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Direct and indirect assortative mating: a multivariate approach to plant flowering schedules

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Abstract

This paper develops methods to partition the phenotypic correlation between mates for a focal trait – the standard measure for assortative mating – into a direct component and additional indirect components. Indirect assortative mating occurs when a nonassorting trait is correlated within individuals to a directly assorting trait. Direct and indirect assortative mating is assessed for flowering phenology in *Brassica rapa*. The flowering time of pollen recipients (mothers) was strongly correlated ($\rho = 0.67$) to that of potential pollen donors (fathers). Similarly, recipients and donors were correlated for duration of their flowering periods ($\rho = 0.32$) and stem diameters ($\rho = 0.52$). A partitioning of between-mate correlations revealed direct assortative mating for flowering time and period duration. However, assortment for stem diameter is explained solely through its correlation to flowering time. Examination of standard quantitative genetic theory shows that indirect assortative mating inflates genetic variance in a focal trait and the genetic covariance between focal and phenotypically correlated traits.

Introduction

Assortative mating can be defined as a phenotypic correlation between mates (Pearson & Lee, 1903; Wright, 1921). When like mates with like, gametic disequilibrium inflates the genetic variance for the assorting trait in the offspring generation (i.e. the frequency of individuals with many alleles adding to the trait value and that of individuals with many alleles subtracting from the trait value, will be greater than random). Hence, assortative mating makes a trait more responsive to selection (Felsenstein, 1981; Jorjani *et al.*, 1997; Lynch & Walsh, 1998). When assortment occurs simultaneously for two or more traits, gametic disequilibrium may also inflate the genetic covariance between them. This covariance inflation can be due to disequilibrium at loci that contribute pleiotropically to both traits, or due to disequilibrium between loci that contribute to the traits independently (Gianola, 1982; Kirkpatrick *et al.*, 2002).

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This paper considers the structure of assortative mating generally and develops an analytical structure to study flowering phenology in order to determine the likely effects of assortment on the variance/covariance structure among phenological traits and other traits correlated to phenology.

Pearson & Lee (1903) recognized that populations can assortatively mate for more than one trait at a time. In the simplest case, assortment for each trait operates independently. For instance, in human couples, husbands and wives are correlated to one another in both physical stature (Silventoinen *et al.*, 2003) and liability for substance abuse (Vanyukov *et al.*, 1996). In plants, different components of pollinator foraging behaviour could cause simultaneous assortment due to petal colour (Jones & Reithel, 2001) and inflorescence height (Levin & Kerster, 1972). In cases like these, the assorting traits may be uncorrelated within individual mates (e.g. tall people are neither more nor less prone to substance abuse). As a result, the correlation between mates for one trait will be independent of that for the other, and so the cross-correlation between mates is expected to be zero. By extension, the genetic consequences of assortative mating will be independent for the two traits.

In other cases, phenotypic correlations within individuals will lead to mutually assortative mating for two or more traits (Pearson & Lee, 1903). For instance, Brown (1993) found that males of the beetle *Trirhabda canadensis* who courted females that were appreciably larger or smaller then themselves did not successfully engage their copulatory organ. Thus, body size was correlated in successful pairs. Body size, in turn, could be correlated with other traits involved in successful engagement, say if leg length affected the male's mechanical advantage in grasping females of given girth. However, body size may also be correlated to suites of traits with no direct effect on mating, such as larval growth rate. Any trait that has a within-individual correlation to a trait causing assortment will also be correlated between mates, whether it contributes mechanistically to the assorting process (e.g. leg length) or not (e.g. larval growth rate). A multivariate approach is needed to decompose the correlation between mates for any focal trait into its direct component and additional indirect components that emerge from the within-individual correlations with the other traits. Additionally, the cross-correlation between mates (say, the correlation between male leg length and female growth rate) can also be decomposed into direct and indirect components. The inflationary effects of assortative mating on the genetic variances for individual traits can thus be partitioned into those caused by direct and indirect assortment. Inflation in the genetic covariance between traits can be partitioned into the direct and indirect components of cross-assortative mating.

The flowering schedule in plants (i.e. the number of active flowers open on each day of the season) is a multivariate trait (Mallo, 2002), with component traits such as the day of first, peak and last flowering. Variation between individuals in the flowering schedule causes phenological assortative mating (Fox, 2003; Weis & Kossler, 2004). Early-blooming plants mate predominantly with other early plants, while late bloomers mate predominantly with other late plants (Gutierrez & Sprague, 1959; Ennos & Dodson, 1987; Weis & Kossler, 2004). However, the distinction between early and late bloomers can be more complex than it may first appear. Fox (2003) made a distinction between 'conventional' and 'cryptic' phenological assortative mating. Conventional assortment results when individuals differ in the beginning and end dates of their flowering periods such that at any one time in the flowering season only a subset of individuals are sexually active. Assortative mating will also occur cryptically. Even if all individuals start and stop flowering on the same dates assortment will arise if individuals vary in the way their flowers are dispersed over the season; plants reaching peak flowering early are more likely to exchange pollen with each other than with late-peaking plants. Put another way, conventional phenological assortative mating is caused by variation in the 'temporal position' of the flowering schedule while cryptic assortment emerges from variation in schedule 'shape'. Position and shape can be correlated within individuals, e.g. plants that flower early may take longer to reach their peak of flower production. Thus the genetic variance in either one of these traits will be inflated directly by assortative mating for that trait and indirectly by assortative mating in the other trait. Further, the genetic covariance between the traits can be inflated, and this inflation also will occur through direct and indirect components of cross-assortative mating.

