

# The demography of slow aging in male and female *Drosophila* mutant for the insulin-receptor substrate homologue *chico*

Meng-Ping Tu, Diane Epstein and Marc Tatar

Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island, USA

## Summary

**Hypomorphic mutants affecting the *Drosophila* insulin/IGF signal pathway are reported to increase longevity in females but not in males. To understand this sex-difference, we conducted a large-scale demographic study with three new isogenic strains of alleles at *chico*, the insulin-receptor substrate homologue. We verify that female dwarf homozygotes ( $ch^1/ch^1$ ) and normal-sized heterozygotes ( $ch^1/+$ ) are long-lived, as originally reported. We find for the first time that male heterozygotes are long-lived relative to wildtype, by about 50%. The life span of male  $ch^1/ch^1$  is similar to that of wildtype but these dwarf males age at a slow demographic rate. The levels of demographic frailty and of age-independent mortality are elevated in  $ch^1/ch^1$  males, counteracting the effect of slow aging upon life expectancy. Mortality deceleration occurs amongst the oldest-old wildtype adults, as seen in many organisms. Remarkably, in similarly sized cohorts of male and female  $ch^1/ch^1$  and of male  $ch^1/+$  mortality deceleration is absent. Mortality deceleration is a phenotype of *chico*. Key words: demographic aging; frailty; Gompertz; insulin; mortality.**

## Introduction

Mutations that affect the insulin/IGF signal pathway increase adult survival in *Caenorhabditis elegans* and in *Drosophila melanogaster*. As originally reported in flies, mutants of genes in this pathway increase longevity in females but not in males (Clancy *et al.*, 2001; Tatar *et al.*, 2001). This pattern raises the question, are the genes of the insulin/IGF signal pathway sex-limited in their effects upon aging? Alternatively, these loci might reduce aging in males and females but differentially induce counterbalancing age-independent mortality. Both cases can produce mutant females with extended longevity and mutant males with ordinary life spans. To distinguish between these alternatives we present an intensive demographic analysis

of *chico*, the *D. melanogaster* gene encoding the insulin-receptor substrate homologue (Bohni *et al.*, 1999).

Insulin/IGF signalling appears to be a conserved regulatory system of aging (Kenyon, 2001). In *Drosophila*, mutants of the insulin-like receptor (*InR*) and of the insulin-receptor substrate homologue (*chico*) extend female longevity by 36–85% (Clancy *et al.*, 2001; Tatar *et al.*, 2001). In males these same alleles produce no net improvement in mean longevity, although *InR* heteroallelic mutants increase life expectancy measured at age 20 days. In *C. elegans*, hypomorphic mutants affecting insulin/IGF signalling extend life span in hermaphrodites and in males (Johnson, 1990; Kenyon *et al.*, 1993; Gems & Riddle, 2000). In both species, aging is modified by insulin through cell-nonautonomous signals (Apfeld & Kenyon, 1998; Tatar *et al.*, 2001), presumably in conjunction with secondary hormones and gonadal endocrine feedback (Arantes-Oliveira *et al.*, 2002). This being so, sex differences in longevity might be expected since endocrine integration of reproductive and somatic physiology is inherently sex-specific. Alternatively, the insulin/IGF mutants may similarly modulate aging in males and females but still produce differences in longevity if they have sex-limited effects upon development and growth. Growth and body size are sexually dimorphic in *D. melanogaster* where males mature quickly and with relatively small size. Since insulin/IGF regulates cell growth and proliferation (Chen *et al.*, 1996; Stocker & Hafen, 2000), male development and subsequent adult age-independent viability may be especially vulnerable to insulin hypomorphism.

These alternatives can be resolved, in part, through parametric analysis of mortality. Here we apply this approach to *chico* genotypes. Cohorts with extensive demographic and genetic replication are used to estimate age-specific mortality for all genotypes within each sex. A new system to control the effect of genetic backgrounds among *chico* alleles is introduced. We replicate the original finding of Clancy *et al.* (2001) –  $ch^1$  extends female survival both as a heterozygote and as a homozygote. Furthermore, we now see that male heterozygotes outlive wildtype. From the parametric analysis of mortality we find that male and female mutants age at equally slow rates, that  $ch^1$  has a countervailing, age-independent impact upon adult mortality, and that mortality deceleration at advanced ages is a phenotype of *chico*.

