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Molecules and Cells

Reproductive Fitness and Dietary Choice Behavior of the Genetic Model Organism *Caenorhabditis elegans* under Semi-Natural Conditions

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Laboratory breeding conditions of the model organism *C. elegans* do not correspond with the conditions in its natural soil habitat. To assess the consequences of the differences in environmental conditions, the effects of air composition, medium and bacterial food on reproductive fitness and/or dietary-choice behavior of *C. elegans* were investigated. The reproductive fitness of *C. elegans* was maximal under oxygen deficiency and not influenced by a high fractional share of carbon dioxide. In media approximating natural soil structure, reproductive fitness was much lower than in standard laboratory media. In semi-natural media, the reproductive fitness of *C. elegans* was low with the standard laboratory food bacterium *E. coli* (γ-Proteobacteria), but significantly higher with *C. arvensicola* (Bacteroidetes) and *B. tropica* (β-Proteobacteria) as food. Dietary-choice experiments in semi-natural media revealed a low preference of *C. elegans* for *E. coli* but significantly higher preferences for *C. arvensicola* and *B. tropica* (among other bacteria). Dietary-choice experiments under quasi-natural conditions, which were feasible by fluorescence *in situ* hybridization (FISH) of bacteria, showed a high preference of *C. elegans* for *Cytophaga-Flexibacter-Bacteroides*, Firmicutes, and β-Proteobacteria, but a low preference for γ-Proteobacteria. The results show that data on *C. elegans* under standard laboratory conditions have to be carefully interpreted with respect to their biological significance.

INTRODUCTION

The soil-dwelling nematode *Caenorhabditis elegans* is one of the most important genetic model organisms. Under laboratory conditions, *C. elegans* is usually kept in monoxenic culture on agar-solidified peptone medium with *E. coli* as bacterial diet (Brenner, 1974). In natural soil, *C. elegans* requires freshly rotting organic material (compost) to reach maturity during development (Kiontke and Sudhaus, 2006). Alternatively, Dauer larvae are formed. In any case, both soil and compost possess quite different properties to standard laboratory media. Mineral and organic constituents form the skeleton of the 'soil system' (Gobat et al., 2003). The respective proportions of these constituents (texture) affect its mode of organization (structure), and both influence the volume and arrangement of soil voids (Skvortsova and Utkaeva, 2008). Depending on moisture content, soil voids are filled with air or water of variable salinity (Stotzky, 1972). Due to its sensitivity to desiccation, *C. elegans* preferentially lives at the interface between air and water (Cheung et al., 2005). The filling of soil voids with air or water also influences the degree of oxygen provision for the nematode. The fraction of oxygen in soil air may vary between 0% and 21% over fairly short periods or distances depending on soil texture and structure, and volume and arrangement of soil voids (Cheung et al., 2005). In addition to abiotic factors, soil biota also affect soil air. The fraction of carbon dioxide in soil air, for example, may rise up to 10% depending on the presence of plant roots, and aerobic microflora and fauna (Bretscher et al., 2008). Gas exchange in *C. elegans* takes place via diffusion with the cuticle representing the main diffusive barrier (Lee and Atkinson, 1976).

Soil contains prokaryotes in high abundance and diversity (Fierer et al., 2007) as well as fungi, algae, below-ground parts of plants and numerous animals from protozoa to mammals. As a bacteriophage, *C. elegans* competes with other bacterivorous soil organisms for food and consequently, allelopathic interactions may occur between competitors (Neidig et al., 2010). *C. elegans* owns numerous sensory mechanisms (Bargmann, 2006), which enables the worm to detect food bacteria. Subsequent to the ingestion of bacteria by *C. elegans*, food passes the pharyngeal lumen to reach the terminal bulb (grinder), where bacteria are homogenized, before the chyme enters the intestinal lumen (Avery and Thomas, 1997). *C. elegans* is able to discriminate between different food bacteria (Abada et al., 2009; Avery and Shtonda, 2003; Shtonda and Avery, 2006). In addition, a negative behavioral response to potential pathogens, such as *Pseudomonas aeruginosa* and *Bacillus thuringiensis*,

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has been shown (Hasshoff et al., 2007; Laws et al., 2006).

As conditions in natural soil differ markedly from standard laboratory conditions, this study investigates the effects of more natural environments on the reproductive fitness of *C. elegans.* We focused on major environmental determinants, which are likely to affect life-history parameters of *C. elegans* including air composition (air partial pressure), habitat structure and diet (bacterial species). Since food requirements affect the preference behavior of organisms, we also studied the dietary choice of *C. elegans* under semi- and quasi-natural conditions.