This paper re-examines data presented by Weis & Kossler (2004) on phenological assortative mating in a greenhouse population of *Brassica rapa*. I first extend and simplify Weis & Kossler's method to estimate the phenotypic correlations and cross-correlations between mates for phenology by restating it in matrix notation. Then, I develop a general framework for evaluating the intensities of the direct and indirect components of assortative and cross-assortative mating and apply it to the *B. rapa* data set. Finally I discuss some consequences of multivariate assortative mating on genetic variance-covariance structure.

Methodological background

Estimating the potential for phenological assortative mating

Finding the correlation in flowering time between pollen recipients and their donors presents some methodological difficulties. Plants typically mate repeatedly, promiscuously and cryptically. Identifying the individual donors to a particular recipient plant can be accomplished by paternity analysis, using genetic markers (e.g. Devlin & Ellstrand, 1990; Campbell, 1989; Jones & Ardren, 2003). Although useful in small, genetically defined experimental populations, the effort required to genotype all potential pollen donors and numerous offspring per recipient makes it impractical for large natural populations.

Weis & Kossler (2004) presented an alternative method; it measures the *potential* for assortative mating in flowering time (days from germination to anthesis) by assessing the overlap in the flowering schedules of pollen recipients and potential donors. Even if mating is random among the flowers open within each day, it will be assortative across the season because of the shifting composition of the mating pool (Fox, 2003; Weis *et al.*, 2004). The Weis–Kossler method estimates ρ, the correlation between maternal flowering time and flowering times of the potential fathers, weighted by the mating probability between each mother–father pairing. This correlation is a prospective estimate of assortment because it is based on mating opportunities. Weis & Kossler (2004) validated the prospective method with standard principles of quantitative genetics. Specifically, the regression of offspring flowering time over maternal

flowering time is expected to equal $0.5h^2$ when mating is random, but $0.5h^2(1 - \rho')$ when mating is assortative, where ρ' is the actual phenotypic correlation between mates. By comparing the slope of the offspring-maternal regression to an independently derived heritability estimate, they showed their prospective estimate of ρ for *B. rapa* flowering time strongly agreed with ρ', the retrospective estimate.

This section extends and simplifies the prospective method by restating in matrix notation. The presentation applies to a highly promiscuous, hermaphroditic, self-incompatible mating system. Adapting it to most other mating systems will be straightforward.

The first step to estimating the phenotypic correlation between mates for phenological traits is to use flowering schedule data to construct Φ, the mating matrix. Φ contains the mating probabilities for all potential mother–father pairings in a sample of individuals. It is the product of two matrices; the first contains the flowering schedules of the plants in their role as pollen recipients (mothers) and the second as pollen donors (fathers). The following $p \times d$ matrix represents flowering schedules of mothers:

$$\mathbf{M} = N^{-1}\begin{pmatrix} n_{11} & n_{12} & \cdots & n_{1d} \\ n_{21} & n_{22} & \cdots & n_{2d} \\ \vdots & \vdots & \ddots & \vdots \\ n_{p1} & n_{p2} & \cdots & n_{pd} \end{pmatrix},$$

where n_{ik} is the number of open flowers on individual i open on census day k of the flowering season, and N is the total number of flowers censused over the season (i.e. $\Sigma_{k=1}^{d}\Sigma_{i=1}^{p} n_{ik}$). Each element of $\mathbf{M}$ is the proportion of all opportunities for pollen receipt in the entire sample of p plants over the entire d days of the flowering season that were held by individual i on census day k; the elements sum to 1. The flowering schedules of the same plants (which are hermaphrodites) acting as fathers can be expressed in the following $d \times p$ matrix:

$$\mathbf{F} = \begin{pmatrix} v_{11} & v_{12} & \ldots & v_{1p} \\ v_{21} & v_{22} & \cdots & v_{2p} \\ \vdots & \vdots & \ddots & \vdots \\ v_{d1} & v_{d2} & \cdots & v_{dp} \end{pmatrix},$$

where v_{kj} is the proportion of all flowers in the population open on census day k that were on individual j (i.e. $v_{kj} = n_{kj}/\Sigma_{j=1}^{p} n_{kj}$); the elements sum to d, the number of census days. The mating matrix is then

$$\Phi = MF \qquad (1)$$

a $p \times p$ matrix wherein each element, Φ_{ij} is the proportion of all mating opportunities for the population in which plant i is the mother and plant j the expected father; the elements sum to 1.

As a point of reference, consider Φ for a sample of p mating pairs drawn from a monogamous, dioecious population; all elements along the leading diagonal would be $1/p$ and all other elements would be zero. In a promiscuous system, all elements can be greater than zero. The Φ could also be established retrospectively using paternity analysis (Jones & Ardren, 2003). Each matrix element would be the proportion of all offspring in the population attributable to the specific mater–paternal combination.

Self-incompatibility can necessitate adjustments to Φ. No adjustment is needed if the goal is to characterize a large population from a sample. If so, the values for the diagonal elements (Φ_{ij}, where $i = j$) represent matings between the sampled individual and other individuals in the larger population with the same phenotype (assuming that the self-incompatibility locus is not in linkage disequilibrium with loci of large effect on the traits of interest). If the goal is to measure assortment in a self-contained population of p plants, as in an experimental setting, the diagonal elements are set to zero, and the remaining elements rescaled.

