

Comparable textural features are observed in granites. For example, feldspar crystals often display rapakivi texture with an alkali feldspar core mantled by a rim of plagioclase feldspar¹⁹. The plagioclase mantle could form from quenching of hotter mafic magma abruptly mixed with felsic magma²⁰; convective self-mixing, however, could also be responsible. Mantled and unmantled feldspar crystals are observed in the same thin section²¹ which could result from localized thermal variations produced by self-mixing. Granites can display, on a local scale, grains of the same phase with contrasting textures, such as large phenocrysts of quartz near to small interstitial grains. There are skeletal and acicular crystal shapes, which are indicative of rapid undercooling and growth, and are consistent with rapid fluctuations in magma conditions²². Orogenic granites are also commonly associated with mafic syn-plutonic intrusions and related mafic inclusions^{23–25}, showing that the necessary conditions for heating and convective self-mixing are widespread.

Convective self-mixing does not require direct involvement of another more mafic magma to generate the complex textures commonly observed in porphyritic orogenic intermediate and silicic volcanic rocks, although mafic magma emplaced at the chamber base is the probable heat source. The concept of self-mixing reconciles otherwise puzzling petrological features, and also supports the notion that magma chambers can convect vigorously. □

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Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of *Dechloromonas*

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Benzene contamination is a significant problem. It is used in a wide range of manufacturing processes and is a primary component of petroleum-based fuels. Benzene is a hydrocarbon that is soluble, mobile, toxic and stable, especially in ground and surface waters. It is poorly biodegraded in the absence of oxygen. However, anaerobic benzene biodegradation has been documented under various conditions. Although benzene biomineralization has been demonstrated with nitrate¹, Fe(III)^{2–5}, sulphate^{6,7} or CO₂^{8,9} as alternative electron acceptors, these studies were based on sediments or microbial enrichments. Until now there were no organisms in pure culture that degraded benzene anaerobically. Here we report two *Dechloromonas* strains, RCB and JJ, that can completely mineralize various mono-aromatic compounds including benzene to CO₂ in the absence of O₂ with nitrate as the electron acceptor. This is the first example, to our knowledge, of an organism of any type that can oxidize benzene anaerobically, and we demonstrate the potential applicability of these organisms to the treatment of contaminated environments.

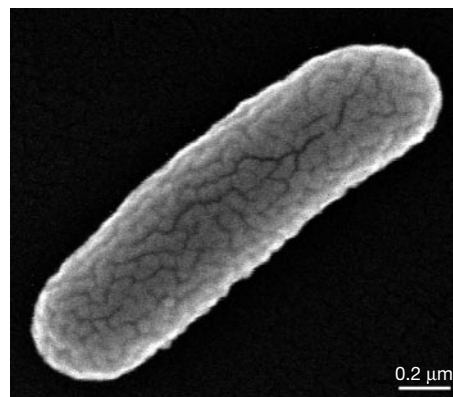


Figure 1 Scanning electron micrograph of anaerobically grown cells of strain RCB.

Benzene is one of the most prevalent organic contaminants in groundwaters¹⁰ and is of major concern owing to its toxicity and relatively high solubility. Numerous benzene-degrading aerobic microorganisms have been identified, the most notable of which are the *Pseudomonas* species, which may account for up to 87% of the petrol-degrading microorganisms in contaminated aquifers¹¹. However, soils and sediments contaminated with benzene frequently develop extensive anaerobic zones^{10,12,13}. As a result, much attention has focused on anaerobic benzene degradation. In the last decade, studies have demonstrated that the hydrocarbon-degrading capacity of anaerobes is far greater than previously assumed^{2,6,14–16}. Pure cultures of anaerobic organisms that can degrade hydrocarbons such as toluene, hexadecane and naphthalene have now been described^{17–20}. However, organisms capable of anaerobic benzene degradation have been elusive and this metabolism was observed only in sediment studies^{2–6,9,15,16} or with microbial enrichments^{1,7,8,21}. Organisms capable of anaerobic benzene degradation had not been isolated. Furthermore, even though anaerobic benzene degradation has been demonstrated under various conditions in the absence of O₂ (refs 1–3, 8, 9, 15, 16), no specific organisms or genera had been associated with the metabolism. Although Fe(III)-reducing sediments that degrade benzene^{4,5} are enriched with organisms of the family Geobacteraceae²², there is no direct evidence to show that members of this family are capable of benzene degradation.