MATERIALS AND METHODS

Keeping of *Caenorhabditis elegans*

As described by Brenner (1974), *C. elegans* (Bristol strain N2; Caenorhabditis Genetic Center, USA) was kept at 20°C on NGM agar plates using *E. coli* OP50 (Caenorhabditis Genetic Center, USA) as food. All experiments were carried out at 20°C with 1.12×10^8 cells of *E. coli* OP50 or other bacterial species as food source. To enable the addition of a defined quantity of food just by the volume of bacterial suspension, the number of cells in diluted suspensions of each bacterial species was counted using a Neubauer counting chamber and related to the respective absorbance at 595 nm. Experimental media were NGM agar plates (∅ = 30 mm), petri dishes (∅ = 30 mm) filled with 800 µl liquid medium (S-medium; Hoppe, 1999; Sulton and Brenner, 1974) or petri dishes (∅ = 30 mm) filled with 800 µl S-medium and 2.33 g autoclaved solids (corresponding to a water content of 34% (m/m); quantities according to ASTM standard; ASTM, 2006). The solids were either glass beads (∅ = 1 mm; A. Hartenstein, Germany) or quartz sand (mean ∅ = 1 mm; Euroquarz, Germany). The surface of the liquid medium did not exceed the height of the solid phase (approximately 2 mm).

Experiments at different air partial pressures

To study effects of air composition (air partial pressure) on the reproductive fitness of *C. elegans*, air with different oxygen content (oxygen partial pressure, Po_2) at normocapnia (i.e. atmospheric CO_2 content) or different carbon dioxide content (carbon dioxide partial pressure, Pco_2) at normoxia (i.e. atmospheric O_2 content) was generated by mixing oxygen, carbon dioxide and nitrogen with gas-mixing pumps (Wösthoff, Germany). After passing three thermostat-controlled humidifiers in series and a microbial filter, water-saturated gas of a specific composition flowed through a thermostat-controlled sealable animal chamber ($25 \times 20 \times 20$ cm polystyrol container) at a rate of 500 ml min^{-1} via an inlet and an outlet attached to the opposite lateral sides of the container (cf., Föll et al., 1999). For all connections, PVC hoses were used, and the tightness of the setup was monitored by observing the gas outflow bubbling through water in a vessel. Experiments started with the insertion of NGM agar plates covered with stretched Parafilm® into the animal chamber with each plate containing one L4 larva (food quantity: 1.12×10^8 cells of *E. coli* OP50). After three days at specific air partial pressures, reproductive fitness (i.e. the number of developed offspring per worm) was evaluated.

Experiments with different experimental media

Effects of experimental media on *C. elegans* reproduction were studied by keeping one L4 larva per treatment for three days in different experimental media (NGM agar plate, S-medium, S-medium with glass beads or quartz sand) under atmospheric air (food quantity: 1.12×10^8 cells of *E. coli* OP50), and evaluating reproductive fitness (i.e. the number of developed offspring per worm) after extracting the worms by flotation if necessary (S-medium with solids). For the extraction procedure, LUDOX® HS-40 stock solution (Sigma-Aldrich) was diluted with water (1:1, v/v) to a specific density of 1.15 g cm^{-3} matching the density of the worms. Experimental media with *C. elegans* were mixed with 1 ml of this solution, and after 1 min floating animals were collected with a pipette and transferred to an empty petri dish (cf. ASTM, 2006; Donkin and Dusenbery, 1993). This procedure was repeated to collect worms that were left.

Experiments using different bacterial food

To study food-quality effects on reproductive fitness and dietary-choice behavior, different food bacteria were used including soil isolates of *Azospirillum brasilense*, *Burkholderia tropica*, *Herbaspirillum* spec., *Pseudomonas fluorescens*, and *Serratia liquefaciens*, wild-type strains of *Bacillus subtilis* and *Cytophaga arvensicola* as well as *Escherichia coli* OP50 (Table 1).

Effects of food quality (bacterial species) on reproductive fitness were studied by keeping one L4 larva per treatment for three days at different bacterial diets in S-medium with glass beads under atmospheric air (food quantity: 1.12×10^8 bacterial cells), and evaluating reproductive fitness (i.e. the number of developed offspring per worm) after extracting the worms by flotation (see above).

