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Multivariate intralocus sexual conflict in seed beetles

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Intralocus sexual conflict (IaSC) is pervasive because males and females experience differences in selection but share much of the same genome. Traits with integrated genetic architecture should be reservoirs of sexually antagonistic genetic variation for fitness, but explorations of multivariate IaSC are scarce. Previously, we showed that upward artificial selection on male life span decreased male fitness but increased female fitness compared with downward selection in the seed beetle *Callosobruchus maculatus*. Here, we use these selection lines to investigate sex-specific evolution of four functionally integrated traits (metabolic rate, locomotor activity, body mass, and life span) that collectively define a sexually dimorphic life-history syndrome in many species. Male-limited selection for short life span led to correlated evolution in females toward a more male-like multivariate phenotype. Conversely, males selected for long life span became more female-like, implying that IaSC results from genetic integration of this suite of traits. However, while life span, metabolism, and body mass showed correlated evolution in the sexes, activity did not evolve in males but, surprisingly, did so in females. This led to sexual monomorphism in locomotor activity in short-life lines associated with detrimental effects in females. Our results thus support the general tenet that widespread pleiotropy generates IaSC despite sex-specific genetic architecture.

KEY WORDS: **Life history syndromes, metabolism, pleiotropy, rate of life, sexual dimorphism, sexually antagonistic.**

Selection often operates differently in males and females, resulting in sex-specific optima for many fundamental life-history traits (Trivers 1972; Wedell et al. 2006; Bonduriansky et al. 2008). Intralocus sexual conflict (IaSC) occurs when such sexually antagonistic (SA) selection acts on traits that have a shared genetic basis in males and females such that alternative alleles at a given locus have opposing fitness effects in the two sexes (Lande 1980; Rice 1984; Chippindale et al. 2001; Rice and Chippindale 2001; Pischedda and Chippindale 2006; Bonduriansky and Chenoweth 2009).

Theory predicts that IaSC may be partly resolved if loci under SA selection are translocated to the sex chromosomes, thus permitting sex-specific expression (Rice 1984) and the evolution of sexual dimorphism (Lande 1980). However, recent studies suggest that most SA loci reside on the autosomes and that modifiers associated with sex-determining loci may instead regulate the expression of many autosomal loci (Lemos et al. 2008; Fry 2010; Innocenti and Morrow 2010), which should lead to wide-ranging pleiotropy and epistasis of SA genes (Stewart et al. 2010; Innocenti and Chenoweth 2013; Perry et al. 2014). Pleiotropy and gene interactions may constrain the evolution of sexual dimorphism in individual traits (Walsh and Blows 2009; Conner 2012). For this reason correlated traits, such as suites of life-history characters, sharing common regulation through integrated

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physiological processes, should be hotspots for standing SA genetic variation (Mank et al. 2008; Bonduriansky and Chenoweth 2009; Harano et al. 2010).

Sexually dimorphic life histories are ubiquitous, but whether it is males or females that show the faster life history likely depends on idiosyncrasies related to the mating system and ecology of the organism (Maklakov and Lummaa 2013). Because life-history variation is caused by integrated physiological processes, sex-specific fine tuning of multiple correlated processes and functions is required for sexual dimorphism in reproductive schedules to evolve. For example, it has since long been realized that metabolism is associated with variation in many aspects of physiology, life history, and behavior (e.g., Pearl 1928; MacArthur and Baillie 1929; Kleiber 1947; more recently reviewed in Hochachka and Somero 2002; Brown et al. 2004; Biro and Stamps 2010; Dell et al. 2011). It is thus likely that pleiotropic effects of genes that regulate metabolic networks will amalgamate the evolution of sex differences in metabolism with those in individual behavioral and life-history traits, unless the multivariate genetic constraints are broken (Wagner and Altenberg 1996; Walsh and Blows 2009).

Sex differences in multivariate genetic architecture (the B-matrix; Lande 1980) of life-history characters are common (Barker et al. 2010; Wyman et al. 2013; Gosden and Chenoweth 2014). Although sex-specific genetic (co)variances are a prerequisite for IaSC resolution, expression of sexual dimorphism both at the gene transcript (Stewart et al. 2010; Griffin et al. 2013; Innocenti and Chenoweth 2013; Hollis et al. 2014; Perry et al. 2014) and phenotypic level (reviewed in Cox and Calsbeek 2009) has often only partly resolved IaSC, suggesting that multivariate genetic constraints may set fundamental limits to sex-specific adaptation. The few studies that have quantified IaSC over multiple functionally related traits support this claim, with pronounced IaSC despite observed sexual dimorphisms in the studied phenotypes (Prasad et al. 2007; Kwan et al. 2008; Abbott et al. 2010; Lewis et al. 2011; Gosden et al. 2012; but see Hunt et al. 2006; Bedhomme et al. 2011). Nevertheless, with the limited empirical data at hand we still lack a firm understanding of multivariate IaSC. Moreover, the data are heavily biased toward studies on *Drosophila* and is almost exclusively restricted to traits with narrow physiological span and close functional relatedness, such as size- and growth-related life-history components on the one hand (e.g., Prasad et al. 2007; Kwan et al. 2008; Abbott et al. 2010) or chemical signaling traits on the other (e.g., Bedhomme et al. 2011; Delcourt et al. 2012; Gosden et al. 2012).

Here, we aim to provide an assessment of the hypothesis that genetic constraints impede the resolution of IaSC over multiple correlated characters in seed beetles (Bruchidae), a model system for the study of sexual dimorphism in life-history traits (e.g., Fox et al. 2004, 2006, 2007; Maklakov et al. 2009; Bilde et al. 2009a; Fritzsche and Arnqvist 2013) and IaSC (e.g., Rankin and Arnqvist 2008; Bilde et al. 2009b; Arnqvist and Tuda 2010; Berg and Maklakov 2012; Berger et al. 2014). By studying the covariation between behavioral, physiological, and life-history traits, we suggest that a more complete understanding of IaSC over optimal reproductive schedules can be gained against the rich backdrop formed by previous research on sexual selection and life-history evolution in seed beetles. In a recent study, we applied artificial sex-limited selection on male life span over multiple generations in the seed beetle, *Callosobruchus maculatus*, to demonstrate IaSC: downward selection for short male life span increased male fitness but decreased female fitness relative to fitness in lines selected for long male life span (Berg and Maklakov 2012). To provide a more detailed understanding of the SA responses in lifetime reproductive success that resulted from this artificial selection, we here use these selection lines to study the correlated evolution of four traits (body mass, life span, metabolic rate, and locomotor activity) that collectively define the core of a sexually dimorphic life-history syndrome in many polygynous species where males start reproducing earlier and live shorter than females (e.g., Promislow and Harvey 1990; Fox et al. 2004, 2006; Wedell et al. 2006; Blanckenhorn et al. 2007, but see Promislow et al. 1992), an effect ascribed to males pursuing a "live-fast-die-young" strategy due to competition over access to females (Trivers 1972; Vinogradov 1998; Bonduriansky et al. 2008; Maklakov and Lummaa 2013).

