Comparing G Matrices: Are Common Principal Components Informative?

Jason G. Mezey[1] **and David Houle**

Department of Biological Science, Florida State University, Tallahassee, Florida 32306-1100

Manuscript received November 4, 2002
Accepted for publication April 28, 2003

ABSTRACT

Common principal components (CPC) analysis is a technique for assessing whether variance-covariance matrices from different populations have similar structure. One potential application is to compare additive genetic variance-covariance matrices, **G**. In this article, the conditions under which **G** matrices are expected to have common PCs are derived for a two-locus, two-allele model and the model of constrained pleiotropy. The theory demonstrates that whether **G** matrices are expected to have common PCs is largely determined by whether pleiotropic effects have a modular organization. If two (or more) populations have modules and these modules have the same direction, the **G** matrices have a common PC, regardless of allele frequencies. In the absence of modules, common PCs exist only for very restricted combinations of allele frequencies. Together, these two results imply that, when populations are evolving, common PCs are expected *only* when the populations have modules in common. These results have two implications: (1) In general, **G** matrices will not have common PCs, and (2) when they do, these PCs indicate common modular organization. The interpretation of common PCs identified for estimates of **G** matrices is discussed in light of these results.

COMPARISON of additive genetic variance-covariance matrices (the **G** matrices) of different populations is an important goal in evolutionary quantitative genetics (Steppan *et al.* 2002). Such comparisons identify commonalities (*e.g.*, Kohn and Atchley 1988; Phillips and Arnold 1999; Roff 2000) or summarize differences (*e.g.*, Shaw *et al.* 1995; Paulsen 1996; Steppan 1997) in the structure of **G**. The broad motivation behind such analysis is clear: **G** is a key component for predicting trait evolution under directional selection and genetic drift as well as for retroactively estimating the selection gradient (Lande 1979; Lande and Arnold 1983). Comparison of **G** therefore reveals whether differences in genetic variation may have played a role in divergent evolutionary trajectories (Price *et al.* 1993). Furthermore, as the structure of **G** depends on pleiotropic effects of segregating alleles, commonalities may also contain information on genetic architecture shared by populations (Phillips and Arnold 1999). Comparison of **G** matrices has been the primary goal of many studies (Arnold 1981; Lofsvold 1986; Kohn and Atchley 1988; Wilkinson *et al.* 1990; Atchley *et al.* 1992; Brodie 1993; Shaw *et al.* 1995; Paulsen 1996; Podolsky *et al.* 1997; Steppan 1997; Arnold and Phillips 1999; Camara and Pigliucci 1999; Badayaev and Hill 2000; Pfrender and Lynch 2000; Roff, 2000, 2002; Service 2000; Waldmann and Anderson 2000; Phillips *et al.* 2001). The results have been used to address issues in areas as diverse as evolution of predation patterns (Arnold 1981), covariance patterns resulting from mutation (Camara and Pigliucci 1999), and change in the **G** matrix itself (see, *e.g.*, Wilkinson *et al.* 1990; Pfrender and Lynch 2000).

Although the utility of comparing **G** matrices seems clear, which methods will furnish informative conclusions is not (Steppan *et al.* 2002). Difficulty arises in any case where trait number is greater than one. For n traits, the **G** matrix includes $n(n + 1)/2$ variance and covariance elements, and each of these may be larger or smaller than its corresponding element in other **G** matrices. Moreover, the number of factors that potentially influence the values of the $n(n + 1)/2$ variances and covariances is considerable, including the specific pleiotropic effects of segregating alleles, the frequency of these alleles, gametic-phase disequilibrium, nonadditive effects, and the effects of mutation. Therefore, it is not easy to define a statistic that both summarizes structure shared by **G** matrices and provides a clear interpretation of the implications of shared structure.

Of the many multivariate statistical techniques proposed for comparison of **G** matrices (reviewed in Steppan *et al.* 2002), common principal components (CPC) analysis (Flury 1987, 1988) is fast becoming the method of choice (Arnold and Phillips 1999; Camara and Pigliucci 1999; Phillips and Arnold 1999; Pfrender and Lynch 2000; Roff 2000). The goal of CPC analysis is to summarize the structure of two (or more) matrices in terms of common principal components, those principal components (PCs) that have the same direction, and to test for differences from this common model (Flury 1987, 1988). The statistical models of CPC ar-

[1]*Corresponding author:* Department of Biological Science, Florida State University, Tallahassee, Florida 32306-1100.
E-mail: mezey@bio.fsu.edu

ranged in order of increasing amount of common structure are the following: no similarity among matrices; PCPC(1), where matrices have a single common PC; PCPC(2); . . . ; PCPC($n - 2$); CPC(All); matrix proportionality; and matrix equality. The m common PCs referred to in PCPC(m) may refer to any m PCs shared among matrices, and common PCs referred to in the CPC models need not be associated with eigenvalues of the same rank. A number of approaches are possible for determining which CPC model correctly describes matrix structure (Flury 1988; Phillips and Arnold 1999). The software implementations used by almost all CPC analyses to date (Phillips 1998a,b,c) employ a maximum-likelihood approach and a hierarchy of hypothesis tests (the Flury hierarchy) to determine whether matrices have common principal components (see Phillips and Arnold 1999 for a discussion of the alternatives when implementing the Flury hierarchy).

The hierarchy of CPC models provides a valuable descriptive summary of matrix structure. However, the biological meaning of the results is unclear (Houle *et al.* 2002). In this article, we address the problem of interpreting common PCs by deriving the conditions under which we expect common PCs among **G** matrices. We perform the analysis for both a two-locus, two-allele model and the model of constrained pleiotropy (Wagner 1989). The analyses demonstrate that common PCs are expected only when pleiotropic effects are constrained to a modular organization (Wagner and Altenberg 1996). When populations being compared have a modular organization in common, they have a common PC. As is discussed, this latter result provides a biological interpretation of common PCs when power is sufficient to reveal differences in the direction of PCs among **G** matrices.

PLEIOTROPIC MODULARITY

Wagner and Altenberg (1996) defined a modular organization as a case in which "pleiotropic effects of the genes fall mainly among members of the same character complex and less frequently between members of different complexes" (p. 971). A modular organization is therefore defined in terms of pleiotropic effects when considering a specific set of traits. In this article, we address an extreme case of modular organization, in which $x > 2$ nonoverlapping sets of pleiotropic effects can be defined in which all pleiotropic effects in a set are orthogonal (oriented at 90°) to all other pleiotropic effects. Each of these x sets is a "perfect" module in the sense that, for the n measured traits, an orthogonal rotation can cause the pleiotropic effects of each module to fall *entirely* on a subset of the new axes and *not at all* on new axes affected by the other modules. Distinct populations have a perfect module in common if the same orthogonal rotation also results in perfect modules. The hypothetical case of perfect modules is used to illustrate the point that considerable constraints on pleiotropic effects are required for common PCs to be expected among **G** matrices.

Modularity can be defined both in terms of pleiotropic effects of alleles segregating in a population and in terms of the pleiotropic effects that may be introduced into the population by mutation (Wagner and Altenberg 1996). Modularities at these two levels are clearly related. If the pleiotropic distribution of possible mutations is modular, then the pleiotropic effects of segregating alleles will also be modular. Although cases are possible where segregating variation is modular while the distribution of mutations is not, such cases are expected to be transient because mutations introduce nonmodular variation (Wagner and Altenberg 1996; Mezey *et al.* 2000). Modularity of segregating variation is therefore expected to be a strong indicator of modularity in the distribution of mutations. Our treatment concerns cases in which the distribution of mutations is modular.

TWO-LOCUS, TWO-ALLELE MODEL

The goal of discussing this simple model is to provide an intuitive illustration of the relationship between modules and common PCs that also applies to the more general model of constrained pleiotropy (Wagner 1989). In a population, all genetic variation in $n = 2$ traits is assumed to be determined entirely by alleles segregating at the $N = 2$ loci. We assume complete additivity of allelic effects (no dominance or epistasis), no disequilibrium (gametic-phase or otherwise), no maternal effects, no sex linkage, and no genotype-environment covariance or genotype-environment interactions. We also assume random mating among diploid individuals. In this case, the additive effect on the traits associated with allele k at locus j can be expressed as a vector,

$$\boldsymbol{\alpha}_{jk} = [\alpha_{jk.1}, \alpha_{jk.2}], \tag{1}$$

where the ith element of the vector $\alpha_{jk.i}$ is the additive effect of allele k associated with trait i (Lynch and Walsh 1998). This model can also be expressed in terms of the average effect of an allelic substitution at locus j:

$$\tilde{\boldsymbol{\alpha}}_j = [\tilde{\alpha}_{j.1}, \tilde{\alpha}_{j.2}]. \tag{2}$$

The relationship between these two vectors is $p_{jk}\tilde{\boldsymbol{\alpha}}_{j.i} = \boldsymbol{\alpha}_{jk.i}$, where p_{jk} is the frequency of allele k at locus j. Because there are two alleles at each locus and no nonadditive effects, each $\tilde{\boldsymbol{\alpha}}_j$ is constant, and the direction of each $\boldsymbol{\alpha}_{jk}$ is constant where $\boldsymbol{\alpha}_{j1} \propto -\boldsymbol{\alpha}_{j2}$. An allelic substitution at locus j has a pleiotropic effect in this model if both of the elements of the allelic vectors are nonzero: $\tilde{\alpha}_{j.i} \neq 0$, $\alpha_{jk.i} \neq 0$. In this model, forward and backward mutations occur at each locus j at the same rate μ_j, where a mutation changes an allele's identity to the other possible allelic state. The structure of the **G** matrix depends on the $\tilde{\boldsymbol{\alpha}}_j$ (the $\boldsymbol{\alpha}_{jk}$) vectors and on the allele frequencies at the two loci (appendix a). Note that,

