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Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect

C. W. FOX*, M. E. CZESAK† & W. G. WALLIN*

*Department of Entomology, Agricultural Science Center North, University of Kentucky, Lexington, KY, USA
†Department of Biology, Vassar College, Poughkeepsie, NY, USA

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Abstract

Evolutionary responses to selection can be complicated when there is substantial nonadditivity, which limits our ability to extrapolate from simple models of selection to population differentiation and speciation. Studies of *Drosophila melanogaster* indicate that lifespan and the rate of senescence are influenced by many genes that have environment- and sex-specific effects. These studies also demonstrate that interactions among alleles (dominance) and loci (epistasis) are common, with the degree of interaction differing between the sexes and among environments. However, little is known about the genetic architecture of lifespan or mortality rates for organisms other than *D. melanogaster*. We studied genetic architecture of differences in lifespan and shapes of mortality curves between two populations of the seed beetle, *Callosobruchus maculatus* (South India and Burkina Faso populations). These two populations differ in various traits (such as body size and adult lifespan) that have likely evolved via host-specific selection. We found that the genetic architecture of lifespan differences between populations differs substantially between males and females; there was a large maternal effect on male lifespan (but not on female lifespan), and substantial dominance of long-life alleles in females (but not males). The large maternal effect in males was genetically based (there was no significant cytoplasmic effect) likely due to population differences in maternal effects genes that influence lifespan of progeny. Rearing host did not affect the genetic architecture of lifespan, and there was no evidence that genes on the Y-chromosome influence the population differences in lifespan. Epistatic interactions among loci were detectable for the mortality rate of both males and females, but were detectable for lifespan only after controlling for body size variation among lines. The detection of epistasis, dominance, and sex-specific genetic effects on *C. maculatus* lifespan is consistent with results from line cross and quantitative trait locus studies of *D. melanogaster*.

Introduction

Much of the focus of quantitative genetic studies of life history evolution has been on additive genetic variation in life history traits and genetic trade-offs between traits (measured as additive genetic correlations) as a constraint on the independent evolution of those traits. Nonadditivity, which includes dominance (interactions among alleles at a single locus) and epistasis (interactions among loci), greatly complicates quantitative genetic theory and is thus generally assumed to be absent (Roff, 1997). However, the presence of substantial nonadditivity can reduce our ability to extrapolate from simple

Correspondence: Charles W. Fox, Department of Entomology, S-225 Agricultural Science Center North, University of Kentucky, Lexington, KY 40546-0091, USA.
Tel.: 859 257 7474; fax: 859 323 1120; e-mail: cfox@uky.edu

models of selection to population differentiation and speciation (Johnson, 2000; Wolf *et al.*, 2000; Wade, 2002). For example, nonadditivity can cause the additive effects of alleles to change as the genetic composition of the population changes (i.e. the genetic variance–covariance matrices change in response to selection) and, as a result, the alleles that are favoured by selection, and the genetic relationships between traits, can change as the genetic background evolves (Goodnight, 2000; Barton & Keightley, 2002). Epistasis also allows the co-evolution of groups of alleles (at different loci), creating co-adapted gene complexes. Disruption of these gene complexes can lead to outbreeding depression. Thus, understanding evolutionary responses to selection and the eventual fate of non-neutral alleles requires an understanding of the importance of nonadditive genetic effects, but the relative contribution of dominance and epistasis to variation has not been well studied.

The evolution of lifespan, mortality rates and patterns of senescence is of substantial interest both because there is tremendous variation in these traits at all taxonomic levels (e.g. Promislow, 1991; Tatar, 2001) and because of the medical implications of genetic analyses of these traits. Studies on mice, *Drosophila* and *C. elegans* have identified numerous genes that influence lifespan and/or rates of senescence (Harshman, 2002). Recent quantitative trait locus (QTL) studies of lifespan in *D. melanogaster* indicate that both dominance and epistasis may have significant effects on variation in lifespan (reviews in Harshman, 2002; Mackay, 2002; Spencer *et al.*, 2003). These studies also show that the genetic architecture (number of genes and degree of allelic and genic interactions) differ between the sexes and depend on the environmental conditions in which individuals are reared. However, little is known about the genetic architecture of lifespan and senescence for organisms other than *D. melanogaster* and *C. elegans*.

Here we report on line cross analyses examining the relative contribution of additive genetic, nonadditive genetic, and maternal effects to differences in lifespan between two populations of the seed beetle, *Callosobruchus maculatus*. These two populations differ in a large number of traits including body size, adult lifespan, larval competitiveness, oviposition behaviour, and amount of paternal investment (e.g. Savalli *et al.*, 2000; Fox *et al.*, 2004a), many of which have arisen due to differences in the properties of their host species (seeds, Messina & Karren, 2003). Previous line cross analyses have demonstrated significant nonadditive effects on population differences in *C. maculatus* oviposition behaviour and that the genetic architecture of population differences depend on both rearing and oviposition environments (Fox *et al.*, 2004b). Because previous QTL studies with *D. melanogaster* have shown that the loci affecting lifespan differ between males and females and vary among rearing environments (Nuzhdin *et al.*, 1997; Vieira *et al.*, 2000; Harshman, 2002; Mackay, 2002; Spencer *et al.*, 2003) we examine how the genetic architecture of lifespan in *C. maculatus* differs between males and females and between environments. We show that, like *D. melanogaster*, the genetic architecture of lifespan differences between populations differs substantially between males and females; there was a large maternal effect in males but not females, and substantial dominance of long-life alleles in females but not males. However, unlike *D. melanogaster*, we did not detect effects of epistasis on the lifespan of hybrid beetles (although epistatic interactions did affect the mortality rate of hybrids), nor did we detect an effect of rearing host on the genetic architecture of population differences in lifespan.