## Results

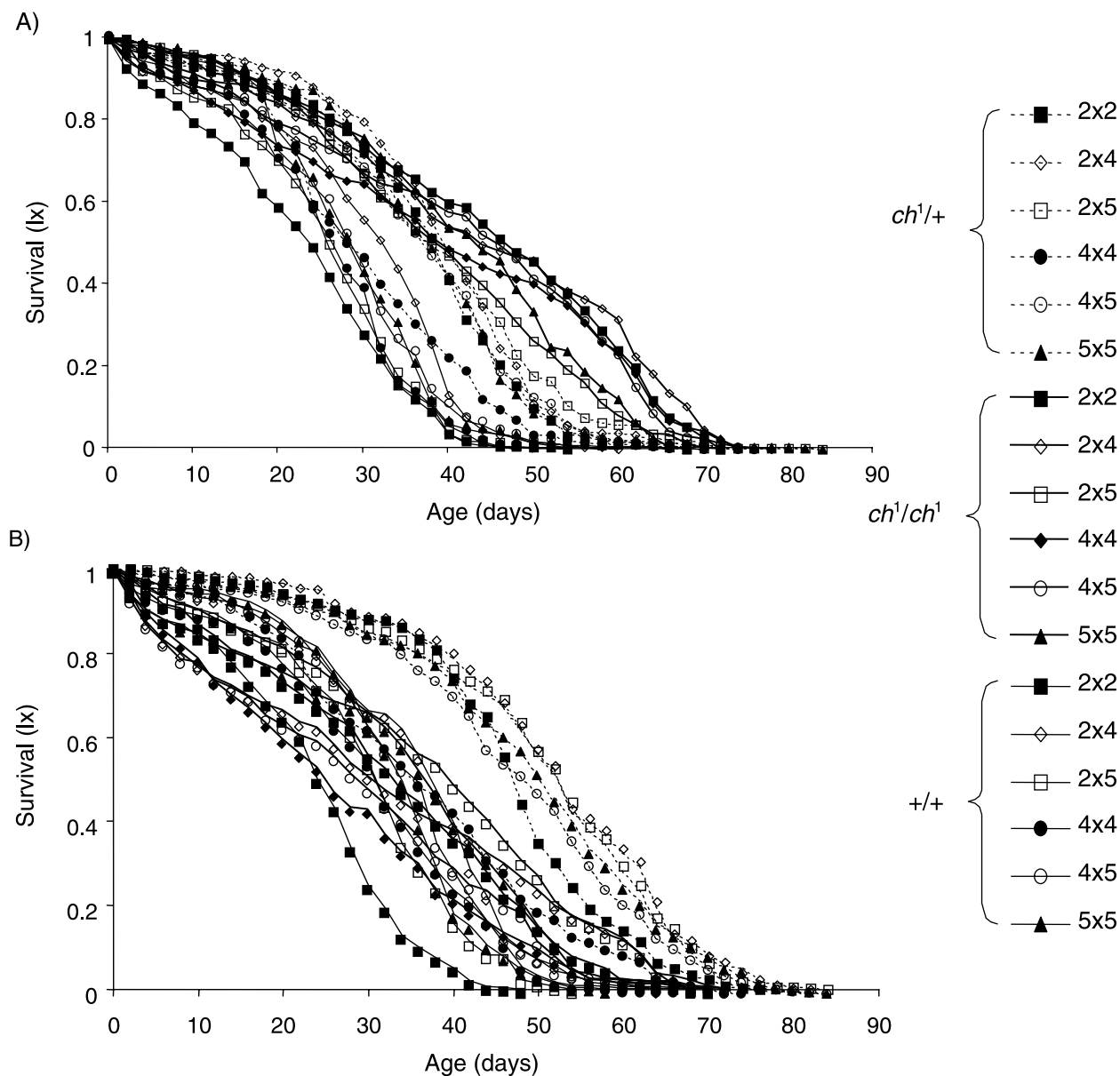
### Non-parametric survival analysis and life tables

