

# Natural selection on body size in *Tribolium*: possible genetic constraints on adaptive evolution

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To determine whether genetic constraints on adaptive evolution were operating in a laboratory population of a flour beetle, *Tribolium castaneum*, we first estimated the direct selection acting on each of several body size traits. Strong selection in males for an increase in pupal weight and a decrease in the ratio of adult to pupal weight occurred. In addition, a non-significant trend for a decrease in adult width was found. No significant selection on females was detected, although there were trends toward an increase in pupal weight and a decrease in adult width. These estimates were then combined with estimates of the genetic variances and covariances of the traits to predict the multivariate response to selection, that is, the evolutionary change in the traits across one generation. These projections showed only a small predicted change in male pupal weight in spite of the strong selection on pupal weight, and a relatively large predicted increase in width in spite of the possible negative direct selection on this trait. Both of these results were due in part to the positive genetic covariance between pupal weight and width, and they therefore suggest the possibility of genetic constraints on adaptive evolution of these traits.

**Keywords:** evolutionary constraint, genetic correlation, natural selection.

## Introduction

A major area of controversy in evolutionary biology has been the extent to which constraints prevent natural selection from producing optimally adapted phenotypes (Alberch, 1982; Mayo, 1983; Maynard Smith *et al.*, 1985; Loeschcke, 1987; Gould, 1989). Two kinds of genetic constraint can slow adaptive evolution. First, low levels of genetic variance limit change across generations in a trait even if there is strong selection within a generation on the trait. Second, genetic correlations can cause constraint through correlated responses to selection (Lande, 1979; Cheverud, 1984; Via & Lande, 1985; Clark, 1987). For example, when two traits are selected in opposite directions, a positive genetic correlation between them can cause constraint by slowing the evolution of the most adaptive combination of the two traits. If only one trait is under selection and the other

is neutral, a genetic correlation between them can cause an evolutionary change in the neutral trait. Although this latter case is not really a constraint *per se*, it is a case of non-adaptive evolution. Few empirical examples illustrating these possibilities exist.

The rate and direction of evolution of a set of phenotypic traits are determined by both the selection on the traits and the additive genetic variances and covariances among them. Therefore, estimates of both are needed to study genetic constraints acting in present-day populations. Selection gradients estimate the direct selection acting on each of a suite of correlated traits, and can be calculated by regressing fitness on all the traits simultaneously (Lande & Arnold, 1983). These selection gradients suggest the combination(s) of traits (within the range of phenotypes measured) that are associated with higher fitness, that is, the combination of traits that are adaptive (for examples see Price, 1984; Grant, 1985; Koenig & Albano, 1987; Conner, 1988; Grant & Grant, 1989). To date, most studies that have estimated selection on several traits simultaneously have not estimated the genetic variances and covariances of the traits.

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Estimates of the additive genetic variances and covariances among traits are important because they indicate the degree to which the changes caused by selection within a generation will be passed on to the next generation. Genetic variances and covariances (or correlations) have now been estimated for a variety of ecologically important traits in a number of organisms (e.g. Via, 1984; Lofsvold 1986; Futuyma & Philippi, 1987; Billington *et al.*, 1988; Garland, 1988; Kohn & Atchley, 1988; Kalisz, 1989), and most studies include at least a qualitative interpretation of the evolutionary effects of the correlations. Most of these studies, however, have not included estimates of the selection acting on the traits.

The estimates of selection and of the genetic variances and covariances can be combined in an equation that predicts the changes in the traits across one generation (Lande & Arnold, 1983):

$$\Delta\bar{z} = G\beta,$$

where the vector of predicted differences between the parental and offspring generations in the mean values of the traits ( $\Delta\bar{z}$ ) is the product of the genetic variance-covariance matrix ( $G$ ) and the vector of selection gradients ( $\beta$ ). A constraint is suggested if the predicted change across a generation for a trait differs from the change within a generation due to direct selection on that trait (estimated by the selection gradient), such that the trait is not evolving in a way that would produce the greatest possible rate of increase in fitness. (It is possible that the rate of adaptive evolution of a trait could be enhanced, rather than constrained, by covariances among traits; see the discussion and Garland, 1988 for possible examples of this.) In other words, if  $\Delta\bar{z}$  (standardized) is smaller in absolute magnitude or differs in sign from  $\beta$  (also standardized), then this suggests a constraint.