Methods

Natural history and study population

Callosobruchus maculatus (F.) is a cosmopolitan pest of stored legumes (Fabaceae), particularly beans of the genus *Vigna*. Larval development and pupation are completed entirely within a single seed of their host species. Larvae require no other sources of food or water, making large-scale experiments practical. Females mate and begin to lay eggs within hours of emerging from the seed in which they developed. Adults are facultatively aphagous, i.e. they require neither food nor water in the adult stage. The absence of adult food is typical for *C. maculatus* in nature; the species has evolved to use dry seeds, most recently having evolved in a storage environment (as an agricultural pest), and is able to mate and complete reproduction using only metabolic water and the resources acquired during larval development (i.e. they are capital breeders; Messina & Slade, 1997). Access to adult resources does improve adult lifespan (e.g. Tatar & Carey, 1995) but adults have no access to food or water in a storage environment (they cannot feed externally on seeds) and there is little evidence that they feed as adults outside of a storage environment.

Life span and mortality rates of *C. maculatus* have been examined in numerous previous studies (Møller *et al.*, 1989; Tatar *et al.*, 1993; Tatar & Carey, 1994a, b, 1995; Fox *et al.*, 2003a, b, 2004a).

We examined the inheritance of differences in adult lifespan between two populations that had been collected from, and subsequently maintained on, different legume hosts. The South India (SI) population was collected in 1979 from infested pods of mung bean, *Vigna radiata* (L.) Wilczek, and the closely related black gram, *V. mungo* (L.) Hepper, in Tirunelveli, India (Mitchell, 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of cowpea, *V. unguiculata* (L.) Walp., in Ouagadougou, Burkina Faso (Messina, 1993). These two populations differ in body size, lifetime fecundity, patterns of egg dispersion, oviposition preference, and

adult longevity. Both populations were maintained in laboratory growth chambers on seeds of *V. radiata* (SI) or *V. unguiculata* (BF) at >1000 adults per generation for >100 generations (BF) or >200 generations (SI) prior to this experiment.

Experimental design

The SI and BF beetles were mated to produce F_1, F_2 and backcross progeny. The crosses were created over three generations so that all beetles were scored for their lifespan simultaneously. All matings were performed reciprocally. For example, F_1 offspring were obtained from both SI ♀ × BF ♂ and BF ♀ × SI ♂ crosses (these are designated F_1 and F_{1R}, respectively). In total we created 14 crosses; purebreds (SI ♀ × SI ♂ and BF ♀ × BF ♂), two F_1 crosses (F_1 and F_{1R}), two F_2 crosses (F_1♀ × F_1 ♂, F_{1R} ♀ × F_{1R} ♂), four SI backcrosses (SI ♀ × F_1 ♂, SI ♀ × F_{1R} ♂, F_1 ♀ × SI ♂, F_{1R} ♀ × SI ♂) and four BF backcrosses (BF ♀ × F_1 ♂, BF ♀ × F_{1R} ♂, F_1 ♀ × BF ♂, F_{1R} ♀ × BF ♂). The reciprocal crosses allowed us to test for the presence of maternal genetic effects and cytoplasmic effects on line cross mean values.

Because the two populations of beetles are adapted to different host species, we established two independent sets of crosses. In one set all larvae were raised on mung (*V. radiata*), the host of the SI population, throughout the three generations of crosses. In the second set of crosses, all larvae were raised on cowpea (*V. unguiculata*), the host of the BF population. This allowed us to test for effects of rearing hosts on the genetic architecture of lifespan.

All crosses were set up with a minimum of 120 pairs created with unmated beetles that emerged from isolated seeds. Matings were performed in 35-mm Petri dishes containing ≈20 cowpea seeds or ≈35 mung seeds. Females were allowed to oviposit, with males present, for 48 h, after which the parents were discarded. Four larvae per family were raised to the adult stage at one egg per seed (excess eggs were scraped off), at 25 °C, 15 : 9 light : dark, in a single reach-in growth chamber. The positions of the dishes in the growth chamber were rotated daily.

Emerging beetles were collected twice daily, at 12 h intervals, and weighed. All adults were confined individually in a sterile 35-mm Petri dish, at 25 °C, L : D 15 : 9, until death. Beetles were scored twice daily for whether they had died.

In total, adult lifespan was scored for 2984 females and 3191 males (1457 females reared on cowpea, 1527 females reared on mung; 1557 males reared on cowpea, 1634 males reared on mung).

Mortality rates

To describe the shape of the mortality curves we estimated the parameter values of a logistic mortality model of the form:

$$u(t) = \frac{a\mathrm{e}^{bt}}{\left[1 + \left(\frac{as}{b}\right)(\mathrm{e}^{bt} - 1)\right]},$$

where a is the intercept of the relationship $u(t) = a\mathrm{e}^{bt}$ (often referred to as the initial or baseline mortality rate), b is the rate of exponential increase in mortality at young ages, and s describes the degree of deceleration in mortality with increasing age (Vaupel, 1990; Pletcher, 1999a). This is similar to a Gompertz mortality model except that it incorporates a term (s) to account for the slowing of the increase of mortality rate with age (Pletcher *et al.*, 2000); when $s = 0$ the logistic model reduces to the Gompertz model. Parameters were estimated using the maximum likelihood estimation procedure of WinModest (Pletcher, 1999b). A Cox proportional hazards model was used to test for overall differences in $u(t)$ (Allison, 1995; Parmar & Machin, 1995). We used the log-likelihood ratio test of WinModest (Pletcher, 1999b) to test whether individual parameter estimates (a, b and s) differed significantly between males and females and between the two rearing environments.

