EVIDENCE FOR STRONG INTRALOCUS SEXUAL CONFLICT IN THE INDIAN MEAL MOTH, *PLODIA INTERPUNCTELLA*

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Males and females share a genome and express many shared phenotypic traits, which are often selected in opposite directions. This generates intralocus sexual conflict that may constrain trait evolution by preventing the sexes from reaching their optimal phenotype. Furthermore, if present across multiple loci, intralocus sexual conflict can result in a gender load that may diminish the benefits of sexual selection and help maintain genetic variation for fitness. Despite the importance of intralocus sexual conflict, surprisingly few empirical studies conclusively demonstrate its operation. We show that the pattern of multivariate selection acting on three sexually dimorphic life-history traits (development time, body size, and longevity) in the Indian meal moth, *Plodia interpunctella*, is opposing for the sexes. Moreover, we combined our estimates of selection with the additive genetic variance–covariance matrix (G) to predict the evolutionary response of the life-history traits in the sexes and showed that the angle between the vector of responses and the vector of sexually antagonistic selection was almost orthogonal at 84.70°. Thus, G biases the predicted response of life-history traits in the sexes away from the direction of sexually antagonistic selection, confirming the presence of strong intralocus sexual conflict in this species. Despite this, sexual dimorphism has evolved in all of the life-history traits examined suggesting that mechanism(s) have evolved to resolve this conflict and allow the sexes to reach their life-history optima. We argue that intralocus sexual conflict is likely to play an important role in the evolution of divergent life-history strategies between the sexes in this species.

KEY WORDS: Antagonistic selection, genetic covariance matrix, intralocus sexual conflict, life-history, *Plodia interpunctella*.

Intralocus sexual conflict occurs whenever selection on shared phenotypic traits in one sex displaces the other sex from its phenotypic optimum (Lande 1980; Bonduriansky and Chenoweth 2009). It occurs because many shared traits have a common genetic basis in the sexes, but are subject to contrasting patterns of selection. The potential implications of intralocus sexual conflict to the evolutionary process are widespread: it may help preserve genetic variation in a population (Foerster et al. 2007; Prasad et al. 2007), diminish the benefits of sexual selection (Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Brommer et al. 2007), increase the risk of population extinction (Kokko

and Brooks 2003), and/or drive speciation (Rice 1996; Parker and Partridge 1998; Gavrilets and Hayashi 2005). Despite the importance of this process, however, surprisingly little conclusive empirical evidence exists supporting its operation (Bonduriansky and Chenoweth 2009). Thus, although it is tempting to implicate intralocus sexual conflict as a fundamental evolutionary process, we are in a dire need of more concrete empirical examples before this can and should be done.

Intralocus sexual conflict will occur whenever the fitness surfaces for the sexes differ (Bonduriansky and Chenoweth 2009). However, it is theoretically predicted to be at its strongest

whenever selection acting on shared phenotypic traits is directly opposing in the sexes and the genetic correlation for the shared trait(s) is strong and positive between the sexes (i.e., the intersexual genetic correlation, r_{MF}). This contrasts the negative r_{MF} for fitness that is predicted to occur during the acute and attenuated phases of intralocus sexual conflict (Bonduriansky and Chenoweth 2009). Although a number of studies have shown a negative genetic correlation for fitness between the sexes (Chippindale et al. 2001; Rand et al. 2001; Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Brommer et al. 2007; Foerster et al. 2007; Mainguy et al. 2009), these studies typically do not locate the shared trait(s) that mediate the conflict. Moreover, because the sign and strength of r_{MF} for fitness may change during the evolutionary progression of intralocus sexual conflict, not detecting a significant r_{MF} for fitness does not necessarily preclude the existence or past operation of intralocus sexual conflict in a population (see Box 2 in Bonduriansky and Chenoweth 2009).

Shared life-history traits are particularly likely to be subject to intralocus sexual conflict because they have a strong relationship to fitness and frequently exhibit different optima in the sexes (Wedell et al. 2006). Few studies have, however, explicitly examined intralocus sexual conflict over life-history traits (Bonduriansky and Chenoweth 2009). Merilä et al. (1997, 1998) showed sexually antagonistic natural selection on body size in the collared flycatcher (*Ficedula albicollis*) and that the r_{MF} for this trait is one. More recently, Long and Rice (2007) showed that adult locomotory activity in *Drosophila melanogaster*, an important component of a male's life-history strategy for locating potential mates, experiences opposing linear selection in the sexes and that there is a strong positive r_{MF} for this behavior. Similarly, Wedell et al. (2009) demonstrated in the green-veined white butterfly (*Pieris napi*) that male sperm production and female sperm storage, which determines female mating rate, are antagonistically selected (many sperm are good for males but bad for females), and that the r_{MF} for sperm production and sperm storage is strongly positive. All of these studies, however, have taken a univariate approach to demonstrating intralocus sexual conflict. Yet selection rarely acts on single traits in isolation and traits are seldom genetically independent (Lande and Arnold, 1983). Consequently, it is possible that taking a univariate approach may underestimate the biological complexity of intralocus sexual conflict, particularly for life-history traits that often show a high degree of covariation.

Lande (1980) proposed a general model for the evolution of shared phenotypic traits in the sexes when subject to contrasting regimes of multivariate selection. In this model, the additive genetic variance–covariance matrix ($\mathbf{G}$) for the shared traits is partitioned into four submatrices that describe the additive genetic (co)variance within males ($\mathbf{G_m}$) and females ($\mathbf{G_f}$) and the additive genetic covariance between the sexes ($\mathbf{B}$ and its transpose $\mathbf{B^T}$)(see

Equation 2 in Methods). Although the genetic covariance in all of these submatrices is generated by pleiotropy and/or linkage disequilibrium, a major difference is that the genetic covariance present in $\mathbf{B}$ is not expressed in any subset of individuals in the population. Consequently, it is predicted that $\mathbf{G_m}$ and $\mathbf{G_f}$ should be exposed to stronger multivariate stabilizing selection and therefore be more stable over evolutionary time than $\mathbf{B}$, a prediction recently supported with empirical data from populations of garter snakes (*Thamnophis elegans*)(Barker et al. 2010). Nevertheless, Lande's (1980) model showed that $\mathbf{B}$ is central to how phenotypic traits evolve in the sexes. Most importantly, whenever there is high genetic covariance between shared traits in the sexes, males and females cannot evolve independently and this may considerably slow rate of sexual divergence in the population (Lande 1980). In fact, with knowledge of selection operating on the shared traits in the sexes and $\mathbf{G}$, Lande (1980) showed that the evolutionary response of traits in the sexes could be predicted (see eq. 3 in Methods). This presents an alternate way of characterizing the magnitude of intralocus conflict within a multivariate framework. If $\mathbf{G}$ is preventing antagonistic selection from driving the sexes to their phenotypic optima, then the angle between the predicted response to selection and the actual direction of selection will approach 90°, whereas if $\mathbf{G}$ does not constrain the operation of antagonistic selection than this angle will approach 0°. Although this angle has been used before as a general measure of genetic constraint within the sexes (Blows and Walsh, 2007; Hansen and Houle 2008; Chenoweth et al. 2010), it has not, to our knowledge, been applied to the study of intralocus sexual conflict.

