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Seasonal variation in life history traits in two *Drosophila* species

E. L. BEHRMAN*, S. S. WATSON†, K. R. O'BRIEN*‡, M. S. HESCHEL§ & P. S. SCHMIDT*

*Department of Biology, University of Pennsylvania, Philadelphia, PA, USA
†Department of Mathematics, Massachusetts Institute of Technology, Cambridge, MA, USA
‡School of Biological Sciences, University of Nebraska, Lincoln, NE, USA
§Department of Organismal Biology & Ecology, Colorado College, Colorado Springs, CO, USA

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Abstract

Seasonal environmental heterogeneity is cyclic, persistent and geographically widespread. In species that reproduce multiple times annually, environmental changes across seasonal time may create different selection regimes that may shape the population ecology and life history adaptation in these species. Here, we investigate how two closely related species of *Drosophila* in a temperate orchard respond to environmental changes across seasonal time. Natural populations of *Drosophila melanogaster* and *Drosophila simulans* were sampled at four timepoints from June through November to assess seasonal change in fundamental aspects of population dynamics as well as life history traits. *D. melanogaster* exhibit pronounced change across seasonal time: early in the season, the population is inferred to be uniformly young and potentially represents the early generation following overwintering survivorship. *D. melanogaster* isofemale lines derived from the early population and reared in a common garden are characterized by high tolerance to a variety of stressors as well as a fast rate of development in the laboratory environment that declines across seasonal time. In contrast, wild *D. simulans* populations were inferred to be consistently heterogeneous in age distribution across seasonal collections; only starvation tolerance changed predictably over seasonal time in a parallel manner as in *D. melanogaster*. These results suggest fundamental differences in population and evolutionary dynamics between these two taxa associated with seasonal heterogeneity in environmental parameters and associated selection pressures.

Introduction

Understanding how populations adapt to environmental variability is a fundamental interest in evolutionary biology. Environmental heterogeneity is commonly partitioned into two basic axes: variation in space and in time. Although parallels between spatial and temporal environmental parameters may exist, greater emphasis has been placed on evaluating spatial variation in evolutionary dynamics of natural populations (Endler, 1977, 1986; Slatkin, 1987; Kingsolver *et al.*, 2001). Inferences regarding spatial variation in selection pressures can be evaluated using population samples collected at a single point in time, whereas determining the significance of temporal variation often requires longitudinal studies over various timescales (Hendry & Kinnison, 1999; Grant & Grant, 2002; Carroll *et al.*, 2007; Siepielski *et al.*, 2009). Temporal variation can be evaluated over short timescales using environmental parameters that change predictably as a function of time across seasons within a year. This includes change in abiotic factors, such as temperature and photoperiod, as well as biotic variation such as ecological interactions within and among taxa. The traits and selection pressures associated with high fitness may be quite distinct between seasons that are favourable for reproduction and population expansion (e.g. summer) and those that are not and must be endured (e.g. winter). Such alternating selection pressures across seasons may be integral in the maintenance

Correspondence: Emily Behrman, 216 Leidy Laboratories, Department of Biology, University of Pennsylvania, 3740 Hamilton Walk, Philadelphia, PA 19104, USA.
Tel.: +1 2158987356; fax: +1 2158988780; e-mail: bemily@sas.upenn.edu

of genetic variation in natural populations (Levene, 1953; Dempster, 1955; Haldane & Jayakar, 1963; Gillespie, 1973; Ewing, 1979; MacKay, 1980; Turelli, 1981; Ellner & Hairston, 1994; Hedrick, 1995, 2002). However, there is limited empirical work on how populations respond ecologically and evolutionary to seasonal changes in environmental parameters, and therefore, there is a need for longitudinal studies in natural populations across seasonal time.

For organisms that have multiple generations each year (multivoltine), there are several predicted outcomes in response to the seasonal environmental differences experienced by subsequent generations; the null hypothesis is that populations either do not respond or exhibit only stochastic differences across seasonal time. Alternatively, changes in traits over generational time may occur at an individual level as direct result of the different environments experienced (e.g. phenotypic plasticity) or they may reflect differences in the genetic composition of the population due to differential fitness over generational time. Phenotypic plasticity is a commonly predicted response to short-term environmental changes over seasonal timescales (Brakefield & Reitsma, 1991; Bradford & Roff, 1993) as seen in the change in body size throughout the summer in the dung fly *Sepsis cynipsea* (Blanckenhorn *et al.*, 1999) and seasonal shifts in frequency of colour morphs of the ladybird beetle *Adalia bipunctata* (Brakefield, 1985). By comparison, seasonal change in the genetic composition of the population due to differential fitness across the changing environments has been less well studied. Although seasonal changes have been documented at the genetic level in annual cycling of *Drosophila pseudoobscura* chromosomal arrangements (Dobzhansky, 1943, 1948) and *Drosophila melanogaster* allele frequencies (Cogni *et al.*, 2013, 2014; Bergland *et al.*, 2014; Paaby *et al.*, 2014), the phenotypic basis for seasonal cycling in allele frequencies remains largely unknown. Reproductive diapause incidence in *D. melanogaster* is one example of change over seasonal time for both phenotype (Schmidt & Conde, 2006) and underlying allele frequencies (Cogni *et al.*, 2013). Here, we use the numerous seasonal changes in allele frequencies in *D. melanogaster* as a point of departure to investigate phenotypic change over seasonal time for a subset of traits that have been implicated in the adaptive response of *D. melanogaster* to spatially variable selection.