Life tables were contemporaneously constructed for each *chico* genotype,  $ch^1/ch^1$ ,  $ch^1/+$ ,  $+/+$ . All genotypes segregated as sibs

Correspondence

Marc Tatar, Tel.: +1 401 863 3455; e-mail: Marc\_Tatar@Brown.edu

Accepted for publication 11 June 2002



**Fig. 1** Cumulative adult survival of *chico* genotype cohorts in (A) females and (B) males. Each line represents the combined offspring of replicate cages from within each cross. The six  $F_1$  background replicate cohorts are denoted by symbols, the genotypes at *chico* are categorized by heavy, thin and broken lines.

from *inter se* crosses among three independent backcross strains (*de-2*, *de-4*, *de-5*) and from self-crosses within strains. Thus, each genotype at *chico* was represented in six independent, highly co-isogenic backgrounds.

Survival among the  $F_1$  cohorts within each genotype is strikingly consistent (Fig. 1A,B). Wildtype adults are uniformly the shortest lived. Among heterozygotes, five of the six  $ch^1/+$  cohorts have similar survival proportions; the *de-4*  $\times$  *de-4* cross is shorter lived. Survival is also consistent among dwarf  $ch^1/ch^1$  cohorts within both sexes. Survival of female  $ch^1/ch^1$  is superior at median and late ages but similar to that of  $ch^1/+$  at early adulthood. At early ages the survival of male  $ch^1/ch^1$  is similar to wildtype but superior at later ages.

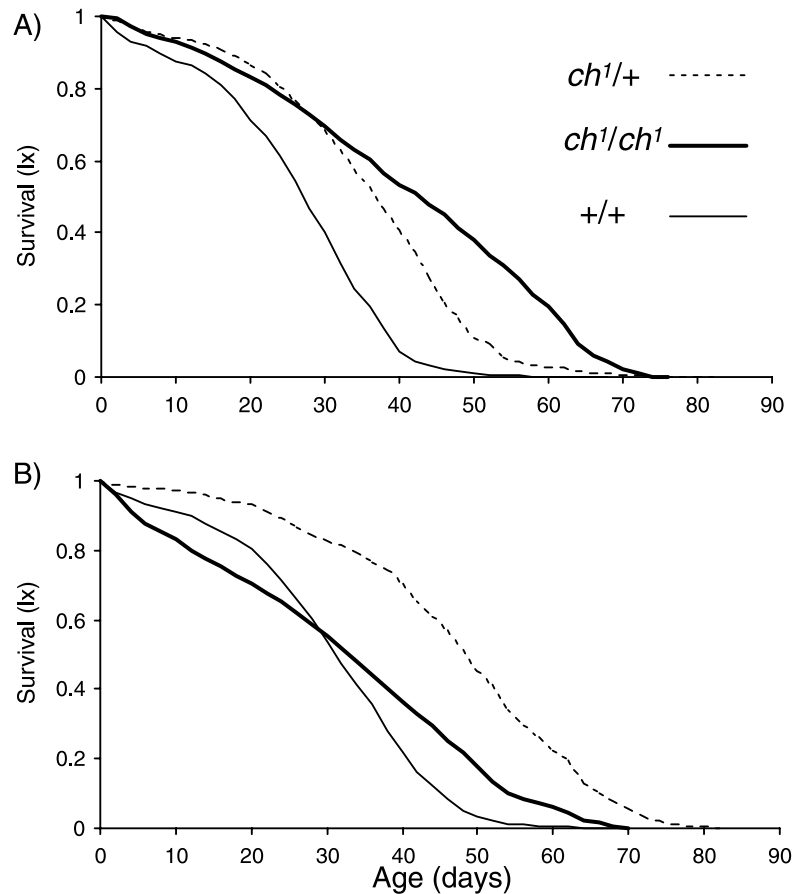
We combine data among the  $F_1$  replicates to statistically evaluate life tables of each genotype (Supplementary material). Female survival (Fig. 2A) and life expectancy (Table 1) is greatest in  $ch^1/ch^1$ , intermediate in  $ch^1/+$  and least in  $+/+$ . In males, overall survival is highest in  $ch^1/+$  (Fig. 2B). Life expectancy of male  $+/+$  and  $ch^1/ch^1$  are similar and less than seen in heterozygote (Table 1).