The prospective estimate of the covariance between mates for a given trait z can be calculated as

$$\rho = \mathbf{z}'\Phi\mathbf{z}, \qquad (2)$$

where $\mathbf{z}$ is a vector of length p containing the normalized phenotypic value, ζ_i, for each parent (i.e. $\zeta = \{z_i - \bar{z}\}s^{-1}$, where s is the standard deviation of z), and the prime indicates transposition. Standardizing the phenotypic values yields a standardized covariance, i.e. a correlation coefficient.

By extension, the correlations between mates for a set of q traits, and the cross-correlations between mates for pairs of traits, can be represented by the $q \times q$ matrix

$$\mathbf{R} = \mathbf{Z}'\Phi\mathbf{Z} \qquad (3)$$

where $\mathbf{Z}$ is a matrix wherein each column contains the normalized phenotypic value for each of the p individuals for one of the q traits. The leading diagonal of $\mathbf{R}$ contains the correlations between mates for each trait, ρ_i, while the off-diagonals are the cross-correlations for pairs of traits, ρ_{ij}. Cross-correlations are symmetrical (i.e. $\rho_{ij} = \rho_{ji}$) in this case because parents are hermaphrodites, but this need not be so when considering other mating systems. When I present cross-correlations, the first subscript will denote the trait in the pollen recipient and the second will denote the trait in the donor.

Direct and indirect assortment and cross-assortment

The concept of direct and indirect assortative mating is similar to that of direct and indirect phenotypic selection first recognized by Pearson (1903) and formally defined by Lande & Arnold (1983). Briefly, the selection differential on a quantitative trait is the covariance between the phenotypic value and relative fitness (Price, 1970). This covariance can be decomposed into a component that accounts for the direct action of selection on the focal trait, plus additional components due to

selection on correlated traits. The direct component, termed the selection gradient, is the partial regression coefficient of relative fitness over phenotypic value (Lande & Arnold, 1983). Each of the indirect components is the product of the selection gradient on another trait and its phenotypic correlation to the focal trait.

The phenotypic covariance between mates can be similarly decomposed if one adopts an alternative interpretation of ρ. Rather than a correlation coefficient, it can be considered a standardized regression coefficient (Weis & Kossler, 2004; Weis *et al.*, 2004) – the maternal phenotype (independent variable) *predicts* the paternal phenotype (dependent variable). (In some biological situations it may be appropriate for maternal and paternal phenotypes to exchange roles as dependent and independent variables.) However, when the trait of interest is correlated to other assorting traits, the regression of father over mother can be decomposed into a network of underlying components.

Figure 1 shows a path diagram for the covariance between mates for traits z and y. The correlation between females and males for trait z consists of the direct component, depicted by path coefficient α_z, which is the partial regression of the paternal value of z over the maternal value. The indirect component, acting through trait y, is the product of the partial regression of paternal z over maternal y, α_{yz}, and the within-mate phenotypic correlation between z and y, r_{zy}. Extending to a suite of q traits, the phenotypic correlation between mates for trait z will be

$$\rho_z = \alpha_z + r_{yz}\alpha_{yz} + r_{xz}\alpha_{xz} + \cdots + r_{qz}\alpha_{qz}. \quad (4)$$

The correlation between mates for traits y to q also can be decomposed by the corresponding path coefficients. Reciprocal cross-regression coefficients, α_{ij} and α_{ji}, will not usually be equal; the covariance between i and j is divided by σ_i^2 to get one coefficient and by σ_j^2 for the other.

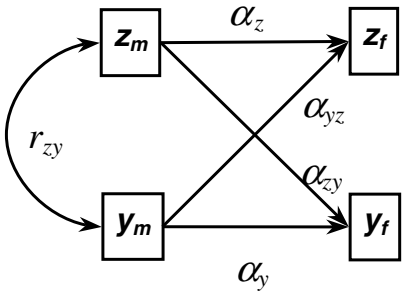

Fig. 1 Path diagram illustrating direct and indirect assortative mating for traits z and y. Subscripts m and f stand for mother and father, respectively. The phenotypic correlation between mates for $z(\rho_z)$ consists of a direct component (α_z) & an indirect component acting through trait y ($r_{zy}\alpha_{yz}$). The correlation between mates for y is decomposed by complementary relationships ($\rho_y = \alpha_y + r_{zy}\alpha_{zy}$).

To illustrate the use of predictive path analysis, Fig. 2 compares it to the correlation structure between mates described by Pearson & Lee (1903). The total correlation between mates for trait z is divided into direct (Fig. 2a) and indirect (Fig. 2b) components under the assumption of no direct cross-correlation between mates for z and y. The cross-regression coefficient α_{yz} in the path model subsumes the direct correlation between mates for y and the phenotypic correlation within fathers for y and z (Fig. 2b).

The power of the path model approach is apparent in decomposing the cross-correlation between mates, ρ_{ij}, which can inflate the genetic covariance between z and y (Gianola, 1982; Hall *et al.*, 2000). Mates will be correlated for z and y when there is a direct mechanistic relationship between the first trait in the mother and second in the father (Fig. 2c). There will also be and indirect component to the cross correlation if assortment occurs in trait y, and y is phenotypically correlated to z (Fig. 2d). Note that the cross-regression between these traits, α_{yz}, is included in both the direct and indirect components of assortment; this is because the path model subsumes the direct correlation between mates for trait y and the correlation between y and z within fathers (see Fig. 2b). The part of the cross-regression due strictly to direct cross-assortment, which I denote as α^*_{yz}, can be found as

$$\alpha^*_{yz} = \rho_{yz} - (r_{yz}\alpha_z + r_{zy}\alpha_y) \quad (5)$$

This formulation assumes that the phenotypic correlation within individuals is the same for males and females, which will hold for hermaphrodites, but not necessarily for dioecious species. It also assumes the covariance between the two focal traits z and y and all other traits make negligible contribution to α_{yz}. Reciprocal direct cross-assortment coefficients are symmetrical (i.e. $\alpha^*_{yz} = \alpha^*_{zy}$). The discussion explains how this decomposition of the cross-regression allows the inflationary effects of assortative mating on genetic covariance to be decomposed into components due to gametic disequilibrium at pleiotropic versus nonpleiotropic loci.

