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Photoperiod modulates gut bacteria composition in male Siberian hamsters (*Phodopus sungorus*)

Michael T. Bailey [a,b,*,1], James C. Walton [c,1], Scot E. Dowd [d], Zachary M. Weil [c,2], Randy J. Nelson [b,c]

[a] *Section of Oral Biology, College of Dentistry, The Ohio State University, Columbus, OH 43210, USA*
[b] *Institute for Behavioral Medicine Research, College of Medicine, The Ohio State University, Columbus, OH 43210, USA*
[c] *Department of Neuroscience, The Ohio State University, Columbus, OH 43210, USA*
[d] *Research and Testing Laboratory and Medical Biofilm Research Institute, Lubbock, TX 79407, USA*

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ABSTRACT

Seasonal changes in day length (i.e., photoperiod) provide animals with a reliable environmental cue to determine time of year, and many physiological changes occur in laboratory animals simply by extending or shortening day length. Male Siberian hamsters (*Phodopus sungorus*) housed in long summer-like day lengths have significantly elevated body and fat masses compared to short-day hamsters. Because others have demonstrated that the intestinal microbiota of humans and rodents promotes host adiposity, we hypothesized that photoperiod-induced changes in body and fat masses could be associated with changes in the microbial composition in the intestines. We used bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) to assess microbial diversity in the cecal contents of hamsters; long days significantly increased the relative abundance of bacteria in the phylum Proteobacteria. This effect was primarily due to a significant increase in the abundance of the genus *Citrobacter*, with both the abundance of Proteobacteria and *Citrobacter* spp. significantly correlated with body mass and with inguinal fat mass. In general, the abundance of the Firmicutes phylum was inversely associated with body mass. These data indicate that the intestinal microbiota are responsive to changes in photoperiod and suggest that these changes may in part influence photoperiodic changes in body and fat masses.

1. Introduction

Individuals of nontropical animal species are exposed to seasonal changes in food availability and energy expenditures. Reduced food availability often coincides with energetic requirements for thermogenesis and therefore winter is a particularly difficult time for small animals to breed and survive. To cope with this energetic challenge, several seasonal adaptations have evolved including adjustments in reproduction, immune function, and metabolism (Atcha et al., 2000; Bartness, 2002; Nelson, 2004; Wade and Bartness, 1984). The timing of the onset of many seasonal adaptations is provoked by the annual change in daily photoperiod (day length) (Goldman, 2001). In mammals, day length information is transduced from an environmental factor into a physiological signal by the nightly duration of elevated melatonin secretion (Lincoln et al., 2006; Lincoln, 2006). Melatonin is secreted in proportion to night length; thus, relatively long duration of melatonin signals short day lengths. Short days inhibit reproduction, bolster certain features of immune function, increase gut efficiency, reduce metabolic rate, and alter body mass (Bartness and Wade, 1984; Dark and Zucker, 1984; Drazen et al., 2001; Mercer et al., 2000).

It is common for many species to gain body fat prior to the winter and then slowly utilize those stored fuels while environmental conditions are harsh, however, other species such as the Siberian hamsters (*Phodopus sungorus*), utilize a different strategy. These animals gain body mass to support the energetically costly processes associated with reproduction in long days and then reduce body mass and particularly body fat prior to winter and run their metabolic systems at a much lower rate (Dark and Zucker, 1984; Heldmaier et al., 1981; Wade and Bartness, 1984). Several central and peripheral mechanisms coordinate this autumnal transition into a lean phenotype including alterations in neuropeptide systems such as leptin and agouti-related peptide that regulate feeding and alterations in autonomic regulation of adipocyte lipolysis (Bowers et al., 2005; Demas and Bartness, 2001; Mercer et al., 2000; Weil et al., 2009). Additionally, some studies have reported short day-induced reductions in food intake in Siberian hamsters, whereas others have reported that the weight loss is independent

* Corresponding author. Address: Section of Oral Biology, College of Dentistry, The Ohio State University, 305W. 12th AVE., Columbus, OH 43210, USA.
E-mail address: bailey.494@osu.edu (M.T. Bailey).
[1] Both authors contributed equally to this work.
[2] Present address: Laboratory of Neurobiology and Behavior and Laboratory of Neuroendocrinology, Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.

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of food intake (Jethwa et al., 2009; McElroy et al., 1986) or the weight loss precedes changes in food intake (Wade and Bartness, 1984).

Siberian hamsters, in common with all vertebrates exist in commensal harmony with many microbial organisms in residence in their guts. The presence of these microbes benefits the host species, at least in part, because these organisms express glycoside hydrolases and polysaccharide lysases, that are not expressed in mammals, and thus allow the energetic utilization of otherwise indigestible polysaccharides (Sonnenburg et al., 2005; Xu et al., 2003). Therefore the composition and activity of gut microbial populations can alter the energetic yield of a diet. Indeed, mounting evidence suggests that the relative abundance of different microbial genera is associated with obese phenotypes in humans and animal models and the existence of extensive coevolution between gut microbes and their hosts (Ley et al., 2006). Germ-free mice are resistant to diet induced obesity and when inoculated with a normal gut microbiome increase body mass rapidly without increasing food intake (Backhed et al., 2004; Backhed et al., 2007). Leptin deficient mice (OB/OB) also exhibit a shift in the ratio of Fermicutes to Bacteroidetes as assessed by shotgun sequencing and transplantation of the gut microbiome from OB/OB mice to lean wildtype conspecifics leads to the development of an obese phenotype (Ley et al., 2005; Turnbaugh et al., 2006). In humans, obesity is characterized by a reduction in bacterial diversity, altered representation of bacterial metabolism related genes, and the same shift in Fermicutes to Bacteroidetes ratio (Ley et al., 2008).