A few studies have estimated both the magnitude of selection acting on suites of correlated traits and the genetic variances and covariances of the same traits in the same population (Boag, 1983; Price *et al.*, 1984; Lynch, 1985; Berenbaum *et al.*, 1986; Schluter & Smith, 1986; Schluter, 1986; Rausher & Simms, 1989; Simms & Rausher, 1989), but in only one of these (Lynch, 1985) were quantitative predictions of phenotypic changes made. More studies of this type are needed to assess the importance of genetic constraints on evolutionary change.

One reason for this lack of information is that these kinds of studies are difficult, particularly in the field. This paper reports predictions of evolutionary change in three body size traits in a laboratory population of the flour beetle *Tribolium castaneum*. Clearly, an understanding of evolutionary constraints also requires

field studies, but for a laboratory study this system offers many advantages. *Tribolium* has been a stored-grain pest for thousands of years (Sokoloff, 1972), and both the strain we used and its progenitors have been maintained under similar laboratory conditions for hundreds of generations (see below). Therefore, the laboratory environment is closer to 'natural' for *Tribolium* than for most species. Another advantage of *Tribolium* is its semi-dominant body-colour polymorphisms (Sokoloff *et al.*, 1960; Wade, 1984). These genetic markers make it possible to obtain excellent estimates of fitness in a population context. Finally, *Tribolium* is easy to rear in the large numbers necessary for experimental estimates of genetic variances and covariances, and is well known ecologically and genetically (see King & Dawson, 1972; Sokoloff, 1972, 1974, 1977 for reviews).

## Methods

### General

All beetles were maintained on whole wheat flour with 5 per cent brewer's yeast added, and were kept in an environmental chamber at 30°C and 70 per cent relative humidity (RH). All test individuals were from the 'cSM' strain. This outbred strain has been maintained at population sizes > 500 (Wade, 1977, 1984) since it was synthesized in 1973 by crossing four lines developed in the mid-1950s (Park *et al.*, 1961); at least some of the progenitors of Park's lines had been maintained in the laboratory under the same conditions since the mid-1930s (Park, 1937). Assuming between five and six generations per year, the ancestors of the beetles used in this experiment have been maintained under the same standard conditions for 300 generations. Background populations for the selection estimates (see below) were drawn from cultures of a black colour morph of this strain (Wade, 1984; the wild type is red, and heterozygotes are an intermediate dark red).

### Estimates of selection

*Creating the experimental population.* Four body size traits were measured: live pupal weight, adult dry weight, elytral (wing cover) length, and the width of the pronotum, which covers the dorsal surface of the first thoracic segment. The last two traits were chosen as repeatable measures of body length and width. The direct selection on these traits was estimated from the multiple regression of fitness on the traits (Lande & Arnold, 1983). To enhance statistical power (see Schluter, 1988), we attempted to increase the number of individuals in the tails of the body size distribution

by rearing larvae at five different densities: 5, 10, 20, 40, and 60 larvae/g flour (higher density vials produced smaller beetles). Within 48 h of pupation, pupae were sifted out of the flour and weighed to  $\pm 0.8\mu\text{g}$  with a Cahn 31 microbalance. After weighing, the pupae were placed in individual vials of flour for 6–10 days (depending on when they pupated) to complete development before the experiment began.

We chose 50 male pupae and 30 female pupae to represent as wide and uniform a distribution of weights as possible for the estimates of selection. After log-transformation, however, neither distribution was significantly different from normal (PROC UNIVARIATE, SAS Institute, 1985). We re-calculated the regression models with the rearing density included as an additional (categorical) independent variable to determine if the different rearing densities affected the estimates of selection. In these models, the effect of rearing density was not significant, and the coefficients for the body size traits were unaffected.

*Measuring fitness.* The fitnesses (reproductive success) of the 50 red males were estimated by placing each male in a population of the black morph. One red test male was placed in a vial (containing 10 g of flour) with nine black males and 10 black females, randomly chosen. Thus the test vials were all at very low density (two beetles/g flour), below the density that affects reproduction in *T. castaneum* (Sonleitner, 1961); this is also similar to the adult densities at the beginning of each generation in laboratory culture ( $\sim 1\text{--}2/\text{g}$ ). We were able to distinguish the offspring of the test male because they were the only heterozygotes (with intermediate body colour) produced in the vial. In test crosses, wild type and heterozygotes are produced in Mendelian ratios, indicating no viability differences between these two morphs (Sokoloff *et al.*, 1960; M. Wade, personal communication).

