LETTERS

Experimental demonstration of chaos in a microbial food web

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Discovering why natural population densities change over time and vary with location is a central goal of ecological and evolutional disciplines. The recognition that even simple ecological systems can undergo chaotic behaviour has made chaos a topic of considerable interest among theoretical ecologists[1–4]. However, there is still a lack of experimental evidence that chaotic behaviour occurs in the real world of coexisting populations in multi-species systems. Here we study the dynamics of a defined predator–prey system consisting of a bacterivorous ciliate and two bacterial prey species. The bacterial species preferred by the ciliate was the superior competitor. Experimental conditions were kept constant with continuous cultivation in a one-stage chemostat. We show that the dynamic behaviour of such a two-prey, one-predator system includes chaotic behaviour, as well as stable limit cycles and coexistence at equilibrium. Changes in the population dynamics were triggered by changes in the dilution rates of the chemostat. The observed dynamics were verified by estimating the corresponding Lyapunov exponents. Such a defined microbial food web offers a new possibility for the experimental study of deterministic chaos in real biological systems.

Apart from the intuitive understanding that external (extrinsic) stimuli influence the variability of abundances, mathematical models have made it apparent that the internal (intrinsic) qualities of a population give rise to population dynamics with large and (at certain parameter ranges) even chaotic fluctuations of abundances, even under wholly constant and predictable conditions[5]. Predator–prey interactions have been considered as a possible driving force of population dynamics since the beginning of ecological studies[6,7]. In his analysis of mathematical models, May[1] found that even simple processes of population growth can show (for a certain range of parameters) an unpredictable behaviour driven by intrinsic mechanisms. May's studies marked the beginning of an intensive debate on the question of whether or not natural systems are characterized by chaotic behaviour. In this context, the term 'deterministic chaos' can be defined as bounded aperiodic fluctuations with sensitive dependence on initial conditions[4]. Under chaotic conditions, population abundances never show a precisely repeated pattern over time; such patterns are only observable in populations at equilibrium or at stable limit cycles. Theoreticians can clearly define parameter ranges of mathematical models that create chaotic behaviour in idealized biological systems[3,8–10]. However, only a very few experiments indicating that bifurcations of dynamic behaviour might occur in the real world have been conducted (for example, ciliate–bacteria interactions[11], flour beetle (*Tribolium castaneum*) dynamics[12,13] and rotifer–algae interactions[14]). Indications of chaotic dynamics under controlled conditions have so far been reported for one-species systems only[13]. A robust tool to verify observed dynamics is estimations of Lyapunov exponents from time series, which test for the exponential divergence of nearby trajectories. Mathematically, stable (convergent) systems show negative Lyapunov exponents, whereas chaotic (divergent) systems have at least one positive Lyapunov exponent[4].

The aim of the present study was to verify the biological relevance of chaotic behaviour in a real multi-species system. The long generation durations of most organisms and the complexity of natural environments have generally made the explanation of underlying ecological mechanisms difficult[15]. However, experiments using microbial populations propagated in controlled environments reduce ecosystem complexity to the point at which understanding simple processes in isolation becomes possible. The rapid reproduction of bacteria and protists is one of the main advantages of working with microorganisms as model organisms[7,16,17]. In addition, the community structure can be exactly defined; for example, single strains of bacteria and protists can be selected. Microorganisms can also be cultured under chemostat conditions. This has the great advantage that extrinsic factors are negligible and changes in population dynamics can be attributed to intrinsic factors. In terms of predation and interspecific competition, one of the simplest systems imaginable is a three-species system with two prey organisms and one predator. Several theoretical studies have been made of such model systems[8–10,18]. Generally, different patterns of population dynamics are predicted by models; for example, the extinction of one or two species and the coexistence of all three species. Assuming that the two prey populations compete with each other and assuming that the better competitor is the preferred prey, three patterns may occur: coexistence at equilibrium, coexistence at stable limit cycles, and coexistence at chaos[8–10,18].

Our study was aimed at identifying these different patterns of coexistence in controlled experiments in a chemostat. We used the dilution rate as the bifurcation parameter in the experiments, because the dynamical behaviour of chemostat models can change with dilution rate[9,10,14]. We constructed one-stage chemostat systems consisting of axenic cultures of three species: a predator (the ciliate *Tetrahymena pyriformis*) and two coexisting prey bacteria, the rod-shaped *Pedobacter* and the coccus *Brevundimonas*. The effective consumption of these bacteria by the ciliate and its food preference was analysed by immunofluorescence techniques. The ciliate can establish stable populations when feeding on either bacterium, but it dies off in the highly diluted organic medium when bacteria are absent. The growth conditions for the bacteria and the mortality of the ciliate are determined by the dilution rates (controlled by peristaltic pumps). *Brevundimonas* was always outcompeted in chemostat experiments containing both bacterial strains without a predator. Thus, *Pedobacter* was considered to have a better fitness. In

