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THE PHENOTYPIC AND GENETIC COVARIANCE STRUCTURE OF DROSPHILID WINGS

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Evolutionary constraint results from the interaction between the distribution of available genetic variation and the position of selective optima. The availability of genetic variance in multitrait systems, as described by the additive genetic variance–covariance matrix (G), has been the subject of recent attempts to assess the prevalence of genetic constraints. However, evolutionary constraints have not yet been considered from the perspective of the phenotypes available to multivariate selection, and whether genetic variance is present in all phenotypes potentially under selection. Determining the rank of the phenotypic variance–covariance matrix (P) to characterize the phenotypes available to selection, and contrasting it with the rank of G, may provide a general approach to determining the prevalence of genetic constraints. In a study of a laboratory population of *Drosophila bunnanda* from northern Australia we applied factor-analytic modeling to repeated measures of individual wing phenotypes to determine the dimensionality of the phenotypic space described by P. The phenotypic space spanned by the 10 wing traits had 10 statistically supported dimensions. In contrast, factor-analytic modeling of G estimated for the same 10 traits from a paternal half-sibling breeding design suggested G had fewer dimensions than traits. Statistical support was found for only five and two genetic dimensions, describing a total of 99% and 72% of genetic variance in wing morphology in females and males, respectively. The observed mismatch in dimensionality between P and G suggests that although selection might act to shift the intragenerational population mean toward any trait combination, evolution may be restricted to fewer dimensions.

KEY WORDS: **G-matrix, evolutionary potential, genetic constraints, matrix rank, P-matrix, sexual dimorphism, wing shape.**

The concept that relationships among traits might impose constraints on evolution has a long history (Fisher 1930; Dickerson 1955), and was formalized in Lande's (1979) theoretical work on multivariate adaptation. A key parameter in studies of evolutionary constraint is the matrix of additive genetic variances and covariances, **G**, which summarizes the additive genetic contributions to a suite of traits (Lande 1980). Analysis of the structure of **G** can indicate the extent to which traits are influenced by independent allelic variation, and therefore whether the organization of genetic variation could constrain the multivariate direction of evolution (Lande 1976, 1979, 1982; Cheverud 1982, 1984; Pease and Bull 1988; Zeng 1988; Arnold 1992; Price et al. 1993; Wagner and Altenberg 1996; Mezey et al. 2000; Orr 2000; Arnold et al. 2001; Steppan et al. 2002; Blows and Hoffmann 2005; McGuigan 2006). Of particular interest is the rank of **G**, that is, the number of independent dimensions of variation described by the traits under consideration (Fisher 1930). If **G** is of less than full rank there are trait combinations without additive genetic variance, which may be interpreted as evidence of absolute constraints on phenotypic evolution (Kirkpatrick and Lofsvold 1992; Mezey and Houle 2005; Hine and Blows 2006).

Evolutionary constraint results from the interaction between the distribution of available genetic variation and the position of selective optima (Lande 1979; Lande and Arnold 1983). Therefore, although identifying **G** of less than full rank suggests genetic constraints may exist, it is insufficient for concluding that

the genetic basis of the traits will restrict evolution. Associating **G** with estimated vectors of selection is one way to determine whether there is a genetic constraint on evolution toward a specific optimum (e.g., Blows et al. 2004; Hall et al. 2004; Blows and Hoffmann 2005). However, we are often interested generally in the evolvability of a population, rather than the response to specific, well-characterized optima (e.g., Reed and Frankham 2001). The matrix of phenotypic variances and covariances, **P**, describes the current distribution of a population in phenotypic space, and plays an important role in defining the multivariate phenotype under directional selection (Lande and Arnold 1983; Steppan 1997; Arnold and Phillips 1999)

$$\beta = \mathbf{P}^{-1}\mathbf{s},$$

where β is the vector of directional selection gradients, and ***s*** is the vector of directional selection differentials. In extreme cases of multicollinearity, **P** will be singular (or nearly so), and it will not be possible to estimate β (Lande and Arnold 1983). Similarly, it has been suggested that the minimum requirement of a good analytical trait should be that it describes some variance that is independent of the variance associated with other traits (Lewontin 1978; McCollum 1999; Fristrup 2001). In the context of uncovering genetic constraints, it is important to consider the multicollinearity in the traits under study. For example, if a **G** of less than full rank was found, but **P** was also of less than full rank, then we need to consider the possibility that the multicollinearity in both matrices is a consequence of poor trait choice.

Dimensionality analyses of **P** represent an approach for determining which phenotypic dimensions have statistical support, and therefore for investigating both whether the traits chosen to characterize phenotype are appropriate, and whether the range of phenotypes available to selection is restricted to particular trait combinations. Assuming sensible choice of traits, if **P** is of less than full rank we have evidence of evolutionary constraint, irrespective of the rank of **G**, because not all trait combinations can be sorted by selection. In this way, the rank of **P** can be considered as an upper limit on the dimensionality of **G**, and thus a useful calibration for the investigation of genetic constraint.

The degree of similarity of covariance structure between **G** and **P** is a longstanding controversy in evolutionary biology (Cheverud 1988; Willis et al. 1991; Roff 1995, 1996). **P** is determined both by **G**, and by the environmental trait (co)variances, and therefore **P** and **G** may be expected to be similar either if there is relatively little influence of the environment or if environment and genotype influence traits through the same developmental pathways (Hegmann and DeFries 1970; Cheverud 1984, 1988; Klingenberg and Leamy 2001). Matrix comparisons suggest that **P** and **G** typically describe similar relationships among morphometric traits (Roff 1995, 1996; Koots and Gibson 1996; Klingenberg and Leamy 2001), although not always so (Baker and Wilkinson 2003; Hadfield et al., 2006). One consistently observed difference has been that trait correlations are often of greater magnitude in **G** than in **P** (Cheverud 1988; Waitt and Levin 1998; Arnold and Phillips 1999). This stronger trait covariation coupled with lower total variance may lead to traits contributing less independent information to **G** than to **P**. That is, **G** may consistently be of lower rank than **P**, and therefore genetic constraints on evolution may be common.

