Thermal adaptation of cellular membranes in natural populations of *Drosophila melanogaster*

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Summary

1. Changes in temperature disrupt the fluidity of cellular membranes, which can negatively impact membrane integrity and cellular processes. Many ectotherms, including *Drosophila melanogaster* (Meigen), adjust the glycerophospholipid composition of their membranes to restore optimal fluidity when temperatures change, a type of trait plasticity termed homeoviscous adaptation.

2. Existing data suggest that plasticity in the relative abundances of the glycerophospholipids phosphatidylethanolamine (PE) and phosphatidylcholine (PC) underlies cellular adaptation to temporal variability in the thermal environment. For example, laboratory populations of *D. melanogaster* evolved in the presence of temporally variable temperatures have greater developmental plasticity of the ratio of PE to PC (PE/PC) and greater fecundity than do populations evolved at constant temperatures.

3. Here, we extend this work to natural populations of *D. melanogaster* by evaluating thermal plasticity of glycerophospholipid composition at different life stages, in genotypes isolated from Vermont, Indiana and North Carolina, USA. We also quantify the covariance between developmental and adult (reversible) plasticity, and between adult responses of the membrane to cool and warm thermal shifts.

4. As predicted by physiological models of homeoviscous adaptation, flies from all populations decrease PE/PC and the degree of lipid unsaturation in response to warm temperatures. Furthermore, these populations have diverged in their degree of membrane plasticity. Flies from the most variable thermal environment (Vermont, USA) decrease

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1: Supplementary methods and results for degree-day model

Table S1. Summary of weather data and degree-day model estimates for each population

Table S2. Magnitude of cellular responses to adult thermal shifts

Figure S1. Developmental reaction norms for lipid saturation

PE/PC to a greater extent than do other populations when developed at a warm temperature, a pattern that matches our previous observation in laboratory-evolved populations. We also find that developmental plasticity and adult plasticity of PE/PC covary across genotypes, but that adult responses to cool and warm thermal shifts do not.

5. When combined with our previous observations of laboratory-evolved populations, our findings implicate developmental plasticity of PE/PC as a mechanism of thermal adaptation in temporally variable environments. While little is known about the genetic bases of plastic responses to temperature, our observations suggest that both environmentally sensitive and environmentally specific alleles contribute to thermal adaptation of membranes, and that costs of plasticity may arise when the adult environment differs from that experienced during development.

Keywords

acclimation; cellular membranes; *Drosophila melanogaster*; homeoviscous adaptation; phenotypic plasticity; thermal adaptation

Introduction

Changes in temperature alter the fluidity of cell membranes through thermodynamic effects on the fatty acid hydrocarbons that comprise the membrane bilayer. In response to changes in temperature, cells dynamically remodel membrane composition. This plastic response maintains fluidity and reduces the deleterious effects of temperature on membranes, such as phase transitions, increased cellular permeability, and compromised function of membrane bound proteins (Sinensky 1974; Hazel 1995; Hochochka & Somero 2002; Montooth, Siebenthall & Clark 2006; Overgaard *et al.* 2008; Cooper *et al.* 2012). Changes in the relative abundance of particular glycerophospholipid (GPL) head groups (e.g., phosphatidylethanolamine (PE) and phosphatidylcholine (PC)) and/or lipid saturation enable homeostasis of membrane fluidity. Physiological models predict that relatively higher concentrations of PC and lipid saturation enable membrane homeostasis in warm environments, while higher concentrations of PE and of unsaturated lipids maintain membrane homeostasis in cool environments (Sinensky 1974; Hazel 1995). This homeoviscous adaptation describes both the adaptation of membrane GPL composition to the mean thermal environment experienced through evolutionary time (i.e., cellular specialization), and the acclimation of membrane GPL composition to the current environment (i.e., cellular generalization or plasticity).

Evolutionary models predict that environments that vary in time or space favor generalist strategies that maintain fitness across a range of environments (Levins 1968; Lynch & Gabriel 1987; Gilchrist 1995). Generalists with fixed phenotypes may have reduced fitness relative to specialists in their home environments due fitness tradeoffs across environments (Whitlock 1996; Reboud & Bell 1997; Kassen 2002). In contrast, generalists with plastic phenotypes can match their phenotypes to the current environment, and should be favored in variable environments when genetic variation for plasticity exists and the costs of plasticity are low relative to the benefits (Via & Lande 1985; van Tienderen 1991; Gomulkiewicz &

Kirkpatrick 1992). Models of plasticity predict that environments that vary greatly across generations favor developmental responses, whereby the developmental environment determines the adult phenotype (Gabriel & Lynch 1992), while environments that vary greatly within generations favor reversible responses (e.g., within the adult life stage) (Gabriel *et al.* 2005). Evolutionary models generally assume genetic independence of developmental and reversible responses, but shared physiological mechanisms for traits like membrane remodeling could generate covariance between these types of plasticity. In holometabolous insects, the extent to which metamorphosis in the pupal stage decouples larval and adult responses to temperature remains unknown. Such decoupling could enable selection to act independently on thermal physiologies across life stages (Moran 1994).