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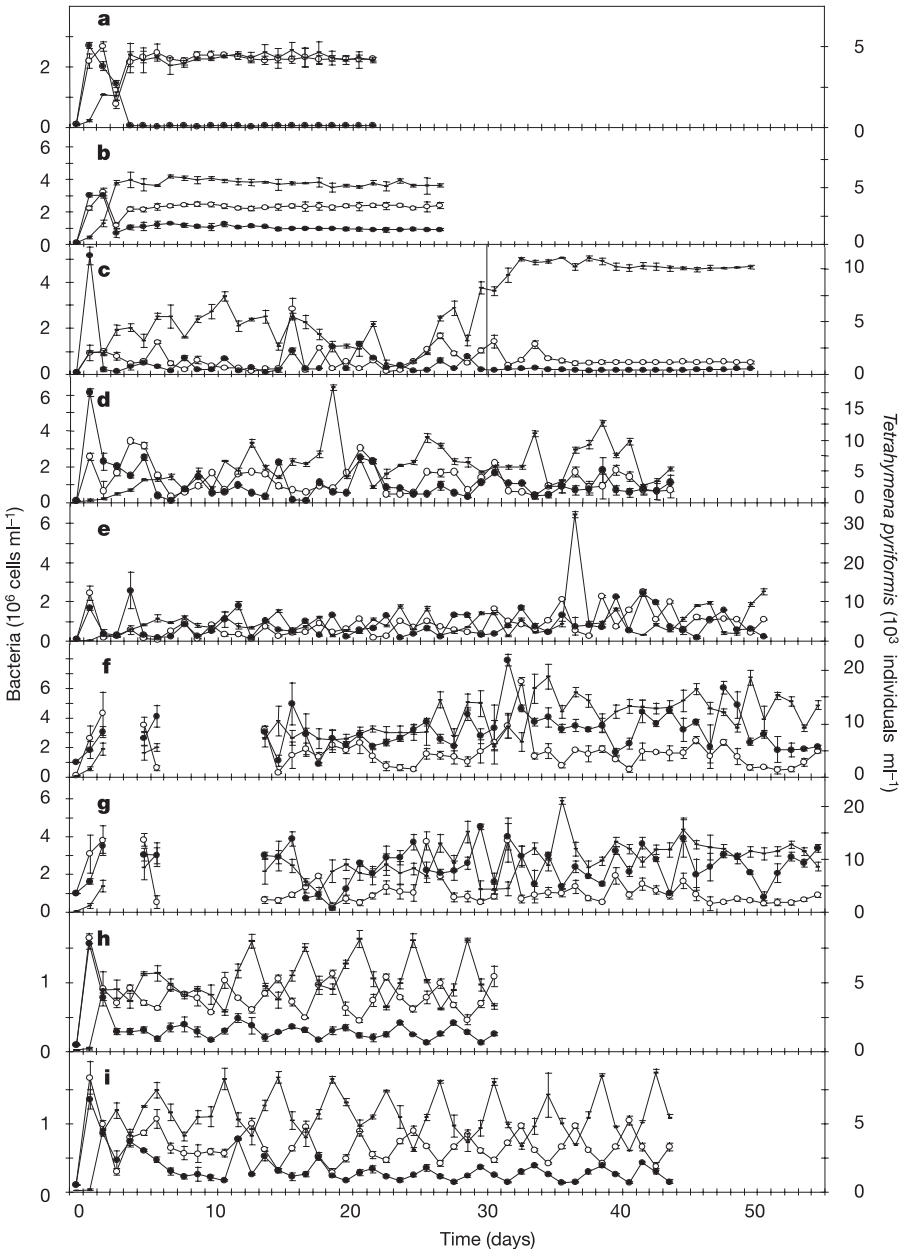

Figure 1 | Experimental results showing the population dynamics of bacteria–ciliate chemostat systems. Dilution rates D were as follows: **a**, 0.90 d^{-1}, **b**, 0.75 d^{-1}; **c**, 0.50 d^{-1} (the line indicates the change to 0.75 d^{-1} at day 30); **d**–**g**, 0.50 d^{-1} (replicate experiments; no sampling took place on days 3, 4 and 7–13 in **f** and **g**); **h**, **i**, 0.45 d^{-1} (replicate experiments). Open circles, *Pedobacter* (preferred prey); filled circles, *Brevundimonas* (less-preferred prey); horizontal bar, *Tetrahymena* (predator). Vertical bars represent the s.d. of triplicate samples taken separately from one chemostat.

contrast, our grazing experiments revealed that *Pedobacter* is preferred as prey by the ciliate over *Brevundimonas* by a factor of four (see Supplementary Information). Experiments were performed with dilution rates of 0.90, 0.75, 0.50 and 0.45 d^{-1}. These dilution rates were selected on the basis of preceding model calculations.