The Indian meal moth, *Plodia interpunctella,* is an ideal species for investigating intralocus sexual conflict. The major determinant of female fitness is fecundity, which is often correlated with large body size in the Lepidoptera (e.g., Jones et al. 1982; Tammaru et al. 1996) and frequently is traded against development time (Nylin and Gotthard 1998). In contrast, male fitness is dependent on achieving successful matings and on gaining high paternity in sperm competition, which is largely unaffected by body size in this species (Cook et al. 1997). However, rapid development time is associated with increased mating success in male *P. interpunctella* (Lewis 2005). This difference in the optimal values for these shared life-history traits between the sexes should, in theory, generate intralocus sexual conflict in this species. *Plodia interpunctella* is also a suitable model as it is a pest of stored products meaning that there is a high degree of similarity between the conditions under which it is reared in the laboratory and its natural environment. Hence, it is likely that measurements of fitness and conflict in the laboratory will reflect those experienced in the wild in this species.

Here, we document the existence of strong intralocus sexual conflict for shared life-history traits in *P. interpunctella*. We started by calculating multivariate selection operating on three

important sexually dimorphic life-history traits (development time, body size, and longevity) in the sexes and then statistically compared the fitness surfaces to show that selection operating on these life-history traits is directly opposing in the sexes. Next, we estimated the genetic (co)variances within the sexes ($\mathbf{G_m}$ and $\mathbf{G_f}$), as well as the genetic covariances between the sexes ($\mathbf{B}$), for these shared life-history traits and combined them with our estimates of antagonistic selection to predict evolutionary responses of these traits in the sexes. We calculated the angle between this predicted evolutionary response in the sexes and the direction of antagonistic selection, as a measure of the degree to which $\mathbf{G}$ constrains the life-history traits in the sexes from reaching their phenotypic optima. Our results suggest that intralocus sexual conflict is likely to have played an important role in the evolution of divergent life-history strategies between the sexes in this species.

Materials and Methods
SOURCE AND REARING OF STOCK POPULATIONS
A large, outbred stock population of approximately 1000 *P. interpunctella* per generation was established from several hundred individuals collected in Perth, Western Australia in 2001. Larvae were reared on a diet of bran midlings, yeast, honey, and glycerol, in an incubator set to $28 \pm 1°C$ with a 16L: 8D photoperiod (Gage and Cook 1994). Each generation, the effect of larval overcrowding during rearing was removed by rearing larvae with excess food at constant densities (400 mL medium per 100 eggs). At the time of our experiment, our stock populations had undergone approximately five generations of breeding in the laboratory.

ESTIMATING MULTIVARIATE SEXUAL SELECTION ON LIFE-HISTORY TRAITS IN THE SEXES
To estimate the strength and form of sexual selection operating on the shared life-history traits (development time, longevity, and body size) in the sexes, we measured these life-history traits and fitness on individual males and females taken at random from our large stock population. Eggs were collected on a piece of filter paper from the stock population and on the day they hatched, first-instar larvae were transferred into single 10-mL vials containing 4 mL of larval medium. Upon reaching the fifth instar, vials were checked daily for eclosion to determine development time, measured as the number of days between hatching from an egg and eclosion to adulthood. We measured male fitness as lifetime mating success. Starting on the day of eclosion, each male was presented daily with a single, unrelated 1-day-old virgin female. The pair was observed for the length of the 8-h dark phase and we recorded whether or not they mated. This was later confirmed by dissection of all females to ensure spermatophore transfer. This process was continued for the life span of the male. We measured

female fitness as lifetime fecundity. On the day of eclosion, each female was mated once to an unrelated virgin male of the same age. Each female was then moved to an individual 10-mL vial. As females prefer to oviposit in the presence of larval medium, a small amount of food was placed in the bottom of each vial, separated from the female by a cone of filter paper. This stimulated oviposition and allowed eggs to be collected easily on the filter paper and counted daily for the remainder of the female's life span. The mating partners used to measure male and female fitness were isolated at random from the stock population as fifth-instar larvae and housed individually in 10-mL vials containing 4 mL of larval medium to ensure they were virgins at mating and of known age. Upon death, adult body size was estimated in each sex by measuring the length of the forewing. To render the veins of the wings visible, the right forewing of each individual was detached from the body using fine forceps and the scales removed by immersion in 90% alcohol, followed by 10% hydrochloric acid and then bleach (Reid, 1976). The wings were then washed in distilled water, mounted on a slide, and allowed to dry. The length between the vein one junction and point of wing insertion was then measured under a microscope using the Micromeasure (version 3) computer program. In total, we had 120 males and 153 females available for our multivariate selection analysis.

QUANTITATIVE GENETIC BREEDING DESIGN
We used a paternal half-sib breeding design to estimate the genetic (co)variance for the three life-history traits within the sexes, as well as the genetic covariances between these traits across the sexes (Falconer and Mackay 1996). On the day of eclosion, 25 virgin males (sires) were paired individually in a 10-mL vial with a randomly allocated, virgin female (dam). Following copulation, the female was removed and this procedure was repeated twice more on consecutive days, so that each sire eventually mated with three dams. After mating, each dam was transferred individually to a 10-mL vial to oviposit and eggs were collected on filter paper as outlined above. On the day that eggs hatched, three larvae of each sex per dam were selected at random and transferred to an individual 10-mL vial provided with 4 mL of larval medium. Male larvae can be easily sexed as their pigmented testes are visible at the posterior end of the larva. On reaching the pupal stage, individuals were checked daily for eclosion to determine development time. Adults were maintained in these individual vials until their death, after which their body size was measured following the protocol outlined above.