To measure the test males' contributions to each population, periodic samples of the eggs laid in these vials were taken. One sample per week was made for the first month of the beetles' lives, and one every other week for the second month for a total of six samples (all the test males were alive at the end of the experiment). These samples were made by sifting each population out of its vial and placing it in a new vial of flour. After 24 h of mating and oviposition in the sample vials, the populations were returned to their original vials and the sample vials were incubated until the offspring matured. Thus, male fitness was a combination of sexual selection (number of females mated) and fertility (number offspring sired per mating).

The absolute fitness estimate for each test male was calculated as the total number of heterozygotes pro-

duced over the six samples divided by the total number of beetles produced by that individual's population, that is, the proportion of heterozygotes produced. This corrects for differences in productivity of populations, allowing us to determine the fitnesses of the red males relative to the other red males. While random differences among the populations of black males placed in each vial could cause frequency-dependent selection, this would only increase the random error in our models and make the tests for selection conservative. Relative fitness for each test male was calculated as the proportion of heterozygotes produced by the male divided by the mean proportion of heterozygotes produced by all 50 males.

To measure female fitness (egg production), the 30 red females were each maintained in a vial with 5 g flour and two black males. Six 24-h egg samples were taken as described above, except the second sample included 48 h of oviposition due to a power failure (eliminating this sample from the analyses did not change the results). Only the single females were transferred to the sample vials; thus the only egg cannibalism occurring in these vials was females eating their own eggs. Egg cannibalism was likely to be very low anyway, because on average, individual *T. castaneum* eat less than 2 per cent of the eggs available to them in 24 h (Sonleitner, 1961). After the 24-h oviposition period, the females were returned to the original vials containing the males. The eggs laid in the sample vial were sifted out of the flour, counted, and weighed as a group on the microbalance. The relative fitness for each female was calculated as the total number of eggs laid by the female in the six samples divided by the average number of eggs laid by all 30 females.

These experiments estimated one fitness component: the relative number of offspring produced in the first 2 months for each sex. This is likely to be a good estimate of total adult fitness for these populations because the life span of these beetles in the laboratory rarely exceeds 2 months (since the establishment of the cSM strain in 1973, the laboratory cultures have been changed to new flour and most or all of the previous generations discarded every 1.5–2 months).

There are two potential problems with these fitness estimates. The first is that offspring production during the first 2 months of life were not corrected for timing of reproduction. In an expanding population with overlapping generations, offspring produced earlier contribute more to future generations. This causes individuals with early reproduction to have higher fitness relative to individuals reproducing later (Charlesworth, 1980; Lande, 1982; Travis & Henrich, 1986). If, in our populations of *Tribolium*, different-sized

beetles produced the majority of their offspring at different times, then estimates of selection on body size, based on total numbers of offspring, would be incorrect. This was not the case, however. Both large and small males and females produced a constant fraction of their offspring across the six samples (Table 1).

The second potential problem with these fitness estimates is that male fitness was based on the number of offspring surviving to maturity, not the number of zygotes produced (genotype at the body colour locus cannot be determined in the egg; Sokoloff, *et al.*, 1960). In the models we used to estimate selection, offspring survival is a component of offspring, not parental, fitness (Arnold & Wade, 1984). However, because survival from egg to adulthood under the conditions used was high (91 per cent in the parental rearing vials of the same density) any differential juvenile survival that was correlated with the adult traits measured would not have had a major effect on the selection estimates.

At the end of the experiment the test individuals were frozen for later size measurements. To measure dry weight, test beetles were dried for several days in

an oven at 60°C and weighed on the microbalance. The length of the left elytron and the width of the pronotum were measured (to  $\pm 0.012$  and  $\pm 0.007$  mm, respectively) using video-digitizing equipment.

