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A highly pleiotropic amino acid polymorphism in the *Drosophila* insulin receptor contributes to life-history adaptation

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Finding the specific nucleotides that underlie adaptive variation is a major goal in evolutionary biology, but polygenic traits pose a challenge because the complex genotype–phenotype relationship can obscure the effects of individual alleles. However, natural selection working in large wild populations can shift allele frequencies and indicate functional regions of the genome. Previously, we showed that the two most common alleles of a complex amino acid insertion–deletion polymorphism in the *Drosophila* insulin receptor show independent, parallel clines in frequency across the North American and Australian continents. Here, we report that the cline is stable over at least a five-year period and that the polymorphism also demonstrates temporal shifts in allele frequency concurrent with seasonal change. We tested the alleles for effects on levels of insulin signaling, fecundity, development time, body size, stress tolerance, and life span. We find that the alleles are associated with predictable differences in these traits, consistent with patterns of *Drosophila* life-history variation across geography that likely reflect adaptation to the heterogeneous climatic environment. These results implicate insulin signaling as a major mediator of life-history adaptation in *Drosophila*, and suggest that life-history trade-offs can be explained by extensive pleiotropy at a single locus.

KEY WORDS: **Adaptation, cline, *InR*, pleiotropy, seasonality.**

Many organisms display intraspecific variation in life-history traits, including differences in reproductive timing and allocation, development, body size, stress tolerance, and life span. Alternative life-history strategies have long been hypothesized to represent adaptive responses to variable selection (Stearns 1992), but examples with known causal nucleotides are rare (Flatt and Heyland 2011). However, sequence polymorphisms have been identified recently for flowering time in *Arabidopsis thaliana* (Méndez-Vigo et al. 2013) and adaptation to freshwater environments by marine stickleback fish, which includes changes in life history as well as in physiology and morphology (Jones et al. 2012). One reason that identification of molecular targets of selection is valuable is because it can illuminate mechanisms of multitrait correlation. For example, the gene *Catsup* in *Drosophila melanogaster* affects longevity, locomotor behavior, and bristle number, but individual polymorphisms within the locus act on the traits independently and do not show pleiotropic effects (Carbone et al. 2006). Likewise, coat color in deer mice is a multiphenotype trait associated with the *Agouti* locus, but mutations within the gene appear to have been targeted independently by selection with minimal pleiotropy (Linnen et al. 2013). These examples suggest that fitness-related traits, even those affected by the same gene, may be functionally independent such that natural populations might achieve any genetic and phenotypic combination. Alternatively,

EVOLUTION
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life-history traits routinely exhibit strong genetic correlations, including negative associations between life span and reproduction and positive associations between life span and stress resistance (Reznick 1985; Stearns 1991; Partridge et al. 2005; Harshman and Zera 2007; Vermeulen and Loeschcke 2007; Toivonen and Partridge 2009). These associations mirror pleiotropic effects of laboratory-derived mutations and can be difficult to break in artificial selection experiments (Patridge et al. 1999; Leroi et al. 2005; Anderson et al. 2011a; but see Khazaeli and Curtsinger 2010; Khazaeli and Curtsinger 2013). Consequently, pleiotropy, and specifically antagonistic pleiotropy, in which a genetic element encodes both positive- and negative-fitness phenotypes, remains an important component in the discussion of how life histories evolve (Williams 1957; Flatt and Heyland 2011).

Drosophila melanogaster are distributed across environments that range from temperate to tropical, and exhibit variation in life history that appears adaptive. At high latitudes, populations exhibit higher incidence of reproductive diapause, larger body size, higher cold stress tolerance, and sometimes longer life span and lower fecundity, relative to low-latitude populations (Capy et al. 1993; Mitrovski and Hoffmann 2001; De Jong and Bochdanovits 2003; Schmidt et al. 2005a; Trotta et al. 2006). There is substantial genetic variance for these traits and pervasive genetic correlations among them, indicating that selection in the local environment may act on some phenotypes but drive expression of others through trade-offs (David and Bocquet 1975; Anderson et al. 2003; De Jong and Bochdanovits 2003; Schmidt et al. 2005b; Rako et al. 2007; Schmidt and Paaby 2008). Tolerance to environmental stress may be especially important, and the ability to resist desiccation, starvation, and temperature stress correlates with climatic environment (Hoffmann and Harshman 1999; Hoffmann et al. 2001, 2005, 2007). This framework suggests a hypothetical selection regime: at high latitude, cold winters impose seasonal stress and favor genotypes that confer stress tolerance and overwintering ability; correlated traits such as larger body size, longer life span, slower development, and lower fecundity may evolve as coadapted responses to the same selection regime or by indirect selection via pleiotropy (Paaby and Schmidt 2009).

Few loci have been found to explain the observed genetic variance for *D. melanogaster* life history (De Luca et al. 2003; Carbone et al. 2006; Paaby and Schmidt 2008; Schmidt et al. 2008; Bergland et al. 2012; Remolina et al. 2012; Sgrò et al. 2013). Genes in the insulin/insulin-like growth factor signaling (IIS) pathway are good candidates because experimental manipulation of IIS mirrors the life-history trade-offs observed in natural populations (Clancy et al. 2001; Tatar et al. 2001, 2003; Partridge and Gems 2002; Giannakou and Partridge 2007; Grönke et al. 2010), and some IIS pathway members show evidence of adaptive response in the wild (Dantzer and Swanson 2012; Jovelin et al. 2014). We previously evaluated sequence variation at the *Insulin-like Receptor* (*InR*) in wild populations of *D. melanogaster* and observed striking reciprocal clines in the northern and southern hemispheres for the common alleles of a complex amino acid indel polymorphism (Paaby et al. 2010). The polymorphism disrupts a region of glutamine–histidine repeats in the first exon (Guirao-Rico and Aguadé 2009). In both North America and Australia, the most common allele is at low frequency in tropical and subtropical populations and increases in frequency with latitude, showing highest frequency in temperate populations; the second most common allele exhibits the inverse cline. These patterns appear to be responses to similar but independent selection pressures between the continents: fly populations in North America and Australia were founded at different times and from different source populations (Bock and Parsons 1981; David and Capy 1988), and nucleotide variants on either side of the polymorphism show neutral patterns across geography.

Initial tests showed that the two common alleles, which differ in length by two amino acids and are herein designated InR^{short} and InR^{long}, demonstrated significant effects on fitness traits. InR^{short}, the allele common at high latitudes, was associated with greater stress tolerance; InR^{long}, which is common at low latitudes, was associated with greater fecundity (Paaby et al. 2010). Here, we show that the allele frequency cline in North America persists five years later and that temporal changes in allele frequency within a single population correlate with fluctuations in the seasonal environment, which mimic the temperature and resource availability differences associated with latitudinal climate. We also find that the *InR* locus in general exhibits elevated patterns of clinality and seasonality relative to the rest of the genome. We more thoroughly tested the functional effects of the complex polymorphism by measuring levels of IIS and a spectrum of life-history phenotypes in InR^{short} and InR^{long} lines with randomized genetic backgrounds. We find that the alleles associate predictably with the tested traits, suggesting that the complex amino acid indel polymorphism at *InR* is an important target of contemporaneous selection in wild *D. melanogaster* populations, is highly pleiotropic, and contributes to observed trade-offs in *D. melanogaster* life history.

Methods

ALLELE FREQUENCY ESTIMATES

As described in Bergland et al. (2014), between 50 and 200 isofemale lines were established from six populations spanning Florida to Maine along the North American east coast (latitudes 25.5°N, 30.9°N, 33.0°N, 35.5°N, 39.9°N, 44.1°N). The population from Linvilla Orchard in Media, Pennsylvania (39.9°N, 74.4°W) was additionally sampled in the spring and fall in 2009, 2010, and 2011. Within five generations, a single male from each

line was pooled into population samples, which were sequenced on an Illumina (San Diego, CA) HiSeq2000 using 100-bp paired-end reads. Reads were aligned to the *D. melanogaster* reference genome version 5.39 using *bwa* version 0.5.9-r16 (Li and Durbin 2009). The indel polymorphism at *InR* was called using *UnifiedGenotyper* from GATK version 3.1–1 (McKenna et al. 2010), which identified multiple discrete polymorphisms within the complex indel haplotypes that we previously characterized (Paaby et al. 2010). That characterization identified many haplotypes of different lengths, two of which were common (herein InR^{short} and InR^{long}); the reference genome to which the new pooled samples were mapped has a haplotype of intermediate length (previously referred to as InR^{251}). Thus, the complex haplotypes, previously identified by their lengths, were decomposed into sets of shared or distinct alleles at discrete polymorphic sites.

We used our prior Sanger sequence data (see GenBank accessions GQ927177–GQ927244) to assign alleles of the discrete polymorphisms to the InR^{short} and InR^{long} allele classes. Here, we report those classifications (polymorphisms are denoted by their position in the *D. melanogaster* reference genome version 5.48). Three discrete polymorphisms in the pooled sequencing data discriminate unambiguously between InR^{short} and InR^{long}: 17405631–7, 17405651–4, and 17405784. In each case, the reference genome allele matches InR^{short}. Only one of the discrete polymorphisms, SNP 17405614, was variable within an allele class; the alternate and reference alleles are both associated with InR^{short} such that the association between the discrete allele identities and the larger haplotypes in which they are embedded (InR^{short} and InR^{long}) are approaching equilibrium ($R^2 = 0.45$). SNP 17405619 segregates at low frequency and was invariant in our Sanger sequencing data such that the InR^{short} and InR^{long} haplotypes we observed shared the same discrete allele. Both the alternate allele of SNP 17405631 and the alternate allele of 17405634–7 match both InR^{short} and InR^{long}. For polymorphism 17405637, two alternate alleles were called; the six-nt insertion matches InR^{long}, but the three-nt insertion is confounded with the alternate allele of 17405634–7 (which is called consistently). Thus, we ignored the three-nt insertion allele at 17405637.