The results revealed a different dynamic behaviour of the experimental system, depending on the applied dilution rate (Fig. 1). At the highest dilution rate ($D = 0.90$ d^{-1}), *Brevundimonas* had died off by the sixth day; the remaining species existed in stable coexistence at equilibrium (Fig. 1a). The establishment of constant, equilibrium population densities of all species was achieved after about 5 days at $D = 0.75$ d^{-1} (Fig. 1b). To check the robustness of the stable equilibrium, we repeated this experiment with a preceding 30-day period of aperiodic dynamics at $D = 0.50$ d^{-1} (Fig. 1c). Although similar dynamic behaviour was observed after a transition period of 5 days, the abundances reached by the three species were different from those of the previous experiment. One possible explanation for these differences in abundances of the rapidly reproducing microbes (30 days represents about 240 generations in the experiments) might be a potential evolutionary shift in population structure[19]. Obviously stable limit cycles were established in the two parallel chemostat systems after a period of about 8 days at a dilution rate of 0.45 d^{-1} (Fig. 1h, i). Maxima and minima for all three species recurred during the whole observation period. Slight differences can be attributed to the sampling interval, which was kept constant at about 24 h. The cycles started with a maximum abundance of the preferred bacterium, followed by a peak of the less-preferred bacterium and the predator. Aperiodic oscillations were always obtained when dilution rates were set to 0.50 d^{-1} (Fig. 1d–g). All four trials showed different patterns in their dynamics. The observed aperiodic oscillations of the chemostat populations were analysed for possible chaotic behaviour by using estimates of corresponding Lyapunov exponents (Fig. 2; see

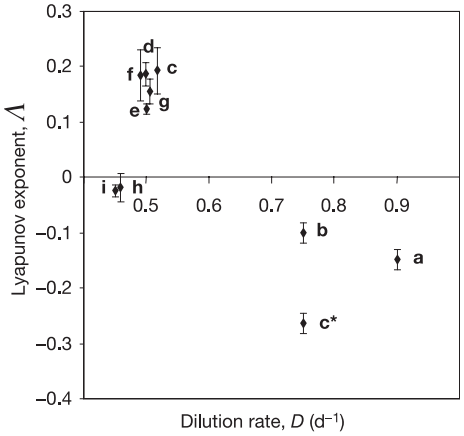

Figure 2 | Relationship between trajectory stability of data sets from Fig. 1 and the corresponding dilution rates. Lyapunov exponents were determined by the method of Rosenstein *et al.*[20] by fitting the rate of exponential separation of initially close trajectories. The error bars correspond to the asymptotic errors in the fit. Letters correspond to panels in Fig. 1 (**c*** is the Lyapunov exponent calculated for the second part of the time series in Fig. 1c; $t = 31$–50 days). Note that for dilution rates of 0.45 d^{-1} and 0.5 d^{-1}, data points were spread slightly along the *x* axis for visual clarity.

Supplementary Information). They were determined from each time series with a previously published algorithm[20], which tests directly for the exponential divergence from nearby trajectories and provides a very robust method for also dealing with small data sets (see Methods). According to general theoretical expectations, the data sets with extinction of the less-preferred prey species (Fig. 1a) and with coexistence at stable equilibrium (Fig. 1b) revealed negative Lyapunov exponents. This also holds true for the second part of the time series in Fig. 1c (after a change in the dilution rate to 0.75 d^{-1}). All experiments with $D = 0.5\ d^{-1}$ (Fig. 1c, left of the vertical line, and Fig. 1d–g) have positive Lyapunov exponents. Thus, we obtained strong experimental evidence for the existence of chaos in a real multi-species system. Note that for small data sets the range of the confidence interval generally increases. The stable sustained oscillations (Fig. 1h, i) have Lyapunov exponents close to zero (Fig. 2). Their absolute value is at least one order of magnitude smaller than all the other exponents. The exponential divergences of nearby trajectories show strong, sustained oscillations as well. There is a large asymptotic standard error in the fit of the Lyapunov exponents because of the strong oscillations. We conclude that the underlying dynamics are stable limit cycles. The observed dynamics in the experiments changed as predicted by a model[10] (stable coexistence at high dilution rates, chaos at intermediate dilution rates, and stable limit cycles at low dilution rates).

