A note on measuring natural selection on principal component scores

Veronica K. Chong[1], Hannah F. Fung[1], John R. Stinchcombe[1,2]

1. Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada
2. Koffler Scientific Reserve, University of Toronto, Toronto, ON, Canada

Author for correspondence: John Stinchcombe <john.stinchcombe@utoronto.ca>

Key words: selection gradients, multicollinearity, principal component analysis, principal component regression, life history, trade-offs, flowering time

Abstract

Measuring natural selection through the use of multiple regression has transformed our understanding of selection, although the methods used remain sensitive to the effects of multicollinearity due to highly correlated traits. While measuring selection on principal component scores is an apparent solution to this challenge, this approach has been heavily criticized due to difficulties in interpretation and relating PC axes back to the original traits. We describe and illustrate how to transform selection gradients for PC scores back into selection gradients for the original traits, addressing issues of multicollinearity and biological interpretation. We demonstrate this approach with empirical data and examples from the literature, highlighting how selection estimates for PC scores can be interpreted while reducing the consequences of multicollinearity.

"Unfortunately, the biological meaning of principal components or discriminant functions is not always obvious." – Endler (1986, p 192)

"… it is difficult to translate from selection on principal components to selection on the original traits."—Brodie et al. (1995)

Understanding how natural selection acts on correlated traits remains a fundamental challenge in evolutionary biology (Lande and Arnold 1983). Most ecologically important traits that influence an organism's fitness are correlated with other features of their morphology, life history, and behavior. The canonical approach to estimating selection on correlated traits is selection gradient analysis (Lande and Arnold 1983). By regressing estimates of relative fitness on multiple traits, the direct effects of individual traits can be distinguished from the effects of selection on correlated traits. Widespread application of the Lande-Arnold framework has revolutionized our understanding of natural selection, and has given empiricists a straightforward tool for evaluating the fitness consequences of phenotypic variation.

One challenge to the Lande-Arnold approach, however, has remained especially stubborn: regressing fitness estimates on correlated traits can become problematic when traits exhibit multicollinearity (Mitchell-Olds and Shaw 1987). If the correlation between traits is high enough, it becomes impossible to reliably estimate their separate contributions to relative fitness. Typical recommendations for multicollinearity include larger sample sizes, dropping or summing variables, principal component analysis, or alternative approaches like partial least squares. While these methods have valuable statistical properties, their widespread adoption has been limited. In many instances estimating selection on highly correlated traits simultaneously is the object of study, and thus traits cannot be dropped. For partial least squares, interpretability of the coefficients or the factors identified can remain problematic, as does their lack of correspondence to selection gradients that can be used to predict evolutionary responses.

The merits of measuring selection on principal component scores, in particular, has been debated since the advent of selection gradient analysis. Lande and Arnold (1983) themselves measured selection on PC scores when analyzing selection on the Bumpus (1899) house sparrows dataset. They presented their use of principal components as a data reduction technique for analyzing 9 traits with 49 female birds. They noted that while directional selection was detected on some of the original traits, they failed to detect directional selection on PC1 scores. One of the major advantages of selection gradient analysis—measuring and comparing the magnitude of direct selection on correlated traits—can thus be lost, as one obtains an estimate of selection on a composite trait that may not be the same in sign or statistical significance as the original traits. PC scores are constructed based on their patterns of variation and covariation, regardless of their importance for fitness, and can be hard to interpret biologically, especially when they encompass many traits (see Mitchell-Olds and Shaw 1987 for an extended discussion). For these interpretive reasons, selection estimates on PC scores have been criticized (e.g., Endler 1986; Mitchell-Olds and Shaw 1987; Conner 2007; Hunt et al. 2007).

Here we present and illustrate a simple approach that circumvents the interpretation problem of measuring selection on principal components. By projecting selection estimates for PC's back into the original trait space, their interpretability is preserved. We illustrate this with an empirical example in the mouse ear cress, *Arabidopsis thaliana,* as well as examples from the literature where previous authors have presented selection estimates for PC scores.

Projecting Selection Gradients for PCs back into trait space

For most incarnations, traits will have separate units, and we assume that PCA has been performed on the correlation rather than the covariance matrix. A vector of selection gradients on PC scores (i.e., regression coefficients of relative fitness on PC scores) can be projected into the original trait space by:

$$\boldsymbol{\beta} = \mathbf{E}\,\mathbf{A} \qquad (1)$$

Where $\boldsymbol{\beta}$ is a vector of reconstituted selection gradients, **E** is a matrix with eigenvectors from the original PCA as columns, and **A** is the vector of regression coefficients obtained from regressing relative fitness on PC scores (Jolliffe 2002, pg 169). The coefficients in **A** describe the relationships between relative fitness and PC scores, while the elements of **E** describe the relationships between the PC scores and the original traits; combining them yields the relationship between traits and relative fitness. Using all the eigenvectors of a PCA for **E** and regression with all PC scores as traits for **A** with (1) returns ordinary least squares regression coefficients (Jolliffe 2002; Wilks 2005), and does not eliminate multicollinearity. Using a subset of PCs can reduce multicollinearity, and estimate selection on the original traits. To estimate uncertainty in the elements of $\boldsymbol{\beta}$, we estimate their variance using equation 6 of Lafi and Kaneene (1992), and from that standard errors. If one wishes to perform hypothesis testing on these reconstituted selection gradients, parameter estimates and standard errors could be used to estimate t-statistics, and P-values. Prior to illustrating the use of (1) with our own data and literature examples, we note two important items that we return to below. First, using (1) necessarily involves a decision about how many PC axes to include, with attendant costs and benefits. Second, the elements of $\boldsymbol{\beta}$ estimated by (1) represent selection within the sub-space represented by the columns of **E**—e.g., if the columns of **E** represent 70% of the phenotypic variation, the elements of $\boldsymbol{\beta}$ represent selection on the traits in the sub-space describing 70% of the phenotypic variance.