Recent analytical developments have opened the way for extending the analysis of the eigenstructure of symmetrical matrices to explicitly test hypotheses of matrix rank (Amemiya 1985; Amemiya et al. 1990; Anderson and Amemiya 1991; Kirkpatrick and Meyer 2004; Meyer and Kirkpatrick 2005; Mezey and Houle 2005; Hine and Blows 2006). For example, Mezey and Houle (2005) bootstrapped eigenvalues of covariance matrices to determine the number of eigenvectors associated with nonzero variance. Hine and Blows (2006) explored the utility of two alternative approaches, one based on factor-analytic modeling within a restricted maximum-likelihood framework (Kirkpatrick and Meyer 2004), and the other approach based on multivariate general linear modeling (Amemiya 1985; Amemiya et al. 1990; Anderson and Amemiya 1991). These studies provide a conflicting picture of populations that either have evolutionary access to all dimensions of phenotypic space (Mezey and Houle 2005), or are largely constrained to a subset of the phenotypic dimensions (Kirkpatrick and Lofsvold 1992; Meyer and Kirkpatrick 2005; Hine and Blows 2006). However, the dimensionality of phenotypic space was not explicitly estimated in any of these studies so it is not clear if there is any mismatch between the range of phenotypes available to selection, and the genetic variation available for evolution.

The Drosophilid wing has emerged as an important model in evolutionary biology (e.g., Weber 1996; Zimmerman et al. 2000; Phillips et al. 2001; Mezey and Houle 2005). Although specific functional effects of subtle variation in size and shape of *Drosophila* wings require further investigation (but see Ennos 1988a,b; Combes and Daniel 2003; Wootton et al. 2003), there is clear evidence that wing size and shape are under selection (Gilchrist et al. 2000; Hoffmann and Shirriffs 2002; Gilchrist et al. 2004; Sisodia and Singh 2004). By studying the wings of *Drosophila bunnanda* we have two experimental aims in this paper. First, we propose a method for estimating the dimensionality of **P** based on repeated measures of traits, a type of data that are commonly used for estimating measurement error in morphometric analyses (e.g., Klingenberg and McIntyre 1998). Using a half-sibling breeding design we estimate **G** for the same 10 wing traits as considered in **P**. Second, we employ factor-analytic modeling to determine the dimensionality of both **P** and **G** to estimate the relative rank of these two covariance matrices.

Materials and Methods

BREEDING DESIGN AND SAMPLE COLLECTION

Drosophila bunnanda is a recently discovered cryptic species endemic to the wet forests of northeast Australia and belonging to the *Drosophila serrata* species complex (Kelemen and Moritz 1999; Schiffer et al. 2004; Schiffer and McEvey 2006). *Drosophila bunnanda* populations have been identified through molecular marker analyses, with a subsequent morphometric comparison revealing divergence in wing size and shape between *D. bunnanda* and the sympatric or parapatric *D. serrata* and *D. birchii* (Schiffer et al. 2004). Like many drosophilids, *D. bunnanda* wings are sexually dimorphic (Schiffer et al. 2004; K. McGuigan, unpubl. data).

Eight female *D. bunnanda* were collected from Cairns, north Queensland, Australia, in May 2002. Isofemale lines were maintained for four generations during which species identity was confirmed based on cuticular hydrocarbon characteristics (M. Higgie, pers. comm.). Isofemale lines were then mixed and the stock kept in two bottles of several hundred individuals, tipped (and mixed) every two weeks. Flies lived on a standard laboratory yeast media (Rundle et al. 2005) at 25°C with 12-h days. To estimate the additive genetic basis of wings we used a paternal half-sibling breeding design in which 122 virgin males were each mated to four virgin females. Wings from two male and two female offspring of each dam were removed with forceps and placed on double-sided tape on a microscope slide. Sampling order was random with respect to sire, dam, and sex. For the purposes of a separate study flies were vortexed in hexane prior to the removal of wings.

Wing images were captured from a Leica MZ6 microscope using a Panasonic digital video camera and the software Video Trace. Nine landmarks were recorded (Fig. 1) using tpsDig2 (Rohlf 2004). Although morphometric data sets are often analyzed using geometric approaches based on aligned landmark coordinates (e.g., Klingenberg and McIntyre 1998; Mezey and Houle 2005), we instead analyzed interlandmark distances for two key reasons. First, aligning landmark coordinates (necessary for analyses of among-individual variation) involves transformations that result in the loss of four degrees of freedom (dimensions) from the data (Rohlf and Slice 1990; Rohlf 1999). This procedural constraint resulting in fewer dimensions than traits seemed an unnecessary complication in the estimation of dimensionality. Second, analysis of nine X-Y coordinate pairs would involve 18 traits. This large number of traits results in prohibitive computational time for the factor-analytic modeling approach taken in this paper. If fewer landmarks were used we lost all information on some wing regions. We note that Mezey and Houle (2005) observed very similar results for analyses of aligned landmark coordinates and of interlandmark distances.

Morpheus (Slice 1998) was used to infer interlandmark distances from the unaligned landmark coordinates. In this paper we analyze 10 traits that describe wing variation. This is a situation in which multicollinearity can arise through measuring the same "trait" several times. Therefore, our aim in the choice of traits was to maximize the opportunity to observe **P** of full rank, avoiding multicollinearity resulting from inappropriate characterization of phenotype. We estimated the linear distance between all pairs of landmarks and from this set of 36 interlandmark distances selected 10 based on the joint criteria of minimizing intertrait correlations (average Pearson's correlation among the chosen 10 interlandmark distances was 0.37) and maximizing coverage of the wing. These linear measures were normally distributed, and analyses were conducted on untransformed data.

One of the traits included in our analysis, trait F (Fig. 1), is typically treated as an estimator of wing (and body) size in *Drosophila* (Partridge et al. 1987). We also estimated centroid size, the geometric morphometric size variable (Rohlf and Slice 1990; Rohlf 1999), using Integrated Morphometrics Package (IMP) (Sheets 2001). Centroid size was not included in the estimation of **P** or **G**, but was reserved as an independent variable to aid in the interpretation of size and shape information contained in eigenvectors of these matrices.