The holometabolous *Drosophilid* flies inhabit a wide range of thermal environments from the relatively constant tropics to the highly seasonal temperate latitudes (David & Capy 1988; Hoffmann, Sørensen & Loeschcke 2003; Cooper, Czarnoleski & Angilletta 2010). Yet, we know relatively little about how membrane composition and plasticity have diverged between species or among populations. Species of *Drosophila* from different latitudes in Japan have diverged in two GPL components (monoenoic and dienoic fatty acids), but not in overall levels of unsaturation (Ohtsu, Kimura & Katagiri 1998). *Drosophila melanogaster* (Meigen) changes the PE to PC ratio (PE/PC) in response to developmental temperature as predicted by physiological models of homeoviscous adaptation (Overgaard *et al.* 2008), but the contribution of this GPL plasticity to surviving short, cold bouts of thermal stress is less clear (Overgaard *et al.* 2005; Overgaard *et al.* 2006; MacMillan, Guglielmo & Sinclair 2009). Laboratory evolution experiments using *D. melanogaster* found no evidence for GPL specialization to constant 16° and 25°C environments, but demonstrated that temporal variation in temperature across generations selectively maintained greater developmental plasticity of PE/PC (Cooper *et al.* 2012). Populations with greater plasticity of PE/PC also have higher fecundity across a range of temperatures, suggesting that this cellular response impacts components of fitness (Condon *et al.* in press). Yet, the extent to which natural populations have diverged in membrane plasticity, whether this plasticity covaries across life stages, and the evolutionary genetic basis for this important physiological adaptation remain unknown.

Here, we investigate patterns of cellular membrane adaptation to temperature among populations and across life stages of *D. melanogaster*. We characterize membrane composition and plasticity in populations of *D. melanogaster* from Vermont (VT), Indiana (IN), and North Carolina (NC), USA estimated to experience both different mean temperatures and different amounts of temporal variation in temperature (see Appendix S1 and Table S1 in Supporting Information), which should favor GPL specialization and plasticity, respectively. We tested 1) whether flies that experience the lowest mean temperatures (VT) have greater PE/PC and greater lipid unsaturation regardless of thermal treatment, 2) given our previous results in the laboratory, whether flies from the most variable thermal environment (VT) more strongly decrease PE/PC when developed at a warm temperature, and 3) whether plasticity of PE/PC covaries across life stages, as would be expected if there was a shared genetic basis of this trait throughout ontogeny.

Methods

Populations of D. melanogaster

In the fall of 2011, we sampled genotypes from East Calais, Vermont, USA; Bloomington, Indiana, USA; and Raleigh, North Carolina, USA. These populations reside at different latitudes and, as a result, experience different mean temperatures and variances in temperature, which we verified using a degree-day model (see Table S1) (Cooper, Czarnoleski & Angilletta 2010; Nilsson-Ortman *et al.* 2012). Captured females were placed into individual vials and allowed to lay eggs. For the following two generations, one virgin female was paired with one male sibling to establish isofemale genetic lines. These lines have since been maintained at very small densities in culture vials to minimize genetic variation within lines with standard Bloomington Drosophila cornmeal-yeast medium at 20.5°C on a 12:12 light cycle.

Experimental treatments

We quantified the developmental and adult plasticity of membrane GPL composition in 13, 14, and 12 isofemale lines from VT, IN, and NC, respectively. During the experiment, 0–3 day old mated females from each line were placed into yeasted food vials for conditioning. Three days later, twenty females from these vials were split between two yeasted vials and allowed to lay eggs for 24 hours at 20.5°C. Eggs from each line were then placed into 16° and 26°C constant environments and allowed to develop to adults. To evaluate adult responses, subsets of adults from each line were shifted on the first day of adult emergence from their respective developmental environments to the opposite thermal environment for 72 hours (i.e., larvae developed at 16°C were shifted upon adult emergence to 26°C, and vice versa). In total, we exposed a median of 4 replicates of each genotype from each population to four treatments: 1) development at 16°C and maintained at 16°C, 2) development at 26°C and maintained at 26°C, 3) development at 16°C with an adult shift to 26°C, and 4) development at 26°C with an adult shift to 16°C.

Extraction, separation and quantification of membrane GPL composition

We used a lipid-profiling assay that we previously developed to quantify the GPL composition of *D. melanogaster* membranes (Hammad *et al.* 2011; Cooper *et al.* 2012). We weighed replicate groups of twenty 2–5 day old male flies from each treatment and extracted their GPLs in ice-cold chloroform-methanol (2:1) (Folch, Lees & Stanley 1957; Kostal & Simek 1998). We centrifuged each homogenate at 5,000 *g* for 10 minutes at 4°C and extracted the supernatant. After this process was repeated, the supernatants were combined with 0.2 ml of 0.9% NaCl, vortexed and centrifuged again. We then dried the lower organic phase under nitrogen and stored samples at -80°C. Whole-fly extracts represent the entire pool of available GPLs, but preclude the measurement of tissue heterogeneity or other membrane structures (e.g., lipid rafts). Nevertheless, PE and PC comprise the bulk of *Drosophila* membranes (Jones *et al.* 1992), and both PE/PC and lipid saturation measured from whole-fly extracts change in response to temperature in *D. melanogaster* (Overgaard *et al.* 2008; Cooper *et al.* 2012).

