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EVOLUTIONARY ADAPTATION TO TEMPERATURE. VIII. EFFECTS OF TEMPERATURE ON GROWTH RATE IN NATURAL ISOLATES OF *ESCHERICHIA COLI* AND *SALMONELLA ENTERICA* FROM DIFFERENT THERMAL ENVIRONMENTS

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Abstract.—Are enteric bacteria specifically adapted to the thermal environment of their hosts? In particular, do the optimal temperatures and thermal niches of the bacterial flora reflect seasonal, geographic, or phylogenetic differences in their hosts' temperatures? We examined these questions by measuring the relationship between the temperature-dependent growth rates of enteric bacteria in a free-living ectothermic host. We sampled two species of enteric bacteria (*Escherichia coli* and *Salmonella enterica*) from three natural populations of slider turtles (*Trachemys scripta elegans*) seasonally over two years. Despite pronounced differences in turtle body temperatures at different seasons and in different locations, we found no evidence that the thermal growth profiles of these bacteria mirrored this variation. Optimal temperatures and maximal growth rates in rich medium were nearly the same for both bacterial species (35–36°C, 2.5 doublings per hour). The thermal niche (defined as the range of temperatures over which 75% of maximal growth rate occurred) was slightly higher for *E. coli* (28.5–41.0°C) than for *S. enterica* (27.7–39.8°C), but the niche breadth was about the same for both. We also measured the thermal dependence of growth rate in these same bacterial species isolated from mammalian hosts. Both bacterial species had temperatures of maximal growth and thermal niches that were about 2°C higher than those of their respective conspecifics sampled from turtles; niche breadths were not different. These data suggest that these bacterial species are thermal generalists that do not track fine-scale changes in their thermal environments. Even major differences in body temperatures, as great as those between ectothermic and endothermic hosts, may result in the evolution of rather modest changes in thermal properties.

Key words.—Bacteria, ectotherm, endotherm, *Escherichia coli*, growth rate, *Salmonella enterica*, thermal adaptation.

Analysis of adaptation to the thermal environment has been a principal and persistent theme in physiological ecology (e.g., Prosser 1973; Hochachka and Somero 1984; Cossins and Bowler 1987). It has also played an important role in experimental and theoretical studies of adaptation in evolutionary biology (e.g., Levins 1968; Huey and Slatkin 1976; Gilchrist 1995; Crill et al. 1996). Both fields have focused on temperature as a key environmental variable because it exerts a controlling influence on nearly all physiological rate processes and thereby affects such important biological factors as growth and reproduction. Although considerable attention has been directed to studying thermal properties in some groups of organisms (e.g., vertebrates and insects), others have been relatively ignored. One poorly studied group of particular interest from a thermal point of view is the enteric bacterial flora. The thermal environment of these organisms depends on the body temperature of their hosts, which may vary enormously temporally, geographically, or phylogenetically. Although the thermal responses of many laboratory strains of originally enteric bacteria have been studied (e.g., Bennett et al. 1990; Bennett and Lenski 1993; Mongold et al. 1996), there is almost no information about the thermal biology and responses of naturally occurring enteric bacterial populations. Evolutionary responses of bacteria to environmental change may be quite different in vivo and in vitro (e.g., Björkman et al. 2000; Bull and Levin 2000). Do natural enteric floras evolutionarily adapt to the specific thermal regimes of their hosts? Do they, for instance, track seasonal differences in host body temperature by changing the thermal dependence of their growth (and thus reproductive) rates? Are there major differences in the thermal properties of bacteria that live in the very different thermal environments of the intestines of ectothermic and endothermic vertebrates? These questions are almost completely unexplored.

Here, we examine the thermal properties of two enteric bacterial species isolated from natural populations of turtles. We focus on these ectothermic hosts because their body temperatures, and therefore the microclimate experienced by the bacteria, track water temperature changes (Thornhill 1982; Spotila et al. 1990; Weisrock and Janzen 1999), which vary greatly seasonally. The goal was to address whether the optimal growth temperature and thermal niche (sensu Huey and Stevenson 1979) in the enteric bacterial flora corresponded to changes in host body temperatures, both seasonally and among populations, indicating thermal adaptation on this fine scale. We also measured these growth characteristics in the same bacterial species sampled from mammals and compared them to those of the turtle isolates. These are the first direct comparisons of the thermal responses of enteric bacteria from ectothermic and endothermic hosts.