MATERIALS AND METHODS

Size and phenology in *Arabidopsis thaliana*

The mouse ear cress, *Arabidopsis thaliana* (Brassicaceaae) is a selfing annual rosette plant. Our goal was to test the hypothesis that natural selection favoring early flowering, which is commonly observed in experiments even with low mortality (Austen et al. 2017), could be explained as selection on correlated traits, such as flowering duration and branch number. The logic behind our hypothesis was that early flowering plants could potentially have longer flowering durations, and more opportunity to produce branches which could themselves support flowers and fruit. We used F3 genetic lines from a cross developed by Stock (2015). She crossed two parental lines collected from a single population in Doylestown, PA, (USA) that had alternative alleles at the flowering time genes *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC);* progeny from this cross thus have all allelic combinations at these two genes. *FRI* and *FLC* are important determinants of flowering time in *A. thaliana* (Caicedo et al. 2004; Shindo et al. 2005). Stock (2015) scored flowering time and gathered seed from ~500 F2 individuals, and we used 5 replicates from 50 lines chosen to evenly span the flowering time distribution.

We synchronized germination by stratifying seeds in 0.15 mg / 100 mL agar solution for six days at 4°C (Stock et al. 2015). We planted each seed in a standard conetainer with saturated Sunshine Mix #1 soil (Sun Gro Horticulture, Agawam, Massachusetts, USA). We arranged plants into cone-tainer racks in a randomized, blocked design, where the two shelves of a growth chamber represented experimental blocks, with plants of each line evenly divided between shelves. We maintained a light-temperature regime of 16 hour days (22°C) followed by nights of 20° C (Suter & Widmer 2013; Stock et al. 2015). We bottom-watered every two days by placing conetainers in trays of water for approximately three hours. After three months, we gradually reduced watering to simulate a terminal end of season drought, and all plants senesced after 4 months.

We scored bolting date, first and last flowering date throughout the experiment; at harvest, we measured branch number, rosette diameter, and counted the number of rosette leaves. We collected and counted fruits as a proxy for fitness (Stinchcombe et al. 2004). We measured flowering time as the number of days between planting and the first flowering date, and flowering duration as the number of days between first and last flowering dates. Analyses were performed using Rv 3.1.2 (R Foundation 2014) and SAS v9.4 (Cary, NC, USA).

Trait correlations and selection

We calculated line means prior to analysis of correlations between traits and selection (Rausher 1992). We then used Pearson correlation coefficients as estimates of the line mean correlations among the traits. We first estimated selection differentials using simple univariate regressions regressing relative fruit number on individual traits, using line means and with traits standardized to a mean of zero and variance of one. We then estimated selection gradients for all traits, by regressing relative fruit number on them in a multiple regression. Strong multicollinearity between flowering time and duration lead to high

variance inflation factors. To circumvent this, we used equation (1) above with 4 PC's, which explained 99% of the variation among line means.

Literature examples

We searched for examples measuring selection on PCs to illustrate potential use of equation (1). Our goal was to find illustrative examples rather than a comprehensive meta-analysis. We describe new findings obtained through the application of (1).

RESULTS AND DISCUSSION

Arabidopsis experiment

The traits showed a range of correlations, ranging from rosette diameter and flower duration which were uncorrelated (r_g = 0.06, P = 0.647) to flowering time and duration, which were highly correlated (r_g = -0.95, P< 0.0001; Figure 1). Flowering time showed strong correlations with branch number (r_g = -0.74, P<0.001) and rosette leaf number (r_g =0.70, P <0.0001); flowering duration had essentially the opposite correlation pattern (r_g = 0.72 and r_g = -0.73, respectively, for branch number and rosette leaf number; Table 1). Principal components analysis showed that flowering time and flowering duration always had opposite signs in their loadings on PC1-PC4; rosette diameter, branch number, and rosette leaf number loaded heavily on PC2, PC3, and PC4.

Selection on flowering time and flowering duration

Univariate estimates of selection suggest that early flowering, long flowering durations, more branches, and fewer rosette leaves are favored (Table 2a, Figure 2a-d). Interpreting these selection differentials is complicated by the strong correlations among traits: positive selection for branch number, for example, could be due to indirect selection for early flowering time (branch number is negatively correlated) or selection for longer flowering durations (branch number is positively correlated). Traditional multiple regression, while presented, is difficult to interpret: the high variance inflation factors for flowering time (12.54) and flowering duration (11.32) suggesting caution in interpreting these gradients. While both flowering time and flowering duration have large and significant selection differentials, neither are significant in the multiple regression, and both have standard errors approximately 3-4 fold larger than the standard error for the differentials, again because of multicollinearity.

