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TEMPERATURE-DEPENDENT FECUNDITY ASSOCIATES WITH LATITUDE IN *CAENORHABDITIS BRIGGSAE*

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Populations of organisms separated by latitude provide striking examples of local adaptation, by virtue of ecological gradients that correlate with latitudinal position on the globe. Ambient temperature forms one key ecological variable that varies with latitude, and here we investigate its effects on the fecundity of self-fertilizing nematodes of the species *Caenorhabditis briggsae* that exhibits strong genetically based differentiation in association with latitude. We find that isogenic strains from a Tropical phylogeographic clade have greater lifetime fecundity when reared at extreme high temperatures and lower lifetime fecundity at extreme low temperatures than do strains from a Temperate phylogeographic clade, consistent with adaptation to local temperature regimes. Further, we determine experimentally that the mechanism underlying reduced fecundity at extreme temperatures differs for low versus high temperature extremes, but that the total number of sperm produced by the gonad is unaffected by rearing temperature. Low rearing temperatures result in facultatively reduced oocyte production by hermaphrodites, whereas extreme high temperatures experienced during development induce permanent defects in sperm fertility. Available and emerging genetic tools for this organism will permit the characterization of the evolutionary genetic basis to this putative example of adaptation in latitudinally separated populations.

KEY WORDS: Adaptation, biogeography, fecundity, population structure, reproductive strategies.

Adaptation to differing local environmental conditions through natural selection forms the principal mode of phenotypic differentiation envisioned by Darwin (1859). Some of the most well-documented examples of adaptive evolution involve latitudinal clines and differentiation among latitudinally separated populations of organisms. For example, repeated clines on different continents in *Drosophila melanogaster* manifest in northerly populations tending to be larger, more tolerant to ethanol, and with consistently skewed frequencies of allozymes and inversions (Robinson et al. 2000; de Jong and Bochdanovits 2003; Fry et al. 2008). Analysis of pine tree populations also demonstrates the role of latitudinally varying factors in local adaptation (reviewed in Garcia-Gil et al. 2003). Flowering time and other life-history traits exhibit clines in *Arabidopsis thaliana*, for which much about the underlying genetic basis of this local adaptation has been elucidated (Stinchcombe et al. 2004). Although a great variety of ecological factors vary consistently from the equator to the poles of the Earth, here we focus on the gradient in temperature, and explore its potential to generate heritable differences in fitness. Although some nematode species in the genus *Caenorhabditis* have latitudinally restricted ranges, the genetic model organisms *C. elegans* and *C. briggsae* are distributed across much of the globe (Kiontke and Sudhaus 2006; Sudhaus and Kiontke 2007). Here, we investigate the potential for local adaptation to have occurred in response to temperature differences among latitudinally separated phylogeographic groups of *C. briggsae*.

The bactivorous nematode *C. briggsae* exhibits phylogeographic patterning such that mitochondrial and nuclear loci both

reveal consistently distinct genetic groups of individuals separated by latitude, forming so-called Temperate and Tropical clades (Graustein et al. 2002; Cutter et al. 2006; Dolgin et al. 2008; Howe and Denver 2008; Cutter et al. 2010). No shared ancestral variation and little-to-no evidence of recent gene flow between these geographic regions is apparent from the molecular data, which underlies the strong genetic differentiation. *Caenorhabditis briggsae* is androdioecious (populations comprised primarily of selfing hermaphrodites, with exceptionally rare males capable of outcrossing), and has low levels of genetic variation compared to obligately outbreeding relatives (reviewed in Cutter et al. 2009). The genetic diversity among Temperate-clade individuals, in particular, is very low, possibly indicating colonization and/or expansion in temperate latitudes around the globe within the last few hundreds or thousands of years (Cutter et al. 2006). Despite comparably low levels of genetic variation, ostensibly similar habitats, and equivalent mode of reproduction by selfing hermaphrodites, *C. briggsae*'s better-known cousin *C. elegans* does not exhibit the striking correspondence between genotype and geography that *C. briggsae* does (Barrière and Félix 2005; Haber et al. 2005; Cutter 2006), nor have any obvious geographic associations with phenotype been documented for *C. elegans* (Hodgkin and Doniach 1997; Palopoli et al. 2008).

Compared to *C. briggsae*'s exceptional molecular tools (Baird and Chamberlin 2006; Winston et al. 2007), published genome annotation (Stein et al. 2003), and genetic map (Hillier et al. 2007), our understanding of ecologically and evolutionarily important trait variation lags behind. However, several features of *C. briggsae* biology in relation to temperature are well-understood in a general sense. Like other nematodes and ectotherms, embryonic and larval development proceeds faster at warmer temperatures in *C. briggsae*. *Caenorhabditis briggsae* also tolerates higher temperatures than its cousin *C. elegans* and, unlike *C. elegans*, is not induced to enter the stress-resistant, "nonaging" dauer stage of the life cycle in response to high temperature (Fodor et al. 1983; Inoue et al. 2007; M. Ailion and J. Thomas pers. comm.). In addition, different isogenic strains of *C. briggsae* exhibit phenotypic variation in the morphology of male tail reproductive structures (Baird 2001; Baird et al. 2005) and in vulva cell fate specification (Delattre and Felix 2001; Dolgin et al. 2008), as well as variation in fecundity under benign conditions (Fodor et al. 1983; Dolgin et al. 2008; Howe and Denver 2008). However, many open questions remain: how phenotypically variable in fitness-related traits are individuals in this species, does phenotypic variation associate with ecological and environmental factors and with the demographic and genealogic history of populations, and might local adaptation provide a plausible driver of phenotypic differences?