Siberian hamsters undergo seasonal changes in body mass. Because gut microbiota affect body mass, we hypothesized that photoperiod might affect body mass by adjusting the composition of the gut bacterial contents. If true, we predicted that the community structure of the intestinal microbiota would be significantly different as a function of photoperiod, and that microbiota abundance would be associated with body and fat mass. We used bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) to characterize the microbiota in the cecal contents of hamsters to test the hypothesis that photoperiod changes the composition of the intestinal microbiota.

2. Methods and materials

2.1. Animals

Siberian hamsters (*Phodopus sungorus*) were bred in our colony maintained at the Ohio State University. Twenty male hamsters were weaned at 21 days of age and singe housed in polycarbonate cages (28 × 17 × 12 cm) on free-standing racks until they reached sexual maturity (>60 days). Six weeks post-weaning, animals were randomly assigned to either long-day (n = 10, LD; 16L:8D) or short-day (n = 10, SD; 8L:16D) conditions (light intensity ~600 lux) (see Table 1 for experimental timeline). All animals were maintained at constant temperature (21 ± 4 °C), relative humidity (50 ± 5%), and given *ad libitum* access to filtered tap water and food (Harlan Teklad 8640, Indianapolis, IN, USA), and were cared for by The Ohio State University Laboratory Animal Resource staff in accordance with USDA animal welfare regulations for the duration of the experiment. All procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and are in compliance with guidelines established by the National Institutes of Health published in *Guide for the Care and Use of Laboratory Animals* (1996).

2.2. Food intake

After 8 weeks in photoperiod, each animal was weighed and total available food in the cage was weighed. Food was weighed daily 3 h prior to the dark phase for 7 days.

2.3. Sample collection

After 9 weeks in a long or short photoperiod, 3 h prior to the onset of the dark phase, hamsters were deeply anesthetized with isoflurane vapors and killed by rapid decapitation. Reproductive tissues, abdominal fat stores, spleen, and adrenals were collected and weighed. Using sterile technique, the cecum was removed and the contents were flash frozen in sterile microfuge tubes on dry ice and held at −80 °C for microbial diversity sequencing.

2.4. Microbial diversity assessment with bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP)

2.4.1. DNA extraction

Total genomic DNA was extracted from the cecal samples as described previously (Dowd et al., 2008) using a Tissuelyser and using a QIAmp stool DNA mini kit per manufacturer directions (Qiagen, Velencia, CA). DNA samples were quantified using a Nanodrop spectrophotometer (Nyxor Biotech, Paris, France).

2.4.2. PCR to create tag encoded amplicons

A 100 ng aliquot of each sample's DNA was used for a 50 μl step 1 PCR reaction. Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) was performed as described previously (Dowd et al., 2008) at the Research and Testing Laboratory (Lubbock, TX). The new bacterial tag-encoded FLX-Titanium amplicon pyrosequencing (bTEFAP) approach is based upon similar principles but utilizes Titanium reagents and Titanium procedures including a one-step PCR, mixture of Hot Start and HotStar high fidelity taq polymerases, and amplicons originating from the 27F region numbered in relation to *E. coli* rRNA.

2.4.3. Bacterial diversity data analysis

Following sequencing, all failed sequence reads, low quality sequence ends and tags were removed and sequences were depleted of any non-bacteria ribosome sequences and chimeras using custom software described previously (Dowd et al., 2008) and the Black Box Chimera Check software B2C2 (described and freely available at http://www.researchandtesting.com/B2C2.html). Sequences less than 350 bp were removed. To determine the identity of bacteria in the remaining sequences, sequences were first queried using a distributed BLASTn.NET algorithm (Dowd et al., 2005) against a database of high quality 16s bacterial sequences derived from NCBI. Database sequences were characterized as high

Table 1
Timeline of experimental procedures.

Weaning	In photoperiod	Food intake	Samples taken
⬇	⬇	⬇	⬇
3 weeks	9 weeks	17 weeks	18 weeks

Experimental manipulation (above) with corresponding age of each animal at time of manipulation (below).

quality based upon the criteria of RDP ver 9 (Cole et al., 2005). Using a .NET and C# analysis pipeline, the resulting BLASTn outputs were compiled, validated using taxonomic distance methods, and data reduction analysis performed as described previously (Acosta-Martinez et al., 2008). Rarefaction, ace, and Chao1 to estimate mathematically predicted diversity and richness in the treatments using 350 bp trimmed, non-ribosomal sequenced depleted, chimera depleted, high quality reads was performed as described previously (Acosta-Martinez et al., 2008).