As in most insects (Honek 1993; Blanckenhorn 2000; Berger et al. 2012), female body size is associated with high fecundity in *C. maculatus* (e.g., Fox 1993; Fox et al. 2007). In contrast, direct selection on body size in males is weak (Fritzsche and Arnqvist 2013). There is no detectable large male advantage either in premating (Savalli and Fox 1999) or postmating (Eady 1994) sexual selection and females are the larger sex (Fox et al. 2007). The direction of sex-specific selection on locomotor activity is reversed in seed beetles and males are more active than females in *C. maculatus* (e.g., Gay et al. 2009). Species in the genus *Callosobruchus* show scramble competition polygyny (Fritzsche and Arnqvist 2013) and active males gain more matings (e.g., Nakayama and Miyatake 2010a,b). In contrast, elevated activity in females expends energy without obvious reproductive benefits. Finally, indirect selection for high metabolic rate in seed beetle males is likely reinforced both from the fact that early sexual maturation is beneficial to males, because females are most fecund and receptive to mating when young and virgin (Pushpinder 1986; Arnqvist and Tuda 2010), and from the production and renewal of sperm and seminal fluid proteins that play a key role for male reproductive success (Rönn et al. 2008; Yamane et al. 2010a,b; Hotzy et al. 2012).

PREDICTIONS

We predict that metabolic rate, locomotor activity, life span, and body mass should show correlated evolution as a result of shared regulation by the same integrated physiological pathways

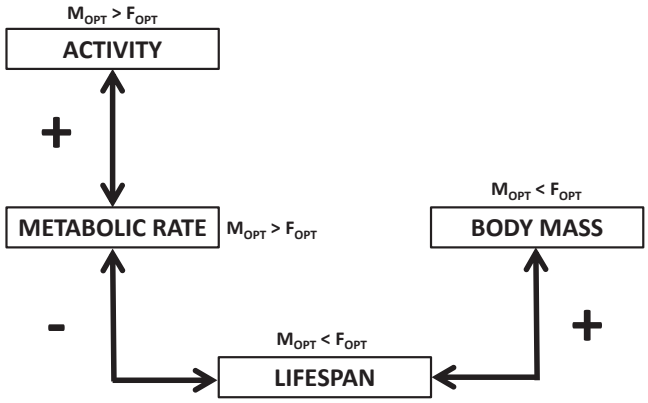

Figure 1. Hypothesized multivariate architecture of sexually antagonistic genetic variation in *Callosobruchus maculatus*. In this simplified chart, sign symbols represent the shared genetic covariance between traits. Because sex-specific selection favors a "live-fast-die-young" life style in males but a "grow-large-die-old" life style in females, the shared dependency between metabolic processes and these key life-history traits should result in sexual antagonism. Here, direct selection for high activity and metabolic rate in males will result in indirect selection for a short life and a small body mass. In contrast, direct selection for large body size and a long life in females will result in indirect selection for low metabolic rate and low activity. Males and females thus show distinct optimal life-history phenotypes (denoted M_{opt} and F_{opt}).

(see Fig. 1). Specifically, metabolic rate and locomotor activity should be positively correlated because high activity increases metabolism and, reversibly, a high metabolism permits higher activity (Reinhold 1999; Biro and Stamps 2010). High metabolic rate is, however, predicted to shorten life span through increased energy expenditure (Hochachka and Somero 2002; Brown et al. 2004). Finally, adult body mass is positively correlated with adult life span in most species (Blanckenhorn 2000). This is true also in *C. maculatus*, as a large soma carries more resources promoting survival in this capital breeder (e.g., Fox 1993; Fox et al. 2007). We therefore predict that male-limited artificial selection on life span in *C. maculatus* has led to correlated responses in metabolic rate, body mass, and locomotor activity in both sexes through a shared genetic architecture (see Fig. 1). In sum, we predict that IaSC emerges as a result of (1) an integrated genetic architecture of life-history variation, signified by genetically correlated responses in the four traits, and (2) limits to sex-specific expression of these traits, signified by correlated evolution in males and females.

Methods

STUDY SYSTEM

Callosobruchus maculatus is a common pest of stored legumes. Females lay their eggs on the surface of beans. Once the larvae hatch, they burrow into the bean and complete their development there, emerging as reproductively mature adults after some 21–29 days at 30°C. *Callosobruchus maculatus* are facultative capital breeders, obtaining all of the resources required for adult survival and reproduction during the larval stage (Fox 1993). The long- and short-life selection lines used in our experiment were derived from an outbred population obtained from C. W. Fox at the University of Kentucky, USA. Originally collected in 1979 from infested mung beans (*Vigna radiata*) in Tirunelveli, India (Mitchell 1991), the stock population has been maintained in our laboratory for over 80 generations at a population size of 400–1000 adults. The beetles have been cultured exclusively on mung beans (*V. radiata*) and kept in climate chambers at 30°C, 50% relative humidity, and a 14:10 h light-dark cycle.

ARTIFICIAL SELECTION ON MALE LIFE SPAN

For a full account of the artificial selection protocol, we refer to Berg and Maklakov (2012). Briefly, we used bidirectional artificial selection on male life span (i.e., sex-limited selection) to create four "long-life" and four "short-life" selection lines. Selection was applied during generations 1–5, followed by relaxed selection during generation 6–20 and renewed selection during generation 21–24, totaling nine generations of selection. The assays reported by Berg and Maklakov (2012) were performed during generation 6. After the 15 generations of relaxed selection, the difference in male life span was reduced from 27% to 8%. After reselection, the difference increased again to 23%. We performed new assays of life span, as well as body size, activity, and metabolism in both sexes at generation 27. There was a strong correlation between sex-specific life spans at generation 6 and generation 27 across the eight populations (see Results).

