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GENETIC VARIANCE FOR DIAPAUSE EXPRESSION AND ASSOCIATED LIFE HISTORIES IN *DROSOPHILA MELANOGASTER*

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Abstract.—The dipteran *Drosophila melanogaster* can express a form of reproductive quiescence or diapause when exposed to low temperature and shortened photoperiod. Among natural populations in the eastern United States, the frequency of lines that express reproductive diapause in the laboratory varies substantially and predictably with latitudinal origin. The goals of the present study were twofold: (1) to examine the impact of genetic variance for diapause expression on multiple traits associated with organismal fitness; and (2) to evaluate the potential for fitness trade-offs between diapause and nondiapause phenotypes that may result in the observed cline. Even prior to diapause entry or expression, inbred lines that express and do not express reproductive diapause in laboratory assays were constitutively distinct for life span, age-specific mortality rates, fecundity profiles, resistance to cold and starvation stress, lipid content, development time, and egg-to-adult viability. Furthermore, estimates of genetic correlations based on line means revealed significant differentiation for genetic variance/covariance matrices between diapause and nondiapause lines. The data indicate the potential for life-history trade-offs associated with variation for the diapause phenotype. The observed cline in diapause incidence in the eastern United States may be generated by these trade-offs and the associated spatial and/or temporal variation in relative fitness of these two phenotypes in natural populations.

Key words.—Diapause, *Drosophila*, genetic correlation, life history, trade-off.

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Environmental heterogeneity can play a fundamental role in shaping the ecological and evolutionary dynamics of specific phenotypes in natural populations. *Drosophila melanogaster*, while tropical in origin, has become established in temperate regions on multiple continents over a historical time period (David and Capy 1988). This provides a replicated system in which to investigate the process of adaptation to climatic heterogeneity at broad geographic scales. Across environmental gradients spanning temperate to neotropical habitats, a number of stress-response and reproduction-related traits in this species demonstrate strong latitudinal clines on different continents (e.g., Boulétreau-Merle et al. 1982; Karan et al. 1998; Hoffmann and Harshman 1999; Mitrovski and Hoffmann 2001; Hoffmann et al. 2002; Frydenberg et al. 2003; Schmidt et al. 2005).

Although latitudinal clines may be generated by selection, such patterns may result from genetic structure and the demographic history of populations across these gradients. Two lines of evidence suggest that clinal variation for particular life-history traits in *D. melanogaster* populations may not simply reflect demography in the absence of selection. First, traits may vary in parallel among species (e.g., Karan et al. 1998), among continents (e.g., body size: Coyne and Beecham 1987; Capy et al. 1993; James et al. 1997), or between sampled years (Gockel et al. 2001). Second, DNA markers that are presumably neutral with respect to fitness have consistently shown no significant or predictable variation with latitudinal origin of populations on multiple continents (Hale and Singh 1991; Berry and Kreitman 1993; Schmidt et al. 2000; Sezgin et al. 2004). One exception to the pattern of spatial homogeneity for neutral DNA markers is the data of Gockel et al. (2001), who observed clines in allele frequency for a subset of microsatellite loci analyzed. In that study, however, the effects of population demography alone were insufficient to explain the observed cline in body size; the data support selection on this or on a correlated trait. It should be noted, however, that demography under nonequilibrium conditions can generate distinct patterns of spatial variation among loci (e.g., Nei and Maruyama 1975; Robertson 1975).

Complementary to the analysis of geographic variation for functionally distinct markers or traits, a critical component in the analysis of adaptation to climatic variation in *D. melanogaster* relates to population dynamics in the field: that is, whether or not temperate populations are truly resident by means of an overwintering mechanism. This is a very basic issue that has profound implications for the dynamics of *D. melanogaster* populations in the wild. Evidence suggests that *D. melanogaster* can overwinter as an adult (Izquierdo 1991; Mitrovski and Hoffmann 2001; Boulétreau-Merle and Fouillet 2002; Hoffmann et al. 2003), which is consistent with inferences of long-term population continuity in some temperate populations (Ives 1945, 1970). The mechanism by which adult flies overwinter may relate to temporal shifts in the timing of reproduction (Mitrovski and Hoffmann 2001; Boulétreau-Merle and Fouillet 2002).

In addition, a common mechanism for overwintering in insects is the expression of the diapause syndrome, a well-characterized adaptation to seasonality (e.g., Tauber et al. 1986). As in endemic temperate drosophilids (e.g., Lumme and Lakovaara 1983; Kimura 1988), *D. melanogaster* exposed to reduced temperatures (<14°C) and short photoperiods (<12 h light) can undergo a form of reproductive quiescence or diapause in which oogenesis is arrested in previtellogenic stages (Saunders et al. 1989; Williams and Sokolowski 1993). Diapause expression in *D. melanogaster* and other *Drosophila*

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results in life-span extension, elevated resistance to multiple forms of environmental stress, and reduced rates of age-specific mortality (Tatar et al. 2001; Tatar 2004). Unlike many systems, however, the expression of diapause in *D. melanogaster* is highly variable within populations: when individual lines are exposed to the standard diapause-inducing assay in the laboratory, some lines become reproductively quiescent, whereas others proceed with vitellogenesis and reproductive development. Along the east coast of the United States, the frequency of isofemale lines that express diapause in these assays varies predictably with latitude, ranging from 30% in neotropical habitats to greater than 90% in temperate ones (Schmidt et al. 2005).