Here we report on the isolation and characterization of *Dechloromonas* strains RCB and JJ, which oxidize benzene to CO₂ in the absence of O₂. These organisms were enriched and isolated from two diverse environments on the basis of very different metabolic abilities.

Strain RCB was enriched as a hydrocarbon-oxidizing chlorate reducer. Enrichments were initiated at 30 °C in anoxic artificial medium²³ with 4-chlorobenzoate (0.5 mM) as the electron donor and chlorate (10 mM) as the electron acceptor. Inocula were taken from sediments collected from the Potomac River, Maryland, USA, which contains an active microbial population that can reduce chlorate or perchlorate ((per)chlorate)^{24,25} and an anaerobic hydrocarbon-degrading population (J.D.C., unpublished observations). Positive enrichments were identified by microscopic observation of increasing cell density over time. After 8 d, cell numbers increased by at least one log unit. The cultures were transferred (10% v/v) into freshly prepared media. After several transfers in chlorobenzoate–chlorate medium, the enrichments were inoculated into an anoxic agar dilution series^{23,24} of the same medium amended with 2% noble agar. Colonies of strain RCB were isolated after six weeks' incubation.

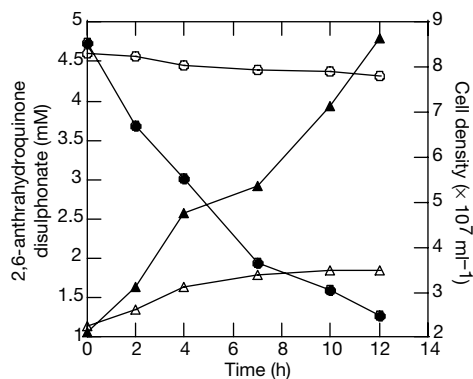


Figure 2 Growth of strain RCB (mean of triplicate determinations) with AHDS (5 mM) as the electron donor and chlorate (10 mM) as the electron acceptor. Acetate (0.1 mM) was added as a suitable carbon source. Filled circles, AHDS concentration in growth culture; open circles, AHDS concentration in heat-killed controls; filled triangles, cell density in growth culture; open triangles, cell density in heat-killed controls.

Strain JJ was isolated as a humic-substances-oxidizing nitrate reducer. Enrichments were initiated at 30 °C in anoxic artificial medium²³ with 2,6-anthrahydroquinone disulphonate (AHDS) (5 mM) as the electron donor and nitrate (5 mM) as the electron acceptor with 0.1 mM acetate as a carbon source. AHDS medium was prepared from anoxic freshwater medium containing 2,6-anthraquinone disulphonate reduced with palladium-coated aluminium chips and H₂ (ref. 26). Inocula were taken from sediments collected from Campus Lake, Southern Illinois University. Positive enrichments were identified by colour change in the media (red to tan) as the AHDS was oxidized. The cultures were transferred (10% v/v) into freshly prepared anoxic AHDS–nitrate medium twice over three weeks and then inoculated into an anoxic agar dilution series^{23,24} of the same medium amended with 2% noble agar. Colonies of strain JJ were isolated after three weeks' incubation.

Cells of strains RCB and JJ are gram-negative rods 1.8 × 0.5 μm (Fig. 1) that are facultatively anaerobic, completely oxidizing and non-fermenting. Both strains grew with air or nitrate as electron acceptors. Nitrate was reduced to N₂ gas. Strain RCB also grew with chlorate or perchlorate, completely reducing them to chloride. Both strains coupled growth to the complete oxidation of various simple organic acids with nitrate as the sole electron acceptor, including formate (10 mM), acetate (10 mM), propionate (5 mM), butyrate (5 mM), lactate (10 mM), succinate (5 mM), pyruvate (10 mM) and benzoate (0.5 mM). Both strains also oxidized toluene (1 mM). Both strains grew anaerobically on nitrate with the reduced analogue for humic substances, AHDS, as the electron donor and acetate (0.1 mM) as the carbon source (Fig. 2). Only slight growth was observed if the AHDS was omitted, which was probably due to the presence of the small amount of acetate (0.1 mM) added as a carbon source (Fig. 2). No AHDS oxidation occurred in the absence of an electron acceptor.