Allele frequencies of SNPs at *InR* and across other sites on chromosome 3R were called from the resequencing data using CRISP (Bansal 2010) as described in Bergland et al. (2014). To test whether the *InR* locus demonstrates elevated clinality or seasonality relative to the rest of the genome, we calculated the proportion of clinal and seasonal SNPs at *InR* and compared them to random genome regions matched for length, chromosome, and inversion status. Clinality and seasonality was estimated by regressing allele frequencies on latitude or season ("spring" vs. "fall"), respectively; technical details of this analysis are available in the Supporting Information.

GENOTYPING

To genotype flies for the experimental assays, the identity of the complex *InR* indel was determined by measuring its amplified fragment length following polymerase chain reaction (PCR) with a fluorescent-tagged primer on an Applied Biosystems (Foster City, CA) 3100 capillary sequencer. PCR conditions, including primer sequences, are described in Paaby et al. (2010). That paper reported the distribution of alleles across geography and referred to the alleles by their PCR fragment lengths, which we erroneously stated as 248 bases for the high-latitude allele and 254 bases for the low-latitude allele. The actual fragment lengths using the cited primers are five bases longer. To avoid confusion, in this article we refer to these two alleles as InR^{short} (previously InR^{248}) and InR^{long} (previously InR^{254}). It should be noted that although fragment-length genotyping does not discriminate between the SNP alleles we characterized in or near the complex indel polymorphism (described above), only one of those SNPs (17405614) appears to be independent of the InR^{short} and InR^{long} allele classes. All evidence to date indicate that fragment-length genotyping unambiguously discriminates between consistent, but complex, haplotype classes (see Fig. 1A, B).

FLY STOCKS

To determine whether InR^{short} and InR^{long} alleles have different effects on phenotype, we generated stocks with InR^{short} or InR^{long} on the third chromosome, and for which the X and second chromosomes were replaced so that they were isogenic within and across lines. The X chromosome was derived from stock 2475 and the second chromosome was derived from stock 6326 from the Bloomington Stock Center. The genome regions surrounding *InR* on the third chromosome were randomized, from lines originally genotyped as InR^{short} and InR^{long}, across lines. To generate the stocks, we selected InR^{short} and InR^{long} parental strains without the *In(3R)Payne* inversion, crossed them, and allowed the offspring to recombine for four generations so that genetically variable regions from the parental third chromosomes were distributed as randomized blocks across lines. Fourteen isogenic stocks were subsequently established per allele by homozygosing the third chromosome via balancer extraction and genotyping the *InR* indel polymorphism. This entire scheme was performed twice, using lines derived from two independent populations: Mount Sinai, New York (40.95°N, 72.84°W) and Bowdoin, Maine (44.01°N latitude, 69.90°W longitude). Replicating across populations provided two sets of randomized genetic backgrounds in which to test the *InR* genotypes. Although four generations of laboratory-imposed recombination are unlikely to have isolated the *InR* alleles completely from the parental backgrounds, the rapid decay of linkage disequilibrium surrounding the polymorphism (Paaby et al. 2010) means that genetic variation on 3R is already randomized in natural populations.

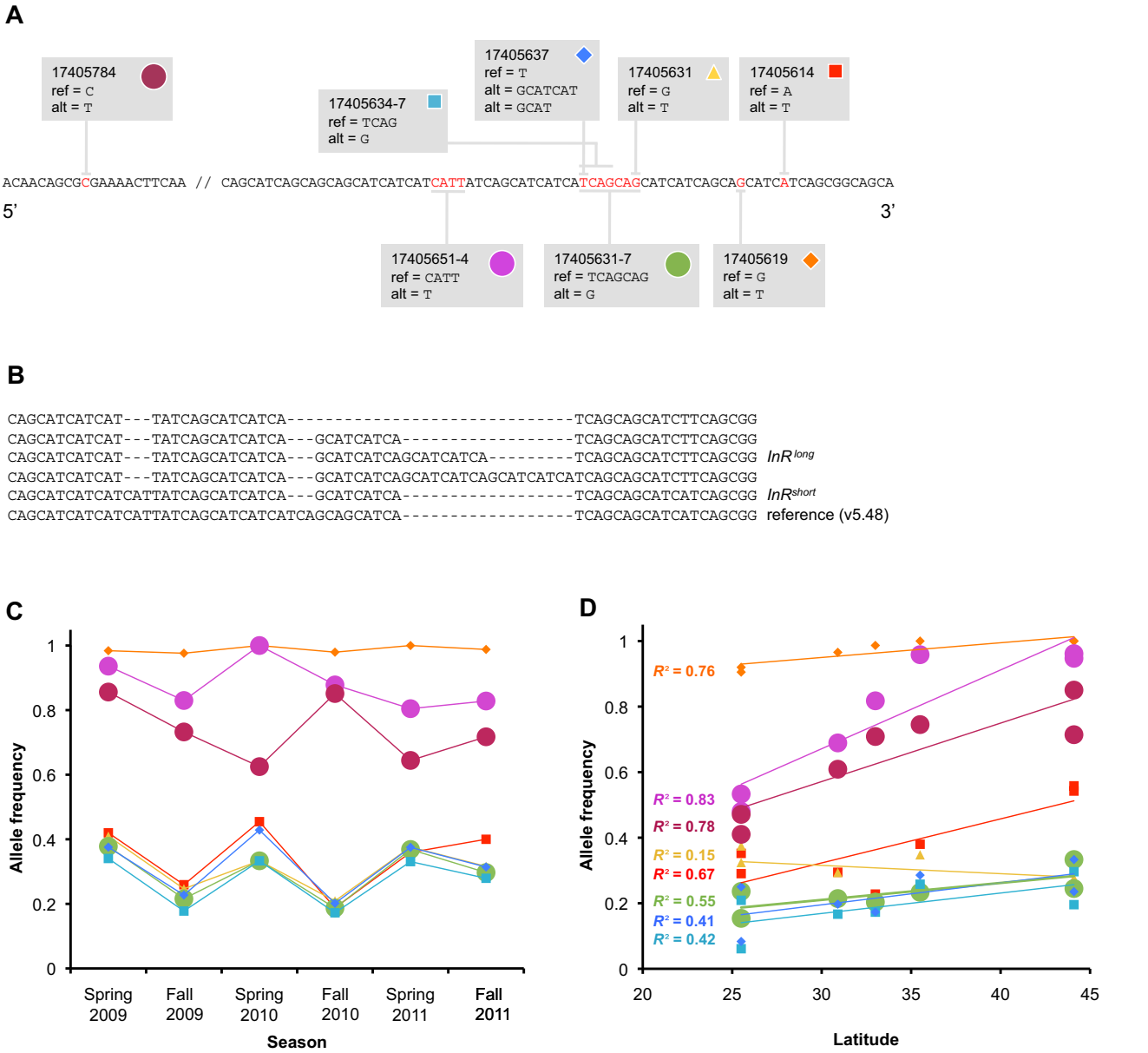

Figure 1. From pooled sequencing data, we identified eight discrete polymorphisms associated with the complex indel in the first exon of *InR* (A). Each polymorphism is labeled according to position on chromosome 3R relative to the published *D. melanogaster* reference genome (version 5.48). The discrete polymorphisms describe the complex indel haplotypes we previously characterized, which include two alleles, InR^{short} and InR^{long}, that are common at high and low latitudes, respectively (B). Polymorphisms that unambiguously discriminate between the InR^{short} and InR^{long} alleles are denoted with a large dot; in each case, the allele present in the reference genome identifies InR^{short}. (See Methods for relationships between the other discrete polymorphisms and InR^{short} and InR^{long}.) All eight of the discrete polymorphisms demonstrate some degree of seasonality, but four exhibit especially strong patterns in which the plotted allele increases in frequency by approximately 20% over the winter for three consecutive years (C). Alleles assigned to the InR^{short} allele class (large dots) generally show higher frequency in the spring and lower frequency in the fall, suggesting that overwintering may impose selection pressures consistent with high latitudes. As in our earlier report (Paaby et al. 2010), alleles assigned to the InR^{short} allele class also increase in frequency with latitude (D). The plotted alleles are all reference genome alleles.

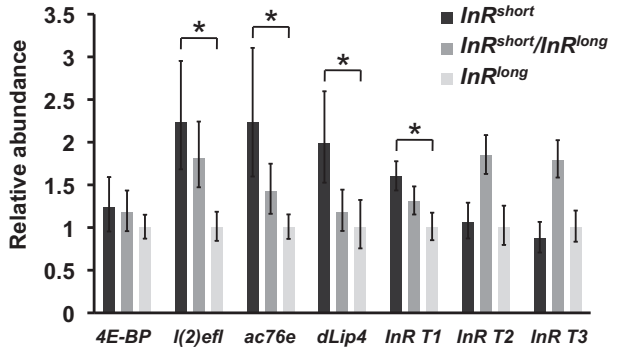

Figure 2. Relative abundance of seven transcriptional targets of dFOXO, a central transcription factor in the IIS pathway that is repressed by InR activity. Increased abundance of these targets indicates reduced IIS; transcript abundance for *l(2)eft*, *ac76e*, *dLip4*, and *InR T1* were significantly greater ($P < 0.05$) in InR^{short} samples compared to InR^{long}. These results are consistent with the expectation that InR^{short} is associated lower IIS than InR^{long}. Error bars show 95% confidence intervals.

InR^{short} and InR^{long} alleles were evaluated as homozygotes and heterozygotes, as well as with *InR* mutant and wild-type alleles derived from a laboratory strain. The *InR* mutant was the hypomorphic InR^{p5545} allele generated by P-element insertion in exon 1 (Tatar et al. 2001) balanced over the *TM3* chromosome (stock 11661 from the Bloomington Stock Center, ry^{506} $P\{PZ\}InR^{p5545}/TM3$, ry^{RK} Sb^{1} Ser^{1}). The *TM3* balancer contains a wild-type *InR* allele (herein InR^{TM3}) that is intermediate in length between InR^{short} and InR^{long}: it is three nucleotides longer than InR^{short}, corresponding to an additional histidine, and three nucleotides shorter than InR^{long}, which contains an additional glutamine, as determined by Sanger sequencing.