Here, we aim to address these questions by quantifying heritable variation in hermaphrodite self-fecundity for isogenic strains representing previously described phylogeographic clades of *C. briggsae*. Specifically, we test for differences in fecundity in response to high- and low-temperature extremes. We demonstrate that strains from the Tropical phylogeographic clade have substantially more progeny when reared at extreme high temperature, and fewer progeny at extreme low temperature, than do strains from the Temperate phylogeographic clade. Laboratory experiments implicate effects on both sperm function and oogenesis as the targets of temperature-dependent fecundity, and different reaction norms in these traits plausibly underlie the heritable differences among strains. We hypothesize that the correspondence between geographic origin and fitness in response to temperature regime might represent the product of local adaptation.

Methods

CAENORHABDITIS BRIGGSAE STRAINS AND FECUNDITY ASSAYS

We included nine strains of *C. briggsae* in this study, representing three phylogeographic clades ("Temperate," "Tropical," and a genetically distinct locality in equatorial Nairobi, Kenya) identified previously as exhibiting strong genetic differentiation (Cutter et al. 2006; Dolgin et al. 2008; Howe and Denver 2008) (Table 1). These iso-hermaphrodite strains from this highly selfing species essentially are isogenic, so phenotypic differences can be attributed directly to genotypic differences between them. We obtained winter (January for northern hemisphere, July for southern hemisphere) and summer mean maximum and mean minimum temperatures for time series >30 years for nearby locations to collection sites (U.S. Department of Commerce 1991).

We then quantified lifetime fecundity for each of these strains at eight rearing temperatures, from a lower extreme of 10°C to an upper extreme of 32°C. First, populations of each strain were developmentally synchronized by using a hypochlorite bleach solution to liberate eggs from gravid hermaphrodites. The eggs were then placed on NGM-lite agar plates devoid of food medium overnight to allow hatching and subsequent arrested development at the first larval stage (L1). We then transferred individual L1 worms onto NGM-lite plates spotted with *E. coli* OP50 as a food resource, and allowed them to develop to adulthood at one of the following temperatures: 10°C, 12°C, 14°C, 16°C, 20°C, 28°C, 30°C, or 32°C. For a given temperature treatment, strains on separate plates were intermingled arbitrarily on a single shelf of an incubator; thermometers placed on the shelf were used to confirm ambient temperature within the incubators. We used a block design with two to five replicates per strain on a subset of temperature treatments per block. Gravid adult hermaphrodites were transferred to new plates daily until oviposition ceased; progeny were left to develop at the same temperature at which they were laid. Finally, progeny counts were conducted on these daily transfers

Table 1. Summary of strain information used in this study.

Strain	Locality of origin	Phylogeographic group	Latitude	Approximate elevation (m)	Mean winter temperature (°C min, max)[1]	Mean summer temperature (°C min, max)[1]
EG4181	Salt Lake City, Utah, USA	Temperate	40°42′N	1,290	−7.8, 2.8	15.6, 34.4
HK104	Okayama, Japan[2]	Temperate	34°40′N	30	0, 8.3	22.8, 30.6
JU439	Reykjavic, Iceland	Temperate	64°08′N	16	−2.2, 2.2	8.9, 14.4
PB826	Ohio, USA[3]	Temperate	39°34′N	270	−6.1, 2.8	17.8, 30
AF16	Ahmedabad, India	Tropical	23°01′N	50	14.4, 29.4	26.1, 33.9
ED3083	Johannesburg, S. Africa[4]	Tropical	26°10′S	1,750	2.8, 18.9	15.6, 27.2
VT847	Hawaii, USA	Tropical	20°57′N	930	18.9, 26.1	22.8, 29.4
ED3092	Nairobi, Kenya	Nairobi	01°19′S	1,660	10.6, 20.6	12.2, 25
ED3101	Nairobi, Kenya	Nairobi	01°19′S	1,660	10.6, 20.6	12.2, 25

[1]Winter temperature data: January for northern hemisphere, July for southern hemisphere; Summer temperature data: July for northern hemisphere, January for southern hemisphere.
[2]Osaka temperature data.
[3]Indianapolis, IN temperature data.
[4]Pretoria temperature data.

when the progeny reached a late larval or young adult stage. We included in analysis an average of 15 replicates per strain per temperature (range: 6–29) after excluding any replicates with excessive foreign bacterial contamination or in which individuals died prematurely from crawling up the sides of the plates (Table S1). For analysis, we constructed a multiple regression model in JMP with lifetime fecundity described as a function of rearing temperature (14°C–30°C), phylogeographic clade (Temperate, Tropical, Nairobi), strain nested within region, and a region × temperature interaction (experimental block did not contribute significantly, and was removed from the final model for analysis). We then performed post hoc contrasts of Tropical-clade versus Temperate- and Nairobi clades with the least-squares means within each temperature treatment, applying a Bonferroni correction to resulting *P*-values. We report the least-squares mean lifetime fecundity values for regions (which accounts for other sources of variation in the model), but for each strain considered separately, we report raw mean lifetime fecundity for ease of interpretation.

DIRECT QUANTIFICATION OF SPERM PRODUCTION

To test for reduced sperm production at extreme temperature as a potential cause of reduced self-fecundity, we quantified the number of sperm produced by hermaphrodites of AF16 ("Tropical") and HK104 ("Temperate") reared at 14°C and 30°C, respectively. Young adult animals were fixed and stained with 4′,6-diamidino-2-phenylindole (DAPI) as described previously (Sulston and Hodgkin 1988; Cutter 2004), for which the condensed sperm nuclei can be counted easily to determine sperm numbers. We assessed sperm numbers within ≥7 gonad arms of each strain at each temperature.