To apply the predictive approach, the matrix containing the partial regression and cross-regression coefficients between mates can be obtained as

$$\mathbf{A} = \mathbf{R}\mathbf{P}^{-1}, \quad (6)$$

where $\mathbf{P}^{-1}$ is the inverse of the matrix of within-mate correlations among the traits. The leading diagonal of this $q \times q$ matrix contains the direct partial regression coefficients for the measured traits, and the off-diagonal elements are the cross-trait partial regressions.

Materials and Methods

Weis & Kossler (2004) collected detailed data on the flowering schedules in a greenhouse population of

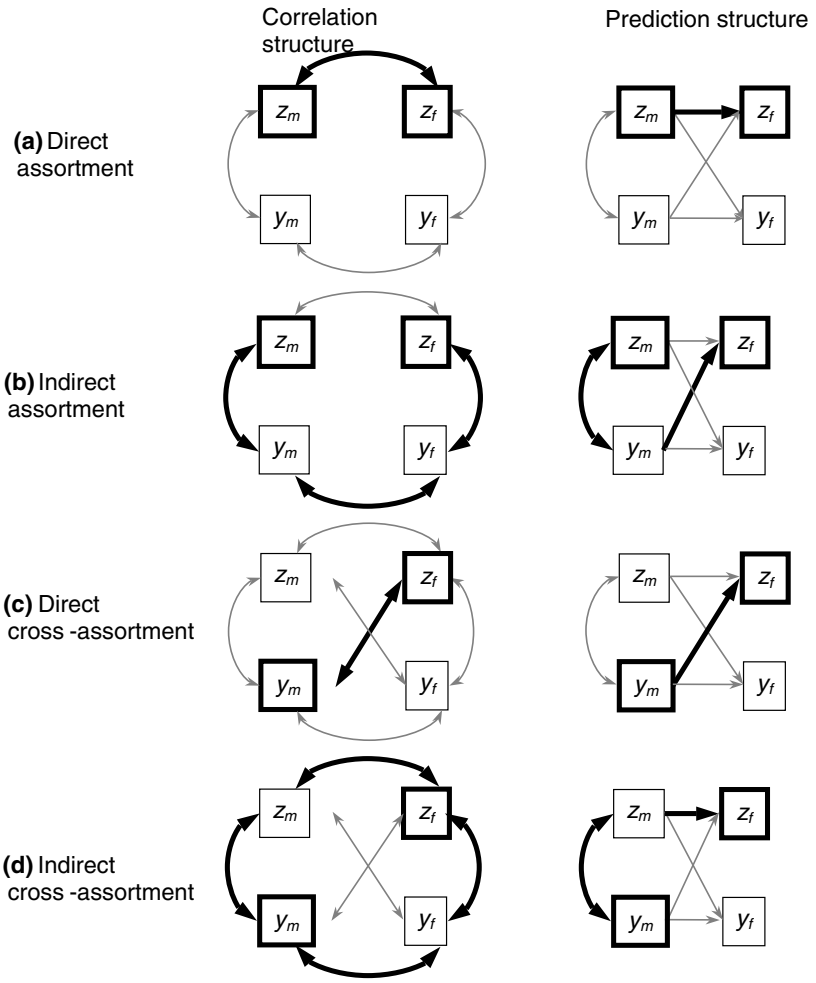

Fig. 2 The types of assortative mating, depicted by correlation structure of Pearson & Lee (1903) and the prediction structure developed in this paper. Dashed lines indicate coefficients assumed to be zero, and the solid lines indicate nonzero coefficients. (a). Direct assortative mating: phenotypic values of z for mothers and fathers are correlated because similarity in z contributes mechanistically to mating probability. (b). Indirect assortative mating: mothers and fathers are correlated for z, but it is because z is correlated within-individuals to y, and in turn, similarity in y contributes mechanistically to mating probability. (c). Direct cross-assortative mating: Phenotypic values of y for mothers are correlated to z values in fathers because concordant trait combinations contribute mechanistically to mating probability. The asterisk indicates the regression of paternal phenotype on maternal phenotype has no components caused by within-individual correlations between z and y. (d). Indirect cross-assortative mating: Phenotypic values of y for mothers are correlated to z values in fathers because, (1) the two traits are correlated within individuals (2) similarity in each trait independently contributes mechanistically to mating probability.

48 *B. rapa* plants. They recorded the day of first flowering and measured stem height and diameter on that day for each individual. They also censused the entire population every third day throughout the flowering season and recorded the number of open flowers per individual. Their goal was to compare the prospective estimate of ρ for flowering time to a retrospective estimate, and so they treated the 48 plants as a self-contained population, making adjustments for self-incompatibility. For present purposes, I treat them as a sample from a larger population, and so make no adjustment; as a result, correlation coefficients reported here are expected to be slightly greater than those in Weis & Kossler (2004).