Estimating selection gradients via equation 1 using four PCs showed strong directional selection for earlier flowering (β ± s.e. = -0.17 ± 0.05), and for extended flowering durations (β = 0.17 ± 0.06). The sign of these selection gradients makes sense relative to their selection differentials and the sign and magnitude of the trait correlation; standard errors are approximately 1/3 to 1/4 observed in the multiple

regression. These data suggest that selection is acting in approximately equal strength on flowering time and flowering duration, in opposite directions. The quantitative similarity of these selection gradients is likely due to the strong correlation between them, and reflects the two traits having approximately equal and opposite loadings on PC1-PC4 (Table 1). These data suggest that accounting for selection on correlated traits such as duration, branch number, size, and leaf number did not eliminate the observed selection favoring early flowering time, even if longer flowering durations are favored.

The estimated selection gradients for branch number, rosette diameter, and rosette leaf number are all approximately equal to their standard errors, suggesting that within the space of PC1-4, direct selection on these traits was weak. In addition, the reconstituted selection gradients for branch number, rosette diameter, and rosette leaf number (and their standard errors) are of roughly the same magnitude as in the original multiple regression, suggesting that calculations from (1) accurately characterized selection on them.

Literature examples

Pollinator-mediated selection: Campbell (1989) measured pollinator-mediated natural selection on floral traits in scarlet gilia (*Ipomposis aggregata*) through both male and female function. She noted in her methods that she addressed multicollinearity in one year by eliminating a trait, and that in other years she used PCs because of high correlations among traits. She summarized 6 floral traits (proportion pistillate, stigma exsertion, corolla length, corolla width, maximum anther distance, minimum anther distance) and one life history trait (flowering date) with 3 PC's that explained 83% of the phenotypic variance. She found significant selection on PC1 using estimates of male fitness and a 1-tailed test, while PC3 was under selection using female fitness.

Our reanalysis of her data suggests that flowering date is under negative selection using male fitness ($\beta = -0.1542 \pm 0.124$), while corolla length, corolla width, and maximum and minimum anther distance are under positive selection (β ranging from 0.1477 ± 0.094 to 0.1987 ± 0.117). For female fitness, the most strongly selected characters are proportion pistillate ($\beta = 0.1425 \pm 0.080$), stigma exsertion ($\beta = 0.1812 \pm 0.0778$), and flowering date ($\beta = 0.1854 \pm 0.1073$). Interestingly, the traits under strongest selection differed markedly between male and female fitness.

Size and emergence in damselflies: Anholt (1991) measured selection on body size components in the damselfly *Enallagma boreale*. Several features of this study stand out. First, it was repeated across two years, and second, it included selection separately for males and females. Third, sample sizes were already large, and Anholt reported that he used PCs because of multicollinearity, as diagnosed by variance inflation factors. Anholt (1991) summarized the date of emergence, wing length, abdomen length, and mass at emergence with PCA, performing four separate PCAs, corresponding to each year-sex combination. He estimated selection differentials for PC1 and PC2 (which accounted for ~85% of the

variance, depending on sex-year combination), using both relative survival and relative mate acquisition as fitness estimates. We focus here on the survival data, as sample sizes were much larger (565 to 993 – vs- 14 to 40). Because PC1 and PC2 are by definition uncorrelated, separate regressions of fitness on each PC to estimate selection differentials are equivalent to multiple regression of fitness on both PCs simultaneously. Consequently, we used the differentials for PC scores to calculate selection gradients.

Anholt interpreted PC1 as a size axis (due to the positive loadings of the size and mass characters), with the negative loadings of date of emergence on PC1 as reflecting the common observed trade-off between size and emergence date. In contrast, PC2 was interpreted as a date of emergence axis because date loaded most heavily, although wing length and abdomen showed moderate loadings in the same direction. As a consequence, PC1 represents a trade-off between date and size, while PC2 represents moderate positive associations between date and size. Because date of emergence figures in two PC axes in opposite directions vis a vis three other traits, and because the two PC axes were under selection in opposite directions in 3 of 4 cohorts, calculating selection gradients allows a straightforward assessment of the net pattern of selection on these traits without keeping track of the relative sign of multiple eigenvector elements and selection coefficients for PC scores across years and sexes. Our recalculations for the 1985 cohort suggest that selection in males favors early emergence dates ($\beta = -0.35 \pm 0.15$) and large mass ($\beta = 0.3321 \pm 0.14$), while in females the pattern is more variable on emergence date ($\beta = -0.27 \pm 0.19$) and almost absent on mass ($\beta = -0.065 \pm 0.17$). For the 1986 cohort, our calculations suggest that the dominant pattern in both males and females was for later emergence dates ($\beta_{males} = 0.472 \pm 0.18$; $\beta_{females} = 0.533 \pm 0.26$).

