doi:10.1111/j.1558-5646.2008.00351.x

REPRODUCTIVE DIAPAUSE AND LIFE-HISTORY CLINES IN NORTH AMERICAN POPULATIONS OF *DROSOPHILA MELANOGASTER*

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Received July 20, 2007
Accepted January 29, 2008

Latitudinal clines are widespread in *Drosophila melanogaster*, and many have been interpreted as adaptive responses to climatic variation. However, the selective mechanisms generating many such patterns remain unresolved, and there is relatively little information regarding how basic life-history components such as fecundity, life span and mortality rates vary across environmental gradients. Here, it is shown that four life-history traits vary predictably with geographic origin of populations sampled along the latitudinal gradient in the eastern United States. Although such patterns are indicative of selection, they cannot distinguish between the direct action of selection on the traits in question or indirect selection by means of underlying genetic correlations. When independent suites of traits covary with geography, it is therefore critical to separate the widespread effects of population source from variation specifically for the traits under investigation. One trait that is associated with variation in life histories and also varies with latitude is the propensity to express reproductive diapause; diapause expression has been hypothesized as a mechanism by which *D. melanogaster* adults overwinter, and as such may be subject to strong selection in temperate habitats. In this study, recently derived isofemale lines were used to assess the relative contributions of population source and diapause genotype in generating the observed variance for life histories. It is shown that although life span, fecundity and mortality rates varied predictably with geography, diapause genotype explained the majority of the variance for these traits in the sampled populations. Both heat and cold shock resistance were also observed to vary predictably with latitude for the sampled populations. Cold shock tolerance varied between diapause genotypes and the magnitude of this difference varied with geography, whereas heat shock tolerance was affected solely by geographic origin of the populations. These data suggest that a subset of life-history parameters is significantly influenced by the genetic variance for diapause expression in natural populations, and that the observed variance for longevity and fecundity profiles may reflect indirect action of selection on diapause and other correlated traits.

KEY WORDS: Aging, clines, diapause, *Drosophila*, life span, trade-offs.

The evolutionary response of populations to environmental variability has long been of fundamental interest in organismal biology. Given the future potential for pronounced environmental fluctuations and climate change, understanding the mechanisms and dynamics of selection in heterogeneous environments remains significant from both basic and applied perspectives. Clines have widely been used to examine the dynamics of selection over environmental gradients at a variety of spatial scales, ranging from meters (e.g., Johannessen et al. 1995) to hundreds of kilometers (e.g., Gilchrist et al. 2004). Furthermore, clines can be very responsive to changes in environmental variables over short timescales, thus providing a tractable method for assessing evolutionary response in natural populations (Bradshaw and Holzapfel 2001; Umina et al. 2005).

In *Drosophila melanogaster,* latitudinal clines have been documented for a large number of traits and loci on multiple continents (e.g., Berry and Kreitman 1993; Karan et al. 1998; Robinson et al. 2000; Hoffmann et al. 2002; Sezgin et al. 2004; Trotta et al.

2006). *Drosophila melanogaster* is native to tropical Africa and has established populations in both Australia and North America in the relatively recent past (David and Capy 1988); in the context of substantial gene flow among populations on both continents (Coyne and Milstead 1987; Gockel et al. 2001; Kennington et al. 2003), significant clines have been interpreted as resulting from adaptation to the climatic variation that exists among habitats in these regions (e.g., Sezgin et al. 2004; Hoffmann and Weeks 2007). However, genetic correlations among traits may result in patterns that reflect the indirect, rather than direct, action of selection; suites of genetically correlated traits may covary with geography and environment, resulting in multiple clines that are not independent. Although the work on stress resistance variation among Australian populations has served as a model for the comprehensive dissection of latitudinal patterns (Hoffmann et al. 2001, 2002; Bettencourt et al. 2002; Frydenberg et al. 2003), the specific environmental variables and mechanism of selection remain elusive for many documented *Drosophila* clines.

The general population dynamics of *D. melanogaster* in the wild, which are integral to both the generation and maintenance of clines, are not fully resolved. Although a tropical endemic, there is increasingly abundant information that suggests adults can overwinter in the temperate habitats they now occupy on multiple continents (e.g., Saunders et al. 1989; Mitrovski and Hoffmann 2001; Bouletreau-Merle et al. 2003; Hoffmann et al. 2003; Schmidt and Conde 2006). This supports inferences of temporal population continuity in temperate regions (Ives 1970; Gravot et al. 2004). The occurrence of an overwintering strategy in this tropical insect would have widespread impacts on the ecology of *D. melanogaster* populations and the dynamics of selection in the wild. Here, we focus on the potential impacts of differential overwintering success on the generation and maintenance of latitudinal clines in North American populations. This connection is based on the following assumptions: (1) the failure to successfully overwinter would be associated with low organismal fitness in temperate habitats, depending on dispersal potential and the occurrence of refugia in these regions; (2) traits associated with overwintering survivorship are variable within and among populations; and (3) the traits associated with overwintering success are genetically correlated with other life-history traits in *Drosophila* (Schmidt et al. 2005b) and are associated with fitness trade-offs (Hoffmann et al. 2003; Schmidt and Conde 2006).

