeated the experimental work described above using different initial spatial configurations and starting strain frequencies (see Methods). In the cases considered, we observed dynamics very similar to those observed in our original experiment—that is, chasing of types, coexistence of all three types for at least 66 generations when ecological processes were localized, and displacement of S and C and persistence by R when processes were not localized (data not shown). These observations suggested that our results were not due to the particular starting conditions chosen in our initial experiments.

The work described here lies at the crossroads of two recent trends in the study of biodiversity. The first is increasing interest in the role of the spatial scale of ecological processes[2,4,9,17]. It has been shown theoretically that simply allowing interaction and dispersal to occur locally can promote diversity within a community[2,4–9,11]. Our experimental results confirm these predictions. The second trend concerns the role of non-hierarchical competitive relationships[3,6,11,28,29]. Recent theory suggests that the number of species coexisting on a limited number of resources can be astonishingly high when there are non-hierarchical relationships among the species[28,29]. Our study system represents a very simple non-transitive triplet: a game of rock–paper–scissors. Our experimental results are consistent with the prediction that non-hierarchical competitive relations can promote diversity. However, in our case, the localization of ecological processes is also necessary.

Localized processes may be important for coexistence in other natural communities as well. For many relatively sessile organisms such as plants, some marine invertebrates and the microbial constituents of biofilms, dispersal and interaction tend to occur over small spatial scales. Communities with such organisms may also exhibit non-transitive relationships. For instance, if one species produces a toxin (a widespread phenomenon, occurring in many species of plants[30], marine invertebrates[13], fungi[30], and essentially every major bacterial lineage[27]) there will be the potential for non-transitivity. This can occur if toxin production is costly, both sensitive and resistant species exist, and costs associated with resistance are less than those associated with toxin production. However, the relative magnitude of these costs will be critical to the prospects for coexistence. Specifically, if the costs of resistance or allelopathy are either too great or too small, the community can collapse, even if the members maintain a non-transitive relationship (for example, see the dark portions of Fig. 1e). If the relative costs in our experimental community are representative of those in natural communities, then we might expect the scale of ecological processes to determine the likelihood of biodiversity maintenance in vegetation with allelopathic plants, coral reefs with toxic sessile invertebrates, and microbial communities with antibiotic-producing microbes. Such communities may be ideal systems to study further the role of local dispersal and interaction in species coexistence. □

Box 1
Lattice-based simulation

We embedded our virtual C–S–R community in a 250 × 250 regular square lattice with periodic (or 'wrap-around') boundaries. To start the simulations shown in Fig. 1, every lattice point was randomly and independently assigned one of the following states: occupation by a C, S or R cell or the 'empty state'. We used an asynchronous updating scheme, which consisted of sequentially picking random focal points and probabilistically changing their states. The state transition probabilities of a focal point depend both on its current state and the states of points within its neighbourhood. The size of a neighbourhood represents the spatial scale of ecological processes. To explore the role of spatial scale, we simulated our community using two different neighbourhood sizes. The 'local' neighbourhood consisted of the eight lattice points directly surrounding a focal point; in such a case, interaction (that is, killing and competition for space) and dispersal (that is, 'birth') are local. The 'global' neighbourhood consisted of every point in the grid outside of a focal point; in this case, interaction and dispersal are no longer exclusively local, and the community behaves like a well-mixed system. For either neighbourhood, if an empty lattice point is selected for updating, the probability that it is filled with a cell of type i (with $i \in \{C,S,R\}$) is given by f_i, the fraction of its neighbourhood occupied by strain i. If an occupied lattice point in state i is selected, it is killed with probability Δ_i. Although Δ_C and Δ_R are fixed values, Δ_S is not; it is equal to $\Delta_{S,0} + \tau(f_C)$, where $\Delta_{S,0}$ is the probability of death of an S cell without any neighbouring C cells, and τ measures the toxicity of neighbouring C cells.

Methods

The experimental system

We used the *E. coli* colicin E2 system: strains BZB1011 (S), $E2^C$-BZB1011 (C), and $E2^R$-BZB1011 (R). C was marked with resistance to T6 coliphage and S with resistance to T5 coliphage. Other than the non-conjugative col plasmid and markers, C was isogenic to S. R bacteria were generated by selecting marked S mutants able to grow in the presence of the colicin[24]. All types were counted by selective plating, with S counted by subtraction. Periodically, we checked the markers by verifying that T6-resistant bacteria produced colicin E2 and that T5-resistant bacteria did not.

Determining strain relationships

The relative fitnesses of the strains were determined by growing two competing strains in pairwise competition, both in 10 ml Luria–Bertani (LB) liquid culture and in 4 ml of soft agar on the surface of agar plates. The fitness (w) of strain x relative to that of strain y is

$$w(x,y) = \frac{\ln(x_F/x_0)}{\ln(y_F/y_0)}$$

where x_0 and y_0 are initial densities and x_F and y_F are densities after 24 h of growth.