FLIES FOR ASSAYS

Once the stocks were established, we used three methods to generate flies for the phenotype assays (derived from one or both of the independent populations). The first two tested the effects of InR^{short} and InR^{long} in a wild-type background; the third tested InR^{short} and InR^{long} effects while paired with either the *InR* hypomorphic allele or a wild-type allele in the *TM3* balancer chromosome. In the first method, we exerted nominal control over the background variation in the stocks by crossing 14 InR^{short} and 14 InR^{long} stocks in a round-robin design to generate a total of 42 lines from which we collected flies for phenotyping (14 each of InR^{short}/InR^{short}, InR^{long}/InR^{long}, and InR^{short}/InR^{long}). The crosses were conducted in standard media vials with seven virgin females (two- to five-day old) and four young males (three- to eight-day old). This design produced nonindependent replication within each genotypic class (in that chromosomes were shared across lines), and because test stocks were never mated to themselves, also ensured that no individuals were strictly isogenic at the third chromosome. In the second method, we combined stocks (minimum of five) carrying the same allele (InR^{short} or InR^{long}) but otherwise randomized variation on the third chromosome in bottles, permitting the stocks to continue recombining freely. To rear flies for the assays, 40 virgin females (two- to five-day old) and 20 young males (three- to eight-day old) were collected and mated in fresh bottles, either within or across genotype class, to generate the two homozygous and the heterozygous genotypes. Unless otherwise specified, the phenotype assays employing this method were replicated via 10 mating bottles for each genotypic class. In the third method, we crossed 12 InR^{short} stocks and 12 InR^{long} stocks to the hypomorphic $InR^{p5545}/TM3$ stock (in vials as in method 1). From these 24 lines, we collected progeny carrying both the *InR* hypomorphic allele and wild-type allele in the balancer chromosome for phenotyping.

PHENOTYPE ASSAYS

For all assays, flies were reared and assays were performed on standard cornmeal-molasses media at room temperature and subject to ambient light cycles. Larval density was kept low to limit overcrowding. We tested InR^{short} and InR^{long} alleles derived from Mount Sinai in all assays; we tested InR^{short} and InR^{long} alleles from Bowdoin in a subset of the assays. All InR^{short} and InR^{long} alleles tested in the presence of the *InR* hypomorph (InR^{p5545}) or wild-type balancer (InR^{TM3}) were from Mount Sinai. All statistics were performed using JMP version 7 (SAS Institute, Cary, NC). Within analyses, we used planned comparisons to test for significant differences between InR^{short} and InR^{long} genotypes. We measured 11 different traits, including life span, lifetime fecundity, early fecundity, development time, body weight, lipid content, body size, chill coma recovery, cold shock tolerance, starvation resistance, and heat shock resistance; assay details can be found in the Supporting Information.

QUANTITATIVE PCR

To test whether *InR* alleles affect IIS, we used quantitative PCR (qPCR) to determine the relative abundance of seven transcriptional targets of dFOXO, a central transcription factor within the IIS pathway (Jünger et al. 2003; Puig et al. 2003; Wang et al. 2005; Casas-Tinto et al. 2007; Flatt et al. 2008; Vihervaara and Puig 2008; Matilla et al. 2009). Total RNA was prepared from flies generated by method two from flies derived from Mount Sinai. Thirty virgin females from each of the three genotypic classes were collected over 24 h, aged on standard media, and snap frozen. RNA was extracted using RNeasy (Qiagen) and reverse transcribed using iScript cDNA synthesis kit (Bio-Rad), following the manufacturer's protocol. Relative abundance of transcript levels was determined using an ABI 7500 Fast Real-Time PCR machine and SYBR Green PCR Master Mix (Applied Biosystems) by the DDC_T relative quantitation method. Four technical

replicates were used for each sample and relative abundance was normalized by using *GAPDH2* as an endogenous control. Other work has demonstrated that *GAPDH2* is an appropriate control for measuring relative levels of IIS (Hwangbo et al. 2004; Flatt et al. 2008). Primer sequences for the qPCR reaction are listed in the Supporting Information.

Results

CLINAL AND SEASONAL ESTIMATES OF ALLELE FREQUENCY

Previously, we showed that *InR* allele frequencies change in association with latitude on two continents, and hypothesized that this was in response to climate-driven selection (Paaby et al. 2010). Now, we ask whether the allele frequency cline in North America persists five years later and whether allele frequencies fluctuate seasonally in a single temperate population. Our previous survey identified haplotypes; here, we interrogated allele frequencies for the discrete polymorphisms that comprise those haplotypes using resequencing data. We find strong evidence of both clinality and seasonality for the indel polymorphism at *InR*, and support for a high degree of complexity in this system given that discrete sites within the haplotypes appear to respond differently to geographical and seasonal pressures (Fig. 1).

We identified all variants (SNPs or small indels) that comprise the complex indel itself and three SNPs –11, –16, and +130 nucleotides from the indel that segregate at frequencies above 5% (Fig. 1A, B). Four of these discrete polymorphisms show clear seasonality, with repeating fluctuations in allele frequency of approximately 20% between spring and fall time points over three consecutive years (Fig. 1C). The other four discrete polymorphisms are suggestive of seasonality: they demonstrate either comparable changes in amplitude but a deviation in the frequency pattern at a single time point, or a repetitive pattern at low amplitude. The discrete polymorphisms also show clines in allele frequency across latitude (Fig. 1D). We were able to unambiguously assign allele identities for three of the discrete polymorphisms to the InR^{short} or InR^{long} allele classes (Table S1), which exhibited strong allele frequency clines previously (Paaby et al. 2010). In each of these cases, changes in allele frequency across latitude replicated our earlier observations, and seasonal fluctuations in frequency for these discrete polymorphisms support the hypothesis that alleles favored at high latitude are favored during the winter.

Compared to the rest of the genome, the *InR* locus exhibits elevated patterns of allele frequency change across latitude and seasonal time. The region surrounding *InR* contains significantly more clinal polymorphisms relative to other regions of chromosome 3R matched for size and inversion status (42% vs. 20%, respectively; $\Pr(\%InR > \%\text{control}) = 0.986$). Relative to matched control regions, the *InR* locus also contains slightly more seasonal polymorphisms (6% vs. 4.7%; $\Pr(\%InR > \%\text{control}) = 0.8$). Previous examination of linkage disequilibrium across *InR* (Paaby et al. 2010) indicates that the complex indel polymorphism is largely independent of variation elsewhere in the gene, which implies that many sites within *InR* may be targets of spatially and temporally varying selection.

ALLELE EFFECTS

We evaluated the effects of *InR* alleles on levels of IIS and multiple phenotypes and found that individuals carrying the InR^{short} allele, which is prevalent in high-latitude, temperate environments, exhibited lower levels of IIS, better survived cold temperature stresses and starvation, and showed sex-specific evidence for longer life span. Flies carrying the InR^{long} allele, which is common in low-latitude, warm environments, were associated with increased signaling and exhibited higher rates of fecundity, larger body mass, faster development time, and better survival of heat shock. These phenotypic differences are consistent with experimental reduction of IIS as well as life-history trade-offs observed in natural populations, and suggest that a single locus can act pleiotropically on a suite of fitness-related traits. We replicated some of the assays by using InR^{short} and InR^{long} alleles derived from two independent populations (Bowdoin and Mount Sinai); the results are reported separately. We also evaluated the effects of InR^{short} and InR^{long} in an *InR* hypomorphic background with reduced InR kinase activity. We found that the effects of InR^{short} versus InR^{long} are generally subtler than those of the wild-type versus hypomorph, and are consistent with our other observations of InR^{short} and InR^{long} and with prior work describing *InR* mutant alleles; a summary of these findings, including additional figures and tables (Figs. S2–S4 and Table S2), is available in the Supporting Information.

LEVELS OF INSULIN SIGNALING

To test whether the observed phenotypic differences between InR^{short} and InR^{long} are mediated by different levels of IIS, we used qPCR to measure relative abundance of seven transcriptional targets of dFOXO, a central transcription factor in the IIS pathway that is repressed by InR activity. Decreased InR activity increases target abundance (Puig et al. 2003), and transcripts for five of these (*4E-BP*, *l(2)efl*, *ac76e*, *dLip4*, and *InR T1*) were highest in InR^{short}, intermediate in the heterozygote, and lowest in InR^{long}; all but *4E-BP* showed statistical significance ($P < 0.05$) between InR^{short} and InR^{long} (Fig. 2). This pattern is robust considering that the InR^{short} genotype exhibited at most twofold higher levels relative to InR^{long}: a difference that may be biologically meaningful, and realistic for wild-type genotypes, but close to the limit of detection by qPCR methods. Two other targets, transcripts