The potential role of genetic variance for diapause expression in the adaptation of *D. melanogaster* to climatic variation has not been addressed. The steep cline in diapause incidence and the absence of clines for presumably neutral DNA variants in these same sampled populations (Schmidt et al. 2000; Sezgin et al. 2004) implicates selection on this and/or on genetically correlated traits. While the potential fitness advantages of diapause expression for individuals exposed to long-term environmental stress are clear (Tatar et al. 2001), it is unknown whether other factors, or perhaps fitness trade-offs, are associated with the observed clinal pattern. The environmental cues that elicit diapause expression, as well as the degree of seasonality and severity of winter stress, vary substantially among populations in the eastern United States. Thus, to test the hypothesis that the cline in diapause incidence is generated by fitness trade-offs, it is necessary to examine variation in fitness when diapause is expressed as well as in the absence of diapause expression.

Here, we examined multiple fitness-associated traits for sets of inbred *D. melanogaster* lines that express diapause in laboratory assays and sets of lines that do not. Line means were then used to generate estimates of genetic correlations among these traits for both sets of lines. The data demonstrated that under environmental conditions routinely employed in *Drosophila* maintenance (25°C, 12:12-h photoperiod), diapause and nondiapause lines constitutively differed for every trait measured except body size, phenotypic associations among life-history traits for diapause and nondiapause phenotypes indicate departures from phenotypic correlations observed in some artificial selection studies, and the genetic variance/covariance matrices for fitness related traits were significantly distinct between diapause and nondiapause genotypes.

Materials and Methods

Line Establishment and Phenotyping

Approximately 400 gravid *D. melanogaster* females were collected from the Clark's Cove Farm population in Walpole, Maine (44°03′N, 69°49′W) in September 2001 by sweep-netting under apple trees. Individual isofemale lines were established in the field on standard banana-molasses medium with topical yeast addition; species identity was confirmed by examining resulting male progeny. Each line was subsequently inbred by 25 generations of full-sib mating and maintained in the laboratory in replicate 175-ml bottle cultures at 25°C and 12:12-h photoperiod. A subset of lines was then karyotyped to evaluate segregation for cosmopolitan inversions. After inbreeding, approximately 100 females were simultaneously collected in the same generation from each line within 2 h posteclosion and placed on banana-molasses medium at 11°C under a 10:14 light:dark photoperiod. Four weeks later, ovaries were dissected in PBS and characterized according to King (1970). A line was considered a diapause (D) line if ovarian development in all replicate females was arrested prior to vitellogenesis (the most advanced oocyte was $\leq$ stage 7). A line was considered to be a nondiapause (ND) line if all replicate females contained mature, stage 14 follicles in each ovary. Thirty D lines and 30 ND lines were selected for subsequent analysis.

Reference Markers

One potential concern was that the lines sampled from the Walpole population may represent recent admixture of different source populations that are distinct for diapause and other life-history phenotypes. The inbred D and ND lines were genotyped for two markers that were previously observed to exhibit substantial geographic variation in allele/haplotype frequencies among *D. melanogaster* populations in the eastern United States: *methuselah* (*mth*; Schmidt et al. 2000; Duvernell et al. 2003) and alcohol dehydrogenase (*Adh*, E.C. 1.1.1.1; Oakeshott et al. 1982). Preliminary data has also indicated that *Drosophila* insulin receptor homologue (*dInR*) haplotypes also vary with geography in eastern North America (A. B. Paaby and P. S. Schmidt, unpubl. data). Following the methodologies given in Schmidt et al. (2000) and Duvernell et al. (2003), the inbred lines were genotyped for five single nucleotide polymorphism (SNP) sites across the *mth* genomic sequence by polymerase chain reaction (PCR) amplification and digestion with the appropriate restriction enzymes. *Adh* genotype was determined by cellulose acetate electrophoresis. *InR* haplotype, here defined by two SNPs, was determined by amplifying with primers InR 323 (5′-ATT TGA CGA AGT GGA GAC-3′) and InR 114923 (5′-GAT ATA ATC TGT GAC CTC G-3′) and digesting the resulting product with *Hae*III and *Mbo*I.

Life-History Trait Measurements

For all of the experiments outlined, all lines were maintained at 25°C under a 12:12-h photoperiod; at no time was any individual exposed to diapause-inducing cues. Each inbred line was used to establish four replicate cultures in 175-ml bottles. All lines were maintained in replicate bottle culture for two generations at a relatively constant density of 200 $\pm$ 20 eggs/bottle. Upon eclosion in the next generation, 20 flies of each sex were randomly selected among the replicates for each line over an 8-h window. These flies were placed in groups of four males and four females in five replicate 8-dram vials with standard banana-molasses medium. Flies were transferred to fresh food daily; mortality events were recorded and the number of eggs counted. 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 be-

tween time x and time $x + 1$, and N_x is the number of live flies at time x.