TEMPERATURE-SHIFT EXPERIMENTS

To test for reduced gametogenesis or defective gametes as a cause of lower self-fecundity at extreme temperatures, we conducted temperature-shift experiments with strains AF16 and HK104. Spermatogenesis is specified in the third-stage (L3) hermaphrodites and initiates in fourth-stage (L4) hermaphrodites in *C. elegans* (Kimble and Ward 1988; Barton and Kimble 1990; Lamont and Kimble 2007) and *C. briggsae* (Hill et al. 2006), and is followed irreversibly by oogenesis in adults. Worms were reared at 30°C (or at 14°C) until young adulthood, at which point they were transferred to an intermediate temperature (20°C) to score fecundity. We also performed the reciprocal experiment, in which worms were shifted from the intermediate temperature (20°C) to one of the temperature extremes (14°C or 30°C). For the 30°C to 20°C down-shift, we conducted an additional treatment in which individual hermaphrodites were allowed to mate over the course of 24 h with six males that had been reared at 20°C as larvae. Samples of 10–18 replicates were performed for each strain-treatment. If sperm defects during spermatogenesis or spermiogenesis are responsible for temperature-dependent fecundity variation, then we expect animals that experience temperature extremes during larval stages to have permanently reduced self-fecundity, and for hermaphrodite fecundity to be restored upon mating with males that were reared at moderate temperature. If worms modulate the rate of oogenesis in response to temperature, then we expect shifted animals to facultatively restore their self-fecundity. Defective self-sperm produced at extreme temperatures also would be implicated by restored fecundity of animals upon mating with males that had been reared at moderate temperature, whereas a lack of fecundity rescue from mating would implicate problems in oogenesis. We tested for significant

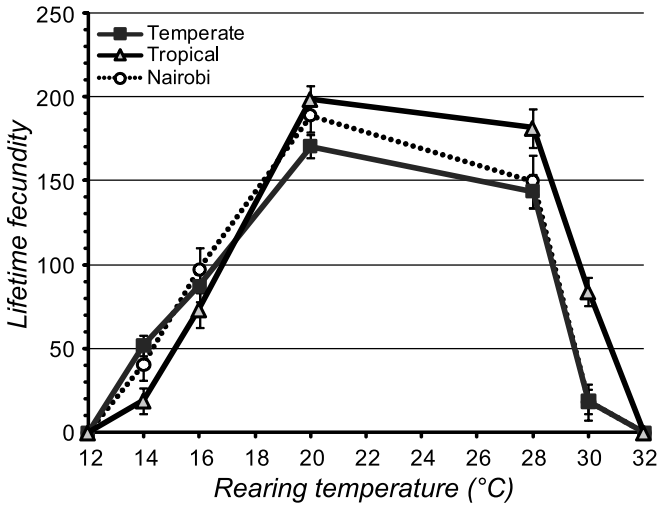

Figure 1. ***Caenorhabditis briggsae*** **self-fecundity as a function of rearing temperature, with strains partitioned by phylogeographic clade. Values represent the least-square means (±1 SEM) from a multiple regression model that also accounts for variation among strains and temperature. Tropical-clade strains have significantly more progeny when reared at 30°C than other strains, and significantly fewer progeny when reared at 14°C.**

differences in fecundity between constant rearing temperatures with a shifted-temperature treatment using one-way ANOVA, applying Bonferroni correction for multiple tests.

Results

HERITABLE, TEMPERATURE-DEPENDENT VARIATION IN SELF-FECUNDITY

We find that the lower and upper thermal limits for fertility in *C. briggsae* lie between 12–14°C and 30–32°C, respectively. When reared at 10°C, worms showed no progression from the first larval stage (L1) over the course of 40 days of observation, despite abundant food. At 12°C, worms grew to adulthood, but no egg laying occurred in any of the strains. We observed egg laying in all strains at all intermediate temperatures from 14°C to 30°C. However, at 32°C, worms again grew to adulthood, but produced no progeny. These data are consistent with the observations of M. Ailion and J. Thomas (pers. comm.).

In addition to species-wide thermal limits on fecundity, we find significant heritable variation in fecundity among strains and among phylogeographic clades. Our multiple-regression model explained 56% of the variance in lifetime fecundity ($F_{20,673} = 44.2$, $P < 0.0001$), with significant effects of rearing temperature ($P < 0.0001$), phylogeographic clade ($P = 0.003$), strain (nested within clade; $P < 0.0001$), and clade × temperature interaction ($P < 0.0001$). Post hoc contrasts of fecundity between the Tropical and the other two clades within each of the rearing temperature treatments identified significant differences only at 14°C ($F_{1,673} = 8.53$, $P_{cor} = 0.018$) and at 30°C ($F_{1,673} = 36.3$, $P_{cor} < 0.0001$), after Bonferroni correction.

At 16°C, 20°C, and 28°C, strains from the Tropical, Temperate, and Nairobi clades demonstrated fecundities that were statistically indistinguishable (Fig. 1; Table S1). However, at the upper and lower observed limits of fecundity, under thermal stress, we identified significant differences in the lifetime fecundity of Tropical-clade strains from other strains (Fig. 2). When reared at 14°C, Temperate-clade (and Nairobi) strains exhibited significantly higher fecundity than Tropical-clade strains (least-squares means: Tropical 19.0 ± 7.4 [±1 SEM]; Temperate 52.1 ± 6.2; Nairobi 40.4 ± 9.3). Note that Temperate-clade strains exhibit a high degree of variation among strains, with this low-temperature