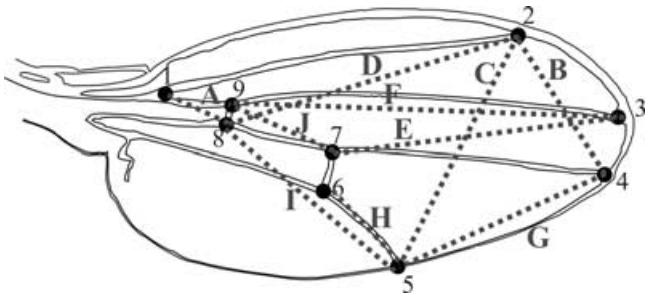

Figure 1. The nine wing landmarks (1–9) recorded to infer the 10 interlandmark distances (grey interrupted lines labeled A–J) analyzed in this study. Wing image is oriented with anterior up, posterior down, distal to the left, and proximal to the right.

PHENOTYPIC COVARIANCE AND THE DIMENSIONALITY OF PHENOTYPIC SPACE

We adopted a repeated measures approach to estimate the phenotypic space described by the 10 wing traits. A total of 16 slides, each with approximately 14 wings, were randomly selected and each wing measured three times. Restricted maximum likelihood (REML) implemented within Proc Mixed in SAS (SAS Institute, Inc., Cary, NC, ver. 9.1) was used to estimate **P** with the model:

$$Y_{ij} = \mu + I_i + R_{j(i)}$$

where individual (I) and replicate measure nested within individual (R) were the sources of variance, and **P** was the individual-level variance–covariance matrix. The eigenstructure of **P** was

determined by principal components analysis (PCA). We took a factor-analytic modeling approach to determining the dimensionality of **P** (Kirkpatrick and Meyer 2004; Hine and Blows 2006). The individual covariance matrix was constrained to be from 10 through one dimensions. A series of nested likelihood-ratio tests were used to determine whether removing a dimension resulted in significant worsening of the fit of the model to the data (Hine and Blows 2006).

P (and the dimensionality of **P**) was estimated separately for males and females. Although *D. bunnanda* wings exhibit sexual dimorphism in trait means, the sexual dimorphism of **P** itself was unknown. We determined whether males and females occupied the same phenotypic space using a likelihood-ratio test (Shaw 1991). The individual-level covariance matrix was constrained to the number of dimensions with the best model fit (as determined above), and the analysis was rerun with data from both sexes. The individual-level covariance parameters were estimated independently for each sex, or constrained to be the same for both sexes. If **P** differs between the sexes then forcing a shared covariance structure will result in a significantly worse fit of the model.

Although this likelihood analysis is a rigorous test of identity of eigenstructure it does not allow us to explore the nature of any identified difference (i.e., differences in eigenvalues vs. eigenvectors: Phillips and Arnold 1999). We compared the eigenvectors of **P** between the sexes using a Krzanowski subspace comparison (Blows et al. 2004). The first five eigenvectors of male and female **P** were used to create the subspaces **A** and **B**, respectively, from which we calculated the matrix $\mathbf{S} = \mathbf{A}^{\mathrm{T}}\mathbf{B}\mathbf{B}^{\mathrm{T}}\mathbf{A}$. **S** finds the minimum angle (i.e., maximum similarity) between the two sets of orthogonal vectors, and the similarity of the two matrices is assessed by examining the sum of the eigenvalues of **S** ($\sum \lambda_{S_i}$), which ranges from zero (no similarity) up to the number of input vectors (identical vectors in **A** and **B**) (Blows et al. 2004). Best linear unbiased predictors (BLUPs) were estimated for each individual for the 10 interlandmark distances using the multivariate unconstrained model, and for centroid size using an unconstrained univariate model. The among-individual variance in principal component scores that was attributable to variation in size was determined by regression analyses.

GENETIC COVARIANCE AND THE DIMENSIONALITY OF GENETIC SPACE

Additive genetic variances and covariances (**G**) were estimated as the sire-level variance component by REML using Proc Mixed in SAS (ver. 9.1) with the model:

$$Y_{ijk} = \mu + S_i + D_{j(i)} + \varepsilon_{ijk},$$

where sire (S) and dam nested within sire (D) were the sources of variance. Again, males and females were analyzed separately, and the eigenstructure of each **G** was determined by PCA. Factor-analytic modeling, in which **G** (the sire-level covariance matrix) was constrained to be 10 through 1 dimensions, was again applied through a nested series of likelihood-ratio tests to determine whether removal of a dimension significantly worsened the model fit (Hine and Blows 2006). Males and females differed in the number of dimensions supported by the factor-analytic modeling (see Results), and we therefore did not use the likelihood-ratio test to determine whether males and females differed in their eigenstructure. However, individual vector correlations were estimated, and a Krzanowski subspace comparison was again made between male and female **G**.

We determined the size variance contained in principal components of **G** through regression of sire BLUPs for the 10 interlandmark distances (multivariate unconstrained model), on sire BLUPS for centroid size (univariate unconstrained model). This analysis was conducted in females only because in males no additive genetic variance for wing size was detected (see Results). We tested whether major eigenvectors of male and female **P** were associated with additive genetic variance by first calculating principal component scores for each offspring from the trait loadings of the two major eigenvectors of **P**. We then used likelihood-ratio tests to compare model fit when the sire variance for the phenotypic PC was estimated versus constrained to be zero. We also estimated the heritability of the major eigenvectors of **P** using the equation

$$h^2 = \frac{v'\mathbf{G}v}{v'\mathbf{T}v},$$

(Kennedy et al. 1993) where $\boldsymbol{v}$ is the column vector of interest (here, PC1 or PC2 of **P**), **G** is the additive genetic variance (four times the sire [co]variance matrix), and **T** is the total variance (sum of sire, dam, and error [co]variance matrices).