Size traits in turtles: Janzen (1993) studied selection on body size in hatchling snapping turtles (*Chelydra serpentine*). After releasing 112 hatchlings, he recaptured 66 individuals and estimated selection. Janzen summarized 5 size-related traits with PCA (initial egg mass, hatchling mass, carapace length, carapace width, and plastron length), using all of them as predictors of relative survival in a regression with other traits including locomotor performance, the size of the clutch that eggs came from, and water potential of the substrate eggs were incubated on (which affects size and hatching times). We used the first four PCs, which explained 99.1% of the phenotypic variance, and confine our attention to size traits used to construct the PCs. We note that subsequent work by Janzen (1998) suggests using logistic regression these cases, but we calculate selection gradients from multiple regression on PCs to illustrate the differences in interpretation.

When we projected selection estimates for the four PCs back into the original trait space, we found directional selection against hatchling mass ($\beta = -0.360 \pm 0.17$), and in favor of greater carapace width ($\beta = 0.370 \pm 0.18$) and plastron length ($\beta = 0.309 \pm 0.15$). Two features of our reanalysis differ from the original results. First, Janzen reported positive directional selection on PC1, which he logically interpreted as an axis of size (because all elements of PC1 were positive for size-related traits). Our findings illustrate selection on the component traits of PC1 is not always in the same direction, even when

all traits load on PC1 with similar sign and PC1 is under selection. The largest gradient we calculated was for decreased mass. The overall patterns of selection against mass, and in favor of carapace width and plastron length, come from selection on PC3 and PC4. PC3, which was under positive selection, had strong loadings of plastron length. PC4 had strong and nearly opposite loadings hatchling mass and carapace width, which showed selection gradients of opposite sign when recalculated. While the overall pattern of selection on the individual traits make sense in light of selection on the PCs and the loadings of the overall traits, the converse is not true: without calculation, it is difficult if not impossible to infer net patterns of selection on individual traits from regression coefficients for PCs.

Tadpole morphology: Kraft et al. (2006). Kraft and colleagues raised tadpoles of the striped marsh frog (*Limnodynastes peroneii*) in the presence and absence of dragonfly predators (*Anax* sp.), and measured the plastic response of morphological traits and their subsequent effects on survival. They measured 9 morphological traits (total length, tail height, tail muscle height, body length, body height, tail area, tail muscle area, body width, and tail muscle width), and performed PCA on log transformed data. They found that PC's 4 and 5 (explaining 1.3 and 0.9% of the phenotypic variance, respectively) differed significantly between predator-induced and control tadpoles. They measured selection on all 9 PC's in a multiple regression, finding significant negative selection on PC2, and positive selection on PC4 and PC5 for both the control and predator-induced individuals. Because sign is arbitrary within a PC axis, negative selection on PC2 is difficult to interpret: PC2 in their study had negative loadings of tail muscle width and positive loadings of body height. Individuals to the extreme of PC2 thus had either narrow tail muscles and large body heights, *or, equivalently*, wide tail muscles and small body heights. Which of these character combinations had lower fitness is would make dramatic differences in the interpretation of selection.

When we projected selection coefficients for PC1-5 (representing 98.4% of the phenotypic variance), we found that selection was acting most strongly on body width in the control individuals ($\beta = -0.0395$), while most strongly on tail muscle height in the induced individuals ($\beta=0.0426$). (Standard errors for the regression coefficients for PCs were not presented, precluding estimation of standard errors of the reconstituted selection gradients). The case of tail muscle width and body height is also clarified: in both treatments, selection favors wider tail muscles and short body heights.

Flower volatiles and size: Schiestl et al. (2011). Schiestl and colleagues measured selection on 11 floral volatiles and inflorescence size in the orchid *Gymnadenia odoratissima*. They performed several steps to facilitate selection analysis: first they reduced the number of floral volatiles analyzed from >40 to the 11 most abundant compounds in addition to inflorescence size. They summarized these variables with 4 principal components which were subjected to a varimax rotation, explaining 84% of the variation. The varimax rotation is meant to facilitate interpretation by creating a set of factors where each factor has predominant loadings from a reduced set of the original traits.

Projecting their selection coefficients for the factors back into the original trait space revealed appreciable selection on 5 measured traits: inflorescence size ($\beta = 0.3208 \pm 0.065$), phenylacetaldehyde ($\beta = 0.1580 \pm 0.066$), eugenol ($\beta = 0.1290 \pm 0.061$), (z)-isoeugenol ($\beta = -0.1733 \pm 0.066$), and phenylethyl ($\beta = 0.1669 \pm 0.061$). Natural selection within the sub-space described by the first four PCs was approximately twice as strong on inflorescence size as on the volatile traits. Shiestl et al. detected negative selection on the 3rd factor (most associated with α-pinene and (Z)-Isoeugenol after varimax rotation), yet α-pinene does not appear to be under selection when the coefficients are projected back into the original trait space.

Flower volatiles, display size, and phenology: Parachnowitsch et al. (2012) also measured selection on floral volatiles, in their case in the beardtongue *Penstemon digitalis*. They measured 30 traits, and faced power challenges in estimating selection gradients for volatiles and other traits. Their solution was to only include traits in a multiple regression if they showed significant or marginally significant selection differentials. They noted several inherent challenge in measuring selection on floral volatiles: there are many compounds within a floral bouquet; simply measuring selection differentials cannot distinguish direct and indirect selection; if more than one trait loads on a PC, it is difficult to determine which trait is under selection if that factor is significant; and measuring selection on principal components or varimax-rotated factors inhibits comparisons to previous reports of selection on other floral traits.