Drosophila melanogaster has long been used as a model system to evaluate the role of environmental heterogeneity and spatially variable selection on evolutionary pattern and process. The species is native to sub-Saharan Africa and, as a human commensal, has colonized temperate habitats on multiple continents (David & Capy, 1988; Andolfatto, 2005) on which they exhibit latitudinal clines for a variety of traits (Capy *et al.*, 1993; James & Partridge, 1995; James *et al.*, 1997; Azevedo *et al.*, 1998; Karan *et al.*, 1998; Robinson *et al.*, 2000; Mitrovski & Hoffmann, 2001; Hoffmann *et al.*, 2002; De Jong & Bochdanovits, 2003; Schmidt *et al.*, 2005; Trotta *et al.*, 2006; Schmidt & Paaby, 2008) as well as allele frequencies at specific loci (Berry & Kreitman, 1993; Verrelli & Eanes, 2000; Bettencourt *et al.*, 2002; Frydenberg *et al.*, 2003; Sezgin, 2004; Tauber *et al.*, 2007; McKechnie *et al.*, 2010; Paaby *et al.* 2010; Cogni *et al.*, 2013; Paaby *et al.*, 2014). Although such patterns of spatial variation may reflect aspects of demography and colonization history (Roesti *et al.*, 2014), the latitudinal clines in *D. melanogaster* are commonly interpreted as an adaptive result of spatially variable selection (Verrelli & Eanes, 2000; Mitrovski & Hoffmann, 2001; Bettencourt *et al.*, 2002; Sezgin, 2004; Schmidt & Paaby, 2008). As many of the climatic factors that change over latitudinal gradients also vary seasonally in temperate environments (e.g. temperature, photoperiod, humidity), *D. melanogaster* populations may also respond adaptively to environmental heterogeneity over short seasonal timescales. Thus, we can use these parallels with latitudinal clines to make concrete predictions about how traits will change over seasonal time: as temperature and associated parameters increase from spring to summer, we predict that traits will change in the same pattern as from high to low latitudes.

We compare two closely related species to identify generality in seasonal response and to dissect particular aspects unique to each species. In temperate North American orchards, the closely related species *D. melanogaster* and *Drosophila simulans* co-occur both over seasonal time and with geography. The species share common ecologies and exhibit at least some degree of parallel response with respect to phenotypic (McKenzie & Parsons, 1974; Watada *et al.*, 1986; Gibert *et al.*, 2004; Arthur *et al.*, 2008; Van Heerwaarden *et al.*, 2012), allozyme (Anderson & Oakeshott, 1984) and transcriptional clines (Zhao *et al.*, 2015), although there is at least one instance of an opposing phenotypic cline between these species (Van Heerwaarden *et al.*, 2012). However, phenotypic and allele frequency clines in *D. simulans* are less abundant and shallower than those observed in *D. melanogaster* (McKenzie & Parsons 1974; Watada *et al.*, 1986; Capy *et al.*, 1993; Arthur *et al.*, 2008) and *D. simulans* has less physiological tolerance to cold and starvation stresses when compared to *D. melanogaster* (Hoffmann & Harshman, 1999); this suggests distinct aspects of demography, physiology or selective response to environmental variance associated with the latitudinal extremes. Additionally, these sibling species exhibit different patterns of relative abundance over seasonal time. In temperate North American orchards, *D. melanogaster* are first evident in the late spring (Schmidt & Conde, 2006) and the population appears persistent over time. In contrast, *D. simulans* populations appear in midsummer and expand throughout the agricultural growing season to outnumber *D. melanogaster* by autumn, but are not present the following spring. The ecological parallels lead to the prediction that the sister taxa will have similar response to seasonal

change, but the less robust clinal patterns in *D. simulans* and different frequencies over seasonal time suggest that their seasonal response may similarly be of reduced magnitude compared to *D. melanogaster*.

Natural orchard populations of both species were sampled from spring through autumn to assess seasonal changes in ecological and evolutionary population dynamics and life history traits. Seasonal changes in ecological parameters are documented using wild-caught individuals and their offspring to measure a subset of ecologically relevant traits: age distribution, reproductive output and development time. Population age structure is a fundamental component in population dynamics in the wild (Cole, 1957) and has been shown to change across seasonal time in other insect species (Carey *et al.*, 2008). It is predicted that adults overwintering in a temperate location will emerge from dormancy fairly synchronously in response to environmental cues, which will result in a single young cohort in the spring that becomes more heterogeneous in age as nonoverlapping generations reproduce throughout the summer (Tauber *et al.*, 1986). In temperate North America, *D. melanogaster* populations appear persistent over time (Ives, 1970; Bergland *et al.*, 2014), potentially due to the expression of an adult reproductive diapause that is associated with overwintering (Saunders *et al.*, 1989; Izquierdo, 1991; Mitrovski & Hoffmann, 2001); therefore, it is predicted that the post-dormancy populations will be young and age heterogeneity will increase across seasonal time.

We examine seasonal genetic change for a subset of traits previously shown to vary with latitude in *D. melanogaster* using wild-derived isofemale lines reared in a common laboratory environment for several generations. We predict traits favoured for survival at high latitudes will also be favoured during the winter because of similarities in their environments; likewise, parallels are predicted in low latitude and summer traits. In North America, adaptation to northern environments is associated with increased investment in stress resistance (Capy *et al.*, 1993; Hoffmann *et al.*, 2001; De Jong & Bochdanovits, 2003; Schmidt *et al.*, 2005; Schmidt & Paaby, 2008). Therefore, we predict that winter environments also select for increased stress tolerance and that early-season generations in the spring will be characterized by elevated stress resistance. As the environment becomes more conducive to population growth throughout the summer, we predict generalized stress tolerance to decline, due to correlations and trade-offs with other aspects of fitness (Roff, 1992).

Materials and methods

Samples

Drosophila melanogaster and *D. simulans* were collected from Linvilla Orchards in Media, PA (39.884179°N, −75.411227°E], using baited traps and aspiration at four timepoints spaced approximately every 8 weeks: 1–4 June, 31 July, 26 September and 9 November 2011. Under light carbon dioxide anaesthetic, flies were sorted to species subgroup and allowed to recover on standard cornmeal molasses food. Isofemale lines were established by placing gravid females into individual vials of standard medium, and the species were identified through examination of the posterior lobe of male offspring.

Characteristics of the natural populations were measured on wild-caught flies: demography, reproductive output and F1 development time. Isofemale lines were maintained in a common laboratory environment (25 °C, 12:12 L:D, standard cornmeal molasses food) for four generations to remove environmental effects so that any difference in traits among the collections represented evolutionary change in the genetics of the population. After the generations in the laboratory, heat knockdown, chill recovery, starvation resistance and development time were measured under standard laboratory conditions.

Age distribution

The age distribution of the sampled populations was estimated at each collection timepoint to assess whether demography changes across seasonal time as it does in other taxa (Carey *et al.*, 2008). Age structure was estimated utilizing the deconvolution model (Müller *et al.*, 2007; Carey *et al.*, 2008) that compares the post-capture survivorship of wild individuals to the full lifespan of their offspring to back-calculate the age distribution of the wild population. This model relies on the assumption that the age of an individual caught in the wild is reflected in post-capture survival, with young individuals surviving proportionally longer than old individuals in the laboratory. By extension, a relative or absolute change in estimated age distribution of the population between collection timepoints indicates a shift in population age structure. All flies were reared in individual vials of standard cornmeal medium that were changed every day for the first ten days in captivity and every three to five days thereafter. Mortality was recorded daily. The deconvolution model was implemented using MatLab (Math Works, Natick, MA, USA). Kaplan–Meier survivorship curves for the post-capture survivorship of wild individuals and the full lifespan of the F1 offspring was graphed using the 'survival' package (Therneau, 2012) in the R statistical analysis software (R Core Team 2012).