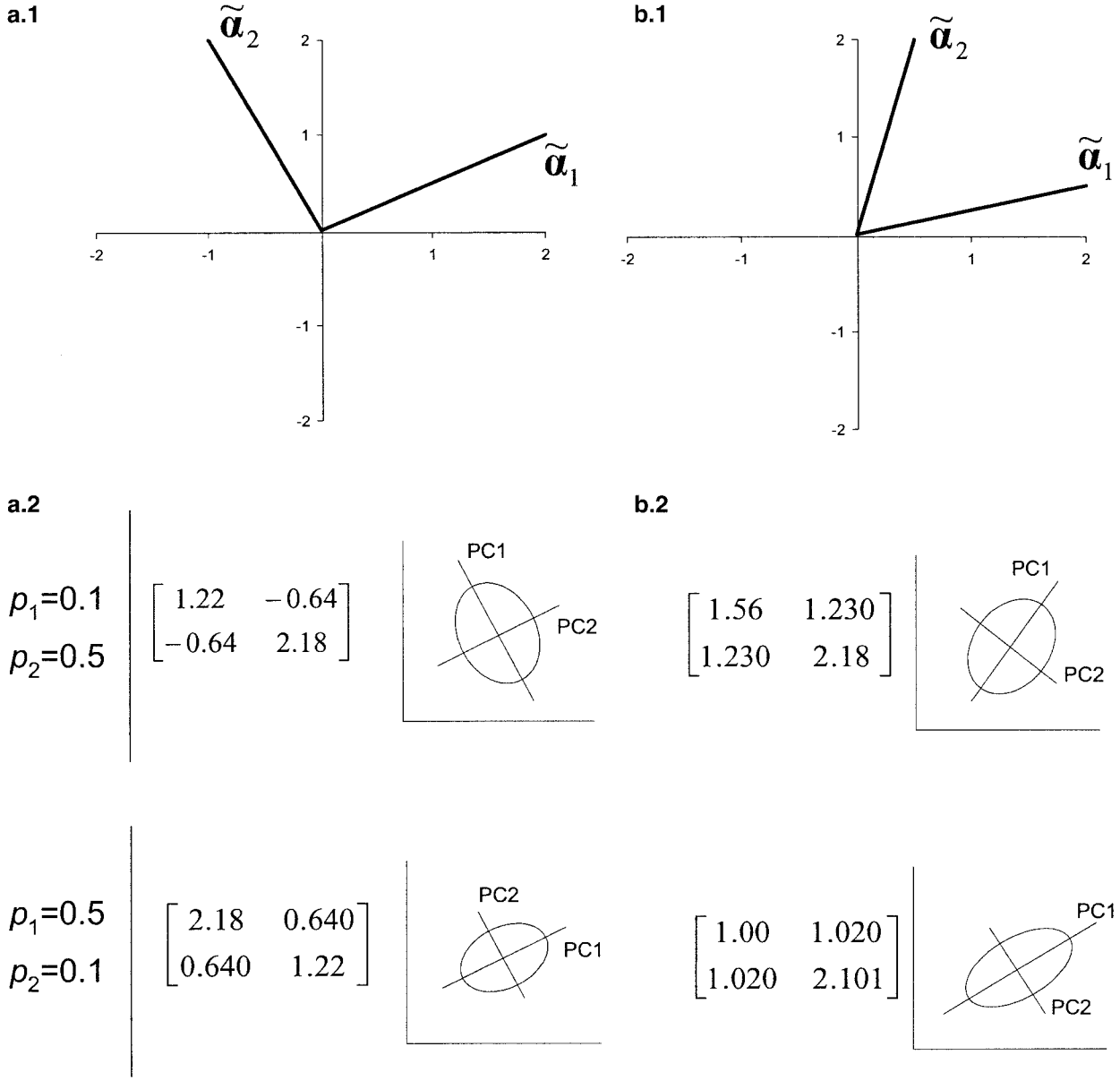

FIGURE 1.—Cases of (a.1) two modules and (b.1) no modules for the two-locus, two-allele model. The $\tilde{\alpha}_j$ are vectors of the average effect of an allelic substitution at locus j. The orientation of the $\tilde{\alpha}_j$ reflects the relative effects on the first and second traits (x-axis and y-axis, respectively). Two modules exist in a.1 because $\tilde{\alpha}_1$ is orthogonal to $\tilde{\alpha}_2$, and no modules exist in b.1 because the vectors are not orthogonal. The diagrams of a.2 and b.2 are the **G** matrices and PCs for different allele frequencies if we assume the vectors of a.1 and b.1, respectively. PC1 and PC2 indicate the PCs corresponding to the larger and smaller eigenvalues, respectively, where length of the PC reflects the relative size of the eigenvalue. Note that in a.2, different allele frequencies lead to different forms of **G**, but the direction of the PCs is the same. In contrast, in b.2, the different allele frequencies lead to different forms of **G** and different directions of PCs.

in the following, we assume that **G** can be estimated without error. We return to sampling issues in the DISCUSSION.

In this two-locus, two-allele model, existence of a module depends on the orientation of the allelic vectors associated with each locus. If the allelic vectors at one locus are orthogonal to the allelic vectors at the other locus, two perfect modules are present, because the pleiotropic effects can be divided into two groups that do not have overlapping effects. As an example, consider the case diagrammed in Figure 1a.1, where the allelic vectors of the loci are orthogonal to one another. Rotating the trait axes to the direction of the allelic vectors associated with each locus produces two new traits, f_1 and f_2, where the effects of allelic substitutions at each of the two loci are limited entirely to one of the two traits. Both of these new traits, f_1 and f_2, therefore define perfect modules. Figure 1b.1 diagrams a case without perfect modules. Because the allelic vectors are not orthogonal, two modules cannot be defined by a

rotation of the axes. However the axes are rotated, allelic substitutions at both loci have effects on both new traits. Note that a modular organization is possible in Figure 1b.1 if a nonorthogonal rotation is used, but such transformations do not result in modules in an evolutionary sense; *i.e.*, directional selection cannot be applied to such a "module" without resulting in a correlated response. Such nonorthogonal modules will be the subject of another article (J. G. Mezey and D. Houle, unpublished results). We confine the discussion here to modules that can be defined by rotations of the trait axes.

In this two-locus, two-allele model, the existence of modules places a major constraint on the possible orientations of **G** matrix PCs. When modules exist, the PCs of the **G** matrix *have the direction of the modules, regardless of allele frequencies and changes in allele frequencies* (appendix a). To visualize this relationship between PCs and modules, again consider Figure 1. Figure 1a.2 diagrams the **G** matrix and PCs associated with two populations, both of which have the modules diagrammed in Figure 1a.1. The populations have different allele frequencies at the two loci, and as a result, the **G** matrices of the populations differ. Although the PCs of **G** are associated with different eigenvalues, the PCs have the same direction as the modules in both populations. Further, all variation attributable to the allelic substitutions defining an individual module is described by a single PC and its associated eigenvalue. Contrast this case with that diagrammed in Figure 1b.2, which diagrams **G** and the PCs for two populations where allelic vectors are described by Figure 1b.1. In this case, the different allele frequencies correspond to different structures of **G** *and* PCs that have different directions. In such cases, there is no simple relationship between PCs and the allelic effects associated with each locus.

For an individual population, the directions of **G** matrix PCs are always the same regardless of allele frequencies only if perfect modules exist (appendix a). Therefore, if distinct populations have such modules in common (the modules have the same direction), the **G** matrices of the populations *will always have common PCs*, regardless of allele frequencies. Note that this relationship depends entirely on the direction of the modules and not the specific allelic effects defining the modules. Populations with different allelic effects at the two loci always have common PCs as long as both populations have modules in the same direction. In contrast, if the populations being compared have no modules, *only a restricted subset of allele frequencies* results in common PCs (appendix a), even if the allelic vectors are the same in the populations being compared.

The cases diagrammed in Figure 2 illustrate these concepts. Figure 2a diagrams two populations (*A* and *B*) that have modules in common. For these populations, Figure 2a.1 diagrams in gray the allele (heterozygote) frequencies in population *A* that result in common PCs, given fixed allele frequencies in population *B*. Figure 2a.2 provides the equivalent diagram for population *B* given fixed allele frequencies in population *A*. Note that every possible allele frequency results in common PCs, regardless of the allele frequency in the other population. Contrast this situation with the case diagrammed in Figure 2b.1, where the populations have the same allelic effect vectors, but no modules are present. For these populations, the only allele frequencies for which common PCs occur are described by the dashed and dotted lines in Figure 2, b.1 and b.2, respectively. The allele frequencies that do not fall on these lines result in no common PCs. Therefore, only a very constrained set of allele frequencies results in common PCs when no modules are present.

The implication of these results is that, when comparing evolving populations, we should not expect common PCs *unless* there are common modules. Without modules, the allele frequencies required for common PCs are so constrained that they are unlikely to occur given the stochastic effects of mutation and genetic drift. As an example, consider a case in which populations *A* and *B* have the same allelic vectors but no modules exist (as in Figure 2a.1). As demonstrated in appendix a, common PCs occur in these two populations when the following constraint is satisfied,

$$\frac{H_{1.B}}{H_{2.B}} = \frac{H_{1.A}}{H_{2.A}}, \tag{3}$$

where $H_{j.P} = 2p_{jk}p_{jl}$ is the heterozygote frequency at locus j in population P and p_{jk} and p_{jl} are the frequencies of the alleles k and l. Note that, even if this constraint is satisfied at some point, any change in allele frequencies at the loci in one of the populations must be exactly balanced by a specific change in allele frequencies in the other population to preserve the ratios in (3). Any other changes in allele frequencies in the other population result in no common PCs. Stochastic changes are therefore not expected to preserve the necessary ratios. Of course, situations can be constructed in which the probability of common PCs is high even in the absence of modules. For example, infinite populations with the same allelic-effect vectors that have reached the same mutation-selection equilibrium would be such a case, but barring such extreme conditions, we should generally expect common PCs only when populations have common modules.