Lipids were separated and quantified by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS). Internal PE and PC standards were used to account for biased detection of GPLs with different head groups (Hammad *et al.* 2011). Dried GPL samples were reconstituted in 1 mL methanol, further diluted by a factor of fifty in methanol, and the internal standards (1,2-dimyristoyl-*sn*-glycero-3- phosphoethanolamine and 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine) were added to a final concentration of 200 pg/μl. We analyzed our samples using a Dionex 3000 Ultimate LC system (Dionex, Sunnyvale, CA) interfaced to a QTRAP 4000 triple quadrupole instrument (ABI Sciex, Foster City, CA). A 1-μL aliquot of the reconstituted lipid extract was injected onto a Kinetex C18 column (100 mm × 2.1 mm, 2.6 μm particle size) maintained at 25°C. Mobile phase A consisted of 10 mM ammonium acetate in H_2O:methanol (10%:90%, v:v). Mobile phase B consisted of 10 mM ammonium acetate in isopropanol:methanol (50%:50%, v:v). The gradient conditions were 30% B to 65% B from 3–10 minutes followed by 3 minutes at 100% B.

We converted analyte peak areas into mole fraction percentages and calculated the percent abundance of each PE and PC GPL in the total pool of PE and PC (Overgaard *et al.* 2008; Cooper *et al.* 2012). Percentages were corrected using the peak areas of the internal standards in each sample. For each sample, we then calculated PE/PC as the proportion of GPLs containing PE divided by the proportion of GPLs containing PC. We assigned the degree of unsaturation of each GPL as the sum of the number of double bonds across its two acyl chains, and calculated the proportion of monounsaturated (one double bond) and polyunsaturated (>1 double bond) GPLs in each sample.

Statistical analyses

We used mixed model analyses of variance to test our hypotheses using the nlme library in the software package R version 2.12.1 (R Core Team 2008). We evaluated the fixed effects of population, temperature treatment, LC-MS/MS block, their interactions, and the random effect of isofemale line on the membrane GPL traits, *PE/PC* and *Saturation*. LC-MS/MS block accounts for technical effects of changing the LC column. Samples from all lines were randomized across blocks, with every population represented in each block, and samples from all treatments for any included line were represented within the same block. Because the LC column can only be used for ~ 100 samples, not all lines can be represented within a single block. This causes sampling effects between blocks that can result in population by block interaction effects (Table 1). Because of this, we used a high level of biological replication for each line that resulted in in 46, 57, and 35 measures of each trait in each of the four treatments for VT, IN, and NC populations, respectively, for a total of 552 samples for lipid quantification. This level of replication (median of 4 biological replicates per line per treatment) exceeds that of other recent lipid profiling studies using MS/MS in *Drosophila* (e.g., Scheitz et al. 2013). A significant effect of treatment indicates that the trait is plastic in response to temperature, while a significant treatment by population interaction indicates that populations differ in the degree of plasticity. To test for specialization of membrane GPL traits across populations (e.g., population differences in PE/PC regardless of treatment), we evaluated the fixed effects of population, LC-MS/MS block, their

interactions, and the random effect of line on membrane traits within temperature treatments.

To quantify covariance between plasticity traits across life stages, we calculated correlations between isofemale line means for each trait. These correlations approximate the combined additive and non-additive components of the genetic correlation. The degree of developmental plasticity (*PE/PC* and *Saturation*) was calculated for each genetic line as the difference between the mean trait values between flies developed at 16°C and at 26°C, and not including adults that were shifted to different temperatures. The degree of adult plasticity was calculated in two ways: (1) the difference between adults developed at 16°C, and adults developed at 16°C that were then shifted to 26°C for 72 hours (upward thermal shift) and (2) the difference between adults developed at 26°C that were then shifted to 16°C for 72 hours, and adults developed at 26°C (downward thermal shift). We used paired t-tests to compare PE/PC after development and following adult shifts for each genetic line to test whether reversible plasticity in adults recovers the developmental trait values when adult environments shift.