All trait measurements were transformed to natural logarithms before analysis. The ratio of adult to pupal weight was calculated by subtracting log pupal weight from log adult weight. This ratio was used in place of adult weight in the regression to aid in interpretation and to reduce multicollinearity problems (Mosteller & Tukey, 1977, p. 395). With two closely related and highly correlated variables like pupal and adult weight as independent variables in a multiple regression, the regression coefficients estimate the slopes of the relationships of the dependent variable and each independent variable after removing the effects of the other correlated dependent variable. By using the ratio of adult/pupal weight in the regression instead of raw adult weight, we eliminated one highly correlated variable (adult weight was highly correlated to the other three variables whereas the ratio was not; Table 2), thereby reducing multicollinearity problems. This also makes the interpretation more obvious: the regression coefficient for the ratio estimates the strength of selection on adult weight *relative* to pupal weight.

There are some potential problems with using a ratio and the denominator of a ratio in the same analysis. Atchley *et al.* (1976) found a negative correlation between the ratio  $X/Y$  and  $Y$  when  $X$  and  $Y$  are uncorrelated, because  $X$  remains constant as  $Y$  increases, so the ratio decreases. However, this is not necessarily the case if  $X$  and  $Y$  are highly correlated (as adult and pupal weight were in our experiment), because as  $Y$  increases, so does  $X$ , and the ratio stays fairly constant (Hills, 1978). This is demonstrated in our data by the small, non-significant correlation between the ratio adult wt/pupal wt and pupal weight (Table 2). The other major problem with using a ratio in linear models is that ratios are not linear functions of their components; however, the difference between two log-transformed variables *is* a linear function of the transformed variables (Hills, 1978). Using raw adult weight in the regression instead of the ratio did not qualitatively change the results.

The variables were then standardized (mean = 0 and variance = 1) to produce selection gradients expressed in units of phenotypic standard deviations. To model selection on the four traits, the 'complete' quadratic models (four linear terms, four quadratic terms, and six cross-product terms) were fitted using PROC RSREG in SAS (SAS Institute, 1985) using relative fitness as the dependent variable.

PROC RSREG provides tests of the significance of each of the two types of higher-order terms (quadratic and

**Table 1** Comparison of timing of reproduction by small versus large beetles. Small and large refer to the smallest 20 and largest 20 individuals by pupal weight out of 50 total for males, and the smallest and largest 10 out of 30 for females. Values given are the proportion of the total reproduction of the size classes in each week sampled. First half is the proportion of each size classes' offspring produced in the first three samples, and first third and last third are the proportions for the first two and last two samples respectively. For example, both small and large males produced 31 per cent of their offspring in the first two samples, and 30 per cent in the last two; this indicates that the size classes did not differ in their timing of reproduction. The number of eggs laid by females in week two were divided by two before calculating the fractions because the females oviposited for 48 h instead of 24 h in that week

Week	Males		Females	
	Small	Large	Small	Large
1	0.12	0.15	0.15	0.17
2	0.19	0.16	0.21	0.20
3	0.20	0.19	0.17	0.15
4	0.19	0.20	0.10	0.17
6	0.14	0.13	0.18	0.14
8	0.16	0.17	0.19	0.17
First half	0.51	0.50	0.53	0.52
First third	0.31	0.31	0.36	0.37
Last third	0.30	0.30	0.37	0.31

**Table 2** (a) Descriptive statistics (means with standard deviations) for both raw and log-transformed values of the four traits measured in the selection experiment.  
(b) Phenotypic correlations among the traits

(a)	Males ( $n = 50$ )		Females ( $n = 30$ )	
	Raw	Log	Raw	Log
Pupal weight	2.52 (0.32)	0.92 (0.13)	2.61 (0.33)	0.95 (0.12)
Elytral length	2.13 (0.12)	0.76 (0.05)	2.19 (0.12)	0.78 (0.05)
Pronotal width	1.01 (0.05)	0.01 (0.04)	1.03 (0.05)	0.03 (0.05)
Adult dry weight	0.90 (0.12)	-0.12 (0.13)	0.97 (0.14)	-0.04 (0.14)

  

(b)	Pupal weight	Elytral length	Pronotal width
Males			
Elytral length	0.72*		
Pronotal width	0.93*	0.71*	
Adult wt/pupal wt	-0.19	-0.07	-0.20
Females			
Elytral length	0.79*		
Pronotal width	0.94*	0.75*	
Adult wt/pupal wt	0.00	0.28	-0.04

Significance levels corrected for multiple comparisons using a sequential Bonferroni technique (Rice, 1989).