We used the 8 PC's presented by Parachnowitsch et al. (2012) which explained 72% of the variance. In addition to volatiles, they included morphological and life history traits, including flower size, corolla pigment, date of first flower, daily floral display size, number of days of flowering, and flower number. They observed very strong selection on PC3, which was associated with cis-6-nonenal, linalool, unknown-1, and daily display. By projecting selection estimates for 8 PCs into the original trait space, we see that while selection was observed for all traits ($\beta = 0.0569 \pm 0.0167$; $\beta = 0.0468 \pm 0.01286$; $\beta = 0.114 \pm 0.0161$; $\beta = 0.1708 \pm 0.01918$, respectively), it was by far the strongest on daily display size. Interestingly, the one compound that was significant in the multiple regression performed by the authors, linalool, has an intermediate magnitude selection gradient when estimated in this manner.

Prospects and Caveats

Our use of PCA in combination with selection gradient analysis for our own data and examples from the literature entailed benefits and costs that are worth considering before wider application. From the standpoint of comparing strengths of selection on different traits, the approach is useful for cases of multicollinearity where the hypotheses being tested preclude omitting traits.

Numerous authors have suggested caution in measuring selection on PC scores, although regression coefficients for PCs are rarely if ever projected back into the original trait space. Lande and Arnold (1983) noted that selection on PC scores can be diluted if traits under selection show strong phenotypic covariances with those unimportant for fitness. Our literature review revealed the converse problem: several cases in which seemingly straightforward selection on the PC axes was in fact a complex pattern of selection on the underlying traits. Endler (1986), Mitchell-Olds and Shaw (1987), Conner (2007), and Hunt et al. (2007) have all criticized selection estimates for PC scores on the grounds of biological interpretability, especially when a PC captures contributions from many traits.

By projecting the selection gradient for PC scores back into the original trait space, the biological interpretation challenge is reduced: the reconstituted selection gradients describe how changes in traits lead to changes in relative fitness. While in some cases PCs have clear biological interpretations (e.g., the early flowering / small –vs- late flowering / large axis studied by Colautti et al. [2010] and Colautti and Barrett [2010]), for more complicated cases like the ones analyzed here, it is quite difficult to interpret PC axes and selection on them. The algebra required to project any selection estimate for PCs and standard errors obtained with PCs back into the original biological context and units is simple, and the biological interpretation of the original traits is preserved. For cases in which the biological hypotheses or interpretability requires selection gradients for highly correlated traits, projecting selection estimates on PC's back into the original trait space may be a valuable approach. It is important to recall, however, that these reconstituted selection gradients estimate how selection is acting on the portion of multivariate trait space spanned by the PCs that were originally included. Including more PCs captures more of the variation in the original traits measured, and is closer to the typical goal of selection gradient analysis.

Principal component regression, however, also has several potential drawbacks. First, the reconstituted regression coefficients are no longer unbiased estimators like least-squares estimates (Wilks 2005, Jolliffe 2002). By omitting PC's that explain relatively little variance, one reduces the variance in the estimated regression coefficients caused by multicollinearity (and their associated variance inflation factors), but at the expense of statistical bias (Jolliffe 2002). Jolliffe (2002, page 170-171) shows how multicollinearity is driven by PCs with small eigenvalues, and accordingly, that regression coefficients for the predictor traits that load heavily on those axes will be estimated imprecisely in the original regression (see also Wilks 2005, page 505). While omitting these PCs eliminates multicollinearity, Mitchell-Olds and Shaw (1987) criticized the assumption that trailing PCs (trait combinations with less variance) were not predictive of relative fitness and could be omitted. Likewise, Joliffe (1982) described several cases outside of evolutionary biology where PC's explaining relatively little variance were still predictive of the response variable. In contrast, Blows and and McGuigan (2015) and Sztepanacz and Blows (2017) show how the distribution of even *leading* eigenvalues from phenotypic and genetic data can be strikingly difficult to distinguish from sampling variation, suggesting that trailing principal components might primarily represent sampling variation. One practical approach to addressing this would be to evaluate whether trailing PC's, which are responsible for multicollinearity, are

predictive of relative fitness. If they are not (as in our empirical example), omitting these PC's and then projecting selection estimates back into the original trait space seems to be a viable option. In the statistical literature, numerous approaches have been developed for determining how many PCs to use in principal component regression; Jolliffe (2002, pgs 173-177 and Hill et al. (1977) provide overviews. Jolliffe (2002, pgs 178-179) and Blair et al. (2006) also outline similarities between principal component regression and other biased regression techniques that can reduce the effects of multicollinearity. Fundamentally, some judgement of the investigator will be required to decide how many PC's to include without reintroducing multicollinearity while still capturing a sufficient portion of phenotypic variance to evaluate biological hypotheses about selection.