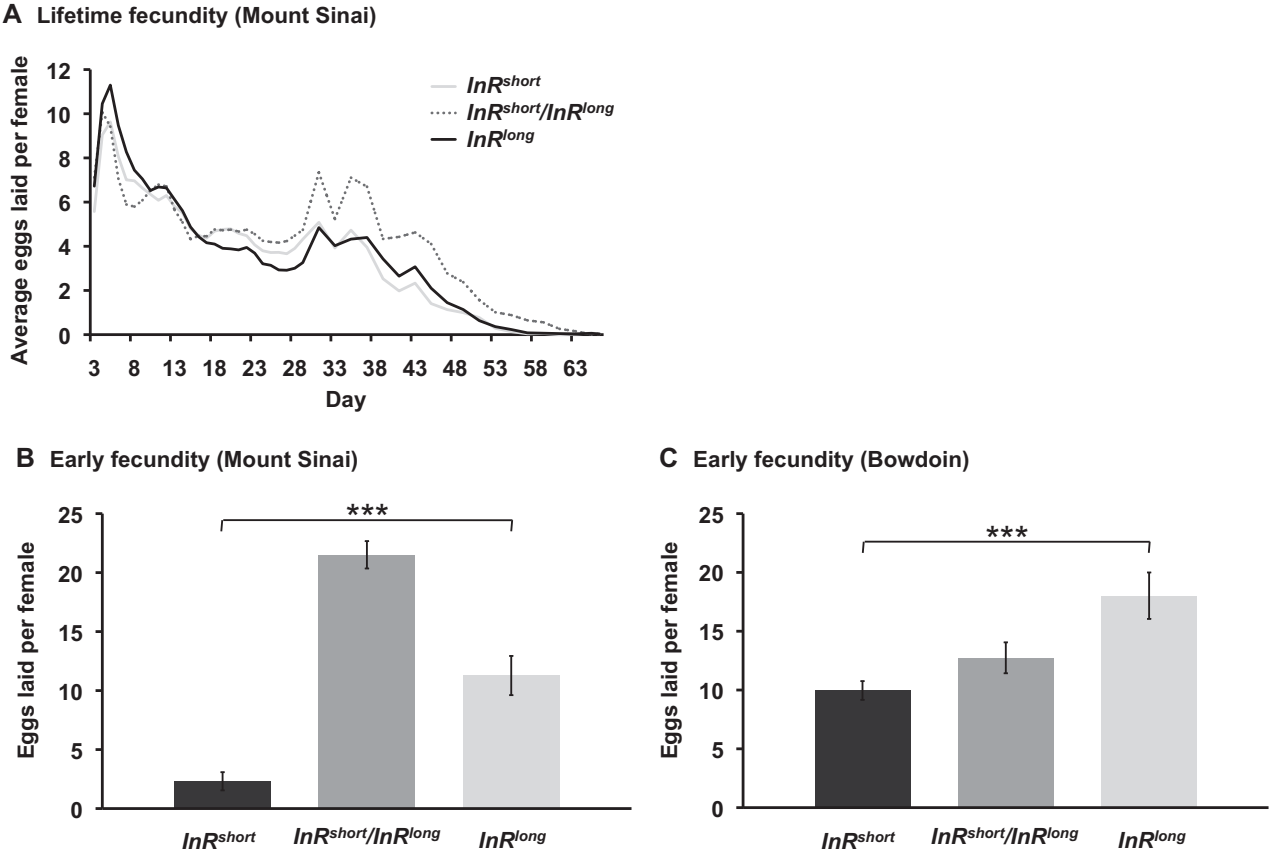

Figure 3. **Average eggs laid per female over lifetime and in the first 12 h after mating. Lifetime fecundity was not significantly different between any genotypes except between InR^{short} and the heterozygote (A). However, InR^{long} females laid many more eggs in the first 12 h after mating than InR^{short} females, in comparisons from both populations (C). Error bars show 95% confidence intervals.**

of *InR* itself, show no difference in abundance level between the homozygote genotypes, but significantly higher abundance in the heterozygote.

FECUNDITY

Surprisingly, the homozygous *InR* genotypes showed no significant differences in total fecundity: a fly carrying the InR^{long} allele laid on average only 5.3 more eggs over her lifetime than a fly carrying InR^{short} ($P = 0.6949$, Table 1). This was unexpected because we routinely observed substantially greater population numbers in our InR^{long} bottle cultures, and previous results showed that the InR^{long} genotype is more fecund (Paaby et al. 2010). Examination of the eggs laid per day, however, revealed that the InR^{long} genotype lays more eggs early in life (Fig. 3A). We performed another assay to explicitly measure early fecundity, or how many eggs young mated females laid upon first access to fresh food. Here, we found that the InR^{long} genotype is nearly six times more fecund than InR^{short} in the first 12 h of egg laying ($P < 0.0001$, Table 1 and Fig. 3B). This result was again observed in a replicate assay using flies derived from the second population ($P = 0.0005$, Table 1 and Fig. 3C).

DEVELOPMENT TIME

From egg to emerging adult, flies carrying the InR^{long} allele developed faster than flies carrying InR^{short}. This result was replicated in flies derived from two populations: in the first, males and females carrying InR^{long} developed an average of 6.0 h faster than those carrying InR^{short} ($P < 0.0001$); in the second population, development time was not significantly different among male genotypes, but InR^{long} females emerged an average of 18.2 h ahead of InR^{short} females ($P = 0.0029$, Table 1 and Fig. 4).

BODY WEIGHT AND SIZE

Because reduction in IIS reduces body size (Clancy et al. 2001; Tatar et al. 2001), we predicted that individuals carrying the InR^{long} allele would be larger than individuals carrying InR^{short}. Indeed, the average dry body weights of InR^{long} flies were heavier than InR^{short} flies. This was true for alleles tested from both populations: InR^{long} females and males derived from Bowdoin were 26.1% and 18.6% heavier, respectively ($P < 0.0001$ for both), whereas InR^{long} females and males derived from Mount Sinai were 15.5% and 10.9% heavier ($P = 0.0002$ and $P = 0.0004$, Table 1 and Figs. 5, S1). The higher mass associated with the

Table 1. Statistical results for planned comparisons and other analyses explicitly testing for significant differences between InR^{short} and InR^{long}.

	DF	SS	*F*	*P*
Mount Sinai				
Lifetime fecundity	1, 39	200.18	0.1561	0.6949
Early fecundity	1, 21	299.77	22.79	0.0001
Development time	1, 3006	17049.77	113.23	<0.0001
Dry body weight (f)	1, 45	1.10×10^{-6}	16.73	0.0002
Dry body weight (m)	1, 44	2.22×10^{-7}	14.99	0.0004
Lipid weight (f)	1, 44	0.0013	0.1361	0.7140
Lipid weight (m)	1, 43	0.0303	1.4110	0.2414
L2 wing vein (f)	1, 230	0.0157	9.0749	0.0029
L2 wing vein (m)	1, 212	0.0005	0.2759	0.5999
Chill coma (f)	1, 187	246,318.61	2.14	0.1448
Chill coma (m)	1, 207	534,075.8	16.31	<0.0001
Bowdoin				
Early fecundity	1, 27	324.82	15.55	0.0005
Development time (f)	1, 244	9383.14	9.07	0.0029
Development time (m)	1, 278	1652.35	1.42	0.2341
Dry body weight (f)	1, 37	1.42×10^{-6}	103.96	<0.0001
Dry body weight (m)	1, 39	4.03×10^{-7}	63.17	<0.0001
Lipid weight (f)	1, 36	0.0767	11.0854	0.0020
Lipid weight (m)	1, 38	0.3759	27.3649	<0.0001
L2 wing vein (f)	1, 117	0.3969	297.3210	<0.0001
L2 wing vein (m)	1, 123	0.3281	35.8582	<0.0001
Wing:thorax ratio (f)	1, 117	0.0599	35.8776	<0.0001
Wing:thorax ratio (m)	1, 123	0.0627	5.4002	0.0218
Mount Sinai	DF	χ^2	*P*	Risk ratio
Lifespan (f, het excluded)	1	0.0188	0.8909	0.9870
Lifespan (m, het excluded)	1	6.7336	0.0095	0.7665
Mount Sinai	Odds ratio	Reciprocal		
Cold shock (f)	0.2613	3.8275		
Cold shock (m)	0.4231	2.3636		
Starvation (f)	0.7493	1.3346		
Starvation (m)	0.5503	1.8173		
Heat shock	15.2938	0.0654		

Where sex was significant in the full model (see Tables S2 and S3), females and males were evaluated separately.

InR^{long} genotype is partially explained by lipid content. For both populations, the InR^{long} allele was associated with greater lipid mass than InR^{short} (Figs. 5, S1); however, only alleles derived from Bowdoin demonstrated a statistically significant difference when nonlipid mass was included as a covariate ($P = 0.0066$ for females, $P < 0.0001$ for males, Table 1). Wings of InR^{long} flies were also larger than those for InR^{short}, at least in the Bowdoin population. In that comparison, the average L2 wing vein length was 0.24 mm longer in InR^{long} females ($P < 0.0001$) and 0.12 mm longer in InR^{long} males ($P < 0.0001$, Table 1 and Fig. 5). In flies derived from Mount Sinai, InR^{short} females had the larger wings, but only by 0.02 mm ($P = 0.0029$), and InR^{short} and InR^{long} males were not different from each other ($P = 0.5999$, Table 1 and Fig. S1).

An association between large size and InR^{long}, the low-latitude allele, is predictable in terms of insulin signaling, but unpredictable given the observation that *Drosophila* are larger at higher latitude (Huey et al. 2000; De Jong and Bochdanovits 2003). Others have shown that proportionally larger wings relative to thorax size are associated with better flying in *Drosophila* (Hoffmann et al. 2007) and that the wing:thorax ratio increases with latitude (Karan et al. 1998), possibly because muscles in colder temperatures generate less power and proportionally larger wings can compensate for this (Gilchrist and Huey 2004). We measured thorax width to see if bigger wing:thorax ratios were associated with the high-latitude InR^{short} allele, but they were not. InR^{short} flies averaged smaller wing:thorax ratios relative to InR^{long} flies ($P < 0.0001$ for females, $P = 0.0218$ for males,