Fecundity and development time

Reproductive output was measured through daily transfers and egg counts of wild females during the first ten days post-capture. We analysed fecundity in two ways: the mean fecundity is a function of the population and is affected by the age distribution (e.g. Tatar *et al.*,

1996; Novoseltsev *et al.*, 2003), and the maximum fecundity is a function of the individual.

Vials containing eggs laid during the first 24 h of captivity were used to measure development time in the F1 post-capture generation. At three timepoints per day, the number of puparia and eclosed adults was recorded to determine the time to pupation and time to eclosion. After four to five generations in standard laboratory culture, development time to eclosion was again measured in the same way. Thus, the full development time from egg to eclosion was estimated at two timepoints for each line: in the F1 generation and after several generations in common-garden laboratory culture. The measurements conducted on F1 generation reflected a combination of genetic, environmental and associated effects, whereas the measurements conducted in the common laboratory environment primarily reflected genetic variance.

Stress tolerance

Tolerance to a variety of stressors was examined for each isofemale line in the common laboratory environment after four to five generations of culture. For starvation resistance, groups of 12 individuals per sex for each line were sorted under light carbon dioxide anaesthesia and recovered on food for 24 h before transfer to vials that contained a cotton ball and 2 mL of deionized water. The number of live and dead flies was observed at three standardized timepoints every 24 h until all experimental flies died.

Thermal stress assays measuring response to high and low temperatures used DAM2 activity monitors (TriKinetics, Waltham, MA, USA) to record locomotor activity every 10 s. Eight flies per sex per line were placed into individual glass tubes and given an hour to recover from the carbon dioxide anaesthetic. To examine response to low temperature, groups of flies were buried in ice, placed in a 4 °C incubator for 2 h and then transferred to 25 °C; chill coma recovery time was estimated at the time required for each fly to resume an upright stance and locomotor activity. To evaluate response to high temperature, collections were placed at 25 °C in a Percival I36VL incubator programmed to increase temperature by 1 °C per min to 37 °C. The temperature remained constant at 37 °C, and time to thermal knockdown was recorded as the time at which locomotor activity ceased.

Statistical analysis of life history traits

Mixed-model ANOVAs were used to assess seasonal change in all phenotypic traits with month and sex as fixed effects and line[month] as a random effect. Species were analysed separately to address the species-specific question of changes over seasonal time and because the absence of *D. simulans* in June made the data and models nonorthogonal. When both species were present, a direct comparison was made between the two species using *t*-tests corrected for multiple comparisons using Tukey's honestly significant difference tests. All statistical analyses were conducted in JMP v10.0.0 (SAS Institute, Cary, NC, USA).

Genetic variance/covariance estimates

For each species, genetic correlations among stress tolerance traits for all seasonal collections were calculated using Pearson's product-moment coefficients. Genetic correlations were estimated using isofemale lines to generate line means (Via, 1984; Roff, 1997). Sample sizes were as follows: *D. melanogaster*, $n = 66$, 83, 47 and 43 isofemale lines per timepoint, in chronological order; *D. simulans*, $n = 0$, 50, 87 and 58 isofemale lines, respectively. Significance probabilities indicate whether a particular genetic correlation differed from zero; *P*-values were obtained by treating the test statistic as coming from a *t* distribution. To test whether line mean genetic variance/covariance (G) matrices were statistically different among species by season combinations, MANOVA was used on jack-knifed genetic variance/covariance values (Roff, 2002). Comparisons of variance/covariance matrices with this MANOVA method have been shown to produce the same statistical results as the Flury method (e.g. Phillips & Arnold, 1999) for comparing G matrices, but environmental effects are easier to incorporate into the MANOVA approach. For every trait pair, genetic variance/covariance pseudovalues were created by jack-knifing. Each set of variance/covariance pseudovalues was coded for species and season of collection. MANOVA was then used to test whether sets of variance/covariance values significantly differed between seasons for each taxon (Roff, 2002); for example, chill recovery variance, heat knockdown variance and chill/heat covariance pseudovalues were treated as multiple response variables in a MANOVA that included seasonal timing (early vs. late) as a predictor variable. *F*-tests and associated *P*-values were calculated from Wilks' lambda values.

Results

Over seasonal time, the relative abundance of *D. melanogaster* and *D. simulans* changed dramatically. In the spring, the flies we sampled were exclusively *D. melanogaster*, but by the end of the autumn, *D. simulans* outnumbered *D. melanogaster* five-fold (Table 1). This suggests fundamental differences in population dynamics between the two taxa.

Age structure

Multiple measurements of population age distribution suggest that the early *D. melanogaster* collection was unimodal and young; in contrast, the collections throughout the rest of the year contained more individuals

Table 1 Post-capture survival of wild-caught *Drosophila* and full lifespan of offspring measured in days.

		Drosophila melanogaster					*Drosophila simulans*				
Month	Sex	*n*	Mean	SE	Med.	Max.	*n*	Mean	SE	Med.	Max.
Wild fly post-capture survival											
June	F	59	45.92	2.59	44	82	–	–	–	–	–
	M	96	43.9	1.74	40	75	–	–	–	–	–
July	F	114	24.62	1.48	19	68	78	30.94	1.75	37	57
	M	110	24.67	1.39	22	61	92	34.09	1.43	34.5	61
September	F	34	29.53	3.07	30	70	126	25.93	1.4	30	60
	M	136	20.57	1.49	16	75	114	29.69	1.62	32.5	60
November	F	56	26.39	1.75	24	62	239	26.76	0.77	27	52
	M	94	21.89	1.51	21	58	122	31.36	1.18	30	56
F1 full lifespan											
June	F	68	44.44	1.82	46	73	–	–	–	–	–
	M	63	57.54	1.61	62	74	–	–	–	–	–
July	F	96	47.15	1.30	48.5	67	66	47.79	1.46	46.5	67
	M	81	46.99	1.71	52	66	68	46.37	1.66	52	65
September	F	45	52.78	2.56	56	81	151	44.75	0.98	44	73
	M	49	53.73	2.10	56	83	130	56.65	1.18	58	84
November	F	47	46.06	2.04	43	73	194	43.88	0.78	44	77
	M	45	52.16	2.35	54	75	193	54.22	1.03	58	78

inferred to come from older age classes. This is seen in the population age structure distributions estimated using the deconvolution model (Fig. 1). The Kaplan–Meier survivorship curves also demonstrated the same pattern: the wild-caught flies in June were inferred to be young because their post-capture survivorship curve was so similar to the full-lifespan survivorship curve of the corresponding F1 flies (Fig. S1). The other collections are inferred to be older: when caught, their post-capture survival was truncated compared to the full F1