Acknowledgements. Our work is supported by NSERC Canada (JRS, VKC) and the Department of Ecology and Evolutionary Biology. We thank Ben Gilbert, Don Jackson, Dave Punzalan, Jessica Forrest, and Corlett Wood for discussion.

References

Austen, E. J., L. Rowe, J. R. Stinchcombe, and J. R. K. Forrest. Explaining the apparent paradox of persistent selection for early flowering. New Phytologist:n/a-n/a.

Anholt, B. R. 1991. Measuring Selection on a Population of Damselflies with a Manipulated Phenotype. Evolution 45:1091-1106.

Bair, E., T. Hastie, D. Paul, and R. Tibshirani. 2006. Prediction by Supervised Principal Components. Journal of the American Statistical Association 101:119-137.

Blows, M. W., and K. McGuigan. 2015. The distribution of genetic variance across phenotypic space and the response to selection. Molecular Ecology 24:2056-2072.

Brodie, E. D., A. J. Moore, and F. J. Janzen. 1995. Visualizing and quantifying natural selection. Trends in Ecology & Evolution 10:313-318.

Bumpus, H. 1899. The elimination of the unfit as illustrated by the introduced sparrow, *Passer domesticus*. Biological lectures from the Marine Biological Laboratory of Woods' Hole, Mass. Ginn, Boston.

Caicedo, A. L., J. R. Stinchcombe, K. M. Olsen, J. Schmitt, and M. D. Purugganan. 2004. Epistatic interaction between *Arabidopsis FRI* and *FLC* flowering time genes generates a latitudinal cline in a life history trait. PNAS 101:15670-15675.

Campbell, D. R. 1989. Measurements of selection in a hermaphroditic plant: Variation in male and female pollination success. Evolution 43:318-334.

Colautti, R. I., C. G. Eckert, and S. C. H. Barrett. 2010. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. Proceedings of the Royal Society B: Biological Sciences 277:1799-1806.

Colautti, R. I., and S. C. H. Barrett. 2010. Natural Selection and Genetic Constraints on Flowering Phenology in an Invasive Plant. International Journal of Plant Sciences 171:960-971.

Conner, J. K. 2007. A tale of two methods: putting biology before statistics in the study of phenotypic evolution. Journal of Evolutionary Biology 20:17-19.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, NJ, USA.

Hill, R. C., T. B. Fomby, and S. R. Johnson. 1977. Component selection norms for principal components regression. Communications in Statistics - Theory and Methods 6:309-334.

Hunt, J., J. B. Wolf, and A. J. Moore. 2007. The biology of multivariate evolution. Journal of Evolutionary Biology 20:24-27.

Janzen, F. J. 1993. An experimental analysis of natural selection on body size of hatchling turtles. Ecology 74:332-341.

Janzen, F. J., and H. S. Stern. 1998. Logistic regression for empirical studies of multivariate selection. Evolution 52:1564-1571.

Jolliffe, I.T. (1982). A note on the use of principal components in regression. Appl. Statist., 31, 300–303.

Jolliffe, I. T. 2002. Principal Component Analysis. Springer, NY, NY, USA.

Kraft, P. G., C. E. Franklin, and M. W. Blows. 2006. Predator-induced phenotypic plasticity in tadpoles: extension or innovation? Journal of Evolutionary Biology 19:450-458.

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

Lafi, S.Q. and J.B. Kaneene. 1992. An explanation of the use of principal components analysis to detect and correct for multicollinearity. Preventive Veterinary Medicine 13: 261-275.

Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. Evolution 41:1149-1161.

Parachnowitsch, A. L., R. A. Raguso, and A. Kessler. 2012. Phenotypic selection to increase floral scent emission, but not flower size or colour in bee-pollinated Penstemon digitalis. New Phytologist 195:667-675.

R Core Team. 2014. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Rausher, M. D. 1992. The measurement of selection on quantitative traits: Biases due to environmental covariances between traits and fitness. Evolution 46:616-626.

Schiestl, F. P., F. K. Huber, and J. M. Gomez. 2011. Phenotypic selection on floral scent: trade-off between attraction and deterrence? Evolutionary Ecology 25:237-248.

Shindo, C., M. J. Aranzana, C. Lister, C. Baxter, C. Nicholls, M. Nordborg, and C. Dean. 2005. Role of *FRIGIDA* and *FLOWERING LOCUS C* in determining variation in flowering time of *Arabidopsis*. Plant Physiology 138:1163-1173.

Stinchcombe, J.R., L.A. Dorn, and J. Schmitt. 2004. Flowering time plasticity in Arabidopsis thaliana: a reanalysis of Westerman & Lawrence (1970). Journal of Evolutionary Biology 17:197-207.

Stock, A. J. 2015. Evolution of Arabidopsis thaliana flowering time in response to water availability post-introduction. MSc Thesis. University of Toronto, Toronto, ON, Canada.

Stock, A. J., B. V. McGoey, and J. R. Stinchcombe. 2015. Water availability as an agent of selection in introduced populations of Arabidopsis thaliana: impacts on flowering time evolution. PeerJ 3:e898.

Sztepanacz, J. L., and M. W. Blows. 2017. Accounting for Sampling Error in Genetic Eigenvalues Using Random Matrix Theory. Genetics.