Flowering schedules

To describe the flowering schedule as a multivariate trait, I fitted the flower census data to the exponential sine function (Mallo, 2002), and treated parameters as component traits. This curve describes the beginning, duration and shape of the flowering schedule with the function:

$$n_d = n_{\max}\left(\sin\left(\pi\left(\frac{d-d_F}{D}\right)^S\right)\right)^K,$$

where n_{d} (dependent variable) is the number of open flowers on census day d (independent variable). Function parameters are as follows: $n_{\max}$, is the number of open flowers at peak flowering, d_{F} is the day of first flowering and D is the duration (number of days) of the flowering period. The two exponential terms, S and K, describe curve shape. When $S < 1$, flower production is concentrated toward the beginning of the flowering period (skewed to the right) and when $S > 1$, it is concentrated toward the end (skewed to the left). Similarly, when $K \ll 1$, the flower production is even over the flowering period (platykurtic), but when $K \gg 1$, most flowers are produced on the peak day (leptokurtic).

I used the NLIN procedure of SAS (SAS Institute, 1990) to find the best-fit curve for each individual. Although all five parameters could be estimated by this iterative procedure, I entered the observed values of d_{F} as

constants in the model statement to improve computation efficiency. The procedure was run at least three times for each individual, with prior values of 0.5, 1.0 and 2 for the S and K parameters, and the prior value for D set as the number of days between first flowering and the last census day with nonzero flower production. If the three runs did not converge to the same solution, additional runs were performed on a wider set of priors until the set of parameter estimates with smallest residual sum of squares was found.

The day of peak flowering, d_P, can be estimated by setting the first derivative of the function to zero and then solving for d (Mallo, 2002):

$$d_P = d_F + \sqrt{D^{1/s}}.$$

Day of peak flowering, d_P, is thus by necessity correlated with day of first flowering. To examine the potentially independent component of peak flowering I used the number of days from first to peak flowering ($P = d_P - d_F$) in the analysis. Three plants showed secondary flowering peaks late in their flowering periods; in these cases S and K were fitted to the first peak, and D was calculated directly from the raw data.

Statistical analysis

I constructed a program in the 'R' language to compute the Φ, **R**, **P** and **A** matrices (see Methodological Background). The following seven phenotypic traits were analysed: flowering time ($F = d_F$ – day of germination), days to peak, flowering period duration, schedule skew and kurtosis, and the height and diameter of the stem on day of first flowering.

Hypothesis testing for the prospective estimates of the correlations between mates, ρ, is problematic (Weis & Kossler, 2004). There are p^2 elements in the mating matrix, Φ, but these are estimated from only p mating schedules. Thus, matrix elements are not independently estimated and conventional standard errors for the correlation coefficients estimated with Φ could be artificially small. I used a bootstrapping procedure to circumvent this problem. The base input for the procedure was a data matrix with 48 rows, one for each individual. The first seven columns contained the phenotypic scores for the traits and the remaining 26 columns contained the number of open flowers produced on each of the census days. For each of the 1000 bootstrap iterations, 48 rows were sampled with replacement from the data matrix, and the analysis repeated. Confidence intervals (99%) were derived for elements of the **R**, **P** and **A** matrices from the 0.5 and the 99.5 percentiles of the distribution of their bootstrap values. The **R** matrix had 28 elements and the **A** had 49, and thus a full analysis involves multiple tests. I restrict statistical inferences to the diagonal elements of these matrices (the total correlation between mates, and its direct component) and consider the off-diagonal elements as descriptive statistics. Program code for the analysis can be obtained by e-mail from the author.

Results

Flowering schedules for four representative individuals are shown in Fig. 3. The first plant flowered at 36 days (post-germination) and the last at 72 days. Mean time to first flower was 51.8 days (SD = 10.7). After flowering, it took an average of 24.9 days to reach peak flower production (SD = 4.5). On average, plants produced flowers for 36.5 days (SD = 6.4). They tended to concentrate flower production later in their flowering period ($\bar{S} = 2.3$, SD = 1.1), and schedules tended to have moderately sharp peaks ($\bar{K} = 1.4$, SD = 0.8).

Within individuals, flowering time had a strong negative correlation with days to peak flowering, and a positive correlation to schedule skew (Table 1). Flowering period duration was not strongly correlated with any

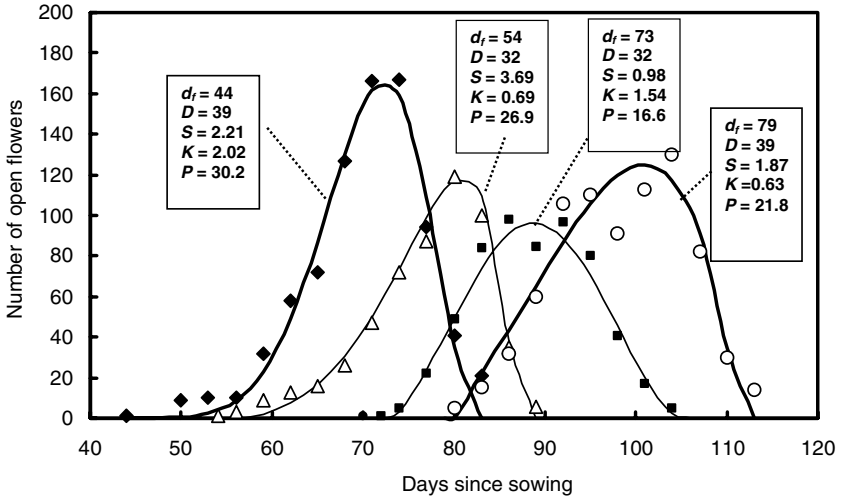

Fig. 3 Flowering schedules for four representative *B. rapa* individuals. Curves are the fitted exponential sine functions for each plant (see text). Boxes contain the estimated parameter values for the function (d_f is day of first flower, D is duration of the flowering period, S and K are the skew and kurtosis in the curve, respectively, and P is the number of days from first to peak flowering).