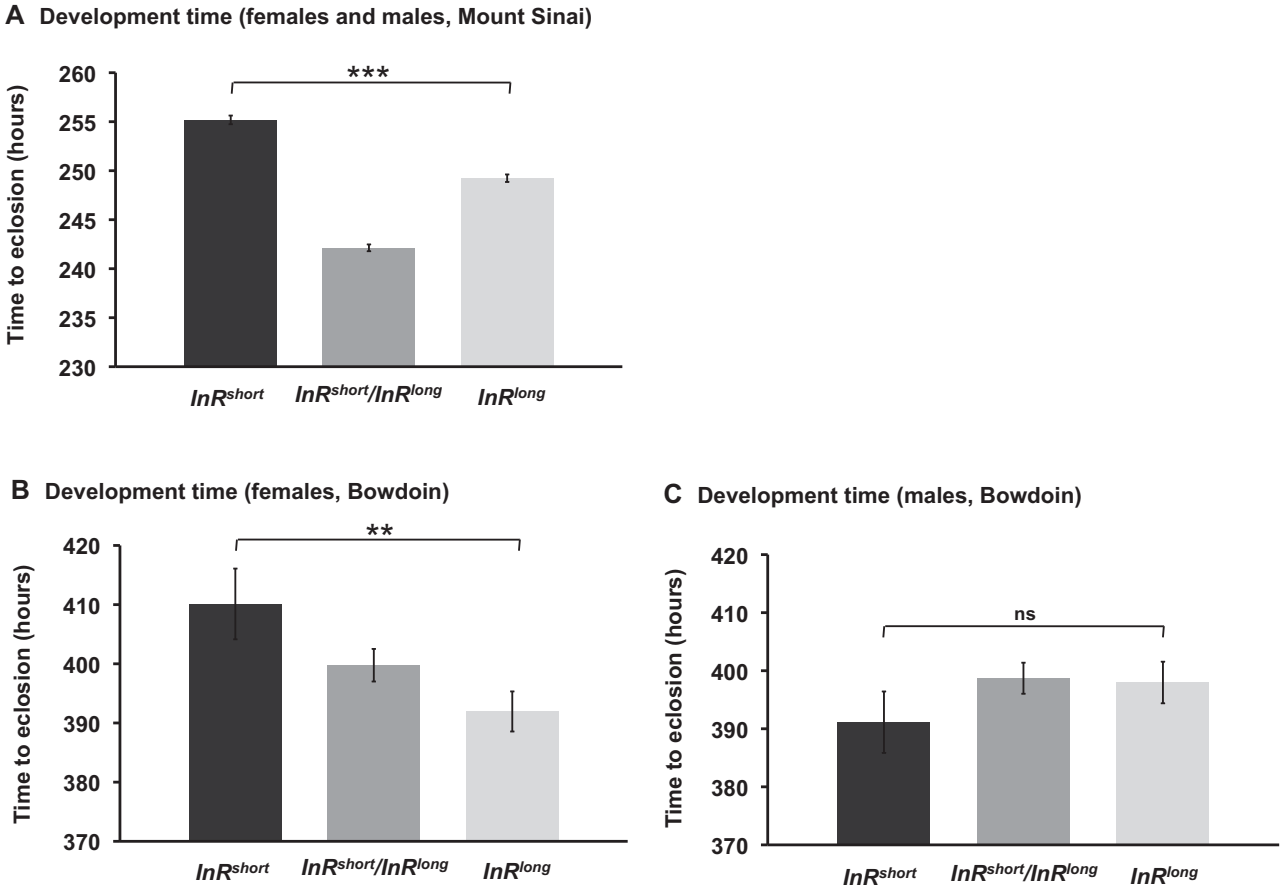

Figure 4. Average development time, from the time the egg was laid to eclosion of the adult. Flies derived from the Mount Sinai population showed no significant differences in development time by sex, and overall InR^{long} flies developed faster than InR^{short} flies (A). However, InR^{long} females from Bowdoin developed faster than InR^{short} females (B), whereas male genotypes were not significantly different (C). Error bars show 95% confidence intervals.

Table 1 and Fig. 5). For all samples in which we measured the L2 wing vein, we also measured the L3 vein, and observed qualitatively identical results (data not shown).

STRESS TOLERANCE

Reduction in IIS confers increased resistance to stress (Giannakou and Partridge 2007), and results for three of our four stress assays accord with these findings and our hypothesis that the high-latitude InR^{short} allele mediates increased stress tolerance via reduced signaling (Fig. 6). Females carrying the InR^{short} allele recovered from chill coma on average 89 sec faster than females carrying InR^{long}; InR^{short} males recovered 123 sec faster (although this comparison was only statistically significant for males; $P = 0.1448$ and $P < 0.0001$, respectively, Table 1 and Fig. 6). Likewise, InR^{short} females and males exhibited 3.8 and 2.4 times the odds of surviving cold shock and 1.3 and 1.8 times the odds of surviving starvation, respectively, compared to InR^{long} flies. However, the InR^{short} allele did not correlate with higher tolerance of heat shock: instead, InR^{short} flies showed 15.3 times the odds of dying in the assay compared to InR^{long} flies (Table 1 and Fig. 6). All four of these outcomes are consistent with presumed selection pressures in natural populations, as flies in high-latitude, temperate environments, where the InR^{short} allele is common, likely face cold temperatures and scarce food availability during the winter, whereas flies in low-latitude, semitropical environments, where InR^{long} is common, should face thermal stress from higher temperatures. However, if levels of IIS mediate these stress phenotypes, the heat shock outcome provides a unique example of correlation reversal between signaling and stress. Nevertheless, the phenotypic correlations across these traits, and the correlations between the phenotypes and latitude, are patterns consistent with other examples of reciprocal tolerance for environmentally imposed thermal stresses (Hoffmann et al. 2001, 2005).

LIFE SPAN

Insulin signaling has been established as a major regulator of longevity in many animals, including *Drosophila*: hypomorphic alleles of *InR* increase life span relative to wild-type controls

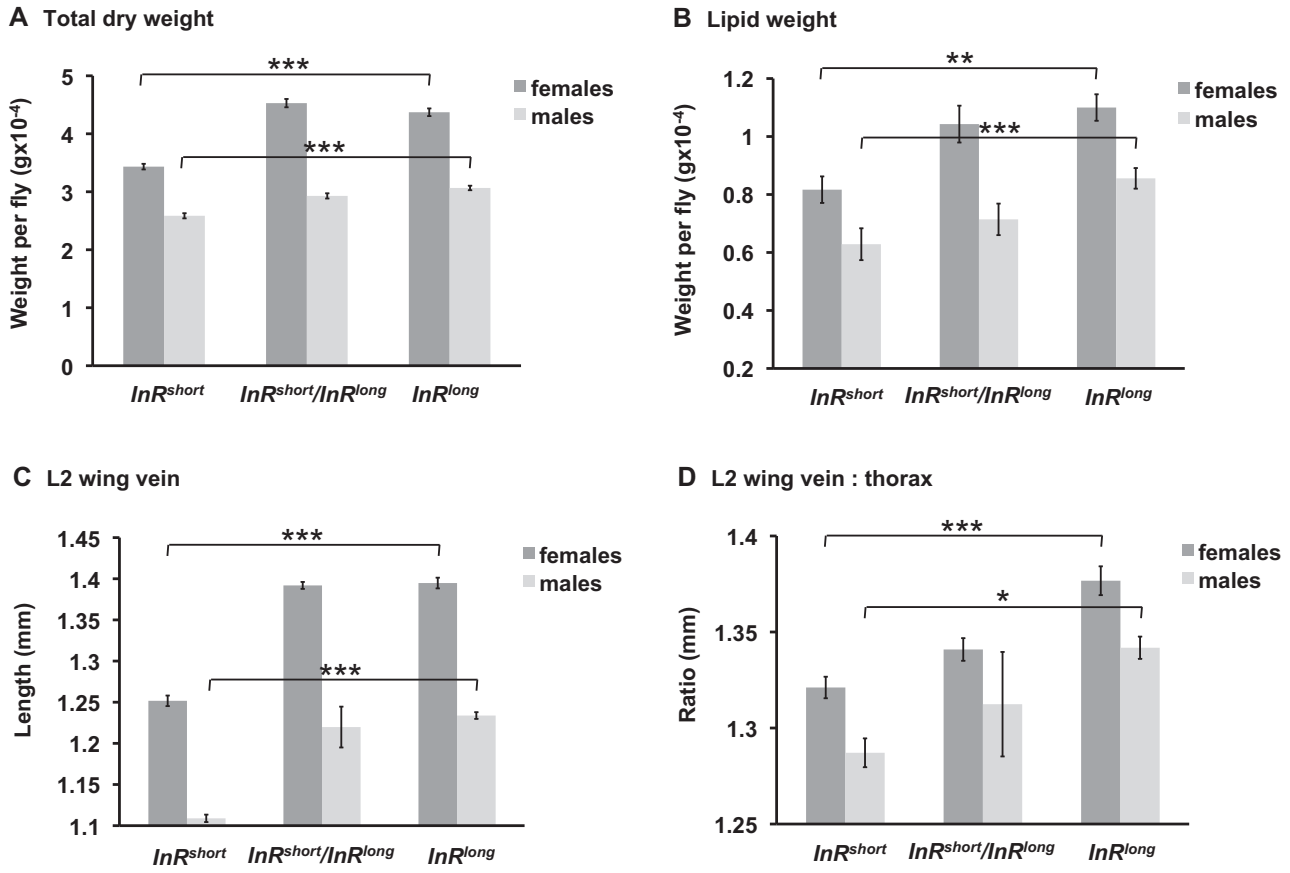

Figure 5. Average dry body weight, lipid weight, length of the L2 wing vein, and wing:thorax ratio. *InR*^{short} flies had lower dry weight (A) and lower lipid content (B) than *InR*^{long} flies. *InR*^{short} flies were also smaller, as measured by the L2 wing vein (C), and had a smaller wing:thorax ratio (D). These results are from flies derived from Bowdoin; similar findings, but of lower magnitude, were observed in flies derived from Mount Sinai (Figure S1). Error bars show 95% confidence intervals.

(Tatar et al. 2001), as do other manipulations that reduce IIS (Clancy et al. 2001; Hwangbo et al. 2004; Grönke et al. 2010). Consequently, we hypothesized that *InR*^{short} might increase life span relative to *InR*^{long}. Flies carrying the *InR*^{short} and *InR*^{long} alleles showed little difference in rates of aging over the majority of life span, although *InR*^{short} males exhibited reduced mortality late in life (Fig. 7). The lifetime survivorship of *InR*^{short} males was significantly longer than that of *InR*^{long} males, with a mortality risk ratio of 1.3 for *InR*^{long} over *InR*^{short} ($P = 0.0095$). The female mortality risk ratio for *InR*^{long} over *InR*^{short} was only 1.01, with no significant difference in longevity between these genotypes ($P = 0.8909$). Interestingly, the heterozygote of both sexes lived significantly longer ($P < 0.0001$) than either of the homozygote genotypes (Table S3). We hypothesize that the heterozygote longevity is a result of heterosis across the extended *InR*-embedded locus, a consequence of the fact that the homozygotes carried identical alleles with haplotypes extending beyond *InR*. Any general increase in fitness associated with greater heterozygosity was probably not mediated by IIS, as the heterozygotes were associated with intermediate levels of gene expression for the majority of IIS targets.