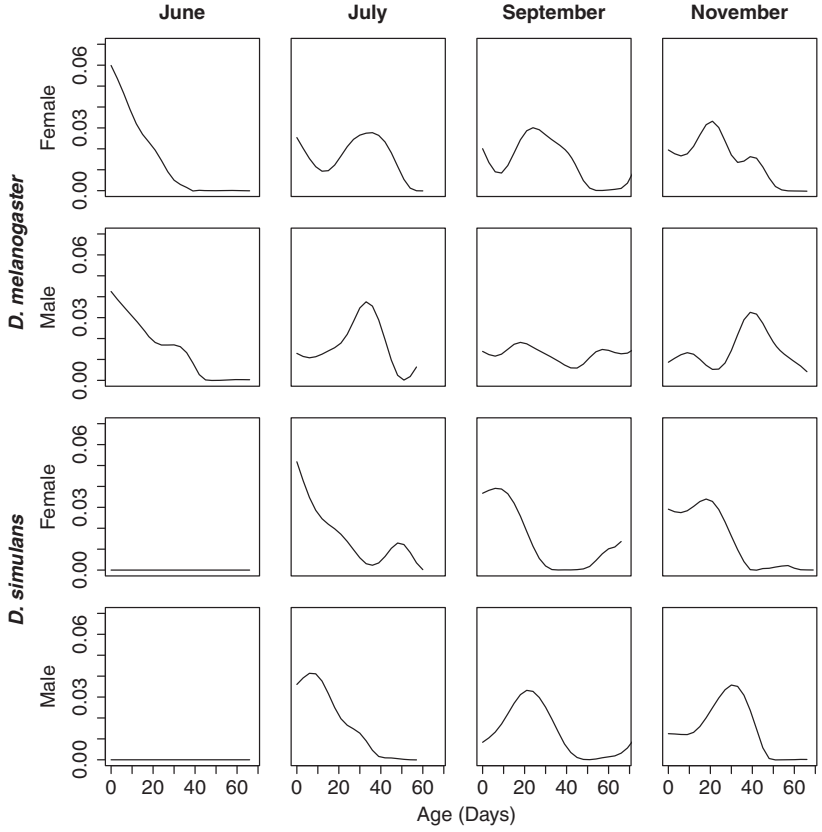

Fig. 1 Seasonal changes in age distribution of wild *Drosophila* estimated using the deconvolution model. Each graph plots the density of the estimated age distribution of the population in nature vs. age in days. Using this method, the June population of *Drosophila melanogaster* is inferred to be relatively young and is distinct from the subsequent collections that are more heterogeneous and contain older flies. Not collected in June, *Drosophila simulans* consistently contained flies of old age classes when the species was present in the orchard.

lifespan. Consistent with these patterns, the median post-capture survival time of wild flies was shorter than the mean in the earliest collection and shifted towards the mean in the later months as the age heterogeneity increased (Table 1). Together, these data provide a consistent picture of a population that appears uniformly young early in the spring, but increases in age heterogeneity as time progresses.

In contrast, the same demographic measurements yield distinct patterns for the *D. simulans* populations. Based on the inferences using the deconvolution model, *D. simulans* populations contained flies of older age classes when the species first appeared in July and there was much less change in age structure across the rest of the year relative to *D. melanogaster* (Fig. 1). The Kaplan–Meier survivorship curves for *D. simulans* exhibited minimal change across seasonal time, suggesting that the age distribution remained consistent across the measured timepoints (Fig. S1). The age distribution of wild flies shifted slightly younger as seasonal time progressed; the median survival time was initially skewed left of the mean and subsequently shifted towards the mean age throughout the autumn (Table 1). These data together depict a population that is consistently age-structured and does not have pronounced shifts in age distribution across seasonal time.

Seasonal change in natural population

The measurements on wild females and their F1 offspring include multiple components, including maternal effects influenced by habitat quality and the environment. In both *Drosophila* species, reproductive output of wild-caught females declined over seasonal time (Fig. 2). The mean number of eggs laid declined, whereas the maximum showed a bimodal pattern with more eggs laid by flies collected in the first half of the study (June and July) than later (September and November). Although there was no difference between species in the early collections, the autumn *D. melanogaster* laid more eggs than *D. simulans* (Table 2).

The development time from egg to adult for F1 offspring differed by month for both species (Table 3), but there was no directionality in these changes: the development time oscillated around 220 h across the collection timepoints (Fig. 3). Early-season *D. melanogaster* developed faster than *D. simulans*, but a difference between taxa was not evident in the autumn collections (Table 3). Larval development time (egg to pupation) mirrored that of the total development time (egg to eclosion) although the time in the puparium did not change by species or season (data not shown).

Seasonal change in genetic composition

Drosophila melanogaster demonstrated a consistent and strong pattern of directional decline in performance