Genetic analyses

All mean values were averaged across beetles within a family and then across families within a cross to control for nonindependence of sisters. We estimated the composite genetic effects explaining the differences between the line mean values as described in Lynch & Walsh (1998, chapter 9). We used the genetic model of Kearsey & Pooni (1996), which has parameterization as described in Table 1 of Gilchrist & Partridge (1999) and uses the expected mean of F_∞ offspring as a point of reference. The parameterization of this model differs only slightly from that described by Lynch & Walsh (1998), who use the expected phenotype of F_2 offspring as a point of reference, although the two models can be easily translated to alternate parameterizations (Basford & De Lacy, 1979).

We tested for goodness of fit to genetic models using the weighted error sums of squares (Lynch & Walsh, 1998; Bradshaw & Holzapfel, 2000). The weighted residual sums of squares is:

$$\mathrm{RSS_w} = \sum_{i=1}^{k} \frac{e_i^2}{\mathrm{SE}_i^2},$$

where k is the number of crosses, e_i is the difference between the observed and predicted composite genetic effects, and SE_i are the standard errors of the estimated composite genetic effects (Lynch & Walsh, 1998; Bieri & Kawecki, 2003). For normally distributed data $\mathrm{RSS_w}$ is chi-square distributed with degrees of freedom equal to the number of lines minus the number of parameters in the model. A significant χ^2 indicates that the fitted model was inadequate to explain the observed line cross mean values.

Table 1 Parameter values for the logistic mortality model, $u(t) = a^{bt}/[1 + (as/b)(e^{bt}-1)]$.

	Parameter estimates (95% CI)		
	a	*b*	*s*
SI population			
Female offspring			
Cowpea	0.00116 (0.00056–0.00241)	0.221 (0.183–0.269)	0.386 (0.168–0.884)
Mung	0.00068 (0.00032–0.00144)	0.235 (0.197–0.280)	0.590 (0.335–1.040)
Male offspring			
Cowpea	0.00108 (0.00036–0.00327)	0.373 (0.276–0.504)	1.426 (0.783–2.597)
Mung	0.00024 (0.00007–0.00084)	0.447 (0.354–0.563)	1.434 (0.901–2.282)
BF population			
Female offspring			
Cowpea	0.00235 (0.00016–0.3421)	0.223 (0.133–0.373)	0.000 (0.000–0.000)
Mung	0.00104 (0.00044–0.00243)	0.276 (0.222–0.341)	0.444 (0.191–1.034)
Male offspring			
Cowpea	0.00005 (0.00001–0.00021)	0.902 (0.756–1.077)	1.739 (1.285–2.354)
Mung	0.00020 (0.00006–0.00066)	0.637 (0.522–0.776)	1.427 (0.969–2.101)

a is the initial mortality rate (intercept), *b* is the rate of exponential increase in mortality at young ages (slope), and *s* describes the degree of deceleration in mortality with increasing age.

Because we are interested in nine different parameters [additive (α), dominance (δ), additive × additive epistasis (α^2), additive × dominance epistasis ($\alpha\delta$), dominance × dominance epistasis (δ^2), an additive genetic maternal effect (am), a dominance genetic maternal effect (dm), a cytoplasmic effect (c), and a Y chromosome effect (Y)] there are $2^9 = 512$ possible models. Traditional joint-scaling techniques avoid the need to compare all models by adding parameters sequentially starting with additivity, then adding dominance, then epistasis, etc., until the line mean values predicted by the model no longer differ from the observed line mean values (based on the comparison of RSS_w to a chi-square distribution, as described above; Mather & Jinks, 1982; Bradshaw & Holzapfel, 2000). However, the order in which terms are introduced into the model affects the ability to detect significant terms added later and this technique does not always produce the most parsimonious model. We used Akaike's Information Criterion (AIC) to find the most parsimonious model (following Bieri & Kawecki, 2003; see Burnham & Anderson, 1998). This technique chooses a model that is the best compromise between the amount of variance explained and the number of parameters in the model. The technique is explained thoroughly by Bieri & Kawecki (2003) and only briefly summarized here. The model with the lowest AIC is the most parsimonious, where $AIC = -2 \ln(L) + 2K$, in which L is the log-likelihood of the model given the data and K is the number of parameters fitted in the model. Bieri & Kawecki (2003) show that $AIC = RSS_w + 2K +$ constant. The constant is the same for all models and thus need not be calculated to compare different genetic models.