Table 1 Phenotypic correlations within plants for the seven traits measured in *B. rapa* – the **P** matrix.

	Flower	Peak	Duration	Skew	Kurtosis	Height	Diameter
Flowering time	1						
Time to peak	**−0.88**	1					
Schedule duration	0.09	−0.02	1				
Schedule skew	**−0.61**	**0.66**	−0.22	1			
Schedule kurtosis	−0.09	−0.03	0.06	**−0.53**	1		
Stem height	**0.75**	**−0.70**	−0.08	**−0.40**	−0.29	1	
Stem diameter	**0.75**	**−0.66**	0.16	**−0.37**	−0.14	**0.55**	1

The 99% confidence intervals for the coefficients in bold face did not include zero.

other trait. Stem height and diameter were strongly correlated to flowering time, days to peak, and schedule skew, and to each other (Table 1).

All seven traits showed significant potential for assortative mating, as indicated by the correlation between mates (Table 2, first column). Assortment was strongest for flowering time, followed by the days to peak flowering and stem diameter. The cross-correlations involving flowering time tended to be strong (Table 3).

Although there was a strong potential for assortment in most traits, only flowering time and flowering period duration exhibited significant direct components for the correlation between mates (Table 2, second column). Given that the days-to-peak flowering, schedule skew, and the stem dimensions were highly correlated with flowering time, it is not surprising that most of the covariance between mates for these traits was explained by the indirect effect through flowering time – day of peak, skew, stem height and stem diameter of the donor can be predicted because theses traits are correlated to flowering time in the recipient (Table 2, column 3). However, even in aggregate, these traits do not contribute reciprocal indirect effects on flowering time (Table 2, column 4, row 1). The cross-regression coefficients (Table 4) indicate that although recipient flowering time predicts of donor peak, skew, height and diameter, the reciprocal is not true – recipient peak, skew, height and diameter are not good predictors of donor flowering time. The cross-regression of all paternal traits over maternal period duration (Table 4, row 3) were comparatively weak, except flowering time and time to peak. However, after accounting for indirect effects of flowering time, there was little residual covariance between mates for any trait explainable by duration of the flowering period (Table 2, column 4); this was because duration did not strongly correlated with any other trait within plants (Table 1).

There is little evidence for any direct components to cross-assortment. For instance, the direct cross-regressions (α^*_{ij}) of time to peak, schedule duration, and stem diameter over flowering time are −0.02, 0.22 and −0.05, respectively. Although there is a hint of a direct component for cross-assortment for flowering time and schedule duration, the overall cross-correlation is not significant (Table 3), and neither is the within-individual phenotypic correlation, which together argue against a simple direct effect and against a direct effect that is nullified by indirect effects of opposite sign.

Discussion

Variation in flowering schedules created the potential for assortative mating in a greenhouse population of *B. rapa*.

Table 2 Correlation between potential mates for seven traits in *B. rapa*.

	Correlation between mates (99% CL) ρ_i	Direct effect (99% CL) α_i	Indirect effect, through flowering time (99% CL) $r_{iF} \times \alpha_{Fi}$	Indirect effects, through all other traits $\Sigma(r_{ij} \times \alpha_{ji})$
Flowering time	**0.67** (0.51, 0.85)	**0.57** (0.37, 0.79)	–	0.10
Time to peak	**0.51** (0.34, 0.69)	0.07 (–0.04, 0.25)	**0.41** (0.27, 0.59)	0.01
Schedule duration	**0.32** (0.11, 0.53)	**0.29** (0.13, 0.45)	0.03 (–0.05, 0.16)	–0.01
Schedule skew	**0.24** (0.13, 0.41)	–0.02 (–0.07, 0.05)	**0.21** (0.11, 0.37)	0.06
Schedule kurtosis	**0.08** (0.02, 0.19)	0.07 (0.00, 0.18)	0.01 (–0.01, 0.07)	0.01
Stem height	**0.33** (0.16, 0.61)	0.01 (–0.04, 0.09)	**0.25** (0.13, 0.43)	0.08
Stem diameter	**0.52** (0.25, 0.81)	0.18 (–0.01, 0.35)	**0.31** (0.10, 0.55)	0.06

The first column contains the correlation coefficients (diagonal of the **R** matrix). The second column contains the direct portion of the correlation, which is the partial regression coefficient of predicted pollen donor phenotype over recipient phenotype (diagonal of the **A** matrix). The third column is the indirect effect of flowering time on the correlation between donors & recipients (products of the r_{iF} & α_{Fi} from the **P** & **A** matrices, respectively). The fourth column is the sum of all indirect effects acting through tratis other than flowering time. The 99%confidence intervals for the coefficients in bold face did not include zero.

Table 3 Phenotypic cross-correlations between mates; the off-diagonal elements of the **R** matrix.

	Pollen donor trait						
Recipient trait	Flower	Peak	Duration	Skew	Kurtosis	Height	Diameter
Flowering time	–						
Time to peak	**−0.58**	–					
Schedule duration	0.30	−0.24					
Schedule skew	**−0.39**	**0.34**	−0.17	–			
Schedule kurtosis	−0.12	0.09	−0.12	0.04	–		
Stem height	**0.47**	**−0.41**	0.21	**−0.26**	−0.10	–	
Stem diameter	**0.57**	**−0.49**	0.24	**−0.31**	−0.11	**0.41**	–

The 99% confidence intervals of coefficients in bold face did not include zero.