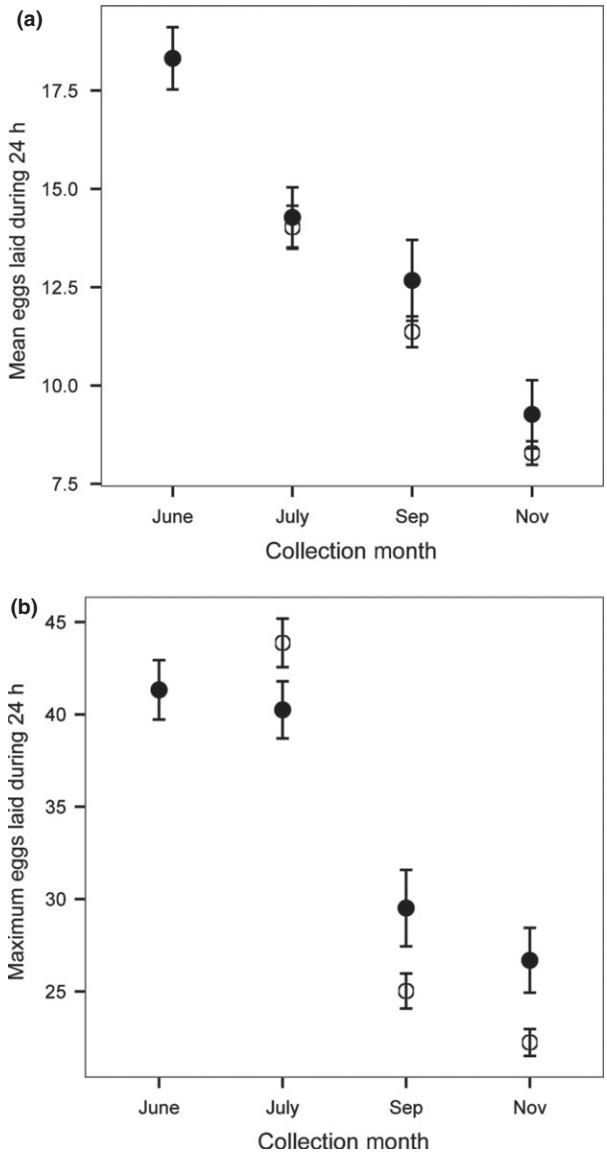

Fig. 2 Wild flies of both species have a seasonal decline in mean (a) and maximum (b) number of eggs (±SE) laid per day during the first 10 days of captivity. *Drosophila melanogaster* are indicated in filled circles and *Drosophila simulans* in empty circles. There is no difference between species in the mean reproductive output; however, *D. simulans* has a higher maximum fecundity compared to *D. melanogaster* in July but lower during the rest of the season.

over seasonal time for the phenotypes assayed in the common garden: tolerance to heat, cold and starvation stressors was highest in the early-season collection and declined predictably over time (Fig. 4, Table 3). Similarly, development time in the common garden increased linearly in *D. melanogaster* such that the last collection took nearly three full days longer to eclose than the earliest, with mid-season collections being intermediate (Fig. 3).

Table 2 Mixed-model ANOVAS for environmental and common-garden traits measured across seasons for each species with month and sex as fixed effects and line[month] as a random effect.

	Drosophila melanogaster			*Drosophila simulans*		
	DF	SS	*F* ratio	DF	SS	*F* ratio
Environment						
Mean eggs per day						
Month	3	26 222	20.479***	2	18 315.9	49.623***
Line[month]	236	100 785	3.3052***	389	71 992.5	2.5087***
Maximum eggs per day						
Month	3	9870.932	18.749***	2	23 027.047	105.639***
F1 development time						
Month	3	264 618	81.784***	2	439 181	98.709***
Sex	1	14 097.8	87.686***	1	30 101.3	166.695***
Month × sex	3	1074.77	2.283	2	606.947	1.6806
Line[month]	281	696 134	15.409***	430	1 244 342	16.025***
Common garden						
Development time						
Month	3	1 317 083	172.41***	2	1 362 253	286.562***
Sex	1	1863	4.481*	1	4521.36	11.796**
Month × sex	3	58 446	46.859***	2	41 179.3	53.717***
Line[month]	227	1 058 577	11.216***	397	1 992 859	13.096***
Heat knockdown						
Month	3	7.23E+09	27.061***	2	6.66E+08	14.409***
Sex	1	1 812 750	0.771	1	93 538.8	0.055
Month × sex	3	8 405 978	1.192	2	3.42 + 06	0.999
Line[month]	228	2.20E+10	40.636***	208	6.51E+09	18.327***
Chill recovery						
Month	3	2.51E+07	3.773*	2	6.74E+07	11.517***
Sex	1	2 015 167	2.593	1	4 525 210	4.726*
Month × sex	3	1 452 574	0.623	2	2 497 510	1.304
Line[month]	237	5.36E+08	2.908***	430	6.01E+08	3.171***
Starvation resistance						
Month	3	1 375 073	138.684***	2	226 248	67.8033***
Sex	1	2 474 462	7062.638***	1	1 070 297	3779.517***
Month × sex	3	85 167.5	81.0287***	2	33 365.4	58.9112***
Line[month]	227	1 919 109	10.0876***	397	465 053	6.0823***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$.

In contrast to patterns observed for *D. melanogaster*, *D. simulans* did not demonstrate a consistent change in thermal tolerance over seasonal time, although the linear decline in starvation tolerance paralleled that observed for *D. melanogaster* (Fig. 4). Compared to *D. melanogaster*, *D. simulans* were more susceptible in the chill and starvation assays, taking longer to recover from the chill and surviving a shorter time without food; however, there was no difference between the species in heat tolerance (Table 3).

Genetic variance/covariance

The estimated variance–covariance matrices significantly differed between species and between the early and late endpoints of the collections; furthermore, the change in the G matrix over seasonal time was distinct between *D. simulans* and *D. melanogaster* (Table 4, Table S1). For either species, only a single variance/covariance estimate (between starvation tolerance and development time in *D. melanogaster*) did not significantly vary between the early and late collections. In *D. melanogaster*, knockdown time under heat stress and recovery time from chill coma demonstrated a significant positive correlation both in the early and in the late season collections, although the correlation was significantly stronger in the former. The positive correlation indicated a negative functional association, as increasing values for high-temperature knockdown were indicative of increased stress tolerance, whereas decreasing values were indicative of the same for tolerance to cold. This suggests the potential for a pronounced trade-off between performance under high and low temperatures. In contrast, the correlation between development time and high-temperature knockdown did not indicate a functional trade-off, as increased resistance to heat stress was associated with a faster rate of development; this was evident in the

Table 3 Mean and standard error by species for traits measured in the common garden and T-test comparing the trait performance when both species were present.