Because the number of possible models is so large (512), we first reduced the number of candidate models by pooling both the three forms of digenic epistasis into E ($\alpha^2 + \alpha\delta + \delta^2$) and the two forms of maternal effects into M ($m_\alpha + m_\delta$). This left us with 64 possible models including all combinations of additivity (α), dominance (δ), epistasis ($\alpha^2 + \alpha\delta + \delta^2$), maternal effects ($m_\alpha + m_\delta$), cytoplasmic effects ($c$), and a Y-chromosome effect. We chose the model with the lowest AIC as the most parsimonious. Only if the most parsimonious model included E did we expand our model into all possible models including the three forms of digenic epistasis. Likewise, only if the most parsimonious model included M did we expand our model into all possible models including the two forms of maternal effects. For example, if the additive–epistasis model was most parsimonious, we expanded this into the following seven models, $\mu_0 + \alpha + \alpha^2$, $\mu_0 + \alpha + \alpha\delta$, $\mu_0 + \alpha + \delta^2$, $\mu_0 + \alpha + \alpha^2 + \alpha\delta$, $\mu_0 + \alpha + \alpha^2 + \delta^2$, $\mu_0 + \alpha + \alpha\delta + \delta^2$, and $\mu_0 + \alpha + \alpha^2 + \alpha\delta + \delta^2$, and again chose the model with the lowest AIC as our most parsimonious. None of the most parsimonious models included a significant cytoplasmic or Y-chromosome effect, so we reduced the 14 crosses into nine crosses by pooling the two F_2 crosses (F_1♀ × F_1 ♂, F_{1R} ♀ × F_{1R} ♂) into a single F_2, SI ♀ × F_1 ♂ and SI ♀ × F_{1R} ♂ into BC_1 (backcross$_1$), F_1 ♀ × SI ♂ and F_{1R} ♀ × SI ♂ into BC_{1R}, BF ♀ × F_1 ♂ and BF ♀ × F_{1R} ♂ into BC_2 and F_1 ♀ × BF ♂ and F_{1R} ♀ × BF ♂ into BC_{2R}. This provided better estimates of the line mean values for use in the reduced models (those lacking cytoplasmic and Y-chromosome effects).

Because it is possible that the most parsimonious model will include parameters that contribute little, we tested whether the removal of individual terms significantly reduced the fit of the model to the observed line mean values using a likelihood ratio test (Lynch & Walsh, 1998). The degree of reduced fit of the model is estimated

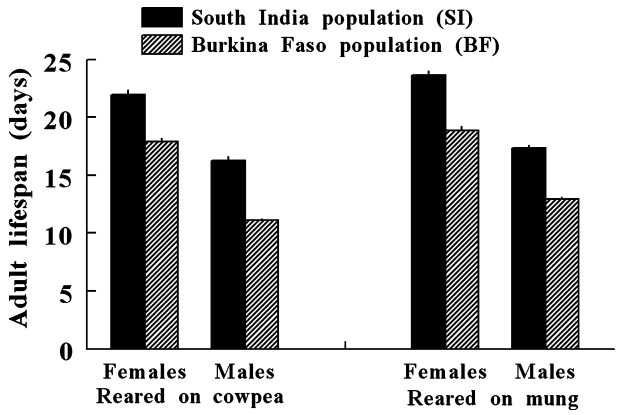

Fig. 1 The mean adult lifespan (±SEM) of SI and BF beetles reared from mung and cowpea seeds.

as $\Lambda = \mathrm{RSS}_{\mathrm{w(reduced\ model)}} - \mathrm{RSS}_{\mathrm{w(full\ model)}}$. The parameter Λ is chi-square distributed at large sample sizes, with degrees of freedom equal to the difference in the number of parameters in the two models.

To compare composite genetic effects between treatments and sexes, we calculated the Wald chi-square statistic in which $\chi^2 = (b_1 - b_2)^2/[\mathrm{SE}(b_1)^2 + \mathrm{SE}(b_2)^2]$, where b_1 and b_2 are the composite genetic effects in each environment or sex and $\mathrm{SE}(b_1)$ and $\mathrm{SE}(b_2)$ are the standard errors of those composite genetic effects (Allison, 1995). The sum of the Wald χ^2 provides a test of whether two models are different; the sum is χ^2 distributed with k degrees of freedom, where k is the number of parameters in each model. Because the parameter estimates are sensitive to which parameters are included in the model, we compared models with the same parameterization, e.g. if a parameter was significantly different from 0 in only one of the two models to be compared, it was nonetheless included in both models for the purpose of hypothesis testing. In addition, because mean lifespan differed between hosts and sexes, we did not include the overall mean when calculating the Wald χ^2.

Results

Descriptive patterns – lifespan and mortality curves of the parental populations

The SI beetles lived longer than BF beetles by over 4 days in all sex–host combinations (Figs 1 and 2a,b; analysis of variance, $F > 201$, $P < 0.001$; for both sexes) and females outlived males by over 5 days in all population–host combinations (Figs 1 and 2a,b; $F > 239$, $P < 0.001$ for both populations). Although the effect was small, beetles reared from mung seeds lived longer than beetles reared from cowpea seeds (by 1–2 days for both populations and sexes; Fig. 1; $F > 4.5$, $P < 0.05$ for all host–sex combinations). All of these effects are consistent with previous results for these two populations of *C. maculatus* (Fox *et al.*, 2004a).

The SI beetles were significantly larger than BF beetles regardless of sex or rearing host ($F > 741$, $P < 0.001$) and in all population–host–sex combinations adult lifespan was positively correlated with adult body mass (R^2 between 0.04 and 0.20; $P < 0.010$ for each).

