

Selection for independence of floral and vegetative traits: evidence from correlation patterns in five species

Jeffrey K. Conner and Andrea Sterling

Abstract: Underlying developmental and genetic relationships cause positive correlations among the sizes of a variety of plant traits. Selection for functional independence among traits, however, can reduce these correlations over evolutionary time. In 1960, R.L. Berg hypothesized that the sizes of flowers in insect-pollinated plants should be selected to remain constant regardless of the size of vegetative structures, so that flowers match the sizes of their pollinators for effective pollination. This hypothesis of functional independence of floral sizes from the size of the rest of the plant predicts that correlations between floral and vegetative traits should be reduced relative to correlations within trait groups. We measured correlations in five species of insect-pollinated plants, including four Brassicaceae and *Phlox divaricata*. Our results support the hypothesis. The correlations among floral traits and the correlations among vegetative traits were significantly greater than the correlations across these two groups of traits in all five species.

Key words: phenotypic correlations, natural selection, functional independence, pollination, floral evolution.

Résumé : Les relations génétiques et développementales sous-jacentes sont responsables de corrélations positives dans les amplitudes de la variété des caractères des plantes. Cependant, la sélection visant à obtenir une indépendance fonctionnelle entre les caractères peut réduire ces corrélations au cours de l'évolution. En 1960, R.L. Berg a formulé l'hypothèse que les dimensions des fleurs chez les plantes pollinisées par les insectes devraient être sélectionnées de manière à les garder constantes, indépendamment des dimensions des structures végétaives, afin que les fleurs aient des dimensions qui concordent avec leurs pollinisateurs, assurant ainsi la fécondation. Cette hypothèse sur l'indépendance fonctionnelle des dimensions florales par rapport au reste de la plante prédit que les corrélations entre les caractères floraux et végétatifs devraient être réduits par rapport aux corrélations à l'intérieur des groupes de caractères. Les auteurs ont mesuré ces corrélations chez cinq espèces pollinisées par des insectes, incluant quatre Brassicaceae et le *Phlox divaricata*. Les résultats confirment l'hypothèse. Les corrélations entre les caractères floraux et les corrélations entre les caractères végétatifs sont significativement plus grandes que les corrélations couvrant l'ensemble des deux groupes de caractères, chez les cinq espèces.

Mots clés : corrélations phénotypiques, sélection naturelle, indépendance fonctionnelle, pollinisation, évolution florale.
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Introduction

Patterns of correlations among traits can provide clues concerning past selection on those traits (Berg 1960; Kingsolver and Wiernasz 1987, 1991; Armbruster 1991). Extremely high correlations among traits may be evidence that selection has increased correlations to promote functional integration among traits (Olsen and Miller 1958; Cheverud 1982, 1984; Conner and Via 1993). Alternatively, traits can be positively correlated due to pleiotropic effects of genes affecting size,

in the absence of any selection on the correlations. In this case, selection for functional independence among traits may reduce correlations.

A hypothesis of pollinator-driven decoupling of traits was advanced by Berg (1960). Berg (1960) found that species that are wind-pollinated or pollinated by less-specialized insects had positive correlations among all traits, including positive correlations between floral and vegetative traits. This is the pattern expected with no selection on correlations, because all the traits measured were related to size. In contrast, Berg found that in species with relatively specialized insect pollinators, floral traits were positively correlated with each other, as were vegetative traits, but the two groups of traits (floral and vegetative) were not correlated. This pattern was also found in wild radish (Conner and Via 1993).

Berg (1960) hypothesized that plants whose floral sizes matched the sizes of their pollinators, regardless of the over-

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J.K. Conner¹ and A. Sterling. Department of Ecology, Ethology, and Evolution, University of Illinois, Shelford Vivarium, 606 East Healey Street, Champaign, IL 61820, U.S.A.

¹ Author to whom all correspondence should be addressed.

all size of the plant, would be pollinated most effectively. This would result in stabilizing selection on floral size, which could reduce the correlation between flower size and the size of the rest of the plant in species with more specialized pollinators. This hypothesis does not imply that flowers pollinated by the wind or by very unspecialized insects are not functionally integrated (floral traits in all species were found to be positively correlated), but rather that they have not evolved to match the sizes of a particular group of pollinators. Note that Berg's (1960) hypothesis is relevant only to correlations within populations, not to correlations across populations and species.

This paper reports a more statistically rigorous test of Berg's (1960) hypothesis than has been done previously (Berg did not test for differences statistically), using five species of plants, only one of which was studied by Berg (1960). All the species are pollinated by relatively specialized insects, so we predicted that all would show the Berg (1960) pattern of reduced correlation between floral and vegetative traits. Because we did not examine any species pollinated by the wind or extremely unspecialized insects, our test of Berg's (1960) hypothesis is incomplete; however, the a priori expectation is that all size-related traits will be positively correlated.