PHENOTYPING

We measured body mass, metabolic rate, locomotor activity, and life span of virgin male and female beetles from the four short-life lines and four long-life lines in generation 27. Upon hatching, all individuals were housed separately prior to testing. Each sample contained a total of 30 male or female beetles. This allowed us to assess group measures of the traits relevant to natural conditions as high densities and interactions between individuals are common in this species. Thus, individuals were allowed to trigger each other's behaviors within each sample, and our measures of locomotor activity thus incorporate both walking, running, and wrestling between individuals, and our group metabolic rate measures are a product of these activities. We measured three different age classes (separately). Beetles were either one-day old (i.e., one day after hatching), seven days old, or 13 days old. For each sex and age class, two replicate samples were taken from each of the eight selection lines (total $N = 96$ samples). For comparison, we also collected measures from replicate samples of the unselected base

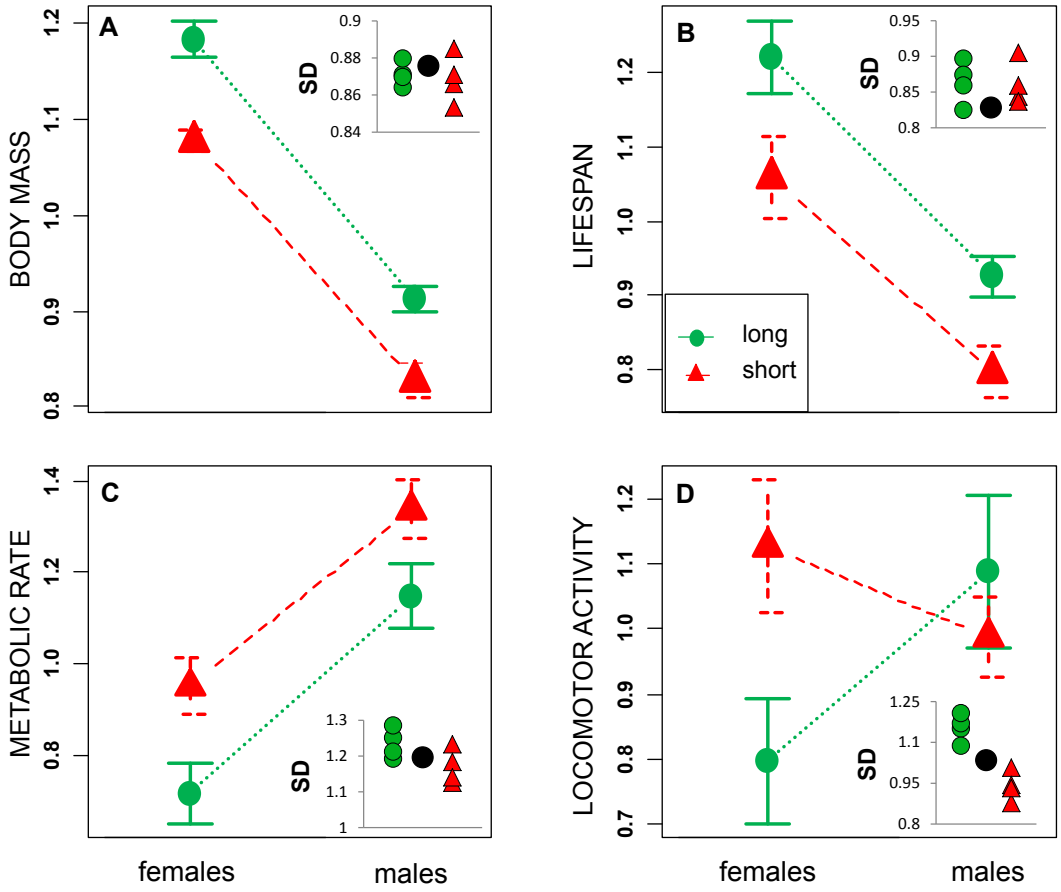

Figure 2. Sex-specific means in long-life (green circles) and short-life (red triangles) male selection regimes, for (A) body mass, (B) life span, (C) body mass corrected metabolism, and (D) locomotor activity. Here, the effect of age was blocked out by the use of mean-standardized data per age class. Differences are thus represented in proportion to a grand mean equal to 1. Means ± 95% CIs. Small panels show sexual dimorphism (SD) for respective trait in each of the eight populations, calculated as male value / (male value + female value). The black circle represents the (unselected) base population.

population in the same manner. These data were not included in the statistical analyses reported below but are included in Figures 2 and 3 for illustrative purposes.

A group measure of metabolic rate from the samples of 30 beetles was quantified using a Sable Systems® (Las Vegas, NV) flow-through respirometry system (Lighton 2008). This system pumps air at a very precisely regulated flow rate through a sealed chamber containing the animals. Downstream gas analyzers are then used to measure the amount of CO_2 produced and O_2 consumed by the beetles, and these measures then provide estimates of metabolic rate. Briefly, a LiCor 7000 infrared gas analyzer (Lincoln, NE), a Sable systems FC-2 differential oxygen analyzer (Las Vegas, NV), and a RH-300 water vapor pressure meter (Sable Systems) were attached to two Sable Systems RM8 eight-channel multiplexers. Respirometry chambers (RC-M, Sable Systems; ø = 2 cm, length = 4 cm) were housed inside a Sanyo MIR-153 incubator with temperature held at 30°C. One of the 16 chambers was left empty and was measured at repeated occasions during the recordings to control for instrumental drift. Inflowing air was pumped using a SS-4 pump (Sable Systems) and flow was regulated to 50 mL/min using a Model 840 mass flow control valve (Sierra Instruments, Monterey, CA). Each individual respirometry chamber was placed inside an activity detector (AD-2; Sable Systems), which provides a precise and continuous measure of locomotor activity of the subjects by using reflective infrared light technology, making sure that movement is monitored in all places inside the chamber. All analogue input data were acquired at 1 Hz via a UI2 analog-digital interface (Sable Systems). Data acquisition and data analyses were performed in ExpeData Pro 1.5.6 (Sable Systems).