Dietary choice was studied by keeping worms (initially L1 larvae, 50-100 specimens) for three days at a mixed diet of fluorescence-labeled test bacteria (Hoechst 33342; Molecular Probes) and fluorescent control bacteria (GFP-expressing *E. coli*, Table 1) in equal amounts (total amount: 1.12×10^8 bacterial cells) in S-medium with glass beads under atmospheric air. Test and control bacteria differed in fluorescence properties: UV excitation with blue emission (test bacteria); blue excitation with green emission (GFP-expressing *E. coli*). Hoechst 33342 staining was carried out according to the following protocol. From overnight cultures, volumes containing 0.56×10^8 bacterial cells were centrifuged (1 min, $2300 \times g$), and re-suspended in 400 µl of supernatant and 150 µl of a 10 µg ml^{-1} Hoechst 33342 solution. After staining (at least two hours; T: 20°C) and centrifugation (1 min, $2300 \times g$), the supernatant was discarded, and the stained cells were re-suspended in 400 µl S-medium. After three days at mixed food supply (with the petri dishes covered with aluminium foil to prevent bleaching of the dye), the food preference of *C. elegans* was assessed by evaluating the type of bacteria within the pharynges of approximately 30 randomly chosen adult worms via digital fluorescence microscopy (Zeiss Axiovert 100; Canon EOS 350D digital camera). For this, all contents of a petri dish were emptied over a NGM agar plate, and about 30 worms were transferred into a droplet (12.5 µl) of levamisol (1 mmol L^{-1}) on a microscope slide for anaesthetization. After putting a cover slip on the slide, the worms were classified according to the following criteria: no bacteria, only test bacteria, only *E. coli*, or both test bacteria and *E. coli*.

Fluorescence *in situ* hybridization

To study the food preference of *C. elegans* under quasi-natural conditions, a fluorescence *in situ* hybridization (FISH) assay was established based on the following DNA probes: EUB338 I-III for all Eubacteria (Amann et al., 1990; Daims et al., 1999), CFB560 for the *Cytophaga-Flexibacter-Bacteroides* group (O'Sullivan et al., 2002), LGC354B for Firmicutes (Meier et al., 1999), BET42a for β-Proteobacteria, and GAM42a for γ-Proteobacteria (Manz et al., 1992).

After transferring worms into a droplet of M9 medium (Stiernagle, 2006) on a microscope slide coated with dried poly-L-lysine (20 µl per slide), a cover slip was used to immobilize but not crush the worms; then the slide and cover slip were im-

Table 1. List of soil bacteria used in this study

Species	Origin	Strain	Temperature, medium
Azospirillum brasilense	Institute of Zoology, TU Darmstadt	Soil isolate	37°C, TSA
Bacillus subtilis	Institute of Molecular Microbiology and Biotechnology, WWU Muenster	IMMB No. 227	30°C, LB
Burkholderia tropica	Institute of Zoology, TU Darmstadt	Soil isolate	37°C, TSA
Cytophaga arvensicola	DSMZ, Braunschweig	DSM No. 3695	30°C, Cytophaga
Escherichia coli	Caenorhabditis Genetics Center, University of Minnesota, USA	OP50	37°C, LB
Escherichia coli	Caenorhabditis Genetics Center, University of Minnesota, USA	OP50-GFP	37°C, LB
Herbaspirillum spec.	Institute of Zoology, TU Darmstadt	Soil isolate	37°C, TSA
Pseudomonas fluorescens	Institute of Zoology, TU Darmstadt	Soil isolate	37°C, LB
Serratia liquefaciens	Institute of Zoology, TU Darmstadt	Soil isolate	37°C, TSA

mersed in liquid nitrogen. Cover slips were then removed, and the worms were fixed with 100% methanol (first at -20°C and then at 20°C; 5 min each). Rehydration was done via a descending methanol series (1 min each): 90%, 70% and 50% (v/v) methanol in 1 × PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2). After washing twice (5 min each) in PTw [0.1% (v/v) Tween20 in 1× PBS], proteins were digested with 1 μg ml^{-1} proteinase K in PTw (15 min). Then the slides were washed; first in PTw containing 2 mg ml^{-1} glycine (2 min) and then in PTw (2 × 5 min). After a next fixation step (3.7% (v/v) formaldehyde in 1× PBS with 80 mM HEPES pH 6.9, 1.6 mM $MgSO_4$, and 0.8 mM EGTA added; 20 min), the slides were washed (twice in PTw, once in PTw containing 2 mg ml^{-1} glycine, and three times in PTw; 5 min each). Prehybridization was done by sequential incubations; first in a 1:1 mixture of hybridization buffer (0.1% (v/v) Tween20 in 50% (v/v) formamide with 100 μg ml^{-1} autoclaved salmon sperm DNA, 50 μg ml^{-1} heparin, 750 mM NaCl, and 75 mM trisodium citrate-$2H_20$ added) and PTw (10 min), then in pure hybridization buffer (10 min), and finally at 37°C in hybridization buffer (1-2 h), which had been heated beforehand in order to denature the salmon sperm DNA. After putting 20 μl of DNA probe (1.5 ng μl^{-1} probe in hybridization buffer) and a cover slip on each slide, hybridization was done overnight at 37°C in a humid chamber. After removing the cover slips, the slides were washed in hybridization buffer at 37°C (10 min) and in TTBS (150 mM NaCl, 50 mM Tris/HCl pH 7.8, 0.1% (w/v) BSA, 1% (v/v) Tween20) at 20°C (2 × 10 min). After adding 20 μl mounting medium (Sigma) and the cover slips, the slides were used for fluorescence microscopy.