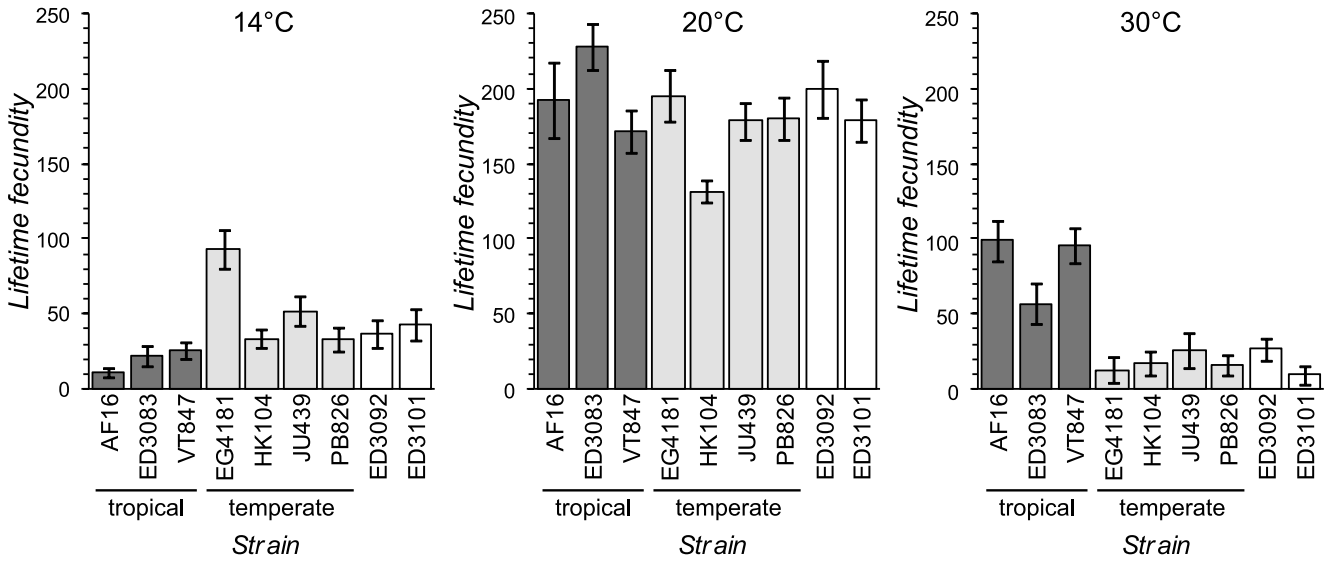

Figure 2. Per-strain lifetime fecundity profiles at extreme rearing temperatures (14°C and 30°C) and at a moderate rearing temperature (20°C). Error bars indicate ±1 SEM.

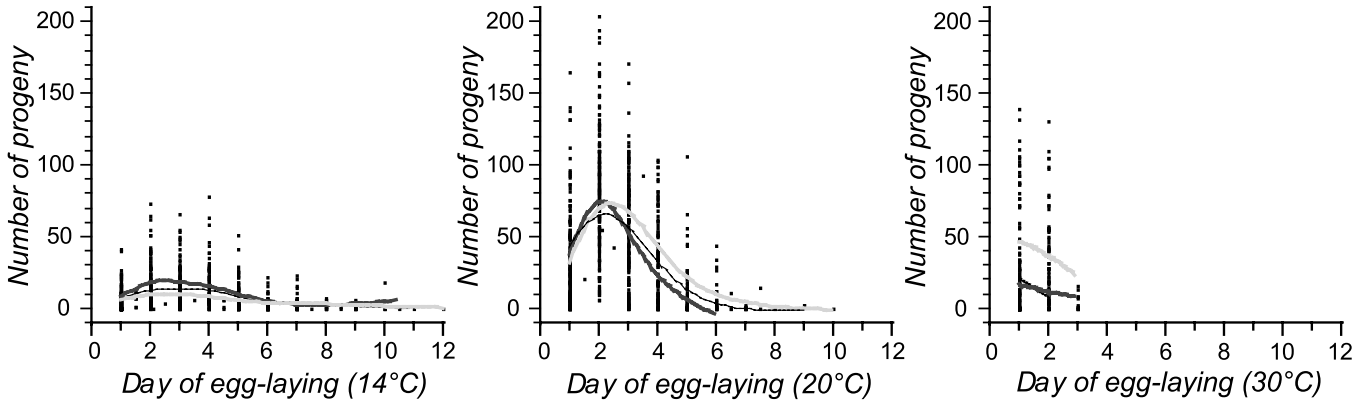

Figure 3. **Daily fecundity counts for extreme rearing temperatures (14°C and 30°C) and a moderate rearing temperature (20°C). Curves show spline fits to the data, for strains grouped by phylogeographic clade; Tropical-clade strains with light gray line, Temperate-clade strains with dark gray line, Nairobi strains with thin black line.**

pattern most obvious for two of them (Fig. 2). Inversely, Tropical-clade strains reared at 30°C showed significantly higher fecundity than either Temperate-clade or Nairobi strains (least-squares means: Tropical 84.2 ± 8.7; Temperate 18.5 ± 7.5; Nairobi 18.2 ± 10.8). This high-temperature pattern appears robust across all strains from a given phylogeographic clade (Fig. 2). At 14°C, the strains from the Temperate clade not only have higher lifetime fecundity, but also nominally higher daily fecundity when compared with the Tropical isolates, whereas we observed the opposite trend at 30°C (Fig. 3). Visual inspection of the response curve of fecundity to rearing temperature suggests that the fecundity profile for the Tropical-clade strains appears slightly right-shifted relative to the Temperate and Nairobi strains (Fig. 1), in which case increased experimental replication might permit detection of significant differences for mid-range temperatures as well.

QUANTIFICATION OF HERMAPHRODITE SPERM PRODUCTION

AF16 and HK104 strains were reared at 14°C or 30°C until adulthood, at which point we fixed and stained hermaphrodites to quantify directly the number of self-sperm in their spermathecae. The number of sperm nuclei per gonad arm did not differ significantly between worms reared at either of these temperatures, for both strains (Fig. 4). Moreover, these data indicate that the total number of sperm produced by hermaphrodites at the extreme temperatures (~200 sperm) is very similar to the total self-fecundity of animals that are reared at moderate temperatures, and much greater than the self-fecundity of animals reared at these same extreme temperatures (Figs. 1 and 2).