STATISTICAL ANALYSIS
Sexual dimorphism in life-history traits
To examine the degree of sexual dimorphism in the shared life-history traits in *P. interpunctella*, we analyzed the phenotypic data from the selection analysis using a multivariate analysis of

variance (MANOVA), including sex as the main effect (fixed) and the shared life-history traits as the response variables. Post-hoc univariate ANOVAs were conducted to determine how each of the individual life-history traits differed across the sexes.

Multivariate selection analysis

Fitness surface estimation.—We calculated the multivariate individual fitness surface for development time, longevity, and body size independently in the sexes. For each sex, we transformed absolute fitness scores (i.e., lifetime fecundity in females and lifetime number of matings in males) to a mean of one (i.e., relative fitness) and all life-history traits to zero means and unit variances as suggested by Lande and Arnold (1983). We then fitted a linear regression including all three life-history traits to estimate the vector of linear selection gradients, $\boldsymbol{\beta}$, for each sex (Lande and Arnold 1983). A quadratic regression model incorporating all linear, quadratic, and cross-product terms was then used to estimate the matrix of nonlinear selection gradients, $\boldsymbol{\gamma}$, for each sex. Quadratic regression coefficients are known to underestimate the stabilizing and/or disruptive selection gradients by a factor of 0.5 and therefore we doubled the quadratic selection gradients as recommended by Stinchcombe et al. (2008).

It is possible to underestimate the strength of nonlinear selection if one interprets the size and significance of the $\boldsymbol{\gamma}$-coefficients individually (Phillips and Arnold 1989; Blows and Brooks 2003). We therefore explored the extent of nonlinear selection by performing a canonical analysis to find the major axes of the fitness surface for each sex (Phillips and Arnold 1989). The strength of nonlinear selection along each of the eigenvectors ($\mathbf{m}_i$) of the response surface is given by their eigenvalue, $\boldsymbol{\lambda}_i$.

To estimate selection gradients in a way that is relevant to equations of evolutionary change, no transformations of the fitness measure apart from conversion to relative fitness should be made (Lande and Arnold 1983). However, violation of distributional assumptions does create problems for testing significance for estimated gradients (Draper and Smith 1981; Mitchell-Olds and Shaw 1987). Thus, to assess the significance of our linear and nonlinear selection gradients, we used a resampling procedure in which fitness scores were randomly shuffled across individuals in the dataset to obtain a null distribution for each gradient where there is no relationship between trait and fitness. Probabilities are the number of times (out of 9999 permutations) in which the gradient pseudo-estimate was equal to or less than the original estimated gradient. We conducted separate randomization analyses for the multiple regression models for directional selection (i.e., model containing only the linear terms) and for the full quadratic model (i.e., model containing linear, quadratic, and correlational terms). We used the same resampling procedure to assess the significance of linear and nonlinear selection on each of the eigenvectors extracted from canonical rotation of $\boldsymbol{\gamma}$ (Bisgaard

and Ankenman 1996). In each instance, we held each of the eigenvectors constant and shuffled fitness at random across individuals in the dataset to obtain a null distribution with no relationship between the eigenvector and fitness. Thus, our approach differed to that of Reynolds et al. (2010) that conducts a new canonical rotation for each permutation so that that the same eigenvectors are not necessarily derived for each permutation.

Fitness surface visualization.—We used thin-plate splines (Green and Silverman 1994) to visualize the major axes of the fitness surface extracted from the canonical rotation for each sex. Thin-plate splines are a nonparametric approach that provides a less-constrained view of the surface than the best quadratic approximation (Blows et al. 2003). We used the Tsp function in the *fields* package in R (available via http://www.r-project.org) to fit a spline surface using the value of the smoothing parameter, $\boldsymbol{\lambda}$, that minimized the generalized cross-validation (GCV) score. We then plotted the surface in R using both the perspective and contour map views.

Comparing multivariate selection between the sexes.—As male and female fitness were measured in different currencies (i.e., lifetime mating success vs. lifetime fecundity), it was necessary to standardize relative fitness across the sexes by dividing the relative fitness of each sex by the mean relative of both sexes combined (i.e., average male and female fitness was the same). We then used a sequential model building approach (partial F-test) to compare strength and form of selection on life-history traits between the sexes (Draper and John 1988). Chenoweth and Blows (2005) provide a detailed description of this approach to compare selection gradients across the sexes. Although this approach statistically tests for differences in the magnitude of selection gradients between the sexes, it does not provide information on the direction of this difference in a multivariate space. Therefore, we also calculated the angle ($\boldsymbol{\theta}$) between the vector of standardized linear selection gradients and the dominant vectors of nonlinear selection for males and females as:

$$\boldsymbol{\theta} = \cos^{-1}\left(\frac{a \bullet b}{\|a\| \, \|b\|} \right), \qquad (1)$$

where $\|a\| = \sqrt{a \bullet a}$ and $\|b\| = \sqrt{b \bullet b}$, respectively. In the case of linear selection, a represents the vector of standardized linear selection gradients for males ($\boldsymbol{\beta_m}$) and b represents this same vector for females ($\boldsymbol{\beta_f}$). In the case of nonlinear selection, a represents the dominant vector of $\boldsymbol{\gamma}$ for males ($\boldsymbol{\lambda_m}$) and b the same vector for females ($\boldsymbol{\lambda_f}$).

Estimating the genetic architecture of life-history traits

We estimated the additive genetic variance–covariance matrix ($\mathbf{G}$) for the shared life-history traits within and between the sexes using restricted maximum likelihood in a nested multivariate mixed

effects model that included sex as a fixed effect and sire and dams nested within sires as random effects, as implemented in the MIXED procedure of SAS (version 9.1.3, SAS Institute, Cary, NC). Due to the modest number of sires used in our breeding design ($n = 25$), we used a factor analytic approach to estimate the (co)variance matrices of the random effects at the dam and sire level: a total of six factors were included in our final model. Of particular importance is the sire-level covariance matrix because, when multiplied by four, it provides an estimate of $\mathbf{G}$:

$$\mathbf{G} = \begin{pmatrix} \mathbf{G_m} & \mathbf{B} \\ \mathbf{B^T} & \mathbf{G_f} \end{pmatrix}, \tag{2}$$

where $\mathbf{G_m}$ and $\mathbf{G_f}$ are the within sex additive genetic (co)variance matrix for males and females, respectively, and $\mathbf{B}$ represents the additive genetic covariance between these sexes with the superscript ^{T} denoting matrix transposition. We determined the significance of $\mathbf{G}$ using a likelihood ratio test in which we fit the multivariate model including the sire term and compared this to a reduced model with the sire term excluded (Fry 2004).