Reproductive diapause was described in *D. melanogaster* by Saunders et al. (1989); as diapause is a classic adaptation to seasonality in a variety of insects and other arthropods (Denlinger 2002; Danks 2006b), the expression of diapause may be associated with overwintering survivorship in temperate habitats. Subsequent work on diapause in *D. melanogaster* supports this. Induction of the trait by exposure to low temperature and shortened daylength results in reproductive quiescence, delayed senescence, and life span extension, and increased stress resistance (Tatar et al. 2001). Similarly, the propensity of a given genotype to express diapause is genetically correlated with a number of life-history traits including life span, mortality rates, stress resistance, development time, and fecundity profiles (Schmidt et al. 2005b). The incidence of diapause expression in natural populations exhibits both a significant latitudinal cline in the eastern United States (Schmidt et al. 2005a) and varies with season in temperate orchard populations (Schmidt and Conde 2006). Thus, diapause expression may be a critical component of *Drosophila* life histories in the novel climatic environments this insect now experiences. The inference of strong selection on the variance for diapause expression, and the distinct genetic correlations among life-history traits for diapause and nondiapause genotypes, suggests that reproductive diapause may also be an important determinant of some latitudinal clines among habitats in eastern North America.

Here, we investigate patterns of basic life-history variation among six populations along a latitudinal gradient in the eastern U.S. Despite the extensive documentation of latitudinal clines in this species, there is relatively little information regarding how life span, rates of aging, and patterns of reproduction vary with environment or geography (but see Boulétreau-Merle et al. 1982; Mitrovski and Hoffmann 2001; Hoffmann et al. 2003; Trotta et al. 2006). Each of the populations assayed here was variable for diapause incidence, allowing the partitioning of the observed variance for life histories into the effects of geography, diapause genotype, and the interaction term. It was of particular interest for the current study to examine the relative effects of diapause genotype versus population source on any observed variance for basic life-history components. It should be noted that in a study such as this that uses isofemale lines recently derived from nature, the effects of diapause genotype cannot be separated from that of genetically correlated traits; however, the effects of independent suites of correlated traits, which vary with geography, can be differentiated. For two relative measures of stress resistance and reproductive profiles, both diapause genotype and the geographic locations of the source populations were found to have a significant effect; however, estimates of longevity and mortality rates over male and female life span were discretely partitioned between diapause genotypes whereas the effects of geographic source were negligible.

Materials and Methods

ISOFEMALE LINE COLLECTIONS

Drosophila melanogaster females were collected over a two-week period from all populations by a combination of baiting/sweeping and trapping. As diapause incidence, and presumably correlated traits, vary temporally, it was deemed critical to sample populations over a narrow temporal interval. Individual gravid females were placed in single vials in the field to establish isofemale lines, and the resulting male offspring were examined for species designation. The logistics of this sampling and the resulting life-history analyses precluded sampling an extensive series of populations across the latitudinal gradient in the eastern U.S. as was done previously (Schmidt et al. 2005a); sampling efforts were instead focused on three geographic regions that are differentiated with respect to a number of traits (e.g., Verrelli and Eanes 2001; Sezgin et al. 2004). Two replicate populations were collected from each of the three sampled geographic regions, which were labeled north, mid-Atlantic, and south. It should be noted that the two populations sampled within each region may not represent truly independent populations, as they were separated by less than 30 km. The northern populations sampled were from Rocky Ridge Orchards (Bowdoinham, ME, 44°2'N, 69°52'W) and Biscay Farms (Damariscotta, ME, 44°1'N, 69°31'W). Southern populations sampled were from the Fruit and Spice Park (Homestead, FL, 25°31'N, 80°24'W) and from the Farmer's Market (Homestead, FL 25°22'N, 80°28'W). These sampled regions approximate the northern and southern range limits for *D. melanogaster* along the latitudinal gradient in the eastern United States. The mid-Atlantic region was represented by isofemale lines sampled from Terhune Orchards (Princeton, NJ, 40°21'N, 74°39'W) and Solebury Orchards (New Hope, PA, 40°22'N, 74°57'W). This mid-Atlantic region was selected for sampling because it is intermediate with respect to various temperature-related climatic factors (e.g., the 40-year mean number of days/year that the minimum temperature is at or below 0°C; www.ncdc.noaa.gov) that may affect adult overwintering. Previous examination of diapause incidence in populations in the eastern U.S. also suggested that this region represented a step point in the observed diapause incidence cline, again being intermediate with respect to the populations from southern Florida and mid-coastal Maine (Schmidt et al. 2005a).

LIFE HISTORIES

Isofemale lines from all populations were passed through two generations of culture on standard cormeal-molasses *Drosophila* medium with a 14-day generation time at 25°C and a photoperiod of 12L:12D. In the third generation, flies were transferred to fresh vials and females allowed to oviposit for 4 h before being transferred again to fresh vials; density was standardized among lines as 20 ± 5 eggs/vial. Fifty lines from each of the six populations were randomly selected for longevity and fecundity measurements. Once flies began to eclose from the low-density vials, virgin males and females were collected over a 4-h period from each line. One male and one female from each line were placed in a single vial and transferred to fresh vials every 2 days. The remaining flies of the cohort from each line were used for replacement of either a male or female upon a mortality event. Vials were transferred until both original flies had died, and age of each fly at death was recorded. The total number of eggs produced was counted over each transfer interval for the life span of each original female.

Survivorship data were analyzed with a proportional hazards model for factorial analysis of main and interaction effects. Mortality rate (m_x), was calculated as $-\ln(1 - q_x)$, where $q_x = d_x/N_x$, d_x is the number of dead flies observed over the period between time x and time $x + 1$, and N_x is the number of live flies at time x. Total fecundity was analyzed with a mixed model analysis of variance. For per capita fecundity, the data are longitudinal and individual female egg counts among the time intervals are nonindependent; however, a standard repeated measures analysis could not be used due to the widespread female mortality during the experiment. To account for nonindependence in the data, only a random subset (1 in 4) of time intervals were used for a given female. The data were then analyzed with an analysis of covariance, with female age as the covariate; the interaction terms were then used to evaluate whether patterns of fecundity differed over the life span among regions and/or between genotypes. All statistical analyses were conducted in JMP version 5 (SAS Institute, Cary, NC).