#### Parametric mortality analysis

Differences in the shape of survival functions in Figs 1 and 2 suggest that patterns of mortality are altered by mutation of *chico*. We use the combined  $F_1$  data to assess variables of the

**Table 1** Mortality and survival statistics cohorts of *chico* with best-fit mortality models. Parameters of the nested Logistic–Makeham–Gompertz model are frailty ( $\lambda$ ), rate of demographic aging ( $\gamma$ ), age-independent mortality ( $c$ ) and mortality deceleration ( $s$ ). Estimates are provided for parameters that correspond to the best-fit model. Within sex, parameters are compared among all pairs of cohorts; differences in superscripts within columns indicate significance between parameters based on the log-likelihood test ( $P < 0.001$ ). Adult life expectancy is estimated from eclosion; superscripts indicate significant differences within each sex for overall survival (log-rank test,  $P < 0.001$  in all cases).

	Model	$\lambda$	$\gamma$	$c$	$s$	Initial cohort size	Adult life expectancy (days)
Female							
+/+	Logistic	0.00286 <sup>a</sup>	0.137 <sup>a</sup>		0.467 <sup>a</sup>	2057	27.1 <sup>a</sup>
<i>ch</i> <sup>1</sup> /+	Logistic	0.00175 <sup>b</sup>	0.110 <sup>b</sup>		0.493 <sup>a</sup>	2084	36.9 <sup>b</sup>
<i>ch</i> <sup>1</sup> / <i>ch</i> <sup>1</sup>	Gompertz–Makeham	0.00119 <sup>b</sup>	0.0770 <sup>c</sup>	0.00606		1933	42.7 <sup>c</sup>
Male							
+/+	Logistic	0.00250 <sup>a</sup>	0.117 <sup>a</sup>		0.413	1494	31.1 <sup>a</sup>
<i>ch</i> <sup>1</sup> /+	Gompertz	0.00163 <sup>b</sup>	0.0723 <sup>b</sup>			1859	46.7 <sup>b</sup>
<i>ch</i> <sup>1</sup> / <i>ch</i> <sup>1</sup>	Gompertz–Makeham	0.00461 <sup>c</sup>	0.0612 <sup>b</sup>	0.00439		1619	32.8 <sup>c</sup>