Estimating the predicted response to selection and the degree of genetic constraint

We estimated the predicted evolutionary response of the shared life-history traits in males and females following Lande (1980) as:

$$\begin{pmatrix} \Delta \bar{z}_m \\ \Delta \bar{z}_f \end{pmatrix} = \frac{1}{2} \begin{pmatrix} \mathbf{G_m} & \mathbf{B} \\ \mathbf{B^T} & \mathbf{G_f} \end{pmatrix} \begin{pmatrix} \beta_m \\ \beta_B \end{pmatrix}, \tag{3}$$

where $\Delta \bar{z}_m$ and $\Delta \bar{z}_f$ represent the predicted response of male and female life-history traits, respectively, measured in units of phenotype standard deviations. The factor of $^1/_2$ appears because the selected male and female parents are assumed to make equal autosomal contributions to the offspring of both sexes (Lande 1980). We calculated the angle (θ) between the vector of predicted responses and the vector of linear selection gradients using equation (1). An angle approaching $90°$ suggests that $\mathbf{G}$ is constraining the predicted response of life-history traits in the sexes away from the direction of selection, whereas an angle approaching $0°$ indicates an alignment of these vectors and therefore no constraint to the evolution of life-history traits in the sexes (Blows and Walsh 2007; Hansen and Houle 2008; Chenoweth et al. 2010).

In addition, we also examined the effect that $\mathbf{B}$ had on the predicted response to selection on life-history traits in the sexes. This was done by calculating the response to selection using equation (3) when $\mathbf{B}$ was estimated from our breeding design (R_B) versus when it was set to zero ($R_{B=0}$) to reflect the case where genetic covariance between the sexes does not constrain the evolution of shared life-history traits (Agrawal and Stinchcombe

2009). We calculated the percentage reduction in the response to selection due to $\mathbf{B}$ for each life-history trait in the sexes as:

$$\% = \frac{R_{B=0} - R_B}{R_{B=0}} \times 100 \tag{4}$$

where a value above 100% indicates that $\mathbf{B}$ biases the evolutionary response in the opposite direction to that predicted by selection alone, whereas a value below 100% indicates that $\mathbf{B}$ permits an evolutionary response in the same direction, but of smaller magnitude, relative to when there is no genetic covariance between the sexes.

Results
SEXUAL DIMORPHISM IN LIFE-HISTORY TRAITS
There is clear sexual dimorphism in life-history traits in *P. interpunctella* (Table 1). On average, females develop faster, have a reduced longevity and are larger in body size at eclosion to adulthood than males (Table 1).

SEXUAL SELECTION ON MALE LIFE-HISTORY TRAITS
Standardized linear, quadratic, and correlational selection gradients for life-history traits in males and information regarding the significance from randomizations tests are presented in Table 2. There is significant linear sexual selection favoring males with shorter development times and increased longevity, but not on male body size (Table 2). There is also significant nonlinear sexual selection operating on life-history traits in males including stabilizing selection (i.e., a negative γ) on development time and longevity and negative correlational selection on these two life-history traits (Table 2). There is also significant positive

Table 1. MANOVA examining the differences in life-history traits (development time (DT), longevity and body size) across the sexes. The mean (±1SE) for each life-history trait is also provided for the sexes.

	MANOVA		
	Pillai's trace	$F_{3,269}$	P
Sex	0.72	234.57	0.0001
	Univariate analyses		

	Males	Females	ANOVA	
			$F_{1,271}$	P
DT (days)	27.88±0.44	26.44±0.24	9.37	0.002
Longevity (days)	8.26±0.16	5.83±0.12	156.53	0.0001
Size (mm)	4.50±0.02	5.13±0.03	371.54	0.0001

Table 2. The vector of standardized linear selection gradients (β) and the matrix of standardized quadratic and correlational selection gradients (γ) for life-history traits in (A) male and (B) female *Plodia interpunctella*. DT refers to development time. The subscripts *m* and *f* refer to males and females, respectively. Randomization test: *P<0.05, **P<0.01, ***P<0.001.

A Traits	β_m	γ_m		
		DT	Longevity	Size
DT	−0.074**	−0.047*		
Longevity	0.114***	−0.050*	−0.044*	
Size	0.050	0.077*	0.036	−0.005

B	β_f	γ_f		
		DT	Longevity	Size
DT	0.080*	0.018		
Longevity	−0.113**	0.139*	−0.037	
Size	0.086*	−0.061	0.081*	−0.004

correlational selection on development time and body size (Table 2).

We conducted a canonical rotation of the **γ** matrix presented in Table 2 to locate the major axes of nonlinear sexual selection. The resulting **M** matrix of eigenvectors and their associated eigenvalues are presented in Table 3. Two of the three eigenvectors experienced significant nonlinear sexual selection (m_{2m} and m_{3m}) and both had negative eigenvalues characteristic of stabilizing selection (Table 3). The eigenvector with the strongest nonlinear selection operating on it (m_{3m}) largely represents the trade-

Table 3. The M matrix of eigenvectors from the canonical analysis of γ for life-history traits in (A) male and (B) female *Plodia interpunctella*. The linear (θ_i) and quadratic (λ_i) gradients of selection along each eigenvector are given in the last two columns. The quadratic selection gradient (λ_i) of each eigenvector (m_i) is equivalent to the eigenvalue. The subscripts *m* and *f* refer to males and females, respectively. DT refers to development time. Randomization test: *P<0.05, **P<0.01, ***P<0.001.