Results

Patterns of GPL plasticity support physiological models

Membrane GPL composition responded strongly to both developmental and adult temperature treatments in all populations. Both PE/PC and lipid unsaturation were significantly increased at 16°C relative to 26°C treatments in all populations (Table 1; Table 2; Fig. 1; Fig. S1), consistent with the predictions of homeoviscous adaptation. While populations did not differ in the degree of developmental plasticity of lipid unsaturation (Table 1; Fig. S1), populations did differ in the degree of developmental plasticity of PE/PC, as indicated by a significant population by treatment interaction (Table 1; Fig. 1). There was not a significant population by treatment by block interaction (Table 1), indicating that this population by treatment interaction was robust to any technical effects of block. The significant population by treatment interaction was driven by differences among populations after development at 26°C. Following development at 16°C, PE/PC did not differ significantly among the three populations (Fig. 1; $F_{2,36} = 0.68$, $P = 0.513$). However, when developed at 26°C, flies from the three populations differed significantly in PE/PC ($F_{2,36} = 3.46$, $P = 0.04$) with VT having lower PE/PC than IN or NC flies (Fig. 1).

In contrast to the population divergence we observed in developmental plasticity of PE/PC, there were no significant population by treatment interactions for GPL composition at the adult stage (Table 2). Rather, across the three populations the degree of plasticity differed depending on the direction of the adult shift (i.e., whether adults were shifted to cooler versus warmer temperatures relative to their developmental temperature). Adults changed PE/PC more strongly following a shift to 26°C, relative to the shift to 16°C ($t_{paired} = 5.21$, $df = 2$, $P = 0.035$), while the change in lipid unsaturation was greater following shifts to 16°C, relative to the shift to 26°C ($t_{paired} = 11.72$, $df = 2$, $P = 0.007$)(Table S2).

Plasticity of PE/PC diverges rather than other membrane GPL traits

Populations did not differ in the plasticity of GPL traits other than PE/PC (Tables 1 and 2), and there was no evidence of physiological specialization of membrane GPL composition across populations. Specialization to the mean thermal environment would predict increased PE/PC or lipid unsaturation in the colder VT population regardless of developmental environment. Populations did not differ in PE/PC after development at 16°C (Fig. 1); and at 26°C the greater response of VT flies resulted in a lower ratio of PE/PC relative to other populations (see results above), a result in the opposite direction of that predicted for specialization of GPL composition. Similarly, we found no evidence of greater lipid unsaturation in populations from cooler mean thermal environments. Population had no significant effect on lipid unsaturation during development at 16°C ($F_{2,36} = 1.21$, $P = 0.311$) or 26°C ($F_{2,36} = 0.05$, $P = 0.956$).

Plasticity of PE/PC covaries across, but not within, life stages

We used the correlation among the isofemale line means for *PE/PC* to estimate the covariance between developmental and adult plasticity, and between plastic responses of adults to downward and upward thermal shifts. The degree of developmental plasticity of PE/PC was significantly and positively correlated with the degree of adult plasticity (Fig. 2*A,B*). However, the plastic responses of adults to downward and to upward thermal shifts were not significantly correlated (Fig. 2*C*).

Adult plasticity of PE/PC fails to recover developmental trait values

We compared adult-shifted and developmental trait values of each isofemale line at particular temperatures to determine the degree to which adult plasticity recovered developmental trait values. Across all populations, adults changed PE/PC more strongly when shifted to 26°C, relative to the shift to 16°C (Table S2). As a result of this reduced adult response to cooler temperature, no populations recovered their 16°C developmental values when adults developed at 26°C were shifted to 16°C, as evidenced by significant differences in the developmental and adult PE/PC ratios at 16°C (Fig. 3*A*). In contrast, when shifted to a warmer temperature, adult flies from IN and NC recovered their 26°C developmental trait values, but genotypes from VT did not (Fig. 3*B*). This lack of recovery of the developmental value of PE/PC in VT flies indicates a potential cost of greater developmental plasticity experienced by adults when their thermal environments shift from cooler to warmer temperatures.

Discussion

Remodeling the GPL composition of membranes enables ectotherms to maintain membrane fluidity and ultimately fitness when temperatures change (Sinensky 1974; Hazel 1995; Hochochka & Somero 2002; Condon *et al.* in press). Consistent with previous observations of laboratory lines (Overgaard *et al.* 2008; Cooper *et al.* 2012), we found that North American *D. melanogaster* decreased PE/PC and lipid unsaturation when developed at 26°C and when adults were shifted to 26°C, relative to exposure to 16°C. While populations shared similar developmental responses of GPL saturation and similar adult responses in all GPL traits, flies from the most temporally variable thermal environment (VT) more strongly

remodeled PE/PC in response to warm developmental temperatures, relative to other populations. This pattern recapitulates observations from replicated laboratory-evolved populations, where high variance in temperature across generations favored greater developmental response of PE/PC to warm temperatures (Cooper *et al.* 2012). These populations with greater membrane plasticity also had greater female fecundity than did populations with lower PE/PC plasticity when developed in a warm environment (Condon *et al.* in press). Finally, the magnitude of developmental PE/PC plasticity that we observed in natural populations was the same as that of laboratory populations evolved in the presence of thermal variability, but was greater than that of populations evolved at constant temperatures (Cooper *et al.* 2012). This suggests that thermal variability maintained membrane plasticity, while evolution in constant thermal environments relaxed selection on plasticity. This result supports the predictions of evolutionary models of genes that are conditionally expressed (Van Dyken & Wade 2009), and suggests that alleles for membrane plasticity may be costly during bouts of thermal stasis (*sensu* van Tienderen 1991). While we cannot make conclusions about the specific magnitude of temporal variation in temperature in nature that generates a selective advantage for PE/PC plasticity, we can conclude that when the multiple populations of *D. melanogaster* that we have measured diverge in this plastic trait they do so via the same mechanism – populations from more thermally variable environments more strongly decrease PE/PC in response to warm developmental environments.