The mortality curves (hazard functions) for males and females were nonproportional within both populations, indicating that the mortality curves were not simply shifted between the sexes but that they actually differ in shape (Fig. 2c,d; differ in slope or deceleration; Cox proportional Hazards, sex × lifespan interaction, $\chi^2_1 > 7.8$, $P < 0.010$ for both populations). For the SI population, males had both a faster rate of increase in mortality (b) and greater deceleration (s), but the baseline mortality rate (a) did not differ between the sexes ($\chi^2_1 > 6.0$,

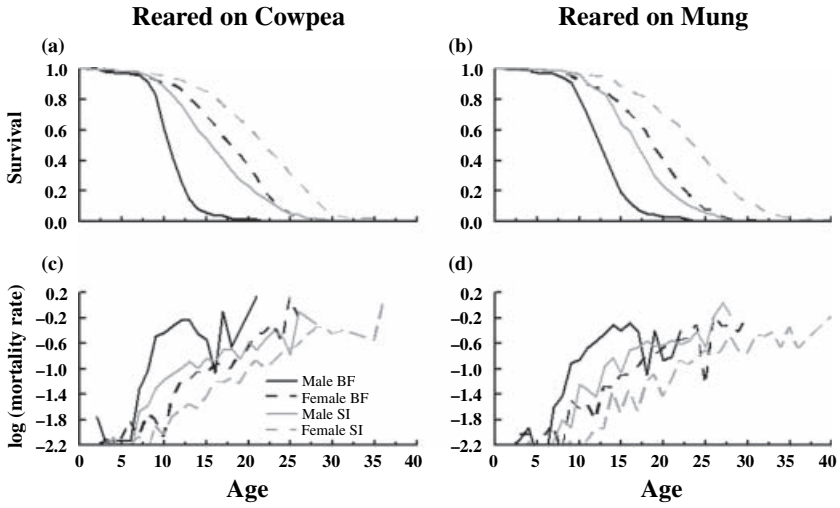

Fig. 2 Survivorship (a, b) and log-transformed mortality curve (c, d; log $[u(t)]$) for male and female *Callosobruchus maculatus* from the SI and BF populations.

$P < 0.05$ for b and s; $\chi^2_1 > 2.3$, n.s. for b). For the BF population, all three parameters of the logistic mortality curve differed between the sexes; males had a lower baseline mortality rate (a), but had a higher rate of increase in the mortality rate (b) and more rapid deceleration (s) than did females (Table 1; log-likelihood ratio test performed using WinModest, Pletcher, 1999b; $\chi^2_1 > 5.4$, $P < 0.05$ for each parameter and both rearing hosts).

For all sex–host combinations except females reared on cowpea, the slope of the mortality curve (b) was significantly greater for BF beetles than for SI beetles (Table 1; $\chi^2_1 > 5.4$, $P < 0.05$). For females reared on cowpea the only detectable difference in the mortality curves was in the rate of deceleration (s), although SI females outlived BF beetles by about 4 days. The intercept (a) differed significantly between populations for males reared on cowpea (a was lower for BF males, $\chi^2 = 10.7$, $P < 0.01$) but not for any of the other between population comparisons ($\chi^2 < 2.5$, n.s. for each).

Inheritance of line differences in lifespan

The additive model was not adequate to explain the distribution of hybrid mean lifespan for either sex reared on either host (Table 2; Fig. 3). Interestingly, which nonadditive effects were most important differed substantially between males and females (Wald $\chi^2_4 > 58$, $P < 0.001$). In females, long lifespan alleles were generally dominant over short lifespan alleles – lifespan of hybrid female offspring resembled the lifespan of SI females. However, dominance was detected in only one of the two analyses for male lifespan (males reared from cowpea) and the composite genetic effect for dominance was smaller in both groups of males than in either group of females (Wald $\chi^2_1 > 11.3$, $P < 0.0001$).

In contrast, the lifespan of male *C. maculatus* was influenced by a large maternal effect – male offspring whose mother was an SI (for the F_1 and BC_{SI}) or whose mother was an F_1 (for the BC_{BF} cross) had significantly longer lifespan than the reciprocal of these same crosses (detected as a highly significant value of m_α in males; confirmed using ANOVAS for each pair of crosses, F_1 vs. F_{1R}, etc.). This is evident in Fig. 3c,d – points above the line all have mothers that have more SI genes than do their fathers. In contrast, the AIC most parsimonious model for female lifespan did not include m_α (Table 2; Fig. 3a,b), and the Wald chi-square test indicated that m_α was significantly greater for males than for females ($\chi^2_1 > 16.5$, $P < 0.0001$). The large maternal effect observed in males is not due to the inheritance of mitochondria or other cytoplasmic factors; the cytoplasmic effect (c) was not present in any of the AIC most parsimonious models. In addition, inclusion of a cyto-

	Females reared on		Males reared on	
	Cowpea	Mung	Cowpea	Mung
Lifespan				
μ_0	20.3 ± 0.3	21.1 ± 0.3	13.6 ± 0.2	15.1 ± 0.1
α_1	−2.4 ± 0.3	−2.5 ± 0.3	0.8 ± 0.3	0.7 ± 0.3
δ_1	3.4 ± 0.6	2.7 ± 0.5	0.9 ± 0.4	–
α^2	–	–	–	–
$\alpha\delta$	–	–	–	–
δ^2	–	–	–	–
m_α	–	–	1.5 ± 0.3	1.4 ± 0.2
m_δ	–	–	−0.6 ± 0.3	–
χ^2	10.2	5.7	10.6	3.9
Slope of the mortality rate, b				
μ_0	0.16 ± 0.03	0.25 ± 0.02	0.89 ± 0.12	0.67 ± 0.04
α_1	–	0.05 ± 0.03	−0.24 ± 0.05	–
δ_1	–	–	–	–
α^2	0.05 ± 0.03#	–	−0.26 ± 0.13	−0.11 ± 0.06
$\alpha\delta$	–	−0.16 ± 0.08	–	−0.40 ± 0.26#
δ^2	0.13 ± 0.05	–	−0.30 ± 0.16#	–
m_α	–	–	–	−0.10 ± 0.03
m_δ	0.07 ± 0.03	–	–	–
χ^2	1.0	6.8	4.6	3.9

Model parameters: μ_0, overall mean; α_1, additive; δ_1, dominance; α^2, additive–additive epistasis; $\alpha\delta$, additive–dominance epistasis; δ^2, dominance–dominance epistasis; m_α, the additive genetic maternal effect; m_δ, the dominance genetic maternal effect; χ^2, goodness of fit of the model to the real data – a lower chi-square statistic indicates a better fit of the model to the data.