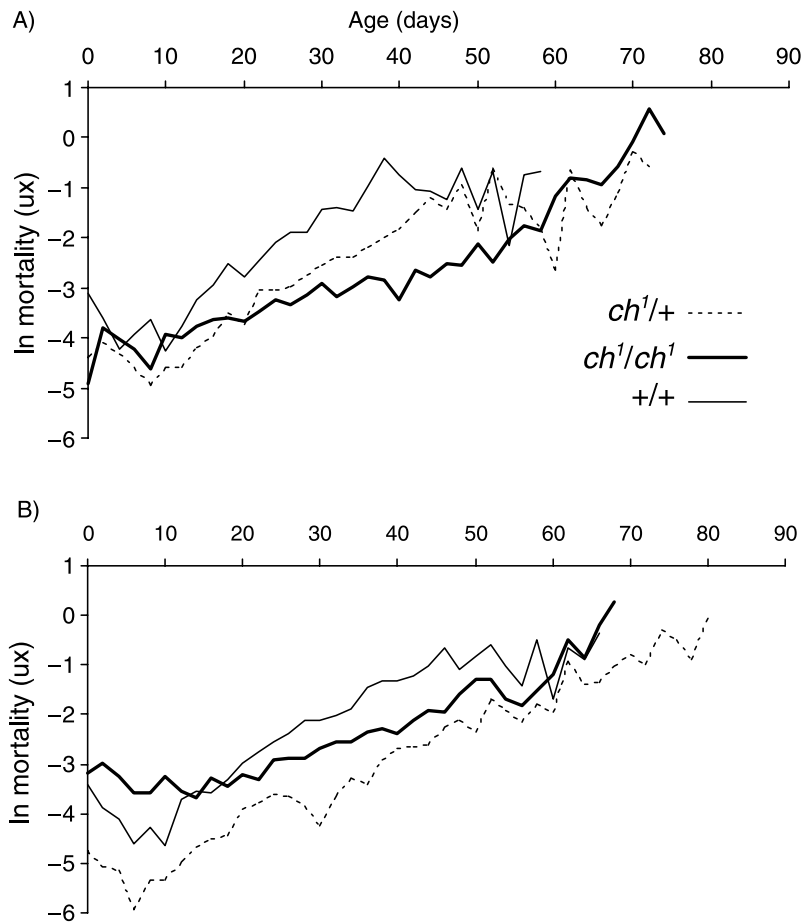


**Fig. 2** Cumulative adult survival of *chico* genotype cohorts in (A) females and (B) males with the F<sub>1</sub> background replicate cohorts within each genotype combined. The genotypes at *chico* are categorized by heavy, thin and broken lines.

nested Logistic–Makeham–Gompertz model (Pletcher, 1999). In the simplest case, the Gompertz model assumes that mortality rate  $\mu_x$  at age  $x$  is  $\mu_x = \lambda e^{\gamma x}$ . The parameter  $\lambda$  represents demographic frailty or baseline mortality (Sacher, 1977; Vaupel *et al.*, 1979). The parameter  $\gamma$  represents the rate of change in mortality with age, the demographic rate of aging. Two mortality features can be added to this model. When change in mortality decelerates at advanced ages, the Logistic

model applies,  $s$  is non-zero and  $\mu_x = \lambda e^{\gamma x} [1 + (\lambda s / \gamma)(e^{\gamma x} - 1)]^{-1}$ . When cohorts experience age-independent mortality, denoted as  $c$ , the Gompertz–Makeham model applies and  $\mu_x = c + \lambda e^{\gamma x}$ . When both cases hold, the Logistic–Makeham model applies as  $\mu_x = c + \lambda e^{\gamma x} [1 + (\lambda s / \gamma)(e^{\gamma x} - 1)]^{-1}$ .

Deaths through day 4 are left censored to remove incidental mortality associated with initiation of the demography cages. We test for improved goodness-of-fit relative to the simple Gompertz



**Fig. 3** Mortality rate of *chico* genotype cohorts in (A) females and (B) males with the  $F_1$  background replicate cohorts within each genotype combined. The genotypes at *chico* are categorized by heavy, thin and broken lines.

model by comparing the log-likelihood when  $s$  and  $c$  are fixed at zero relative to when  $s$ ,  $c$ , or  $s$  and  $c$  are freely estimated. The more parameterized model provides a better fit if twice the difference in log-likelihood is greater than 3.84 (log-likelihood test, d.f. = 1). Table 1 summarizes model choice, parameter estimates and statistics for genotypes within each sex.

Mortality among wildtype and heterozygote females is best described as Logistic. Mortality at older ages presents a strong plateau near  $\mu_x = 0.38$  (Fig. 3). To compare parameters among genotypes, we estimate all variables based on the most complex model of the pair and then assess the log-likelihood under this case relative to the case when the tested variable is estimated as common to both cohorts (Table 1). Following this procedure,  $s$  does not differ between wildtype and heterozygote females but  $\lambda$  and  $\gamma$  are reduced in the heterozygote. The demographic rate of aging ( $\gamma$ ) is further reduced in dwarf females. Mortality deceleration ( $s$ ) is not significant in dwarf females. Since similar sized cohorts are studied for all genotypes, the absence of  $s$  in  $ch^1/ch^1$  (or in  $ch^1/+$  males) is not likely to be an artefact of insufficient demographic power. Dwarf females also present a significant value for the Makeham term ( $c$ ), unlike wildtype and heterozygote females.