Results

PHENOTYPIC COVARIANCE AND THE DIMENSIONALITY OF PHENOTYPIC SPACE

Three repeated measures of wing landmarks were obtained for 96 males and 115 females, and **P** was estimated (Table 1). Measurement error associated with the 10 traits ranged from 3.7% to 18.0% (mean: males = 9.5%; females = 8.9%). Factor-analytic modeling supported the hypothesis of 10 dimensions in the individual phenotypic (co)variance matrices (Table 2; difference between 10 and nine dimensions: males $\chi^2 = 28.9$, df = 1, $P < 0.001$; females $\chi^2 = 58.4$, df = 1, $P < 0.001$). That is, each wing trait contributed some information about the phenotypic distribution of the population that was independent of the information provided by all other traits.

Table 1. Male (above the diagonal) and female (below the diagonal) phenotypic variances (in bold) and covariances. Refer to Figure 1 for traits.

a	b	c	d	e	f	g	h	i	j
12.80	2.94	7.00	0.74	2.30	0.91	4.57	−1.78	−2.97	−0.87
	14.07	6.63	−3.36	7.59	5.74	3.70	1.82	0.22	−1.13
18.17		**27.31**	14.29	9.81	11.09	15.85	2.12	5.25	3.14
2.84	**10.82**		**34.89**	12.33	25.48	7.22	6.26	17.86	12.10
7.29	6.95	**30.03**		**27.96**	19.39	14.09	12.68	6.27	−8.67
0.38	1.82	23.44	**45.69**		**30.36**	13.75	8.06	17.71	10.14
2.28	4.78	14.85	26.74	**36.75**		**29.61**	−0.50	1.60	1.11
1.10	7.26	17.93	40.92	32.43	**49.95**		**15.19**	9.83	−5.20
5.88	6.06	22.43	20.71	21.33	25.17	**33.95**		**21.15**	11.15
−0.55	0.39	0.45	5.16	10.04	6.01	−3.26	**12.56**		**18.82**
−1.27	2.56	6.12	20.79	10.74	24.06	3.50	7.83	**21.44**	
−0.33	3.16	5.34	14.82	−3.46	17.83	5.62	−3.94	13.52	**21.47**

Fitting the restricted maximum-likelihood models to sexes separately significantly improved the model fit over constraining the sexes to share the same covariance structure at the individual level ($\chi^2 = 149.8$, df $= 55$, $P < 0.001$). The eigenvalues of male and female **P** were similar, although greater for females on the first eigenvector (Table 3). Comparing a five-dimensional subspace using Krzanowski's method, the eigenvalues of **S** summed to 4.41 of a possible 5.00. Thus, very similar information was contained in the five-dimensional subspace of male and female **P** matrices. These first five vectors accounted for 91% of the phenotypic variance in males, and 93% in females.

Many eigenvectors of male **P** could be readily identified in the female **P** (or visa versa). In particular, the first two eigenvectors had intersex vector correlations of 0.99 and 0.91, respectively, and

Table 2. Model fit statistics of the number of dimensions of the individual-level covariance matrix, P, and the sire-level covariance matrix, G.

Level	No. parameters	Males		Females	
		−2LL	AIC	−2LL	AIC
P					
fa0(10)	110	11738.6	11958.6	14110.3	14330.3
fa0(9)	109	11767.5	11985.5	14168.7	14386.7
fa0(8)	107	11820.1	12032.1	14300.5	14514.5
fa0(7)	104	11943.3	12151.3	14487.1	14693.1
fa0(6)	100	12116.8	12316.8	14693.7	14893.7
fa0(5)	95	12348.6	12536.6	15041.3	15229.3
fa0(4)	89	12653.4	12831.4	15379.1	15557.1
fa0(3)	82	13042.4	13206.4	15757.2	15921.2
fa0(2)	74	13491.7	13639.7	16278.7	16426.7
fa0(1)	65	14027.4	14157.4	16897.2	17027.2
G					
fa0(10)	165	31697.3	32025.3	39315.4	39639.4
fa0(9)	164	31708.3	32032.3	39315.4	39639.4
fa0(8)	162	31708.3	32032.3	39315.4	39635.4
fa0(7)	159	31708.3	32030.3	39315.4	39631.4
fa0(6)	155	31708.3	32026.3	39317.6	39621.6
fa0(5)	150	31708.5	32018.5	39320.6	39620.6
fa0(4)	144	31709.8	32009.8	39333.3	39617.3
fa0(3)	137	31712.4	32000.4	39357.9	39625.9
fa0(2)	129	31717.7	31991.7	39377.9	39631.9
fa0(1)	120	31724.9	31982.9	39402.7	39642.7

Table 3. First two principal components of P and of G for males and females (refer to Fig. 1 for traits).

	P PC1		**P** PC2		**G** PC1		**G** PC2	
	Male	Female	Male	Female	Male	Female	Male	Female
λ	101.71	155.55	45.08	41.09	6.51	15.71	3.81	12.66
%	43.81%	55.39%	19.41%	14.63%	45.23%	44.05%	26.49%	35.48%
a	0.05	0.04	0.20	0.31	0.27	−0.01	0.00	−0.24
b	0.09	0.08	0.26	0.11	0.22	0.20	−0.46	−0.16
c	0.33	0.30	0.25	0.39	0.50	0.31	0.25	−0.17
d	0.49	0.50	−0.35	−0.18	0.20	0.35	0.76	0.50
e	0.37	0.39	0.41	0.23	0.47	0.61	−0.27	−0.23
f	0.51	0.54	−0.10	−0.22	0.07	0.44	−0.17	0.36
g	0.31	0.34	0.42	0.49	0.47	0.33	0.03	−0.20
h	0.17	0.07	0.05	−0.12	0.02	0.15	−0.19	−0.14
i	0.32	0.25	−0.36	−0.45	−0.20	0.13	0.05	0.33
j	0.14	0.17	−0.47	−0.40	−0.32	−0.13	0.12	0.53

these vectors are interpreted as being the same in both sexes. The first eigenvector of **P** described contributions of all 10 traits in the same direction (Table 3), a characteristic of a vector describing size. This interpretation of a size vector was further supported by the strongest contribution coming from trait F, a commonly used indicator of wing (and body) size (Partridge et al. 1987; Table 3). Regression analysis of the individual BLUPs revealed almost all of the variation in PC1 could be explained by variation in centroid size (males: $F_{1,94} = 2019.84$, $r^2 = 0.955$, $P < 0.001$; females: $F_{1,113} = 2453.57$, $r^2 = 0.956$, $P < 0.001$). Centroid size did not account for a significant amount of variation in any other principal component. The second major axis of phenotypic variation in both males and females was determined by strong contrasting contributions from several traits (Table 3), apparently describing variation in the shape of the distal part of the wing. The posterior wing tip is expanded or contracted at the expense of the proximal region of the wing.