Beetles (N = 30 beetles in each sample) were weighed to the nearest 0.00001 g (Sartorius® Genius ME 235P), placed in a respirometry chamber and were allowed to acclimatize for 70 min prior to the data collection session. Each session lasted for four consecutive 70-min cycles, where data were recorded from each of 11 chambers (eight selection line samples, two base population

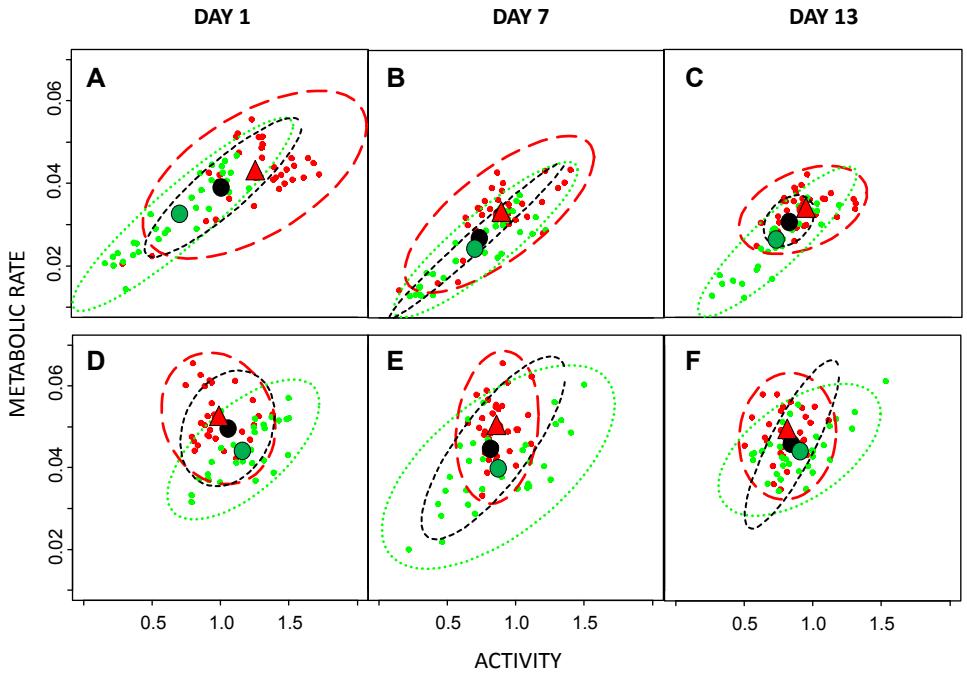

Figure 3. **Metabolic rate and activity. Body mass corrected metabolic rates (mL CO_2/min/mg$^{1.27}$) regressed on activity for females (A–C) and males (D–F) from the long-life (green circles, dotted lines) and short-life (red triangles, hatched lines) male selection regime. Ellipses show 95% bivariate confidence limits. The black circles and hatched ellipses represent the (unselected) base population for comparison. Small datapoints represent single recordings of activity and metabolic rate (four per sample), and large symbols represent selection regime averages.**

samples, and one empty blank) for 5 min in each cycle. Within each cycle, we included four 5 min blank baseline recordings. We ran two recording sessions (each including a separate set of beetles) per day, one in the morning and one in the afternoon.

Following the respirometry assays, beetles from each sample were transferred to a Petri dish and returned to a climate chamber and maintained at 30°C, 50% relative humidity, and a 14:10 h light-dark cycle. Average life span of each sample was measured by recording the number of individuals that were found dead each day after the respirometry analysis until all beetles were dead.

STATISTICAL ANALYSES

Differences between selection treatments and sexes were assessed using linear mixed models implemented in the lme4 package (Bates et al. 2011) of the statistical software R version 2.14.1 (R Core Team 2012). Statistical significance was evaluated by log-likelihood ratio tests of models including effects of interest and models where a specific effect had been removed, using a type-II sums-of-squares approach.

For all analyses we performed two complementary versions to efficiently control for effects of body mass on metabolic rate. Because each datapoint in our analyses represented an average of 30 individual beetles, the variance in body mass within each cell (i.e., treatment:sex:age combination) was very low, depressing the precision of the estimates for the independent effect of body mass on metabolic rate across cells. In our analysis using both within- and between-group variation in body mass, the scaling exponent was estimated to be 1.27 (CI = ±0.72) such that $CO_2 = c + \text{body mass}^{1.27}$ (where c is a scaling coefficient). To ensure that our results were not biased due to an inappropriate body mass compensation, we also ran all analyses using a fixed scaling exponent of 0.75, which is the theoretical expectation based on metabolic theory (Brown et al. 2004). Both of these alternative scaling exponents are within the range of scaling exponents found in populations of *C. maculatus* (D. Berger, E. Immonen., unpublished data). However, we note here that the complementary analyses showed that the choice of scaling exponent in no case affected results or conclusions qualitatively (Supporting Information S1).

We first analyzed differences in group metabolic rate corrected for body mass (i.e., respiration per unit mass) and locomotor activity. We included activity, body mass, and age as covariates that were first log-transformed to homogenize variances across selection treatments and sexes, and mean-centered to allow evaluation of differences in mean metabolic rates independent of covariates. Sex, selection treatment, and their interaction were included as fixed effects. We also included three-way interactions between sex × treatment × age or activity. In all analyses, we included selection line as a sex- and treatment-specific random effect, including estimation of random slopes for the covariates

age and activity. This allows for significance testing of main effects using the correct level of replication (i.e., eight lines in total). In cases where the line variance in random regression slopes for age and activity approached zero, these random effects were removed and inferences were based on the simplified model. The use of the simpler model was in all cases supported by lower Akaike Information Criterion scores. In addition, we blocked out effects of the time of day each run was performed (morning vs. afternoon), spatial effects of the two shelves of the climate cabinet holding the animal chambers during respirometry (top vs. bottom), and differences between the four serial respirometry cycles (acclimation effect).

We also analyzed overall differences in metabolic rate between the sexes and selection treatments without using activity as a covariate. This analysis gives the overall rate of respiration per unit mass, a measure that is predicted to correlate negatively with life span (Brown et al. 2004).