Phylogenetically, strains RCB and JJ are closely related to each other (98.1% 16S ribosomal DNA sequence similarity) and both are members of the *Dechloromonas* subgroup in the β subclass of the Proteobacteria²⁷. Their closest relative is *Ferribacterium limneticum* (Fig. 3), which is an anomaly in this subgroup²⁸ as it is a strictly anaerobic Fe(III) reducer. All other members of the subgroup are facultatively anaerobic (per)chlorate reducers^{27,28}.

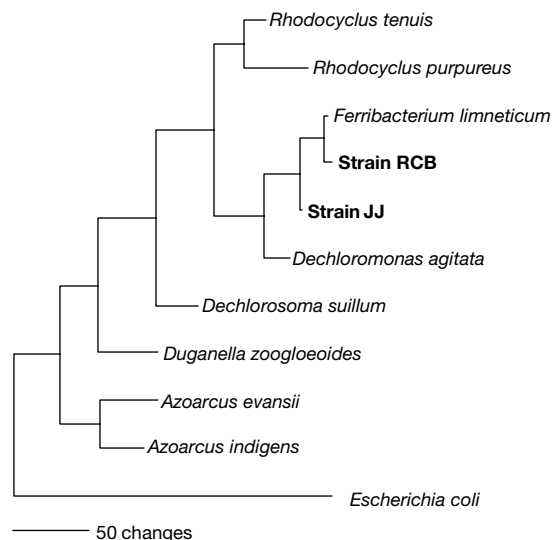


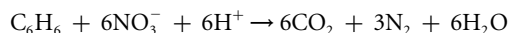
Figure 3 Phylogenetic tree of the 16S rDNA sequences of strains RCB and JJ and their closest relatives resulting from a heuristic search with parsimony analysis. The same topology was obtained from distance and from maximum likelihood, and was supported by bootstrap analysis.

Both strains RCB and JJ oxidized benzene anaerobically with nitrate as the electron acceptor (Fig. 4a). ¹⁴C-labelled benzene was oxidized to ¹⁴CO₂. No [¹⁴C]benzene oxidation occurred if the electron acceptor was omitted (Fig. 4a). In the case of strain RCB, once ¹⁴CO₂ production ceased, 45% of the original ¹⁴C label could be accounted for as ¹⁴CO₂. Analysis by high-performance liquid

chromatography (HPLC) indicated that 47% of the initial benzene remained undegraded in the culture medium, confirmed by comparison with heat-killed controls. This result accounts for 92% of the initial ¹⁴C label, showing that benzene is completely oxidized to CO₂. In support, HPLC analysis coupled to liquid scintillation counting indicated that the residual ¹⁴C label in the aqueous phase was present only as benzene.

In parallel incubations with the aerobic benzene-oxidizer *Pseudomonas* strain JS-150, ¹⁴CO₂ production was not observed unless O₂ was added (data not shown), demonstrating that benzene oxidation by strains RCB and JJ was anaerobic and not the result of O₂ contamination. Because of the similar morphology, phylogeny and physiology of these two strains, strain RCB was selected for a more detailed characterization of anaerobic benzene oxidation coupled to nitrate reduction.

With nitrate as the sole electron acceptor, the oxidation of 163 ± 19 μM benzene (± standard deviation, n = 3) by strain RCB resulted in the reduction of 843 ± 64 μM nitrate (± standard deviation, n = 3). This represents greater than 86% of the theoretical ratio for benzene oxidation coupled to nitrate reduction according to:



In an active benzene-degrading culture of strain RCB with nitrate as the electron acceptor, increase in cell number was concomitant with benzene disappearance (Fig. 4b). Minimal growth occurred in the absence of benzene (Fig. 4b) and was probably the result of carry-over from the inoculum. The small amount of benzene lost in the heat-killed controls was due to absorption into the butyl-rubber stoppers^{8,16}. When the active culture from the growth curve shown in Fig. 4b was used to inoculate fresh medium, benzene was again rapidly degraded and cell increase was concomitant with benzene removal (Fig. 4c), demonstrating that strain RCB could grow and be transferred in media with benzene as the sole electron donor and nitrate as the sole electron acceptor. When strain RCB was grown anaerobically with nitrate and universally labelled (UL)-[¹⁴C]benzene, 2% of the ¹⁴C label was associated with the retentate after the culture was filtered through a filter with a pore size of 0.2 μm. A similar filtration of a heat-killed control retained 0.8% of the ¹⁴C label, suggesting that 1.2% of the ¹⁴C label was incorporated into biomass. In contrast, 3.0% of the ¹⁴C label was incorporated into the biomass in an aerobically grown culture. These low values of incorporation of ¹⁴C label into biomass are similar to values reported previously for a nitrate-reducing, benzene-oxidizing

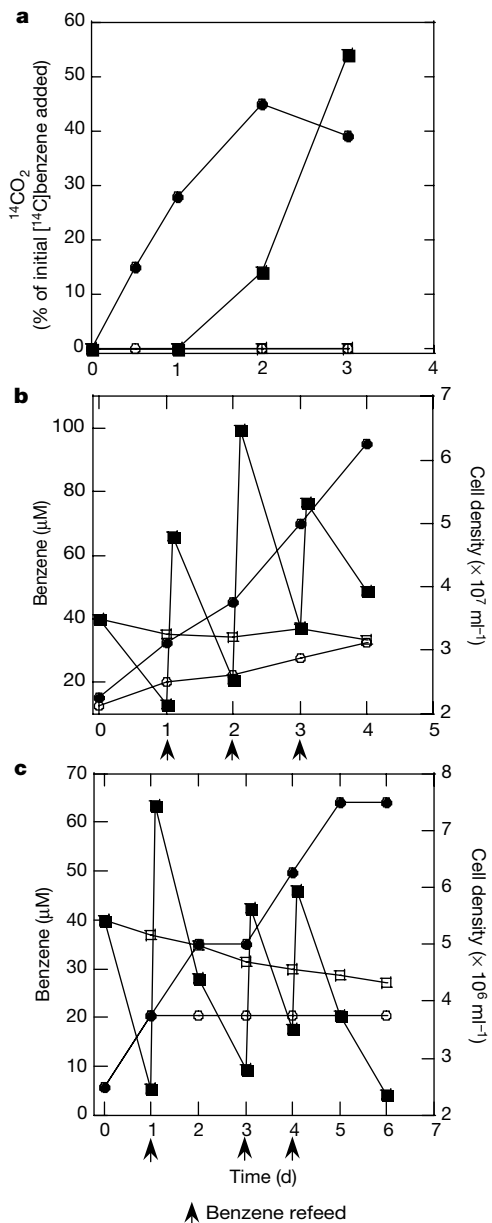


Figure 4 Anaerobic oxidation of benzene by strains JJ and RCB (mean of triplicate determinations). **a**, [¹⁴C]benzene is completely oxidized to ¹⁴CO₂ by strains RCB (filled circles) and JJ (filled squares) in the absence of oxygen with nitrate as the electron acceptor. No ¹⁴CO₂ production was observed with either strain (RCB, open circles; JJ, open squares) in the absence of nitrate or if the cells were heat-killed. **b**, Anaerobic growth and benzene oxidation by strain RCB with nitrate as the electron acceptor (filled squares, benzene concentration in the growth culture; open squares, benzene concentration in the heat-killed controls; filled circles, cell numbers in the growth culture; open circles, cell numbers in the absence of benzene). **c**, Growth and benzene oxidation in fresh anoxic culture media with nitrate as the electron acceptor after inoculation (10% v/v) from the active growth culture tubes from **b** (filled squares, benzene concentration in the growth culture; open squares, benzene concentration in the heat-killed controls; filled circles, cell numbers in the growth culture; open circles, cell numbers in the absence of benzene).