GENETIC COVARIANCE AND THE DIMENSIONALITY OF GENETIC SPACE

A total of 1384 flies were characterized for the 10 wing traits, consisting of 625 males from 103 sires and 759 females from 114 sires. Additive genetic (co)variances were estimated separately for males and females (Table 4). Factor-analytic modeling revealed support for only two of the possible 10 dimensions of the sire effect space in males (Table 2; from two to one dimensions: $\chi^2 = 19.6$, df = 9, $P < 0.021$). However, in females the hypothesis of five dimensions was accepted (Table 2; from five to four dimensions: $\chi^2 = 12.7$, df = 6, $P = 0.048$). The supported eigenvectors of **G** explained 72% of the variance in males, and 99% in females (Table 3).

Eigenvalues of **G** were an order of magnitude lower than those of **P**, and eigenvalues of the female **G** were about three times those of the male **G** (Table 3). Intersex differences in eigenvectors were also greater in **G** than **P**, but substantial similarity in trait relationships was still indicated by a sum of the eigenvalues of **S** of 3.65 out of 5.00. These first five eigenvectors of **G** accounted for 96% of the variance in males, and 99% in females. Intersex correlations were relatively low for the major eigenvectors of **G** (0.76 and 0.51 for PC1 and PC2, respectively), so we interpret these vectors differently for each sex.

In females, regression of sire principal component scores (estimated from the 10 trait sire BLUPs) onto the centroid size sire BLUPs indicated some variation in both PC1 and PC2 could be attributed to variation in wing size (PC1: $F_{1,112} = 159.70$, $r^2 = 0.584$, $P < 0.001$; $F_{1,112} = 7.63$, $r^2 = 0.055$, $P = 0.007$). However, substantial variation on these two principal components remained unexplained by size variation (42% on PC1 and 95% on PC2). No other PCs described size variation. Shape variation associated with the first PC of **G** describes increased (or decreased) length of the wing tip without change in the proximal region, whereas PC2 described variation involving increased (decreased) depth of the wing tip, with little or opposing change in the proximal region (Table 3).

Although PC1 of **P** describes almost no shape variation while PC1 of **G** describes shape and size variation the correlation between PC1 of **G** and PC1 **P** was 0.90, and the correlation between **G** PC2 and **P** PC2 was 0.82. The similarity index from the five-dimensional Krzanowski subspace was 3.89, suggesting that considerable trait information was shared between **P** and **G** in females. Both of the first two eigenvectors of the female **P** were associated with additive genetic variance (PC1: $\chi^2 = 19.8$, df = 1, $P < 0.001$; PC2: $\chi^2 = 40.2$, df = 1, $P < 0.001$) corresponding to heritabilities of 0.535 and 0.820, respectively.

In males there was no additive genetic variance for the wing size trait F (Table 4), or for the first principal component of

Table 4. Male (above the diagonal) and female (below the diagonal) genetic variances (in bold) and covariances. Refer to Figure 1 for traits.

a	b	c	d	e	f	g	h	i	j
1.48	0.27	0.88	0.14	0.65	−0.30	0.44	−0.21	−0.59	−0.79
	1.43	0.37	−0.82	1.15	0.71	0.47	0.49	−0.14	−0.45
1.03		**2.13**	1.31	1.10	−0.06	1.52	0.14	−0.39	−0.93
0.10	**2.07**		**2.55**	−0.22	−0.45	0.69	−0.59	−0.21	−0.11
0.47	1.42	**2.57**		**1.63**	0.06	1.54	0.00	−0.97	−1.37
−1.55	−0.97	0.58	**6.17**		**.00**	0.82	−0.33	−0.38	−0.11
0.65	2.20	3.11	2.12	**6.84**		**2.01**	0.05	−0.35	−0.45
−1.08	1.15	1.28	4.22	3.15	**4.94**		**0.48**	−0.01	−0.36
0.59	1.79	2.57	0.18	3.38	1.54	**3.58**		**0.25**	0.45
0.39	0.70	0.58	0.43	2.22	0.21	−0.02	**1.55**		**1.05**
−1.01	−0.08	−0.39	2.62	0.48	2.63	−0.42	−0.02	**1.86**	
−1.59	−0.74	−1.36	1.90	−3.12	1.89	−1.25	−1.83	1.96	**4.68**

P ($\chi^2 = 0.40$, df = 1, $P = 0.527$). The vector correlation between PC1 of **P** and PC1 of **G** was 0.54; however, the correlation between PC2 of **P** and PC1 of **G** was 0.77, and this second eigenvector of **P** was associated with significant additive genetic variance ($h^2 = 0.448$; $\chi^2 = 9.5$, df = 1, $P = 0.002$). Thus, the major axis of additive genetic variance in male wings does not describe variation in total wing size, rather is influenced by expansion of the posterior wing tip at the expense of the rest of the wing (or visa versa: Table 3). The correlation between PC2 of **G** and PC3 of **P** in males was weaker (0.42: no improvement through vector order rearrangement), and this phenotypic eigenvector was not associated with additive genetic variance ($\chi^2 = 1.1$, df = 1, $P = 0.294$). Despite this, the five-dimensional Krzanowski subspace shared between **G** and **P** was 3.76, indicating the matrices shared similar trait relationships. The second vector of the male **G** was dominated by trait D, and appeared to describe variation in the depth of the centre of the wing at the expense of the distal and proximal regions (Table 3).