STRESS RESISTANCE

In the third generation of culture, each of the 300 isofemale lines was split into eight replicate vial cultures. In the following generation, flies from all replicates were transferred to fresh vials, females allowed to oviposit for 1 h, and adults subsequently transferred again. Upon eclosion of flies in the replicate low-density vials, virgin males and females were collected in 4 h cohorts over a 12-h window across all replicates for each isofemale line. For each line, experimental flies were pooled across replicates and placed in mixed sex groups of 20 in fresh vials. Flies were aged to 5 days and then assayed for stress resistance. In the cold shock assay, 20 flies of each sex from each line were exposed to −15°C for a period of 45 min. Flies were then transferred to fresh vials and allowed to recover at 25°C for 24 h, at which time mortality events were tabulated for each sex and isofemale line combination (total $N = 10{,}936$ flies scored). All assayed isofemale lines were assayed for cold shock resistance simultaneously. The assay for heat shock resistance consisted of exposure to 38°C for 105 min; pilot studies indicated that this combination of temperature and duration of exposure resulted in informative patterns of mortality across the sampled populations. Twenty flies of each sex from each line were transferred to empty culture vials and placed in circulating water baths. After the stress exposure, flies were transferred to fresh culture vials and allowed to recover for a period of 24 h; mortality data were then recorded ($N = 11{,}498$). All lines were again assayed simultaneously for heat shock resistance. Mortality data for both heat and cold shock were analyzed with nested analyses of variance in JMP version 5 (SAS Institute). In all life-history trait analyses, both population within region and isofemale line were treated as random predictor variables.

DIAPAUSE PHENOTYPING

After establishment of the life history and stress assays, all lines were phenotyped for reproductive diapause in the sixth generation of laboratory culture. Approximately 20 virgin females were collected from each of the 300 isofemale lines within 2-h post-eclosion and placed at 11°C with a photoperiod of 10-h light: 14-h dark. Four weeks after exposure to the diapause-inducing conditions of low temperature and short days, females were dissected and the development of the ovaries assessed. A fly was scored as diapausing if the most advanced oocyte was ≤ stage 7 according to King (1970). Because the experimental lines were isofemale collections with an unknown number of segregating chromosomes within each line, some of the isofemale lines were segregating for both diapause and nondiapause alleles. In these instances, a line was scored as a diapause genotype if the majority of dissected flies were nonvitellogenic (≤ stage 7) and as a nondiapause genotype if the majority of females were vitellogenic. The inclusion of these variable lines was predicted to inflate the error variance associated with each genotypic class by region combination; thus, this was considered to be a more conservative approach, relative to the exclusion of variable isofemale lines, in determining the effect of diapause genotype on life-history profiles.

All life-history analyses were conducted on experimental individuals that were cultured and maintained under normal rearing conditions (25°C, 12L:12D): flies were not exposed to environmental cues that would elicit diapause expression. Thus, the experiments were not designed to assess the effects of actual diapause expression (i.e., diapause phenotype) on life histories. Rather, the emphasis was on evaluating the effects of the underlying genetic variance for diapause expression (i.e., diapause genotype) in a series of natural populations on constitutive life-history profiles.

Results

LIFE SPAN

Mean life span for both males and females varied predictably with geography, and varied inversely with total lifetime fecundity (Fig. 1). Similarly, survivorship curves for both males and females were highly variable among regions, with the survivorship profiles being most distinct between the northern and southern collections (Fig. 2). Ignoring the predictor variable of diapause genotype, analysis of life span with a proportional hazards model revealed significant variation among geographic regions (df = 2, LR $\chi^2 = 21.92, P < 0.001$), between replicate populations within region (df = 3, LR $\chi^2 = 13.56, P < 0.004$) and between sexes (1 df = 1, LR $\chi^2 = 6.79, P < 0.01$). However, diapause incidence varied significantly and predictably with geographic origin of the sampled populations, as expected. For the lines assayed here, the frequency of diapause genotypes pooled across replicate populations was 0.802 for the northern, 0.561 for the mid-Atlantic, and 0.310 for the southern region. When diapause genotype was included in the proportional hazards model, the main effect of diapause genotype was highly significant whereas the effect of geographic region was not (Table 1). In addition, longevity again varied between replicate populations sampled within regions as well as between males and females.

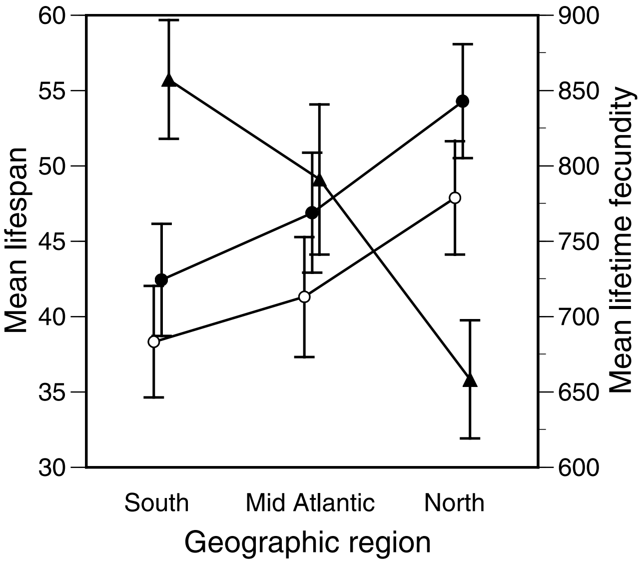

Figure 1. Mean (± SE) life span for males (filled circles) and females (open circles) in the three sampled geographic regions. Mean (± SE) lifetime reproductive output is plotted on the secondary y-axis, and is denoted by triangles. Lines simply connect points for graphical presentation.