Figure 3 provides a summary of the four possible cases that can arise when two populations are compared for the two-locus, two-allele model: (1) The populations have modules in common (Figure 3a), (2) both populations have modules but the directions are different (Figure 3b), (3) one population has a module and the other does not (Figure 3c), and (4) neither population has modules (Figure 3d). Only the case in Figure 3a will always have common PCs. For the cases in Figure 3, b–d, the vast majority of allele frequencies will result in

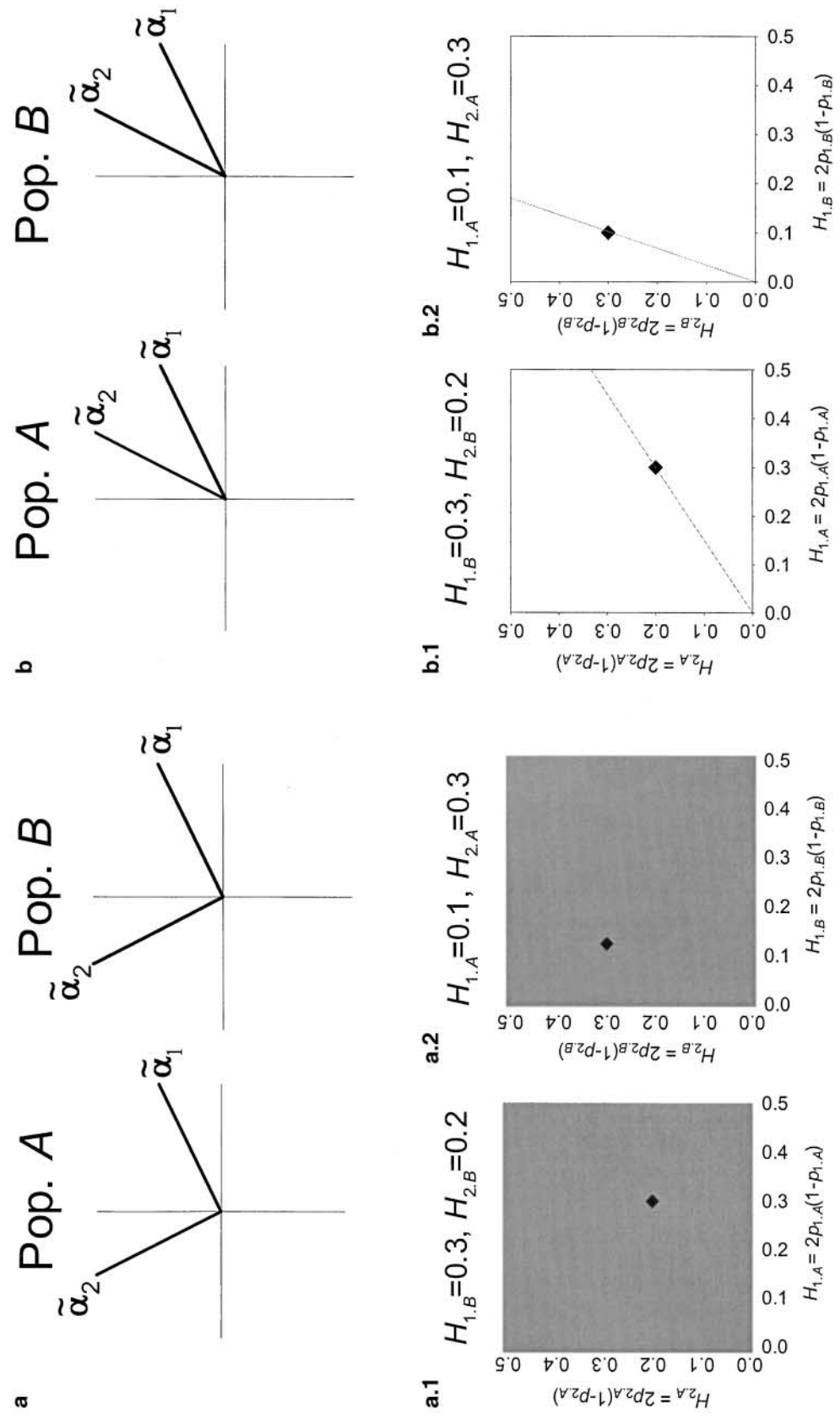

FIGURE 2.—Allele (expressed as heterozygote) frequencies for which populations *A* and *B* have the same PCs. (a) Populations *A* and *B* have common modules. a.1 depicts in gray the heterozygote frequencies for which population *A* has the same PCs as population *B*, fixing the heterozygote frequencies in population *B* at $H_{1.B} = 0.3$ for locus 1 and $H_{2.B} = 0.2$ for locus 2 (the black diamond indicates the frequencies in population *B*). a.2 is the corresponding graph for population *B*, fixing the heterozygote frequency in population *A* at $H_{1.B} = 0.1$ and $H_{2.B} = 0.4$. Note that in both cases any heterozygote frequencies result in the same PCs. (b) Populations *A* and *B* have no modules. b.1 and b.2 depict the heterozygote frequencies for which PCs will be the same when heterozygote frequencies are fixed in the other population. In this case, the heterozygote frequency must fall on the lines in b.1 and b.2 for the two populations to have the same PCs.

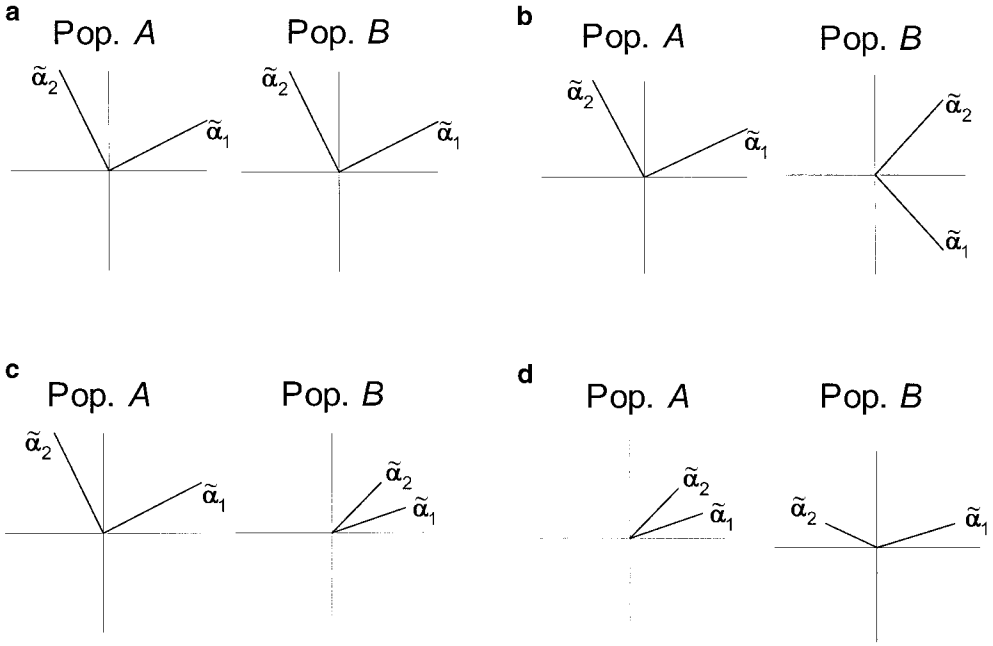

FIGURE 3.—Four cases that may arise in a comparison of two populations described by the two-locus, two-allele model: (a) The populations have common modules; (b) the populations each have modules but have no common modules; (c) population *A* has modules and population *B* has no modules; (d) neither population has modules.

no common PCs, and we should not expect to find common PCs when the populations are evolving.

Note that, if the allelic vectors in the two populations approximate a perfectly modular case (they are almost but not quite 90°), only very constrained allele frequencies result in common PCs as in Figure 3d. This result may seem strange. The reason for it is that the PCs in each **G** matrix must have *exactly* the same direction for common PCs to exist. In the absence of perfect modules, the vast majority of allele frequencies result in slight differences in the directions of the PCs in the populations and therefore in no common PCs. This is not to say that we would be able to determine that the PCs are different in such a case when analyzing *estimates* of the **G** matrices. The effects of sample size will tend to obscure such subtle differences, so cases that approximate perfect modules will be indistinguishable from perfect modules in practice. We return to this issue of how sample size affects the expectation of finding common PCs in the DISCUSSION.

MODEL OF CONSTRAINED PLEIOTROPY

Constraints on pleiotropic effects are the key to whether common PCs are expected. The model of constrained pleiotropy (WAGNER 1989) formalizes a type of constraint that can result in common PCs. The conceptual underpinning of the model is the assumption that allelic variation at a given locus additively affects variation in a physiological property associated with a gene product of the locus. The relationship between variation in the property and the genetic variation in n phenotypic traits is assumed to be linear and is expressed as a matrix transformation (hence the model is sometimes referred to as the "*B*-matrix" model). These assumptions constrain mutations at a given locus to have the same pleiotropic effects on the n traits, although the magnitudes of the pleiotropic effects associated with particular allelic substitutions at the locus may differ. In this way, the model differs from the more general additive pleiotropic model presented by LANDE (1980), where the alleles at a locus may have different pleiotropic effects.

In a quantitative genetic formulation, the model of constrained pleiotropy makes the assumption that the absolute values of the additive-effect vectors of any alleles k and l at a locus j are proportional:

$$|\boldsymbol{\alpha}_{jk}| \propto |\boldsymbol{\alpha}_{jl}|. \tag{4}$$

Any number of alleles may be segregating at each locus, and mutations may introduce new alleles at a locus, although the effects of all alleles conform to the constraint of Equation 4. The structure of the **G** matrix depends on the effects and frequencies of the alleles segregating in a population. As in the two-locus, two-allele model, we consider the exact structure of **G** and assume no disequilibrium (gametic-phase or otherwise), no maternal effects, no sex linkage, no genotype-environment covariance or genotype-environment interactions, and random mating among diploid individuals.