Discussion

Identification of constraints on the potential for a population to respond to future selection is a central endeavor in evolutionary biology and related disciplines. To date, research on constraint has focused on trait interactions from the perspective of bivariate genetic correlations or the relationship between additive genetic variance and specific selection vectors (Blows and Hoffmann 2005). In this paper we followed a more inclusive approach, inferring constraint based on the rank of phenotypic and genetic covariance matrices (Kirkpatrick and Lofsvold 1992; Mezey and Houle 2005; Hine and Blows 2006). We addressed the related questions of whether a laboratory population of *D. bunnanda* was limited in the directions that selection could act, and whether there was genetic variance available for an evolutionary response to selection in any direction of phenotypic space. We observed full rank of **P**-matrices suggesting that selection could sort among flies based on any combination of individual trait values, and that irrespective of the position of an adaptive optimum selection could shift the population toward that peak. However, we were unable to demonstrate the availability of additive genetic variance for all dimensions of the phenotypic space, suggesting that some directions of phenotypic space were not evolutionarily accessible.

Our choice of traits in this study was made to maximize the opportunity to observe a **P** of full rank. We achieved this aim, but it remains an open question whether **P** are typically of full rank, and what factors will influence the dimensionality of phenotypic space. Our intention in directing our research toward a full rank **P** was to maximize the potential for detecting a difference in rank between **P** and **G**. Our results again suggest we achieved this aim, but two aspects of our approach caution against concluding very low dimensionality of the *D. bunnanda* **G**, particularly in males. First, the simulation results of Hine and Blows (2006) suggested factor-analytic modeling may underestimate dimensionality when heritabilities are low to moderate, and when sample sizes are small. We detected only two genetic dimensions in males, as opposed to five in females. This result is consistent with our lower statistical power in males (smaller sample size, and lower heritabilities: average was 0.51 in females and 0.23 in males). Second, although we can statistically demonstrate the existence of some dimensions of **G**, we cannot achieve the converse and statistically prove the absence of variation for some trait combinations (see Mezey and Houle 2005). We were able to demonstrate statistical support for 99% of the additive genetic variance in females, leaving only 1% of the variance to be accounted for by the remaining five dimensions. In males, we demonstrated support for 72% of the additive genetic variance. If the remaining variance is evenly distributed

among the remaining eight dimensions in males each dimension would be associated with only 3.5% of the additive genetic variance. We interpret our results as providing evidence that **G** is likely to be of less than full rank, at least in the functional sense that some trait combinations are likely to display relatively little additive genetic variance (as distinct from a strict statistical sense in which some eigenvalues are demonstrably zero).

Two other studies of **G** have reported lack of support for matrices of full rank (Kirkpatrick and Lofsvold 1992; Hine and Blows 2006). In contrast, Mezey and Houle (2005) determined wing shape in a Florida population of *D. melanogaster* was associated with **G** of full rank. Hine and Blows (2006) suggested that the dimensionality-estimating bootstrapping method employed by Mezey and Houle (2005) would overestimate the number of independent traits because of inconsistency between bootstrap replicates in the estimated eigenvectors, and the reordering of eigenvectors associated with similar amounts of variance. Nevertheless, Mezey and Houle's (2005) sampling design was large (149 sires, 567 dams, and 16,615 offspring), and it is therefore possible the high rank observed in their study was partly a consequence of being able to detect dimensions associated with very small genetic variances. Conversely, our lack of support for full rank of **G** may reflect lack of statistical power, or population history (our population was initiated from eight females). More studies are needed to determine whether **G** typically underlies all, or only part of the phenotypic space.

A limitation of our approach to characterizing genetic constraints is that it assumes selection will act on phenotypic variants already in the population. It is possible that the fittest trait mean lies outside the current phenotypic distribution of the population. Such a situation can only be addressed by measuring selection through approaches that incorporate phenotypic manipulations (e.g., Moore and Moore 1999). In this paper we have considered standing phenotypic and genetic variation of our population. In natural populations, novel phenotypic variation is generated through mutation or migration. If shared developmental processes preclude independent mutational targets for each trait then **G** will always describe fewer dimensions than traits. Such absolute constraints on trait evolution could be demonstrated through testing the dimensionality of the matrix of additive mutational variances and covariance, **M**. If the dimensionality of **M** is lower than that of **P** then multivariate evolution over any time frame cannot shift the population toward particular optima. Higher rank of **M** than of **G** is consistent with depletion of additive genetic variance through drift or selection (Turelli 1988; Phillips et al. 2001). Estimating **M** is a serious challenge in evolutionary biology (Lynch and Walsh 1998), and our ability to rigorously test the dimensionality of estimates of **M** is likely to be extremely limited. Comparison of the dimensionality, as well as the eigenstructure, of **G** among naturally evolving or manipulated populations is a feasible alternative approach for exploring the evolution of genetic constraints (Steppan et al. 2002; McGuigan 2006).

In our population of *D. bunnanda* males and females differed only subtly in the phenotypic space they occupied, with more pronounced sex differences in additive genetic variance. Although sex-specific wing **G** might occur, our observed differences could be readily attributed to the experimental design. Estimating **G** from a paternal half-sibling in *Drosophila* ignores the contribution of X-linked loci in males, but not in females. Our estimate of genetic variance for longitudinal vein 3 (Trait F) was zero in males, but nonzero in females, and PC1 of **P** (a multitrait estimate of wing size) had a heritability of 54% in females, but 9% in males. Substantial X-linked additive genetic variance in wings size (but not shape) has been observed in *D. melanogaster* (Cowley et al. 1986), suggesting our observations were due to our breeding design. Kellermann et al. (2006) detected significant additive genetic variance in wing size for *D. bunnanda* using a parent–offspring breeding design, but because their analysis was conducted on pooled males and females it is not possible to conclusively attribute our observed lack of variance for male wing size to our half-sibling breeding design.

We detected both phenotypic and genetic variation for wing size and shape in *D. bunnanda*, with much of the shape variation associated with changes in the wing tip, rather than in more proximal regions. Changes in the wing tip account for much of the wing shape variation and divergence reported for *Drosophila* (e.g., Gilchrist et al. 2000; Klingenberg and Zaklan 2000; Hoffmann and Shirriffs 2002). Although **P** and **G** described similar subspaces, variation in wing size was greater in **P** than **G**, consistent with wing size being highly sensitive to environmental variance (e.g., French et al. 1998). In addition, size and shape variation were relatively independent in **P** (PC1 vs. PC2), but not in **G** (both PC1 and PC2 of the female **G** contain size and shape variation), which further suggests strong environmental, but not genetic, effects on total wing size. We are continuing to explore factors influencing the evolution of wings in *D. bunnanda*.