Development temperature more strongly affected PE/PC than did adult thermal shifts, with the consequence that thermally shifted adults were not able to recover developmental values of PE/PC. The stronger developmental, but similar adult plasticity of VT flies resulted in VT flies being further from their 26°C developmental PE/PC value following shifts to 26°C as adults. If developmental values reside near the physiological optimum, this would indicate a greater cost of matching cellular physiology to the developmental environment for VT flies when adult environments change (i.e., a cost of developmental plasticity realized by adults). Our interpretation of this putative cost assumes that the 3-day exposure of adults was a long enough period of time for a complete membrane response. While more experiments are needed to determine the timing of membrane turnover in *D. melanogaster*, theory predicts that selection should favor the evolution of reversible responses so long as the response time does not exceed the rate of environmental change (Gabriel *et al.* 2005). Three days represents an extensive amount of time for adaptive membrane turnover when we consider that adult flies experience thermal variation on much finer timescales. Yet, the way in which *D. melanogaster* experiences variation in the thermal environment differs across life stages. Larvae develop in fermenting fruit with limited capacity to buffer thermal variation, while flight enables adults to behavioral avoid thermal extremes within their lifetime (Feder, Blair & Figueras 1997; Huey, Hertz & Sinervo 2003; Dillon *et al.* 2009). If adults can behaviorally buffer the thermal environment, then adult flies may never experience a cost of decreased performance when temperatures shift (Angilletta *et al.* 2006).

Despite the fact that the natural populations we sampled experience different mean thermal environments, we found no evidence for specialization of membrane GPL composition. While not consistent with predictions of homeoviscous adaptation, this result also matches observations from laboratory-evolved populations, where evolution in constant

environments did not lead to specialization of membrane GPL composition (Cooper *et al.* 2012). *Drosophila* species that inhabit different latitudes in Japan have diverged in the percentage of monoenoic and dienoic acids in their membranes, but in a way that maintains a similar degree of lipid saturation across species (Ohtsu, Kimura & Katagiri 1998). Thus, it remains unclear whether this species divergence is adaptive or a result of compensatory evolution to maintain the overall degree of GPL saturation. The populations that we sampled may have specialized GPL composition in ways that our assay does not capture. For example, cholesterol affects membrane dynamics (Robertson & Hazel 1995; Robertson & Hazel 1997; Shreve, Yi & Lee 2007), and higher order membrane structures (e.g., lipid rafts) can change in response to environmental temperature (Zehmer & Hazel 2003). Alternatively, effective developmental plasticity in a species like *D. melanogaster* with a relatively short life span, combined with behavioral thermoregulation in adults (Dillon *et al.* 2009), may decrease selection pressure to specialize membrane composition across different mean thermal environments.

Across genotypes, we observed a positive correlation between developmental and adult responses of PE/PC to temperature. This correlation could arise through correlated selection (Agrawal & Stinchcombe 2009) on distinct genes and mechanisms that underlie developmental and adult plasticity, although theory suggests that distinct types of environmental heterogeneity (across- versus within-generation variance) favor these two types of plasticity (Gabriel & Lynch 1992; Gabriel *et al.* 2005). Alternatively, the covariance across life stages may reflect shared genes and mechanisms that affect PE/PC responses in larvae and adults. In contrast to this covariance across life stages, we found no correlation between the magnitudes of adult change in PE/PC to upward and downward thermal shifts. This indicates a degree of genetic decoupling of membrane remodeling in response to warm versus cool temperatures within the adult life stage. Our data provide some evidence for regulation of different GPL membrane components in response to upward and downward thermal shifts in adults; PE/PC responded more strongly to upward thermal shifts, while the degree of lipid saturation responded more strongly to downward thermal shifts. Distinct biochemical pathways underlie PE/PC modification and lipid desaturation (Miller, Yates & Geer 1993; Dobrosotskaya *et al.* 2002; Montooth, Siebenthall & Clark 2006), providing potential mechanisms by which adult responses to different thermal shifts may be genetically and physiologically independent.

We previously found support for the prediction that temporal variability in temperature favors alleles with greater thermal sensitivity (Cooper *et al.* 2012). We find similar support from natural populations. Selection favoring alleles specific to different mean environments would result in population specialization. The fact that we observed greater developmental plasticity in the absence of specialization suggests that divergence in developmental plasticity of PE/PC occurs via alleles with greater environmental sensitivity (*sensu* Via 1993). Models of conditional gene expression predict that genotypes expressed in specific environments experience less effective selection than do those expressed more ubiquitously (Kawecki 1994; Whitlock 1996; Van Dyken & Wade 2009). Thus, plasticity may more likely diverge among populations via environmentally sensitive genotypes that affect phenotypes across a range of environments. Nevertheless, responses of the membrane to

upward and downward thermal shifts in adults were not correlated, suggesting environment-specific responses in this life stage.