Males show a similar progression from Logistic to Gompertz–Makeham with increasing dose of the  $ch^1$  allele, although  $ch^1/+$  is best described by the simple Gompertz. The dwarf male,

as with the female, has Gompertz–Makeham mortality. As in females, the demographic rate of aging ( $\gamma$ ) in both  $ch^1/+$  and  $ch^1/ch^1$  is reduced relative to wildtype. Dwarf males have significant age-independent mortality ( $c$ ) and the largest observed value of demographic frailty ( $\lambda$ ). As a result, although mortality accelerates with a slow rate in male  $ch^1/ch^1$ , this genotype is not long-lived.

## Discussion

As reported by Clancy *et al.* (2001), the  $ch^1$  allele extends adult life span. We independently replicate this result in a new genetic background. Our strains have useful features for the demographic analysis of a single locus mutation. First, the replicate backcross lines have a total of 54 meiotic events to reduce linkage disequilibrium about the *chico* locus. Second, crosses among isogenic lines produce  $F_1$  offspring with reproducible backgrounds. Finally, all genotypes segregate within sibships and alleles within each stock perpetually retain background similarity since we do not rely upon balancer chromosomes.

We found remarkable agreement among the replicate life tables within genotypes but note that a strain can occasionally produce a stray result (i.e.  $de-4 \times de-4$ ). Overall, we verify that females are long-lived as dwarf homozygotes (57% increase relative to wildtype) and as normal size heterozygotes (36%

increase relative to wildtype). As reported by Clancy *et al.* (2001), small body size is not a necessary condition for extended longevity mutants affecting insulin/IGF signalling in *D. melanogaster*. Among males, we find that heterozygotes are 50% longer lived than both dwarf and wildtype. The data of Clancy *et al.* (2001) suggested this pattern but inference was limited by cohort size. We resolve these issues and show that *ch<sup>1</sup>* has a clear survival advantage for males as well as for females.

How *ch<sup>1</sup>* differentially affects life span of males and females might be understood from the perspective of mortality patterns (Vaupel, 1986). Life expectancy and the level of mortality acceleration are similarly ranked across genotypes in females. Overall, mortality accelerates the slowest and maintains the lowest level in the *ch<sup>1</sup>/ch<sup>1</sup>* cohort. Female heterozygotes have an intermediate value for  $\gamma$  and share with *ch<sup>1</sup>/ch<sup>1</sup>* a low level of frailty ( $\lambda$ ). In contrast to females, frailty ( $\lambda$ ) modifies the rank order of life expectancy across male genotypes. Dwarf males, like females, have a Makeham–Gompertz mortality trajectory. The Makeham term (*c*) represents a degree of age-independent mortality that is superimposed upon a slow rate of demographic aging. In males the frailty of *ch<sup>1</sup>/ch<sup>1</sup>* is relatively large. As a result, the absolute value of early adult mortality dominates the cohort and reduces life expectancy at eclosion. Wildtype and heterozygote males are free of age-independent mortality and have frailty levels similar to the levels estimated for females. The absence of extended longevity in slowly aging *ch<sup>1</sup>/ch<sup>1</sup>* males is the outcome of sex-by-genotype expression of elevated frailty ( $\lambda$ ) combined with age-independent mortality (*c*).

These data imply that *ch<sup>1</sup>* is pleiotropic for demographic traits. The allele has dominant beneficial effects upon senescence and recessive deleterious effects upon age-independent mortality. A single allele of *ch<sup>1</sup>* reduces the demographic rate of aging and the initial mortality rate but incurs no age-independent mortality; life span is extended in both sexes. Two alleles of *ch<sup>1</sup>* can further reduce the rate of demographic aging but also increase age-independent mortality factors and, in males, frailty. The increase in age-independent mortality may result from strong hypomorphism of insulin/IGF function during development that retards growth and delays pupation. Altered development may be directly deleterious if it produces anomalies in morphology or physiology of adults. As well, slow growth could be indirectly detrimental if it retards escape from a deteriorating larval environment, which in turn may reduce adult fitness. Since males are intrinsically small in size and even more so as dwarfs, they may be especially sensitive to either of these mechanisms.