We tested for differences in activity between the sexes and selection treatments by including age as a covariate and selection treatment and sex, as well as their interaction as fixed effects. Sex– and treatment–specific line effects, including random regression slopes for the age covariate, were included as random effects.

We assessed differences in body mass and life span by including sex-specific and selection treatment specific line differences as random effects and selection treatment crossed by sex as fixed effects. Here, the effect of the initial age (1, 7, or 13 days) on body mass and life span was factored out by adding age as a fixed effect.

Intersexual genetic correlations were estimated by calculating correlations across line means for male and female per unit mass metabolic rate, locomotor activity, life span, and body mass. In addition, we used the estimates of sex-specific reproductive success reported in Berg and Maklakov (2012) for each of the eight lines to estimate genetic correlations with fitness for each trait and sex separately.

Results

BODY SIZE AND LIFE SPAN

Females were larger than males and lived longer as virgins (body size: $\chi^2 = 1608.6$, df $= 1$, $P < 0.001$, life span: $\chi^2 = 136.9$, df $= 1$, $P < 0.001$). More interestingly, males and females from the lines selected for long male life span were both larger ($\chi^2 = 27.1$, df $= 1$, $P < 0.001$) and lived longer ($\chi^2 = 21.0$, df $= 1$, $P < 0.001$) relative to short-life lines. These differences were similar in the sexes (sex × treatment interactions: body size: $\chi^2 = 0.12$, df $= 1$, $P = 0.73$, life span: $\chi^2 = 0.01$, df $= 1$, $P = 0.91$), indicating a shared genetic basis underlying variation in life span (see Berg and Maklakov 2012) and body mass in males and females. In accordance, sexual dimorphism (male value / [male value + female value]) showed no statistical difference between selection treatments (t-test across line means, body size: $P = 0.76$, life span: $P = 0.91$) (Fig. 2 A, B). The correlation between the reproductive life span at generation 6 reported in Berg and Maklakov (2012) and our measured virgin life span at generation 25 was very strong across lines for both males ($r = 0.83$) and females ($r = 0.85$), suggesting that the main characteristics of these lines have been maintained since when they were assessed in the previous study.

Table 1. Tests of fixed effects in the full linear mixed model of variance in metabolic rate, corrected for body mass and locomotor activity.

Effect	χ^2	df	P
Treatment	6.14	1	**0.013**
Sex	4.23	1	**0.040**
Activity	562.42	1	**<0.001**
Age	5.48	1	**0.019**
Body mass	12.25	1	**<0.001**
Time of day	3.90	1	**0.048**
Acclimation	128.86	1	**<0.001**
Spatial effect	5.66	1	**0.017**
Treatment × sex	4.06	1	**0.044**
Treatment × activity	4.90	1	**0.027**
Sex × activity	1.07	1	0.30
Treatment × age	0.17	1	0.68
Sex × age	21.01	1	**<0.001**
Treatment × sex × activity	5.45	1	**0.020**
Treatment × sex × age	15.66	1	**<0.001**

Significant values are given in bold.

METABOLIC RATE AND LOCOMOTOR ACTIVITY

As predicted, activity was positively correlated with metabolic rate (Table 1, Fig. 3). Interestingly, beetles from the short-life lines had higher metabolic rates than beetles from the long-life lines even when controlling for differences in activity and body mass, showing that selection for prolonged male life span decreased mass- and activity-specific metabolic rate. Metabolic rate declined with age. The effect of activity and age on metabolic rate differed between selection treatments and this difference was sex specific (Table 1, Fig. 3), suggesting that the genetic architecture underlying covariation between life span, activity, and metabolic rate differs between males and females.

The analysis that did not use activity as a covariate (thus comparing overall gross metabolic rate per unit mass across sexes and treatments) showed results congruent with the above model (see Table 2). However, all interactions including sex and selection treatment were nonsignificant, indicating that the sex-specific responses picked up in the former model were to a

Table 2. Tests of fixed effects in a linear mixed model of variance in metabolic rate, corrected for body mass but not locomotor activity.

Effect	χ^2	df	*P*
Treatment	7.16	1	**0.007**
Sex	10.30	1	**0.001**
Age	1.81	1	0.178
Body mass	11.13	1	**<0.001**
Time of day	5.94	1	**0.015**
Acclimation	113.25	1	**<0.001**
Spatial effect	4.76	1	**0.029**
Treatment × sex	3.50	1	0.061
Treatment × age	0.15	1	0.690
Sex × age	7.32	1	**0.007**
Treatment × sex × age	0.54	1	0.460

Significant values are given in bold.

Table 3. Tests of fixed effects in a linear mixed model of variance in locomotor activity.

Effect	χ^2	df	*P*
Treatment	2.19	1	0.14
Sex	9.49	1	**0.002**
Age	6.58	1	**0.010**
Time of day	0.96	1	0.33
Acclimation	32.40	1	**<0.001**
Spatial effect	0.22	1	0.64
Treatment × sex	19.96	1	**<0.001**
Treatment × age	2.06	1	0.15
Sex × age	1.88	1	0.17
Treatment × sex × age	4.49	1	**0.034**

Body mass was not included in the model as it did not explain any variance in activity levels within either sex. Significant values are given in bold.

large extent caused by changes in locomotor activity rather than differences in overall metabolic rate, which, as for body mass and life span, showed strongly correlated evolution in males and females (Fig. 2C). Accordingly, there was no significant difference in sexual dimorphism for metabolic rate between selection treatments based on line means (*t*-test, $P = 0.09$; Fig. 2C).

Locomotor activity also decreased with age, but there was no main effect of selection treatment on activity (Table 3). Instead, most variation in activity was explained by an interaction between sex and selection treatment, showing that the sexes did indeed respond differently and in a trait-specific manner to selection on male life span. Although selection for short male life span resulted in the predicted increase in activity in females, this was not true in males (Fig. 2D). This resulted in the evolution of sexual dimorphism for locomotor activity from being heavily male biased in lines selected for long life span, to being sexually monomorphic in lines selected for short male life span (*t*-test, $P = 0.001$; Fig. 2D).