\* $P < 0.001$ .

cross-product) as a group to help decide if these should be retained in the model. The group tests for the quadratic and the cross-product terms were not significant for either males or females, suggesting a lack of stabilizing/disruptive and correlational selection on these traits. (Note, however, that with sample sizes of 50 and 30 our power to detect these forms of selection was low.) Therefore, these terms were dropped from the model and directional selection gradients were calculated as the linear regression coefficients from the multiple regression of relative fitness on the four phenotypic traits using PROC REG in SAS.

Residual plots indicated no serious violations of the assumptions of multiple regression. We checked for multicollinearity using variance inflation factors (VIF), where a VIF above 10 suggests a problem (Neter *et al.*, 1985, p. 392). The VIF for pupal weight in the female fecundity regression was slightly over 10 (10.3); however, eliminating elytral length (which had a regression coefficient of zero) from the model reduced the pupal weight VIF to under nine without changing the regression coefficients of the remaining variables, indicating that these estimates are not seriously affected by multicollinearity. None of the other VIF was over 10.

#### *Estimates of genetic variances and covariances*

A standard quantitative genetic half-sib mating design was implemented in which 20 males (sires) were each mated to four different females (dams). Eggs were collected from the dams at two times (blocks) separated by 6 weeks and six eggs from each dam were raised individually. An average of five offspring per dam survived to adulthood, resulting in an average half-sib family size of 20 and a total of 400 offspring in this analysis. Pupae were weighed and elytral lengths and pronotal widths were measured on the adults as described above (adults were not weighed). The pupae were all weighed at the same stage of development (when eye pigmentation first appeared).

Note that this experiment was conducted separately from the experiment estimating selection described above, using two randomly selected subpopulations from the same laboratory population. [While the beetles used in the selection estimates were chosen based on their size from a larger random sample, their size was virtually random with respect to their genotype due to the different rearing densities (J. Conner & S. Via, unpublished observations).] The means of the

traits measured did not differ significantly between the two populations. All environmental conditions (temperature, humidity, flour medium, etc.) were the same for the two experiments. The only difference between the two experiments was that in the genetic analysis, beetles were all housed singly, not raised at different densities and tested in groups as in the selection estimation experiment. This difference is unlikely to affect our predicted response to selection for two reasons. First, the selection experiments were conducted at a single low density (see above), which is unlikely to be very different from isolation. Second, heritability estimates of body size in *T. castaneum* from beetles housed in group vials agree closely with our estimates from beetles raised in isolation (Enfield *et al.*, 1966; Wong & Boyland, 1970; Enfield, 1980), suggesting that our genetic estimates are valid for the conditions under which we estimated selection.

To test for significant additive genetic variance for the three traits, an ANOVA was performed (PROC GLM, SAS Institute, 1985). The sire variance and covariance components for the three traits within each sex were estimated by equating observed and expected mean squares after the Block effect was removed (PROC NESTED was run on the residuals of a linear model with Block as the only independent variable; SAS Institute, 1985). As the data were nearly balanced, these Type I least-squares estimates should be quite accurate; indeed, the variance components agreed very closely with estimates made using restricted maximum likelihood (REML) methods (Shaw, 1987). We used the least squares method because they enabled us to calculate covariance components using SAS.

The sire covariances across the sexes (e.g. between pupal weight in males and pupal weight in females) were estimated as covariances among half-sib family means. Covariance components cannot be calculated across sexes because each individual can only be one sex, and therefore pairs of traits cannot be measured in the same individual. In this case the phenotypic covariance does not exist, but genetic and environmental covariances do exist and can be estimated (Falconer, 1989, p. 316). This is similar to estimating covariances across environments when each individual experiences only one environment (Via, 1984). All sire variances and covariances were multiplied by four to estimate additive genetic variances and covariances (Falconer, 1989).

The predicted changes in the traits across one generation ( $\Delta\bar{z}$ ) were calculated as the product of the vector of unstandardized selection gradients ( $\beta$ ) and the estimated genetic variance/covariance matrix ( $G$ ). As adult weight was not measured in the genetic analysis, it could not be included in the predictions. There-

fore, for the predictions, the selection gradients were recalculated without the ratio of adult to pupal weight in the model. As this ratio was not highly phenotypically correlated with the other three traits (Table 2), dropping it from the model caused only small changes in the selection gradients for the other three traits. Note that, regardless of statistical significance, all estimated values of the selection gradients were used in the calculation of the predicted change because these values are the best point estimates of the selection on those traits. There is currently no published method to test the statistical significance of the predicted changes in the traits. One possibility is bootstrapping but the proper way to bootstrap this complex model is unknown, because deciding what parameters to resample is not straightforward (Wu, 1986). An even better test of genetic constraint would be to test for differences between  $\Delta\bar{z}$  and  $\beta$ , however, there is no published method for doing this test either.