2.4.4. Bacterial identification

Based upon the above BLASTn derived sequence identity (percent of total length query sequence which aligns with a given database sequence) and validation using taxonomic distance methods, the bacteria were classified at the appropriate taxonomic levels based upon the following criteria. Sequences with identity scores to known or well characterized 16S sequences greater than 96.5% identity (i.e., <3% dissimilarity) were resolved at the species level when possible, between 94.5% and 96.4% at the genus level, between 89.5% and 94.4% at the family and between 80% and 89.4% at the order level. After resolving based upon these parameters, the percentage of each bacterial identification was individually analyzed for each cecal sample providing relative abundance information within and among the samples based upon relative numbers of reads within a given sample. When multiple identifications were found (e.g., *Bacteroides caccae* and *Bacteroides stercoris*) such identifications were resolved arbitrarily to the top hit. Evaluations presented at a given taxonomic level, except species level, represent all sequences resolved to their primary genera identification or their closest relative (where indicated).

2.5. Statistical analyses

Independent-samples *t* tests were used to compare food intake, reproductive tissues, spleen, and adrenal mass between treatment groups. Spleen, paired adrenal mass, and food intake data were corrected for body mass. Clustering methods based upon weighted pairs, Manhattan distances and standard average deviation scaling were performed as described previously (Dowd et al., 2008). Independent-samples *t* tests were also used to compare the relative abundance of specific phyla, families, and genera of bacteria between the long day and short-day hamsters. All mean differences were considered statistically significant at $p < 0.05$.

3. Results

In response to short days, hamsters reduced body mass ($t_{(18)} = 4.71$, $p < 0.001$; Fig. 1A) and reduced inguinal fat pad mass ($t_{(18)} = 8.67$, $p < 0.001$; Fig. 1B). Additionally, hamsters maintained in short days showed regression in mass of the reproductive tissues; paired testes ($t_{(18)} = 15.84$, $p < 0.001$; Fig. 1C), seminal vesicles ($t_{(18)} = 6.55$, $p < 0.001$; Fig. 1D), and epididymides ($t_{(18)} = 7.96$, $p < 0.001$; Fig. 1D). Exposure to short days had no effect on corrected paired adrenal mass ($t_{(18)} = 0.07$, $p = 0.987$; Fig. 1E) or corrected spleen mass ($t_{(18)} = -0.49$, $p = 0.627$; Fig. 1F). Short days had no effect on food intake ($t_{(18)} = 0.88$, $p = 0.39$; not shown) or food intake corrected for body mass ($t_{(18)} = -1.57$, $p = 0.14$; Fig. 2).

The microbiota in the cecal contents of hamsters was comprised of bacteria from 7 phyla, with the highest proportion of bacteria belonging in the Firmicutes phylum, followed by the Bacteroidetes, and Proteobacteria (Fig. 3A). Less than 1% of the bacteria belonged in the phyla Actinobacteria, Tenericutes, Verrucomicrobia, and Spirochaetes (Fig. 3A). We did not detect any bacteria in the phylum Deferribacteres or Fusobacterium. Exposure to short day lengths caused a significant reduction in the relative abundance of bacteria in the phylum Proteobacteria compared to the abundance found in the hamsters exposed to long day lengths ($t_{(18)} = 3.13$, $p < 0.01$; Fig. 3B). This effect of photoperiod did not appear to be due to a significant extinction or bloom of specific species of bacteria because evaluation of diversity and richness estimates based upon rarefaction, chao1, and ace methods, as well as the use of curve fitting equations to predict maximum potential operational taxonomic units (Acosta-Martinez et al., 2008) did not find any significant differences between samples from long- and short-day hamsters (Table 2). Although the change in the Proteobacteria was the only difference that was evident at the phylum level, Fig. 3D provides a summary of 6 genera that differed between the long- and short-day hamsters. The clustering analysis indicated that overall, microbial populations within 8 of the 10 short-day hamsters were more similar to each other than they were to microbial populations found in the long-day hamsters. The primary population leading to this clustering was *Citrobacter* sp., which was found to be significantly lower in the short-day hamsters compared to the long-day hamsters (Fig. 3C).

The relative abundance of bacteria in the Proteobacteria phylum were positively correlated with hamster body mass ($r_{(18)} = 0.55$, $p < .05$; Fig. 4A);. This association was most likely due to the positive association between Proteobacteria and inguinal fat mass ($r_{(18)} = 0.509$, $p < .05$; Fig. 4B). The effects at the phylum level could be traced down to the genus level where it was evident that the relative abundance of *Citrobacter* was also associated with body mass ($r_{(18)} = 0.468$, $p < .05$; Fig. 4C), but was only moderately associated with inguinal fat mass ($r_{(18)} = 0.415$, $p = .08$, Fig. 4D).