MATERIALS AND METHODS

Study Animals and Field Procedures

We trapped and sampled adult male red-eared slider turtles (*Trachemys scripta elegans*) from three study sites located in Randolph County, Illinois within the Kaskaskia State Fish and Wildlife Area (Baldwin Lake, 800 hectares; Dry Lake, 1.5 hectares; and the Kaskaskia River sampled 1 km west of Baldwin Lake). Turtles were trapped in November 1997; May, July, and November 1998; and May 1999 with fyke

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and hoop nets. Body temperatures were measured with a cloacal thermometer (Miller and Weber, Inc., Queens, NY) or temperature reading radio transmitters (Advanced Telemetry Systems, Isanti, MN); water temperature was measured during every sampling period. Three samples were taken from the cloaca of each turtle with sterile swabs and immediately stored in refrigerated screwtop glass tubes of tryptic soy agar.

Laboratory Procedures

Isolation and identification of Escherichia coli and Salmonella enterica

We obtained up to four isolates each of *Escherichia coli* and *Salmonella enterica* per turtle for up to five turtles from every sampling date. Approximately two weeks after each sampling period, for each turtle sampled, we incubated one cloacal swab in Luria broth and one in tetrathionate broth to enrich *E. coli* and *S. enterica*, respectively. We then followed isolation techniques detailed in Orskov (1981) and LeMinor (1981). Isolates were stored in a 25% glycerol, 75% Luria broth mixture at −80°C. All putative *S. enterica* isolates also tested positive in an agglutination test with *Salmonella* O Antiserum Poly A (Fisher Scientific, Pittsburgh, PA).

For further verification of these identifications, additional tests were performed on samples of isolates. A subsample of November 1997 and May 1998 isolates were verified with fatty acid methyl ester (FAME) analysis, which uses gas chromatography to compare the suite of fatty acids produced by the unknown bacterium with a library of known fatty acid groups (MIDI 1996). A subsample of July 1998 isolates were verified by DNA sequencing 827 bases of the malate dehydrogenase gene, which is often used to identify *Escherichia* and *Salmonella* (Boyd et al. 1994).

Escherichia coli were also isolated from the fecal pellets of free-living golden-mantled ground squirrels (*Spermophilus lateralis*) trapped in Inyo County, California in September 1998 (C. Frank, unpubl. data), using the same isolation techniques. A mammalian-derived *S. enterica* (strain 13311) was purchased from ATCC (Manassas, VA). This particular isolate is the type strain for *S. e. typhimurium* isolated from swine.

Thermal sensitivity of growth rate in Escherichia coli and Salmonella enterica

Growth curves were generated for up to three isolates of each bacterium per turtle across a range of temperatures. Maximum doubling rate (i.e., the maximum number of cell divisions per hour during exponential phase growth) was measured spectrophotometrically in Luria broth at 10, 20, 30, 34, 35, 36, 37, 39, 41, 43, and 45°C (Microbiological Workstation Bioscreen Model C, Labsystems, Inc., Franklin, MA). Maximal doubling rate at a temperature was defined as the maximum hourly difference in $\log_2$ optical densities at that temperature. Optimal temperature was defined as that having the peak doubling rate. The thermal niche was calculated as the range of temperatures over which the observed doubling rate equaled or exceeded 75% of the peak doubling rate.

Statistical Analyses

Possible factors influencing bacterial growth included temperature; sampling location (Baldwin, Dry, Kaskaskia) or sampling date (November 1997; May, July, November 1998; May 1999); turtle (nested within sampling location and date); and bacterial isolate (nested within turtle). However, because the isolates of a given bacterial species from a given turtle generally were indistinguishable, we assumed them to be clone-mates and included the isolate within turtle source of variation in the error variance. Because a nonlinear response to temperature was expected, temperature was treated as a categorical fixed independent variable rather than as a covariate. Turtle was a random effect, whereas location and date were fixed effects. Because not all locations were sampled on all occasions, we tested the effect of location at one date and the effect of sampling date in one location. These data were analyzed in a combined mixed-model repeated-measures analysis of variance using the Mixed Model procedure in program SAS (Proc Mixed in SAS vers. 6.12, SAS Institute, Cary, NC; see also Wolfinger and Chang 1999).

Post hoc profile contrasts of bacterial growth along the thermal gradient were used to identify the temperature at which growth was highest (the optimal temperature), and the temperatures at which growth rate varied among temporal or spatial samples. Once the optimal temperature for growth was determined, the thermal niche (75% of the peak growth rate) was calculated by linear interpolation. Variation in these thermal profiles among sampling locations and dates was determined by the interactions: temperature × location and temperature × date.