In the model of constrained pleiotropy, modules exist if, for the N loci that may result in genetic variation in n traits, a subset of M loci ($M < N$) can be defined where the allelic vectors at each of these M loci are orthogonal to the allelic vectors at each of the other $N - M$ loci. In this case, for each $\boldsymbol{\alpha}_{jk(M)}$ that may occur at the M loci and each $\boldsymbol{\alpha}_{jk(N-M)}$ that may occur at the remaining $N - M$ loci,

$$\boldsymbol{\alpha}^{\mathrm{T}}_{jk(M)}\boldsymbol{\alpha}_{jk(N-M)} = 0. \tag{5}$$

Each subset M defines a module because new traits can be defined by a rotation of the n trait axes where genetic variation in the new traits affected by the M loci is independent of genetic variation in the rest of the new

traits. Note that variation associated with a module M need not fall along a single vector. Modules may therefore be multidimensional, but the relationship of such higher-dimensional modules to the PCs of the **G** matrix is more complicated. In this article, we restrict the discussion to modules in which the allelic vectors at all of the M loci defining a module have the same direction; *i.e.*, $|\boldsymbol{\alpha}_{jk(M)}| \propto |\boldsymbol{\alpha}_{mk(M)}|$ for all loci j, m in M. We consider higher-dimensional modules in another article (J. G. Mezey and D. Houle, unpublished results).

Just as in the two-locus, two-allele model, when a one-dimensional module exists in a population, a PC with the same direction as the module will exist regardless of allele frequencies (appendix b). Therefore, when populations have a module in common, they will always have a common PC with the same direction as the modules, regardless of allele frequencies in the populations. Also as in the two-locus two-allele model, if the populations do not have a one-dimensional module in common, very restricted allele frequencies are required for common PCs to exist (appendix b). In the model of constrained pleiotropy, x common modules can exist, $0 < x \leq n$, when n traits are considered. The same reasoning applies to such cases: If populations have x modules in common, $0 < x \leq n$, at least x (excluding $n - 1$) common PCs will exist, and very restricted allele frequencies in the two populations will result in more than x common PCs (appendix b).

Figure 4 illustrates these concepts. It diagrams three different possibilities that may arise when two populations (A and B) are compared when $n = 3$. In Figure 4a, the two populations have three modules in common. The **G** matrices of these populations will always have three common PCs; *i.e.*, all PCs will be common PCs. Note that even if both A and B had three modules but the modules had different directions in the two populations, only very restricted allele frequencies would result in common PCs (appendix b). In Figure 4b, the two populations have a single one-dimensional module in common. In this case, the **G** matrices will always have one PC in common, although the PC may be associated with different eigenvalues in the two populations. For there to be more than a single common PC in case 4b, very restricted allele frequencies are required in the two populations. In Figure 4c, neither population has any modules. Again, only very restricted combinations of allele frequencies would yield common PCs.

In summary, when comparing evolving populations with x common one-dimensional modules, we expect to find exactly x common PCs. The stochastic effects of mutation and genetic drift are very likely to result in allele frequencies where the other PCs differ in their orientiations (appendix b).

DISCUSSION

The goal of the theory developed in this article is to assess whether the CPC model that is the basis of the CPC analysis can be informative for comparing **G** matrices beyond a descriptive summary of matrix similarity. When assessed solely from this perspective, the results are quite positive. Because of the close relationship between common PCs and modular structure, when common PCs do exist they have a biological interpretation: *Common PCs indicate the existence of common modules.* The intuition that common PCs have a biologically meaningful interpretation is therefore well founded (Phillips and Arnold 1999).

The modular structure that is sufficient to create common PCs is quite restrictive. It requires that the genetic effects of some set of loci be orthogonal to those of all other segregating loci. This requirement is equivalent to the requirement that some rotation of the axes in phenotype space that produces traits that are independent of all other traits exists. Given the general assumption that pleiotropy is ubiquitous, which we share, the existence of such extreme modules seems somewhat unlikely. Thus, we expect that the form of modular structure and therefore common PCs is unusual. This is not to say that cases approximating modular organizations are expected to be so rare that the possibility of their existence should be discounted. As discussed by a number of authors (Wagner and Altenberg 1996; Cheverud *et al.* 1997; Rice 2000), pleiotropic distributions that approximate the perfect case (*i.e.*, where pleiotropic effects are "mainly" limited to a particular subset of traits) are not necessarily unexpected, particularly when appropriate sets of traits are considered.

How are we to reconcile these results with those of studies that have applied CPC analysis to **G** matrices and reported many common PCs? For example, Arnold and Phillips (1999) compared **G** matrices for six morphological traits for two populations (inland and coastal) of the garter snake *Thamnophis elegans.* CPC analyses were performed for all possible pairwise comparisons of **G** estimated for both males and females in both populations. For almost all comparisons, the CPC model CPC(All) could not be rejected. Pfrender and Lynch (2000) estimated **G** for life-history traits for a population of *Daphnia pulex* at four different times. CPC analyses were performed for pairwise comparisons among three of these matrices. CPC models including at least one common PC could not be rejected for each of these comparisons.

If the intuition that common modules should be rare is correct, the most likely explanation is that the power to detect differences in the direction of matrix PCs is low for the sample sizes commonly used in estimates of **G**. This explanation seems particularly likely given the results of Houle *et al.* (2002). For example, in the Houle *et al.* (2002) study, matrices were simulated using an additive factor model in which the angle between the directions of the second PCs (corresponding to the second eigenvalue) in two matrices was altered. CPC analysis using the software of Phillips (1998c) was performed on 100 pairs of estimates of the matrices, with

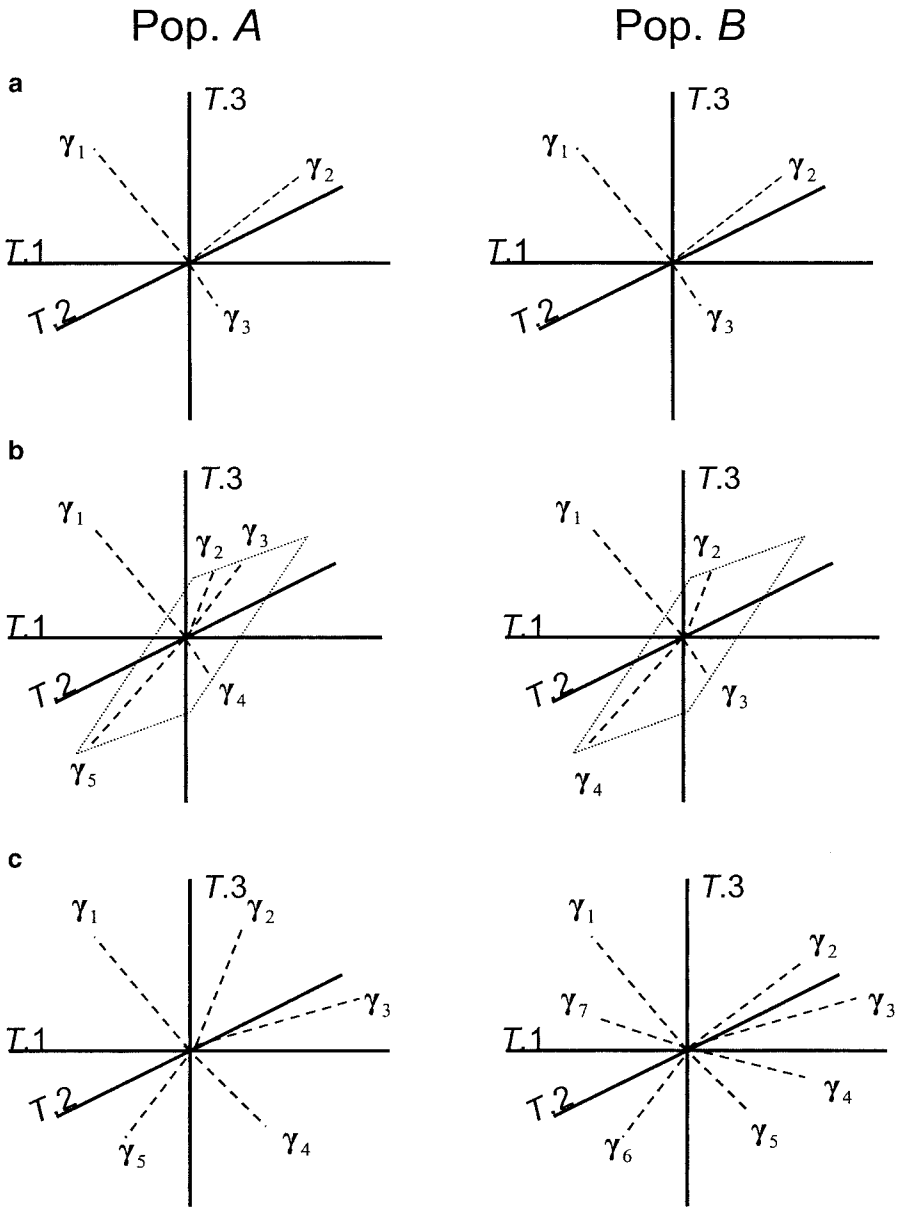

FIGURE 4.—Three cases comparing populations (*A* and *B*) where variation in three traits (*T*.1, *T*.2, and *T*.3) is described by the model of constrained pleiotropy. Each $\boldsymbol{\gamma}_j$ is a vector in the direction of additive allelic-effect vectors associated with a locus *j*. Populations *A* and *B* have (a) three common modules, (b) a single common module, and (c) no modules.

a sample size of 300. For differences in direction of up to 6°, both the jump-up and Akaike information criterion approaches indicated the CPC model equality (all PCs are in common) for as many as 50% of the comparisons. Because the sample sizes for most estimates of **G** are not large (STEPPAN *et al.* 2002), this result indicates that CPC analysis may be indicating more common PCs than actually exist as a result of low sample sizes.

One reason for the inability of CPC analysis to distinguish distinct PCs when sample sizes are low may be the way that position in the Flury hierarchy is assessed (PHILLIPS and ARNOLD 1999). For example, the decision to move up in the Flury hierarchy in the jump-up approach advocated by PHILLIPS and ARNOLD (1999) is based on being unable to reject a hypothesis of common PCs *vs.* a hypothesis that no common PCs exist. One moves up in the hierarchy until a hypothesis of *x* common PCs can be rejected. As PHILLIPS and ARNOLD (1999) stated, such a result should not be interpreted as demonstrating that the matrices have $x - 1$ common PCs, only that the presence of $x - 1$ common PCs cannot be rejected. It is tempting, however, to interpret stopping position as a reflection of matrix similarity. In fact, the results of a CPC analysis reflect both matrix similarity and statistical power.