Although photoperiod did not significantly change the relative abundance of bacteria in the phylum Firmicutes ($t_{(18)} = 1.42$, $p = .17$; see Fig. 3A), the relative abundance of Firmicutes was inversely associated with body mass ($r_{(18)} = -0.564$, $p < .01$, Fig. 4E) and with inguinal fat mass ($r_{(18)} = -0.489$, $p < .05$; Fig. 4F). In this case, animals that had higher levels of Firmicutes tended to have a lower body mass. There was a trend for the relative abundance of bacteria within the genus *Lachnobacterium*, which belongs to the Firmicutes phylum, to be increased in the hamsters exposed to short day length ($t_{(18)} = 1.78$, $p = .09$; data not shown). The relative abundance of *Lachnobacterium* sp. was low (0.12 ± 0.03% for LD hamsters and 0.20 ± 0.03% for SD hamsters), but the inverse association between the Firmicutes phylum and body mass could be traced to this genus of bacteria. The relative abundance of *Lachnobacterium* sp. was inversely associated with body mass ($r_{(18)} = -0.583$, $p < .01$; Fig. 4G) and with inguinal fat mass ($r_{(18)} = -0.521$, $p < .05$; Fig. 4H).

4. Discussion

As previously demonstrated, photoperiod resulted in several phenotypic changes (Navara et al., 2007; Weil et al., 2007). In comparison to hamsters in the long day photoperiod, the body, inguinal fat, epididymides, seminal vesicles, and testes masses were significantly reduced by a short photoperiod. This reduction in mass was organ specific and not widespread across the body because the mass of the spleen and the adrenal glands were not significantly affected by photoperiod. Moreover, the reduction in body and fat masses were not due to a change in diet, because long- and short-day hamsters consumed the same amount of standard laboratory chow. Thus, it is evident that the enhanced masses were due to a difference in the ability of the hamsters to harvest energy from the consumed food.

Several studies have now demonstrated that different community profiles of intestinal microbiota also have differing capacities to harvest energy from food. This appears to be due to an increase