To examine seasonal adaptation, we analyzed data for Baldwin Lake, where we successfully trapped turtles on every visit. To address whether thermal growth profiles varied among locations on a given sample date, we analyzed data for July 1998 because this sampling occasion had the greatest spread in average body temperature of the host turtles. Finally, to compare bacteria isolated from turtles and mammals, we performed two analyses. For *E. coli*, we compared isolates from six different squirrels against all isolates from turtles with repeated-measures analysis of variance (described above). For *S. enterica*, we compared the single swine isolate to those from turtles with a one-sample *t*-test at each temperature.

RESULTS

Variation in Host Body Temperature

Table 1 reports the average water temperature (T_w) during each sampling period and the average cloacal body temperature (T_b) for all turtles sampled. While in the water, turtle body temperature rapidly equilibrates to water temperature and the latter can therefore be used to estimate the former for most of the day (see Table 1). Seasonal differences in water temperature in these locations was very great, ranging between 5°C and 33°C. In addition to this long-term (seasonal) thermal variability, enteric bacteria were also exposed to short-term (daily) thermal variability (unpubl. data). For example, one turtle from Baldwin Lake had a T_b that ranged

TABLE 1. Sample locations, dates, and temperature summaries; *n*, the number of adult males sampled; T_b, the average cloacal temperature of the sampled adult males; T_w, the average water temperature during the several day sampling period.

	Baldwin Lake		Dry Lake		Kaskaskia River	
November 1997	$T_w = 13.6$		$T_w = 5.2$		$T_w = 6.1$	
	$T_b = 14.1$	($n = 5$)				
May 1998	$T_w = 27.9$		$T_w = 25$		$T_w = 24.4$	
	$T_b = 27.0$	($n = 6$)			$T_b = 24.2$	($n = 8$)
July 1998	$T_w = 33.0$		$T_w = 27.3$		$T_w = 29.0$	
	$T_b = 32.9$	($n = 9$)	$T_b = 27.5$	($n = 6$)	$T_b = 29.7$	($n = 7$)
November 1998	$T_w = 17.1$		$T_w = 8.5$		$T_2 = 9.5$	
	$T_b = 17.0$	($n = 5$)				
May 1999	$T_w = 22.5$		$T_w = 18.4$		$T_w = 19.5$	
	$T_b = 23.0$	($n = 10$)	$T_b = 20.6$	($n = 5$)	$T_b = 20.1$	($n = 4$)

from 20°C to 39.5°C over a single day in July and from 12°C to 20°C on a single day in November.

Seasonal Variability in Bacterial Growth Rates

Turtles from Baldwin Lake were sampled on five occasions: November 1997; May, July, and November 1998; and May 1999. Not surprisingly, average temperatures were lowest in the November and highest in the July samples (November $T_b = 14.1$°C and 17.0°C vs. July $T_b = 32.9$°C, see Table 1). We measured thermal growth profiles on 72 isolates of *S. enterica* and 71 isolates of *E. coli* from Baldwin Lake (two or three isolates per five turtles per five sampling dates). The thermal growth profiles for these natural isolates (Fig. 1) are similar to those previously reported for the strains of these species that have been widely used in laboratory studies (Ingraham 1987). Assay temperature had a pronounced effect on growth rate (Table 2); growth approximately doubled between 10°C and 20°C, and doubled again between 20°C and 30°C (temperature quotient over 10° interval, Q_{10}, = 2.3 for *S. enterica* and 2.0 for *E. coli*). Optimal temperatures for growth were 35–37°C for all *S. enterica* isolates and 35–36°C for all *E. coli* isolates, regardless of date sampled, with maximal rates of 2.3–2.5 doublings per hour. Higher temperatures resulted in steep decrements in growth rate, and no detectable growth was observed in most isolates at 43–45°C. Thermal niches, defined as 75% maximal growth rate, were 28.4–39.6°C in *S. enterica* and 28.0–41.4°C in *E. coli*.