From the replicate culture bottles, more than 20 flies of each sex were again randomly collected from each line for both the starvation- and cold-stress assays described below. For the starvation-stress assay, freshly eclosed mixed-sex groups were collected simultaneously for all lines over 8 h, transferred in groups of 10 males and 10 females to fresh food vials, and aged to 5 days. The experimental flies were then placed in sealed 175-ml culture bottles with no food but 10 ml of water in cotton. After a period of 3 days the number of live and dead males and females was determined. For the cold-stress assay, mixed-sex groups were collected and aged to 4 days then transferred to fresh vials and placed at 11°C for 2 h. Following the period of cold hardening, flies were transferred again to empty 8-dram vials and exposed to −20°C for 40 min. Flies were transferred to fresh vials with food and allowed to recover at 25°C for 24 h; patterns of mortality were then determined. Patterns of survivorship following exposure to each form of stress were analyzed with nominal logistic regression.

Total body lipid content was measured using ether extraction (Robinson et al. 2000). From the line replicates, freshly eclosed mixed-sex groups were simultaneously collected over 8 h, and groups of 10 males and 10 females were aged to 5 days in fresh food vials. Samples were then sorted by sex and stored in 1.5-ml microcentrifuge tubes at −80°C until analysis. Each group was dried for 24 h at 60°C and weighed to the nearest 1×10^{-5} g. One milliliter of diethyl ether was added to the tubes, which were then incubated with shaking for 24 h at room temperature. Final fat-free weight was determined following air-drying for 48 h. Log-transformed lipid content was analyzed with ANCOVA, with log (body mass) as the covariate. Results were qualitatively identical to analysis of residuals generated by initial regressions of lipid content on body mass.

To estimate development time and egg-to-adult viability, four replicate groups of five freshly eclosed flies of each sex were randomly collected from each inbred line and transferred to fresh vials. Following a period of mating for 5 days each group was transferred to a separate new vial and females allowed to oviposit for 1 h. Adult flies were then purged and the number of eggs counted; density was standardized among lines and replicates at 30 eggs/vial. The number of larvae at each developmental stage was counted daily; after the formation of pupae, the number of pupae and adults were counted every 4 h until all flies had eclosed. ANOVA was used in the analysis of arcsine-square-root-transformed proportion viability as well as development time.

Genetic Correlations

Genetic correlations were calculated among life-history traits with Pearson product-moment coefficients. When necessary, variables were log-transformed to meet normality assumptions. The data were split by D versus ND condition and by sex. For phenotypic analyses, all replicates within each line were examined; however, to estimate genetic correlations inbred line means were used (Via 1984). Each mean was generated by analysis of a minimum of 20 flies per sex per line per assay. Genetic associations estimated with means have been found to be similar to genetic correlations calculated with variances and covariances (Geber 1990; Schuster et al. 1992; Donovan and Ehrlinger 1994), particularly when more than 25 lines are used (Roff 1997). Significance probabilities indicated whether a particular genetic correlation differed from zero. These *P*-values were obtained by treating the test statistic as coming from a *t* distribution with $n - 2$ degrees of freedom. To test whether line mean genetic variance-covariance (**G**) matrices were statistically different between D and ND lines, MANOVA was used on jackknifed genetic variance-covariance values (Roff 2002). Comparisons of variance-covariance matrices with this MANOVA method have been shown to produce the same statistical results as the Flury method (e.g., Phillips and Arnold 1999) for comparing **G**-matrices, but environmental effects are easier to incoporate into the MANOVA approach. For every trait pair, genetic variance-covariance pseudovalues were created for each diapause condition and sex combination by jackknifing. Each set of correlation pseudovalues was coded for diapause condition and sex. MANOVA was then used to test whether sets of genetic correlation values significantly differed between diapause phenotypes (Roff 2002).

RESULTS

Reference Markers

For all three markers analyzed, allele frequencies were equivalent between the D and ND inbred lines. At *mth*, the common ATATC haplotype, which exhibits a 40% cline in the eastern United States, was present in 18 of 26 D lines (69.2%) and 15 of 26 ND lines (57.7%). Both estimates are consistent with haplotype frequencies in northern populations (Schmidt et al. 2000). At *InR*, both the D and ND lines were dominated by a single haplotype that was present in 23 of 26 (88.5%) and 24 of 26 (92.3%) lines, respectively. Similarly, the frequency of the *Adh-F* allele was 45.5% in the D lines and 54.5% in the ND lines; both estimates are consistent with previous data for temperate populations (e.g., Oakeshott et al. 1982).

Life Histories

D and ND lines assayed at 25°C differed in patterns of age-specific mortality and longevity (Fig. 1). Life expectancies for females were 30.2 days (D) and 25.18 days (ND) and for males 30.49 days (D) and 28.28 days (ND). Analysis of life span indicated significant effects for line within phenotype, phenotype, sex, and the sex-by-phenotype interaction term (Table 1). Flies from D lines lived longer, and the difference in life span between males and females was less pronounced than for flies from ND lines. Age-specific mortality rates generally increased over the life span and were higher for ND relative to D lines, particularly for females and early in life (Fig. 1).