We also analyzed our data separately for males and females to better illustrate sex-specific correlated responses of activity and metabolic rate. In females, there was no difference in metabolic rates between selection treatments when controlling for activity ($\chi^2 = 0.18$, df $= 1$, $P = 0.67$), but a large difference in activity ($\chi^2 = 15.1$, df $= 1$, $P < 0.001$). When activity was excluded from the analysis of metabolism, selection treatments showed significant differences in metabolic rate ($\chi^2 = 7.46$, df $= 1$, $P = 0.006$), suggesting that differences in overall metabolic rate between selection regimes exist in females, but are closely associated with evolved differences in activity (Fig. 3A–C). In contrast, there was no difference in activity between selection regimes in males ($\chi^2 = 0.15$, df $= 1$, $P = 0.70$), but there was a significant difference in metabolic rate ($\chi^2 = 10.4$, df $= 1$, $P = 0.001$). This difference remained when activity was removed as a covariate ($\chi^2 = 10.3$, df $= 1$, $P = 0.001$), illustrating that evolved differences in male metabolic rate was independent of variation in male activity (Fig. 3D–F).

INTERSEXUAL AND CROSS-TRAIT CORRELATIONS

Genetic correlations between female and male life span, body mass, and metabolic rate were positive and strong. However, there was no apparent intersexual correlation for activity (Fig. 4, Supporting Information S2), corroborating the results from the previous analyses indicating that this trait evolved more independently in the two sexes. Accordingly, cross-trait correlations estimated across sexes (i.e., the B-matrix; Lande 1980) for metabolic rate, body mass, and life span were all high and close to unity, whereas only female, but not male, locomotor activity showed high cross-trait intersexual correlations (Supporting Information S2). Female activity was strongly negatively genetically correlated with the estimates of female reproductive success reported in Berg and Maklakov (2012), and female body mass was strongly positively correlated with the estimates of female reproductive success, whereas no male traits showed significant covariation with reproductive success (Fig. 4).

We further investigated the relationships of the four traits underlying the genetically correlated responses to artificial selection. We looked at the correlation structure independently of the genetically correlated responses to male-limited artificial selection by statistically removing the mean effect of selection treatment (short/long) and then estimated trait correlations based on the 48 male and 48 female sample means. We used structural equation modeling to assess the biological hypothesis outlined in Figure 1, and then we tested for sex specificity in trait correlation structure. We built hierarchical models by either including or excluding possible covariance structures and compared likelihoods of these. A full description of methods and results can be found in Supporting

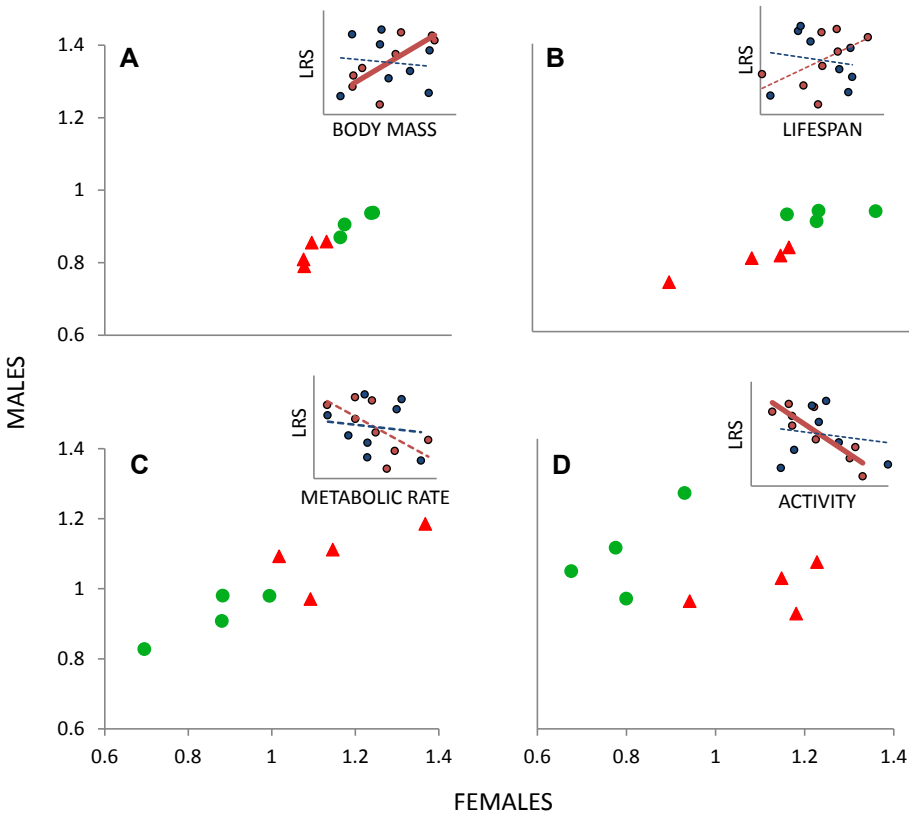

Figure 4. Intersexual genetic correlations. Correlations based on population means across selection treatments (short life = red triangles, long life = green circles). Small panels show the genetic correlation for each trait with reproductive success for males (dark blue) and females (light purple), respectively. The genetic correlation between sexes is strong for body mass (A), life span (B), and body mass corrected metabolic rate (C), but seemingly absent for locomotor activity (D). For females, there is a significant (bold regression lines) negative genetic correlation between the previously reported lifetime reproductive success (LRS) and locomotor activity and a significant positive genetic correlation with body mass. No significant correlations were found for males (dotted regression lines). Correlations with LRS were calculated on mean and unit variance standardized data for each sex separately.

Information S3. In brief, this analysis confirmed the hypothesized relationships in Figure 1: in both sexes, body mass was positively correlated with life span, and activity was positively correlated with metabolic rate, which in turn was negatively correlated with life span (Supporting Information S3). The relationship between activity and metabolic rate was stronger in females than in males and models assuming sex-specific covariance structure always provided a significantly better fit to the data than models constraining trait covariances to be equal (all $P \leq 0.001$; Supporting Information S3).

Table 4. Summary of whether the sex-specific responses to artificial selection on male life span significantly agreed with (√) our predictions (Fig. 1) or not (X).

	Lifespan	Body mass	Metabolism	Activity	LRS
R_F	√	√	√	√	√
R_M	√	√	√	X	√
r_{MF}	+	+	+	0	−

For data on lifetime reproductive success (LRS), see Berg and Maklakov (2012). R_F and R_M denote female and male responses, respectively, and r_{MF} denotes the sign of the documented intersexual genetic correlation.