Discussion

Our results indicate that the complex indel polymorphism in the first exon of *InR* is likely a direct target of natural selection in wild populations of *D. melanogaster*. Allele frequencies exhibit persistent latitudinal clines, change rapidly and cyclically over seasonal time scales, and the two common alleles are pleiotropically associated with multiple traits that correspond to expected pressures in distinct climatic environments. The alleles are also associated with predicted up- and downregulation of IIS, strongly suggesting that they mediate signaling behavior to effect differential trait expression. The fact that the alleles encode different amino acid sequences suggests that this occurs through changes in protein function. Overall, our findings provide an example of life-history trade-offs influenced by a complex, strongly pleiotropic polymorphism.

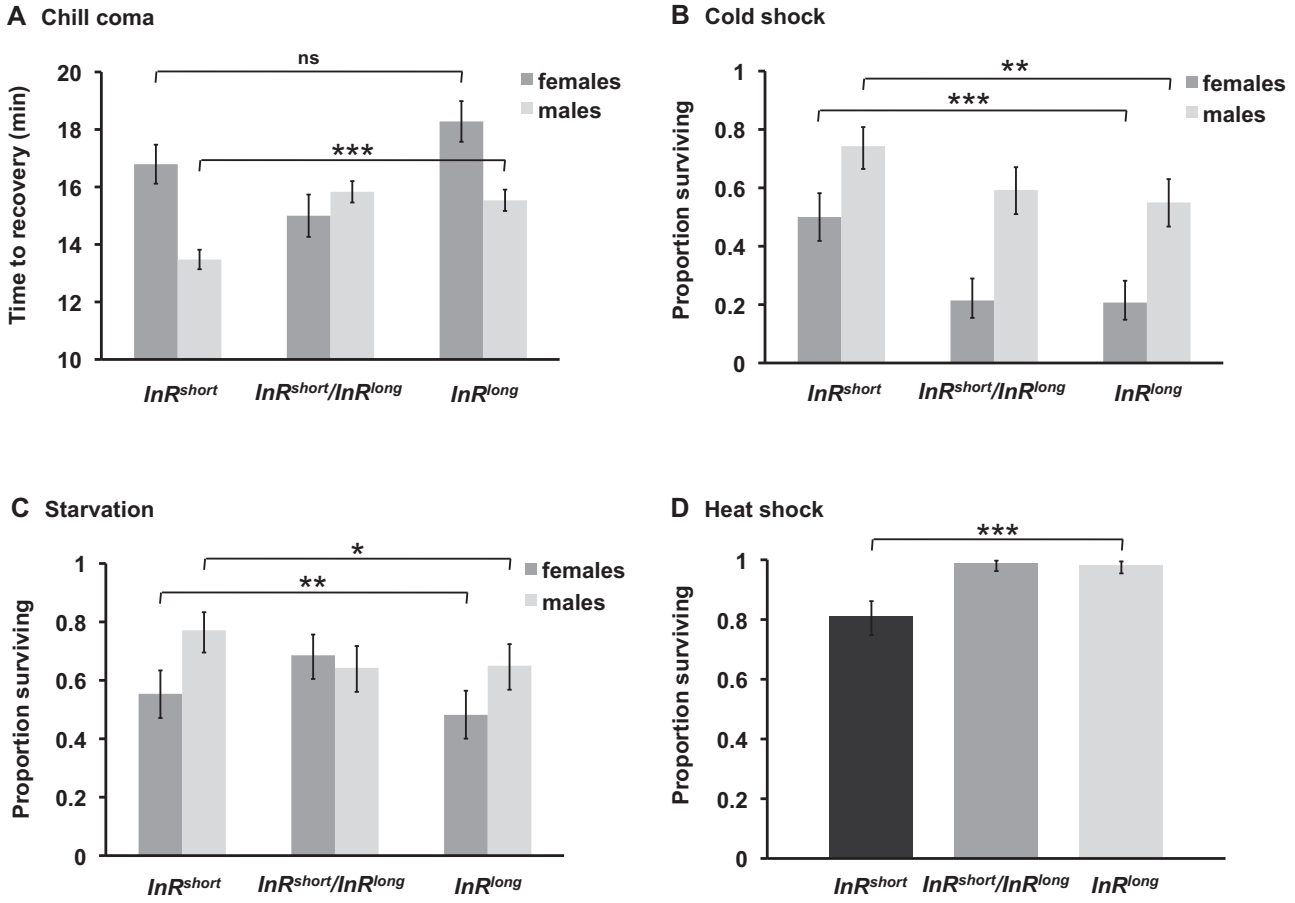

Figure 6. Average time to recovery from chill coma and proportion of flies surviving cold shock, starvation, and heat shock. Although InR^{short} females and males both recovered faster from chill coma than did InR^{long} flies, the effect was only significant for males (A). InR^{short} flies also better survived cold shock (B) and starvation (C), but more InR^{long} flies survived heat shock (D). Error bars show 95% confidence intervals.

Although the phenotypes associated with InR^{short} and InR^{long} were specific and predictive, our assays did not precisely test the effects of the glutamine and histidine changes that account for the length differences in the complex indel polymorphism. This is for two reasons. First, the InR^{short} and InR^{long} alleles themselves contain several other SNPs, each of which exhibits nonrandom patterns of allele frequency across latitude and seasonal time (Fig. 1). Only one of these (a nonsynonymous SNP at position 17405614) has the potential to confound our functional results, however, as the other SNPs did not vary between our InR^{short} and InR^{long} lines. Allele frequencies at 17405614 exhibited substantial seasonal and clinal patterns, although not as strongly as several of the discrete indel variants that comprise the complex indel polymorphism (Fig. 1). Second, genome regions flanking the complex indel polymorphsim were not fully randomized due to the limits of experimentally imposed recombination. Consequently, in our test lines, the InR^{short} and InR^{long} alleles were embedded in haplotypes that extended beyond the *InR* locus. Based on prior estimates of linkage disequilibrium, however, flanking variants should be unlinked to the focal indel polymorphism (Paaby et al. 2010). Although these other variants may be targets of selection—a conclusion supported by our observation that the entire *InR* locus exhibits elevated clinality and seasonality relative to the rest of the genome—the fact that the indel polymorphism exhibits such strong changes in allele frequency across climatic environments provides complementary evidence for allele functionality. This accords with other reports of high recombination and rapid decay of linkage disequilibrium (Mackay et al. 2012), and evidence that selection precisely targets functional loci, including *InR* (Fabian et al. 2012), in natural populations of *D. melanogaster*.

Two of the discrete polymorphisms associated with the complex indel polymorphism, 17405631 and 17405634–7, do not discriminate between InR^{short} and InR^{long} but rather discriminate between the published reference genome genotype (which is of intermediate amino acid length) and InR^{short} or InR^{long}. Both of these discrete polymorphisms show strong seasonal patterns of allele frequency, which suggests that the intermediate allele may be a

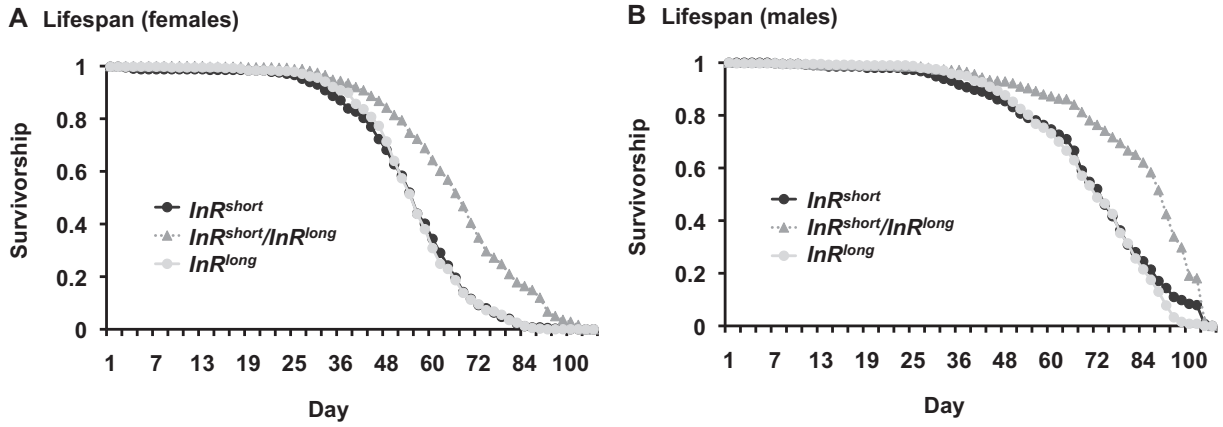

Figure 7. Survivorship curves, for females (A) and males (B). In both sexes, the heterozygote lived significantly longer than either homozygote genotype. In males, the *InR*^{short} genotype exhibited a reduced rate of aging late in life to produce a significant lifespan extension relative to *InR*^{long}, but there was no difference in longevity between *InR*^{short} and *InR*^{long} females.

target of selection as well. The discrete 17405631 and 17405634–7 alleles segregate at substantial frequencies in all populations, and in both North America and Australia the intermediate-length haplotype is the third most common allele class (Paaby et al. 2010). In our previous survey, the intermediate allele showed no cline in North America and a weak cline in Australia. Here, the discrete 17405631 and 17405634–7 alleles again show little evidence of clinality in North America (Fig. 1D and Table S1). These observations support the conclusion that seasonal and geographical environments may impose different evolutionary forces, including aspects of demography and selection, even in the face of generally concordant responses to shared climatic selection pressure (Bergland et al. 2014).