Lifetime fecundity was equivalent between ND and D line females: mean fecundity based on 20 replicate females for ND and D lines was 9928.69 and 9978.89, respectively ($F_{1,52} = 0.0039$, $P > 0.95$). However, patterns of relative reproduction over the life span differed between diapause phe-

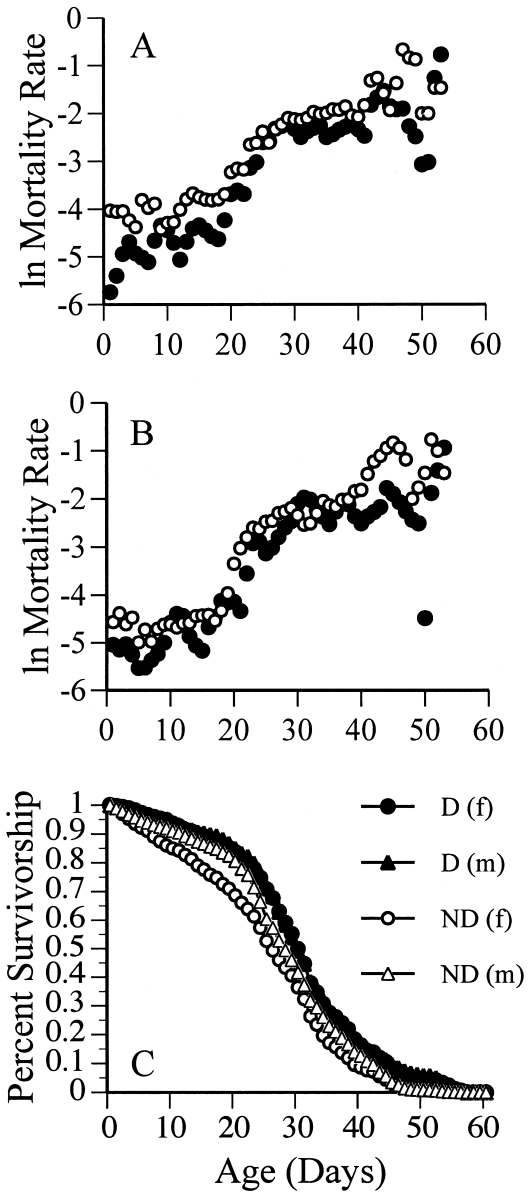

FIG. 1. Log-transformed mortality rates (m_x) for nondiapause (ND, open symbols) and diapause (D, closed symbols) replicate inbred lines as a function of adult age: (A) females, (B) males. (C) Survivorship curves for both sexes and phenotypes. Survivorship is plotted as the percentage alive at a given time point pooled across replicate lines. Curves were smoothed using a sliding window of 3-day means.

notypes (Fig. 2). Per capita fecundity was higher for ND lines in the first 17 days of life; the pattern of differential per capita fecundity was significant by a runs test ($n_1 = 31$, $n_2 = 25$, seven runs, $P < 0.005$, Sokal and Rohlf 1981). This increased level of reproduction for ND females was associated with higher rates of age-specific mortality over the same time period. The inherent differences in early reproduction and associated longevity between D and ND females can be seen in Figure 3. The difference in this fitness proxy, proportional fecundity multiplied by age-specific survivorship, is positive for the first 7 days of life and remains negative from day 14 until the end of the experiment. The pattern of differentiation

TABLE 1. Proportional hazards analysis of life span.

Source	df	L.R. chi square	*P*
Phenotype	1	46.66	0.0001
Line(phenotype)	53	213.23	0.0001
Sex	1	4.86	0.028
Phenotype × sex	1	10.59	0.001

between phenotypes was again nonrandomly distributed over time ($n_1 = 9$, $n_2 = 47$, four runs, $P < 0.005$).

Patterns of survivorship under two environmental stresses were affected by diapause phenotype, sex, and the interaction term (Table 2). Diapause lines demonstrated increased survivorship following exposure to both starvation and cold stress; this pattern was consistent for both males and females, although the difference between sexes was again greater for the ND phenotype (Fig. 4B). These results were not predicted by total body lipid content. Adjusting for mass, lipid content was heterogeneous among lines and also differed between diapause phenotypes and sexes (Table 3). Females were larger and also contained more total lipid than males (Fig. 5). Body size was equivalent between diapause phenotypes and phenotype-by-sex combinations (analysis not shown). However, for both sexes ND lines contained more total lipid per unit body mass than did D lines (Fig. 5). As D lines were characterized by greater stress resistance, this result was contrary to our expectations.

Consistent with the data from other experiments reported here, both egg-to-adult viability ($F_{1,48} = 7.74$, $P < 0.01$) and development time ($F_{1,48} = 8.75$, $P < 0.01$) differed between the D and ND phenotypes (Fig. 4A). Mean time to eclosion was more than 12 h longer for the D relative to the ND phenotype. However, the mean proportion of eggs laid that developed to adulthood was 19% less for ND relative to D lines.

Genetic Correlations

A striking difference between lines of the D and ND phenotypes is the general pattern of differentiation for the es-

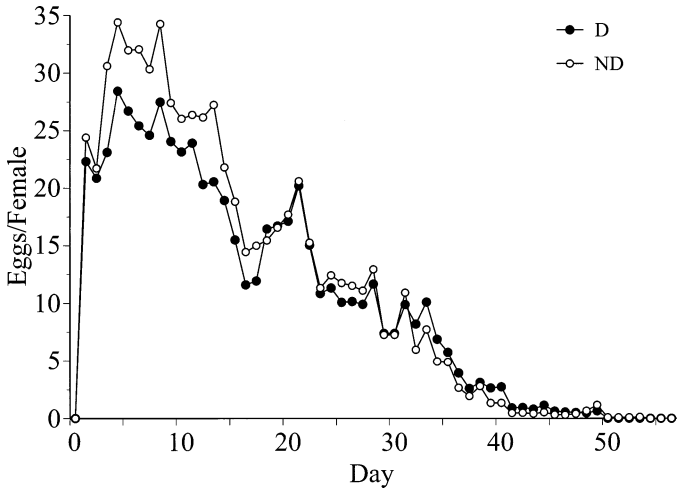

FIG. 2. Per capita fecundity rate for diapause (D) and nondiapause (ND) females over the life span.