PREDICTIONS VERSUS DATA

In summary, in females, all four of the studied traits showed correlated responses to selection on male life span. The previously observed decrease in reproductive success of females from lines selected for short male life span was associated with female phenotypes evolving toward becoming more male-like (reduced life span, higher metabolic rate, higher locomotor activity, and smaller body mass). Conversely, in males, the previously reported decrease in relative reproductive success of males selected for long life span was linked with the evolution of a more female-like multivariate phenotype (increased life span, lower metabolic rate, and larger body mass). Interestingly however, locomotor activity did not respond to the artificial selection in males as it did via correlated evolution in females (Table 4), leading to a reversal of

sexual dimorphism across the selection treatments (Fig. 2D) and fitness consequences in females (Fig. 4D).

Discussion

In recent years, there has been a major effort to assess to what extent IaSC constrains adaptive evolution. The pioneering work by Chippindale et al. (2001), which demonstrated a negative intersexual genetic correlation for fitness in *Drosophila melanogaster*, has been followed by studies documenting IaSC in a wide variety of organisms under both laboratory (Rand et al. 2001; Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Long and Rice 2007; Maklakov et al. 2008; Bilde et al. 2009a,b; Arnqvist and Tuda 2010; Lewis et al. 2011; Mills et al. 2011; Berg and Maklakov 2012; Berger et al. 2014) and field (Brommer et al. 2007; Foerster et al. 2007; Mainguy et al. 2009; Tarka et al. 2014) conditions. Most studies have focused on identifying overall negative intersexual genetic correlations for fitness or on testing for IaSC over a particular trait of interest. However, theory predicts overrepresentation of SA variance in suites of genetically integrated characters. When SA selection targets individual life-history components, a simple resolution of IaSC through sex-specific regulation may thus be unlikely given widespread pleiotropy of life-history genes (Mank et al. 2008, 2010; Stewart et al. 2010; Innocenti and Chenoweth 2013; Perry et al. 2014). Studies of IaSC over multivariate phenotypes are nevertheless few and mainly limited to *Drosophila* and traits such as growth/development rates and resulting body size (e.g., Prasad et al. 2007; Kwan et al. 2008; Abbott et al. 2010) or cuticular hydrocarbons (e.g., Bedhomme et al. 2011; Delcourt et al. 2012; Gosden et al. 2012). Here, we aimed at providing further insight into multivariate IaSC by taking an integrative approach simultaneously estimating behavioral, physiological, and life-history covariation in another insect model system where IaSC has been documented.

Previous work has shown that selection for short male life span leads to decreased female reproductive success compared to selection for long male life span in seed beetles (Berg and Maklakov 2012). We show that females from lines selected for short male life span not only had reduced life span, but were smaller, had higher locomotor activity and a higher mass-specific metabolic rate. Thus, as predicted, selection for short male life span resulted in correlated evolution in females toward a more male-like life-history syndrome (Table 4). As large females in this species have higher fecundity, the reduction in body size in short-life lines will be responsible for a part of the decrease in female reproductive success (Fig. 4A). In addition to this effect, the increased activity level of short-life females could be costly in terms of energy expenditure (Fig. 4D). Correspondingly, selection for short male life span led to elevated reproductive success in male seed beetles (Berg and Maklakov 2012). Our current results show that this response was associated with increased metabolic rate and decreased life span and body mass (Table 4). Again, this describes a more masculinized life-history syndrome. As detailed above (see Introduction and Fig. 1), we suggest that the increase in male fitness seen in Berg and Maklakov (2012) was primarily caused by an increased male reproductive potential early in life (i.e., ability to mate with virgin females; Pushpinder 1986; Arnqvist and Tuda 2010). Overall our results are in line with the suggestion that genetic integration of multiple characters acts to maintain SA genetic variation for this sexually dimorphic and rate-dependent life-history syndrome that defines many polygynous taxa (e.g., Trivers 1972; Promislow and Harvey 1990; Fox et al. 2004, 2006; Wedell et al. 2006; Blanckenhorn et al. 2007; Bonduriansky et al. 2008).

Conditions favoring distinct life-history syndromes in males and females are likely ubiquitous (e.g., Wedell et al. 2006; Maklakov and Lummaa 2013), but whether the pleiotropic nature of life-history genes per se generates widespread IaSC is less clear; genetic correlations can be built or broken by selection (Houle 1991; Shaw et al. 1995; Walsh and Blows 2009; Delph et al. 2011; Roff 2012). In cases when SA selection is targeting life-history syndromes involving suites of coadapted traits, genetic correlations could thus promote adaptive evolution of sexual dimorphism rather than constrain it. In addition to our study, several studies on *Drosophila* (Prasad et al. 2007; Kwan et al. 2008; Abbott et al. 2010; Bedhomme et al. 2011; Delcourt et al. 2012; Gosden et al. 2012) have looked at conflict over multivariate life-history optima in a system where IaSC has been known to operate. Only few of these have provided necessary data on both sex-specific selection gradients and multivariate genetic architectures to separate and quantify the number of focal traits targeted by SA selection from those merely sharing genetic covariance and potentially constraining adaptation (but see Delcourt et al. 2012; Gosden et al. 2012). Nevertheless, the understanding of multivariate IaSC has been greatly facilitated by the rich background information on sex-specific selection available in *Drosophila*, and we took a similar approach here to capitalize on the knowledge of the seed beetle model system. Overall, the existing studies, as well as our own, indicate that IaSC often is present despite pronounced sexual dimorphism in the studied characters, a conjecture in line with both theoretical predictions (e.g., Connallon et al. 2010; Connallon and Clark 2014) and observations of persistent SA selection on sexually dimorphic univariate phenotypes (Cox and Calsbeek 2009).