Table 2 Composite genetic effects contributing to differences in lifespan and the slope of the mortality curve (b) between *Callosobruchus maculatus* populations (South India and Burkina Faso). The values represent the composite genetic effects from the Akaike's Information Criterion (AIC) most parsimonious genetic model. # Indicates a term present in the AIC most parsimonious model but which, when dropped from the model, does not significantly reduce the fit of the model to the data (see Methods for details).

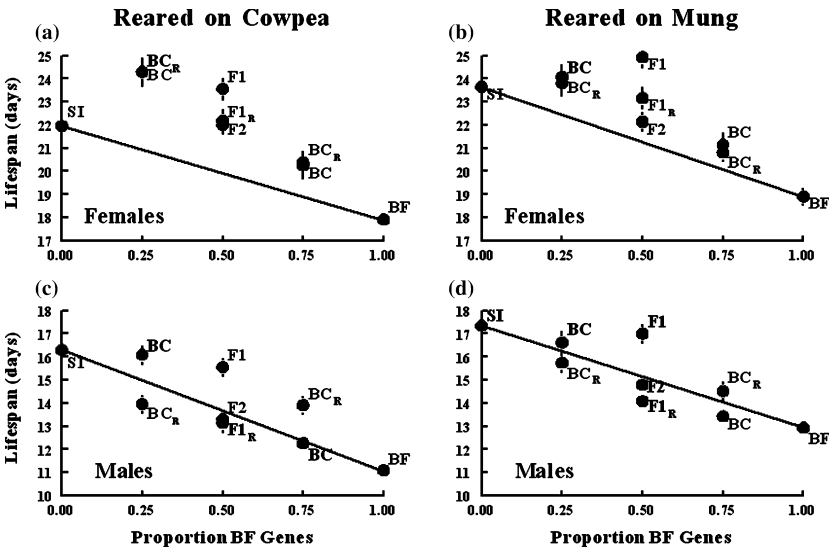

Fig. 3 Adult lifespan (±SEM) of SI, BF and hybrid beetles reared from cowpea or mung seeds. Reciprocal crosses are designated with an R. The line indicates where hybrid mean values should fall if inheritance of the population difference in lifespan is purely additive.

plasmic effect to the most parsimonious model did not significantly improve the fit of the model and did not make any of the previously significant additive–genetic maternal effects (m_α) nonsignificant.

Contrary to previous results for *C. maculatus* oviposition behaviour, the composite genetic effects for lifespan did not differ between the two rearing hosts for either females or males ($\chi^2_4 < 4.5$, n.s.). We also had no evidence of a Y-chromosome effect on the lifespan of males.

We tested whether body size variation among the cross types was adequate to explain the variation in adult lifespan among cross types. Including body mass into the ANOVA as a covariate substantially reduced the mean square for the cross effect ($F_{9,9} = 132$, $P < 0.001$) indicating that much of the variation in lifespan was due to variation in body size among the crosses. However, the cross effect was still significant ($F_{9,5513} = 1.91$, $P < 0.05$) indicating that body mass alone was not adequate to explain all of the variation among lines. To examine whether the variance in body size was adequate to explain the observed dominance in females or the observed maternal effect in males, we calculated the least-square mean values (mean lifespan calculated controlling for body size) using SAS PROC GLM and used these for the genetic analysis. The maternal effect (m_α) remained significant for male lifespan and nonsignificant for female lifespan, and the magnitude of m_α still differed significantly between males and females. Interestingly, the dominance observed in the lifespan of hybrid females was converted to significant epistasis after correcting for body size; dominance was not significant in any AIC most parsimonious model, but epistatic interactions became significant for both sets of female crosses (both α^2 and $\alpha\delta$ were significant for females raised on cowpea, and α^2 and $\delta\delta$ were significant for females reared on mung).

Hybrid beetles had higher rates of increase in their mortality rates (*b*, the slope of the mortality curves in Fig. 2c,d) than expected under a strict additive model ($\mu_0 + \alpha_1$). This is visible in Fig. 4, in which the estimates of *b* for hybrid beetles are above the line of expectation for the additive model for 21 of 28 hybrid crosses (sign test, $P < 0.01$). This initially appears inconsistent with the dominance of long lifespan alleles discussed above (Fig. 3). However, this apparent inconsistency is due to a substantial reduction in baseline mortality rates (*a*, the intercept of the mortality curve) in hybrids; for all except one hybrid cross, the intercept of the mortality curve (*a*) was below the line of expectation for the additive model.

The AIC most parsimonious model for *b* included epistasis for all sets of crosses (both sexes and both rearing hosts) although which form of epistasis was included in the most parsimonious model differed between the four sets of crosses (Table 2). As we observed for lifespan, the genetic architecture of the rate of increase in mortality (*b*) differed between males and females ($\chi^2_7 = 31.3$, $P < 0.001$ for beetles raised on mung, $\chi^2_7 = 13.4$, $P = 0.06$ for beetles raised on cowpea) but did not differ between rearing hosts for either sex ($\chi^2_7 > 12.3$, $P = 0.09$ for both sexes).