In summary, determining the prevalence of genetic constraints in multitrait systems requires knowledge of both the distribution of genetic covariance and the direction in which selection may operate. Unfortunately, in many cases we will be ignorant of the direction of selection under natural conditions. In such cases, determining the dimensionality of **G** will only be informative as an indication of genetic constraint if it can be demonstrated **G** does not span the full phenotypic space of the traits. Thus, using the rank of **P** to characterize the range of phenotypes available to selection, and calibrating the dimensionality of **G** by contrasting it with the rank of **P** potentially provides a general approach to determining how common genetic constraints might be.

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LITERATURE CITED

Amemiya, Y. 1985. What should be done when an estimated between group covariance matrix is not nonnegative definite. Am. Stat. 39:112–117.

Amemiya, Y., T. W. Anderson, and P. A. W. Lewis. 1990. Percentage points for a test of rank in multivariate components of variance. Biometrika 77:637–641.

Anderson, T. W., and Y. Amemiya. 1991. Testing dimensionality in the multivariate-analysis of variance. Stat. Probabil. Lett. 12:445–463.

Arnold, S. J. 1992. Constraints on phenotypic evolution. Am. Nat. 140:S85–S107.

Arnold, S. J., and P. C. Phillips. 1999. Hierarchical comparison of genetic variance–covariance matrices. II. Coastal-inland divergence in the garter snake, *Thamnophis elegans*. Evolution 53:1516–1527.

Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macro-evolution. Genetica 112:9–32.

Baker, R. H., and G. S. Wilkinson. 2003. Phylogenetic analysis of correlation structure in stalk-eyed flies (*Diasemopsis*, Diopsidae). Evolution 57:87–103.

Blows, M. W., S. F. Chenoweth, and E. Hine. 2004. Orientation of the genetic variance–covariance matrix and the fitness surface for multiple male sexually selected traits. Am. Nat. 163:329–340.

Blows, M. W., and A. A. Hoffmann. 2005. A reassessment of genetic limits to evolutionary change. Ecology 86:1371–1384.

Cheverud, J. M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution 36:499–516.

———. 1984. Quantitative genetics and developmental constraints on evolution by selection. J. Theor. Biol. 10:155–171.

———. 1988. A comparison of genetic and phenotypic correlations. Evolution 42:958–968.

Combes, S. A., and T. L. Daniel. 2003. Flexural stiffness in insect wings I. Scaling and the influence of wing venation. J. Exp. Biol. 206:2979–2987.

Cowley, D. E., W. R. Atchley, and J. J. Rutledge. 1986. Quantitative genetics of *Drosophila melanogaster*.1. Sexual dimorphism in genetic parameters for wing traits. Genetics 114:549–566.

Dickerson, G. E. 1955. Genetic slippage in response to selection for multiple objectives. Cold Spring Harb. Sym. 20:213–224.

Ennos, A. R. 1988a. The importance of torsion in the design of insect wings. J. Exp. Biol. 140:137–160.

———. 1988b. The inertial cause of wing rotation in Diptera. J. Exp. Biol. 140:161–169.

Fisher, R. A. S. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford, U.K.

French, V., M. Feast, and L. Partridge. 1998. Body size and cell size in *Drosophila*: the developmental response to temperature. J. Insect Physiol. 64:1081–1089.

Fristrup, K. M. 2001. A history of character concepts in evolutionary biology. Pp. 13–35 *in* G. P. Wagner, ed. The character concept in evolutionary biology. Academic Press, San Diego, CA.

Gilchrist, A. S., R. B. R. Azevedo, L. Partridge, and P. O'Higgins. 2000. Adaptation and constraint in the evolution of *Drosophila melanogaster* wing shape. Evol. Dev. 2:114–124.

Gilchrist, G. W., R. B. Huey, J. Balanya, M. Pascual, and L. Serra. 2004. A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. Evolution 58:768–780.

Hadfield, J. D., A. Nutall, D. Osorio, and I. P. F. Owens. 2006. Testing the phenotypic gambit: phenotypic, genetic and environmental correlations of colour. J. Evol. Biol. 20:549–557.

Hall, M., A. K. Lindholm, and R. Brooks. 2004. Direct selection on male attractiveness and female preference fails to produce a response. BMC Evol. Biol. 4:1.

Hegmann, J. P., and J. C. Defries. 1970. Are genetic correlations and environmental correlations correlated? Nature 226:284–286.

Hine, E., and M. W. Blows. 2006. Determining the effective dimensionality of the genetic variance–covariance matrix. Genetics 173:1135–1144.

Hoffmann, A. A., and J. Shirriffs. 2002. Geographic variation for wing shape in *Drosophila serrata*. Evolution 56:1068–1073.

Kennedy, B. W., J. H. J. Vanderwerf, and T. H. E. Meuwissen. 1993. Genetic and statistical properties of residual feed intake. J. Anim. Sci. 71:3239–3250.

Kelemen, L., and C. Moritz. 1999. Comparative phylogeography of a sibling pair of rainforest *Drosophila* species (*Drosophila serrata* and *D. birchii*). Evolution 53:1306–1311.

Kellermann, V. M., B. van Heerwaarden, A. A. Hoffmann, and C. M. Sgró. 2006. Very low additive genetic variance and evolutionary potential in multiple populations of two rainforest *Drosophila* species. Evolution 60:1104–1108.

Kirkpatrick, M., and D. Lofsvold. 1992. Measuring selection and constraint in the evolution of growth. Evolution 46:954–971.

Kirkpatrick, M., and K. Meyer. 2004. Direct estimation of genetic principal components: simplified analysis of complex phenotypes. Genetics 168:2295–2306.

Klingenberg, C. P., and L. J. Leamy. 2001. Quantitative genetics of geometric shape in the mouse mandible. Evolution 55:2342–2352.