Suter, L., and A. Widmer. 2013. Environmental Heat and Salt Stress Induce Transgenerational Phenotypic Changes in Arabidopsis thaliana. PLoS ONE 8:e60364.

Wilks, D.S. 2005. Statistical methods in the atmospheric sciences. Elsevier, NY, NY, USA.

TABLES

Table 1. Line mean correlations for the Arabidopsis study, with eigenvector loadings for PC1-4.

	Flowering Time	Flowering Duration	Branch Number	Rosette Diameter	PC1	PC2	PC3	PC4
Flowering Time	1				0.522	-0.215	0.154	-0.352
Flowering Duration	-0.95 P<0.0001	1			-0.523	0.157	-0.244	0.426
Branch Number	-0.74 P<0.0001	0.72 P<0.0001	1		-0.476	-0.018	0.856	-0.189
Rosette Diameter	-0.12 P=0.39	0.06 P = 0.67	-0.09 P= 0.54	1	0.045	0.913	-0.047	-0.397
Rosette Leaf Number	0.70 P<0.0001	-0.73 P<0.0001	-0.64 P<0.0001	0.33 P = 0.02	0.473	0.306	0.425	0.708
Percent Variance Explained					65.17%	23%	6.94%	3.5%

Table 2. Selection estimates for the Arabidopsis experiment. Univariate estimates of selection are from regressions of relative fruit production on each trait alone. Selection gradients are estimated from a multiple regression of relative fitness on all traits, although the estimates for flowering time and duration are uncertain because of multicollinearity (indicated by variance inflation factors (VIF)). Principal component regression estimates are regression coefficients for PC1-PC4 projected back into the original trait space.

	Univariate Regressions			Multiple Regression				Principal Component Regression
Trait	s ± se	t	P	β ± se	t	P	VIF	β ± se
Flowering time	**-0.37 ± 0.05**	**6.96**	**<0.0001**	-0.30 ± 0.19	1.57	0.12	12.54	-0.17 ± 0.05
Flowering Duration	**0.35 ± 0.06**	**6.29**	**<0.0001**	0.05 ± 0.18	0.27	0.78	11.32	0.17 ± 0.06
Branch Number	**0.295 ± 0.06**	**4.73**	**<0.0001**	0.07 ± 0.08	0.95	0.35	2.45	0.09 ± 0.08
Rosette Diameter	0.11 ± 0.07	1.52	0.136	0.059 ± 0.07	0.87	0.39	1.60	0.07 ± 0.07
Rosette Leaf number	**-0.218 ± 0.07**	**3.18**	**0.0026**	0.061 ± 0.09	0.63	0.53	3.21	-0.06 ± 0.10