Although there was a significant temperature x date interaction (Table 2) for both species, post hoc analysis indicated that variation in the bacterial thermal profiles did not correspond to differences in turtle body temperature. Adaptive models predict seasonal shifts in the thermal growth curves, such that colder-adapted organisms have left-shifted curves in comparison to warm-adapted organisms (e.g., Precht et al. 1973; Prosser 1973). Therefore, optimal temperatures for growth should show a positive correlation with body temperature. However, optimal temperatures for all isolates range between 34°C and 37°C and are not correlated positively with body temperature in this seasonal series (*S. enterica*: $r = -0.42$, $P = 0.04$, $n = 24$; *E. coli*: $r = 0.20$, $P = 0.33$, $n = 25$). The variation in the growth rates of bacteria sampled in different seasons is therefore apparently random with regard to thermal adaptation. Notice also that most of the significant variation occurred at temperatures higher than those experienced by the bacteria in these populations of turtles (Fig. 1).

Spatial Variability in Bacterial Growth Rates

During July 1998, the average body temperatures of turtles varied across the three sites by 5.4°C (Baldwin Lake $T_b = 32.0$°C, Dry Lake $T_b = 27.5$°C, and Kaskaskia River $T_b = 29.7$°C). We analyzed thermal growth profiles of 50 isolates of *S. enterica* and 51 of *E. coli* from these sites (Fig. 2). For *S. enterica*, bacterial growth rate varied with assay temperature (Table 3); Q_{10} was 2.4 over the range 10–30°C. The temperature of maximal growth was 35°C (thermal niche = 28.2–40.3°C) for all *S. enterica* isolates. The interaction between temperature and location was not significant (Table 3, see also Fig. 2A). Thermal growth profiles for all *E. coli* isolates were similar ($Q_{10} = 1.9$ between 10°C and 30°C, optimal temperature was 35–37°C, and the thermal niche was 28.0–41.4°C). Location and temperature interacted to affect bacterial growth for *E. coli* (Table 3), with growth rates differing among localities at 10°C and 41°C (Fig. 2B). However, there was again no correlation in the predicted direction between growth rates at these extreme temperatures and host body temperature ($P > 0.05$) nor between optimal temperature and host body temperature ($r = 0.078$, $P = 0.92$, $n = 17$). Therefore, in spite of differences in thermal environments among these localities, there was no evidence of local genetic adaptation by the enteric bacteria to these differences.

Variation in Growth Rates of Bacteria Isolated from Ectotherms and Endotherms

Thermal growth profiles for a *S. enterica* isolate from swine and for numerous *E. coli* isolates from ground squirrels are graphed in Figure 3, along with conspecific isolates from turtles. The swine *S. enterica* differed significantly from the turtle isolates in thermal profiles (Table 4); these analyses indicated significant differences in growth rates at 10, 20, 30, 41, and 43°C. The swine *Salmonella* grew more slowly