TEMPERATURE-SHIFT EXPERIMENTS

We shifted AF16 and HK104 hermaphrodites from an extreme larval rearing temperature of 14°C or 30°C to an intermediate temperature (20°C) following the adult molt—as well as the reciprocal intermediate-to-extreme temperature shift—and then quantified the progeny production of individuals. For the 30°C to 20°C downshift, we performed an additional treatment in which we mated males to the temperature-shifted hermaphrodites (males were reared at 20°C). Because sperm production in hermaphrodites occurs during the fourth larval stage (Hill et al. 2006), this allowed us to test for the effects of temperature on sperm fertility and for the ability of temperature shifts and mating to rescue hermaphrodite fecundity.

When reared as larvae at 14°C and then up-shifted to 20°C as young adults, hermaphrodites fully (AF16) or partially

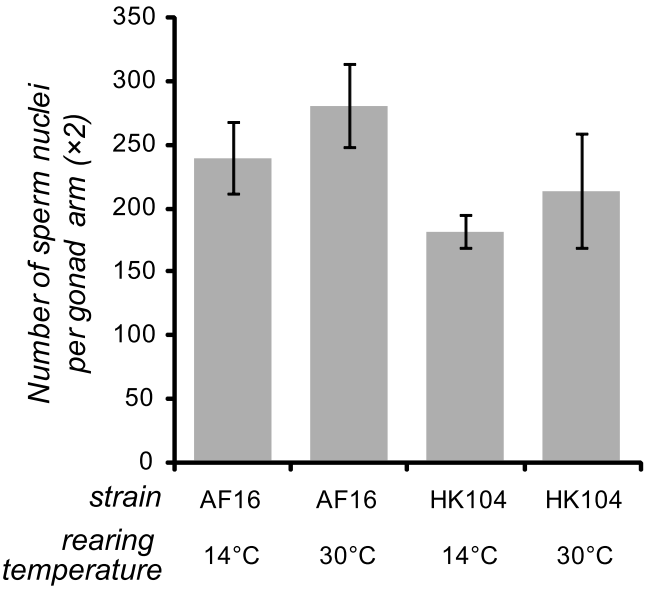

Figure 4. **Counts of sperm nuclei in young-adult hermaphrodite spermathecae exceed the observed lifetime self-fecundity at both 14°C and 30°C (cf. Fig. 2). Nuclei counts were conducted separately for each gonad arm; the figure shows values scaled by a factor of 2 to reflect the total sperm present per animal. Error bars indicate ±1 SEM.**

Table 2. Summary of fecundities from temperature-shift experiments.

Treatment	AF16 mean fecundity (±1 SE)	HK104 mean fecundity (±1 SE)
14°C[1]	45.40 (±14.57)	33.00 (±4.09)
20°C[1]	183.17 (±25.89)	139.83 (±8.66)
30°C[1]	40.72 (±9.96)	10.73 (±3.30)
20°C→14°C	58.00 (±13.07)	51.36 (±7.85)
14°C→20°C	154.10 (±15.81)	66.00 (±7.16)
20°C→30°C	174.80 (±11.32)	112.36 (±13.78)
30°C→20°C	78.00 (±11.88)	6.83 (±3.01)
30°C→20°C+males	200.40 (±18.72)	214.10 (±29.41)

[1]Fecundities differ slightly from those reported for these strains in the Table S1 in these independent experiments.

(HK104) recovered the levels of self-fertility observed at moderate temperatures (Table 2; Fig. 5). Conversely, self-fecundity of hermaphrodites that were down-shifted from 20°C to 14°C reflected the reduced levels observed for individuals that experienced 14°C for their entire lives (Table 2; Fig. 5). Thus, hermaphrodite self-fecundity at cool-to-moderate temperatures depends primarily on the temperature experienced by the post-spermatogenic adult and not by the developing larva.

The effect of extreme high temperature on reduced fecundity, however, manifests during larval development. For neither strain did the 30°C to 20°C down-shift restore self-fecundity above the levels observed when they were reared at 30°C for the duration of their lives (Table 2; Fig. 5), indicating that the larval growth, gonad development, and spermatogenesis experienced at 30°C decreased self-fecundity even after transfer to a benign moderate temperature. And yet, hermaphrodites of both strains that mated with males following their temperature shift from 30°C to 20°C recovered fecundity levels at least as high as those of self-fertilizing 20°C-reared worms (Table 2; Fig. 5). This is consistent with a permanently detrimental effect of high temperature on sperm or spermatogenesis, but not oocytes or oogenesis. Moreover, up-shifted hermaphrodites from 20°C to 30°C exhibit self-fecundities comparable to animals reared at 20°C for their entire lives (Table 2; Fig. 5). This further supports the notion that the temperature experienced by the gonad during spermatogenesis is the critical determinant of self-fecundity at extreme high temperatures, and has no detrimental effects on mature spermatozoa. Similar temperature-shift experiments in *C. elegans* also have