The sensitivity of CPC results to sample size means that, in practice, we cannot necessarily interpret common PCs as a demonstration of common modules. However, CPC analysis could be a useful tool for indicating which sets of traits are likely to have a modular organization, particularly if methods for assessing confidence in the existence of common PCs could be developed. We would not expect to have high confidence in a common PC among **G** matrices unless the populations have ap-

proximately modular organizations in common. The existence of modules would always have to be confirmed by independent means, because even without common modular organization, the allele frequencies required to produce a true common PC among **G** matrices could have occurred by chance.

In the context of identifying which sets of traits may have a modular organization, the reordering option available in the CPC analysis software of Phillips (1998a,b,c) is valuable. The default is that the program estimates the model CPC(All) for the combined data and builds the common PC models of the Flury hierarchy [PCPC(1), PCPC(2), etc.], using the common PCs of CPC(All) in rank order according to the size of their eigenvalues. The reordering option allows the user to designate a different ordering scheme. This flexibility is useful in a case where matrices have a common PC but the common PC is not associated with eigenvalues first in the default rank ordering. In such a case, the default generally causes CPC analysis to indicate no similarity among the matrices (Houle *et al.* 2002), but if the reordering option is used to place the true common PC first in the ordering scheme, CPC analysis should indicate that a common PC exists.

The possibility that CPC analysis could be developed for the detection of modules is a particularly exciting prospect because modules have clearly defined genetic and evolutionary properties. For example, from a genetics perspective, modules represent a specific constraint on how variation at the gene level is related to variation in the phenotype (Bonner 1988; Cheverud *et al.* 1997; Mezey *et al.* 2000). When there is modular organization, the effects associated with groups of genes are entirely limited to distinct aspects of the phenotype. From an evolutionary perspective, modules are units in the sense that variation in the traits defining a module will be uncorrelated with variation in other traits, given appropriate assumptions about random mating and no gametic-phase disequilibrium (Magwene 2001). Because selection can act on traits in a module without causing a correlated response in traits outside the module, identification of modules provides a foundation for constructing hypotheses about the evolutionary properties of a population that could be tested experimentally. Modular organization also plays an important conceptual role in relation to the evolvability of a genetic system (Wagner and Altenberg 1996; Wagner and Mezey 2003). It is therefore of interest to identify whether and to what degree cases of approximate modularity exist in nature.

In conclusion, our results suggest (1) that common PCs are unlikely without modular organization and (2) that there is a biological interpretation of common PCs and a possible role for common PCs in the identification of modular organization. In both cases, interpretation of common PCs will be stymied until a systematic study of the sensitivity of CPC analysis to sample size is performed. If this problem could be addressed, CPC analysis of **G** matrices could provide biologically useful insight beyond a summary of matrix structure. In this role, CPC analysis could be particularly useful for addressing questions that require a relatively complete picture of genetic architecture: Do modules correspond to functional architectures (Houle *et al.* 2002; Steppan *et al.* 2002)? To what extent is the structure of the **G** matrix constrained (Turelli 1988)? How modular is the G-P map (Wagner 1996)?

We thank Kyle Galivan, Thomas F. Hansen, Frances C. James, Eric Klassen, Joseph Travis, Zhao-Bang Zeng, and two anonymous reviewers for their comments on this manuscript. This work was supported by National Science Foundation grant no. 0129219.

LITERATURE CITED

Arnold, S. J., 1981 Behavioral variation in natural populations. I. Phenotypic, genetic and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. Evolution **35:** 489–509.

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.

Atchley, W. R., D. E. Cowley, C. Vogl and T. Mclellan, 1992 Evolutionary divergence, shape change and genetic correlation structure in the rodent mandible. Syst. Biol. **41:** 196–221.

Badayaev, A. V., and G. E. Hill, 2000 The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. Evolution **54:** 1784–1794.

Bonner, J. T., 1988 *The Evolution of Complexity*. Princeton University Press, Princeton, NJ.

Brodie, E. D., 1993 Homogeneity of the genetic variance-covariance matrix for antipredator traits in two natural populations of garter snakes *Thamnophis ordinoides*. Evolution **47:** 844–854.

Camara, M. D., and M. Pigliucci, 1999 Mutational contributions to genetic variance-covariance matrices: an experimental approach using induced mutations in *Arabidopsis thaliana*. Evolution **54:** 1692–1703.

Cheverud, J. M., E. J. Routman and D. K. Irschick, 1997 Pleiotropic effects of individual gene loci on mandibular morphology. Evolution **51:** 2004–2014.

Flury, B. K., 1987 Two generalizations of the common principal component method. Biometrika **74:** 59–69.

Flury, B. K., 1988 *Common Principal Components and Related Multivariate Models*. Wiley, New York.

Houle, D., J. Mezey and P. Galpern, 2002 Interpretation of the results of common principal components analyses. Evolution **56:** 433–440.

Kohn, L. A. P., and W. R. Atchley, 1988 How similar are genetic correlation structures? Data from mice and rats. Evolution **42:** 467–481.

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

Lande, R., 1980 The genetic covariance between characters maintained by pleiotropic mutations. Genetics **94:** 203–215.

Lande, R., and S. J. Arnold, 1983 The measurement of selection on correlated characters. Evolution **37:** 1210–1226.

Lofsvold, D., 1986 Quantitative genetics of morphological differentiation in *Peromyscus*. I. Tests of homogeneity of genetic covariance structure among species and subspecies. Evolution **40:** 559–573.

Lynch, M., and B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

Magwene, P. M., 2001 New tools for studying integration and modularity. Evolution **55:** 1734–1745.

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

Paulsen, S. M., 1996 Quantitative genetics of wing color pattern in

the buckeye butterfly (*Preciscoenia* and *Preceis evarete*): evidence against the constancy of **G.** Evolution **50:** 1585–1597.

Pfrender, M. E., and M. Lynch, 2000 Quantitative genetic variation in *Daphnia*: temporal changes in genetic architecture. Evolution **54:** 1502–1509.

Phillips, P. C., 1998a CPCrand: randomization test of the CPC hierarchy. University of Texas, Arlington, TX (http://darkwing.uoregon.edu/~pphil/software.html).

Phillips, P. C., 1998b H2boot: bootstrap estimates and tests of quantitative genetic data. University of Texas, Arlington, TX (http://darkwing.uoregon.edu/~pphil/software.html).

Phillips, P. C., 1998c CPC: common principal components analysis. University of Texas, Arlington, TX (http://darkwing.uoregon.edu/~pphil/software.html).

Phillips, P. C., and S. J. Arnold, 1999 Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. Evolution **53:** 143–151.

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.

Podolsky, R. H., R. G. Shaw and F. H. Shaw, 1997 Population structure of morphological traits in *Clarkia dudleyana*. II. Constancy of within-population genetic variance. Evolution **51:** 1795–1796.

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

Rice, S. H., 2000 The evolution of development interactions: epistasis, canalization and integration, pp. 82–98 in *Epistasis and the Evolutionary Process*, edited by J. B. Wolf, E. D. Brodie and M. J. Wade. Oxford University Press, New York.

Roff, D., 2000 The evolution of the **G** matrix: Selection or drift? Heredity **84:** 135–142.

Roff, D., 2002 Comparing **G** matrices: a MANOVA approach. Evolution **56:** 1286–1291.

Service, P. M., 2000 The genetic structure of female life history in *D. melanogaster*: comparisons among populations. Genet. Res. **75:** 153–166.

Shaw, F. H., R. G. Shaw, G. S. Wilkinson and M. Turelli, 1995 Changes in genetic variances and covariances: **G** whiz! Evolution **49:** 1260–1267.

Steppan, S. J., 1997 Phylogenetic analysis of phenotypic covariance structure. II. Reconstructing matrix evolution. Evolution **51:** 587–594.

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 genetic variance. Evolution **42:** 1342–1347.

Wagner, G. P., 1989 Multivariate mutation-selection balance with constrained pleiotropy effects. Genetics **122:** 223–234.

Wagner, G. P., 1996 Homologues, natural kinds and the evolution of modularity. Am. Zool. **36:** 36–43.

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

Wagner, G. P., and J. Mezey, 2003 The role of genetic architecture constraints for the origin of variational modularity, in *Modularity in Development and Evolution*, edited by G. Schlosser and G. P. Wagner. Chicago University Press, Chicago (in press).

Waldmann, P., and S. Anderson, 2000 Comparison of genetic (co)variance matrices within and between *Scabiosa canescens* and *S. columbria*. J. Evol. Biol. **12:** 826–835.

Wilkinson, G. S., K. Fowler and L. Partridge, 1990 Resistance of genetic correlation structure to directional selection in *Drosophila melanogaster*. Evolution **44:** 1990–2003.