Survivorship curves and transformed mortality rates for diapause and nondiapause genotypes, pooled across all populations, are plotted in Figure 3. As with the life span data for the geographic regions, these curves are distinct between diapause and nondiapause genotypes; the difference in curve topology is more pronounced between diapause and nondiapause genotypes than it is between any two geographic regions. In addition to mean, median, and maximum life span, age-specific mortality rates were also observed to be highly divergent between diapause and nondiapause genotypes (Fig. 3B). Age-specific female mortality rates between the two genotypic classes were nonoverlapping, and were most distinct early in life; patterns for males were similar.

FECUNDITY

Lifetime reproductive output showed a trend with geography, decreasing with increasing latitude (means plotted in Fig. 1). When

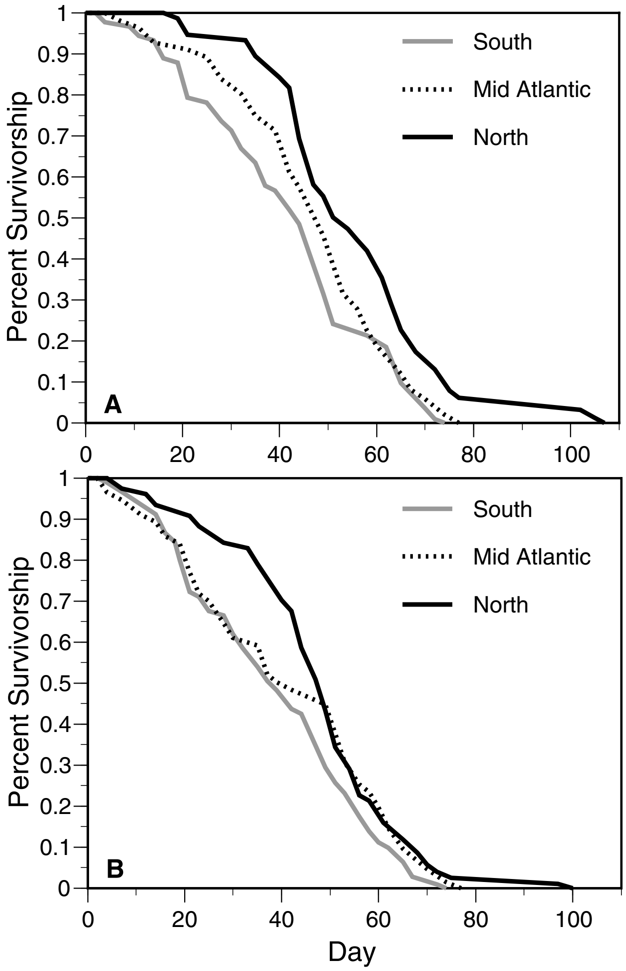

Figure 2. Survivorship curves for isofemale line populations from the three sampled geographic regions; data for males are plotted in panel A, females in panel B. Data were pooled for replicate populations within regions.

total fecundity was analyzed with an analysis of variance, however, none of the predictor variables had a significant effect on lifetime fecundity (Table 2); this was true whether diapause genotype was included as a predictor variable or not. The planned contrast between means for the northern and southern region was also nonsignificant, but marginally so ($F = 5.176$, $P < 0.084$).

Table 1. Proportional hazards analysis of life span.

Source	Df	LR Chi Square	*P*
Region	2	1.38	0.503
Population [region]	3	8.43	0.038
Diapause genotype	1	68.30	0.0001
Sex	1	10.19	0.0014
Region × diapause genotype	2	0.47	0.793
Phenotype × sex	1	0.20	0.658

In contrast to total reproductive output, patterns of per capita fecundity over the life span varied substantially among females from the three geographic regions and between diapause genotypes (Table 3). Females from the southern region laid more eggs per day through day 26 of life, with the magnitude of the difference being most pronounced between females from the northern and southern regions (Fig. 4A). As with the analysis of life span, differences in patterns of per capita fecundity were more consistently distinct between diapause and nondiapause genotypes than they were between any two sampled regions (Fig. 4B). Across populations and regions, nondiapause genotypes laid more eggs per day for the first 51 days of life; after this point, the trend reversed and remained constant through the end of the experiment.

The distinct patterns observed for total lifetime and per capita fecundity reflect two underlying causes. First, although nondiapause genotypes had higher per capita fecundity early in life, early life mortality rates were also elevated in comparison to diapause genotypes. Thus, lifetime reproductive output was statistically equivalent although relative reproductive output across the life span differed between genotypes as well as geographic regions. Second, lifetime fecundity is affected by the timing of female mortality events, whereas per capita fecundity reflects only the reproductive output of females that have survived to a particular time point. Due in part to widespread female mortality, particularly early in life, the observed variance for lifetime fecundity greatly exceeded that associated with per capita fecundity (Tables 2 and 3).

STRESS RESISTANCE

Resistance to both heat and cold shock also exhibited a trend with geography: resistance to both stressors increased positively with latitudinal origin of the sampled populations (Fig. 5). Although geography had a similar effect on stress resistance, the variation between diapause and nondiapause genotypes was quite distinct for heat and cold shock resistance. Resistance to heat stress did not significantly vary among regions, between replicate populations, or between diapause and nondiapause genotypes (Table 4). However, the effects of geography and population were marginally nonsignificant, and northern flies exhibited higher survivorship under heat stress than did flies from southern regions ($F_{1,3} = 15.11$, $P < 0.04$). As expected, there was a great deal of heterogeneity in heat stress resistance among isofemale lines within the sampled populations. Resistance to elevated temperatures was also influenced by sex, with females being slightly but significantly more resistant than males (Table 4).