The data reveal intriguing genotypic differences among the patterns of old-age mortality. Mortality deceleration is a common feature of many animal life tables, including *D. melanogaster* (Vaupel *et al.*, 1998). Cohort heterogeneity with the selective loss of frail individuals can generate mortality deceleration (Vaupel & Yashin, 1985). Alternatively, personal rates of senescence may decline with advancing age. It is difficult to empirically distinguish among these models but genetic approaches may provide a useful tool. Quantitative genetic analysis of *D. melanogaster* reveals heritable variance

for the mortality deceleration parameter *s* of the Logistic model (Promislow *et al.*, 1996). Here we find an explicit gene that influences the parameter *s*. Among females, mortality decelerates in wildtype and heterozygote genotypes. In contrast, mortality among the old dwarf females (*ch<sup>1</sup>/ch<sup>1</sup>*) accelerates beyond this level and with no apparent attenuation. Among males, late age mortality strongly decelerates in the wildtype cohort. Male heterozygotes and homozygotes accelerate beyond this plateau. These data indicate that mortality deceleration is a demographic phenotype of *chico*.

If the *ch<sup>1</sup>* allele decreases variance in frailty, the loss of late-age mortality deceleration in *ch<sup>1</sup>* cohorts may result from reduced opportunities for demographic selection. In this case, sex differences may arise if *ch<sup>1</sup>* more strongly reduces variance for frailty in males. Alternatively, the individual rate of senescence may decelerate within wildtype individuals but continue to accelerate in *ch<sup>1</sup>/ch<sup>1</sup>* individuals even as this genotype slows the overall rate of aging. In this case, sex differences for deceleration may occur if a single *ch<sup>1</sup>* allele in males fully expresses the slow-aging and the loss-of-deceleration phenotypes while a single dose of *ch<sup>1</sup>* in females weakly affects aging and has no impact on deceleration. The genetic features of our *chico* strains offer a new experimental model to study these alternative mechanisms of mortality deceleration.

## Experimental procedures

### Strains segregating alleles of *chico*

The original strain of *ch<sup>1</sup>* P{ry+} was provided by E. Hafen (Zurich), the same source of *ch<sup>1</sup>* used in Clancy *et al.* (2001). We crossed *ch<sup>1</sup>* into an isofemale line with markers *cn/cn; ry<sup>506</sup>/ry<sup>506</sup>*. Females of *ch<sup>1</sup>, cn/cn; ry/ry* were recovered through progeny testing and backcrossed to males of the *cn/cn; ry<sup>506</sup>/ry<sup>506</sup>* line. Recombinant offspring (cinnabar) were retained. Eighteen backcross generations were used to isogenize *ch<sup>1</sup>* in three independent lines (denoted *de-2*, *de-4* and *de-5*). Presently, segregation of *chico* alleles is maintained by propagation of heterozygotes (normal-size, cinnabar). For demography, segregating genotypes among sibs were identified as: *ch<sup>1</sup>/+* normal-size, cinnabar; *ch<sup>1</sup>/ch<sup>1</sup>*, dwarf, cinnabar; *+/+*, normal-size, apricot.