While membrane remodeling is a complex phenotype controlled by many genes, the quantitative divergence that we observed may result from changes at a small number of loci that modify membrane responses to temperature. In *Drosophila*, the sterol regulatory element binding protein (SREBP) regulates the expression of enzymes that synthesize PE and may directly respond to alterations in membrane fluidity Dobrosotskaya *et al.* 2002; Rawson 2003). A number of genes regulated by SREBP, as well as lipid desaturases, change in expression in response to temperature during development and during adult thermal shifts in *D. melanogaster* (Montooth, Siebenthall & Clark 2006), providing a set of candidate genes that enable a mechanistic approach to understanding how cellular plasticity evolves in response to environmental heterogeneity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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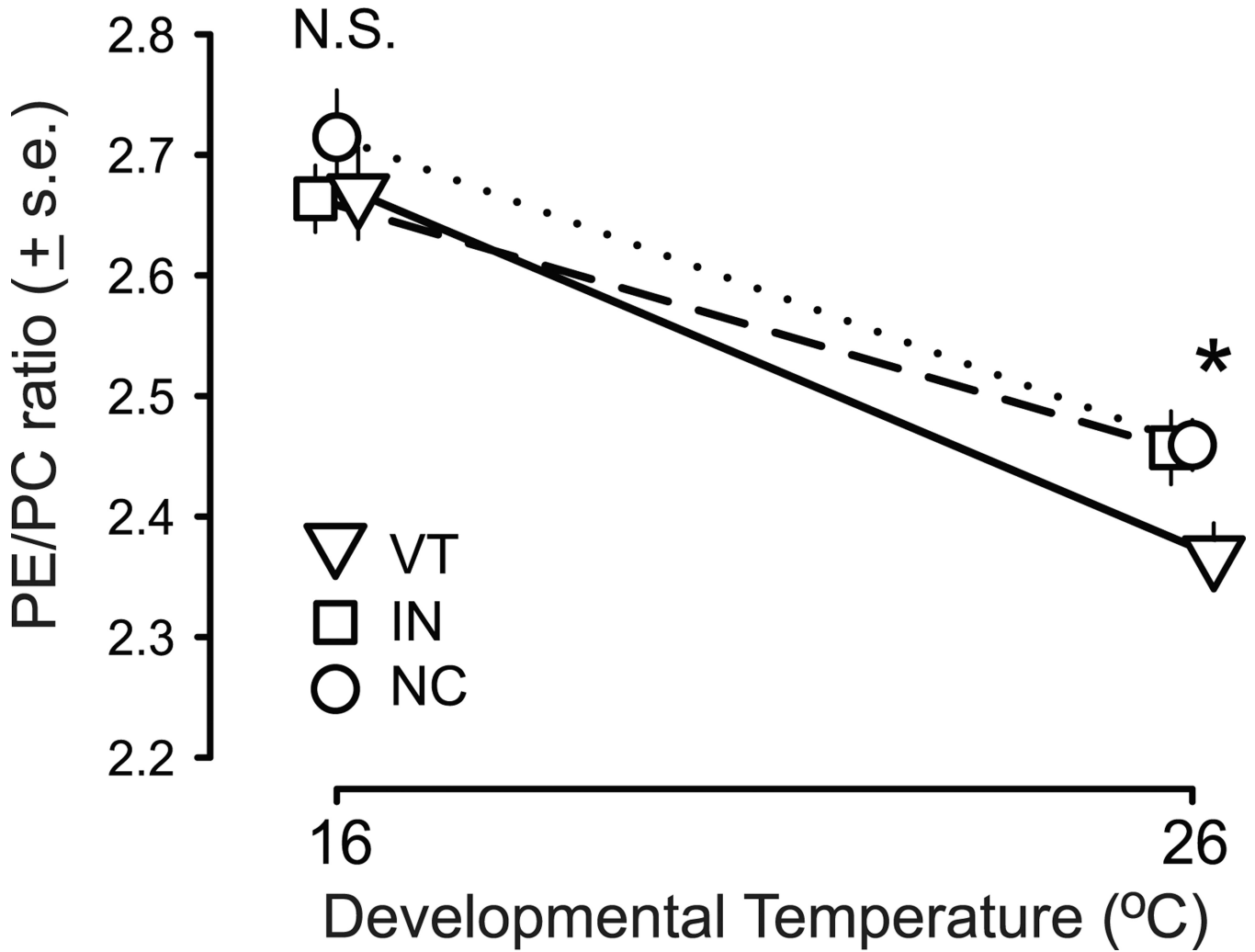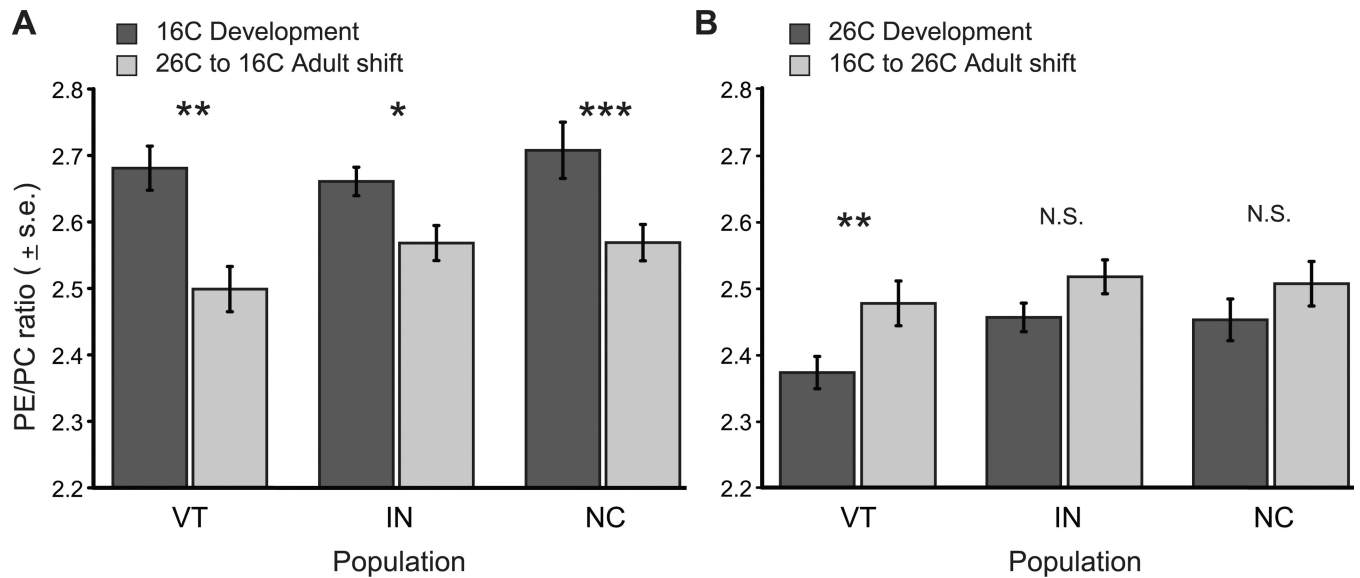
Figure 1.