FIGURES

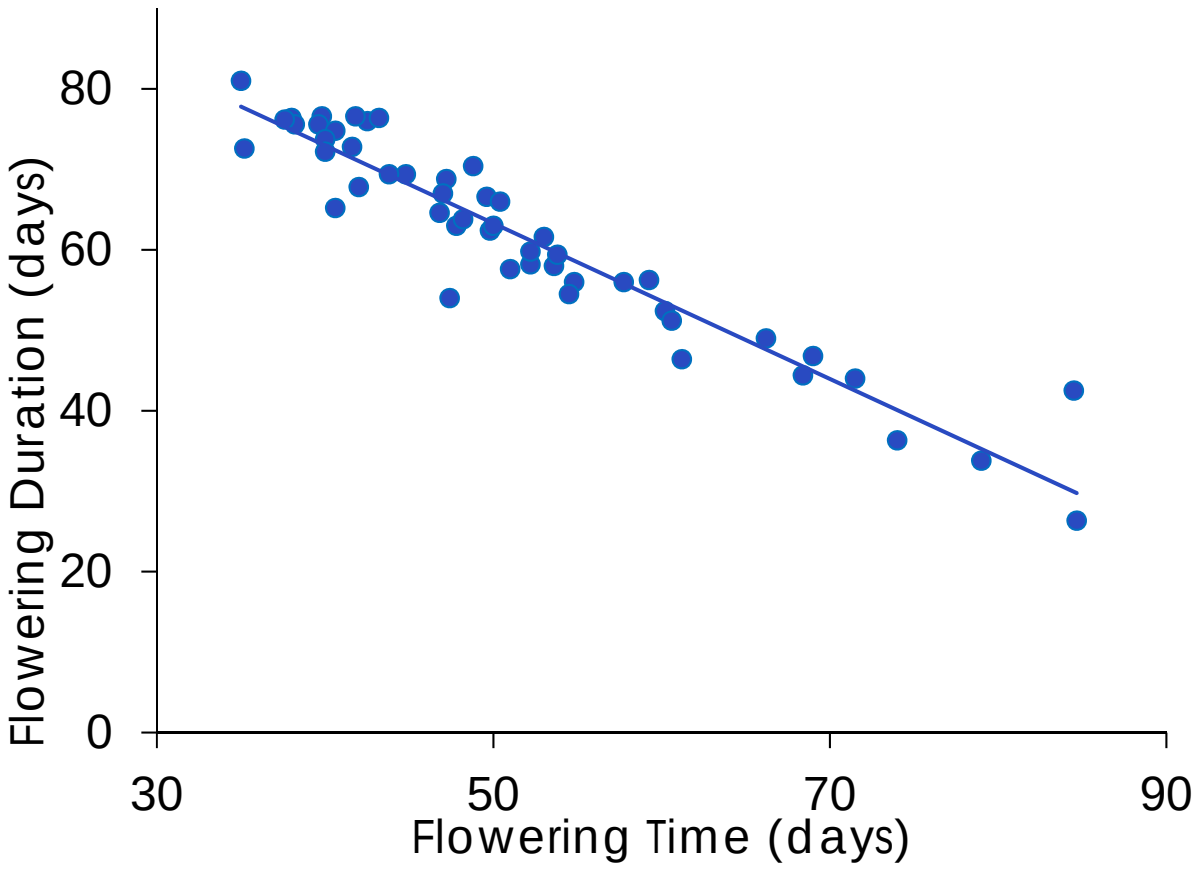

Figure 1. Line mean correlation between flowering time and flowering duration in the Arabidopsis chamber experiment. The inbred line mean correlation is -0.95.

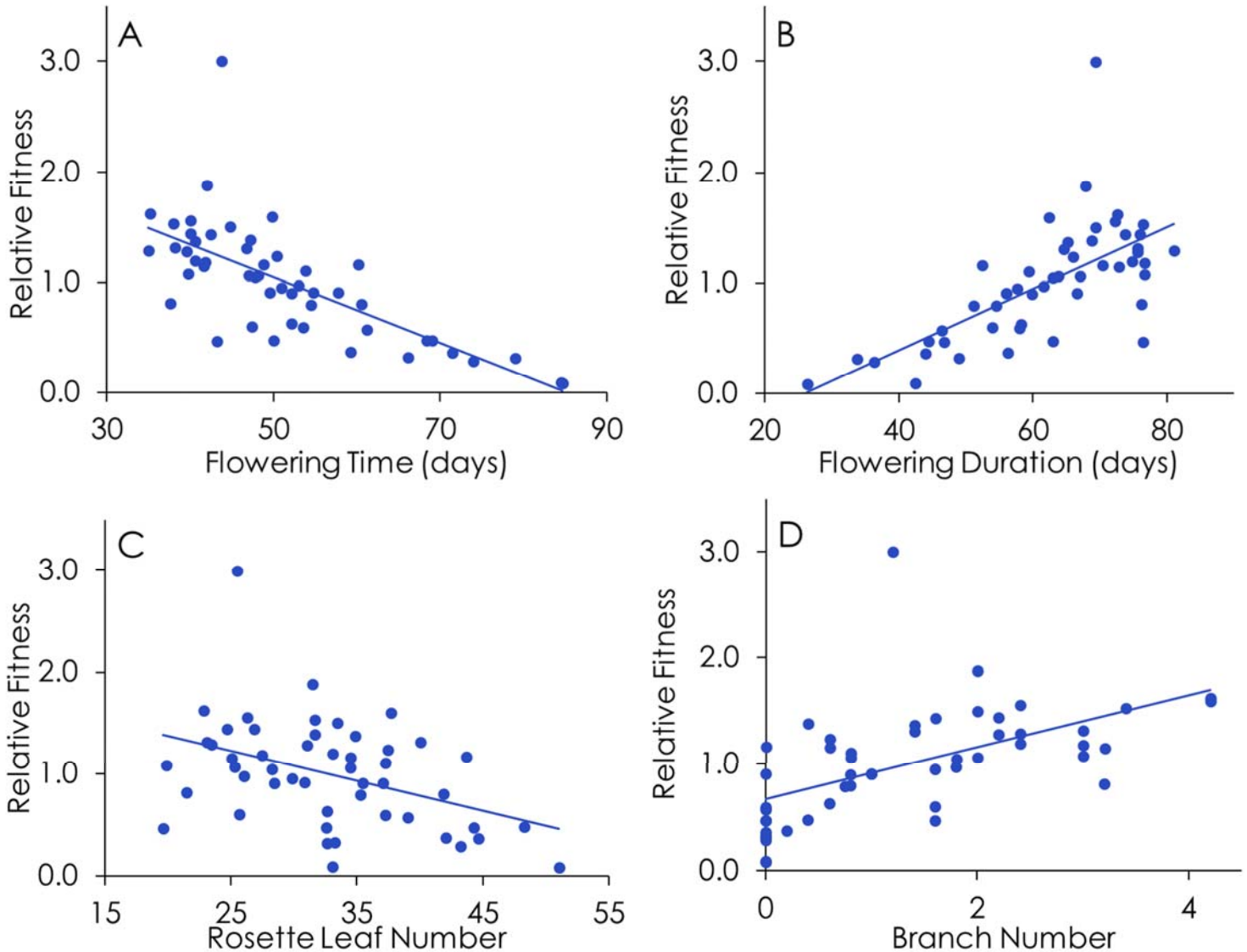

Figure 2. Selection differentials for: (A) flowering time and (B) flowering duration, (C) Rosette leaf number, and (D) Branch Number in the *Arabidopsis* experiment. Differentials are portrayed in the original trait units.