In contrast to resistance at elevated temperature, exposure of experimental flies to low temperatures resulted in patterns of mortality that were nonrandom with respect to geography (Table 5). Most of the observed variance in cold shock resistance, as with heat resistance, was due to variation among isofemale

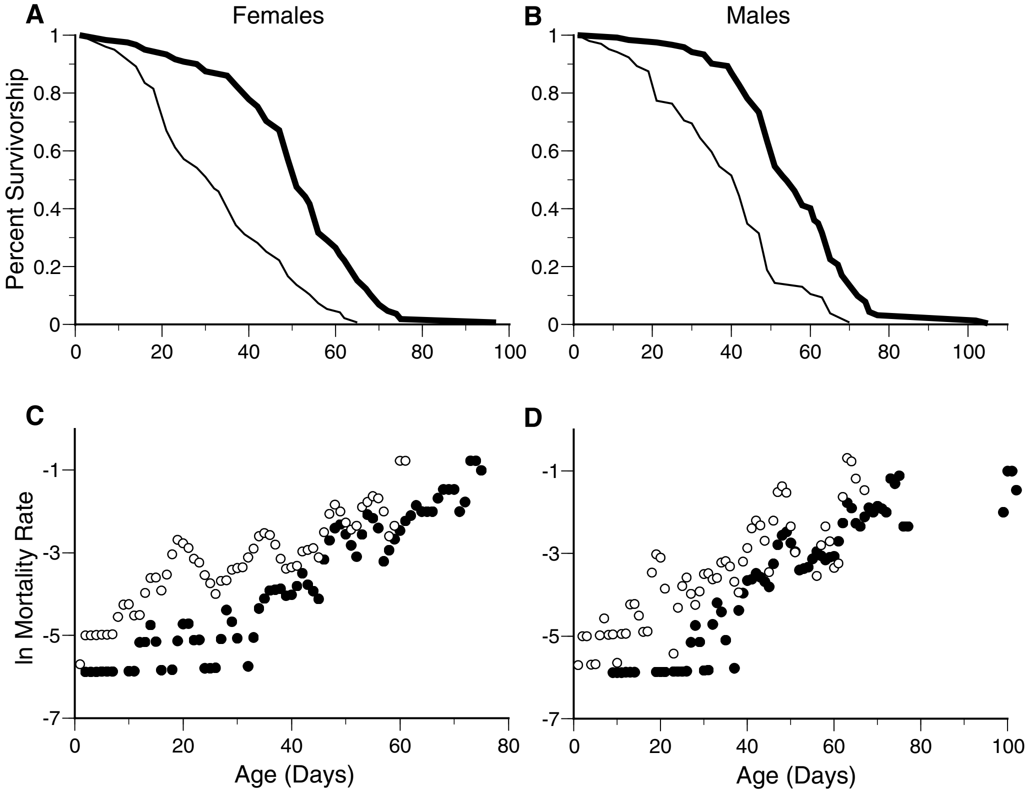

Figure 3. Percent survivorship (A, B) and transformed mortality rates (C, D) as a function of age for diapause and nondiapause genotypes pooled across all sampled populations. Diapause genotypes are denoted by thick lines and filled symbols, nondiapause genotypes by thin lines and open symbols.

lines sampled within populations. Diapause genotype was also observed to have a large impact on cold shock resistance, and the difference between diapause and nondiapause genotypes was variable among the three sampled regions (Table 5). The interactions between population source, diapause genotype, and sex on patterns of stress resistance are depicted in Figure 6. Geography had widespread and consistent effects on patterns of stress resistance, whereas diapause genotype and the interaction between diapause genotype and region were of major effect for cold shock resistance only.

Table 2. Nested analysis of variance for total lifetime fecundity with population as a random effect.

Source	Df	SS	*F*	*P*
Region	2	862,785	2.59	0.188
Population [region]	3	518,709	1.28	0.283
Diapause genotype	1	129,464	0.96	0.329
Region × diapause genotype	2	70,831.3	0.26	0.769
Error	197	26,646,217		

Discussion

STRESS RESISTANCE

Both measured aspects of stress resistance exhibited latitudinal clines, as might be expected given the robust patterns observed in other populations. Along the latitudinal gradient in eastern Australia, high and low temperature resistance demonstrate

Table 3. Analysis of covariance for subsampled per capita fecundity over the female life span.

Source	df	SS	*F*	*P*
Region	2	23,688.7	9.91	<0.0001
Diapause genotype	1	20,756.0	17.36	<0.0001
Age	1	285,214.7	238.59	<0.0001
Region × diapause genotype	2	4006.4	1.68	0.1876
Age × region	2	14,818.0	6.20	0.0021
Age × diapause genotype	1	6743.5	5.64	0.0177
Age × region × diapause genotype	2	3591.3	1.50	0.2231
Error	1140	1,362,781.3		