Methods

Species studied

We chose five species with similar floral morphologies so that the same traits could be measured. Four of the species are annuals in the Brassicaceae and of Eurasian origin. Canola (*Brassica napus*) is an oil-seed crop, and black mustard (*Brassica nigra*), wild radish (*Raphanus raphanistrum*), and dame's rocket (*Hesperis matronalis*) are widespread weeds of disturbed areas. The fifth species is *Phlox divaricata* (Polemoniaceae), a native spring woodland perennial. All of the species are pollinated by bees, butterflies, and (or) flies.

In each species 50–340 plants were measured. All plants measured were growing in the field except for wild radish, in which field-collected seeds from Michigan and New York were grown in the greenhouse (the two populations were analyzed separately). For details of the populations studied, the sampling procedures used, and the biology of these plants see Conner and Sterling (1995).

Traits measured

One to three flowers were measured on each plant; if more than one flower was measured, the mean values for each plant were used. Six floral traits were measured: the length and width of the distal, showy part of the petal (the "limb," outside the corolla tube), the length of the corolla tube (the "claw," the proximal part of the petal), the length of the pistil, and the lengths of the longest and shortest filaments.

In addition, two or four vegetative traits were measured on each plant. These traits were chosen in an attempt to obtain repeatable measurements of a variety of vegetative structures for each species. Some of these reflect overall plant size, while others are dimensions of modular traits. These are all appropriate for testing Berg's (1960) hypothesis, which states that floral dimensions should be decoupled from both overall plant size and the sizes of individual modules, so that the floral size can match pollinator size independent of the rest of the plant. The length and width of the oldest (lowermost) undamaged leaf blade was measured on all species; owing to differences in plant age and (or) rates of leaf senescence, the nodal position of measured leaves varied among individuals. If leaf sizes varied between nodes, this could have reduced the correlations

between leaf traits and both floral and other vegetative traits; therefore, this should not bias our test of Berg's (1960) hypothesis.

The other two vegetative traits measured differed among species, depending on growth form and age of the population. On wild radish from Michigan and on black mustard, we measured the thickness of the main stem directly above the base of the lowest healthy leaf and the height of the entire plant. On dame's rocket and canola, the first two floral internodes (the distance between the pedicel attachment points on the stem) were measured. On phlox, the top two leaf internodes were measured after plant growth had ceased.

All traits were measured with digital calipers except plant height, which was measured with a metre stick. Measurement errors for all traits were <2% (for details on measurement error estimation, see Conner and Via 1993).

Data analyses

Phenotypic correlations among traits were calculated; these are appropriate for evaluating these selective hypotheses because the phenotype determines fitness and adaptation. The correlations among all the traits were divided into two subsets to test Berg's (1960) hypothesis regarding uncoupling of floral and vegetative traits. The mean of the correlations within the floral and vegetative trait groups (i.e., floral–floral correlations and vegetative–vegetative correlations) was compared with the mean of the correlations across the floral and vegetative trait groups (i.e., floral–vegetative correlations) for each species. This comparison tests whether the across-group correlations are lower than the within-group correlations, as predicted by Berg's (1960) hypothesis.

The within-group correlations consisted of the mean of 12 correlations among the floral traits and the 6 correlations among the vegetative traits, whereas the across-group correlations consisted of the mean of all 24 correlations between floral and vegetative trait pairs. The only exception to these numbers occurred in wild radish from New York, which had only 13 within- and 12 across-group correlations because only two vegetative traits were measured. The within-group correlations in each species included all vegetative trait correlations and all floral trait correlations except the three correlations among the filaments and corolla tube, because these do not represent general levels of floral correlations (Conner and Via 1993; Conner and Sterling 1995). This decision resulted in a more conservative test of Berg's (1960) hypothesis, because these three correlations were consistently among the highest of the within-floral group and including them would increase the difference between the within and across groups.

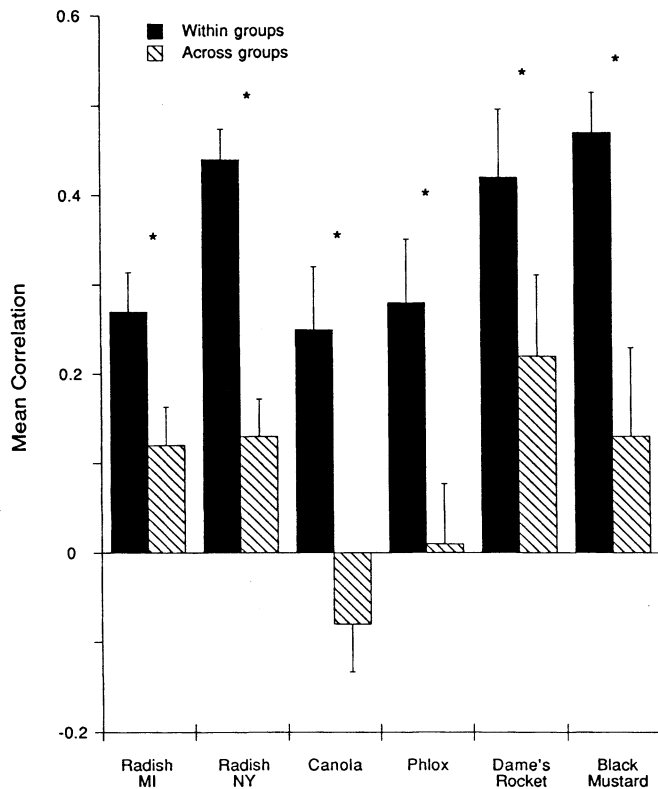
The groups being compared are drawn from the same correlation matrix; therefore, they are not independent and standard statistical tests are inappropriate. For this reason, a bootstrap analysis (Efron 1982) was performed using SAS (SAS Institute Inc. 1989). For details of this technique see Conner and Sterling (1995).