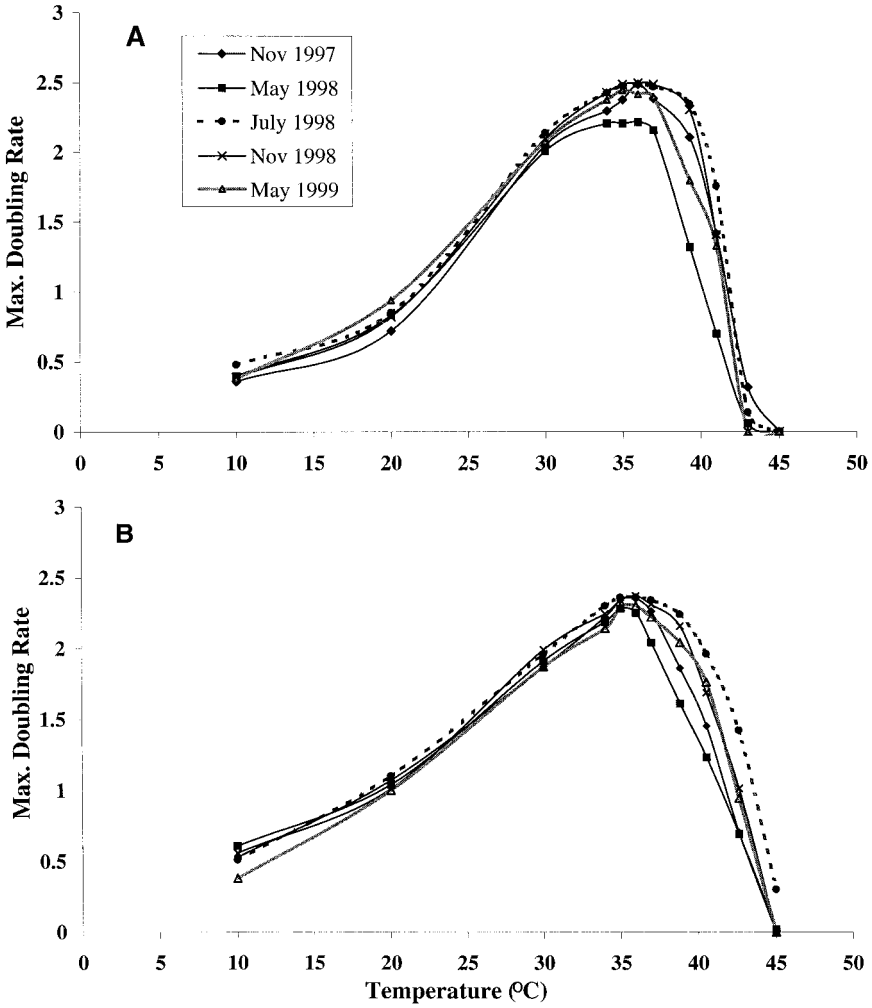

FIG. 1. Mean thermal growth profiles (maximum doubling rate at each temperature, as defined in the Materials and Methods) for each sampling date at Baldwin Lake. (A) *Salmonella enterica* growth rate differed among seasonal profiles at 20, 34, 35, 36, 37, and 39°C based on post hoc contrasts ($P < 0.05$). Optimal temperature for all sampling dates is 35–37°C. (B) *Escherichia coli* growth rate differed among seasonal profiles at 10, 34, 39, and 41°C. Optimal temperature for all sampling dates is 35–36°C.

at 10, 20, and 30°C, and more rapidly at 41°C and 43°C, than did isolates from turtles (Fig. 3A). Optimal temperature for growth was 35–36°C (thermal niche = 27.7–39.8°C) for the turtle isolates and spanned 34–41°C (thermal niche = 30.2–43.2°C) for the swine isolate. Maximal growth rates were greater in the turtle isolates than in the swine isolates (2.4 vs. 2.1 doublings per hour, respectively).

The thermal profiles for turtle and squirrel *E. coli* also differed between the two host types (significant interaction in Table 5). Post hoc analyses indicated that growth differed at 10, 20, 39, 41, 43, and 45°C (Fig. 3B). The squirrel *E. coli* grew more slowly at the lower temperatures (10°C and 20°C) and faster at the higher temperatures (39–45°C). The thermal optimum for turtle *E. coli* was 35–36°C (thermal niche = 28.5–41.0°C), whereas the thermal optimum for the squirrel *E. coli* was 37–39°C (thermal niche = 30–43.3°C). Maximal growth rates were similar in the two sets of isolates (2.4 doublings per hour).

Both of these bacterial species demonstrated a classical adaptive difference in their thermal profiles depending on the body temperatures of their hosts: The turtle isolates are left-shifted in comparison to the mammalian isolates. For the

TABLE 2. Tests of fixed effects on bacterial growth for Baldwin Lake (results from Proc Mixed in SAS).

	Salmonella enterica				*Escherichia coli*			
Source of variation	ndf	ddf	Type III *F*	(Pr > *F*)	ndf	ddf	Type III *F*	(Pr > *F*)
Date	4	20	6.02	0.0024	4	20	3.19	0.0352
Temperature	10	717	1,083	0.0001	10	705	659	0.0001
Temperature × date	40	717	5.27	0.0001	40	705	4.03	0.0001