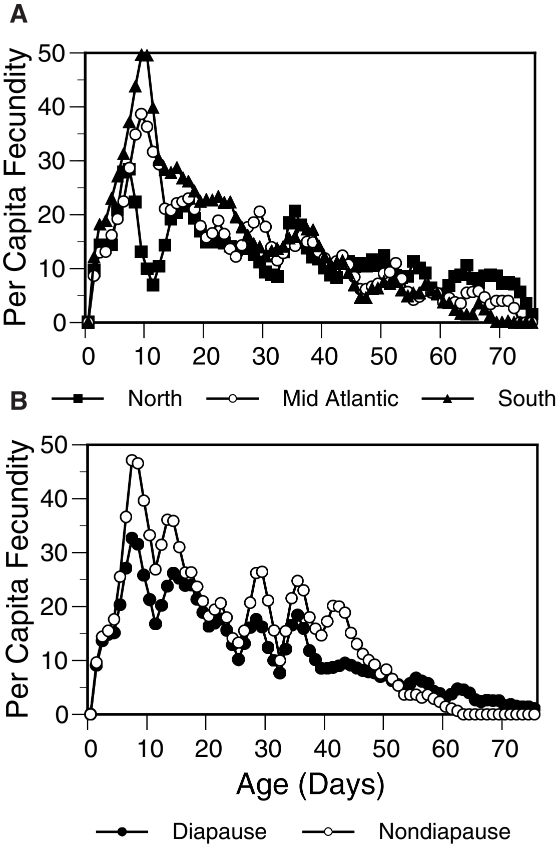

Figure 4. Mean per capita fecundity as a function of age for females sampled from the three geographic regions (A) and for diapause / nondiapause genotypes pooled across all regions (B). Data are plotted as a sliding window of 3-day means.

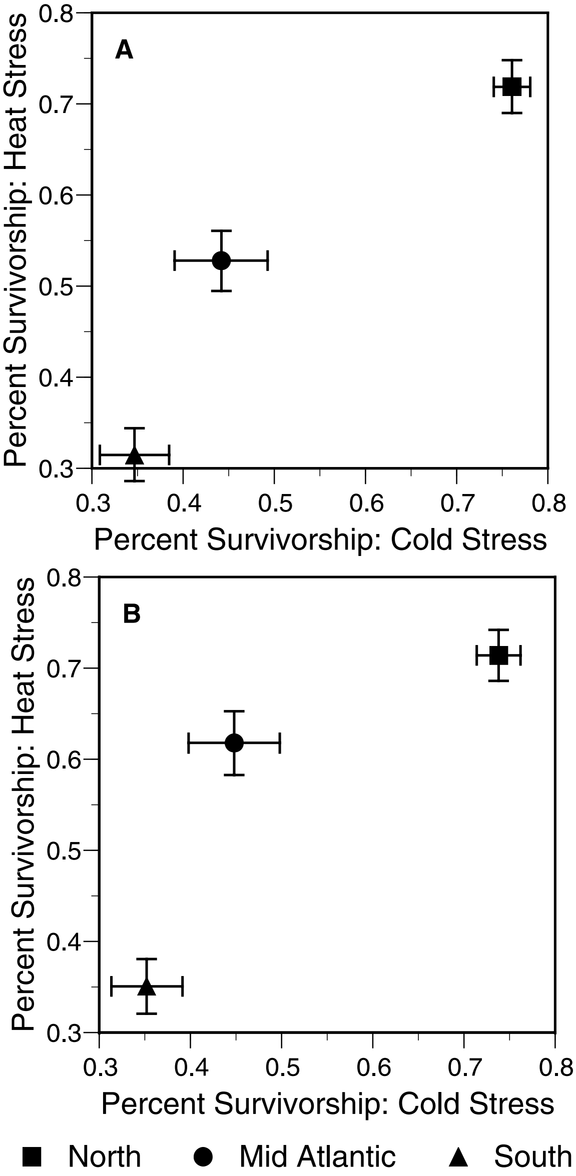

Figure 5. Mean (± SE) percent survivorship in heat and cold stress assays for males (A) and females (B) from the three sampled geographic regions. Data are pooled across replicate populations within regions.

opposing clines; aspects of cold tolerance increase positively with latitude, whereas heat shock resistance decreases (Hoffmann et al. 2002). Similarly, a trade-off has been shown to exist for cold and starvation resistance (Hoffmann et al. 2005). Trade-offs among resistance-related traits have also been documented on the Indian subcontinent, where desiccation and heat resistance exhibit opposing latitudinal clines in three drosophilid species (Karan et al. 1998). Although such traits are quantitative in nature, several factors have been identified as contributing to the observed clines (Bettencourt et al. 2002; Anderson et al. 2003; Frydenberg et al. 2003; Kennington et al. 2006).

In the present study, we observed coincident clines for heat and cold shock resistance. The elevated resistance of temperate populations to heat shock was contrary to our predictions based on patterns observed in other populations (Karan et al. 1998; Hoffmann et al. 2002) and the associations between heat resistance and fitness in distinct habitats (Feder 1999; Michalak et al. 2001). The distinct patterns observed in our data compared to other studies may simply reflect differences in experimental methodologies or idiosyncrasies associated with the populations sampled. However, the increase in heat shock resistance as a function of latitudinal origin may also reflect a general pattern of increased stress resistance in these temperate populations and/or the underlying genetic correlations between high temperature resistance and other traits such as life span. The positive association between longevity and stress resistance has been well described in multiple taxa including *Drosophila* (reviewed in Vermeulen and Loeschcke 2007). Specifically, elevated levels of the heat shock protein Hsp 70 directly result in subsequent life span extension (Tatar et al. 1997).

Regardless, the patterns observed in the current study are clear: diapause genotype did not affect resistance to high

Table 4. Nested analysis of variance for transformed percent survivorship in heat stress assays with population and line as random effects.