	M			Selection	
	DT	Longevity	Size	θ_i	λ_i
A					
m_{1m}	0.494	0.053	0.868	0.013	0.018
m_{2m}	−0.503	0.831	0.236	0.144***	−0.024*
m_{3m}	0.709	0.553	−0.437	−0.011	−0.090***
B					
m_{1f}	0.835	0.548	−0.047	0.001	0.065**
m_{2f}	−0.204	0.388	0.899	0.017	0.020
m_{3f}	−0.511	0.741	−0.435	−0.162***	−0.109*

off between increased development time and adult longevity and decreased body size, whereas m_{2m} largely reflects the trade-off between increased longevity and body size and decreased development time (Table 3). m_{2m} also experienced significant positive linear selection suggesting that the convex curvature of this eigenvector was not symmetrical within the range of life-history traits sampled (Table 3, Fig. 1). m_{1m} had a positive eigenvalue
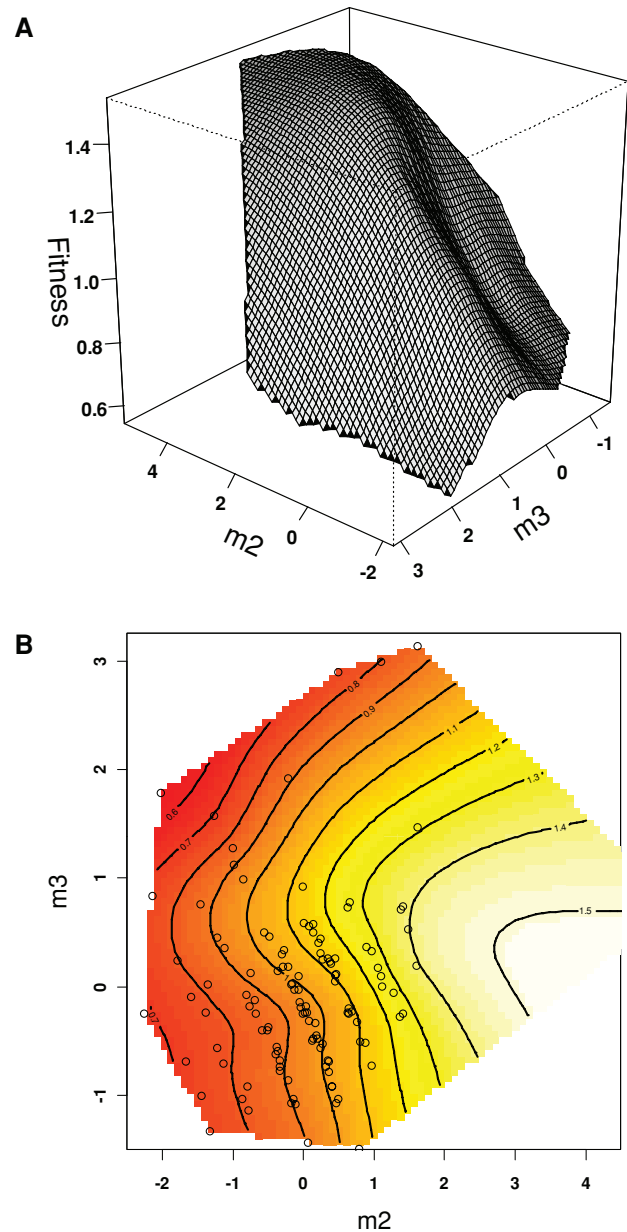

Figure 1. Thin-plate spline (A) perspective-view and (B) contour map visualizations of the fitness surface for the two major axes of nonlinear sexual selection (m_2 and m_3) for male life-history traits in *Plodia interpunctella*. Male fitness is measured as the lifetime number of matings obtained. Actual datapoints for males are overlaid in (B) and yellow color represents regions of high fitness and red color represents regions of low fitness.

characteristic of disruptive selection but selection operating on this eigenvector is not statistically significant. Thus, although the overall pattern of nonlinear sexual selection operating on life-history traits in males was indicative of a multivariate saddle (i.e., a combination of positive and negative eigenvalues), the lack of significant selection on m_{1m} suggests that the fitness surface is not significantly different from a multivariate peak (Fig. 1).

FECUNDITY SELECTION ON FEMALE LIFE-HISTORY TRAITS

Standardized linear, quadratic, and correlational selection gradients for life-history traits in females are presented in Table 2. There is significant linear fecundity selection on all three life-history traits in females favoring females with longer development times, larger body size but reduced longevity (Table 2). There is also significant positive correlational fecundity selection on development time and longevity and on body size and longevity (Table 2).

The **M** matrix, resulting from the canonical rotation of the γ matrix for females, is presented in Table 3. As shown for males, two of the three eigenvectors experienced significant nonlinear sexual selection (m_{1f} and m_{3f}) (Table 3). However, although m_{3f} has a negative eigenvalue characteristic of stabilizing selection, m_{1f} has a positive eigenvalue characteristic of disruptive selection. The strongest eigenvector of nonlinear sexual selection (m_{3f}) represented the trade-off between increased longevity and reduced development time and body size (Table 3). There was also significant negative linear sexual selection operating on m_{3f} demonstrating that the convex curvature of this eigenvector was not symmetrical within the range of life-history traits sampled. In contrast, m_{1f} represented the trade-off between increased development time and longevity and reduced body size (Table 3, Fig. 2). m_{2f} has a positive eigenvalue characteristic of disruptive selection and largely represents the trade-off between increased longevity and body size and reduced development time (Table 3). However, nonlinear sexual selection acting on this eigenvector was not significant (Table 3). Collectively, the combination of positive (m_{1f}) and negative (m_{2f}) eigenvalues demonstrates that the fitness surface for female life-history traits best resembles a multivariate saddle (Fig. 2).