During experiments, approximately 300 non-fed *C. elegans* individuals each were spread over 10 ml fresh garden compost in petri dishes (∅ = 60 mm) and incubated for 12 h under atmospheric air. After spreading out the compost samples on a glass plate (∅ = 200 mm), 20 worms were isolated by hand under a binocular, and subjected to the FISH protocol. As worms were lost during the washing steps of the protocol, 28-55 worms from 3-18 different compost samples could be used for each phylum- or class-specific analysis (specific FISH protocol). The number of worms showing the FISH signal is given as a percentage of the total number of worms remaining (28-55) after carrying out a specific FISH protocol.

Preliminary and control experiments

The chosen bacterial quantity (1.12×10^8 cells) was based on preliminary experiments to determine the optimal quantity of bacteria under the most unfavorable feeding conditions (dietary-choice behavior under semi-natural conditions). During the preliminary experiments, 50-100 worms each (initially L1 larvae) were kept for four days at five different quantities (between 0.28×10^8 and 4.48×10^8 cells) of GFP-expressing *E. coli* OP50 in S-medium with glass beads under atmospheric air. For each treatment (different bacterial quantity), 10-20 worms, which had grown up to become adults during that period, were collected to assess the number of worms with the fluorescent *E. coli* in their pharynges (see dietary-choice experiments). The highest percentage (about 75%) was detected at a bacterial quantity of 1.12×10^8 cells, which then was also used as standard for other experiments (reproduction experiments).

To evaluate potential changes in bacterial density during incubation, the different bacterial species were kept under identical conditions (three days in S-medium with glass beads under atmospheric air) as described for the reproduction test but without the *C. elegans* L4 larva. Bacterial density during the three days of incubation usually declined slightly (-25%), which was at least partly due to bacteria remaining attached to the glass beads.

Statistical analyses

Data are given as means ± standard deviation (SD) with *n* indicating the number of animals. Differences were assessed by a one-way analysis of variance (one-way ANOVA; SigmaStat 3.01, SPSS). In case of statistically significant differences ($p < 0.05$), a 'multiple comparison versus control group' procedure (MCC; Holm-Sidak's method) or an 'all pairwise multiple comparison' procedure (PMC; Student-Newman-Keul's method) was applied ($p < 0.05$) to determine differing values. Graphs were prepared with SigmaPlot 8.0.

RESULTS

Reproductive fitness of *C. elegans* at different air partial pressures

To study effects of air partial pressures on the reproductive fitness of *C. elegans*, the number of developed offspring per worm was determined on NGM agar plates at different oxygen and carbon dioxide partial pressures. Reproductive fitness was maximal (one-way ANOVA, MCC: $p < 0.05$, df = 30) at severe hypoxia (3.6 kPa; Fig. 1A). Towards anoxia, the reproductive fitness decreased. Carbon dioxide partial pressures between 0.03 and 4.91 kPa did not affect the reproductive fitness of *C. elegans* (one-way ANOVA, PMC; Fig. 1B).

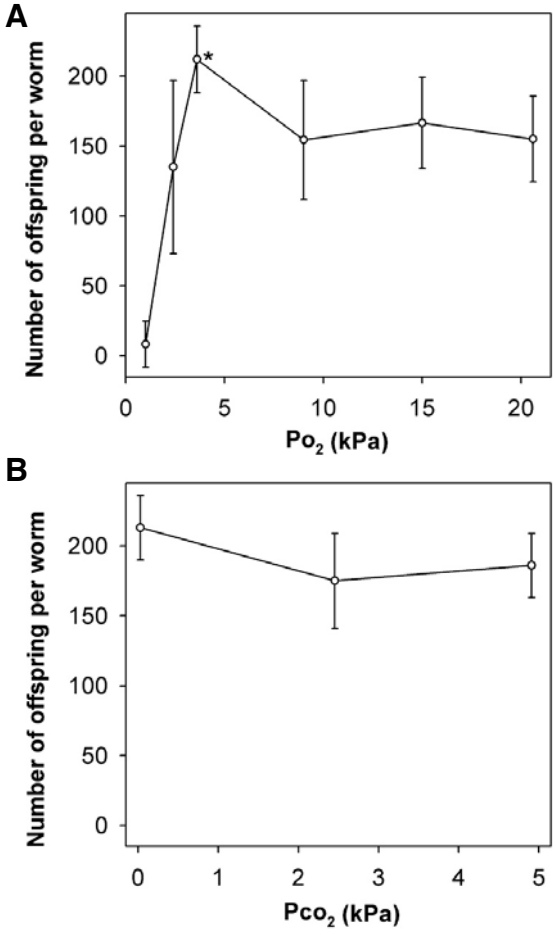

Fig. 1. Reproductive fitness of *C. elegans* at different (A) oxygen (P_{O2}) and (B) carbon dioxide (P_{CO2}) partial pressures (means ± SD, *n* = 3-7 experiments). The number of developed offspring per worm was maximal (asterisk; one-way ANOVA) during hypoxia (P_{O2} = 3.6 kPa).