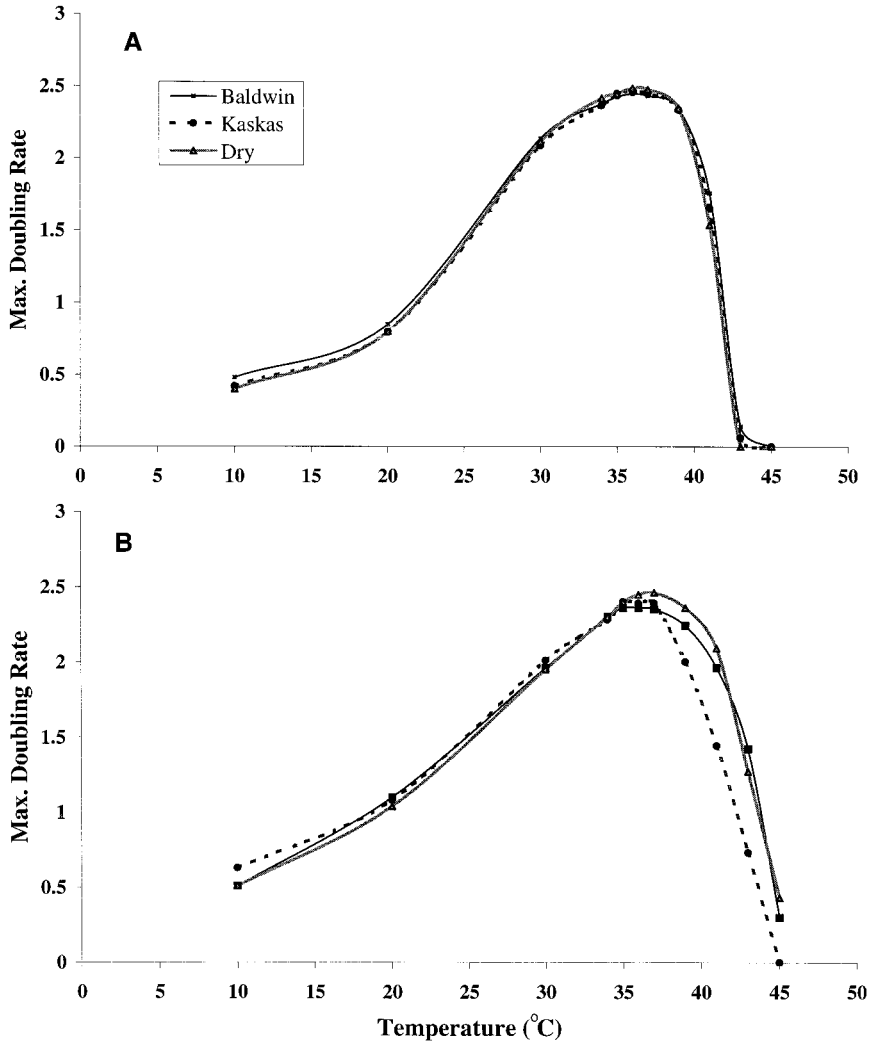

FIG. 2. Mean thermal growth profiles for each location during July 1998. (A) *Salmonella enterica* growth rate did not differ significantly among locations at any temperature. Optimal temperature is 35°C at all locations. (B) *Escherichia coli* growth rate differed among location profiles at 10°C and 41°C. Optimal temperature across all locations is 35–37°C.

cooler-adapted form (the turtle isolates), growth rates at low temperatures are higher and growth rates at high temperatures are lower; moreover, their optimal temperatures and thermal niches are lower than in the warmer-adapted form (the mammalian isolates). These shifts in the thermal growth profiles are, however, fairly modest, being only about 2–3°C for both bacterial species, which is considerably less than the differences in mean body temperatures of their host organisms.

DISCUSSION

The analyses of thermal growth profiles of enteric turtle bacteria fail to provide any evidence that these populations evolve to match temporal or spatial variation in host body temperature, at least on local and seasonal scales. Rather than tracking seasonal or geographic differences in the temperature of their hosts, they maintain a single growth reaction norm that is fairly typical for mesophilic bacteria (Ingraham 1987). This response might be considered typical of a thermal generalist, maintaining a fairly broad thermal niche, rather than a thermal specialist, with a shifting optimal temperature of its growth profile. Given the fact that turtle body temperature may vary as much as 20°C in a single day within these populations, perhaps a generalist, ''jack-of-all-temperatures'' (Huey and Hertz 1984) mode is not too surprising. However, in spite of this large daily variability, turtle body temperature ranges are nonoverlapping in different seasons. There is no doubt that turtles in November are chronically colder than they are in July, yet there was no evidence of an adaptive shift in the growth profiles of their enteric bacteria over that time scale.

We have previously demonstrated experimentally that *E. coli* is capable of rapid and extensive evolutionary thermal adaptation under appropriate selective conditions (e.g., Bennett et al. 1992; Bennett and Lenski 1993; Mongold et al. 1996). Even a rather modest and nonstressful change in culture temperature from 37°C to 32°C is a sufficient selective stimulus to promote evolutionary change. Why, therefore, was a similar response not apparent within natural popula-

TABLE 3. Tests of fixed effects on bacterial growth for July 1998 (results from Proc Mixed in SAS).