Communicating editor: Z-B. Zeng

APPENDIX A: TWO-LOCUS, TWO-ALLELE MODEL

It is assumed that the entirety of the genetic variation in $n = 2$ traits is determined by alleles segregating at $N = 2$ loci where only two alleles are possible at each locus. Forward and backward mutations occur at locus j at the same rate, μ_j. We assume no dominance, epistasis, disequilibrium (linkage or otherwise), maternal effects, sex linkage, genotype-environment covariance, or genotype-environment interactions. We assume random mating among diploid individuals. $\alpha_{jk.i}$ is the additive effect of allele k at locus j associated with trait i, p_{jk} is the frequency of allele k, and $\tilde{\alpha}_{j.i}$ is the average effect of an allelic substitution at locus j on trait i such that $p_{jk}\tilde{\alpha}_{j.i} = \alpha_{jk.i}$ (Lynch and Walsh 1998). Designating $H_j = 2p_{jk}p_{jl}$ as the heterozygote frequency at locus j ($0 \leq H_j \leq 0.5$), the **G** matrix can be written as

$$\mathbf{G} = \begin{bmatrix} \sum_{j=1}^{2} H_j \tilde{\alpha}_{j.1}{}^2 & \sum_{j=1}^{2} H_j \tilde{\alpha}_{j.1}\tilde{\alpha}_{j.2} \\ \sum_{j=1}^{2} H_j \tilde{\alpha}_{j.1}\tilde{\alpha}_{j.2} & \sum_{j=1}^{2} H_j \tilde{\alpha}_{j.2}{}^2 \end{bmatrix}. \tag{A1}$$

Note that, under the assumption of no nonadditive effects, the $\tilde{\alpha}_{j.i}$ are constant, so the structure of **G** is a function of the allele frequencies, which may change as a result of mutation, selection, or genetic drift. A population is defined as having two modules if the vectors describing the average effect of an allelic substitution are orthogonal: $\tilde{\boldsymbol{\alpha}}_1^{\mathrm{T}}\tilde{\boldsymbol{\alpha}}_2 = 0$, where $\tilde{\boldsymbol{\alpha}}_j = [\tilde{\alpha}_{j.1}, \tilde{\alpha}_{j.2}]$. In such a case, each $\tilde{\boldsymbol{\alpha}}_j$ defines a module (see text). The existence of modules can also be written as $\boldsymbol{\alpha}_{1k}^{\mathrm{T}}\boldsymbol{\alpha}_{2l} = 0$ for all alleles k and l at the two loci where $\boldsymbol{\alpha}_{jk} = [\alpha_{jk.1}, \alpha_{jk.2}]$. If these conditions do not apply, no modules exist. Below, we assume that **G** has no multiplicity of eigenvalues and is of full rank unless noted. This latter assumption requires that all $p_{jk} > 0$ and that $\tilde{\boldsymbol{\alpha}}_1$ and $\tilde{\boldsymbol{\alpha}}_2$ have different directions.

Result A1: *If populations have modules with the same direction, the* **G** *matrices have common PCs for all allele frequencies.*

The matrix **G** is a real, 2×2, symmetric matrix. An orthonormal matrix **Q** and a diagonal matrix Λ therefore exist, such that

$$\mathbf{G} = \mathbf{Q}\Lambda\mathbf{Q}^{\mathrm{T}}, \tag{A2}$$

where each column vector **q** of **Q** is a PC, an eigenvector, of **G** ($\mathbf{q}_1 \perp \mathbf{q}_2 = 0$ and $\mathbf{q}_1^{\mathrm{T}}\mathbf{q}_2 = 1$) and each diagonal element of Λ (λ_1 and λ_2) is an eigenvalue. In the absence of a multiplicity of eigenvalues, the spectral decomposition

of **G** exists and is unique. In this case, no other matrix **Q**, defined up to the multiplication of columns by −1 and column permutation, produces a diagonalization of **G** (Flury 1988).

If two modules exist ($\tilde{\boldsymbol{\alpha}}_1 \perp \tilde{\boldsymbol{\alpha}}_2$), the matrix $\tilde{\mathbf{A}}$ can be defined as

$$\tilde{\mathbf{A}} = \left[\frac{\tilde{\boldsymbol{\alpha}}_1}{||\tilde{\boldsymbol{\alpha}}_1||}, \frac{\tilde{\boldsymbol{\alpha}}_2}{||\tilde{\boldsymbol{\alpha}}_2||}\right], \tag{A3}$$

where $||\tilde{\boldsymbol{\alpha}}_j|| = +\sqrt{\tilde{\boldsymbol{\alpha}}_j^{\mathrm{T}}\tilde{\boldsymbol{\alpha}}_j}$. Note that $\tilde{\mathbf{A}}$ is an orthonormal matrix with column vectors that have the same direction as $\tilde{\boldsymbol{\alpha}}_1$ and $\tilde{\boldsymbol{\alpha}}_2$. Also define the diagonal matrix **D** with diagonal elements $d_j = H_j||\tilde{\boldsymbol{\alpha}}_j||^2$. The matrix **G** can be written as

$$\mathbf{G} = \tilde{\mathbf{A}}\mathbf{D}\tilde{\mathbf{A}}^{\mathrm{T}}. \tag{A4}$$

This relation holds with the same orthonormal matrix $\tilde{\mathbf{A}}$ no matter what the allele frequencies in the population. Because this expression is a diagonalization of **G**, the uniqueness of the spectral decomposition implies that $\mathbf{Q} = \tilde{\mathbf{A}}$ (up to column permutation and multiplication of columns by −1). Therefore, if $\tilde{\boldsymbol{\alpha}}_1 \perp \tilde{\boldsymbol{\alpha}}_2$, the matrix **G** has PCs with the same direction as the modules (*i.e.*, the same direction as $\tilde{\boldsymbol{\alpha}}_1$ and $\tilde{\boldsymbol{\alpha}}_2$) regardless of allele frequencies. Also, each eigenvalue λ_j is a function of the allele frequency at a single locus: $\lambda_j = H_j||\tilde{\boldsymbol{\alpha}}_j||^2$. Therefore, in a population with modules, each PC accounts for the entirety of the variation attributable to a single module, which in this case is defined by allelic variation at a single locus. Because the **G** of a population with modules has the same PCs regardless of allele frequencies, if other populations have modules *with the same direction*, the **G** matrices of the populations will have two common PCs (*i.e.*, both PCs will have the same direction) although the eigenvalues associated with the PCs in the two populations may differ.

Note that in the special case where an allele at one locus goes to fixation in one of the populations, the same argument can be used to demonstrate that there will still be two common PCs if the $\tilde{\boldsymbol{\alpha}}_j$ at the other locus has the same direction as a module in the other population. In such a case, a zero eigenvalue will be associated with one of the PCs in the population with the fixed allele. Similarly, if both $\tilde{\boldsymbol{\alpha}}_j$ in one population have the same direction, if the direction is the same as that of a module in the other populations, common PCs will still exist. These results also hold in the case where one or several of the **G** matrices have a multiplicity of eigenvalues. The reason is that the common PC model framework handles such cases where the eigenvector matrix is not unique by choosing the direction of the PCs to correspond to the PCs of other matrices (if possible). Also note that, if populations have modules with different directions, they will never have common PCs, unless allele frequencies are such that a multiplicity of eigenvalues exists. Because the approach used in *Result A2* can be used to demonstrate that a multiplicity of eigenvalues in the **G** matrix occurs only for a very restricted set of the possible allele frequencies, common PCs are not expected when modules are not in common.

Result A2: *If populations A and B have no modules, given heterozygote frequencies in population A, a line intersecting the region bounded by the square of possible heterozygote frequencies in population B ($0 \le H_{j.B} \le 0.5$) describes the frequencies that result in common PCs in $\mathbf{G}_A$ and $\mathbf{G}_B$.*

An intuitive interpretation of this result is that the number of heterozygote (allele) frequencies for which $\mathbf{G}_A$ and $\mathbf{G}_B$ have common PCs is far smaller than the number of heterozygote (allele) frequencies for which the PCs are different. For example, given heterozygote frequencies in population *A* ($H_{1.A}$ and $H_{2.A}$) for every heterozygote frequency $H_{1.B}$ at the first locus in population *B*, a single frequency $H_{2.B}$ at the second locus produces common PCs. All other frequencies at the second locus will result in different PCs.

Assume that there are no modules in population *B*, such that $\tilde{\boldsymbol{\alpha}}_{1.B}^{\mathrm{T}}\tilde{\boldsymbol{\alpha}}_{2.B} \neq 0$. Fix $H_{1.A}$ and $H_{2.A}$ between 0 and 0.5 and assume that alleles are segregating at both loci in population *B*. Define the PC matrices of $\mathbf{G}_A$ and $\mathbf{G}_B$ as $\mathbf{Q}_A$ and $\mathbf{Q}_B$. By the spectral theorem, if population *B* has the same PCs as population *A*, then $\mathbf{Q}_B = \mathbf{Q}_A$ (up to column permutation and multiplication of columns by −1), and we can write

$$\mathbf{G}_B = \mathbf{Q}_A\Lambda_B\mathbf{Q}_A^{\mathrm{T}}. \tag{A5}$$

From (A1), we can rewrite $\mathbf{G}_B$ as

$$\mathbf{G}_B = H_{1.B}(\tilde{\boldsymbol{\alpha}}_{1.B}\tilde{\boldsymbol{\alpha}}_{1.B}^{\mathrm{T}}) + H_{2.B}(\tilde{\boldsymbol{\alpha}}_{2.B}\tilde{\boldsymbol{\alpha}}_{2.B}^{\mathrm{T}}). \tag{A6}$$

Therefore, for population *B* to have the same PCs as population *A*, the following relation must be satisfied:

$$H_{1.B}\mathbf{Q}_A^{\mathrm{T}}(\tilde{\boldsymbol{\alpha}}_{1.B}\tilde{\boldsymbol{\alpha}}_{1.B}^{\mathrm{T}})\mathbf{Q}_A + H_{2.B}\mathbf{Q}_A^{\mathrm{T}}(\tilde{\boldsymbol{\alpha}}_{2.B}\tilde{\boldsymbol{\alpha}}_{2.B}^{\mathrm{T}})\mathbf{Q}_A = \Lambda_B. \tag{A7}$$