Source	df	SS	*F*	*P*
Region	2	0.550	7.73	0.066
Population [region]	3	0.252	2.36	0.072
Line [population, region]	275	64.765	6.62	0.001
Diapause genotype	1	0.002	0.05	0.827
Sex	1	0.365	10.27	0.002
Region × diapause genotype	2	0.029	0.41	0.665
Error	277	110.310		

temperatures, whereas this trait significantly varied with geographic origin of the populations assayed. This suggests that the genetic variance for diapause expression in natural *D. melanogaster* populations does not contribute to the heat shock response. However, these assays were conducted on experimental flies that were not exposed to diapause-inducing conditions. Heat shock proteins are associated with diapause expression (Denlinger 2002) and cold resistance during diapause (Rinehart et al. 2007). Similarly, the induction of diapause in *D. melanogaster* results in increased resistance to subsequent heat shock (Tatar et al. 2001). Thus, although diapause and nondiapause genotypes were not observed to differ in constitutive resistance to elevated temperature, the actual expression of diapause would be predicted to result in phenotypic differentiation between genotypes.

In contrast to heat resistance, the patterns observed for cold shock resistance were in accord with prediction. It was hypothesized that populations in temperate regions are either resident and overwintering or experience selection based on cold resistance in refugia habitats: thus, it was predicted that phenotypes associated with cold stress resistance would increase positively with latitude. As with the assay for heat shock resistance, the cold shock assay was designed as a relative measure that did not accurately mimic stress exposure in natural habitats. Although cold shock resistance and other measures of cold tolerance, such as chill coma recovery, are distinct phenotypes, there is a positive association between them; for example, intermittent selection for chill coma recovery time resulted in increased resistance to cold shock (Anderson et al. 2005).

Table 5. Nested analysis of variance for transformed percent survivorship in cold stress assays with population and line as random effects.

Source	df	SS	*F*	*P*
Region	2	0.610	18.32	0.021
Population [region]	3	0.007	0.15	0.933
Line [population, region]	238	72.880	18.39	0.0001
Diapause genotype	1	1.246	74.81	0.0001
Sex	1	0.0001	0.01	0.935
Region × diapause genotype	2	0.147	4.40	0.013
Error	242	4.030		

Cold shock resistance was observed to vary both with geography and between diapause and nondiapause genotypes. The magnitude of the difference between these genotypes decreased with increasing latitude; in the northernmost populations, where exposure to low temperatures is most extreme and of the longest duration, the observed difference was least. Furthermore, nondiapause genotypes from northern populations were more cold resistant than were diapause genotypes from southern populations. As expected, the data suggest that there are multiple factors, one of these being the genetic variance for diapause expression, contributing to the variation for cold shock resistance in natural populations (Morgan and Mackay 2006). The physiological basis for the cold shock response is not well understood, but observed genetic variance for cold shock resistance may be related to variation in membrane properties and lipid composition (Michaud and Denlinger 2006; Overgaard et al. 2006; Shreve et al. 2007), cytoskeletal fortification (Kim et al. 2006), carbohydrate cryoprotectants (Kimura et al. 1992; Kelty and Lee 2001), and constitutive polyol content (Yoder et al. 2006). The association between diapause and cold resistance has been demonstrated in a variety of insects (Danks 2005, 2006a). In comparison to the heat shock and high temperature stress, the physiological and evolutionary responses to cold stress have not been extensively characterized in *Drosophila*. However, candidate genes for cold resistance traits have been identified (Goto 2001; Anderson et al. 2003; Greenberg et al. 2003; Qin et al. 2005). Our results suggest that some of these may also exhibit latitudinal allele frequency clines in North American populations.

LONGEVITY AND FECUNDITY

Patterns of longevity and fecundity were tightly coupled in the sampled populations. This association has been well documented (e.g., Rose 1984; Tatar et al. 1996; Sgrò and Partridge 1999), suggesting a general negative correlation between early investment in reproduction and subsequent survivorship in later life (e.g., Partridge and Gems 2002). Both of these primary life-history components were observed to vary substantially and predictably with geography, as expected (Mitrovski and Hoffmann 2001; Trotta et al. 2006). What is remarkable, however, was how much of the variance in longevity and fecundity was explained by diapause genotype, and that the effects of geographic origin either became nonsignificant (longevity) or were greatly reduced (fecundity profiles) when this factor was taken into consideration. In *D. melanogaster*, genetic analyses of life span have indicated that the