COMPARISON OF SELECTION BETWEEN THE SEXES

Our sequential model building approach demonstrated that there was an overall significant difference in linear selection between the sexes ($F_{3,265} = 14.67$, $P = 0.0001$) and this was driven predominantly by contrasting selection on longevity in the sexes ($F_{1,253} = 38.41$, $P = 0.0001$). The angle (θ) between the vector of linear selection gradients in males (β_m) and females (β_f) was 127.91° indicating that linear selection was largely opposing in the sexes. There was also a significant difference in quadratic ($F_{3,259} = 4.61$, $P = 0.004$) and correlational ($F_{3,253} = 6.75$,
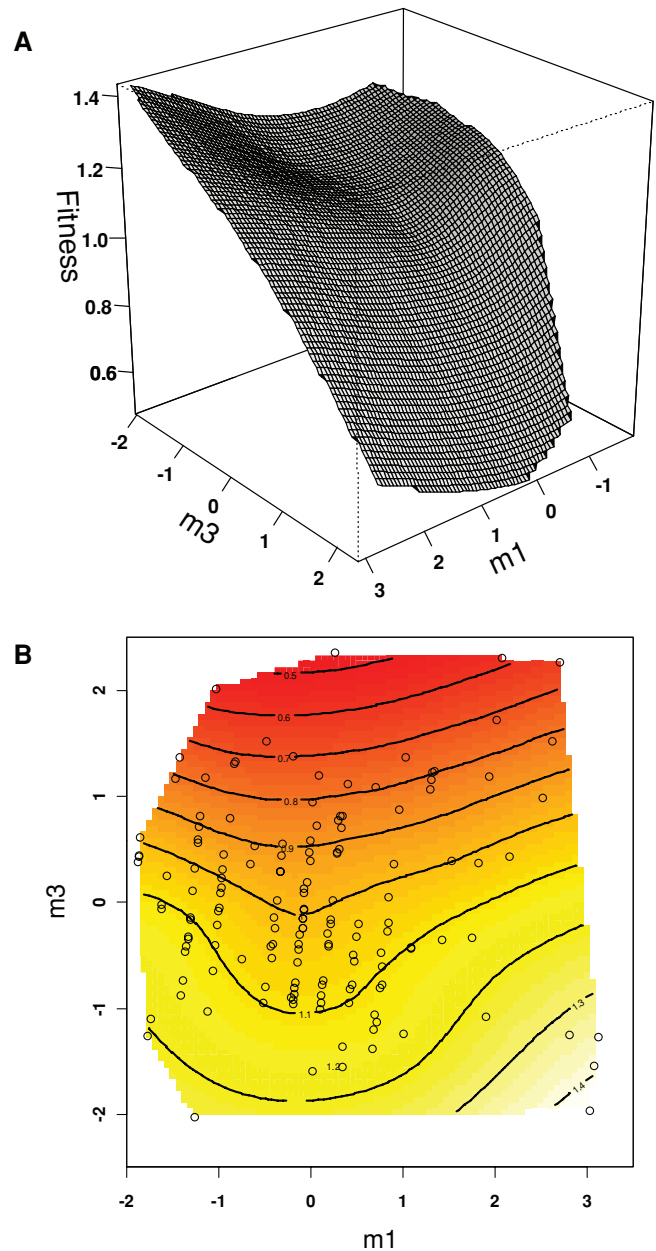

Figure 2. Thin-plate spline (A) perspective-view and (B) contour map visualizations of the fitness surface for the two major axes of nonlinear fecundity selection (m_1 and m_3) for female life-history traits in *Plodia interpunctella*. Female fitness is measured as lifetime fecundity. Actual datapoints for females are overlaid in (B) and yellow color represents regions of high fitness and red color represents regions of low fitness.

$P = 0.0002$) selection operating on life-history traits between the sexes. Inspection of the interaction terms between life-history traits and sex revealed that the overall difference in quadratic selection was driven largely by contrasting selection on development time in the sexes ($F_{1,253} = 5.22$, $P = 0.022$), whereas the overall difference in correlational selection was due to differences in the covariance between development time and longevity

Table 4. Additive genetic variance–covariance matrix (G) for life-history traits in male and female *Plodia interpunctella*. G can be partitioned into four submatrices: the additive genetic (co)variance matrix within males (G_m, top left quadrant) and females (G_f, bottom right quadrant) and the additive genetic covariance between the sexes (*B*, top right quadrant) and its transpose (B^T, bottom left quadrant) (Lande 1980). The subscripts *m* and *f* refer to male and female, respectively, and DT refers to development time.

	DT_m	Longevity$_m$	Size$_m$	DT_f	Longevity$_f$	Size$_f$
DT_m	4.736	0.033	1.520	1.111	−0.180	0.135
Longevity$_m$	0.033	0.124	0.157	−0.780	0.100	−0.125
Size$_m$	1.520	0.157	0.661	0.337	0.013	0.121
DT_f	1.111	−0.180	0.135	0.570	−0.362	0.048
Longevity$_f$	−0.780	0.100	−0.125	−0.362	0.233	−0.035
Size$_f$	0.337	0.013	0.121	0.048	−0.035	0.033

($F_{1,253} = 14.40$, $P = 0.0001$) and development time and body size ($F_{1,253} = 9.44$, $P = 0.002$) in the sexes. The angle (θ) between the dominant eigenvectors of nonlinear selection in males ($\lambda_m = m_{3m}$) and females ($\lambda_f = m_{3f}$) was 76.25°.

GENETIC ARCHITECTURE OF LIFE-HISTORY TRAITS

Table 4 presents **G** for the life-history traits in male and female *P. interpunctella*. Our likelihood ratio test comparing the full multivariate mixed-effect model to a reduced one with the sire term removed showed significant levels of additive genetic (co)variation exists within and between the sexes for these shared life-history traits (difference in −2 log likelihood = 55.20, df = 21, P = 0.00007). Interestingly, we found a large sexual asymmetry in the genetic estimates for development time and body size (Table 4). Most Lepidoptera, including *P. interpunctella*, have a *WZ/ZZ* sex chromosome mechanism with females being the heterogametic sex (Traut and Marec 1996). Thus, whenever the genes affecting a trait are sex linked, the coefficient of relatedness for males is 0.75, whereas it remains 0.5 for females (Lynch and Walsh 1998). Consequently, the high additive genetic variance for development time and body size in males relative to females suggests that these life-history traits may be sex linked in *P. interpunctella*.

PREDICTED RESPONSE TO SELECTION AND THE DEGREE OF GENETIC CONSTRAINT

We combined our estimates of selection and **G** to predict the evolutionary response of life-history traits in the sexes (Table 5). With the exception of female longevity, all other life-history traits were predicted to decrease in response to the observed pattern of sexually antagonistic selection (Table 5). That is, the observed pattern of antagonistic selection and **G** in *P. interpunctella* is predicted to yield shorter development times and smaller body sizes in both sexes but an increase in female longevity and a decrease in male longevity. The angle (θ) between this vector of predicted responses and the actual direction of linear selection was 84.70° suggesting that **G** is preventing antagonistic selection from driving the sexes to their phenotypic optima. This genetic

constraint is also evident when comparing the predicted response to selection of life-history traits when genetic covariance matrix between the sexes (**B**) is estimated directly from our breeding design (R_B) versus when it is set to zero ($R_{B=0}$) to reflect the absence of a genetic constraint (Table 5). In females, all life-history traits were predicted to respond to selection in the opposite direction when there was a genetic constraint versus when this constraint was absent (Table 5). Conversely, with the exception of longevity that showed a similar pattern to females, male life-history traits tended to respond to selection in the same direction with and without **B** posing a genetic constraint, although **B** did greatly reduce the magnitude of this response (Table 5). This suggests that the pattern of sexually antagonistic selection and **B** observed in *P. interpunctella* is likely to drive females further from their phenotypic optima than males.