Table 4 Partial cross-regression of expected donor phenotype over recipient phenotype – the off-digonal elements of the **A** matrix.

	Pollen donor trait						
Recipient trait	Flower	Peak	Duration	Skew	Kurtosis	Height	Diameter
Flowering time	–	−0.07	**0.23**	0.05	−0.05	−0.05	0.09
Time to peak	**−0.47**	–	**−0.19**	−0.04	0.04	0.03	−0.08
Schedule duration	**0.25**	−0.02	–	0.00	−0.10	0.01	−0.02
Schedule skew	**−0.34**	0.08	**−0.13**	–	0.02	0.03	−0.02
Schedule kurtosis	−0.05	0.00	**−0.13**	0.00	–	0.02	−0.03
Stem height	**0.34**	−0.05	**0.17**	0.03	−0.05	–	0.09
Stem diameter	**0.41**	−0.04	**0.19**	0.04	−0.04	0.00	–

The 99% confidence intervals of coefficients in bold face did not include zero.

The five traits comprising the flowering schedule showed significant correlations between pollen recipients and their potential donors, as did two morphological traits. However, only two of the traits, flowering time and flowering period duration, exhibited the potential for direct assortative mating. Assortment in most of the traits appeared to be indirect and caused by their within-individual correlations to flowering time.

This reanalysis of the *B. rapa* data yielded higher correlation coefficients than reported by Weis & Kossler (2004). For instance, they reported $\rho = 0.59$ for flowering time, vs. $\rho = 0.67$ reported here. This difference occurs because they treated the small experiment as an entire population; the possibility of a plant receiving pollen from an identical donor was nil because of self-incompatibility. Here I treat the experiment as a sample from a very large population, where a recipient can receive pollen from many phenotypically identical and compatible donors.

Flowering schedules and phenological assortative mating

The flowering schedules of individual plants will frequently (usually?) differ from one another in both temporal position and shape. Futher, position and shape components may often covary for both genetic and environmental reasons. This covariance makes it difficult to determine the relative roles of what Fox (2003) called 'conventional' and 'cryptic' phenological assortative mating. The multivariate method holds shape statistically constant and asks if there is an effect of position, and vise versa. With regard to the exponential sine function, flowering time denotes position, while time to peak, skew and kurtosis denote shape. Flower period duration contains information on both position and shape. Given the strong direct effects of flowering time and period duration, and the absence of direct effects for other schedule components, assortment in this *B. rapa* population is primarily 'conventional.' It is an empirical matter to determine if this is generally the case.

Further empirical work is needed to determine how often the potential for assortative mating, measured by the prospective estimate of ρ, reflects actual assortment. This can be accomplished with the retrospective method of Weis & Kossler (2004), which is based on the distortion of the parent offspring regression caused by assortative mating. The retrospective method is more feasible for large natural populations than paternity analysis by neutral markers, but it is no trivial exercise either. Yet it is important to do so, because there are several ways in which pollinator behavior can cause the realized level of assortative mating to diverge from its potential. For instance, pollinator service may be lower early and/or late in the season, when only the extreme individuals are in flower (e.g. Schemske, 1977; Ashman & Stanton, 1991). This will reduce the realized correlation between mates by lowering the proportions of early × early and/or late × late matings. Low-pollinator service at the beginning and end of the season would also induce stabilizing selection (as in the absolute preference model of sexual selection of Lande, 1981) thereby

weakening the inflationary effect on genetic variance (Felsenstein, 1981). Other nonrandom patterns in pollinator service, such as preferences for inflorescences with many open flowers (e.g. Mitchell, 1994; Conner & Rush, 1996; Ishii & Sakai, 2002) could also cause estimates of realized assortative mating to differ from one based on flowering schedules.

A major limitation of the prospective method, as developed so far, is the assumption of self-incompatibility. Inbreeding and assortative mating are similar, yet they have quite distinct population genetic consequences (Lewontin *et al*., 1968; Walsh & Lynch, in press). Nevertheless, partial selfing affects the phenotypic correlation between pollen recipients and donors. Procedures to estimate ρ in mixed mating systems will need to incorporate weighted contributions from the mating matrix, Φ, and from a diagonal matrix of selfing rates.

Assortative mating and inflation of genetic variances and covarinces

Standard population genetic theory shows that assortative mating creates gametic phase disequilibrium in an assorting trait. In the absence of selection, allele frequencies do not change under assortment, but alleles of similar phenotypic effect are more likely to co-occur in individual genotypes. This association between alleles of similar effect inflates the additive genetic variance in an assorting trait (Wright, 1921; Felsenstein, 1981; Barton & Turelli, 1991; Lynch & Walsh, 1998). Starting with a randomly mating population, one generation of assortative mating inflates the additive genetic variance in trait z by the amount $0.5\rho_z h_z^2 G_z$, where G_z is the base variance, i.e. the variance in the absence of gametic disequilibrium. The term $\rho_z h_z^2$, the phenotypic correlation between mates × heritability, is the genetic correlation between mates for trait z (the 'marital correlation', Wright, 1921). Sustained assortative mating will generate gametic disequilibrium and thereby inflate the genetic variance to

$$\hat{G}_z = \frac{G_z}{1 - \left[1 - \left(\frac{1}{2N_e}\right)\right]\rho_z \hat{h}_z^2}, \qquad (7)$$

where the circumflex denotes an equilibrium value and N_e is the effective number of segregating loci contributing to the trait. Note that when many loci contribute to the trait, as assumed under the infinitesimal model (Bulmer, 1980), the term in square brackets approaches 1, and the denominator becomes $1 - \rho_z \hat{h}_z^2$. The stronger the phenotypic correlation between mates for trait z, and the greater its heritability, the greater the variance inflation. Based upon the relationships outlined in this paper, the genetic correlation between mates for focal trait z, in a suite of q traits, can be decomposed to

$$\rho_z h_z^2 = \left(\alpha_z + r_{yz}\alpha_{yz} + r_{xz}\alpha_{xz} + \cdots + r_{qz}\alpha_{qz}\right)h_z^2 \qquad (8)$$