Discussion

The genetic architecture of line differences in lifespan differed substantially between males and females of *C. maculatus*. Alleles that increased lifespan of SI females were dominant over the alleles for shorter lifespan of BF females, but there was significantly less dominance detected for alleles affecting male lifespan. In contrast, male lifespan was affected by a large and highly significant maternal effect that was not observed for female

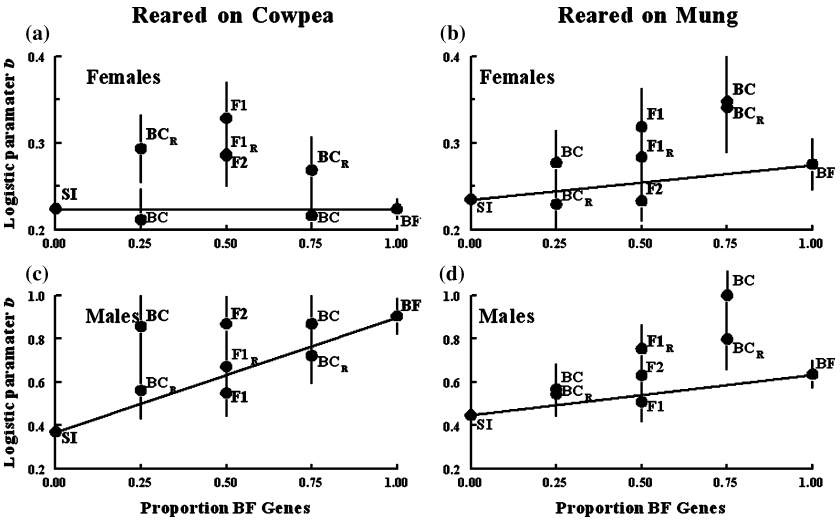

Fig. 4 Slope of the mortality rate (±95% confidence intervals) of SI, BF and hybrid beetles reared from cowpea or mung seeds. Reciprocal crosses are designated with an R. The line indicates where hybrid mean values should fall if inheritance of the population difference is purely additive.

lifespan. Unlike *D. melanogaster*, we did not detect any effects of epistasis on the lifespan of hybrid beetles, nor did we detect an effect of rearing host on the genetic architecture of population differences in lifespan. However, some of the variation in lifespan among lines was explainable by differences in body size and, after correcting for body size variation, epistasis was detectable for the lifespan of females.

The influence of alleles on phenotypes depends on the environment and sex in which those alleles are expressed (Falconer, 1989). QTL studies have identified at least 17 loci that influence lifespan differences between lines of *Drosophila* (reviews in Leips & Mackay, 2000, 2002; Harshman, 2002; Mackay, 2002). The effects of these QTLs are generally both sex and environment specific (Nuzhdin *et al.*, 1997; Vieira *et al.*, 2000; Harshman, 2002; Mackay, 2002; Spencer *et al.*, 2003). Such sex and environmental effects on genetic architecture are of substantial importance when examining traits that have evolved due to environment- or sex-specific selection. In *C. maculatus*, the genetic correlation between the lifespan of males and lifespan of females is much less than 1.0 (e.g. Fox *et al.*, 2004a) indicating that either different genes affect the lifespan of males and females or that these genes have different effects in the two sexes. It is thus not surprising that the genetic architecture of population differences in lifespan of *C. maculatus* differs between males and females.

However, we found no evidence that genetic architecture differed between rearing environments, despite differences in mean lifespan between rearing environments (cowpea vs. mung). The two populations of *C. maculatus* studied here have evolved on different host species and many of the population differences are likely due to host-associated differences in selection. One previous study with these two populations found that changing the rearing host changed both whether epistasis was detectable and the magnitude of the different composite genetic effects for egg dispersion (distribution of eggs among oviposition sites; Fox *et al.*, 2004b). We are currently examining the influence of rearing and oviposition host of the genetic architecture of population differences in body size, fecundity and egg size for these same two populations of *C. maculatus*.

The large maternal effect observed for male lifespan was unexpected. Maternal effects have been observed for a variety of traits in *C. maculatus* that are expressed early in larval development and thus expected to be heavily influenced by egg size or maternal proteins and mRNAs packaged into eggs (e.g. development time and egg-to-adult survival; Fox, 1993, 1994; Mousseau & Fox, 1998). However, the effects of egg size and maternally derived substances tend to decline rapidly as offspring age and are rarely detectable by the time offspring reach maturity (Bernardo, 1996; Fox & Savalli, 1998; Heath *et al.*, 1999; and references therein). The exceptions are typically maternal effects that stimulate large developmental shifts in offspring, e.g. in many insect species females control offspring diapause, flight morph of their offspring, and sexual vs. asexual reproduction in offspring (review in Fox & Mousseau, 1998). Yet we detected a large maternal effect on male *C. maculatus* lifespan indicating that the maternal effect persisted until late in the beetle's life. A few previous studies have also detected maternal effects on adult traits, including offspring lifespan, both within populations (Lynch & Ennis, 1983; Kruuk *et al.*, 2000; Priest *et al.*, 2002) and in population crosses (Antolin, 1992; da Cunha & de Oliveira, 1996; but see Hutchinson & Rose, 1991). In fact, in a previous study of *C. maculatus* we observed a small effect of maternal age on lifespan; offspring of older mothers lived longer than did offspring of younger mothers (Fox *et al.*, 2003a) opposite the pattern commonly observed in other organisms (Priest *et al.*, 2002).