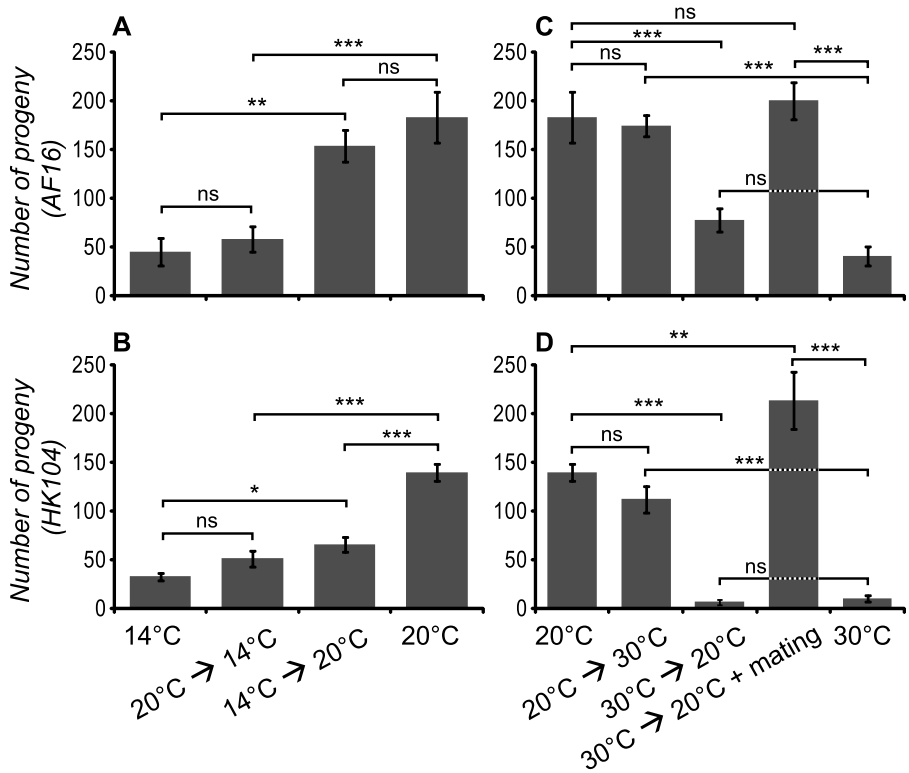

Figure 5. ***Caenorhabditis briggsae*** **fecundity in temperature-shift experiments for strains AF16 (A, C) and HK104 (B, D). Rearing temperature, and shifts following the adult molt, are indicated below the *x*-axis in the bottom panels. Values for 20°C rearing are the same for (A, C) and for (B, D). Post hoc one-way ANOVA results use Bonferroni correction, with significance indicated above tested pairs (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); sample sizes range from 10 to 18. Error bars indicate ±1 SEM.**

found partial-restoration of fecundity (M. Ailion and J. Thomas, personal communication).

Discussion

TEMPERATURE-DEPENDENT VARIATION IN *C. BRIGGSAE* SELF-FECUNDITY IS HERITABLE AND ASSOCIATED WITH GEOGRAPHIC ORIGIN

The study of temperature stress in *Caenorhabditis* has a long history (Fatt and Dougherty 1963; Brun 1965), predating even the seminal work on *C. elegans* that brought it to center stage as a model organism (Brenner 1974). Earlier still, Nigon and Dougherty (1949) reported that both *C. elegans* and *C. briggsae* produce more spontaneous male self-progeny at high temperatures due to sex-chromosome nondisjunction during meiosis. More recently, quantitative genetics approaches have proved enlightening about segregating variation for genotype–environment interactions for gene expression and for various reproductive traits in *C. elegans* (Li et al. 2006; Gutteling et al. 2007a; Gutteling et al. 2007b), with a single polymorphism in *tra-3* being identified that controls the effect of temperature on body size (Kammenga et al. 2007). Here, we quantify the effects of extreme high and low temperatures on *C. elegans'* relative *C. briggsae*, and demonstrate significant heritable differences among strains in their fecundity as a function of rearing temperature regime. Moreover, we find that strains collected across the globe near the Tropic of Cancer or Capricorn produce more progeny at high temperature (30°C) and fewer progeny at cold temperature (14°C) than do strains collected from temperate latitudes or from mesic, equatorial Kenya (Fig. 1). The disparity in lifetime fecundity is particularly robust at high temperature, whereas among-strain variation is more evident when worms were reared at low temperature.

Kawecki and Ebert (2004) emphasize two key criteria for inferring local adaptation: the relative fitness of a genotype should be disproportionately higher when tested within "sympatric" than within "allopatric" habitat and the pattern should be replicated across multiple demes. These *C. briggsae* data are strongly consistent with local adaptation having shaped self-fecundity. However, despite disparate collection localities of strains from temperate and tropical latitudes, strains from tropical latitudes form a single phylogeographic group (Tropical clade). Similarly, *C. briggsae* isolates from temperate regions are each others' closest genetic relatives and form a single phylogeographic group (Temperate clade). Strains from Nairobi, Kenya are genetically distinct from other known strains, and this mesic location most closely resembles temperate regions (Table 1), despite its latitudinal position on the equator (Graustein et al. 2002; Cutter et al. 2006; Dolgin et al. 2008; Howe and Denver 2008; Cutter et al. 2010; Raboin et al. 2010). Thus, available sampling of *C. briggsae* does not provide genetically independent replicates across geographic regions that would be required to conclude definitively that local adaptation is the cause of the temperature-dependent differences in fecundity observed here. Recently, Joyner-Matos et al. (2009) reported that strains of *C. briggsae* from temperate regions have longer life spans than Tropical-clade strains when reared at 20°C, which should motivate tests for temperature-dependent differences in life span among phylogeographic groups of this species. If local adaptation to temperature-dependent ecological differences between temperate and tropical regions has occurred—coupled with the strong and extensive linkage disequilibrium throughout the *C. briggsae* genome that is facilitated by high inbreeding via self-fertilization (Cutter et al. 2009)—then such differential selection would provide a clear mechanism for the maintenance of geographically based genetic differentiation in *C. briggsae*, even in the face of migration that might be mediated by human activity. Moreover, it might suggest *C. briggsae* as a genetically tractable model for studying incipient "ecological speciation" (Rundle and Nosil 2005), despite an apparent lack of intrinsic postzygotic isolating barriers (Dolgin et al. 2008).

Although we characterize the differences among strains as a latitudinal association, it is likely that key ecological variables are the true key features of differentiation. For example, we find that strains collected close to the equator in mesic Nairobi, Kenya (moderate year-round temperature, high elevation; Table 1), which are genetically differentiated from both Temperate- and Tropical-clade strains (Dolgin et al. 2008; Howe and Denver 2008), more closely resemble phenotypically the Temperate-clade strains. We also do not know how microclimatic variation of the habitat used by *C. briggsae* might deviate from air temperature profiles, or whether temperature might interact with other environmental variables in ways that could generate causal genotype–environment effects. All samples of *C. briggsae* included here derive from anthropogenic habitat, so it is conceivable that migration from ancestral locations might have disrupted our inference of true geographic origin of the genetically distinct individuals from the single collection localities, such as Nairobi. In general, however, migration has not compromised the clear genetic and phenotypic signal for Temperate- and Tropical-clade isolates sampled from diverse localities around the globe (Cutter et al. 2006). Future investigation of climatic variables that might associate with the full range of localities at which the phylogeographic clades have been sampled might shed light on particular candidate ecological determinants of differentiation.