Discussion

Whenever the same phenotypic traits are expressed in males and females they are likely to have a common genetic basis that prevents these shared traits from evolving independently in the sexes

Table 5. The predicted response of male and female life-history traits in *Plodia interpunctella* to selection when the additive genetic (co)variance matrix between the sexes (B) is estimated directly from our breeding design (R_B) and when it is set to zero ($R_{B=0}$). Responses are presented in units of phenotypic standard deviations and the calculations for the percentage reduction in response are provided in equation (4).

	Females			Males		
	R_B	$R_{B=0}$	%	R_B	$R_{B=0}$	%
Development time	−0.003	0.045	105.783	−0.032	−0.135	76.056
Longevity	0.002	−0.029	107.706	−0.002	0.010	125.203
Size	−0.003	0.005	163.121	−0.013	−0.030	57.548

(Lande 1980). Consequently, if the selection acting on these shared traits differs between the sexes, intralocus sexual conflict will be generated and this may prevent males and females from reaching their sex-specific phenotypic optima (Lande 1980). Here, we show that the angle between the vector of predicted responses and the vector of linear selection approaches orthogonal (84.70°) demonstrating that **G** biases the predicted response of life-history traits in the sexes away from the direction of sexually antagonistic selection in *P. interpunctella*. Moreover, by comparing the predicted response of life-history traits when the intersexual genetic covariance matrix (**B**) is estimated directly from our breeding design versus when it is set to zero, we showed that **B** significantly alters the predicted response of the sexes to selection. This effect was particularly pronounced for females where **B** led to a predicted response that was in the opposite direction for all life-history traits examined, whereas for males **B** largely reduced the magnitude of the predicted response to selection rather than the direction (with the exception of longevity). Taken together, these findings confirm the presence of strong intralocus sexual conflict in *P. interpunctella*. Despite this, we found that all of the life-history traits we examined were sexually dimorphic suggesting that mechanism(s) are likely to have evolved to resolve this conflict and allow the sexes to reach their life-history optima.

To date, the majority of empirical studies have taken the existence of a negative intersexual genetic correlation (r_{MF}) for fitness as evidence of intralocus sexual conflict (Chippindale et al. 2001; Rand et al. 2001; Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Brommer et al. 2007; Foerster et al. 2007). For example, a negative r_{MF} for fitness has recently been shown in natural populations of red deer (*Cervus elaphus*, Foerster et al. 2007) and the collared flycatcher (*F. albicollis*, Brommer et al. 2007). However, although such a result implies that conflict is occurring, it does not locate the shared trait(s) that are mediating the conflict nor does it allow the role that intralocus sexual conflict has played in the evolution of sexual dimorphism to be determined. Knowledge of sex-specific selection and r_{MF} are needed to address these questions. Furthermore, because the sign and strength of r_{MF} for fitness can change during the evolutionary progression of intralocus sexual conflict, the absence of a negative r_{MF} for fitness does not conclusively prove that intralocus sexual conflict is absent (Bonduriansky and Chenoweth 2009). Thus, caution should always be taken when interpreting the strength and sign of r_{MF} and it is likely that an approach that combines both the r_{MF} for fitness, as well as r_{MF} for the trait(s) mediating the conflict, will add considerable value to future empirical studies (Bonduriansky and Chenoweth 2009).

In a recent review of the published literature on wild animal populations, Cox and Calsbeek (2009) found that the median difference between estimates of linear selection in males and females was 0.17 after controlling for spatial and temporal replication within studies. Although this suggests that males and females often differ substantially in the strength and direction of linear selection operating on shared traits, it is important to note that statistically significant sexually antagonistic selection was actually rare being found in only 3% of studies examined and this was heavily weighted by work on a single species (Cox and Calsbeek 2009). The results we present here provide clear statistical evidence that selection operating on life-history traits in *P. interpunctella* is sexually antagonistic and strong in magnitude. The average difference in linear selection gradients between the sexes in our study was 0.139, a value of similar in magnitude to that reported in Cox and Calsbeek (2009). Moreover, our sequential model building approach showed that the sexes differed significantly in linear, quadratic, and correlational selection and we found that the angle (θ) between the vectors of linear (β_m and β_f) and nonlinear selection (λ_m and λ_f) in the sexes was 127.91° and 76.25°, respectively. This illustrates that the sexes are being selected in different directions in multivariate life-history space favoring the divergence of life-history strategies in the sexes. In particular, male *P. interpunctella* were shown to maximize their fitness by developing more rapidly, having a reduced body size and living longer as an adult. In males, faster development is likely to represent the more efficient utilization of resources and this extends adult longevity that provides males with a greater opportunity for mating. In contrast, female fitness is maximized by a longer development time, larger body size, and reduced adult longevity. Larger females tend to lay more eggs and this pattern is well documented in the Lepidoptera (e.g., Jones et al. 1982; Tammaru et al. 1996; Wedell 2006). It is argued that this occurs because large females have more resources available for egg production (García-Barros 2000). Thus, unlike males that are selected to acquire their resources as quickly as possible, it appears that a longer development is needed for females to accrue the resources required for reproduction. Costs of reproduction, such as those expressed through the trade-off between body size, development time, and longevity are common in female insects (e.g., Chapman et al. 1998; Jervis et al. 2005; Partridge et al. 2005) and are particularly likely to be important in species such as *P. interpunctella* that do not feed as adults.

Using our estimates of the antagonistic selection on life-history traits in the sexes and **G**, we predicted the evolutionary response of life-history traits in the sexes over a single generation and showed that the angle between the resulting vector of responses and the vector of linear selection gradients was 84.70° demonstrating that **G** constrains the predicted response of life-history traits in the sexes away from the direction favored by sexually antagonistic selection. Moreover, as an angle of 90° represents an absolute genetic constraint (Blows and Walsh 2007), the magnitude of the genetic constraint we observe in *P. interpunctella* appears to be particularly strong. We also showed that