## Results

### *Estimates of selection*

The directional selection gradients for the male traits reveal significant directional selection both for increased pupal weight and for a lower adult/pupal weight ratio (Table 3, column 1). The negative coefficient for the ratio means that, for a given live pupal weight, males that had a lower dry weight at the end of the experiment had higher fitness. No selection on length was found, and there was a non-significant trend toward selection for decreased pronotal width.

No significant selection on the female traits was detected, although the standardized selection gradients for pupal weight and pronotal width in females were very similar in magnitude to those measured in males (Table 3, col. 2). This may have been due to the smaller sample size used for the females ( $n = 30$ ). There were no relationships between the four traits in females and the mean weight of eggs laid (Table 3, col. 3).

### *Estimates of genetic variances and covariances*

Results of the quantitative genetic analyses show significant additive genetic variance for pupal weight and width, and marginal significance for elytral length (Table 4). The additive genetic variances and covariances are shown in the centre of Table 5. Note that the additive genetic covariances among all traits are positive except that between weight and length in females, which is essentially zero.

The standardized selection gradients (recalculated without the adult weight/pupal weight ratio) are given

**Table 3** Standardized partial regression coefficients (s.e.) from the regressions of various fitness measures (listed across the top) on four phenotypic traits. The dependent variables are: in the first column, the number of offspring of the males that survived to adulthood; in the second column, the number of eggs laid by females; and in the third column, the mean weight of eggs laid by females. All dependent variables were relative fitness values (raw values/mean). The  $r^2$  gives the proportion of variance explained by all four variables, with asterisks indicating the significance level from the  $F$ -test of the overall regression model

	Male	Female	
	Number of offspring	Number of eggs	Mean egg weight
Pupal weight	0.24 (0.09)*	0.21 (0.14)	0.01 (0.03)
Adult wt/pupal wt	-0.10 (0.03)**	-0.02 (0.05)	0.00 (0.01)
Elytral length	0.02 (0.05)	0.00 (0.08)	-0.01 (0.02)
Pronotal width	-0.14 (0.09)	-0.17 (0.13)	0.02 (0.03)
$r^2$	0.42**	0.12	0.21
d.f.	49	29	29

\* $P < 0.01$ ; \*\* $P < 0.005$ .

**Table 4** Results of the analysis of variance testing for significant additive genetic variance in the three traits (i.e. significant variance among half-sib families, the Sire main effect in the analysis). All factors were tested over the error term except 'Sire', which was tested over the nested effect 'Dam'. Type IV  $SS$  are given (SAS Institute, 1985). The  $h^2$  line is the narrow-sense heritability; that is, the proportion of the total phenotypic variance that was additive genetic. Heritability was calculated from the variance components calculated by PROC NESTED as four times the Sire component divided by the sum of all components

Source	d.f.	Pupal weight			Elytral length			Pronotal width		
		SS	F	P	SS	F	P	SS	F	P
Model	81	1.298	2.0	<0.0001	0.230	2.0	<0.0001	0.225	2.0	<0.0001
Block	1	0.085	10.7	0.0012	0.003	2.1	0.1464	0.003	2.2	0.1392
Sex	1	0.180	22.7	<0.0001	0.068	48.3	<0.0001	0.059	42.8	<0.0001
<b>Sire</b>	<b>19</b>	<b>0.388</b>	<b>1.9</b>	<b>0.0281</b>	<b>0.062</b>	<b>1.7</b>	<b>0.0575</b>	<b>0.066</b>	<b>1.9</b>	<b>0.0351</b>
Dam (Sire)	60	0.635	1.3	0.0646	0.113	1.3	0.0641	0.112	1.4	0.0485
Error	292	2.318			0.412			0.399		
$h^2$			0.24			0.18			0.22	