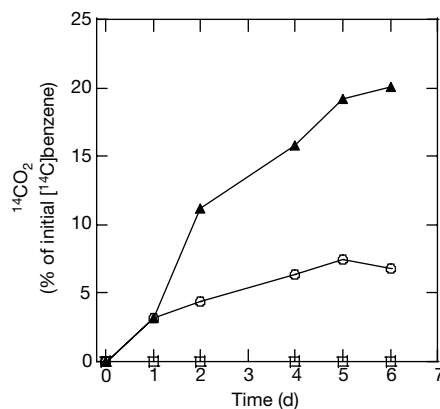


Figure 5 Degradation of [¹⁴C]benzene in anoxic sediments amended with nitrate (10 mM) in the presence and absence of strain RCB (mean of triplicate determinations). Filled triangles, sediment amended with both strain RCB and nitrate. Open circles, nitrate-amended sediment without strain RCB; open circles, unamended sediment without strain RCB.

enrichment culture (5–8%)¹ and are supportive of the low cell yield for anaerobic growth on benzene observed for strain RCB (Fig. 4b).

In order to determine the ability of these organisms to attenuate benzene in the natural environment, anoxic aquatic sediments amended with [¹⁴C]benzene and nitrate were inoculated with an active culture of strain RCB. Geochemical analysis of the sediment revealed that the indigenous nitrate and sulphate concentrations were 22 and 867 μM, respectively. No chlorate was present. The total iron content was 38 mM, and 45% of this (= 17 mM) was present as Fe(II). Determination of the terminal electron-accepting process in these sediments with [¹⁴C]acetate¹⁶ indicated that Fe(III) reduction was predominant (data not shown). Samples amended with both strain RCB and nitrate showed rapid evolution of ¹⁴CO₂ from UL-[¹⁴C]benzene (Fig. 5). Benzene oxidation also occurred in the unamended sediments (Fig. 5). This was unlikely to be due to O₂ contamination because the high concentrations of Fe(II) in the sediment would rapidly remove O₂. The indigenous benzene oxidation was inhibited by the addition of nitrate when strain RCB was omitted (Fig. 5). Similar inhibition by nitrate addition has previously been reported for a benzene-oxidizing, sulphate-reducing sediment²⁹.

Anaerobic benzene oxidation by microbes has been extensively documented in the last decade; however, until now it had only been observed in sediment studies or with enrichment cultures^{1–9,21}. Here we describe two isolates that can couple the anaerobic oxidation of benzene to the reduction of nitrate. Little is known about either microbial oxidation of humic substances or microbial (per)chlorate reduction. Previous studies in our lab have demonstrated that the phylogeny of microorganisms capable of these types of metabolism is diverse^{23,24,26}. Very little is known of their other metabolic capabilities. Both strains JJ and RCB are members of the *Dechloromonas* genus even though these organisms were isolated from two different environments on the basis of very different metabolic capabilities (that is, microbial (per)chlorate reduction and microbial humic-substances oxidation). Previous studies by our group have demonstrated the ubiquity of the *Dechloromonas* species, and members of this genus have been identified in a broad range of environments including pristine and contaminated soils and sediments²⁴, and even in samples collected from Antarctica (L.A.A., unpublished observations). The results presented here indicate that the members of this genus offer great potential not only for the attenuation of perchlorate but also for the treatment of benzene in contaminated environments. □

Methods

Medium preparation

All media and solutions were prepared using strict anaerobic techniques as described^{6,23,24}. All culturing was done in sealed serum vials with N₂/CO₂ (80/20, v/v) in the headspace. Basal medium used for the isolation of strain RCB was amended from a sterile aqueous anoxic stock solution of 4-chlorobenzoate (100 mM) to give a final concentration of 0.5 mM when appropriate. Basal medium used for the isolation of strain JJ was prepared from anoxic freshwater medium containing 2,6-anthraquinone disulphonate reduced with palladium-coated aluminium chips and H₂, as described²⁶.

In general, alternative electron donors tested were added from sterile aqueous anoxic stock solutions. When necessary, benzene was added from an anoxic aqueous stock solution prepared as described⁶. Growth was determined by observation of cell density increase and was confirmed by direct cell counts under oil-immersion phase-contrast microscopy. Results were compared with those of unamended controls. [¹⁴C]Benzene (1 μCi) was added from an anoxic stock solution as described^{25,30}.