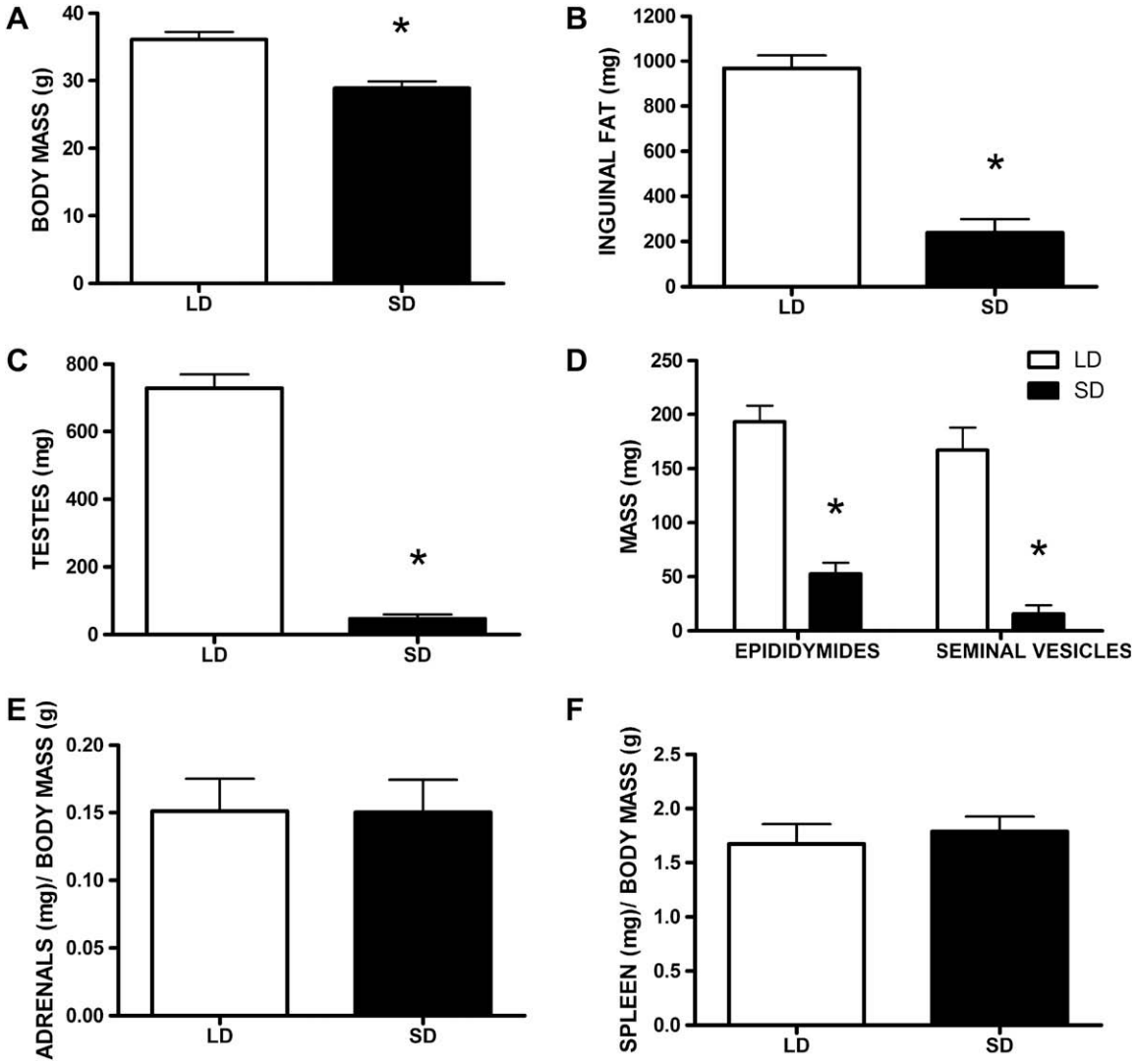

Fig. 1. Photoperiodic responses to short day lengths in Siberian hamsters. Short days reduced overall body mass (A), inguinal fat mass (B), paired testes mass (C), paired epididymal, and paired seminal vesicle mass (D). Short day lengths had no effect on corrected paired adrenal mass (E) or corrected spleen mass (F). All graphs reported as mean ± 1 standard error of the mean (SEM); LD = long-day (n = 10), SD = short-day (n = 10), $^*p < 0.05$.

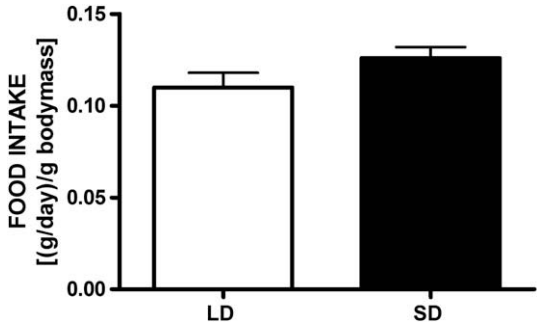

Fig. 2. Photoperiodic changes in food consumption in Siberian hamsters. Short days had no effect on food consumption corrected for body mass after 8 weeks in photoperiod. Mean ± 1 standard error of the mean; LD = long-day (n = 10), SD = short-day (n = 10).

in factors such as glucose absorption and fatty acid production and absorption (Backhed et al., 2004). In our study, photoperiod resulted in significant differences in the composition of the intestinal microbiota. This was primarily manifest as a difference in the relative abundance of bacteria in the Phylum Proteobacteria, with hamsters housed in long day lengths having a higher abundance than hamsters housed in short day lengths. This increase was associated with body mass; hamsters with more Proteobacteria had elevated body and fat masses. Most studies in humans and in rodents report that increased body mass is associated with a significant increase in the relative abundance of bacteria in the phylum Firmicutes, with a concomitant decrease in the relative abundance of Bacteroidetes (Ley et al., 2005, 2006; Turnbaugh et al., 2006; Turnbaugh et al., 2009). However, other studies have reported a significant increase in the relative abundance of the Phylum Proteobacteria. For example, mice fed a high fat diet to induce obesity significantly increased Proteobacteria and Actinobacteria and increased Firmicutes and decreased Bacteroidetes (Hildebrandt et al., 2009). Our finding of an increased abundance of Proteobacteria as a consequence of long day length, and a significant correlation with body and fat masses, suggests that the Proteobacteria are involved with photoperiod-induced increases in body mass.

It was unexpected that photoperiod did not significantly affect the relative abundance of the Firmicutes, because previous reports have linked the relative abundance of the Firmicutes to obesity in humans and in rodents (Ley et al., 2005; Ley et al., 2006; Turnbaugh et al., 2006; Turnbaugh et al., 2009). Changes in Proteobacte-