			D. melanogaster		*D. simulans*		Species comparison		
Assay	Collection month	Sex	Mean	SE	Mean	SE	t-ratio (S-M)	DF	p-value
Chill recovery (minutes)	June	F	52.292	2.819	–	–	–	–	–
		M	58.658	3.173	–	–	–	–	–
	July	F	57.741	2.987	75.602	4.77	17.861	592	**
		M	61.919	3.396	66.37	4.542	4.452	673	
	September	F	62.683	4.555	66.076	3.114	3.393	669	
		M	72.779	5.261	65.181	3.097	7.598	574	
	November	F	85.327	6.18	78.507	5.016	6.820	553	
		M	84.848	6.439	68.161	4.089	16.687	491	*
Heat knockdown (minutes)	June	F	93.586	4.454	–	–	–	–	–
		M	83.755	4.103	–	–	–	–	–
	July	F	40.663	1.056	37.159	1.623	2.961	518	
		M	41.859	1.168	38.692	2.143	3.361	549	
	September	F	57.419	1.533	60.405	1.479	3.133	756	
		M	60.167	1.536	58.984	1.417	1.186	737	
	November	F	49.067	1.367	49.945	1.107	0.489	801	
		M	50.322	1.194	49.175	1.027	1.441	823	
Starvation survival (hours)	June	F	119.664	1.042	–	–	–	–	–
		M	75.152	0.777	–	–	–	–	–
	July	F	112.456	0.917	95.734	0.974	18.963	1714	***
		M	74.328	0.655	58.236	0.631	10.784	1871	***
	September	F	113.886	1.384	84.95	0.779	28.209	686	***
		M	66.187	0.929	47.407	0.397	20.264	610	***
	November	F	72.216	0.886	68.515	0.695	3.701	683	**
		M	47.144	0.374	44.558	0.211	2.586	548	***
Development time (hours)	June	F	214.362	0.494	–	–	–	–	–
		M	216.560	0.455	–	–	–	–	–
	July	F	211.043	0.371	218.704	0.475	1.098	1517	
		M	214.955	0.379	221.856	0.492	0.368	818	
	September	F	234.133	0.605	236.069	0.442	0.397	983	
		M	237.707	0.595	241.151	0.443	2.801	972	*
	November	F	214.839	1.059	215.697	0.720	6.841	1170	***
		M	221.812	1.1502	220.063	0.787	2.766	1057	*

*p<0.05, **p<0.01, ***p<0.0001.

early-season collection only and was distinct from the observed pattern in the late season collection.

Although all variance/covariance matrices were significantly distinct between early and late collections for *D. simulans*, the only individual correlation that approached significance was that between starvation tolerance and chill coma recovery time (Table 4). This correlation was negative: that is, this demonstrated a positive functional association where lines with an increased starvation resistance also recovered more quickly from exposure to cold.

Discussion

Mode of seasonal change

There are several ways in which multivoltine species may respond to changing selection pressures caused by seasonal environmental heterogeneity. Populations may not respond to the seasonal change in environmental parameters, with phenotypes that are either fixed over time or fluctuate in a stochastic, nondirectional manner. Although phenotypic plasticity may be elicited in response to cyclic environmental heterogeneity (e.g. Brakefield & Reitsma, 1991; Bradford & Roff, 1993), in this study the isofemale lines were reared in a common-garden laboratory environment to remove environmental effects that may have reflected phenotypic plasticity. Alternatively, natural selection for traits associated with high fitness in a specific environment may result in a rapid adaptive response if the population contains standing genetic variation for those traits; for example, in *D. melanogaster* seasonal shifts in diapause incidence (Schmidt & Conde, 2006) may result in seasonal change in allele frequencies at the gene *couch potato* (Cogni *et al.*, 2013).

The significant differences in traits across collection timepoints in both species were indicative of some

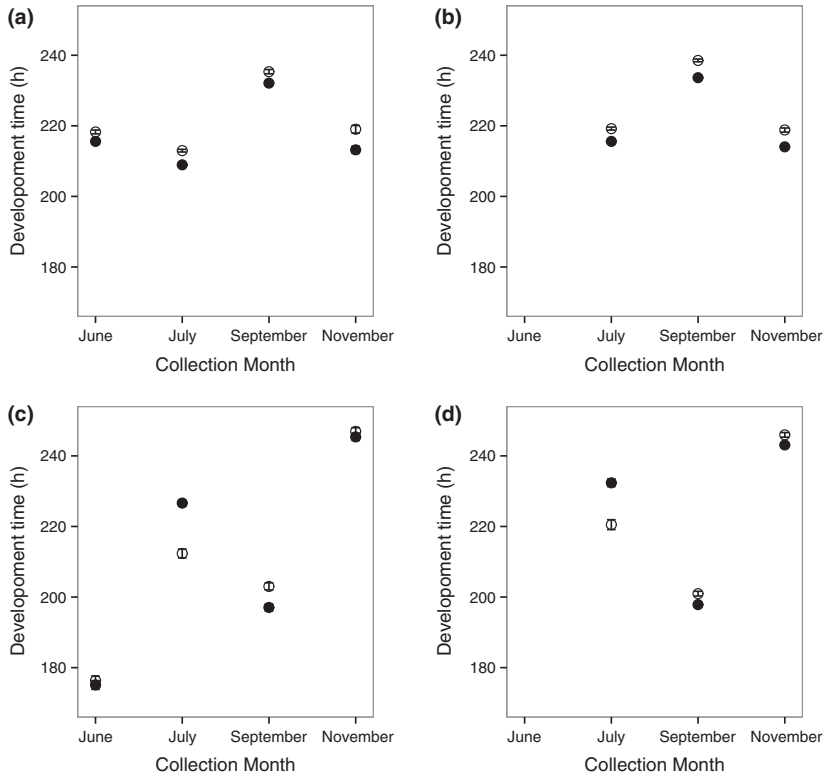

Fig. 3 Mean (±SE) development time in hours for F1 individuals (a and b) and flies that had been in a common-garden laboratory environment (c and d) for *Drosophila melanogaster* (a and c) and *Drosophila simulans* (b and d). Females are indicated in filled circles and males in empty circles. The F1 development time includes maternal effects that may reflect environmental quality and oscillates around the same duration for both species across seasonal time. The common-garden development time removes such environmental effects and increases drastically for *D. melanogaster*; however, it does not have a directional change for *D. simulans*.

degree of seasonal response. Changes were seen in measurements of wild flies (i.e. fecundity) that reflect environmental variation as well as in the traits measured in the common garden (i.e. stress traits) that indicate genetic change in the population. The predictable pattern of decline for some traits suggests that the changes across seasonal time were not due to random chance. Additionally, the traits measured in the common garden showed rapid changes between subsequent collections that were unlikely to be explained by genetic drift. Therefore, it seemed unlikely that the seasonal patterns described were due to random stochasticity, but instead represented deterministic ecological and evolutionary processes.