Because flies derived from high latitudes live longer than those from low latitudes (Schmidt et al. 2005a), and reduction in IIS extends life span (Clancy et al. 2001; Tatar et al. 2001; Hwangbo et al. 2004; Grönke et al. 2010), we predicted that the *InR*^{short} allele might be associated with longer life span than the *InR*^{long} allele. *InR*^{short} males did live significantly longer than *InR*^{long} males, but the magnitude of difference was small compared to other traits and there were no differences between the alleles in females. The comparatively weak longevity effect may reflect decoupling between life span and reproduction that has been observed elsewhere (Khazaeli and Curtsinger 2010)—or it may represent true functional variation. Laboratory-derived *InR* mutations have shown a dramatic effect on life span, but only the heterozygous combination of two hypomorphic alleles, of all genotypes tested, significantly extended life span (Tatar et al. 2001). This suggests that IIS-mediated traits may be determined by precise titration of IIS levels; it may also be the case that within the *InR* locus, some polymorphisms affect some traits more than others (Stern 2000). For both sexes, the heterozygote lived significantly longer than either homozygote genotype, which we interpret as a function of excess homozygosity on chromosome 3R independent of *InR* genotype. *Drosophila melanogaster* suffers severe inbreeding depression, which negatively affects longevity (Swindell and Bouzat 2006); as life span is a highly polygenic, quantitative trait, it stands to reason that it is the most sensitive to heterozygosity of all the traits we evaluated.

In addition to longer male life span, the *InR*^{short} allele was also associated with better cold and starvation tolerance and lower fecundity in females, a classic example of antagonistic pleiotropy (Williams 1957). The differences in fecundity were limited to early life, and represent variation in egg-laying rate, because total lifetime fecundity was not significantly different between the alleles. Modest differences in egg laying in early life could have massive fitness consequences in the wild, particularly on patchy substrates with larval competition, yet the competitive advantage may be undetectable in the laboratory if fecundity is measured over more than one day. Consequently, we emphasize that estimates of reproduction and other quantitative fitness traits should be examined carefully, particularly in the context of decoupling correlated traits. We also note that although the high-latitude *InR*^{short} allele is associated with smaller body size, in general high-latitude flies are bigger (Huey et al. 2000; De Jong and Bochdanovits 2003). If *InR* contributes to selection-mediated differences in body size, it either acts epistatically with other body-size loci or suffers antagonistic selection pressures across multiple fitness axes. This finding, like observations of the pleiotropic polymorphism at *neurofibromin 1* in which the high-latitude haplotype is negatively associated with wing size (Lee et al. 2013), demonstrates the complexity of selection dynamics in natural populations.

One hypothesis for strong genetic correlations among life-history traits, including the trade-off between life span and reproduction, is the presence of pleiotropic alleles. (Williams 1957; Flatt and Heyland 2011). If many genetic variants for life history are pleiotropic, then correlations among traits can never be completely dissolved. Our results implicate extensive pleiotropy, but

the complexity of the *InR* indel polymorphism limits our ability to map phenotype to specific nucleotides. The two alleles we tested, InR^{short} and InR^{long}, differ at four amino acids across a span of 16 residues (Paaby et al. 2010). Even if in isolation these sites could break pleiotropy by functioning independently, over short time scales the distinction between pleiotropy and close linkage may be inconsequential (Paaby and Rockman 2013). However, this region at *InR* clearly experiences sufficient recombination (or mutation) over longer time scales to generate a hypervariable set of alleles on which selection may act. Tests on additional *InR* alleles would help to resolve the functional roles of individual nucleotides. Moreover, our findings in support of pleiotropy may not represent the majority of life-history alleles segregating in natural populations. Other work has shown that recombination can generate genotypes with positively correlated effects for both life span and reproduction, which suggests that life-history variation in nature is likely determined by both pleiotropic and recombining nonpleiotropic loci (Khazaeli and Curtsinger 2013). Sampling genotypes from nature may represent blocks of alleles that produce the commonly observed correlations, but which can be broken under the right circumstances. Finally, although the two major alleles of the *InR* indel polymorphism showed canonical trade-offs in the laboratory, the role of these alleles in the natural environment is unknown. Whether and how they affect trait expression might vary across different environments (Fournier-Level et al. 2013), with the possibility that under some conditions, pleiotropy may present as conditional neutrality (Anderson et al. 2011b). Thus, true understanding of the role of this polymorphism in adaptive response, including fitness consequences and degree of pleiotropy, requires investigation rooted in the natural environment.

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DATA ARCHIVING

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LITERATURE CITED

Anderson, A. R., J. E. Collinge, A. A. Hoffmann, M. Kellett, and S. W. McKechnie. 2003. Thermal tolerance trade-offs associated with the right arm of chromosome 3 and marked by the *hsr-omega* gene in *Drosophila melanogaster*. Heredity 90:195–202.

Anderson, J. L., R. M. Reynolds, L. T. Morran, J. Tolman-Thompson, and P. C. Phillips. 2011a. Experimental evolution reveals antagonistic pleiotropy in reproductive timing but not life span in *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. 66:1300–1308.

Anderson, J. T., J. H. Willis, and T. Mitchell-Olds. 2011b. Evolutionary genetics of plant adaptation. Trends Genet. 27:258–266.

Bansal, V. 2010. A statistical method for the detection of variants from next-generation resequencing of DNA pools. Bioinformatics 26:i318–i324.

Benjamini, B., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 57:289–300.

Bergland, A. O., H. S. Chae, Y. J. Kim, and M. Tatar. 2012. Fine-scale mapping of natural variation in fly fecundity identifies neuronal domain of expression and function of an aquaporin. PLOS Genet. 8:e1002631.

Bergland, A. O., E. L. Behrman, K. R. O'Brien, P. S. Schmidt, and D. A. Petrov. 2014. Genomic evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. PLOS Genet. 10:e1004775.

Bock, I. R., and P. A. Parsons. 1981. Species of Australia and New Zealand. Pp. 291–308 *in* M. Ashburner, H. L. Carson, J. N. Thompson, eds. Genetics and biology of *Drosophila*. Academic Press, Lond.

Capy, P., E. Pla, and J. R. David. 1993. Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *Drosophila simulans*. Evolution 25:517–536.

Carbone, M. A., K. W. Jordan, R. F. Lyman, S. T. Harbison, J. Leips, T. J. Morgan, M. DeLuca, P. Awadalla, and T. F. Mackay. 2006. Phenotypic variation and natural selection at *catsup*, a pleiotropic quantitative trait gene in *Drosophila*. Curr. Biol. 16:912–919.

Casas–Tinto, S., M. T. Marr, P. Andreu, and O. Puig. 2007. Characterization of the *Drosophila* insulin receptor promoter. Biochim. Biophys. Acta 1769:236–243.

Clancy, D. J., D. Gems, L. G. Harshman, S. Oldham, H. Stocker, E. Hafen, S. J. Leevers, and L. Partridge. 2001. Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. Science 292:104–106.

Dantzer, B., and E. M. Swanson. 2012. Mediation of vertebrate life histories via *insulin-like growth factor-1*. Biol. Rev. Camb. Philos. Soc. 87:414–429.

David, J. R., and C. Bocquet. 1975. Evolution in a cosmopolitan species: genetic latitudinal clines in *Drosophila melanogaster* wild populations. Experientia 31:164–166.

David, J. R., and P. Capy. 1988. Genetic variation of *Drosophila melanogaster* natural populations. Trends Genet. 4:106–111.

De Jong, G., and Z. Bochdanovits. 2003. Latitudinal clines in *Drosophila melanogaster*: body size, allozyme frequencies, inversion frequencies, and the insulin-signalling pathway. J. Genet. 82:207–223.

De Luca, M., N. V. Roshina, G. L. Geiger-Thornsberry, R. F. Lyman, E. G. Pasyukova, and T. F. Mackay. 2003. *Dopa decarboxylase* (*Ddc*) affects variation in *Drosophila* longevity. Nat. Genet. 34:429–433.

Fabian, D. K., M. Kapun, V. Nolte, R. Kofler, P. S. Schmidt, C. Schlotterer, and T. Flatt. 2012. Genome-wide patterns of latitudinal differentiation among populations of *Drosophila melanogaster* from North America. Mol. Ecol. 21:4748–4769.

Flatt, T., and A. Heyland. 2011. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford Univ. Press, New York.

Flatt, T., K. J. Min, C. D'Alterio, E. Villa-Cuesta, J. Cumbers, R. Lehmann, D. L. Jones, and M. Tatar. 2008. Drosophila germ-line modulation of insulin signaling and lifespan. Proc. Natl. Acad. Sci. USA 105:6368–6373.

Fournier-Level, A., A. M. Wilczek, M. D. Cooper, J. L. Roe, J. Anderson, D. Eaton, B. T. Moyers, R. H. Petipas, R. N. Schaeffer, B. Pieper, et al. 2013. Paths to selection on life history loci in different natural environments

across the native range of *Arabidopsis thaliana*. Mol. Ecol 22:3552–3566.

Giannakou, M. E., and L. Partridge. 2007. Role of insulin-like signalling in *Drosophila* lifespan. Trends Biochem. Sci. 32:180–188.

Gilchrist, G. W., and R. B. Huey. 2004. Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. Integr. Comp. Biol. 44:461–470.

Grönke, S., D. F. Clarke, S. Broughton, T. D. Andrews, and L. Partridge. 2010. Molecular evolution and functional characterization of *Drosophila* insulin-like peptides. PLOS Genet. 6:e1000857.

Guirao-Rico, S., and M. Aguadé. 2009. Positive selection has driven the evolution of the *Drosophila Insulin-like receptor* (*InR*) at different timescales. Mol. Biol. Evol. 26:1723–1732.

Harshman, L. G., and A. J. Zera. 2007. The cost of reproduction: the devil in the details. Trends Ecol. Evol. 22:80–86.

Hoffmann, A. A., and L. G. Harshman. 1999. Desiccation and starvation resistance in *Drosophila*: patterns of variation at the species, population and intrapopulation levels. Heredity 83:637–643.

Hoffmann, A. A., R. Hallas, C. Sinclair, and P. Mitrovski. 2001. Levels of variation in stress resistance in *Drosophila* among strains, local populations, and geographic regions: patterns for desiccation, starvation, cold resistance, and associated traits. Evolution 55:1621–1630.

Hoffmann, A. A., J. Shirriffs, and M. Scott. 2005. Relative importance of plastic vs genetic factors in adaptive differentiation: geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. Funct. Ecol. 19:222–227.