in the last column of Table 5. While unstandardized gradients were used to calculate the predicted changes, standardized gradients are presented for comparison with the standardized predicted changes in the traits across one generation, which are given in the first three columns. The total predicted change was partitioned into two parts, one due only to direct selection on that trait and one (indirect) due to correlated response to selection on other measured traits:

$$\Delta \bar{z}_x (\text{direct}) = V_A(x) \cdot \beta_x$$

$$\Delta \bar{z}_x (\text{indirect}) = \sum_{x \neq y} Cov_A(xy) \cdot \beta_y$$

where  $V_A(x)$  and  $Cov_A(xy)$  are the additive genetic variances and covariances (modified from Lande, 1979

and Lynch, 1985). Note that these two parts sum to equal the total predicted change. The traits are all predicted to increase between 1.1 and 4.9 per cent of a phenotypic standard deviation in one generation (first column, Table 5).

The possibility that the evolution of these morphological traits is genetically constrained is suggested by a comparison of the selection acting within a generation on the traits and the predicted evolutionary change across a generation in those traits. While selection acted to change the mean male pupal weight by a quarter of a standard deviation (standardized  $\beta = 0.26$ ), the total predicted change in pupal weight was only 0.021 of a standard deviation. The predicted change was lowered relative to the strength of selection by two

**Table 5** Predicted changes in the traits across one generation. In the left section are the standardized predicted changes ( $\Delta\bar{z}$ , in units of phenotypic standard deviations), in the centre section are the additive genetic variances and covariances ( $G$ ), and in the right column are the standardized selection gradients recalculated with only three traits per sex ( $\beta$ ). (Unstandardized selection gradients were used to calculate  $\Delta\bar{z}$ , which was then standardized by dividing by the phenotypic standard deviations estimated from the genetic analysis.) The predicted change is partitioned into two parts, the response to direct selection on that trait (direct) and the correlated response to selection on correlated traits (indirect). All calculation were done using log-transformed values

	$\Delta\bar{z}$			$G(\times 10^{-3})$						$\beta$
	Total	Direct	Indirect	Male weight	Male length	Male width	Female weight	Female length	Female width	
Male										
Weight	0.021	0.048	-0.027	2.27	1.12	1.38	3.28	0.84	1.32	0.26
Length	0.047	0.001	0.046		0.41	0.45	1.32	0.48	0.40	0.00
Width	0.049	-0.030	0.079			0.46	1.36	0.30	0.56	-0.12
Female										
Weight	0.042	0.022	0.020				1.25	-0.01	0.36	0.22
Length	0.011	0.000	0.011					0.02	0.14	-0.01
Width	0.028	-0.018	0.046						0.20	-0.16

genetic factors. One was the relatively small genetic variance for weight; only 24 per cent of the phenotypic variance for this trait was attributable to additive genetic variance, so that the predicted response to direct selection was only 0.048 of a standard deviation. The total predicted response was further reduced by the possible negative selection on male width, which was positively correlated with weight (width was responsible for most of the negative indirect predicted change of -0.027).

The total predicted change in male width is positive in spite of a trend toward negative selection on this trait; in fact, the predicted change is considerably greater than that for male weight, which was under positive direct selection. The main reason for this is that the genetic covariance between width and weight is greater than the variance for male width, so the correlated response to the positive selection on weight outweighed the direct response to the negative selection on width. The total predicted changes for male length and female weight and width were all increased over their small or negative direct predicted changes due mainly to their positive genetic covariances with male pupal weight.

## Discussion

This study revealed possible genetic constraints on adaptive evolution in a *Tribolium* population. The evolution of increased male pupal weight favoured by selection was predicted to be slowed considerably by

low genetic variance and a positive genetic covariance with width (which was under negative selection). Only a few other studies have examined both selection on groups of traits and the genetic variances and covariances of the same traits, and some of these results have also suggested the possibility of constraints. In a laboratory study of *Daphnia* (Lynch, 1985), direct selection acted to decrease age at first reproduction. The predicted response to that selection, however, was greatly reduced by selection to increase clutch size, which was positively genetically correlated with age at first reproduction. In a Darwin's ground finch, *Geospiza fortis*, selection favoured increased beak depth and decreased beak width (Price *et al.*, 1984). While predicted responses to the selection were unreported, the strong positive genetic correlation between these two traits (Boag, 1983) suggests a constraint on the evolution of beak shape. The levels of two defensive chemicals (sphondin and percent bergapten) in the seeds of wild parsnip were both selected to increase, but the negative genetic correlation between these traits is likely to constrain the response to that selection (Berenbaum *et al.*, 1986).