Sediment experiments

Fresh sediments were collected from Campus Lake, Southern Illinois University, and stored under N₂ at 4 °C until used. Analyses of geochemical and terminal electron-accepting processes were performed directly before use as described^{14–16}. All experiments were performed in triplicate with strict anaerobic techniques in sealed 35-ml serum vials amended with 5 g sediment under an N₂ headspace. The inoculum size was 10% v/v of anaerobically grown cells with nitrate (10 mM) as the sole electron acceptor. [¹⁴C]benzene (1 μCi) was added from a stock solution⁶ and benzene concentrations were determined by gas chromatography with gas proportional counting detection (GC-GPC)^{25,30}. Nitrate was amended from an anoxic sterile stock solution.

Electron microscopy

Scanning electron micrographs were prepared from cells grown anaerobically with acetate (10 mM) as the electron donor and nitrate (10 mM) as the electron acceptor as described²⁵ and viewed with a Hitachi S570 scanning electron microscope at 20 kV.

Analytical techniques

Concentrations of ¹⁴CO₂ in the headspace of cultures amended with [¹⁴C]benzene were determined by GC-GPC^{25,30}. Benzene concentrations in growth cultures were determined by gas chromatography of 0.1-ml headspace samples as described⁶. Concentrations of aqueous-phase benzene and potential catabolic intermediates were determined by HPLC with an LC-18 Supelcosil column with ultraviolet detection at 254 nm. The mobile phase was 60/40 (v/v) methanol/water. If [¹⁴C]benzene was used, eluted peaks were collected directly into 5 ml of liquid scintillant and analysed for ¹⁴C label by liquid scintillation counting with a Beckman LS 6000IC. Nitrate concentrations were determined by ion chromatography of aqueous samples with a Dionex DX500 equipped with an AS9-SC column using a mobile phase of sodium carbonate (2 mM) and sodium bicarbonate (7.5 mM) at a flow rate of 2 ml min⁻¹. AHDS concentrations were determined by colorimetric assay at 450 nm as described²⁶. Cell numbers were determined by direct microscopic counting under oil immersion.

Phylogenetic analysis

Polymerase chain reaction, sequencing and analysis of the 16S ribosomal RNA genes was performed as described^{24,27}. Sequence entry and manipulation was performed with the MacVector 6.5 sequence analysis software program (Oxford Molecular Group). Sequences of select 16S rRNAs were downloaded from the Ribosomal Database Project and GenBank into the computer program SeqApp. 16S rDNA sequences of strains RCB and JJ were manually added to the alignment using secondary structure information for proper alignment (alignment available from J.D.C.). Only those regions sequenced in all of the organisms (1,456 nucleotides) were used in the subsequent phylogenetic analyses. Distance, parsimony and maximum likelihood analysis of the aligned sequences was performed with PAUP* 4.0. Bootstrap analysis was conducted on 100 replications using a heuristic search strategy to assess the confidence level of various clades. GenBank accession numbers for sequences represented in Fig. 3 are: *Rhodocyclus tenuis*, D16209; *R. purpureus*, M34132; *Ferribacterium limneticum*, Y17060; *Dechloromonas agitata*, AF047462; *Dechlorosoma suillum*, AF170348; *Duganella zooglooides* (previously *Zoogloea ramigera*), X74913; *Azoarcus evansii*, X77679; *A. indigens*, L15531; and *Escherichia coli*, J01859).

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Life-history traits of voles in a fluctuating population respond to the immediate environment

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Life-history traits relating to growth and reproduction vary greatly among species and populations^{1,2} and among individuals within populations³. In vole populations, body size and age at maturation may vary considerably among locations and among years within the same location^{4–8}. Individuals in increasing populations are typically larger and start reproduction earlier in the spring than those in declining populations^{6–8}. The cause of such life-history variation within populations has been subject of much discussion^{7,9,10}. Much of the controversy concerns whether the memory of past conditions, leading to delayed effects on life-history traits, resides in the environment (for example, predators^{11,12}, pathogens¹³ or food^{14,15}) or intrinsically within populations or individuals (age distribution^{16,17}, physiological state³, genetic¹⁸ or maternal effects^{19,20}). Here we report from an extensive field transplant experiment in which voles were moved before the breeding season between sites that differed in average overwintering body mass. Transplanted voles did not retain the characteristics of their source population, and we demonstrate an over-riding role of the immediate environment in shaping life-history traits of small rodents.