Klingenberg, C. P., and G. S. McIntyre. 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with procrustes methods. Evolution 52:1363–1375.

Klingenberg, C. P., and S. D. Zaklan. 2000. Morphological integration between developmental compartments in the *Drosophila* wing. Evolution 54:1273–1285.

Koots, K. R., and J. P. Gibson. 1996. Realized sampling variances of estimates of genetic parameters and the difference between genetic and phenotypic correlations. Genetics 143:1409–1416.

Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. Evolution 30:314–334.

———. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. Evolution 33:402–416.

———. 1980. The genetic covariances between characters maintained by pleiotropic mutations. Genetics 94:203–215.

———. 1982. A quantitative genetic theory of life history evolution. Ecology 63:607–615.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:110–126.

Lewontin, R. C. 1978. Adaptation. Sci. Am. 239:157–169.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates, Inc., Sunderland, MA.

McCollum, M. A. 1999. The robust australopithecine face: a morphogenetic perspective. Science 284:301–305.

McGuigan, K. 2006. Studying phenotypic evolution using multivariate quantitative genetics. Mol. Ecol. 15:883–896.

Meyer, K., and M. Kirkpatrick. 2005. Restricted maximum likelihood estimation of genetic principal components and smoothed covariance matrices. Genet. Sel. Evol. 37:1–30.

Mezey, J. G., J. M. Cheverud, and G. P. Wagner. 2000. Is the genotype-phenotype map modular? A statistical approach using mouse quantitative trait loci data. Genetics 156:305–311.

Mezey, J. G., and D. Houle. 2005. The dimensionality of genetic variation for wing shape in *Drosophila melanogaster*. Evolution 59:1027–1038.

Moore, A. J., and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. Proc. R. Soc. Lond. B 266:711–716.

Orr, H. A. 2000. Adaptation and the cost of complexity. Evolution 54:13–20.

Partridge, L., A. Hoffmann, and J. S. Jones. 1987. Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. Anim. Behav. 35:468–476.

Pease, C. M., and J. J. Bull. 1988. A critique of methods for measuring life history trade-offs. J. Evolution Biol. 1:293–303.

Phillips, P. C., and S. J. Arnold. 1999. Hierarchical comparison of genetic variance–covariance matrices. I. Using the Flury hierarchy. Evolution 53:1506–1515.

Phillips, P. C., M. C. Whitlock, and K. Fowler. 2001. Inbreeding changes the shape of the genetic covariance matrix in *Drosophila melanogaster*. Genetics 158:1137–1145.

Price, T., M. Turelli, and M. Slatkin. 1993. Peak shifts produced by correlated response to selection. Evolution 47:280–290.

Reed, D. H., and R. Frankham. 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55:1095–1103.

Roff, D. A. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. Heredity 74:481–490.

———. 1996. The evolution of genetic correlations: an analysis of patterns. Evolution 50:1392–1403.

Rohlf, F. J., and D. E. Slice. 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. Syst. Zool. 39:40–59.

Rohlf, F. J. 1999. Shape statistics: procrustes superimpositions and tangent spaces. J. Classif. 16:197–223.

———. 2004. tpsDig2. Department of Ecology and Evolution, State University of New York, Stony Brook, NY. Available at http://life.bio.sunysb.edu/morph/.

Rundle, H. D., S. F. Chenoweth, P. Doughty, and M. W. Blows. 2005. Divergent selection and the evolution of signal traits and mating preferences. PLos Biol. 3:1988–1995.

Schiffer, M., M. E. Carew, and A. A. Hoffmann. 2004. Molecular, morphological and behavioural data reveal the presence of a cryptic species in the widely studied *Drosophila serrata* species complex. J. Evolution Biol. 17:430–442.

Schiffer, M., and S. F. McEvey. 2006. *Drosophila bunnanda*: a new species from northern Australia with notes on other Australian members of the *montium* subgroup (Diptera: Drosophilidae). Zootaxa 1333:1–23.

Shaw, R. G. 1991. The comparison of quantitative genetic parameters between populations. Evolution 45:143–151.

Sheets, H. D. 2001. IMP: Integrated Morphometrics Package. Physics Department, Canisius College, Buffalo, NY. Available at http://www.canisius.edu/~sheets/morphsoft.html.

Sisodia, S., and B. N. Singh. 2004. Size dependent sexual selection in *Drosophila ananassae*. Genetica 121:207–217.

Slice, D. E. 1998. Morpheus et al. Department of Ecology and Evolution, State University of New York, Stony Brook, NY. Available at http://life.bio.sunysb.edu/morph/morpheus/

Steppan, S. J. 1997. Phylogenetic analysis of phenotypic covariance structure .1. Contrasting results from matrix correlation and common principal component analyses. Evolution 51:571–586.

Steppan, S. J., P. C. Phillips, and D. Houle. 2002. Comparative quantitative genetics: evolution of the **G** matrix. Trends Ecol. Evol. 17:320–327.

Turelli, M. 1988. Phenotypic evolution, constant covariances, and the maintenance of additive variances. Evolution 42:1342–1347.

Wagner, G. P., and L. Altenberg. 1996. Complex adaptations and the evolution of evolvability. Evolution 50:967–976.

Waitt, D. E., and D. A. Levin. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. Heredity 80:310–319.

Weber, K. E. 1996. Large genetic change at small fitness cost in large populations of *Drosophila melanogaster* selected for wind tunnel flight: rethinking fitness surfaces. Genetics 144:205–213.

Willis, J. H., J. A. Coyne, and M. Kirkpatrick. 1991. Can one predict the evolution of quantitative characters without genetics? Evolution 45:441–444.

Wootton, R. J., R. C. Herbert, P. G. Young, and K. E. Evans. 2003. Approaches to the structural modeling of insect wings. Philos. T. R. Soc. B 358:1577–1587.

Zeng, Z.-B. 1988. Long-term correlated response, inter-population covariation and interspecific allometry. Evolution 42:363–374.

Zimmerman, E., A. Palsson, and G. Gibson. 2000. Quantitative trait loci affecting components of wing shape in *Drosophila melanogaster*. Genetics 155:671–683.

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