We predicted that selection for short life span in males should result in elevated locomotor activity in both sexes (Fig. 1). Surprisingly however, only females showed such a correlated response to male-limited life span selection (Fig. 2D). We also failed to find any correlation between metabolic rate and activity in males selected for short life span, whereas in males selected for long

life span this correlation was strong and similar to that observed in females (Fig. 3). Our results imply that sex-specific pleiotropy is regulating the expression of locomotor activity to achieve genetic independence in the sexes, consistent with a history of SA selection on this trait in *C. maculatus,* and recently documented IaSC (Long and Rice 2007) and sex-specific quantitative trait loci (Mackay 2009) for locomotor activity in *D. melanogaster*. Given the importance of activity for male mating success in this system (as in many other insect taxa: Husak and Fox 2008), we suggest that latent and strong sexual selection in males may have depleted male-specific genetic variance in locomotor activity, which could explain the lack of response and absent covariance with adult reproductive success in males. It is important to note that, like most behaviors, locomotor activity levels are likely context dependent. Thus, it is possible that differences between selection regimes in other aspects of male activity could have been detected had locomotor activity been measured under other conditions, such as those used when adult reproductive success was assayed (low density and in the presence of females) and found to differ between selection regimes (Berg and Maklakov 2012). Importantly however, female locomotor activity, as measured, still evolved in response to male-limited life span selection, and at an apparent cost to females in short-life lines (Fig. 4D), demonstrating that pleiotropic constraints involving some of the underlying genes regulating locomotor activity still generate IaSC in the face of sex-specific phenotypic expression of the trait in *C. maculatus* (see also Harano et al. 2010).

Asymmetries in the B-matrix have been reported previously (reviewed in Wyman et al. 2013), but are usually not of the magnitude or direction as found here where the cross-trait genetic covariance between life span and locomotor activity was higher between than within sexes. This ultimately resulted in the reversal of sexual dimorphism for locomotor activity across selection treatments as a result of only female activity responding to the male-limited life span selection (Fig. 2D). Chenoweth et al. (2008) reported that experimentally induced sexual selection on males, as predicted, increased sexual dimorphism in cuticular hydrocarbons in *D. serrata*, while natural selection decreased it. However, unexpectedly, the change in sexual dimorphism under sexual selection was only due to a response in females. As an alternative to the hypothesis that sexual selection was working directly on female, but not male cuticular hydrocarbons (Chenoweth et al. 2008), our results highlight another possibility that may explain such counterintuitive responses: namely, that directional sexual selection may not have targeted the focal traits under study in either sex, but instead an unmeasured genetically correlated trait in one of the sexes, leading to a response in the focal traits of the opposite sex. Although strong and persistent SA selection often will lead to the evolution of sex-specific expression of single target traits, we argue that widespread pleiotropy at SA loci will result in multivariate genetic constraints that can commonly generate IaSC via cross-trait, between-sex genetic covariances.

A negative genetic correlation between metabolic rate and life span, such as revealed by our induced artificial selection, is usually taken as support for the rate-of-living hypothesis (ROLH; Pearl 1928) and the involvement of metabolic rate in shaping life-history syndromes (Brown et al. 2004). Oxidative stress theory as an extension of the ROLH provides a direct link between metabolism and rate-dependent life histories by invoking that accumulation of molecular damage that induces ageing is caused by reactive oxygen species (ROS) produced during mitochondrial respiration (Harman 1956). ROS production through increased metabolism would therefore have the potential to mediate life-history trade-offs and shape sexual dimorphism in life histories (e.g., Monaghan et al. 2009; Selman et al. 2012; Archer et al. 2013). However, both the ROLH and oxidative stress theory have received considerable critique questioning the generality of these hypotheses (e.g., Brand 2000; Speakman and Selman 2011; Gems and Partridge 2013).

We suggest that there is a straightforward explanation, which does not need to incorporate ROS production, for why we find experimental support for an evolutionary link between life-history variation and metabolic rate. Metabolic rate inescapably forms the basis of all other biological rates and therefore governs life-history traits, such as growth, development, reproduction, and life span (Brown et al. 2004). Yet there is substantial variation across species and populations in how environmental factors affect metabolism, as well as in how subsequent changes in metabolism affect biological rates (e.g., Hochachka and Somero 2002; Dell et al. 2011), suggesting that metabolic constraints are not fateful and compensatory adaptations can substantially shape life-history syndromes. Seed beetles are usually limited in the amount of adult resources because they typically do not feed as adults. Hence, increased metabolism due to locomotion, egg production, or ejaculate expenditure will invariably deplete the existing pool of resources and trade-off with other biological functions. Selection for long life in such taxa therefore cannot result in compensatory increases in adult resource intake, but is much more likely to result in lower rates of resource expenditure through decreases in metabolic rate and/or metabolically expensive activities in the adults. Thus, although our results provide support for a link between metabolism and rate-dependent life-history syndromes, this link is likely to be weaker in organisms where resources depleted by high metabolic rates can be replenished (e.g., Khazaeli et al. 2005).

Conclusions

Males and females often have different optima for rate-dependent life-history syndromes built by genetically integrated

behavioral and life-history traits that depend on metabolic processes. Pleiotropy is thus likely to play a key role in maintaining IaSC and should affect the evolution of sex specificity in life-history trade-offs. Our experimental data are consistent with the general tenet that IaSC in *C. maculatus* emerges as a result of multivariate genetic constraints on sexually dimorphic life histories. Sex-limited selection for short male life span, which increased male but decreased female reproductive success (Berg and Maklakov 2012), was associated with evolution in females toward a more male-like multivariate life-history phenotype. Conversely, selection for long male life span was associated with evolution in males toward a more female-like phenotype. An exception to this pattern was locomotor activity, which showed sex-limited evolution and sex-specific genetic covariance with metabolic rate. Remarkably, selection on male life span did not affect male activity levels but led to a strong correlated response in females, resulting in a reversal of sexual dimorphism in locomotor activity across selection treatments at the apparent detriment of active females. Thus, our results also demonstrate how multivariate genetic constraints can generate persistent IaSC despite seemingly sex-limited regulation of single traits under SA selection.

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DATA ARCHIVING

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Supporting Information

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

Supporting Information S1. Complementary analyses fixing the allometric exponent for body mass effects on metabolic rate to 0.75.
Supporting Information S2. Intersexual genetic correlations and sex-specific selection differentials (estimated by Pearson's moment correlations).
Supporting Information S3. Sex differences in between-trait covariances.
Figure S1. Path diagram of the relationships between locomotor activity, metabolic rate, body mass, and longevity for males and females estimated within selection treatments.
Figure S2. Graphical depiction of the correlation matrix of locomotor activity, (mass-corrected) metabolic rate, body mass, and longevity for males and females in the long- and short-life selection treatments.