We found $w(R,S) = 0.59 \pm 0.097$ and $w(R,C) = 1.91 \pm 0.399$ in the liquid culture environment and $w(R,S) = 0.73 \pm 0.142$ and $w(R,C) = 1.90 \pm 0.230$ on the plates. We did not compute $w(S,C)$ in either environment, but we observed that the colicin produced by strain C effectively kills S cells (for example, spotting colicin on a S lawn gives large clear plaques). Thus, C kills S, S outgrows R, and R outgrows C.

Tracking community dynamics

In the Static Plate environment, the bacteria grew on the surface of 4 ml of soft LB agar, which had been poured onto a plate with a hard LB agar base. To initialize a plate, 15 μl droplets of pure culture were placed in a 31-point hexagonal lattice (Fig. 3a). The droplet pattern was generated by randomly assigning the identity of the strain at each lattice point according to the probability distribution: $Pr\{C\} = 0.25$, $Pr\{S\} = 0.5$, and $Pr\{R\} = 0.25$. After 24 h of growth at 37 °C, we pressed a fully grown plate on a platform covered with velvet. Then three fresh plates were pressed on the velvet, taking care to preserve orientation. The first plate was used for counting strain densities after the next day's growth. This was done by scraping off the soft agar layer with the bacterial community into

10 ml of saline, vortexing the mixture for several seconds, and then following standard selective plating protocol. The second plate was used for propagating the community (that is, for pressing on the velvet after the next day's growth). The third plate was photographed (see Fig. 3).

The Mixed Plate environment was identical to the Static Plate environment described above with the following exception: at each transfer, the fully-grown plate was (1) pressed lightly on the velvet, (2) turned clockwise at a randomly chosen angle and pressed a second time, (3) turned randomly counter-clockwise and pressed a third time, and (4) turned randomly clockwise and pressed a fourth time. The fresh plates were then pressed on the velvet to initiate this 'mixed up' sample.

Our Flask environment was a 125 ml flask with 33.75 ml of LB broth shaken at 125 rev min^{-1} at 37 °C. After 24 h of growth, 50 μl of the culture was transferred to a flask with fresh media. These volumes guaranteed that the total number of bacterial cells in the flask after a full day's growth, and total number of cells transferred, matched the corresponding numbers of cells from the plate runs.

In all three environments, the densities of each type were determined every 24 h, and all treatments were replicated in triplicate. We terminated the experiments after one week (approximately 66 generations), because by this time we detected a substantial number of resistant mutants derived from the S population (for example, the dense clumps within the C patches in Fig. 3a). Evolution of R from S violates an assumption of our model and can lead to a change in the predicted dynamics, especially if the cost of resistance is much lower in the *de novo* R mutants (see low values of Δ_R in Fig. 1e). We are currently exploring such evolutionary phenomena both theoretically and experimentally. It should be noted that one week suffices for a single 15-μl droplet placed in the centre of a plate to nearly cover the plate under the Static Plate regime.

Additional starting conditions

We manipulated the starting strain frequencies and initial spatial configuration on the plates in two additional sets of runs (data not shown). First, we repeated the experiments as outlined above with the same initial hexagonal lattice pattern, but using another random assignment of the strains (taken from the probability distribution above). Second, rather than initiating the plates with droplets in a hexagonal pattern, we mixed the bacterial strains in soft agar at different starting frequencies (with S in excess and C and R rare) and poured the agar over the plates, resulting in an initially 'unclumped' distribution.

Received 12 December 2001; accepted 27 March 2002; doi:10.1038/nature00823.

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Acknowledgements

We thank M. Munos for help in the laboratory, N.B. Raju for helping with the plate photography, and D. Ackerly, P. Armsworth, C. Boggs, C. Devine, P. Godfrey-Smith, D. Gordon, A. Hirsh, J. Huisman, C. Jessup, S. Levin, D. Petrov, P. Rainey, T. Ricketts, S. Tuljapurkar, K. Walag and V. Walbot for many comments on previous versions of the manuscript.

Competing interests statement

The authors declare that they have no competing financial interests.

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Exceptional sperm cooperation in the wood mouse

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Spermatozoa from a single male will compete for fertilization of ova with spermatozoa from another male when present in the female reproductive tract at the same time[1]. Close genetic relatedness predisposes individuals towards altruism, and as haploid germ cells of an ejaculate will have genotypic similarity of 50%, it is predicted that spermatozoa may display cooperation and altruism to gain an advantage when inter-male sperm competition is intense[2]. We report here the probable altruistic behaviour of spermatozoa in an eutherian mammal. Spermatozoa of the common wood mouse, *Apodemus sylvaticus*, displayed a unique morphological transformation resulting in cooperation in distinctive aggregations or 'trains' of hundreds or thousands of cells, which significantly increased sperm progressive motility. Eventual dispersal of sperm trains was associated with most of the spermatozoa undergoing a premature acrosome reaction. Cells undergoing an acrosome reaction in aggregations remote from the egg are altruistic in that they help sperm transport to the egg but compromise their own fertilizing ability.

Several examples of sperm cooperation have been reported mainly in molluscs and insects[3,4]. A possible exception in Mammalia is the spermatozoa of opossums that conjugate to form pairs during sperm maturation and disengage immediately before fertilization[5]. Sperm will benefit from cooperation if Hamilton's rule[6] is fulfilled. This depends on the probability of sperm survival in terms of