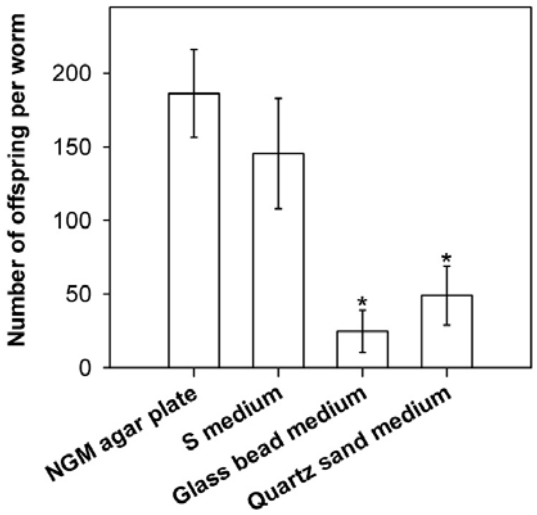

Fig. 2. Reproductive fitness of *C. elegans* in different media (means ± SD, *n* = 3-6 experiments). The number of developed offspring per worm decreased significantly (asterisks; one-way ANOVA) in media containing solids (glass beads, quartz sand).

Reproductive fitness of *C. elegans* in non-structured and structured media

Under atmospheric air, the reproductive fitness of *C. elegans* was tested with standard laboratory media (NGM agar plates, S-medium), and to simulate the natural mineral texture and structure of soil in S-medium containing solids (glass beads or quartz sand). The number of developed offspring per worm was maximal on NGM agar plates and in S-medium and reduced in S-medium containing solids (Fig. 2; one-way ANOVA, PMC: $p < 0.05$, df = 17). Worms incubated in S-medium containing quartz sand showed morphological abnormalities in the pharynx region (not shown). Accordingly, S-medium containing glass beads was used in subsequent experiments.

Food bacteria and reproductive fitness and dietary choice of *C. elegans*

Since *E. coli* (OP50) is unlikely to be the main food of *C. elegans* in the field, we analyzed the reproductive fitness and dietary choice of *C. elegans* with different test bacteria (species from different phyla or classes) in S-medium containing glass beads under atmospheric air. The number of developed offspring was higher both with *B. tropica* (β-Proteobacteria) and *C. arvensicola* (Bacteroidetes) than with *E. coli* OP50 and *S. liquefaciens* (γ-Proteobacteria) as food source (one-way ANOVA, PMC: $p < 0.05$, df = 33; Fig. 3A). By offering differently fluorescing bacteria (GFP-expressing *E. coli* OP50 and test bacteria stained with Hoechst dye) in equal amounts as food source, the dietary choice of *C. elegans* was evaluated by counting the relative number of worms showing none, one or both bacterial species within their pharynges. As *C. elegans* preferred the GFP-expressing *E. coli* strain over Hoechst-stained *E. coli* (Fig. 3B), Hoechst staining probably reduced the food attractiveness of bacteria for *C. elegans*. In addition, Hoechst-stained bacteria alone were found only in a few worms during any experiment. Depending on bacterial species, however, significant differences were found between the relative number of worms showing either both test bacterium and GFP-expressing *E. coli* or GFP-expressing *E. coli* alone in their pharynges. As food ingested together with GFP-expressing *E. coli*, *B. subtilis* (Firmicutes), *B. tropica* (β-Proteobacteria), *C. arvensicola* (Bacteroidetes), and *P. fluorescens* (γ-Proteobacteria) were favored over GFP-expressing *E. coli* alone (one-way ANOVA, PMC: $p < 0.05$; df = 47; Fig. 3B). Hoechst-stained *C. arvensicola* was also the bacterial species, which was most often found exclusively in the worms' pharynges (6.3%).

Fluorescence *in situ* hybridization

To identify bacteria ingested by *C. elegans* in natural compost, a FISH protocol was established based on different kingdom-, phylum- or class-specific DNA probes. Free bacteria as well as bacteria within the pharynges of *C. elegans* could be identified (Fig. 4). After 12 h in garden compost, FISH analyses on subsequently isolated worms revealed food preference (as percentage of worms showing a specific FISH signal in their pharynges) to decrease along the order *Cytophaga-Flexibacter-Bacteroides* (78%), Firmicutes (59%), β-Proteobacteria (53%), and γ-Proteobacteria (29%).