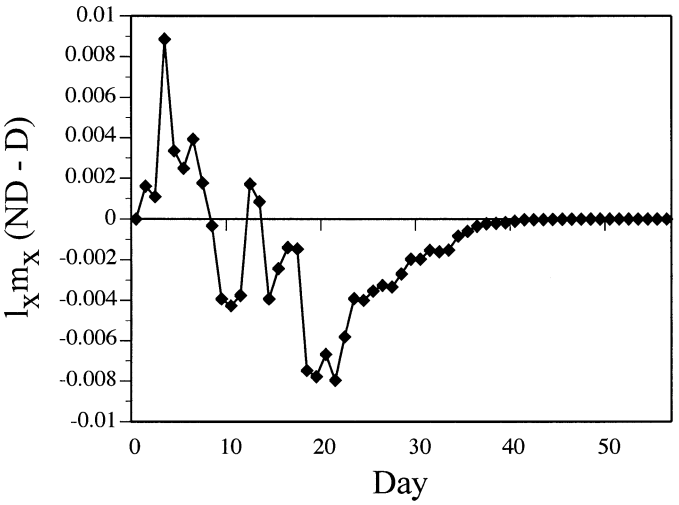

FIG. 3. Differences between nondiapause (ND) and diapause (D) females in proportional reproduction multiplied by cumulative survivorship as a function of time. The data are based on a sliding window of 3-day means.

TABLE 2. Nominal logistic regression for survivorship of diapause and nondiapause lines under cold and starvation stress.

		Cold stress		Starvation stress	
Source	df	Wald chi square	*P*	Wald chi square	*P*
Phenotype	1	51.11	0.0001	507.54	0.0001
Sex	1	22.33	0.0001	0.049	0.826
Phenotype × sex	1	14.31	0.0002	25.95	0.0001

timates of genetic correlations (Table 4). The variance-covariance matrices were significantly different between the two sets of lines according to MANOVA. Furthermore, 17 of 21 pairwise comparisons of correlations between traits were significantly distinct between D and ND lines. The correlations evidenced by ND lines are similar to those reported from other studies and suggest a trade-off between traits linked to survival and those associated with early reproduction (e.g., Luckinbill et al. 1984; Service and Rose 1985; Leroi et al. 1994; Tatar et al. 1996). For example, there was a negative correlation between lipid content and life span, but a positive correlation between lipid content and fecundity early in life. Within the ND phenotype, lines characterized by higher lipid content per unit mass tended to lay more eggs in the first 2 weeks of life but did not live as long. However, estimates of genetic correlations for D lines did not appear consistent with predictions based on relative resource allocation to reproduction and survival. Lipid content was negatively associated with early-life fecundity and positively correlated with life span, and there was a positive association between life span and early-life fecundity. Although none of the correlations was significant by itself, the values are opposite in sign from allocation trade-off predictions as well as from the correlations generated using ND lines.

DISCUSSION

Variance for the Diapause Phenotype

Ovarian diapause was first described in *D. melanogaster* by Saunders et al. (1989). As in other drosophilids (e.g., Kimura 1988), the exposure of Canton-S females to low temperatures and short-day photoperiods results in reproductive arrest, as characterized by the absence of vitellogenic oocytes in the developing ovaries. Diapause is terminated by transfer to higher temperatures or long-day photoperiods; this suggests that *D. melanogaster* exhibits a type of facultative dormancy in which diapause is both initiated and terminated by photoperiod and/or temperature (Saunders et al. 1989). Subsequent analysis demonstrated that *D. melanogaster* females measure night, rather than day, length (Saunders 1990). Exogenous application of juvenile hormone analogs also breaks diapause, and the diapause response is associated with a reduced rate of juvenile hormone synthesis in the corpora allata (Saunders et al. 1990). Studies by Richard et al. (1998, 2001) also implicated a role for ecdysteroids in mediating vitello-

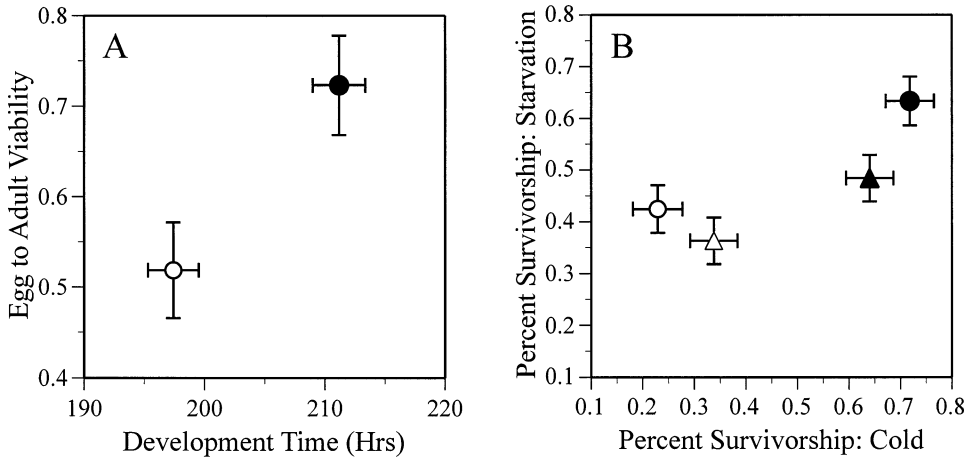

FIG. 4. (A) Phenotypic association between egg-to-adult viability and development time for the diapause (D, filled symbols) and nondiapause (ND, open symbols) phenotypes. The longer development time for D lines is associated with increased viability. (B) Phenotypic association between survivorship following cold and starvation exposure for D (filled) and ND (open) females (circles) and males (triangles). Diapause males and females exhibited higher survivorship than did their ND counterparts.