The off-diagonal elements of the matrix on the left side of (A7) are the same and are equal to zero elements in the matrix on the right side:

$$H_{1.B}(q_{11.A}\tilde{\alpha}_{1.1.B} + q_{12.A}\tilde{\alpha}_{1.2.B})(q_{21.A}\tilde{\alpha}_{1.1.B} + q_{22.A}\tilde{\alpha}_{1.2.B}) + H_{2.B}(q_{11.A}\tilde{\alpha}_{2.1.B} + q_{12.A}\tilde{\alpha}_{2.2.B})(q_{21.A}\tilde{\alpha}_{2.1.B} + q_{22.A}\tilde{\alpha}_{2.2.B}) = 0, \tag{A8}$$

where $\tilde{\alpha}_{j.i.B}$ is the average effect of an allelic substitution at locus j on trait i in population B and $q_{jk.A}$ is element k of column j of matrix $\mathbf{Q}_A$. Note that if we assume population B has no modules, $\tilde{\boldsymbol{\alpha}}_{1.B}^{\mathrm{T}}\tilde{\boldsymbol{\alpha}}_{2.B} \neq 0$, and because $\mathbf{q}_{1.A}^{\mathrm{T}}\mathbf{q}_{2.A} = 0$, if the term in (A8) associated with either $H_{1.B}$ is zero, the other term associated with $H_{2.B}$ is positive. In such a case (A8) can be satisfied only if $H_{2.B} = 0$ (and vice versa). Because we are currently concerned with common PCs and assume alleles are segregating at all loci, it is the case that

$$\begin{aligned}(q_{11.A}\tilde{\alpha}_{1.1.B} + q_{12.A}\tilde{\alpha}_{1.2.B}) \neq 0, \quad (q_{21.A}\tilde{\alpha}_{1.1.B} + q_{22.A}\tilde{\alpha}_{1.2.B}) \neq 0\\ (q_{11.A}\tilde{\alpha}_{2.1.B} + q_{12.A}\tilde{\alpha}_{2.2.B}) \neq 0, \quad (q_{21.A}\tilde{\alpha}_{2.1.B} + q_{22.A}\tilde{\alpha}_{2.2.B}) \neq 0.\end{aligned} \tag{A9}$$

Given (A8) and (A9), for population B to have the same PCs as population A, the heterozygote frequencies at the two loci in population B must satisfy the following relationship:

$$\frac{H_{1.B}}{H_{2.B}} = -\frac{(q_{11.A}\tilde{\alpha}_{2.1.B} + q_{12.A}\tilde{\alpha}_{2.2.B})(q_{21.A}\tilde{\alpha}_{1.1.B} + q_{22.A}\tilde{\alpha}_{2.2.B})}{(q_{11.A}\tilde{\alpha}_{1.1.B} + q_{12.A}\tilde{\alpha}_{1.2.B})(q_{21.A}\tilde{\alpha}_{1.1.B} + q_{22.A}\tilde{\alpha}_{1.2.B})}. \tag{A10}$$

Note that a single set of PCs is associated with each pair of heterozygote frequencies, so if (A10) holds for $H_{1.B}$ and $H_{2.B}$, the equations defined by the diagonal elements of (A7) are satisfied by the eigenvalues of $\mathbf{G}_B$ corresponding to $H_{1.B}$ and $H_{2.B}$:

$$\begin{aligned}H_{1.B}(q_{11.A}\tilde{\alpha}_{1.1.B} + q_{12.A}\tilde{\alpha}_{1.2.B})^2 + H_{2.B}(q_{11.A}\tilde{\alpha}_{2.1.B} + q_{12.A}\tilde{\alpha}_{2.2.B})^2 = \lambda_{1.B}\\ H_{1.B}(q_{21.A}\tilde{\alpha}_{1.1.B} + q_{22.A}\tilde{\alpha}_{1.2.B})^2 + H_{2.B}(q_{21.A}\tilde{\alpha}_{2.1.B} + q_{22.A}\tilde{\alpha}_{2.2.B})^2 = \lambda_{2.B}.\end{aligned} \tag{A11}$$

Therefore, only when the heterozygote frequencies in population B satisfy (A10) are the PCs of $\mathbf{G}_B$ in the same direction as the PCs of $\mathbf{G}_A$ (*i.e.*, two PCs are in common). These heterozygote frequencies can be visualized as falling on a one-dimensional "plane" that cuts through the region bounded by the square of possible heterozygote frequencies in population B where $0 \leq H_{j.B} \leq 0.5$. The ratio of the number of heterozygote frequencies for which common PCs occur to all possible heterozygote frequencies is small. Therefore, the vast majority of the possible allele frequencies in population B result in different PCs in the two populations and similarly for population A when heterozygote frequencies in population B are held constant. Under the special case in which the same alleles are segregating in both populations ($\tilde{\boldsymbol{\alpha}}_{1.A} = \tilde{\boldsymbol{\alpha}}_{1.B}$ and $\tilde{\boldsymbol{\alpha}}_{2.A} = \tilde{\boldsymbol{\alpha}}_{2.B}$), common PCs occur only when

$$\frac{H_{1.B}}{H_{2.B}} = \frac{H_{1.A}}{H_{2.A}}, \tag{A12}$$

which happens when $\mathbf{G}_A \propto \mathbf{G}_B$.

The constraint of (A10) makes common PCs unexpected among the $\mathbf{G}$ matrices of populations A and B if there are no modules. The reason is that, even if this constraint is satisfied at some point, any change in allele frequencies at one locus must be exactly balanced by a change at the other locus that preserves the ratios in (A10). The stochastic changes in allele frequencies due to mutation and genetic drift are therefore not expected to preserve the necessary ratios.

Note that, although two populations are considered in this section, the reasoning can also be extended to multiple populations. Also, the same reasoning can be used to demonstrate that, in the case where an allele at one locus goes to fixation in one of the populations or where both $\tilde{\boldsymbol{\alpha}}_j$ in one population have the same direction, the allele frequencies required for common PCs are highly constrained in the same fashion. The case of multiplicity of eigenvalues does not occur in the special case of the two-locus, two-allele model when the $\tilde{\boldsymbol{\alpha}}_j$ in a population are not orthogonal.

APPENDIX B: THE MODEL OF CONSTRAINED PLEIOTROPY

APPENDIX B extends the framework outlined in *Result A1* and *Result A2* to the model of constrained pleiotropy of WAGNER (1989). The model of constrained pleiotropy assumes that all segregating alleles and all possible mutant alleles at an individual locus j have effects that fall along a single vector. In a quantitative genetic formulation, the absolute values of the additive effect vectors of any alleles k and l at a locus j are proportional: $|\boldsymbol{\alpha}_{jk}| \propto |\boldsymbol{\alpha}_{jl}|$. Mutations are assumed to occur at each locus j at a rate μ_j. Here, n traits are being considered in all populations being compared, although the populations may have different numbers of loci. We assume random mating among diploid individuals in a population. We also assume no dominance, epistasis, disequilibrium (linkage or otherwise), maternal effects, sex linkage, genotype-environment covariance, or genotype-environment interactions.

The additive effect of allele k at some locus j for n traits is

$$\boldsymbol{\alpha}_{jk} = \sum_{l}^{J_j} p_{jl}\mathbf{g}_{kl} - \boldsymbol{\mu}_{\mathrm{g}}, \tag{B1}$$

where p_{jl} is the frequency of allele l, J_j is the number of alleles at locus j, each entry of $\mathbf{g}_{kl} = [g_{kl.1}, \ldots, g_{kl.n}]$ is the mean phenotype of trait i given alleles k, l at locus j, and each entry of $\boldsymbol{\mu}_{\mathrm{g}} = [\mu_{\mathrm{g}.1}, \ldots, \mu_{\mathrm{g}.n}]$ is the mean genotypic value of trait i (Lynch and Walsh 1998). In the absence of dominance $\mathbf{g}_{kl} = (\mathbf{g}_{kk} + \mathbf{g}_{ll})/2$, and making this substitution into (B1), we can write the additive effect of an allele as follows:

$$\boldsymbol{\alpha}_{jk} = p_{jk}\mathbf{g}_{kk} + \frac{1}{2}\sum_{l\neq k}^{J_j} p_{jl}(\mathbf{g}_{kk} + \mathbf{g}_{ll}) - p_{jk}\mathbf{g}_{kk}\sum_{m}^{J_j} p_{jm} - \sum_{l\neq k}^{J_j} p_{jl}\mathbf{g}_{ll}\left(\sum_{m}^{J_j} p_{jm}\right) \tag{B2}$$

$$\boldsymbol{\alpha}_{jk} = \frac{1}{2}\sum_{l\neq k}^{J_j} p_{jl}(\mathbf{g}_{kk} - \mathbf{g}_{ll}). \tag{B3}$$

Under the assumption of constrained pleiotropy, the genotypic values associated with a locus are proportional and this condition requires that $\mathbf{g}_{kl} \propto \mathbf{g}_{qr}$ for all alleles k, l, q, r at locus j. We can therefore write

$$\boldsymbol{\alpha}_{jk} = \frac{1}{2}\left(\sum_{l\neq k}^{J_j} p_{jl}(\Gamma_{kk} - \Gamma_{ll})\right)\boldsymbol{\gamma}_j, \tag{B4}$$

where $\boldsymbol{\gamma}_j = [\gamma_{j.1}, \ldots, \gamma_{j.n}]$ is the unit scaled vector in the direction of the pleiotropic effect associated with locus j, and the Γ are scalars. In the model of constrained pleiotropy, each of the $\boldsymbol{\gamma}_j$ are constant. Note that under the assumed conditions the $\mathbf{G}$ matrix can be written

$$\mathbf{G} = \sum_{j}^{N}\sum_{k}^{J_j} p_{jk}\boldsymbol{\alpha}_{jk}\boldsymbol{\alpha}_{jk}^{\mathrm{T}}. \tag{B5}$$

Setting $\kappa_{jk} = \frac{1}{2}(\Sigma_{l\neq k}^{J_j}\, p_{jl}(\Gamma_{kk} - \Gamma_{ll}))$ and $L_j = 2\Sigma_{k}^{J_j}\, p_k\kappa_{jk}^2$, we can write $\mathbf{G}$ as

$$\mathbf{G} = \sum_{j}^{N} L_j\boldsymbol{\gamma}_j\boldsymbol{\gamma}_j^{\mathrm{T}}. \tag{B6}$$

Below, we assume that $\mathbf{G}$ has no multiplicity of eigenvalues and is of full rank unless noted.

Modules exist in a population if a subset of M loci ($M < N$) exists in which the allelic vectors at each of these M loci are orthogonal to the allelic vectors at each of the other $N - M$ loci. This means that, for each $\boldsymbol{\alpha}_{jk(M)}$ that may occur at the M loci and each $\boldsymbol{\alpha}_{jk(N-M)}$ that may occur at the remaining $N - M$ loci,

$$\boldsymbol{\alpha}_{jk(M)}^{\mathrm{T}}\boldsymbol{\alpha}_{jk(N-M)} = 0. \tag{B7}$$

If no subsets of M loci satisfy this relationship, a module does not exist. Note that in the following, we are concerned only with modules that are one-dimensional where $|\boldsymbol{\alpha}_{jk(M)}| \propto |\alpha_{mk(M)}|$ for all loci j, m in a subset of M loci that define a module.