DISCUSSION

Effects of air partial pressures on the reproductive fitness of *C. elegans*

The fitness of *C. elegans* in terms of growth and reproduction

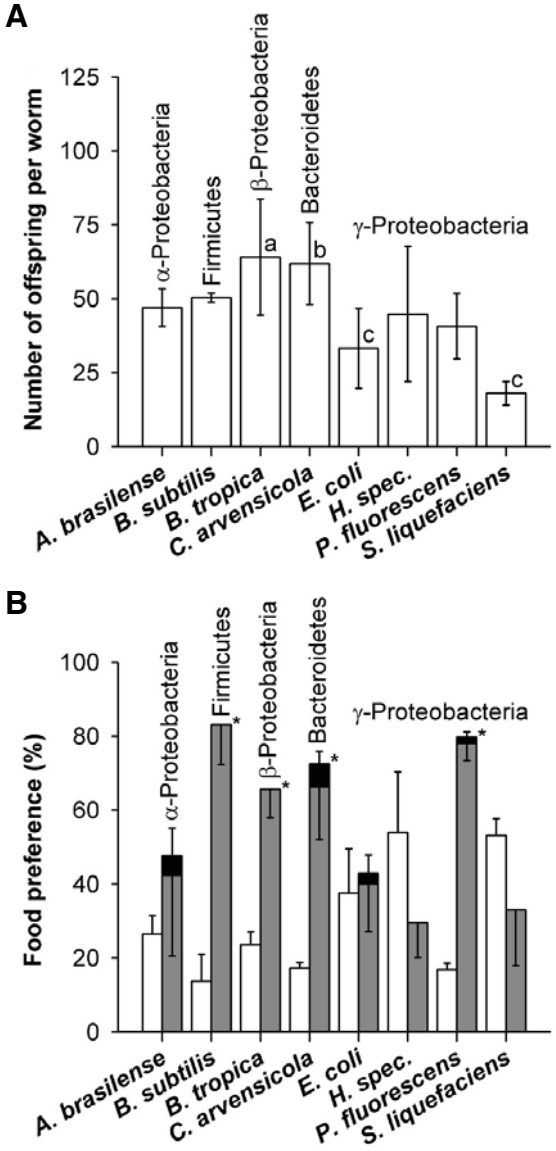

Fig. 3. The effects of different species of bacteria on (A) reproductive fitness (means ± SD, *n* = 3-6 experiments) and (B) food preference (means ± SD, *n* = 3 experiments) of *C. elegans* in S-medium with glass beads. The number of developed offspring per worm was higher with *B. tropica* and *C. arvensicola* than with *E. coli* or *S. liquefaciens* as food (a and b vs. c; one-way ANOVA). Depending on bacterial species, the relative number of worms showing this test bacterium within their pharynges varied (food preference; white bars: only GFP-expressing *E. coli* found, gray bars: test bacterium and GFP-expressing *E. coli* found, black bars: only test bacterium found). In case of *B. subtilis*, *B. tropica*, *C. arvensicola*, and *P. fluorescens*, the number of worms with these test bacteria within their pharynges was significantly higher than those with GFP-expressing *E. coli* alone (asterisks; one-way ANOVA). Phyla or classes of tested bacteria are shown on top.