### Demography

Life tables were generated for F<sub>1</sub> offspring of direct (*de-2* × *de-2*, *de-4* × *de-4*, *de-5* × *de-5*) and *inter se* (*de-2* × *de-4*, *de-2* × *de-5*, *de-4* × *de-5*) crosses. Reciprocal crosses within genotype were combined. Similar egg densities were laid up for all crosses. Larvae developed in standard cornmeal–dextrose–agar–yeast media supplemented with live yeast. From each cross, sibs that emerged within an 8-h period were sorted by genotype under light CO<sub>2</sub> and placed in three demography cages with about 200 flies per cage, mixed sex. Demography cages were 1-L clear food service containers with a screened lid, a gasket-covered opening for access of an aspiration pipe and a short connector

tube the width of a shell food vial. Food vials were fixed to the connector tube by a plastic sleeve. With these cages, flies remained in place day-to-day while fresh food (standard diet plus excess live yeast) was provisioned on alternate days. Dead flies were aspirated from cages when food was changed. Three replicate cages were started for each genotype. Cohorts of all genotypes were synchronously initiated and maintained at 25 °C, 40% r.h and a 12-h light cycle.

Survival was calculated as the proportion remaining alive at day  $x$  relative to the number of adults  $N_0$  initiating the cohort. Data of replicate cages were combined.  $N_0$  was estimated from the extinct cohort method as  $\sum_{x=0}^{\infty} d_x$  where  $d_x$  was the number dead in the 2-day period ending at age  $x$ . Adult life expectancy was estimated from eclosion (adult age 0-days) and calculated by the actuarial method with the census interval of 2 days.

Parameter estimates and hypothesis tests for Gompertz-family mortality models were executed with WinModest (Pletcher, 1999) where a series of nested models can be systematically evaluated with maximum likelihood methods.

## Acknowledgments

Support provided by the Ellison Medical Foundation and the U.S. National Institutes of Health (AG-16632).

## References

- Apfeld J, Kenyon C (1998) Cell nonautonomy of *C. elegans daf-2* function in the regulation of diapause and life span. *Cell* **95**, 199–210.
- Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C (2002) Regulation of lifespan by germline stem cells in *C. elegans*. *Science* **295**, 502–505.
- Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, Andrus BF, *et al.* (1999) Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1–4. *Cell* **97**, 865–875.
- Chen C, Jack J, Garofalo RS (1996) The *Drosophila* insulin receptor is required for normal growth. *Endocrinology* **137**, 846–856.
- Clancy DJ, Gems D, Harshman LG, Oldham S, Stocker H, Hafen E, *et al.* (2001) Extension of life-span by loss of CHICO, a *Drosophila* insulin receptor substrate protein. *Science* **292**, 104–106.
- Gems D, Riddle DL (2000) Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. *Genetics* **154**, 1597–1610.
- Johnson TE (1990) Increased life-span of *age-1* mutants in *Caenorhabditis elegans* and lower Gompertz rate of aging. *Science* **249**, 908–912.
- Kenyon C (2001) A conserved regulatory system for aging. *Cell* **105**, 165–168.
- Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**, 461–464.
- Pletcher S (1999) Model fitting and hypothesis testing for age-specific mortality data. *J. Evol. Biol.* **12**, 430–440.
- Promislow DEL, Tatar M, Khazaeli AA, Curtsinger JW (1996) Age-specific patterns of genetic variance in *Drosophila melanogaster*. I. Mortality. *Genetics* **143**, 839–848.
- Sacher GA (1977) Life table modification and life prolongation. In: *Handbook of the Biology of Aging* (Finch CE, Hayflick L, eds). New York: Van Nostrand Reinhold Co, pp. 582–638.
- Stocker H, Hafen E (2000) Genetic control of cell size. *Current Opinion Genet. Dev.* **10**, 529–535.
- Tatar M, Kopelman A, Epstein D, Tu M-P, Yin C-M, Garofalo RS (2001) A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107–110.
- Vaupel JW (1986) How change in age-specific mortality affect life expectancy. *Population Studies* **40**, 147–157.
- Vaupel J, Carey J, Christensen K, Johnson T, Yashin AI, Holm NV, *et al.* (1998) Biodemographic trajectories of longevity. *Science* **280**, 855–860.
- Vaupel JW, Manton KG, Stallard E (1979) The impact of heterogeneity in individual frailty on the dynamics of mortality. *Demography* **16**, 439–454.
- Vaupel JW, Yashin AI (1985) Heterogeneity's ruses: some surprising effects of selection on population dynamics. *Am. Statistician* **39**, 177–185.