The mechanism of the maternal effect observed in both this and our previous study is unknown, but could indicate that the effects of maternal gene products persist through to the adult stage. It cannot be due to the inheritance of organelles or micro-organisms in the cytoplasm (Antolin, 1992; Korpelainen, 1999); both would be detected as a cytoplasmic effect (*c*) in our analysis (although mitochondria carry genes, and thus can cause genetically based maternal effects, they are inherited uniparentally and thus detected as part of the *c* in the genetic model), but *c* was not significant in any of the line crosses. Instead, the maternal effect was due to the expression of maternal nuclear genes (mothers with more SI genes produced male offspring that lived longer than male offspring produced by mothers containing more BF genes). Likewise, the maternal age effect observed for *C. maculatus* by Fox *et al.* (2003a) is unlikely due to the inheritance of organelles or micro-organisms because it occurs within families, all of which share the same mother, suggesting that some other maternal gene products or their effects must persist until offspring adulthood. A number of maternal effect genes (genes coding for mRNAs that are packaged into eggs) that affect lifespan in *Caenorhabditis elegans* have been identified (e.g. clock genes; Wong *et al.*, 1995; Lakowski & Hekimi, 1996). Although their mRNAs are packaged in the cytoplasm of the egg, these genes are nuclear and thus would be detected as maternal effects (m_α and m_δ) and not cytoplasmic effects (*c*). It is thus likely that some maternal effect genes have effects that persist through to the adult stage of *C. maculatus* with observable effects on lifespan.

Interestingly, the magnitude of the maternal effect observed here differed between males and females. This is consistent with the maternal age effect on lifespan observed in Fox *et al.* (2003a) in which the maternal effect on lifespan was larger for male offspring than for female offspring. Although these two different studies of maternal effects on *C. maculatus* lifespan are consistent, they are contrary to what we would have predicted *a priori*. We have previously used a half-sib breeding design to measure the genetic and environmental variance (V_A and V_E) in lifespan within both the SI and BF populations and found that V_E is substantially higher in females than in males (Fox *et al.*, 2004a). We thus expected that females would be more sensitive to all sources of environmental variance, including maternal effects which, although they are influenced by the maternal genotype (m_α), are environmental effects from the perspective of the offspring. However, our previous estimate of V_E was confounded with dominance (V_D) which this current study suggests may be quite substantial in females.

Previous studies examining the influence of specific loci on lifespan have found that effects depend on the genetic background in which the gene is expressed, indicating that epistatic interactions are important for genes affecting lifespan (Leips & Mackay, 2000; Mackay, 2002; Spencer *et al.*, 2003; but see Hutchinson *et al.*, 1991; Leips & Mackay, 2002). Although we detected no epistasis affecting the mean lifespan of hybrid beetles, epistasis became detectable when the influence of body size was statistically removed from the analysis. We also detected substantial dominance of long-life (SI) alleles over short-life (BF) alleles. Dominance has also been observed in QTL studies of *D. melanogaster* lifespans (Leips & Mackay, 2000; but see Hutchinson & Rose, 1991), including some QTLs exhibiting significant overdominance (e.g. Leips & Mackay, 2002) as observed here for female *C. maculatus*. Interestingly, as in *Drosophila*, the degree of dominance in *C. maculatus* was highly sex specific (Leips & Mackay, 2000, 2002) consistent with our result of substantial overdominance affecting hybrid female lifespan but little dominance detectable on hybrid male lifespan. Other studies (e.g. on pesticide resistance of *Culex* mosquitoes; Bourguet *et al.*, 1996) have shown that the degree of dominance affecting a trait can vary substantially across environmental conditions, but we observed no effect of rearing environment (cowpea vs. mung) on the degree of dominance for *C. maculatus* lifespan.

Hybrid beetles had higher rates of increase in their mortality rates (slope of the mortality curve, *b*, i.e. they senesced faster) than expected under a model of additive inheritance. This is due to nonadditive genetic effects on lifespan (both dominance and epistasis) and may reflect some degree of hybrid breakdown, such as breaking up co-adapted gene complexes. However, it is unclear how to interpret the composite genetic effects for *b* in Table 2. Composite genetic effects are measures of the relative contribution of the various kinds of genetic effects to the trait mean of a population of hybrid individuals mating randomly. However, our estimates of *b* are a property of the entire population of individuals in each cross and are not necessarily the mean of the slopes of all individuals in that cross. Even if we imagine that each individual has a mortality curve describing the probabilities of death at each age, it is unclear that the population-level curve, and thus the parameters of that curve, reflects the mean for the parameters of the curves for all individuals. We thus must be cautious when interpreting these results.

In summary, the genetic architecture of *C. maculatus* lifespan differed between males and females; a large maternal effect influenced male (but not female) lifespan whereas substantial overdominance was detectable for female (but not male) lifespan. The genetic architecture of lifespan was not influenced by rearing host. Interestingly, epistatic interactions among loci were detectable for lifespan only after controlling for variation in body mass, although significant epistasis was detected for the slope of mortality curves. These results are generally consistent with line cross and QTL studies of *D. melanogaster* suggesting that gene interactions (dominance and epistasis) and sex-specific genetic effects will be typical

in genetic studies of animal lifespans. However, we emphasize that we only studied the lifespan of virgin *C. maculatus*. Previous studies have found that patterns of mortality (e.g. Tatar *et al.*, 1993; Messina & Fry, 2003) and the genetic architecture of lifespan (e.g. Leips & Mackay, 2002) both change when individuals are mated, although many loci have similar effects on lifespan regardless of mating status (e.g. Reiwitch & Nuzhdin, 2002). Mating alters the physiology of insects (e.g. changing patterns of resource allocation) reducing the resources available for somatic maintenance and potentially increasing the susceptibility of individuals to external sources of mortality. We thus must be cautious when extrapolating from our results to the genetic architecture that would be observed for reproductively active females.

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