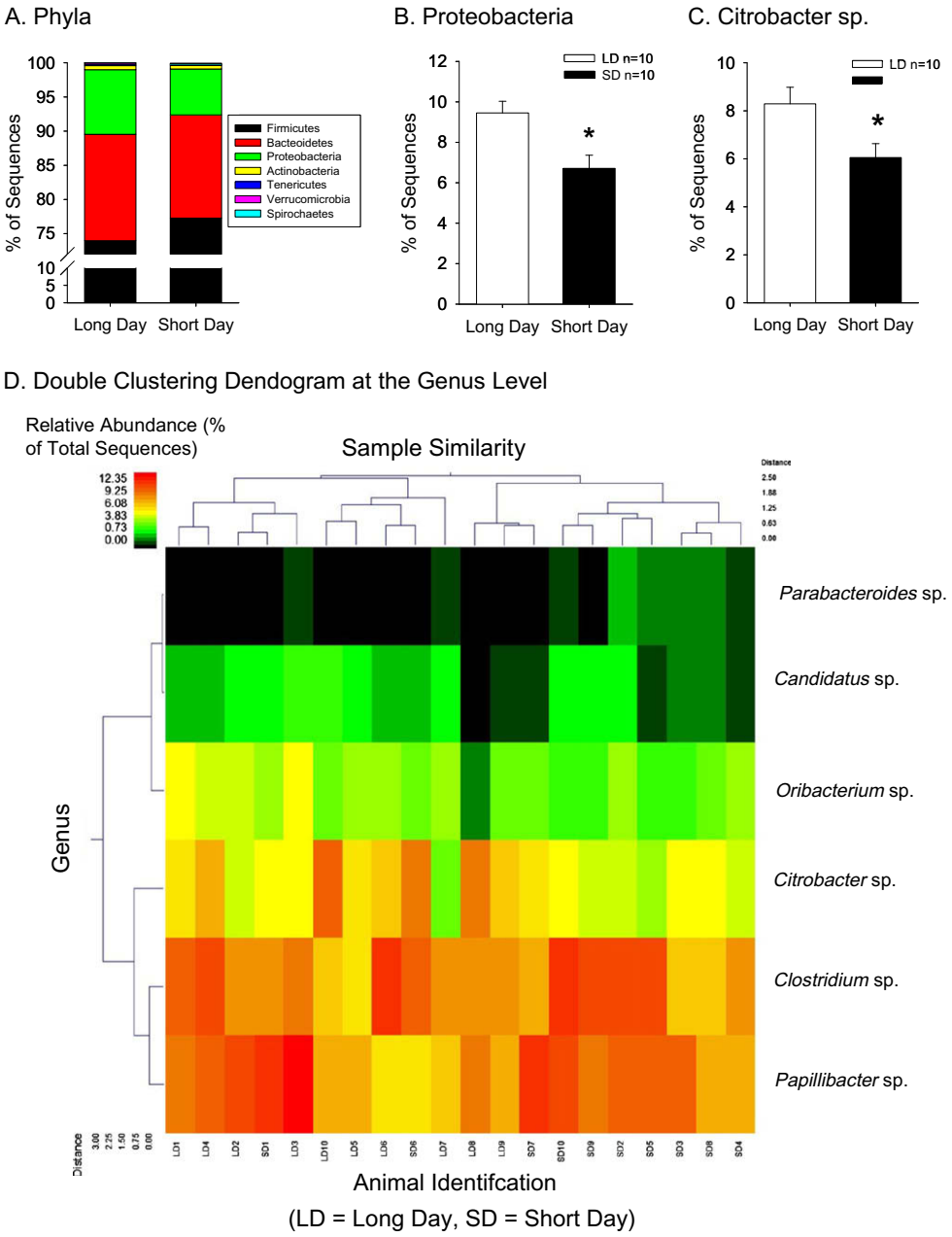

Fig. 3. Photoperiodic changes in the composition of the cecal microbiota. (A) Exposure to short day lengths changed the relative abundance of bacteria in specific phyla presented as a percentage of total sequences derived from the cecal contents of $n = 10$ long-day and $n = 10$ short- day hamsters. (B) Exposure to short day lengths resulted in a significant reduction in the relative abundance of bacteria within the phylum Proteobacteria. Data are depicted as the mean ± SEM of the % of total sequences identified. $^{*}p < .05$. (C) Exposure to short day lengths resulted in a significant reduction in the relative abundance of bacteria with the genus Citrobacter. Data are the mean ± SEM of the % of total sequences identified. $^{*}p < .05$. (D) A double dendogram showing genera of bacteria that differ between long-day and short-day hamsters. The heat map shows the relative abundance of the given genera within each sample with a color legend and scale provided; distance of the individual samples is based upon weighted pair linkage and Manhattan distance methods. The clustering analysis indicated that overall, microbial populations within short-day hamsters were more similar to each other than they were to microbial populations found in long-day hamsters. The bacterial genera and the associated clustering are provided along the *Y*-axis and their associated distance scores indicated. Data are from the cecal contents of $n = 10$ short-day and $n = 10$ long-day hamsters.

ria, however, may be more relevant for seasonal changes in hamsters in natural settings, because during summer months, hamsters prefer to eat seeds that are high in fat and protein content (Fine and Bartness, 1996). This preference for eating high fat seeds may not be simply due to the fact that seeds are more abundant in the summer, because hamsters housed in long summer-like day lengths in the laboratory also show an increased preference for high fat diets in comparison to hamsters housed in short winter-like day lengths (Fine and Bartness, 1996). Blooms of bacteria in the phylum Proteobacteria occur when mice are fed a high fat diet (Hildebrandt et al., 2009), thus an increase in Proteobacteria would be expected to occur during the summer when high fat seeds are available. How photoperiodic changes in Proteobacteria occur in hamsters ingesting comparable amounts of a standardized diet remains unspecified, but may involve changes to gastrointestinal functioning.

Gastrointestinal functioning changes in many mammalian species during different seasons, presumably to maintain energy requirements in the face of seasonal variations in food abundance and quality. In general, many species, including Cavies (*Microcavia*

Table 2
Photoperiod does not impact microbial diversity or richness.

	Rarefaction			OTU		
	5%	3%	1%	5%	3%	1%
Long day	328 ± 8	415 ± 10	587 ± 15	334 ± 8	424 ± 11	601 ± 15
Short day	323 ± 8	412 ± 13	584 ± 23	326 ± 7	417 ± 12	592 ± 21
	ACE			Chao		
	5%	3%	1%	5%	3%	1%
Long day	395 ± 13	543 ± 20	883 ± 31	400 ± 13	551 ± 20	875 ± 26
Short day	397 ± 9	550 ± 18	884 ± 41	399 ± 9	540 ± 17	864 ± 39

Mathematical evaluation of diversity and richness estimates based upon rarefaction, chao, ace, and operational taxonomic units methodology indicated that photoperiod did not affect any estimate of diversity or richness. The data show the calculated richness and diversity from all the samples pooled together, thus it is not possible to calculate variability.