Hoffmann, A. A., E. Ratna, C. M. Sgrò, M. Barton, M. Blacket, R. Hallas, S. De Garis, and A. R. Weeks. 2007. Antagonistic selection between adult thorax and wing size in field released *Drosophila melanogaster* independent of thermal conditions. J. Evol. Biol. 20:2219–2227.

Huey, R. B., G. W. Gilchrist, M. L. Carlson, D. Berrigan, and L. Serra. 2000. Rapid evolution of a geographic cline in size in an introduced fly. Science 287:308–309.

Hwangbo, D. S., B. Gershman, M. P. Tu, M. Palmer, and M. Tatar. 2004. Drosophila dFOXO controls lifespan and regulates insulin signalling in brain and fat body. Nature 429:562–566.

Jones, F. C., M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M. C. Zody, S. White, et al. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. Nature 484:55–61.

Jovelin, R., J. S. Comstock, A. D. Cutter, and P. C. Phillips. 2014. A recent global selective sweep on the age-1 phosphatidylinositol 3-OH kinase regulator of the insulin-like signaling pathway within *Caenorhabditis remanei*. G3 4:1123–1133.

Jünger, M. A., F. Rintelen, H. Stocker, J. D. Wasserman, M. Vegh, T. Radimerski, M. E. Greenberg, and E. Hafen. 2003. The *Drosophila* forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. J. Biol. 2:20.

Karan, D., A. K. Munjal, P. Gibert, B. Moreteau, R. Parkash, and J. R. David. 1998. Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: a study of natural populations from the Indian subcontinent. Genet. Res. 71:31–38.

Khazaeli, A. A., and J. W. Curtsinger. 2010. Life history variation in an artificially selected population of *Drosophila melanogaster*: pleiotropy, superflies, and age-specific adaptation. Evolution 64:3409–3416.

———. 2013. Pleiotropy and life history evolution in *Drosophila melanogaster*: uncoupling life span and early fecundity. J. Gerontol. A Biol. Sci. Med. Sci. 68:546–553.

Lee, S. F., Y. C. Eyre-Walker, R. V. Rane, C. Reuter, G. Vinti, L. Rako, L. Partridge, and A. A. Hoffmann. 2013. Polymorphism in the neurofibromin gene, *Nf1*, is associated with antagonistic selection on wing size and development time in *Drosophila melanogaster*. Mol. Ecol. 22:2716–2725.

Leroi, A. M., A. Bartke, G. De Benedictis, C. Franceschi, A. Gartner, E. S. Gonos, M. E. Fedei, T. Kivisild, S. Lee, N. Kartaf-Ozer, et al. 2005. What evidence is there for the existence of individual genes with antagonistic pleiotropic effects? Mech. Ageing Dev. 126:421–429.

Li, H., and R. Durbin. 2009. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760.

Linnen, C. R., Y. P. Poh, B. K. Peterson, R. D. Barrett, J. G. Larson, J. D. Jensen, and H. E. Hoekstra. 2013. Adaptive evolution of multiple traits through multiple mutations at a single gene. Science 339:1312–1316.

Mackay, T. F., S. Richards, E. A. Stone, A. Barbadilla, J. F. Ayroles, D. Zhu, S. Casillas, Y. Han, M. M. Magwire, J. M. Cridland, et al. 2012. The *Drosophila melanogaster* genetic reference panel. Nature 482:173–178.

Mattila, J., A. Bremer, L. Ahonen, R. Kostiainen, and O. Puig. 2009. *Drosophila FoxO* regulates organism size and stress resistance through an adenylate cyclase. Mol. Cell. Biol. 29:5357–5365.

McKenna, A., M. Hanna, E. Banks, A. Sivachenko, K. Cibulskis, A. Kernytsky, K. Garimella, D. Altshuler, S. Gabriel, M. Daly, et al. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 20:1297–1303.

Méndez-Vigo, B., J. M. Martínez-Zapater, and C. Alonso-Blanco. 2013. The flowering repressor *SVP* underlies a novel *Arabidopsis thaliana* QTL interacting with the genetic background. PLOS Genet. 9:e1003289.

Mitrovski, P., and A. A. Hoffmann. 2001. Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions. Proc. Biol. Sci. 268:2163–2168.

Paaby, A. B., and M. V. Rockman. 2013. The many faces of pleiotropy. Trends Genet. 29:66–73.

Paaby, A. B., and P. S. Schmidt. 2008. Functional significance of allelic variation at *methuselah*, an aging gene in *Drosophila*. PLOS One 3:e1987.

———. 2009. Dissecting the genetics of longevity in *Drosophila melanogaster*. Fly 3:29–38.

Paaby, A. B., M. J. Blacket, A. A. Hoffmann, and P. S. Schmidt. 2010. Identification of a candidate adaptive polymorphism for *Drosophila* life history by parallel independent clines on two continents. Mol. Ecol. 19:760–774.

Partridge, L., and D. Gems. 2002. The evolution of longevity. Curr. Biol. 12:R544–546.

Partridge, L., N. Prowse, and P. Pignatelli. 1999. Another set of responses and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. Proc. Biol. Sci. 266:255–261.

Partridge, L., D. Gems, and D. J. Withers. 2005. Sex and death: what is the connection? Cell 120:461–472.

Puig, O., M. T. Marr, M. L. Ruhf, and R. Tjian. 2003. Control of cell number by *Drosophila* FOXO: downstream and feedback regulation of the insulin receptor pathway. Genes Dev. 17:2006–2020.

Rako, L., M. J. Blacket, S. W. McKechnie, and A. A. Hoffmann. 2007. Candidate genes and thermal phenotypes: identifying ecologically important genetic variation for thermotolerance in the Australian *Drosophila melanogaster* cline. Mol. Ecol. 16:2948–2957.

Remolina, S. C., P. L. Chang, J. Leips, S. V. Nuzhdin, and K. A. Hughes. 2012. Genomic basis of aging and life-history evolution in *Drosophila melanogaster*. Evolution 66:3390–3403.

Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. Oikos 44:257–267.

Schmidt, P. S., and A. B. Paaby. 2008. Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. Evolution 62:1204–1215.

Schmidt, P. S., L. Matzkin, M. Ippolito, and W. F. Eanes. 2005a. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution 59:1721–1732.

Schmidt, P. S., A. B. Paaby, and M. S. Heschel. 2005b. Genetic variance for diapause expression and associated life histories in *Drosophila melanogaster*. Evolution 59:2616–2625.

Schmidt, P. S., C. T. Zhu, J. Das, M. Batavia, L. Yang, and W. F. Eanes. 2008. An amino acid polymorphism in the *couch potato* gene forms the basis for climatic adaptation in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 105:16207–16211.

Sgrò, C. M., B. van Heerwaarden, V. Kellermann, C. W. Wee, A. A. Hoffmann, and S. F. Lee. 2013. Complexity of the genetic basis of ageing in nature revealed by a clinal study of lifespan and *methuselah*, a gene for ageing, in *Drosophila* from eastern Australia. Mol. Ecol. 22:3539–3551.

Stearns, S. C. 1991. Trade-offs in life-history evolution. Funct. Ecol. 3:259–268.

———. 1992. The evolution of life histories. Oxford Univ. Press, New York.

Stern, D. L. 2000. Evolutionary developmental biology and the problem of variation. Evolution 54:1079–1091.

Swindell, W. R., and J. L. Bouzat. 2006. Inbreeding depression and male survivorship in *Drosophila*: implications for senescence theory. Genetics 172:317–327.

Tatar, M., A. Kopelman, D. Epstein, M. P. Tu, C. M. Yin, and R. S. Garofalo. 2001. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. Science 292: 107–110.

Tatar, M., A. Bartke, and A. Antebi. 2003. The endocrine regulation of aging by insulin-like signals. Science 299:1346–1351.

Toivonen, J. M., and L. Partridge. 2009. Endocrine regulation of aging and reproduction in *Drosophila*. Mol. Cell Endocrinol. 299: 39–50.

Trotta, V., F. C. Calboli, M. Ziosi, D. Guerra, M. C. Pezzoli, J. R. David, and S. Cavicchi. 2006. Thermal plasticity in *Drosophila melanogaster*: a comparison of geographic populations. BMC Evol. Biol. 6:67.

Vermeulen, C. J., and V. Loeschcke. 2007. Longevity and the stress response in *Drosophila*. Exp. Geront. 42:153–159.

Vihervaara, T., and O. Puig. 2008. *dFOXO* regulates transcription of a *Drosophila* acid lipase. J. Mol. Biol. 376:1215–1223.

Wang, M. C., D. Bohmann, and H. Jasper. 2005. JNK extends life span and limits growth by antagonizing cellular and organism–wide responses to insulin signaling. Cell 121:115–125.

Williams, G. C. 1957. Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Average dry body weight, lipid weight, and length of the L2 wing vein for flies derived from the Mount Sinai population.

Figure S2. Average early fecundity (A) and development time (B, C) for single copies of InR^{short} and InR^{long}, paired with either an *InR* hypomorphic (InR^{p5545}) or wild-type balancer (InR^{TM3}) allele.

Figure S3. Average dry body weight (A, B) and average lipid weight (C, D) for single copies of InR^{short} and InR^{long}, paired with either an *InR* hypomorphic (InR^{p5545}) or wild-type balancer (InR^{TM3}) allele.

Figure S4. Average length of the L2 wing vein (A, B) and wing:thorax ratio (C, D) for single copies of InR^{short} and InR^{long}, paired with either an *InR* hypomorphic (InR^{p5545}) or wild-type balancer (InR^{TM3}) allele.

Table S1. Characterization of the eight discrete polymorphisms that are associated with, or are nearby, the complex insertion–deletion polymorphism and that are segregating at appreciable frequencies in the seasonal and clinal populations.

Table S2. Statistical results for tests of fecundity, development, body size, and chill coma recovery for *InR* genotypes in a wild-type background.

Table S3. Statistical results for tests of temperature shock, starvation, and life span for *InR* genotypes in a wild-type background.

Table S4. Statistical results for tests of InR^{short} and InR^{long} in an InR^{-} background.