	Salmonella enterica				*Escherichia coli*			
Source of variation	ndf	ddf	Type III *F*	(Pr > *F*)	ndf	ddf	Type III *F*	(Pr > *F*)
Location	2	14	0.17	0.8448	2	14	1.81	0.2002
Temperature	10	495	1,554	0.0001	10	513	290	0.0001
Temperature × location	20	495	1.39	0.1201	20	513	3.97	0.0001

tions? Because this is the first study of this type, we can only speculate on the answer to this question, and these speculations fall into three categories: evolutionary, methodological, and ecological. From the evolutionary viewpoint, it may be that the fluctuations in body temperature on a seasonal basis occur on too fine a scale, that is, are too rapid, to be tracked by evolutionary change in the enteric bacterial populations. Even our laboratory populations of *E. coli*, reproducing at six or seven generations per day, required 800–1000 generations at 20°C to show a significant improvement in fitness at that temperature (Mongold et al. 1996). Natural populations are almost certainly reproducing at substantially lower rates than this, and therefore seasonal temperature fluctuations may be too rapid to permit measurable adaptation

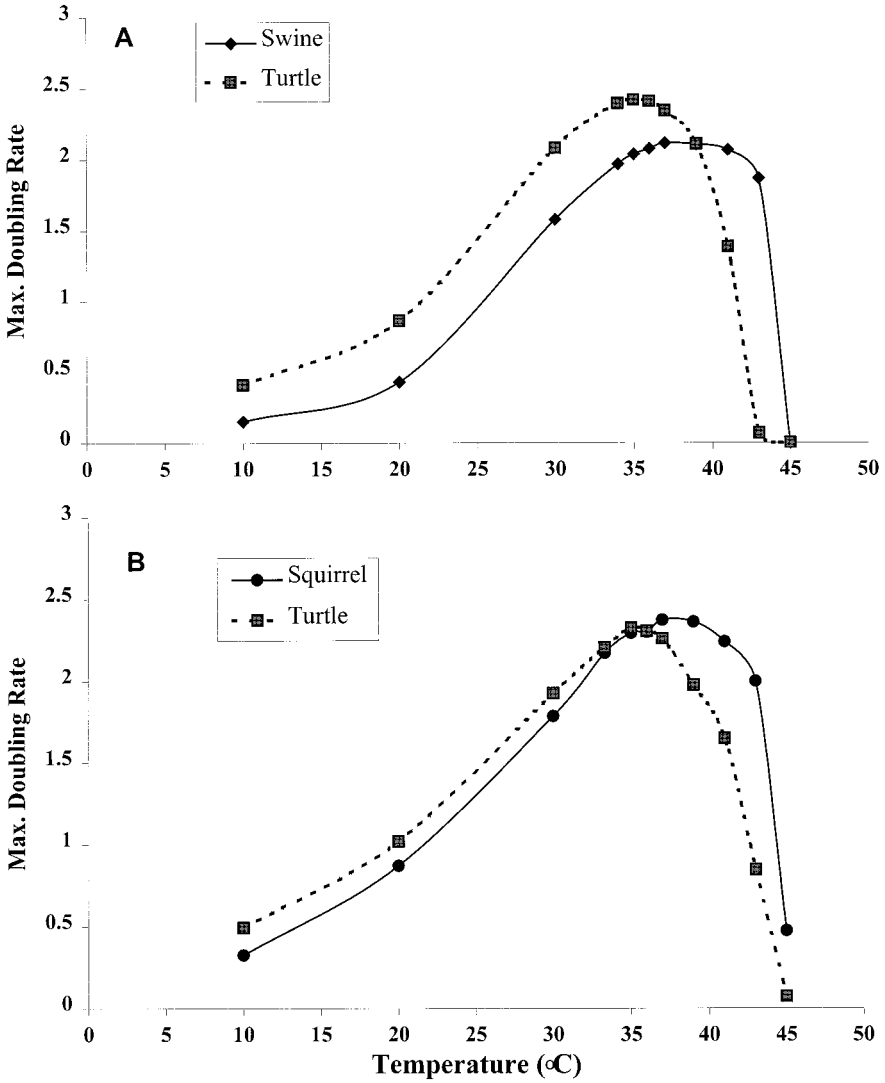

FIG. 3. Mean thermal growth profiles for mammalian and turtle-derived bacteria. (A) *Salmonella enterica* maximum doubling rate as a function of temperature for isolates from swine and turtles. Growth rate differed at 10, 20, 30, 41, and 43°C between swine and turtle *Salmonella*. Temperature of maximum growth spanned 34–41°C for swine *S. enterica*, and 35–36°C for turtle *S. enterica*. (B). *Escherichia coli* maximum doubling rate as a function of temperature for isolates from squirrels and turtles. Growth rate differed at 10, 20, 39, 41, 43, and 45°C between squirrel- and turtle-derived *E. coli*. Temperature of maximum growth is 37–39°C for squirrel *E. coli* and 35–37°C for turtle *E. coli*.