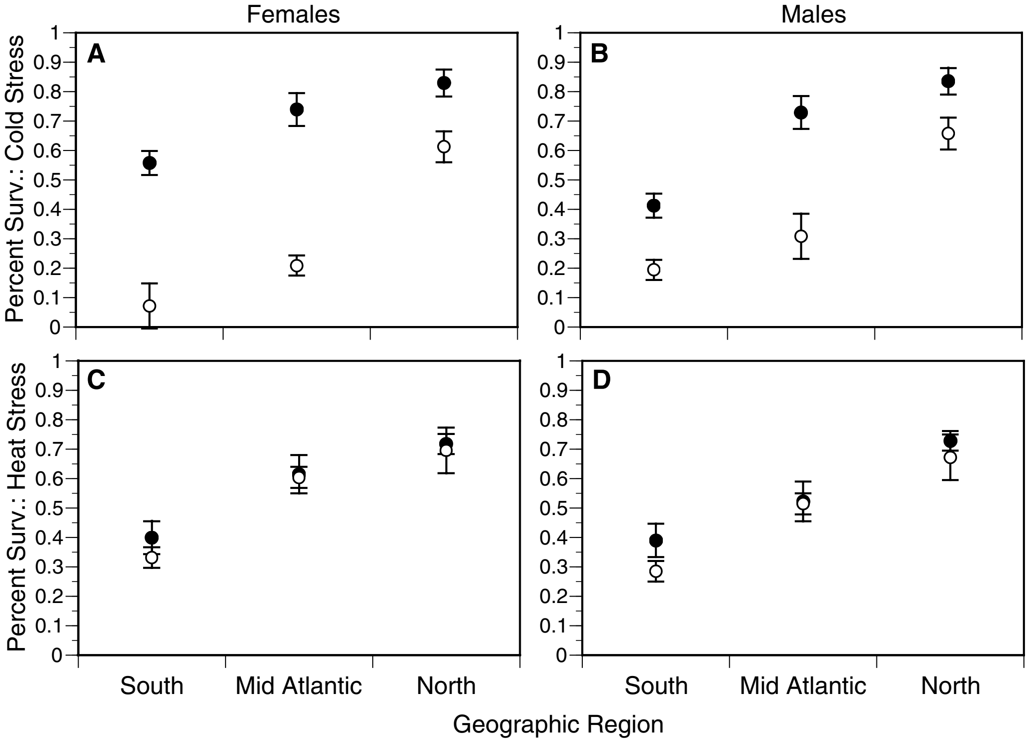

Figure 6. Geographic variation in resistance to cold shock (A, B) and heat (C, D) stress for diapause (filled symbols) and nondiapause (open symbols) genotypes from the three sampled geographic regions. Data are plotted as means (± SE). Female survivorship values depicted in A, C and males are given in B, D.

trait is highly complex, with identified genetic factors exhibiting both sex- and environment-specific effects (Nuzhdin et al. 1997; Wilson et al. 2006). Despite this inherent complexity and variability for longevity phenotypes, survivorship profiles were discretely partitioned between diapause and nondiapause genotypes across all sampled populations; somewhat surprisingly, diapause genotype was a better predictor of life span than was sex. The survivorship curves and mortality rate profiles are reminiscent of comparisons between mutants that confer life span extension and progenitor genotypes (e.g., Li et al. 1998; Rogina et al. 2000; Clancy et al. 2001; Hwangbo et al. 2004).

In fact, evidence suggests that the observed variance for diapause expression may be governed by one to several genes. Reciprocal backcross analyses suggested that high diapause expression segregates as a single autosomal factor (Williams and Sokolowski 1993; Schmidt et al. 2005a). This apparently simple pattern of inheritance is consistent with the hypothesis that diapause in *D. melanogaster* is of recent evolutionary origin (Saunders et al. 1989; Saunders and Gilbert 1990), and may not be characterized by the genetic complexity that often characterizes physiological diapause in other insects (e.g., Denlinger 2002). Although diapause is common among temperate insect taxa, the phenotype, as well as other aspects of seasonal adaptation such as cold tolerance, have evolved multiple times independently (Danks 2006b).

Recently, two candidate genes for diapause in *D. melanogaster* have been identified. A derived allelic variant of the *Drosophila* clock gene timeless has increased in frequency in temperate European populations and is associated with increased diapause expression (Tauber et al. 2007), providing a link between circadian rhythms and seasonal responses that was initially investigated by Saunders et al. (1989). Similarly, Williams et al. (2006) demonstrated that experimental reduction in the expression of *dp110* (PI3-kinase), a gene in the insulin-insulin like signaling pathway, results in an increase in diapause expression. The homolog of *dp110* in *C. elegans*, *age-1*, has pronounced effects on life span and aging (e.g., Morris et al. 1996). The study of Williams et al. (2006) provided a mechanistic link between the dauer phenotype in *C. elegans* and reproductive diapause in *D. melanogaster*, and suggested that diapause may be regulated by insulin-like signaling (Tatar and Yin 2001). The effects of genes in the insulin signaling pathway on aging and correlated phenotypes has been well documented in multiple taxa (reviewed in Tatar et al. 2003); thus, the functional difference between diapause and nondiapause genotypes observed here may be due to underlying variation in insulin signaling.

Together, the genetic analyses of reproductive diapause in *D. melanogaster* suggest that the results observed here may be the result of allelic effects at a small number of loci: genotypes that express diapause are associated with reduced expression of identified candidate genes. Previous studies indicated that the variation for the diapause phenotype is maintained by fitness trade-offs and selection pressures that vary in natural habitats both spatially with geography and temporally with season (Schmidt and Conde 2006). The results of the current study therefore suggest that at least a portion of the variance for longevity and correlated traits may be generated in a manner consistent with the model of antagonistic pleiotropy (Williams 1957). Diapause genotypes are more stress resistant, longer lived, and are at increased frequency following the winter season; however, nondiapause genotypes, which predominate in neotropical locales and increase in frequency over the summer season, are more fecund and are characterized by a faster rate of development (Schmidt et al. 2005b). As diapause genotype is shown here to be a powerful predictor of life-history profiles in natural populations, selection on diapause expression would be predicted to result, indirectly, in substantial variance for the life-history traits assayed. However, the results presented here merely demonstrate that diapause genotype is a predictor of life histories, not necessarily a causative agent in differentiating life histories in natural populations. This mechanistic link would require direct manipulations and analyses of the gene(s) that determine diapause expression.

SUMMARY

Drosophila melanogaster has been widely used as a model system in which to examine the adaptive response to temperature extremes and climatic variation. Here, it is shown that a number of life-history traits vary significantly with geographic origin in recent collections of North American isofemale lines. However, diapause genotype also explained a significant amount of the observed variance for life-history profiles, with the exception of heat shock resistance. These data suggest that the variation for reproductive diapause is a fundamental yet overlooked component of generalized life histories in natural populations of *D. melanogaster*. In particular, the underlying genetic variance for diapause has widespread effects on traits associated with overwintering and low temperature tolerance. Given the recent identification of candidate genes for reproductive diapause, this trait is a promising model system for the comprehensive analysis of the adaptive response to environmental heterogeneity.

ACKNOWLEDGMENTS

We thank A. Gonzalez-Sulser for assistance in performing heat stress assays. This work was supported by grants from the U.S. National Science Foundation and the American Federation for Aging Research.

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Associate Editor: D. Promislow