The change across seasonal time in stress resistance traits measured in the common laboratory environment demonstrated seasonal change in the genetic composition of the population. In *D. melanogaster*, the decline in stress resistance from spring through summer was consistent with the operation of natural selection following the prediction of selection for high stress resistance during the winter and relaxed selection on stress resistance throughout the summer. However, the observed data may have reflected migration because *D. melanogaster* populations at lower latitudes are characterized by reduced tolerance to at least some stressors (Hoffmann *et al.*, 2001; Paaby & Schmidt, 2008) and an influx of migrants from lower-latitude locales throughout the summer would be predicted to result in a decrease in stress tolerance over seasonal time. Such patterns of migration should have affected allele frequency profiles as well as phenotypes. Pooled sequencing of this Pennsylvania orchard population over three successive years, including the collections analysed here, has demonstrated that migration alone is insufficient to explain observed seasonal changes in allele frequencies genomewide (Bergland *et al.*, 2014). By extension, migration from southern regions on the east coast of the United States cannot explain the rapid and pronounced change in phenotypic profiles that we observed and describe here.

The demographic and phenotypic patterns across seasonal time were different for *D. simulans* compared to *D. melanogaster*. *Drosophila simulans* was absent in the earliest collection, and when it appeared in late July, the population was composed of a diversity of age classes that remained consistent throughout the autumn. The absence of *D. simulans* during the first half of the calendar year may be because they are generally considered to be a more tropical taxon than *D. melanogaster* (Capy *et al.*, 1993; Hoffmann & Harshman, 1999) with no overwintering mechanism identified, and therefore, they may be less able to maintain a resident population in temperate climates (Schmidt & Conde, 2006; Schmidt, 2011). The stability of age heterogeneity across seasonal time was consistent with

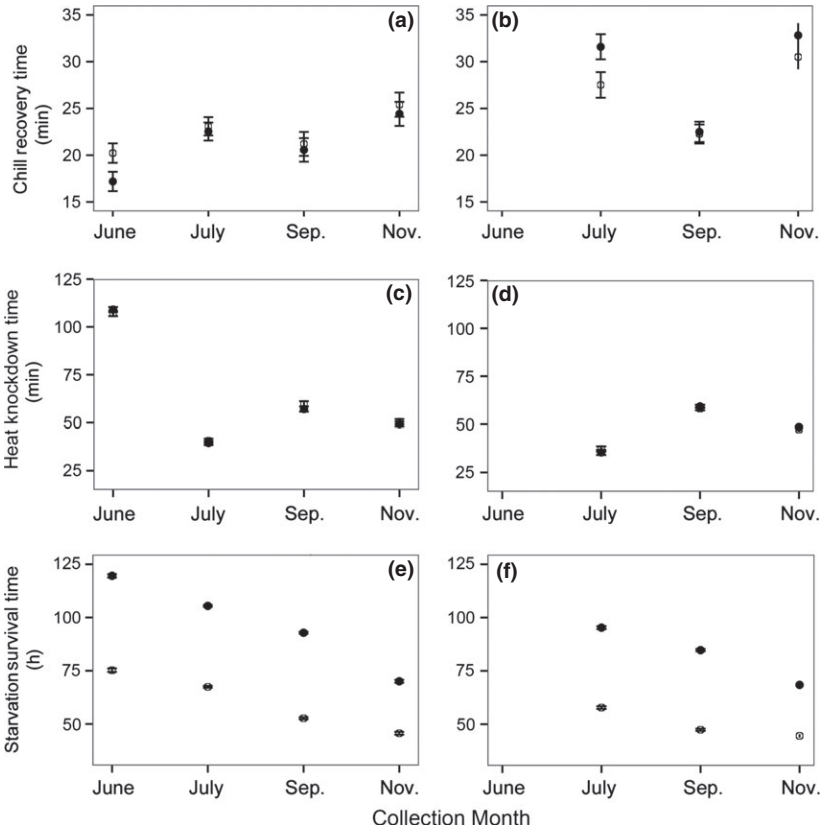

Fig. 4 Mean (±SE) recovery time from chill (a and b), knockdown time from heat (c and d) and survival time without food (e and f) for flies that had been in a common-garden laboratory environment. *Drosophila melanogaster* (a, c, e) shows a seasonal decline in quality for all of these traits, whereas *Drosophila simulans* (b, d, f) demonstrates no clear pattern for thermal traits and a seasonal decline in starvation resistance. Females are indicated in filled circles and males in empty circles.

Table 4 Correlated traits throughout the season with early season (June) above the diagonal and late (November) below the diagonal. Bolded correlations are significantly different than zero at $P = 0.05$ with Pearson's product-moment correlation tests; italicized and bolded correlations are marginally different than zero at $P = 0.10$. Italicized correlations indicate trait variance/covariances that did not significantly differ between months at $P = 0.05$; all other variance/covariances differed between months for a particular trait combination at $P = 0.001$ with MANOVA.

	Chill	Heat	Starvation	Development
Drosophila melanogaster				
Chill	1	**0.48429018**	−0.0880632	0.01198028
Heat	**0.39263658**	1	0.00142993	**−0.2642816**
Starvation	−0.124261	0.00840717	1	*−0.0276306*
Development	−0.0668889	−0.0847142	0.02486902	1
Drosophila simulans				
Chill	1	−0.2277738	***−0.3282139***	0.10125605
Heat	−0.0513712	1	−0.3038521	0.12133322
Starvation	−0.0636857	−0.186429	1	0.10700678
Development	−0.0845394	−0.1804966	0.15166336	1

the hypothesis of either annual recolonization or a longer residence time in refugia. Migration from a southern refuge could have caused the delay in appearance and explained the age heterogeneity, as *Drosophila* of all ages are thought to be transported passively by wind or humans over long distances (Dobzhansky, 1973). Alternatively, the delayed appearance could have been due to a longer residence in local refugia that support continuous populations, such that upon return to the orchard, the flies were a mixture of ages. Both scenarios would result in the patterns collected here; *D. simulans* reappeared in the orchard when environmental conditions were suitable and exhibited less directional phenotypic change in comparison with

D. melanogaster. Only starvation resistance declined from spring to autumn in a parallel way between the two species. Distinguishing between the recolonization and local refuge hypotheses requires a targeted study that involves direct field measurements over time (e.g. mark–release–recapture) or inference from longitudinal sampling and sequencing. However, both hypotheses are consistent with the inference that, relative to *D. melanogaster*, *D. simulans* was less temporally persistent in temperate orchards and may exhibit a relatively weaker adaptive response to seasonal change in environmental parameters.