TABLE 3. Nested ANCOVA for log-transformed total body lipid content of diapause and nondiapause lines; line was considered a random factor with log(mass) as the covariate.

Source	df	SS	*F*
Phenotype	1	0.617	4.456*
Sex	1	1.067	13.847**
Line(phenotype)	46	7.547	2.334*
Line × sex	46	3.232	0.888
Log(mass)	1	4.9	61.908***
Error	342	27.071	

*$P < 0.05$; **$P < 0.001$; ***$P < 0.0001$.

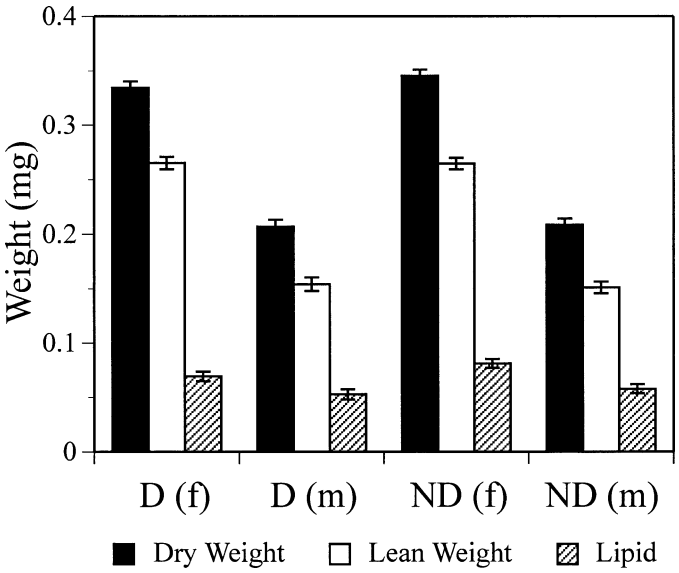

FIG. 5. Body size and lipid content for males and females of the diapause (D) and nondiapause (ND) phenotypes. Histograms depict means (± SE) for replicate inbred lines at 5 days of age.

genesis and the diapause response and further supported that *D. melanogaster* diapause is under neuroendocrine control.

The expression of diapause was shown to vary both among laboratory strains (Saunders and Gilbert 1990) and natural populations (Williams and Sokolowski 1993). Saunders and Gilbert (1990) stated that the characteristics of ovarian diapause in *D. melanogaster* indicate that the trait is of relatively recent evolutionary origin; consistent with this hypothesis, diapause expression has not been observed in either East or West African *D. melanogaster* strains or in *D. simulans* (P. Schmidt, unpubl. data).

The fitness advantages associated with diapause expression have been described for many insect species (reviewed in Tauber et al. 1986). The study by Tatar et al. (2001) extended the life-history analysis of diapause to *D. melanogaster* and suggested that, as in medflies (Carey et al. 1998), this species exhibits dual modes of aging associated with the reproductive (nondiapause) and quiescent (diapause) phenotypes. Several aspects of this study are worth noting here. First, males and females held in diapause for three, six, and nine weeks exhibited postdormancy survivorship curves and age-specific mortality rates that were indistinguishable from freshly eclosed period controls. Thus, diapause expression was associated with negligible senescence over at least a nine-week dormancy period as well as substantial life-span extension. Second, diapausing flies had significantly higher survivorship in thermal and oxidative stress assays than did nondiapausing-period controls. Finally, the exogenous application of the juvenile hormone analog methoprene during exposure to diapause-inducing conditions resulted in increased mortality rates, shortened life span, and reduced resistance to oxidative stress when compared to diapausing controls (application of solvent only).

In the present study, no fly was exposed to a diapause-inducing cue in any of the experiments. Thus, the reported differences in life span and mortality rates, fecundity profiles, stress resistance, lipid content, time to eclosion, and egg-to-adult viability are due to underlying, constitutive differences between lines that express reproductive diapause in the laboratory and lines that do not. Diapause phenotypes are distinct for life-history profiles prior to diapause entry or expression, and these differences become greatly exaggerated when the trait is actually expressed. The fitness advantages of diapause expression in temperate habitats, where food is scarce and temperatures low during a long winter season, are clear (e.g., Lumme and Lakovaara 1983; Tatar et al. 2001). Thus, it was not surprising that the vast majority of isofemale lines sampled from northern U.S. populations expressed diapause in the laboratory (Schmidt et al. 2005). However, it remains unknown why diapause is observed at relatively low frequencies in southern neotropical habitats, where *Dro-*

TABLE 4. Nondiapause (above the diagonal) and diapause (below the diagonal) genotypic mean correlation matrix for females. *P*-values indicate whether a particular correlation was significantly different from zero. Overall **G**-matrices statistically differed between nondiapause and diapause lines (MANOVA, $F = 3.06$, df = 1,52, $P < 0.001$). Bold correlations indicate that the variance-covariance matrix differed between nondiapause and diapause lines for a particular trait combination with MANOVA at $P = 0.001$.