In view of eqn 7, eqn 8 implies that the genetic variance in focal trait z can become inflated by assortative mating even if z has no direct impact on mating patterns (i.e. $\alpha_z = 0$). Take stem diameter as the focal trait and flowering time as the correlated trait. In the case of *B. rapa*, genetic variance in diameter would be inflated even if pollinators visited plants without regard to this character; thick and thin plants flower at different times in the season, and this would cause the stems of pollen recipients to have similar diameters to their donors. This agrees with Gianola (1982) who found that under the simplifying assumptions of the infinitesimal model, the inflation of the equilibrium variance in nonassorting trait z caused by its correlation to assorting trait y is

$$\hat{G}_z = \frac{G_z}{1 - \rho_y \hat{h}_y^2 \hat{g}_{zy}^2}.$$

where $\hat{g}_{zy}^2$ is the within-individual genetic correlation of z to y at equilibrium.

Phenotypic cross-correlations between mates can arise indirectly when one or both of two correlated traits shows direct assortment (Fig. 2b,d). They can also arise by direct cross- assortment, as when mothers with high values of y mate disproportionately with fathers having high values of z (Fig. 2c). Cross-correlations are important because they can inflate the genetic covariance between two traits z and y by gametic disequilibrium (Gianola, 1982). The distinction between indirect and direct cross-assortment is important because the former inflates the genetic covariance between traits correlated through pleiotropy, whereas the later can create genetic covariance even in the absence of pleiotropy.

In a randomly mating population at equilibrium, the within-individual genetic correlation between traits z and y is $g_{zy} = r_{zy}h_z h_y$, where h_i is the square root of heritability (which is also the regression of phenotypic value over genotypic value; Lynch & Walsh, 1998). If, as in the previous section, there is direct assortment for trait y but not trait $z(\alpha_y \neq 0$ but $\alpha_z = 0)$ Gianola (1982) showed that the equilibrium genetic correlation between them is

$$\hat{g}_{zy} = \frac{g_{zy}}{1 - \rho_y h_y^2}\sqrt{\frac{G_z G_y}{\hat{G}_z \hat{G}_y}}.$$

If the base genetic correlation, g_{zy}, is zero, the equilibrium genetic correlation is likewise zero. By definition, the base genetic correlation is that obtained in the absence of gametic disequilibrium, and therefore is the correlation due to pleiotropy alone (absent outside forces like correlated selection or population subdivision generating initial gametic disequilibrium). Thus, indirect cross-assortative mating will inflate the genetic covariance between traits only if they share some common genetic basis. With regard to *B. rapa*, it is reasonable to think that the genetic correlation between flowering time and stem height observed by Dorn & Mitchell-Olds (1991) is rooted in pleiotropy – genes that code for an early switch from

vegetative growth to reproduction will result in a smaller body size at maturity. Phenological assortative mating is likely to increase the flowering time-stem height correlation by creating gametic disequilibrium at loci governing the temporal switch.

This situation is different for direct cross-assortative mating (Fig. 2c); genetic covariance between traits z and y can arise by gametic disequilibrium alone. Gianola (1982) examined the case in which two traits are under cross-assortment only (in the notation used here, $\alpha_z = \alpha_y = 0$ and $\rho_{zy} = \alpha^*_{zy} \neq 0$), again under the assumptions of the infinitesimal model. His results show that so long as the correlation between mates and the base genetic correlation do not have discordant signs (as they would if α^*_{zy} were positive but g_{zy} negative), then the equilibrium within-individual genetic correlation is

$$\hat{g}_{zy} = g_{zy} + \frac{1}{\alpha^*_{zy}\hat{h}_z\hat{h}_y} - \sqrt{g^2_{zy} + \left(\alpha^*_{zy}\hat{h}_z\hat{h}_y\right)^{-2}} - 1.$$

Note that in this relationship the base genetic correlation, g_{zy}, can be zero, yet the equilibrium correlation nonzero. This implies that direct cross-assortment can create a genetic correlation by gametic disequilibrium alone. For instance, starting with a zero genetic correlation, a cross-regression of 0.5 and equilibrium heritabilities of 0.5, the equilibrium genetic correlation between z and y would be 0.127.

The *B. rapa* data set illustrates cases of direct and indirect assortative mating, and indirect cross-assortative mating. However, no evidence for *direct* cross-assortment was found. As a hypothetical example, one could imagine a plant species with a corolla colour di-morphism (red and white) and variation in stem height. Suppose there are two pollinator species, one of which tends to fly high in the canopy and has a partial preference for red, while the other flies lower and prefers white. Even if genetic control for colour were independent of that for height, over time, the population would contain more tall/red and short/white plants than expected by random. Models of sexual selection by female choice (Lande, 1981, Barton & Turelli, 1991, Hall *et al.*, 2000) also show that within-individual genetic correlations are inflated by nonrandom mating. Female preference for a male criterion trait and the criterion trait itself become genetically correlated by gametic phase disequilibrium even if they have independent genetic origins.

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