australis) (Sassi et al., 2007), marmots (*Marmota marmota*) (Hume et al., 2002), and prairie voles (*Microtos ochrogaster*) (Gross et al., 1985) have significantly increased masses of their digestive organs during periods of scarce food or low quality food to maximize the intestinal surface area on which digestion and/or fermentation of foods and absorption of nutrients occurs (Green and Millar, 1987; Toloza et al., 1991). But, these changes in gastrointestinal functioning or morphology are also likely to influence intestinal microbial profiles. For example, in hibernating species, such as 13-lined ground squirrels (*Spermophilus tridecemlineatus*), significant changes in gastrointestinal tract morphology occur during hibernation with a concomitant reduction in viable microbiota (Barnes, 1970; Barnes and Burton, 1970). A single study in Soay sheep has investigated the impact of photoperiod on the microbiota; short chain fatty acids and overall microbial diversity changed as a function of photoperiod. However, this effect could reflect changes in food consumption because sheep exposed to long day lengths consumed more food (McEwan et al., 2005). In our study, the microbiota were affected by photoperiod even though the hamsters consumed the same amounts of chow.

Physical activity influences microbiota composition in both humans and laboratory animals (Matsumoto et al., 2008; Santacruz et al., 2009). Although we did not measure physical activity in the current study, previous studies in our laboratory have demonstrated that photoperiod influences locomotor activity in Siberian hamsters (Weil et al., 2009). In general, hamsters housed under long summer-like day lengths are more active than hamsters housed in short summer-like day lengths. These differences are the most pronounced during the early and late periods of the dark cycle, with no differences in locomotor activity observed during the light cycle (Weil et al., 2009). Although the mechanisms through which physical activity influences the microbiota are not known, it is possible that the association involves the autonomic nervous system which is affected by photoperiod (Weil et al., 2009).

Many endocrine systems are affected by alterations in photoperiod, with the most widely studied hormone being melatonin. This hormone is produced by the pineal gland, which receives photoperiodic information from the retina through the actions of the suprachiasmatic nucleus. Because melatonin production occurs during the dark, melatonin levels are higher in short days than during long days. Melatonin is also produced in the gastrointestinal tract in levels that far exceed levels produced by the pineal gland (Bubenik, 2002). Although a direct impact of melatonin on bacterial growth has not been clearly demonstrated *in vitro*, it is possible that photoperiodic changes in other hormones directly affect microbial populations. There is growing evidence that bacteria can sense and respond to neurotransmitters and hormones in their immediate environment (Clarke and Sperandio, 2005; Lyte, 2004; Lyte and Bailey, 1997). The most well-studied neuroendocrine-bacterial interactions involve growth enhancement by exposure to catecholamine hormones, namely epinephrine, norepinephrine, and dopamine, through iron-dependent and independent mechanisms (Freestone et al., 2007; Hughes et al., 2009; Pacheco and Sperandio, 2009; Sandrini et al., 2009). Moreover, there is a growing appreciation that many different species of bacteria produce a wide range of molecules with striking similarity to mammalian hormones, such as estrogen, thyroid stimulating hormone, insulin,