	Lipid ratio	Life span	No. vitellogenic oocytes	Eggs/female days 1–7	Eggs/female days 8–15	Survival: cold	Survival: starvation
Lipid ratio	1	**−0.438***	**−0.2175**	**0.3439†**	**0.3864***	**−0.1679**	**0.0161**
Life span	**0.1474**	1	**0.0134**	−0.2231	−0.0035	**0.1518**	−0.0113
No. vitellogenic oocytes	**0.2217**	**0.2073**	1	−0.1253	**−0.1747**	**0.2342**	**0.1324**
Eggs/female days 1–7	**−0.1752**	0.1324	−0.0603	1	**0.2828**	**−0.2023**	**0.2124**
Eggs/female days 8–15	**−0.3368†**	0.1957	**−0.4008***	**0.516****	1	**0.1528**	**0.0267**
Survival: cold	**0.2728**	**0.2683**	**0.0285**	**−0.1105**	**−0.253**	1	**0.1586**
Survival: starvation	**0.0486**	0.0638	**−0.146**	**−0.0121**	**−0.0908**	**0.0676**	1

*$P < 0.05$; **$P < 0.01$; † $P < 0.10$.

sophila populations are not exposed to diapause-inducing temperatures for long periods of time. In such locales, diapause expression may not be required for overwinter survivorship of individuals or be associated with population persistence. The results presented here indicate the potential for an increased relative fitness of the ND phenotype under nonstressful environmental conditions, as these lines were characterized by a significantly shorter development time and elevated early-life fecundity. In holometabolous insects such as *D. melanogaster*, development time and early fecundity are major determinants of fitness (e.g., Chippindale et al. 1997). While our results indicate the potential for life-history trade-offs that may result in the maintenance of the observed cline, this hypothesis must be directly evaluated by experimental manipulation in the laboratory and field.

Many of the traits investigated in the present study, such as stress resistance (Hoffmann et al. 2003) and patterns of reproduction (Boulétreau-Merle et al. 1982; Mitrovski and Hoffmann 2001), vary predictably with latitude among natural populations. The results of Hoffmann et al. (2001) also demonstrate that much of the variance for stress resistance and correlated traits is due to variation among strains within local populations. Our data are consistent with this result, as all lines were derived from a single, temperate source in midcoastal Maine. The simple inclusion of a single dichotomous trait, whether or not a line expresses reproductive quiescence when exposed to standardized cues in the laboratory, explained a significant portion of the variance for all traits analyzed.

The genetic variance underlying the phenotypic dichotomy between D and ND lines has clear and extensive pleiotropic effects on *Drosophila* life histories. This is also evident from the genotypic mean correlation matrices: the variance-covariance matrices for D and ND females differ for 17 of the 21 possible trait combinations. Not only are lines of these two phenotypes distinct for all traits investigated, the estimates of genetic correlations between multiple sets of traits are often of equal magnitude but opposite in sign. The results of Williams and Sokolowski (1993) indicated that patterns of inheritance for diapause in isofemale line crosses are consistent with the action of a single autosomal factor. Chromosome introgressions using wild-derived and laboratory lines have demonstrated that the phenotypic variance for diapause is due entirely to a factor or set of factors on the third chromosome (Schmidt et al. 2005). However, the generality of these results beyond the scope of the specific lines used has not been addressed. Multiple series of third-chromosome-specific recombinant lines have been generated and are currently being used to establish a detailed genetic map for the trait.

An alternative hypothesis is that differences in life histories between D and ND lines are due to variation in resource acquisition rather than allocation (e.g., Houle 1991; Harshman and Hoffmann 2000). Similarly, inferences of life-history trade-offs based on negative genetic correlations, or the distinct genetic variance-covariance matrices for diapause phenotypes presented here, may be generated by exposure to novel environments (Service and Rose 1985). However, the lines used in the present experiments were cultured and inbred under laboratory conditions identical to those used in the phenotypic assays. In other drosophilids that express diapause, diapause lines have been observed to accumulate two to five times more triacylglycerol than nondiapause lines prior to the onset of adverse seasonal conditions (Ohtsu et al. 1992). Lipid content is positively associated with survivorship during this period, although other metabolic pools may play a more primary role (Kimura et al. 1992). In contrast, in the present study ND lines had slightly but significantly greater total body lipid content than did D lines, yet body size (measured as dry weight) was statistically equivalent between diapause phenotypes. This suggests that the observed trait differences (e.g., stress resistance) were not due to enhanced resource acquisition of D lines. However, only total body lipid content was measured, and the relative contribution of different lipid sources (e.g., fat body vs. ovaries) was not determined. While the data implicate widespread differences between diapause and nondiapause phenotypes for various fitness components, the potential role of variation in resource acquisition remains to be comprehensively evaluated.