There are two important conclusions to be drawn. First, chaotic dynamics of small, rapidly growing organisms can occur in all microbiotopes. Second, because of the low generation times of microbes (only a few hours), such dynamics may be established before perturbations by external stimuli are effective. Examples of such communities are the tiny, fragmented populations of protists and bacteria that can occur on each grain of sand in a sediment, as well as on each small detritus particle in the pelagic zone of the open ocean or lakes[21,22]. The defined microbial food web that we established under chemostat conditions offers a completely new possibility for the experimental study of deterministic chaos in real biological systems. It is now possible to address many questions previously posed by theoreticians. We have provided a biological system that allows the investigation of the transition between different dynamical states, the analysis of interactions of fragmented populations showing either similar or different dynamic behaviours, the study of resilience and the importance of perturbations under varying dynamical states, and the interplay between complex dynamics and biodiversity[1–4,8,16,18,23]. When combined with molecular techniques, this system would also allow the evolutionary consequences of different dynamic behaviours to be analysed[19].

METHODS

Chemostat experiments. We established cultures of the ciliate *Tetrahymena pyriformis* (axenic culture from CCAP 1630/1W, average length and width 85 μm × 22 μm), the bacterium *Pedobacter* sp. (Cytophaga Flexibacter group, 2 μm × 1 μm) and *Brevundimonas* sp. (α-Proteobacteria, 2.5 μm × 2.5 μm) in 185 ml glass chemostats at 20 ± 1 °C in the dark. Both bacterial species were isolated by K. Beck from Lake Schöhsee, Germany; bacteria were always inoculated from deep-frozen stock cultures. The one-stage chemostat systems were fed continuously with sterile medium (0.2 $g\,l^{-1}$ proteose peptone, 0.025 $g\,l^{-1}$ yeast extract) at different dilution rates and mixed by continuous gentle aeration to ensure an even distribution of organisms. Chemostats were always started with the same inoculum. Sterile syringes were used to take samples daily at about 11:00 from the centre of the chemostats. Living ciliate samples were counted under a phase-contrast microscope immediately after sampling (more than 150 individuals were counted). Samples of bacteria were fixed with formaldehyde and stained with 4′,6-diamidino-2-phenylindole (DAPI)[24] for subsequent counting on membrane filters (pore size 0.2 μm) under an epifluorescence microscope (Zeiss Axioskop) with Zeiss filter set 01. At least 300 bacteria were counted on each filter. Organism abundances were the average of triplicates taken separately from one chemostat. The total volume of water taken from the chemostats during one sampling was 3 ml. Chemostats were checked regularly for the appearance of contaminant bacteria by using strain-specific antibodies against *Pedobacter* and *Brevundimonas* and by non-specific staining of the bacterial community with DAPI. With our present apparatus, the maximum number of samplings possible before contamination or any other technical problem hindered further experimentation was 50–55 days.

Grazing experiments. Experiments were performed to determine the food preference of *Tetrahymena*. A bacterial mixture (1:1) of *Pedobacter* and *Brevundimonas* (each strain at 4×10^6 cells ml^{-1}) was offered as prey in 50-ml vessels at 20 °C. The contents of the vessels were fixed with a buffered paraformaldehyde solution 3 min after inoculation of *Tetrahymena*[25]. The abundances of *Pedobacter* and *Brevundimonas* in the food vacuoles of *Tetrahymena* were determined by immunofluorescence[26] after hybridization with specific Cy3-labelled antibodies (permeabilization was performed with 8% Triton X-100).

Calculation of Lyapunov exponents. The calculations of the Lyapunov exponents by using the algorithm of Rosenstein *et al.*[20] were performed with the TISEAN package[27] (see Supplementary Information). Similarly to the independently published algorithm of Kantz[28], it directly tests the presence of exponential divergence and thus permits a decision on whether it makes sense to compute a Lyapunov exponent for given data. In contrast, the first published and widely used algorithm of Wolf *et al.*[29] makes the *a priori* assumption that there is an exponential divergence of nearby trajectories and is therefore prone to yield finite positive Lyapunov exponents also for stochastic data. This has been criticized in the ecological literature[4,30], and alternative approaches have been proposed that rely on approximating the equations of the underlying dynamics. The exponents are calculated from the jacobian, which resembles the linear part of the dynamics. This method is efficient if the data permit a good reconstruction of the dynamics. However, one has to be careful, because a good approximation of the dynamics does not guarantee well-approximated partial derivatives in the jacobian. However, because the present data stem from constant experimental conditions in a chemostat environment, the algorithm of Rosenstein *et al.*[28] should reveal more reliable estimates. The exponents were calculated by reconstructing the attractor dynamics from the time series of the predator's abundances with appropriate embedding dimensions and reconstruction delays, which robustly exhibited exponential divergence. The Lyapunov exponent was then fitted as the slope of the linear increase in the log-transformed divergence by using the least-squares method.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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