the constraint imposed by **B** resulted in female life-history traits responding in the opposite direction to that predicted by selection alone (i.e., no constraint), whereas male life-history traits were largely predicted to respond in the same direction as when selection acted alone but to a smaller magnitude. Yet despite this, we found clear evidence that *P. interpunctella* is sexually dimorphic for the life-history traits we examined with females, on average, developing faster, having a reduced longevity and being larger in size at adult eclosion compared to males (Table 1). This finding suggests intralocus sexual conflict has at least been partially resolved in this species. The genetic architecture of traits under persistent sexually antagonistic selection should, in theory, evolve to reduce the genetic constraints on the independent evolution of the sexes, allowing each sex to attain their phenotypic optima (Lande 1980). Various mechanisms are known to facilitate this process and have empirical support, including the sex-specific expression of autosomal loci via sex-linked modifiers (Long et al. 1995; Nuzhdin et al. 1997; Foley et al. 2007) or alternate splicing mechanisms (McIntyre et al. 2006), sex-biased gene expression (Vicoso and Charlesworth 2006; Ellegren and Parsch 2007), gene duplication (Partridge and Hurst 1998; Rice and Chippindale 2001, 2002), and sex-linkage (Rice 1984; Rice and Chippindale 2002; but see Fairbairn and Roff 2006; Rhen et al. 2007). Genomic imprinting (Day and Bonduriansky 2004), condition-dependence (Bonduriansky and Rowe 2005; Bonduriansky 2007), and maternal effects (Foerster et al. 2007) can also theoretically contribute to the resolution of intralocus sexual conflict, but these mechanisms have not yet received the same degree of empirical support (Bonduriansky and Chenoweth 2009). Although we do not know the exact mechanism allowing the sexual dimorphism in life-histories to evolve in *P. interpunctella*, evidence for sex-linkage of fitness-related traits (Sperling 1994; Prowell 1998), including body size (e.g., Grula and Taylor 1980; Stillwell and Davidowitz 2010), appears common in the Lepidoptera. Whether this is the also the case for the other life-history traits studied here in *P. interpunctella* remains unknown. We do know, however, that whatever the mechanism(s), this process can be achieved relatively rapidly, as we have observed evolutionary changes in the individual traits that contribute to fitness in *P. interpunctella* in populations evolving under reproductive environments that favor either males or females in as little as 20 generations of experimental evolution (Ingleby et al. 2010).

Most trait-focused studies of intralocus sexual conflict have taken a univariate approach where the pattern of selection and estimates of genetic (co)variances are examined on a trait-by-trait basis (e.g., Price and Burley 1993, 1994; Merilä et al. 1997, 1998; Long and Rice 2007; Poissant et al. 2008). This is surprising given that selection rarely acts on individual traits in isolation and phenotypic traits are more often than not genetically correlated meaning that they cannot evolve independently (Lande and

Arnold 1983). Blows (2007) showed how such a univariate approach may misrepresent the genetic basis of quantitative traits and the pattern of selection acting on them. This raises the possibility that empirical studies adopting a univariate approach may routinely underestimate the strength and/or complexity of intralocus sexual conflict. This problem is further amplified by the fact that most trait-focused studies typically only measure the strength of linear selection operating on traits and ignore important forms of nonlinear selection (Cox and Calsbeek 2009), that are becoming increasingly common in empirical studies that may also hold important implications for intralocus sexual conflict (Lande 1980). Our work highlights the value of applying a multivariate approach to the study of intralocus sexual conflict. We show that nonlinear sexual selection is an important form of selection acting on male and female life-histories in *P. interpunctella* and that this selection not only differs in direction but also in form across the sexes (Figs. 1 and 2). The major axes of nonlinear sexual selection also present a very different picture of selection acting on the sexes than if only the linear selection gradients were interpreted. For example, the trade-off between male body size and longevity and development time is only apparent after canonical analysis. Similarly, our understanding of how the life-history traits in the sexes are predicted to evolve would be considerably different if only r_{MF} for the shared traits were interpreted. With the exception of development time, r_{MF} for the other two life-history traits were positive but relatively low and this could be interpreted as evidence for weak intralocus sexual conflict over these traits if these traits are viewed independently. However, when examined within a multivariate framework, we showed that both **B** and **G** significantly bias phenotypic evolution in the sexes leading to strong intralocus sexual conflict in this species. Whenever data of this nature are available, we urge authors to consider taking a multivariate approach to the study intralocus sexual conflict.

Our study, like most others attempting to measure fitness empirically, is subject to a number of important caveats (Hunt and Hodgson 2010). Female *P. interpunctella* are moderately polyandrous, mating on average 1.79 times in their lifetime (Cook 1999), yet our measure of male fitness did not include any aspects of postcopulatory sexual selection. In addition, males did not experience competition with other males over matings, which is also likely to influence their reproductive success. It is important to note, however, that pre- and postcopulatory sexual selection are reinforcing in this species with males that are successful at obtaining matings either in the absence or presence of rival males also being superior sperm competitors (Lewis 2005). Similarly, female lifetime fecundity is not affected by the number of mating partners (Lewis 2005), suggesting lifetime fecundity of singly mated females is a good surrogate of female fitness. It might also be argued that the use of laboratory populations to study intralocus sexual conflict may not truly represent the level of conflict found in nature. In

addition to the effect that laboratory rearing may have on the estimation of quantitative genetic parameters (Hoffmann and Parsons 1991), a history of selection under a relatively stable laboratory environment may remove naturally selected fitness variation and thus inflate the importance of intralocus sexual conflict (Chapman et al. 2003). Bonduriansky and Chenoweth (2009) caution that artifactual intralocus sexual conflict can also arise if the transfer of the study organism from the field to the laboratory influences fitness (and thus estimates of selection) in a sex-specific manner. However as *P. interpunctella* is a pest of stored products, the laboratory environment in which we have quantified intralocus sexual conflict closely resembles its natural environment, perhaps more so than many other study species. Moreover, our population of *P. interpunctella* has been maintained under the same environmental conditions for over 9 years (∼80 generations), so the transition from the field to the laboratory is not a new one. Consequently, although the way we have quantified male and female fitness in our study is not without limitations, we believe our central finding of strong intralocus sexual conflict in *P. interpunctella* is largely robust to these potential caveats.

In conclusion, we provide strong evidence that there is intralocus sexual conflict over life-history traits in *P. interpunctella*. Moreover, our finding that the angle between the vector of predicted responses of life-history traits in the sexes and the vector of linear selection approached orthogonal suggests that this conflict is particularly intense in this species and should prevent the sexes from reaching their phenotypic optima. However, we show that the life-history traits mediating the conflict in *P. interpunctella* have evolved to be sexually dimorphic, suggesting that intralocus sexual conflict has been resolved, to some degree, in this species. Interestingly, Cox and Calsbeek (2009) did not find a relationship between the strength of sexually antagonistic selection and the degree of sexual dimorphism in their review suggesting that the evolution of sexual dimorphism is not sufficient to totally remove intralocus sexual conflict, a finding that is entirely consistent with the results of our study.

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