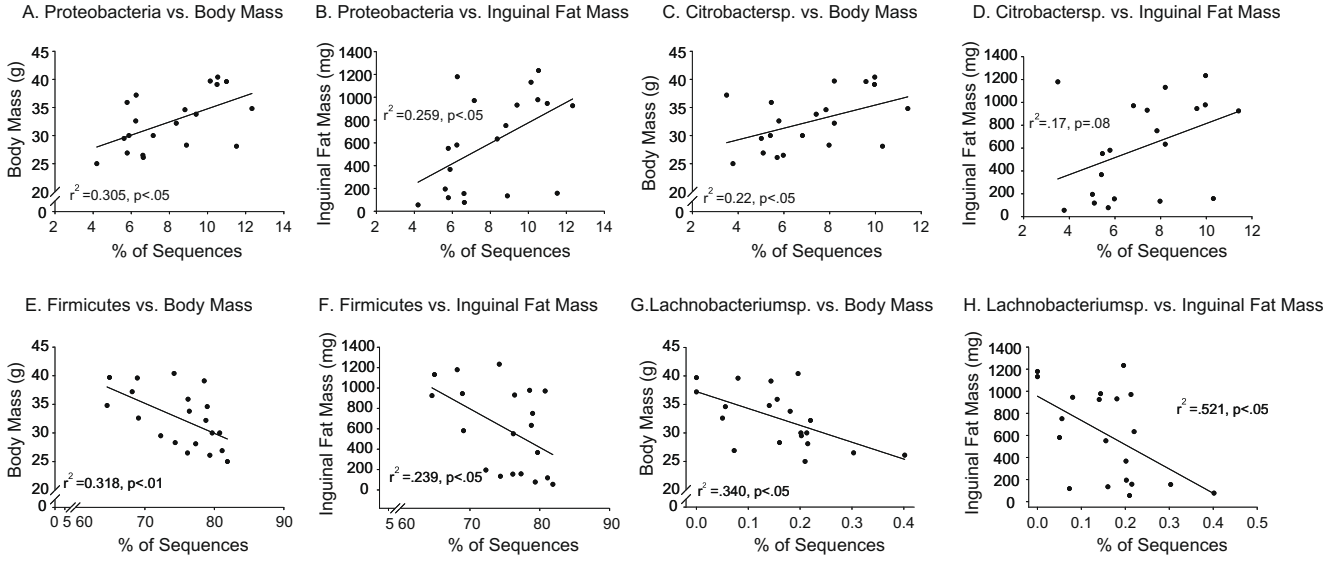

Fig. 4. The relative abundance of bacterial phyla and genera are significantly correlated with body and fat masses. The relative abundance (expressed as a percentage of total sequences) of bacteria in the phylum Proteobacteria is positively associated with body mass (A) and with (B) the mass of the inguinal fat pads. The relative abundance of bacteria in the genus *Citrobacter* is positively associated with body mass (C). There was a trend of the relative abundance of bacteria in the genus Citrobacter to be positively associated with inguinal fat mass ($p = .08$) (D). The relative abundance of bacteria in the phylum Firmicutes was *inversely* associated with body mass (E) and the mass of the inguinal fat pads (F). The relative abundance of bacteria found to be in the genus *Lachnobacterium* was also inversely associated with body mass (G) and the mass of the inguinal fat pads (H). In all cases, the relative abundance of bacteria in the cecum and the masses were collected from the same animals to allow for Pearson's correlation. Data are from $n = 10$ short-day and $n = 10$ long-day hamsters.

and β-endorphin (LeRoith et al., 1985; Roth et al., 1985). Bacteria are also responsive to these hormones, and have been found to contain hormone binding substances that resemble vertebrate-type receptors (Burshell et al., 1984; Josefsson and Johansson, 1979; Loose et al., 1983; Weiss et al., 1983). These hormones are also significantly changed upon photoperiodic cues (Hanon et al., 2008; Hanon et al., 2009; Helwig et al., 2006; Korhonen et al., 2008; Roberts et al., 1985; Tups et al., 2006), and thus could have direct influences on the microbiota. The likelihood of direct neuroendocrine-microbiota affects induced by changes in photoperiod will be the topics of future studies.

Beyond identifying a link between the microbiota and body mass, this study has potential implications for other host systems. Photoperiod has a wide impact on host physiology, and it has been well documented that immune responses of Siberian hamsters (and other mammals) are affected by photoperiod (Nelson, 2004). In general, long day length enhances innate inflammatory responses to antigenic stimulation (Bilbo et al., 2002; Navara et al., 2007; Prendergast et al., 2003), but suppresses delayed type hypersensitivity and other measures of cell mediated immunity (Prendergast et al., 2004; Weil et al., 2007). Although we did not assess immune functioning in the current study, it is possible that the photoperiod-induced changes of the microbiota we measured in the current study may be associated with photoperiod-induced alterations of immunity. It is now well accepted that the intestinal microbiota influence several components of the immune system, including mucosal immunity (i.e., cytokine production, antibody production, and intraepithelial lymphocyte development in mucosal tissue), as well as systemic immunity (reviewed in O'Hara and Shanahan, 2006; Round and Mazmanian, 2009). The effects of the microbiota on systemic immunity are thought to occur in part through their effects on dendritic cell activation and differentiation (Fujiwara et al., 2008) and on the expansion of regulatory T cells (O'Mahony et al., 2008). Because inflammatory responses to LPS and the DTH response to antigenic stimulation are strongly influenced by these cell types, it is possible that the photoperiodic changes in immune reactivity are linked to the changes in the intestinal microbiota.

Our results may also have larger implications for host resistance to intestinal diseases. In our study, a significant increase in *Citrobacter* spp. occurred in response to long day length. The genus *Citrobacter*, which is closely related to the genus *Escherichia*, is in the family Enterobacteriaceae and is a Gram-negative rod. During experimental infection in mice, the levels of intestinal pathogens, such as *Campylobacter jejuni* and *Salmonella enterica*, are positively associated with high levels of Enterobacteriaceae (Lupp et al., 2007; Stecher et al., 2007). Furthermore, it has been argued that the increased Enterobacteriaceae help to provide an intestinal environment that is conducive to pathogen colonization and replication (Pedron and Sansonetti, 2008; Stecher and Hardt, 2008). Given that the peak occurrences of many bacterial foodborne illnesses, such as illness caused by *Campylobacter jejuni* and *Salmonella enterica*, occur during the summer (Ailes et al., 2008; Arshad et al., 2008), it is tempting to speculate that photoperiod-induced increases in Enterobacteriaceae contribute to the seasonal increase in foodborne illnesses.

Studies of the impact of the intestinal microbiota on host physiology have rapidly increased in number during the past five years, and it is now recognized that the intestinal microbiota have a broad impact on host physiology. In fact, many researchers have argued that the intestinal microbiota are akin to and as important as any organ in the body (O'Hara and Shanahan, 2006). Yet, animal models in which to study the impact of the microbiota on host physiology are still somewhat limited, with many studies relying on germ-free and gnotobiotic animals. Because host physiology in seasonal mammals, such as Siberian hamsters, changes in reliable and predictable ways when housed with different day lengths, this animal model will be a valuable tool in which to study associations between host physiology and commensal gut microbes.

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