Seasonal population dynamics

The initial young composition of *D. melanogaster* was consistent with the hypothesis that after overwintering, adults were cued by environmental stimuli to emerge synchronously from dormancy to produce an initial cohort of uniform age composition (Tauber *et al.*, 1986). The June collection analysed here was likely among the first post-dormancy cohorts; based on slower development time at cool spring temperatures (Trotta *et al.*, 2006), it was estimated that the eggs were laid in April, the time at which *D. melanogaster* were first collected in appreciable numbers in Pennsylvania (Schmidt & Conde, 2006). After the initial uniformly young sample, the age composition followed the predicted increase in heterogeneity as the population grew and reproduced throughout the summer (Tauber *et al.*, 1986); such seasonal changes in population age composition have also been documented in the medfly *Ceratitis capitata* (Carey *et al.*, 2008). Together, the seasonal changes in demography of *C. capitata* and *D. melanogaster* suggest that the age composition of many multivoltine species may be dynamic across seasonal time. However, the different seasonal demographic dynamics in *D. melanogaster* and *D. simulans* indicate that seasonal changes in age composition is one component of a suite of traits that may respond to environmental heterogeneity.

Whereas *D. melanogaster* and *D. simulans* are commonly considered to have a short lifespan (~1–6 days) in nature (Rosewell & Shorrocks, 1987), the data presented here suggest that individuals of both species reached old ages of one to 2 months in the wild; as with other short-lived insects (Bonduriansky & Brassil, 2002), senescence may be pronounced in natural populations of *Drosophila*. As many life history traits have age-specific properties (e.g. Minois & Le Bourg, 1999; Nghiem *et al.*, 2000; Zerofsky *et al.*, 2005), any seasonal change over time in the demographic composition of the population may also have significant effects on selection dynamics in the field. Early in the season, the unimodal young *D. melanogaster* population would be expected to have a uniform age-specific response to the environment. In contrast, the increase in age heterogeneity throughout the summer and autumn would lead to a predicted wider range of responses to the same stress.

The change in population age structure across the season in *D. melanogaster* suggests that antagonistic pleiotropy associated with age-specific fitness parameters may add an additional layer of complexity to the population dynamics (Williams, 1957). Antagonistic pleiotropy can maintain additive genetic variation for fitness components and may allow for protected polymorphism in the absence of over dominance in populations with overlapping and nonoverlapping generations (Rose, 1982, 1983, 1985). In this way, it is possible that antagonistic pleiotropy may contribute to adaptive seasonal polymorphism in these populations (Bergland *et al.*, 2014).

Implications of seasonal selection

In *D. melanogaster* populations, traits associated with fitness change in a nonrandom manner along with the environment over seasonal time; this may be due to environmentally mediated selection over short timescales that previously may have been considered evolutionarily static. Whole-genome resequencing of the same population over three consecutive years has demonstrated that hundreds of SNPs consistently oscillate in allele frequency between spring and autumn (Bergland *et al.*, 2014). Taken together, these results suggest that selection in *D. melanogaster* can act in a rapid fashion and that temporal variation in fitness may result in seasonal oscillations at both the phenotypic and genomic levels. However, the rapidity of environmental change may result in maladaptation because of a delay between the traits being selected in the parental environmental conditions that may not have the highest fitness in the subsequent generation.

The rapid response to environmental variables that characterize seasons may result in cyclic selection that maintains diversity in the population. Based on the observed change in traits from spring to autumn, we predict that the distinct selection regimes associated with summer population expansion and winter collapse will produce annual cycles in these traits as seen in reproductive diapause frequency in *D. melanogaster* (Schmidt & Conde, 2006). This alternating selection for winter and summer phenotypes is a special case of microevolution for an intermediate optimum known as fluctuating–stabilizing selection (Wright, 1968; Istock, 1981); this selection for bet hedging can be applied hierarchically to a broad range of evolutionary scales (Simons, 2002). It is expected to maintain phenotypic and genetic variation within a population and, in doing so, seasonal environmental selection may limit or slow evolutionary processes including population divergence (Levins, 1968; Sasaki & Ellner, 1997) and local adaptation (Kawecki & Ebert, 2004).

The phenotypic change we observe here from spring through autumn should be considered in the larger

context of annual cycles of seasonal selection across seasons. Such cycling selection may yield underestimates in the strength or direction of selection by averaging trait values across seasonal time; this may contribute to low estimates of the strength of selection over an entire breeding season (Kingsolver *et al.*, 2001). Additionally, seasonal changes in the variance–covariance matrices demonstrate that strong selection on this timescale effects genetic correlations; this alters the basic assumption of stable covariance over time when making phenotypic evolutionary predictions.

The magnitude and rapidity of the phenotypic change observed over seasonal time leads to the hypothesis that seasonal dynamics may also contribute to the formation and persistence of latitudinal clines. Differential length of seasons could generate latitudinal clines if a favoured phenotype reaches high frequency in the winter but selection against it during the summer decreases its frequency in proportion to the length of the growing season (Rhomberg & Singh, 1986). Our data demonstrate that the range and variance associated with temporal sampling of *Drosophila* in a temperate orchard is equivalent to that previously observed in collections of natural populations spanning 20° latitude in the eastern United States (Schmidt & Paaby, 2008). Given the extent of phenotypic change throughout the climatic period favourable for *Drosophila* population growth and reproduction, temporal variation in selection pressures could be at least partially responsible for the generation of latitudinal clines that appear so pervasive in *D. melanogaster* (e.g. Capy *et al.*, 1993; James & Partridge, 1995; Mitrovski & Hoffmann, 2001; Schmidt *et al.*, 2005; Trotta *et al.*, 2006). Such systematic changes in season length along a latitudinal gradient can generate either simple or 'saw-tooth' clines (Roff, 1980). However, the seasonal phase cline hypothesis and the connection between temporal and spatial evolutionary dynamics of life histories remain to be comprehensively tested in nature.

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Supporting information

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

Figure S1 Kaplain–Meier survivorship curves for wild (solid) and F1 (dashed) *Drosophila* across seasonal time.

Table S1 Correlation matrices between traits by species across time.

Data deposited at Dryad: doi: 10.5061/dryad.2j48p

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