Phenotypic Correlations

The phenotypic associations between life-history traits for diapause and nondiapause phenotypes presented here are not entirely consistent with results obtained from artificial selection studies in *D. melanogaster*. Differences between diapause phenotypes are consistent with a positive correlation between longevity and stress resistance (e.g., Rose et al. 1992), as lines that express reproductive diapause are constitutively longer lived and more stress resistant. In selecting directly on starvation resistance, Chippindale et al. (1996) demonstrated that an increase in starvation resistance is correlated with a reduction in pre-adult viability, an increase in body size and lipid content, and a longer development time. Here, the D phenotype was characterized by both increased starvation resistance and development time; however, these lines were equivalent in size to lines of the ND phenotype, contained less lipid per body weight, and had increased pre-adult survivorship. The results of Chippindale et al. (1996) also highlighted the widespread differences in these life-history traits between the sexes. While our results consistently yielded differences between males and females, diapause phenotype was often a better predictor than was sex: for example, although D females were more stress resistant than D males, males of D lines were also more resistant to both cold and starvation stress than were females of the ND phenotype.

Similarly, selection for an accelerated rate of development has phenotypically correlated responses. An increase in rate of development is associated with reductions in energy expenditure during the larval wandering phase, pre-adult survivorship, body size, and lipid content (Chippindale et al. 1997). Here, the accelerated rate of development and reduced pre-adult survivorship of the ND phenotype also reflected these associations. Again, however, the ND and D phenotypes were equivalent in mean weight and the phenotype with the faster rate of development had significantly increased lipid content. These discrepancies may be due, in part, to the complexity of the fitness associations and trade-offs, the different

source populations used, and widespread pleiotropy in age-structured populations (e.g., Rose 1985; Charlesworth 1990). Furthermore, long-term laboratory culture can impact life histories and their underlying genetic architecture (e.g., Promislow and Tatar 1998), as well as the associations between diapause and aging phenotypes (Tatar and Yin 2001).

Diapause and Overwintering in Temperate Habitats

The relative importance of diapause expression in overwintering and adaptation to climatic variation in natural populations has not been directly addressed in the present study. The D phenotype is assayed by induction of females collected within 2 h of eclosion, prior to upregulation of particular insect hormones (e.g., juvenile hormone) and subsequent reproductive development. However, females at older ages have been observed to express the trait (P. Schmidt, pers. obs.). Rather than being characterized by an absence of any vitellogenic (>stage 7) oocytes, older females of D lines respond to low temperature and shortened photoperiod by suppressing any further deposition of yolk proteins into developing oocytes (P. Schmidt, unpubl. data). What remains to be determined is whether older females that become reproductively quiescent are also characterized by the life-span extension, elevated stress resistance, and reduced rates of age-specific mortality previously observed for males and females collected within a few hours of eclosion (Tatar et al. 2001).

The potential impact of variation for the D phenotype in natural populations on other continents remains unknown. Both for European (Boulétreau-Merle and Fouillet 2002) and Australian (Mitrovski and Hoffmann 2001) populations from temperate habitats, the incidence of reproductive diapause was reported to be relatively low. Although purely speculative, discrepancies in diapause incidence in temperate habitats on different continents may reflect general differences in climate. For example, the average daily temperature during the winter period in the experiment of Mitrovski and Hoffmann (2001), conducted near Melbourne, Australia, ranged from approximately 9°C to 16°C. The 15-year average (1990–2004) winter (21 December to 21 March) temperature for the Portland, Maine, weather station, located approximately 100 km southwest of the Walpole population analyzed here, was −3.97°C (U.S. National Climatic Data Center, www.ncdc.noaa.gov). It would be particularly informative to evaluate geographic variation for diapause incidence and its impact on *Drosophila* life histories on continents other than North America.

Correlations of Diapause and the Cline in Diapause Incidence

The data presented here also suggest a potential mechanism for the previously observed cline in diapause incidence in eastern North America. The estimates of genetic correlations indicate the potential for life-history trade-offs between D and ND phenotypes (Reznick 1985; Harshman and Hoffmann 2000). Nondiapause genotypes are constitutively less stress resistant and have a shorter life span, but they also develop at a faster rate and have significantly increased early-life fecundity. Thus, there may be both an advantage and a cost associated with each phenotype; in the wild, the relative fitness and frequency of diapause and nondiapause lines would be dependent on the environmental conditions experienced in a particular habitat. If diapause is functionally associated with overwintering mechanisms, genotypes that diapause are predicted to be of higher fitness in temperate habitats characterized by a strong degree of seasonality, cold temperatures, and temporally variable food supply. Alternatively, in neotropical habitats that are more temporally homogeneous in terms of temperature and resource availability, diapause expression and overwintering may not influence organismal fitness to the same degree or be associated with population persistence. In such environments, genotypes that do not express diapause are predicted to be of higher fitness due to their faster rate of development and increased early-life fecundity. These predictions are currently being tested in laboratory and field experiments.

The phenotypic and genetic correlation data presented here suggest that the diapause phenotype is a powerful predictor of life-history variation in natural populations. While diapause expression must be induced by exposure to token stimuli, reproductive diapause in *D. melanogaster* cannot be considered simply an inducible trait: the underlying genetic variance for the diapause phenotype has substantial effects on multiple aspects of life history even in the absence of diapause-inducing stimuli. Selection on diapause expression is predicted to result in concomitant changes in correlated traits and vice versa. The genetic variance-covariance matrices make clear predictions regarding geographic variation in life-history traits in North American populations that substantially differ in the frequency of diapause incidence. This system also presents an excellent opportunity to evaluate the relative impacts of genetic background and environmental heterogeneity on the evolutionary dynamics of the **G** matrix itself.

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