The temperature range that *C. briggsae* can tolerate is qualitatively consistent with that observed by others (Fodor et al. 1983; M. Ailion and J. Thomas pers. comm.). The reduced fecundity and developmental rates at low temperatures are not due to low food availability (Schiemer 1982; Goranson et al. 2005), as excess bacteria were present in all temperature treatments. Despite a previous observation that strains of *C. briggsae* with high mitochondrial

heteroplasmy have lower fecundity (at 20°C) (Howe and Denver 2008), we do not observe an association of reported mitochondrial heteroplasmy frequencies with our fecundity measurements. This might be explained by rapid changes in heteroplasmy frequency within laboratory strain populations due to genetic drift, so that the levels of heteroplasmy present in the copies of the strains differ substantially between laboratories.

In this work, we have not explored the potential for transient, extreme heat stress (e.g., ≥35°C) to effect differences between the Temperate- and Tropical-clade strains of *C. briggsae*. Such approaches with *C. elegans* have helped identify the genetic basis for heat acclimation (Treinin et al. 2003), and could similarly be applied in this related species. Because of our focus on extreme rearing temperatures, we excluded a range of temperatures that likely include growth conditions optimal for reproductive output in *C. briggsae* (between 20°C and 28°C). Future work investigating heritable variation in optimal growth conditions should test such intermediate temperatures; indeed, Fodor et al. (1983) observed maximal fecundity at 22.5°C or at 25°C for two strains of *C. briggsae*. We also focused here on the hermaphrodite sex, so the implications of temperature variation for male sperm production and function, and for mating behavior, remain unknown. We predict that male spermatogenesis would be as sensitive to high temperatures as in hermaphrodites, although refutation of this prediction could indicate interesting developmental differences in the process of sperm production. Lastly, by investigating the effects of temperature on fecundity, we did not quantify the ways in which temperature variation might influence more subtle behavioral phenotypes. *Caenorhabditis elegans* thermal preference behavior has been subject to investigation by numerous research groups (Hedgecock and Russell 1975; Mori and Ohshima 1995; Ryu and Samuel 2002; Ito et al. 2006; Anderson et al. 2007; Jurado et al. 2010). Given the striking differences among *C. briggsae* wild isolates in temperature-dependent fecundity and the availability of recombinant inbred line mapping resources (Baird et al. 2005; Hillier et al. 2007), we anticipate that this species will provide many further insights into the genetic basis and evolutionary context of nematode thermotaxis behavior and temperature-sensitive traits.

MECHANISMS OF FECUNDITY VARIATION

To gain some insight into potential mechanisms underlying the temperature-dependence of *C. briggsae* fecundity overall, we conducted several additional experiments that focused on two strains (Tropical-clade AF16, Temperate-clade HK104). Our aim with these experiments was to determine in a broad sense how gametogenesis in *C. briggsae* responds to temperature extremes, as a first step toward understanding the phenotypic and genetic cause(s) of different temperature-sensitivities among strains. In *C. elegans*, fecundity is sperm-limited at the standard laboratory temperature of 20°C, because of protandrous gametogenesis within the ovotestes (Ward and Carrel 1979). Crosses from temperature shift experiments in *C. elegans* implicate the number of functional sperm as a partial cause of infertility in worms reared at high temperatures, although it is not clear whether this is due to production of fewer sperm or to a greater fraction of defective sperm (Harvey and Viney 2007). If sperm-limited fecundity holds true at extreme temperatures, then we would predict that hermaphrodites reared at extreme temperatures would produce fewer sperm, and that this reduced sperm production would underlie the lower fecundities at these temperatures. Consequently, we reared AF16 and HK104 at 14°C and 30°C and quantified sperm numbers in young adults directly by visualizing the compact sperm nuclei within their spermathecae with DAPI staining. We found that both strains produce sperm in substantially greater abundance than their self-fertility would predict if fecundity at extreme temperatures were limited by sperm number (Fig. 4). Thus, the *C. briggsae* gonad of a given strain appears to produce a consistent number of sperm, regardless of rearing temperature. Separate experiments in *C. elegans* indicate that this holds true in both species (Murray 2009).

Given that it appears not to be sperm count per se that limits hermaphrodite fecundity at 14°C and 30°C in *C. briggsae*, at least four alternative hypotheses seem plausible. (i) Extreme temperatures might render sperm permanently defective and incapable of fertilization. (ii) Oogenesis might be impaired permanently by extreme temperatures experienced by the developing gonad. (iii) Hermaphrodites might modulate oogenesis facultatively (actively or passively) in response to temperature, despite the presence of numerous functional sperm in their reproductive tracts. (iv) Extreme temperatures might reversibly disrupt sperm fertility, motility, or oviposition-promoting cell-signaling. In an attempt to distinguish these alternatives, we performed temperature-shift experiments in which animals reared at extreme temperatures were shifted to moderate temperatures as young adults following spermatogenesis (and vice versa). Returning *C. briggsae* hermaphrodites to moderate temperature (20°C) from 14°C following the adult molt restored self-fecundity (although only partially for strain HK104). A down-shift from moderate to cold caused young adults to make few progeny. Thus, fecundity at cool-to-moderate temperatures primarily reflects the conditions experienced as an adult, and not as a developing larva (Fig. 5). By contrast, a shift from 30°C to moderate temperature failed to restore self-fecundity, and hermaphrodites up-shifted from 20°C to 30°C had fecundities comparable to when they were reared at 20°C for their entire lives. Thus, fecundity at high temperatures reflects predominantly the conditions experienced as a developing larva (Fig. 5). Components of male ejaculates (major sperm protein, MSP) stimulate oogenesis in *Caenorhabditis* (Miller et al. 2001), so we mated the 30–20°C shifted hermaphrodites to males that had been reared at 20°C to further decipher the cause of