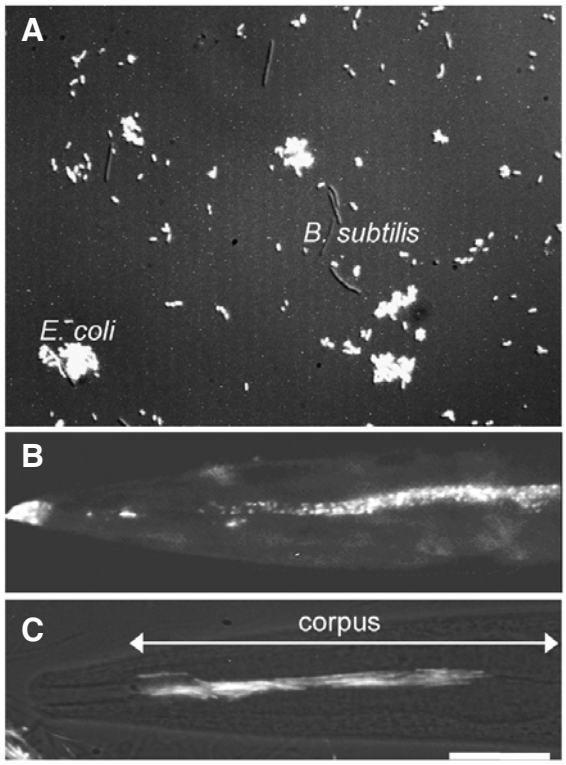

Fig. 4. Fluorescence *in situ* hybridization (FISH) of bacteria. (A) Free bacteria of specifically stained *E. coli* (white) and unstained *B. subtilis* (EcoAlexa; Fuchs et al., 1998) as well as (B) *E. coli* or (C) *B. subtilis* within the pharynx of *C. elegans* stained with a combination of probes (EUB338 I-III) detecting nearly all eubacteria (bar: 25 μm).

depends on the balance between oxygen and food availability and demand. In the natural habitat of *C. elegans* (i.e. compost), oxygen partial pressures (Po_2) and Po_2 gradients are much lower than the Po_2 values under standard laboratory conditions (normoxia; Po_2: about 20 kPa). Consequently, laboratory cultures of *C. elegans* have to be considered as growing under quasi-hyperoxic conditions (Burggren and Warburton, 2005), which may affect biological processes in the worm. On the other hand, no major adverse effects of real hyperoxia (Po_2: 90 kPa) have been found in *C. elegans*, which survived for at least 50 generations under this condition (van Voorhies and Ward, 2000). Concerning reproductive fitness, however, the number of developed offspring was maximal during severe hypoxia (Po_2: 3.6 kPa; Fig. 1A) and not during normoxia, which proves that laboratory oxygen conditions are suboptimal for *C. elegans*. Due to its small body size, the diffusive uptake of oxygen at low Po_2 is not a problem for *C. elegans*. Van Voorhies and Ward (2000) reported the maintenance of normal metabolic rate down to a Po_2 of 3.6 kPa and a near-normal metabolic rate at a Po_2 of 2 kPa. In addition, well-developed anaerobic capacities enable *C. elegans* to survive longer episodes of anoxia (50% mortality after 50 h at anoxia and 10°C, and after 30 h at anoxia and 20°C; Föll et al., 1999). The maximum of reproductive fitness at severe hypoxia indicates optimal fitness of the worm at low oxygen conditions. Consequently, processes related to growth and reproduction such as TOR signaling (Wullschleger et al., 2006), which regulates cell growth (protein synthesis) in response to nutrient and oxygen levels, may behave differently in *C. elegans* under laboratory standard (i.e. normoxia) or field conditions (i.e. hypoxia). In this context, it is interesting that Van Voorhies et al. (2005) reported that *C. elegans* mutants (*Δdaf-2*), which live twice as long as wild-type worms under laboratory conditions, typically die sooner than wild-type worms in natural soil. These authors also showed that the longevity of *C. ele-*

gans wild-type is reduced up to 10-fold under more natural conditions compared with standard laboratory conditions. In addition to reproductive fitness, the behavior of *C. elegans* is adapted to low oxygen conditions. Gray et al. (2004) reported preference of *C. elegans* for low oxygen conditions (5-12% oxygen, which corresponds to a Po_2 between 5 and 12 kPa), and avoidance of higher and lower oxygen levels.

As soil organism being exposed to a broad range of carbon dioxide levels, *C. elegans* maintained reproductive fitness between normocapnia (Pco_2: 0.03 kPa) and severe hypercapnia (Pco_2: 4.91 kPa). However, negative effects, such as aberrant motility and reduced fertility, have been reported at even higher CO_2 levels (9-19% CO_2; Sharabi et al., 2009). In contrast to the maintenance of reproductive fitness at high Pco_2, the behavioral avoidance of CO_2 starts at a much lower level (about 1% CO_2; Bretscher et al., 2008; Hallem and Sternberg, 2008). Information on acid-base control in *C. elegans* to explain how the worm copes with the decrease in pH at increasing Pco_2 (respiratory acidosis) is yet missing.

Effects of different media on the reproductive fitness of *C. elegans*

The natural habitat of *C. elegans* is a complex and structured environment. One process in *C. elegans*, which is known to be influenced by environmental structure, is the mode of locomotion. On the smooth surface of agar plates, worms crawl on their sides and move by propagating sinusoidal waves along their body (Gray and Lissmann, 1964; Park et al., 2008). The surface tension of the thin water layer on the agar plate presses the worm to the surface, forming a shallow channel that restricts lateral movements. The worm moves with very little slip at a speed of up to 0.5 mm s^{-1}. In liquids, *C. elegans* shows fast but less effective swimming motions (speed about 0.1 mm s^{-1}). Applying microfluidic structures (uniformly spaced agar posts), Park et al. (2008) reported fast and effective locomotion of *C. elegans* at a speed of up to 1.3 mm s^{-1} (post spacing 0.425-0.475 mm). Testing reproductive fitness in different media (agar plate, liquid, and liquid with solids) revealed a strongly reduced number of developed offspring in structured environments (glass beads or quartz sand; Fig. 2). Reasons for this reduction in reproductive fitness may include differences in the allocation of energy towards locomotion or growth and reproduction or in the effort to take food in the different media. As mechanosensory mutants move differently to wild-type worms in a microstructured environment (Park et al., 2008), it would be interesting to test if the effects of structure on reproductive fitness also involve mechanosensation.