TABLE 4. One-sample *t*-tests of bacterial growth from a swine versus turtles.

Salmonella enterica			
Temperature (°C)	df	*t*-statistic	(Pr > *t*, two-tailed)
10	148	−3.27	0.001
20	148	−2.25	0.026
30	148	−2.76	0.006
34	148	−1.34	0.18
35	148	−1.41	0.16
36	141	−1.19	0.23
37	142	−0.63	0.53
39	148	+0.07	0.94
41	148	+3.11	0.001
43	148	+7.48	<0.0001
45	148	NA[1]	

[1] Turtle distribution mean and standard deviation equals zero.

to thermal excursions. Further studies examining the thermal properties of enteric bacteria from different species of ectotherms from relatively constant and diverse thermal environments (e.g., tropical vs. temperate) would be helpful to the investigation of this issue. In terms of methodology, there are a few caveats to a broad interpretation of this study. First, growth rates were examined in a rich nutrient broth to determine maximal rates of division. Perhaps differences in growth curves would have been observed in a medium more reflective of the contents of the turtle intestine, but what such a medium might be is unclear. Second, maximal growth rate is only one component of fitness (although it is anticipated to be an important one): Perhaps an examination of other factors (e.g., survival during starvation, efficiency of nutrient conversion to biomass) would have indicated differences among bacterial populations consistent with season or location. Only further investigations could begin to resolve which of these factors are important. From an ecological standpoint, it is unclear whether the bacteria sampled are stable residents within the turtles or transients adapted to living elsewhere. Additional sampling of these bacteria from the water column and other host species living therein might help to clarify this issue, especially if the bacterial population-genetic structure were also analyzed at this scale.

The optimal temperature of growth and the upper and lower niche boundaries were higher for bacteria isolated from mammals than for the same species isolated from turtles. That these differences are genetic is demonstrated by their persistence after being frozen and exposed to identical acclimation procedures. These differences suggest that natural populations of enteric bacteria can evolve and have evolved in response to broad-scale thermal differentiation in their hosts, in contrast to the lack of such adaptation at small temporal and spatial scales in the turtle populations. The comparisons between isolates from mammals and turtles are particularly compelling (despite small samples from the mammalian hosts) because, in both bacterial species, the shifts in the thermal growth profiles were always in a presumptively adaptive direction (positive correlation between optimal temperature and host body temperature, negative correlation between growth rates at temperatures below optimal and host temperature, and positive correlation between growth rates at temperatures above optimal and host temperature). Also of interest is the fact that all isolates, irrespective of host, had nearly equal thermal niche breadth. This result is in contrast to theoretical expectations (Levins 1968; Huey and Hertz 1984; Lynch and Gabriel 1987) that predict the evolution of more broadly adapted genotypes in thermally variable environments (e.g., turtle guts) and specialization in thermally constant environments (e.g., mammalian guts), resulting in possible trade-offs between performance over a narrow range and niche breadth (Futuyma and Moreno 1988). Conservation of thermal niche breadth has also been observed in laboratory lines of *E. coli* adapted to either constant or varying temperatures (Bennett and Lenski 1993; Mongold et al. 1996). This pattern in these natural isolates therefore accords with a shifting or sliding pattern of thermal niche adaptation (see Bennett and Lenski 1993, fig. 1A), which involves a conservation of thermal niche breadth.

We have shown that enteric bacteria do not necessarily evolve to changes in their mean thermal environment over small seasonal and spatial scales, but they may do so when temperature differences are persistent over a longer time scale. These results therefore broadly support recent studies emphasizing the importance of the time scale over which environmental variability is experienced in its influence on evolutionary change (Huey and Hertz 1984; Gilchrist 1995; Crill et al. 1996; Padilla and Adolph 1996).

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TABLE 5. Tests of fixed effects on bacterial growth from turtles and squirrels (results from Proc Mixed in SAS).

	Escherichia coli			
Source of variation	ndf	ddf	Type III *F*	(Pr > *F*)
Host type	1	50	2.74	0.1042
Temperature	10	1,577	140	0.0001
Temperature × host type	10	1,577	10.0	0.0001

several times a year. This research was supported by the National Science Foundation through a Biosciences Related to the Environment postdoctoral fellowship (DBI-9750218) to AMB and AFB, and with grants IBN-9507416 and IBN-9905980 to AFB and REL.

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