Flies from all populations have a strong plastic response of PE/PC to developmental temperature, but flies from the most variable thermal environment (VT) respond more strongly to warm developmental conditions. Following development at 16°C, flies from the three populations did not differ significantly in the mean PE/PC ($F_{2,36} = 0.679$, $P = 0.5134$), but when developed at 26°C, flies from the three populations differed significantly in PE/PC ($F_{2,36} = 3.461$, $P = 0.04$), with VT having lower PE/PC than IN or NC flies.
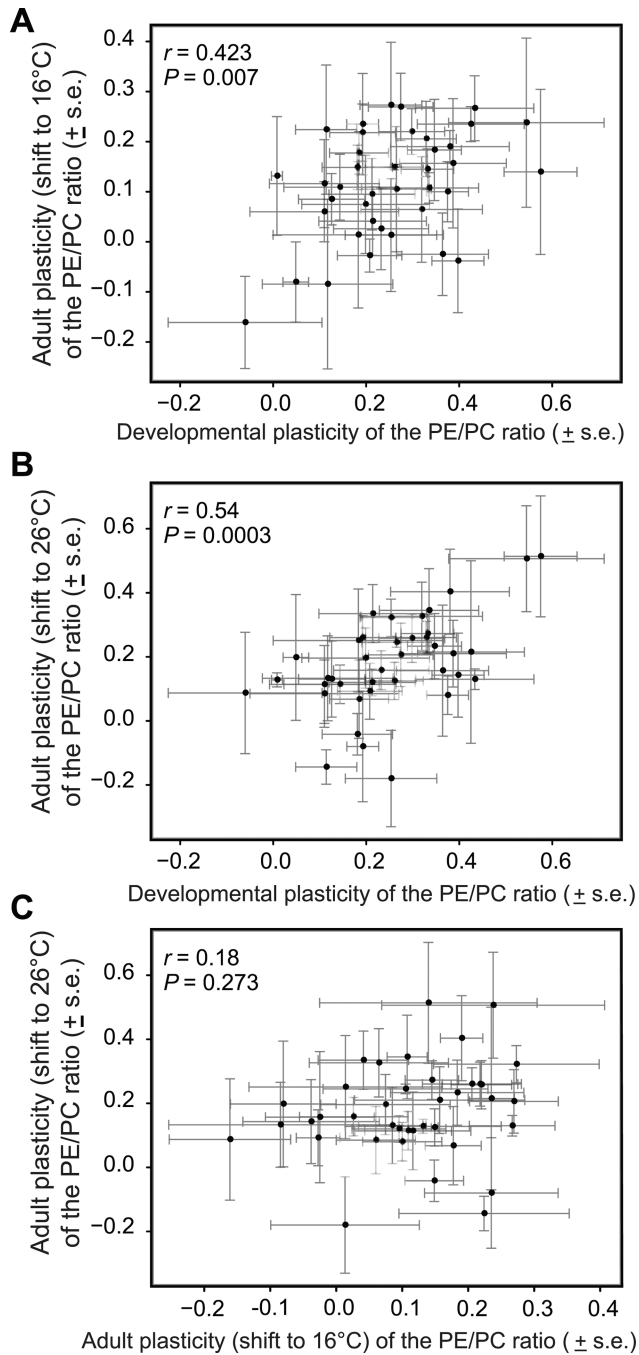
Figure 2.
Developmental and adult responses of PE/PC to temperature covary across but not within life stages. A) Developmental plasticity is significantly and positively correlated with the response of adults shifted to 16°C, and B) with the response of adults shifted to 26°C. C) Adult responses to upward and downward thermal shifts do not significantly covary with one another. Plasticity is calculated as the difference between line means as: developmental plasticity = dev16°C - dev26°C, adult plasticity (shift to 16°C) = adult16°C - dev26°C, and