Effects of food quality on the reproductive fitness and dietary choice of *C. elegans*

In laboratory standard culture, *C. elegans* is fed with the *E. coli* strain OP50 (γ-Proteobacteria), which is highly artificial as *E. coli* is not a soil bacterium in the strict sense. Reproductive fitness was indeed low with *E. coli* (Fig. 3A) but clearly higher with *B. tropica* (β-Proteobacteria) and *C. arvensicola* (Bacteroidetes) as food. Dietary-choice experiments under semi-natural conditions revealed a low preference for *E. coli* (Fig. 3B) but markedly higher preferences for *B. tropica* (β-Proteobacteria), *C. arvensicola* (Bacteroidetes), *B. subtilis* (Firmicutes), and *P. fluorescens* (γ-Proteobacteria). Experiments on dietary-choice behavior under quasi-natural conditions also showed a lower preference for Hγ-Proteobacteria than for *Cytophaga-Flexibacter-Bacteroides*, Firmicutes, and β-Proteobacteria. In addition, Abada et al. (2009) reported a higher preference of *C. elegans* for various soil bacteria including *Bacillus mycoides* and *Bacillus soli* than for *E. coli* in binary choice assays. *B. tropica* and *C. arvensicola* are members of two of the most abundant bacterial groups in soil and therefore represent typical soil bacteria (Fierer et al., 2007). *B. tropica* is a nitrogen-fixing bacterium often associated with plants (Reis et al., 2004). *C. arvensicola* is an aerobic-to-microaerobic organism growing on different carbohydrates (Nakagawa and Yamasato, 1993; Pankratov et al., 2006); it was first isolated from soil (Oyaizu et al., 1982), and later reassigned to the genus *Chitinophaga* (Kämpfer et al., 2006). As *C. elegans* did not grow and reproduce better with *B. subtilis* and *P. fluorescens* as bacterial diet (Fig. 3A), the worm's higher preference for them (Fig. 3B) was probably due to the restriction on only two species (test bacterium and *E. coli*) during the behavioral experiments.

Studying food transport in the *C. elegans* pharynx, Avery and Shtonda (2003) suggested smaller bacteria to be a better food source for *C. elegans* than larger ones. However, *C. elegans* preferred *C. arvensicola* in competition with *E. coli* (Fig. 3B), although *C. arvensicola* has a similar or larger size (age-dependent variation; Pankratov et al., 2006) than *E. coli*. As another example, *S. liquefaciens* has roughly the same size as *E. coli* (Ashelford et al., 2002) but *C. elegans* preferred *E. coli* in comparison with *S. liquefaciens* (Fig. 3B). Consequently, bacterial size cannot be the only decisive factor for the dietary choice of *C. elegans*. Bacterial shape, arrangement, motility or cell wall structure also do not determine the worm's preference behavior (Abada et al., 2009). Since *C. elegans* is able to distinguish between poor and good bacterial food with the latter supporting higher growth rates, and since the dietary choice of *C. elegans* depends on its previous food experience, Shtonda and Avery (2006) suggested chemosensation to be involved in the dietary-choice behavior of *C. elegans*. Based on experiments with chemosensory mutants, Abada et al. (2009) also suggested chemosensation to be a central element of the dietary-choice behavior of *C. elegans*. Chemosensation may also explain the reduced attractiveness of bacteria for *C. elegans* after Hoechst staining (Fig. 3B). However, additional studies are required to causally link bacterial odor, chemoreception, and dietary-choice behavior of the worm.

CONCLUSION

This study shows that the reproductive fitness of *C. elegans* strongly depends on oxygen partial pressure, habitat structure, and bacterial diet. Fitness is higher in an oxygen-poor atmosphere (hypoxia) and lower in structured media. The standard food organism *E. coli* neither supports a high reproductive fitness nor is it preferred by the worm. Our data rather suggest that a suitable diet for *C. elegans* should include major shares of *Cytophaga-Flexibacter-Bacteroides* and β-Proteobacteria but only minor amounts of γ-Proteobacteria. The results of this study also show that data on *C. elegans* under standard laboratory conditions have to be carefully interpreted with respect to their biological significance.

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