Other studies have not found evidence for constraint through genetic correlation, due either to a lack of correlation or to selection acting in the same direction on positively correlated traits. Selection through differential survival acted to decrease tarsus length and increase beak length in song sparrows (Schluter & Smith, 1986a), but since the genetic correlation between these traits was essentially zero (Schluter & Smith,



1986b), a constraint is unlikely. A genetic constraint is also unlikely in the evolution of resistance to different herbivores in morning glories (*Ipomoea purpurea*), because selection acted in the same direction on two traits that were positively genetically correlated (damage by corn earworms and generalist insects; Rausher & Simms, 1989; Simms & Rausher, 1989). In this case the correlation should increase, rather than constrain, the rate of adaptive evolution.

A universal problem with this approach is unmeasured characters, which can affect conclusions about constraints in two ways. The first is that the interpretation of constraints hinges on whether the estimated selection gradient accurately represents a direct, causal relationship between the trait and fitness; that is, adaptive selection. As a number of authors have noted (e.g. Lande & Arnold, 1983; Endler, 1986; Crespi & Bookstein, 1989) this is only true if there are no unmeasured traits that are correlated with the trait of interest and are themselves under selection. Indeed, in this study it is clear that there is not direct selection on male pupal weight through differential adult reproductive success, because pupal weight is not expressed in adults. It is, however, highly correlated with adult body size, and this is probably what the selection gradient for pupal weight reflects. Also interesting in this context is the negative relationship between fitness and adult dry weight relative to pupal weight (discussed further below). Further studies are needed to definitively establish the patterns of adaptive selection on these traits; experimental manipulations of individual traits that leave correlated traits unaltered (if possible) would be especially enlightening (Mitchell-Olds & Shaw, 1987).

Unmeasured traits might also affect the predicted changes themselves. The most important effect may be through selection acting in the larval stage on traits that are genetically correlated with the adult traits of interest. However, measuring the effect on adult traits of selection acting in juvenile stages is a difficult problem in general (Grant, 1986; Conner, 1988; but see Emerson *et al.*, 1988).

#### *Interpretation of selection*

The results showed significant negative selection on the ratio of adult to pupal weight in males. There are several possible interpretations of this result. First, the negative selection could be interpreted as higher reproductive success for those males that lost relatively more weight during the pupal stage and eclosion of the adult. It is not obvious to us why this might be true. Second, pupal weight was a live (wet) weight and adult weight was dry, so the selection could be interpreted as higher

reproductive success for males with a higher percentage of water. Although there could be a physiological advantage to males with higher water reserves in an environment (stored grain) with no free water, we have no direct evidence that this is the case. Finally, pupal weight was measured before the experiment and adult weight after; therefore, a possible interpretation is that those males that had the greatest reproductive effort and thus lost more weight for a given body size had the highest fitness. At the present time we have no data that could distinguish among these hypotheses, so further work is warranted.

The finding of strong directional selection on pupal weight in males is somewhat surprising. As these beetles have been maintained under the same laboratory conditions for hundreds of generations, one might expect weight to have evolved to its optimum. One possible explanation is that our estimates of selection do not reflect the overall selection acting in the past. Another explanation is that unmeasured selection for smaller size at the larval stage is opposing selection for larger size at the adult stage. The overall result could be stabilizing selection for pupal weight, suggesting a population at equilibrium (Endler, 1986). A third explanation is that the evolution of weight is constrained by the genetic factors such as the positive correlation with width, which may be under negative selection. Strong positive correlations, coupled with selection in opposite directions, can theoretically retard evolution to the joint optimum of the two traits for hundreds or even thousands of generations (see examples in Via & Lande, 1985).

Further evidence for these kinds of constraints is provided by studies of artificial selection on pupal weight in *T. castaneum*, which show a decline in population fitness as the population mean pupal weight increases (Enfield, 1980). Population fitness returns to normal in a few generations when selection on pupal weight is relaxed. These results suggest that natural selection may be acting on correlated traits to constrain the evolution of increased pupal weight. More studies that predict the evolutionary change in groups of genetically correlated traits are needed. Particularly valuable would be studies that went on to measure the actual change across several generations to see the effects of genetic constraints in action.

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