Result B1: *For each pair of modules that populations have in common, the* $\mathbf{G}$ *matrices have a common PC with the same direction as the module, regardless of allele frequencies or effects of mutations in the populations.*

In a population in which $M < N$ loci define a module, the $\mathbf{G}$ matrix can be written as

$$\mathbf{G} = \sum_{j}^{M} L_j\boldsymbol{\gamma}_j\boldsymbol{\gamma}_j^{\mathrm{T}} + \sum_{j}^{N-M} L_j\boldsymbol{\gamma}_j\boldsymbol{\gamma}_j^{\mathrm{T}}, \tag{B8}$$

where the first summation is over the M loci defining the module and the second is over the remaining $N - M$ loci. Each summation term is itself a matrix:

$$\mathbf{G} = \mathbf{G}_M + \mathbf{G}_{N-M}. \tag{B9}$$

Because we have assumed that $\mathbf{G}$ is of full rank, at least two alleles are segregating at at least one locus in M. If so, regardless of the specific set of alleles and the frequency of alleles in the population, the allelic vectors defining the module span a one-dimensional space in n, and the rest of the allelic vectors span an $n - 1$-dimensional space that is orthogonal. Correspondingly, the matrix $\mathbf{G}_M$ is of rank 1 and $\mathbf{G}_{N-M}$ is of rank $n - 1$. The spectral decomposition of $\mathbf{G}_{N-M}$ produces a zero eigenvalue:

$$\mathbf{Q}_{N-M}^{\mathrm{T}}\mathbf{G}_{N-M}\mathbf{Q}_{N-M} = \begin{bmatrix} 0 & 0 & \dots & 0 \\ 0 & \lambda_{1.N-M} & & \vdots \\ \vdots & & \ddots & 0 \\ 0 & \dots & 0 & \lambda_{n.N-M} \end{bmatrix}. \tag{B10}$$

Because the allelic vectors of the $N - M$ loci span the $n - 1$ space, the eigenvector corresponding to the zero eigenvalue is orthogonal to the $n - 1$-dimensional space and has the same direction as the module *regardless of allele frequencies or the effects of mutation* in the population. Applying $\mathbf{Q}_{N-M}$ to the matrix $\mathbf{G}_M$ produces

$$\mathbf{Q}_{N-M}^{\mathrm{T}}\mathbf{G}_M\mathbf{Q}_{N-M} = \begin{bmatrix} \lambda_M & 0 & \dots & 0 \\ 0 & 0 & & \vdots \\ \vdots & & \ddots & 0 \\ 0 & \dots & 0 & 0 \end{bmatrix}, \tag{B11}$$

where λ_M corresponds to the eigenvector with the same direction as the module. The orthonormal matrix $\mathbf{Q}_{N-M}$ therefore diagonalizes $\mathbf{G}$,

$$\mathbf{Q}_{N-M}^{\mathrm{T}}\mathbf{G}\mathbf{Q}_{N-M} = \mathbf{Q}_{N-M}^{\mathrm{T}}(\mathbf{G}_M + \mathbf{G}_{N-M})\mathbf{Q}_{N-M} = \Lambda, \tag{B12}$$

and is unique up to column permutation and multiplication of columns by -1, by the spectral theorem. Therefore a PC of $\mathbf{G}$ that has the same direction as the module always exists, and from (B11), this PC also accounts for the entirety of the variation segregating at the loci defining the module. Because the population has a PC that always corresponds to the module regardless of allele frequencies, if several populations have a module with the same direction, these populations will always have a common PC with the same direction as the module. The argument also holds for any number of modules (up to n), so a common PC occurs in the $\mathbf{G}$ matrices corresponding to each pair of modules that the populations have in common. Note that the same argument can be used to demonstrate that populations with modules in common will have common PCs corresponding to the modules even when the $\mathbf{G}$ matrix is not of full rank. Similarly, as explained for the two-locus, two-allele model, common PCs will occur when common modules do, even if $\mathbf{G}$ has a multiplicity of eigenvalues.

Result B2: *For populations A and B with no common modules, given allele frequencies in population A, the allele frequencies that result in common PCs in $\mathbf{G}_A$ and $\mathbf{G}_B$ are described by n overlapping quadratic ($N_B\bar{J}_B - n + 1$)-dimension planes intersecting the $N_B\bar{J}_B$-dimension region describing the possible allele frequencies at each of the N_B loci in population B.*

$\Psi_{(x)}$ indicates a matrix with elements ψ_{ij}, where element ψ_{xx} is a positive value, all other elements in column ψ_{x-} and row ψ_{-x} are zero, and all other elements may or may not be equal to zero. For example, $\Psi_{(1)}$ is an instance of a matrix with the following form,

$$\begin{bmatrix} \psi_{11} & 0 & \dots & 0 \\ 0 & \psi_{2,2} & \dots & \psi_{n,2} \\ \vdots & \vdots & \ddots & \vdots \\ 0 & \psi_{2,n} & \dots & \psi_{n,n} \end{bmatrix}, \tag{B13}$$

where each ψ_{ij} except ψ_{11} may or may not be equal to zero. In this notation, if populations A and B have PC x in common, the following relation holds:

$$\mathbf{Q}_A^{\mathrm{T}}\mathbf{Q}_B = \Psi_{(x)}. \tag{B14}$$

We consider the $\mathbf{G}_A$ and $\mathbf{G}_B$ associated with populations A and B at a given point in time. The populations segregate for N_A and N_B loci, respectively. By the spectral theorem and (B6), for population B to have PC x in common with population A, the following relation must be satisfied:

$$\sum_j^{N_B} L_{j.B}\mathbf{Q}_A^{\mathrm{T}}(\boldsymbol{\gamma}_{j.B}\boldsymbol{\gamma}_{j.B}^{\mathrm{T}})\mathbf{Q}_A = \Psi_{(x)}. \tag{B15}$$

Under the definition of L_j above, the off-diagonal elements of column (or row) x of the matrix on the left side of this relation define a system of $n - 1$ equations of the form

$$2\sum_j^{N_B}\sum_k^{J_{j.B}} p_k\kappa_{k.B}^2(q_{x1.A}\gamma_{j1.B} + \dots + q_{xn.A}\gamma_{j.n.B})(q_{i1.A}\gamma_{j.1.B} + \dots + q_{in.A}\gamma_{j.n.B}) = 0 \tag{B16}$$

for $0 < i \leq n$, $i \neq x$. Note that each κ_k is a linear function of the allele frequencies at locus j, excluding allele k.

The highest-order terms of (B16) are therefore quadratic. The allele frequencies in population B that satisfy the equations of (B16) are described by a quadratic plane with a maximum of $(N_B\bar{J}_B - n + 1)$ dimensions, where $\bar{J}_B$ is the mean number of segregating alleles per locus in population B. The frequencies for which the populations have PC x in common are described by the part of this plane that intersects the region bounded by the $N_B\bar{J}_B$-dimension region describing the possible allele frequencies at each of the N_B loci in population B. The part of this region describing the allele frequencies of the $J_{j.B}$ alleles at a single locus j is a $J_{j.B}$-dimension simplex ($0 \leq p_{k.B} \leq 1$, $(\Sigma_k^{J_{j.B}} p_{k.B}) = 1$). With respect to the allele frequencies of any two alleles from different loci, the region has the form of a square ($0 \leq p_{k.B} \leq 1$ for allele k at each locus). Note that the quadratic plane describing the allele frequencies that result in common PCs is always at least one less than the dimensionality of the figure. Therefore, the ratio of the number of allele frequencies for which PC x is common to all possible allele frequencies is always small and gets smaller the greater the number of traits n.

The constraint of (B16) makes common PCs unexpected among the **G** matrices of populations A and B if no modules exist. The reason is the same as for the two-locus, two-allele model. Even if the constraint is momentarily satisfied, any change in allele frequencies must be exactly balanced by changes in other allele frequencies to satisfy the constraint, and these frequencies represent a small fraction of possible allele frequencies. The stochastic changes in allele frequencies due to mutation and genetic drift are therefore not expected to preserve the constraint in (B16).

If relation (B16) is satisfied, the populations have a single PC x in common, but for n traits, n PCs may be in common. For each of these, a system of $n - 1$ equations of the form of Equation B16 define a quadratic $(N_B\bar{J}_B - n + 1)$-dimension plane (for $n = 2$, the systems are the same). Where these planes intersect the figure, at least one PC is in common, and where these planes overlap, there is more than one common PC. Although more traits define more planes, each plane is of correspondingly lower dimension. Therefore, as n gets larger, the ratio of the number of allele frequencies for which at least one PC is in common to the number of possible allele frequencies gets smaller.

Note that, although two populations are considered in this section, the reasoning can also be extended to multiple populations. Also, for completeness, three special cases must be considered. In the case where populations A and B have x pairs of common modules, at least x common PCs will exist, as discussed above. In this case, the frequencies in population B for which $x + 1$ (where $x < n - 2$) common PCs exist are described by the portion of an $(N_B\bar{J}_B - C_B) - (n + x) + 1$-dimension plane that intersects the region bounded by a $(N_B\bar{J}_B - C_B)$-dimension figure, where C_B is the number of allelic vectors defining the modules in population B. Two other special cases are those in which each population has a module but the modules have different directions. If the modules are orthogonal in the space of n traits, common PCs are again described by the portion of an $(N_B\bar{J}_B - C_B) - (n + x) + 1$-dimension plane that intersects the region bounded by the $(N_B\bar{J}_B - C_B)$-dimension figure. If the modules in the two populations are not orthogonal in the space of n traits, at least two PCs will exist that are not in common unless allele frequencies are such that a multiplicity of eigenvalues occurs.