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The Filter-Feeding Ciliates *Colpidium striatum* and *Tetrahymena pyriformis* Display Selective Feeding Behaviours in the Presence of Mixed, Equally-Sized, Bacterial Prey

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This study examined whether two ciliates could discriminate between equally-sized bacterial prey in mixture and if so, how selectivity might benefit the ciliate population. Live *Klebsiella aerogenes*, *K. ozaenae* and *Escherichia coli*, expressing different coloured fluorescent proteins, were cultured in such a way as to provide populations containing equally-sized cells (to prevent size-selective grazing taking place) and these prey were fed to each ciliate in 50:50 mixtures. *Colpidium striatum* selected *K. aerogenes* over *K. ozaenae* which itself was selected over *E. coli*. *Tetrahymena pyriformis* showed no selectivity between *K. aerogenes* and *E. coli* but *K. aerogenes* was selected over *K. ozaenae* while *E. coli* was not. This apparent selection of *K. aerogenes* over *K. ozaenae* was sustained in ciliate populations with different feeding histories and when *K. aerogenes* comprised only 20% of the prey mixture, suggesting possible optimal foraging behaviour. The metabolic benefits for selecting *K. aerogenes* were identified as possibly being an increase in cell biovolume and yield for *C. striatum* and *T. pyriformis*, respectively. The mechanism by which these ciliates selected specific bacterial cells in mixture is currently unknown but the use of live fluorescent bacteria, in prey mixtures, offers an exciting avenue for further investigation of selective feeding by protozoa.

Key words: protozoa; clearance-rate; ingestion-rate; selective-index; differential-digestion.

Introduction

Flagellated protozoa are regarded as the major consumers of bacteria in aquatic environments but ciliates can also play an important role as bacteriovores (Sherr and Sherr 1987). Considerable effort has been devoted to studying the feeding behaviour of these protozoa yet the

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question of whether they can feed selectively on bacteria is still unresolved. Experiments which have addressed selective feeding in protozoa have used prey species which are easily distinguishable in the mixture, normally eukaryotic as opposed to prokaryotic prey (Lessard and Swift 1985; Mast and Hahnert 1935; Schaeffer 1910). Even so, in all cases the protozoan has demonstrated a clear preference for a particular prey over another, even if the preferred prey was less abundant in the mixture (Mast and Hahnert 1935). Thus there are tantalising pieces of evidence to suggest that protozoa are able to practice selective-feeding, leading researchers to seek the mechanisms behind this behaviour.

There is now a growing body of evidence to show that prey size plays an important role in prey selectivity (González 1996; Jonnson 1986; Šimek and Chrzanowski 1992), but this might not be 'selection' per se; rather a physical constraint on the protozoan for particular prey sizes. However, characteristics other than prey or predator size do appear to play a role in selectivity, such as the presence of feeding receptors on the protozoan (Wootton et al. 2007) and the motility of the prey (Matz and Jürgens 2005), its hydrophobicity (Matz and Jürgens 2001; Monger et al. 1999) and nutritional quality (C:N:P ratio) (Shannon et al. 2007). But, in the latter experiments the cell dimensions of different test prey were not standardised, so more than one variable was essentially being tested.

The evidence for selective feeding in protozoa has recently been reviewed (Montagnes et al. 2008) and what has emerged is that there has not been one publication which has definitively shown that protozoa can selectively feed on bacteria for reasons other than size. Here, we present an experiment where two ciliate species were presented with a mixture of two live, equally-sized, non-motile prey. Each was distinguished from each other in mixture due to the expression of different coloured fluorescent proteins; a technique previously used in ciliate grazing experiments involving one prey species (e.g. Gunderson and Goss 1997; Parry et al. 2001); not with a mixture of prey. The aim of the study was to test the hypotheses that, (i) ciliates can feed selectively on a preferred bacterial species, (ii) that selectivity is maintained when the preferred species is at low concentration in the prey mixture, (iii) that selectivity is not influenced by the feeding history of the ciliate and, (iv) that the preferred prey provides a physiological benefit to the ciliate population.

Results

Bacterial Prey Characteristics

Klebsiella ozaenae and *K. aerogenes* were transformed to express DSred (rfp) and yellow (yfp) fluorescent protein, respectively. These species, along with *Escherichia coli* JM105 expressing either DSred or yellow fluorescent protein (hereafter referred to as *E. coli*^{rfp} or *E. coli*^{yfp}) were grown for 10 d, then washed and stored in Chalkley's medium at 20 °C for 4 d prior to all experiments. This long period of growth and storage led to each bacterial population comprising cells with equivalent dimensions (Fig. 1). The cell sizes within the bacterial populations were stable throughout the growth experiments, which lasted 5 days, thus variation regarding prey cell size between the bacterial species, which were to be used as prey for the ciliate predators, was removed as a variable in grazing experiments.

An experiment was carried out to ensure that the expression of different coloured fluorescent proteins did not affect ciliate ingestion rates. Here, *E. coli* JM105 cells expressing red or yellow fluorescent protein (*E. coli*^{rfp} and *E. coli*^{yfp}, respectively) were mixed in a 50:50 ratio and fed to the ciliates *Tetrahymena pyriformis* and *Colpidium striatum*. No significant difference in instantaneous ingestion rate or clearance rate of the different coloured *E. coli* cells was recorded (Table 1) and the selective index showed no preference. Thus, potential variation in cells expressing different coloured proteins was removed as a variable in experiments and either red or yellow *E. coli* JM105 could be used in subsequent prey mixtures with yellow

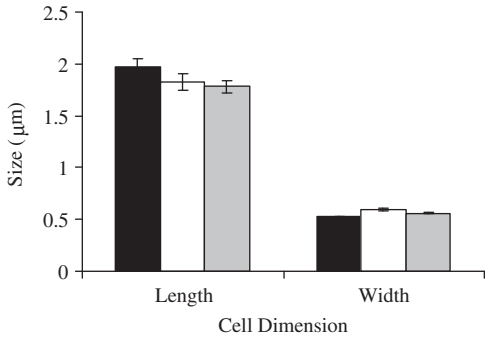

Figure 1. Average cell dimensions (μm) of three fluorescent prey species; *Escherichia coli*^{rfp}/^{yfp} Black; *Klebsiella ozaenae*^{rfp}, White and *K. aerogenes*^{yfp}, Grey. Error bars represent Standard Error of the Mean.