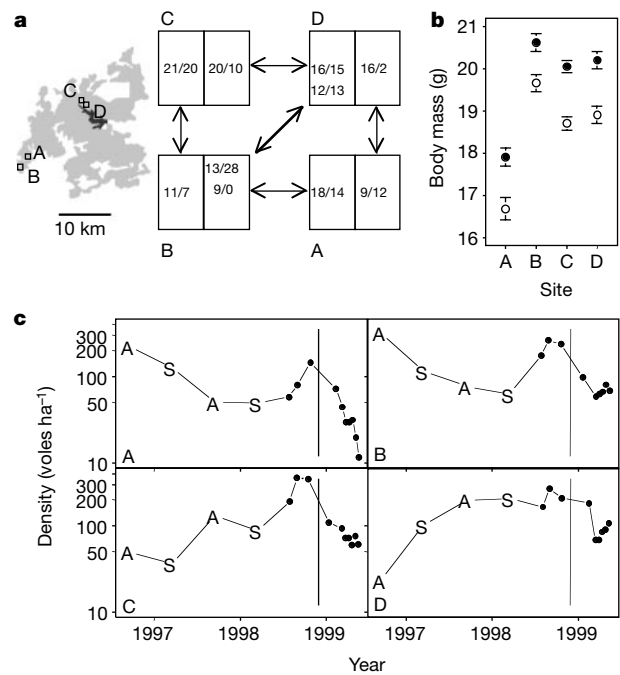


Figure 1 Design of the study. **a**, Groups of voles were swapped between half-sides of four 1-ha unfenced trapping grids in early winter (November/December). Arrows, pairs of transplanted groups. A second transplant was done between two of the sites (B and D) in February because few voles settled in some groups. Numbers in the boxes show number of males/females that were successfully transplanted between grids and captured at least once during the spring. **b**, Average body mass (\pm s.e.) at the time of transplant in November/December among animals that had not previously bred. Open circles, females; filled circles, males. **c**, Density trajectories at the four sites plotted on a log₁₀ scale. Density estimates denoted by S (spring) and A (autumn) are based on vole sign indices²¹. Estimates plotted as filled circles are obtained by the robust design model²⁷ of capture–mark–recapture data. Vertical lines show the time of transplant. Density trajectories in neighbouring control areas showed similar patterns (see Supplementary Information).

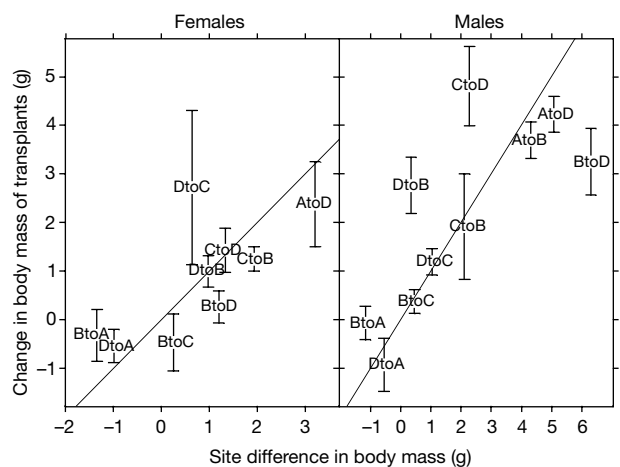


Figure 2 Average (\pm s.e.) individual change in body mass of transplanted voles recaptured in January/February during the first session after transplanting plotted against the difference in average body mass at the sites. The site differences in body mass are calculated as average body mass in the target population during the first trapping session after the transplant minus average body mass of transplants at capture during the transplantation. Voles in the transplant group are excluded from the target population when calculating the site differences. Hence, measurements are independent. Line is the unity slope through the origin, indicating perfect convergence. Plotted labels denote ‘source-to-target’ transplant groups. See Supplementary Information.