Results and discussion

The results of the comparison of the correlations within the floral and vegetative trait groups versus the correlations across the two groups clearly show the pattern reported by Berg (Fig. 1). The mean correlation within floral and vegetative trait groups is significantly greater than the mean correlation across groups in all six populations, despite the fact that different vegetative traits were measured in different species.

Four of these species were in the same family, so these similarities may be due in part to phylogeny. Berg (1960) also reported that *P. divaricata* showed the pattern of reduced correlations between floral and vegetative traits but did not

Fig. 1. Comparisons of the mean correlation among traits within the floral and vegetative trait groups with the mean of the correlations across the two groups for the six populations. Error bars are + 1 bootstrap SD, and asterisks denote significant differences (within vs. across) indicated by the bootstrap analysis.



present the data that supported this conclusion. It is important to note that the positive correlations within the floral and vegetative trait groups do not indicate functional relationships among traits; rather, these correlations are due at least in part to overall size relationships and probably reflect common developmental pathways. Indeed, the interesting result is that the correlations across groups are so weak, because in plants without specialized pollinators the dimensions of all structures are positively correlated (Berg 1960), as expected for size-related traits.

It is not entirely clear, however, whether our five species support Berg's (1960) hypothesis that this pattern is due to stabilizing selection on floral size to match the sizes of pollinators. Some of these species are known to be visited by a wide variety of insects of widely varying sizes (see also Herrera 1995). For example, wild radish is visited by butterflies, bumblebees, honeybees, small solitary bees, and a variety of sizes of syrphid flies (Kay 1976; Stanton et al. 1989; Conner and Rush 1996). It may be that the flowers of the plants we studied are only adapted to a subset of their visitors, and the Berg (1960) pattern is a result of selection to match the sizes of this subset. Alternatively, it may be that reduced correlations between vegetative and floral traits have evolved for reasons other than those suggested by Berg (1960).

To further test Berg's (1960) hypothesis, future studies could measure correlation patterns in plants from a wide range of taxa and with a wide range of pollination systems.

Even more crucial are detailed analyses of selection on floral traits, with particular emphasis on measurements of stabilizing selection on floral size.

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References

- Armbruster, W.S. 1991. Multilevel analysis of morphometric data from natural plant populations: insights into ontogenetic, genetic, and selective correlations in *Dalechampia scandens*. *Evolution*, **45**: 1229–1244.
- Berg, R.L. 1960. The ecological significance of correlation pleiades. *Evolution*, **14**: 171–180.
- Cheverud, J.M. 1982. Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution*, **36**: 499–516.
- Cheverud, J.M. 1984. Quantitative genetics and developmental constraints on evolution by selection. *J. Theor. Biol.* **110**: 155–171.
- Conner, J.K., and Rush, S. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia*. In press.
- Conner, J.K., and Sterling, A. 1995. Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral and vegetative traits in five insect-pollinated plants. *Am. J. Bot.* **82**: 1399–1406.
- Conner, J.K., and Via, S. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution*, **47**: 704–711.
- Efron, B. 1982. The jackknife, the bootstrap, and other resampling plans. Society for Industrial and Applied Mathematics, Philadelphia, Pa.
- Herrera, C.M. 1995. Microclimate and individual variation in pollinators: Flowering plants are more than their flowers. *Ecology*, **76**: 1516–1524.
- Kay, Q.O.N. 1976. Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature (London)*, **261**: 230–232.
- Kingsolver, J.G., and Wiernasz, D.C. 1987. Dissecting correlated characters: adaptive aspects of phenotypic covariation in melanization pattern of *Pieris* butterflies. *Evolution*, **41**: 491–503.
- Kingsolver, J.G., and Wiernasz, D.C. 1991. Development, function, and the quantitative genetics of wing melanin pattern in *Pieris* butterflies. *Evolution*, **45**: 1480–1492.
- Olsen, E.C., and Miller, R.J. 1958. Morphological integration. University of Chicago Press, Chicago, Ill.
- SAS Institute Inc. 1989. SAS/STAT user's guide, version 6 edition. SAS Institute Inc., Cary, N.C.
- Stanton, M.L., Snow, A.A., Handel, S.N., and Berczky, J. 1989. The impact of a flower-color polymorphism on mating patterns in experimental populations of wild radish (*Raphanus raphanistrum* L.). *Evolution*, **43**: 335–346.