reduced fertility of hermaphrodites reared at high temperatures. These mated hermaphrodites exhibited restored fecundity for both AF16 and HK104 when they received sperm from males. Consequently, we conclude that low temperatures largely induce reduced oogenesis in hermaphrodites in a facultative manner, with sperm mostly retaining competence to fertilize oocytes (hypotheses iii and possibly iv), whereas high temperatures cause a permanent detrimental impact on the fertility of developing sperm (hypothesis i), although high temperature does not adversely affect the fertility of mature spermatozoa.

The cool-to-moderate temperature-shift experiments are consistent with a reversible, oogenesis-based mechanism of reduced fecundity (hypothesis iii), but we cannot exclude a contributing role for sperm (hypothesis iv). The slowed oogenesis and overall lower fecundity at low temperatures, which fails to exploit for fertilization all sperm in the reproductive tract, could reflect impaired sperm signaling with the surrounding gonad (Miller et al. 2001). For example, this could occur if major sperm protein (MSP) has temperature-sensitive signaling activity and/or its receptors are less sensitive at low temperatures. Temperature-sensitive genetic mutations that specifically affect spermatids have been characterized in *C. elegans*, but result in immotile cells that do not activate into mature spermatozoa (Minniti et al. 1996). However, the retention of sperm in the reproductive tract of cold-reared animals that can then fertilize successfully upon return to warmer conditions suggests that sperm motility is not compromised to any great extent, because immotile sperm likely would be flushed out of the reproductive tract (Ward and Miwa 1978). The HK104 strain did not recover fertility as well as AF16 following the shift in temperature from 14°C to 20°C, suggesting that genetic differences between these strains might contribute to low-temperature recovery. We also did not observe excess unfertilized oocytes at low temperatures, which would be expected to result if oogenesis proceeded normally, but sperm were impotent.

In a stark contrast to the reversible effects of cold on fecundity, high temperatures effect permanent defects in sperm fertility (hypothesis i). Presumably even more extreme high temperatures could also detrimentally affect oogenesis (hypothesis ii), as seen in *C. elegans* gonads under extreme heat stress (Brun 1965), but this was not evident in our experiments. Further work is required to determine the cell-biological or molecular features that are disrupted by temperature extremes. A flurry of recently characterized temperature-sensitive defects in *C. elegans* sperm development, induced by endogenous small interfering RNA-related factors and other small RNA regulatory pathways (e.g., 21U-RNAs and PRG-1, 26G-RNAs and ALG-3/4), certainly provide provocative candidates for future work (Duchaine et al. 2006; Ruby et al. 2006; Batista et al. 2008; Wang and Reinke 2008; Conine et al. 2009; Gent et al. 2009; Gu et al. 2009; Han et al. 2009; Pavelec et al. 2009). With reference to the patterns we report in *C. briggsae*, *C. elegans* double-mutants of *alg-3* and *alg-4* are especially notable in that hermaphrodites make normal numbers of sperm, but experience severe spermiogenesis defects at high temperatures (Conine et al. 2009). Temperature-shift experiments with these *C. elegans alg-3; alg-4* mutants also yield strikingly similar results to the patterns we report, so it will be useful to test whether the single-copy orthologous candidate gene in *C. briggsae* (CBG09950 = *Cbr-tag-76*) might underlie natural temperature-dependent variation. Similarly, the temperature-dependent effects on self-fecundity for null mutations in *C. briggsae*'s sex-determination pathway gene *she-1* are highly suggestive (Guo et al. 2009).

Two additional potential explanations for reduced fecundity at extreme temperatures include greater rates of sperm loss or expulsion through the vulva and greater mortality among young zygotes. We did not evaluate directly the potential for temperature-dependent sperm expulsion/loss to occur. Temperature-sensitive laboratory mutants of *fer-1* affect sperm fertility in *C. elegans*, and appear to result in disproportionate sperm expulsion at high temperatures as a consequence of the immotility of the sperm (Ward and Miwa 1978). It is conceivable that extreme high temperatures impair sperm motility in nonmutant individuals, with their extrusion from the reproductive tract as a byproduct. We consider it unlikely, however, that fully motile sperm would suffer differential expulsion through the vulva at high temperatures. In addition, the fecundity of hermaphrodites shifted from 20°C to 30°C was normal, suggesting that high temperature disrupts sperm development rather than the function of mature spermatozoa. We did not observe large numbers of fertilized dead eggs that would be indicative of excessive embryonic mortality, so we find differential zygote mortality also to be an implausible explanation for the disparities in fecundity with temperature.

CONCLUSIONS

Latitudinal clines in phenotype provide some of the most striking examples of local adaptation. Here we document heritable, temperature-dependent differences in fecundity between latitudinally differentiated, phylogeographically distinct clades of the nematode *C. briggsae* in a manner consistent with local adaptation. We further determine that fecundity sensitivity has a different basis at low and high extreme temperatures, with high temperatures experienced by the developing gonad resulting in permanent defects in the fertility of developing sperm versus reversible effects of cold on oogenesis and possibly sperm fertility. With the encouraging recent success in other organisms (Abzhanov et al. 2008; Stinchcombe and Hoekstra 2008), this genetically tractable system holds great promise for characterizing the molecular basis for natural variation in temperature-dependent fitness and other traits (Kammenga et al. 2008).

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Supporting Information

The following supporting information is available for this article:

Table S1. Per-strain lifetime fecundity at each rearing temperature.

Supporting Information may be found in the online version of this article.