adult plasticity (shift to 26°C) = dev16°C - adult26°C. Points represent mean plasticity +/−
s.e. for each genetic line.

Figure 3.
Adult plasticity does not always recover developmental values of PE/PC. A) Across all populations, flies developed at 26°C and shifted to 16°C for 72 hours as adults do not recover the 16°C developmental value of PE/PC. B) Flies from IN and NC do recover their 26°C developmental values of PE/PC when shifted as adults to 26°C, while adults from VT do not. Dark bars represent developmental values and light bars represent adult values of PE/PC after being thermally shifted. Data are means +/− s.e. of all genetic lines within each population. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ for paired t-tests

Table 1

Mixed model ANOVAs evaluating the effect of developmental temperature on membrane traits. The random effect of genetic line is included in each model.

Treatments compared	Dependent variable	Fixed effect	num df	den df	F	P-value
Developmental plasticity: 16°C /16°C[1] v. 26°C / 26°C	*PE/PC*	Treatment	1	204	210.96	<0.001
		Population	2	36	1.82	0.177
		Block	5	204	25.83	<0.001
		Population×Treatment	2	204	3.27	0.040
		Population×Block	10	204	3.35	0.001
		Treatment×Block	5	204	1.61	0.158
		Population×Treatment ×Block	10	204	0.69	0.735
	Saturation	Treatment	1	204	90.85	<0.001
		Population	2	36	0.45	0.640
		Block	5	204	16.96	<0.001
		Population×Treatment	2	204	0.75	0.472
		Population×Block	10	204	1.54	0.127
		Treatment×Block	5	204	1.22	0.302
		Population×Treatment ×Block	10	204	0.44	0.926

[1] Larval-to-adult development temperature / adult temperature after eclosion

Table 2

Mixed model ANOVAs evaluating the effect of adult temperature shifts on membrane traits. The random effect of genetic line is included in each model.

Treatments compared	Dependent variable	Fixed effect	num df	den df	F	P-value
Adult plasticity: 26°C / 26°C[1] v. 26°C / 16°C	PE/PC	Treatment	1	204	48.73	<0.001
		Population	2	36	3.12	0.056
		Block	5	204	25.63	<0.001
		Population×Treatment	2	204	0.06	0.940
		Population×Block	10	204	1.62	0.102
		Treatment×Block	5	204	0.72	0.606
		Population×Treatment × Block	10	204	0.82	0.607
	Saturation	Treatment	1	204	50.25	<0.001
		Population	2	36	0.14	0.869
		Block	5	204	13.53	<0.001
		Population×Treatment	2	204	0.42	0.657
		Population×Block	10	204	1.35	0.206
		Treatment×Block	5	204	0.52	0.763
		Population×Treatment×Block	10	204	0.91	0.521
Adult plasticity: 16°C / 16°C v. 16°C / 26°C	PE/PC	Treatment	1	204	80.21	<0.001
		Population	2	36	0.44	0.649
		Block	5	204	19.88	<0.001
		Population×Treatment	2	204	1.06	0.347
		Population×Block	10	204	1.64	0.097
		Treatment×Block	5	204	0.66	0.653
		Population×Treatment ×Block	10	204	0.96	0.477
	Saturation	Treatment	1	204	2.29	0.132
		Population	2	36	1.31	0.282
		Block	5	204	18.60	<0.001
		Population×Treatment	2	204	2.02	0.13
		Population×Block	10	204	1.52	0.136
		Treatment×Block	5	204	5.12	<0.001
		Population×Treatment×Block	10	204	0.46	0.912

[1] Larval-to-adult development temperature / adult temperature after eclosion