Constraints on plant signals and rewards to multiple mutualists?

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Many plants invest substantial resources in signaling to and rewarding two kinds of 'interguild' mutualists, pollinators and seed dispersers. The signals and rewards are expressed via traits of flowers and fruits. Pollinators and seed dispersers could act in synergistic or antagonistic ways to influence selection on these traits. Here, we address the issue of whether plant species might be constrained in signaling to and rewarding multiple mutualists that provide different types of benefits to plants. Specifically, does investment in one type of mutualist limit investment in another? We examined the correlation between flower size and fruit size for 472 plant species spanning three regional floras. Our analyses made the assumption that structure size is related to plant investment in signals and/or rewards. We expect that a constraint due to interguild mutualisms would be evidenced by a negative correlation between flower and fruit size. Instead, we found significantly positive relationships between flower size and fruit size in all three regional floras. These relationships remained robust after correcting for plant evolutionary history using phylogenetically independent contrasts. These patterns may reflect synergies in selection by pollinators and seed dispersers, genetically-based or resource-based constraints on investment in reproductive tissues, and/or an underlying trade-off in structure size versus number.

Introduction

Mutualisms are common in nature, and organisms frequently engage in more than one mutualism at a time.[1-3] For instance, plants can form mutualisms with mycorrhizal fungi, endophytic fungi, nitrogen-fixing bacteria, pollinators and seed dispersers. Yet, the majority of research has focused on pair-wise mutualisms, neglecting the generalized, multi-species interactions that characterize most ecological communities.[4,5] Most studies have examined only one mutualist or functional group of mutualists, potentially overlooking interactions among species that confer different types of benefits.[6,7] These 'interguild mutualists' could act in synergistic or antagonistic ways to influence plant fitness[8] as well as selection on individual plant traits.

A recent consideration of the comparative evolution of flowers and fruits[9] highlighted the fact that many plants invest substantial resources in signaling to and rewarding two kinds of mutualists, pollinators and seed dispersers. Pollinators respond to the visual and olfactory signals produced by flowers, and are typically rewarded by nectar and/or pollen also produced in flowers.[10-12] Signaling to seed dispersers is accomplished by the visual and olfactory signals produced by fruits, and the fruit itself serves as the reward.[11,12] In both cases, signaling is mediated by the size of the display and the presence of pigments such as anthocyanins and carotenoids; rewards are carbohydrates and, to a lesser extent, proteins and lipids. Despite these similarities, pollinators and seed dispersers likely exert different selective pressures on plant structures. For example, because the costs of sharing pollinators are much greater than those of sharing seed dispersers,[13] character displacement and/or ecological sorting is expected to be greater in flowers than in fruits. Indeed, for 472 species in three floras, phylogenetically controlled analyses of nearest-neighbor distances in multidimensional trait space demonstrated that flowers were more divergent from one another than were fruits.[9] These results suggest that flowers and fruits evolve under different regimes, and raise the question of how selection on one structure might influence or constrain morphology of the other structure.

Here, we take a comparative approach to consider whether plant species might be constrained in signaling and providing rewards to both pollinating and seed-dispersing mutualists. Assuming that the size of a structure is positively correlated with energetic investment by the plant, we examine data on flower and fruit size from three floras: the Caribbean island of St. John, U.S. Virgin Islands,[14] Hawaii,[15] and the Great Plains of the U.S.[16] If plant investment in the signal/reward to one mutualist constrains investment in the signal/reward to another mutualist, we expect a negative correlation between flower and fruit size. We examined the relationships between flower and fruit sizes with general linear models, and then followed these analyses with an examination of phylogenetically independent contrasts.[17]
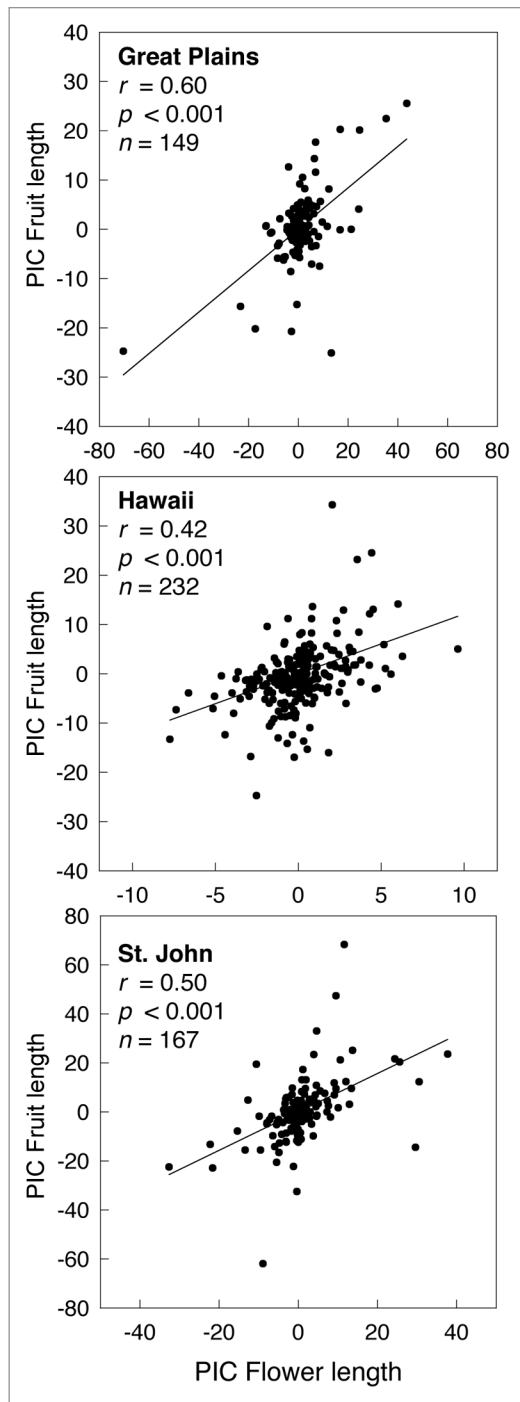
Results

For the raw data analyses, fruit size was positively related to flower size ($F_{1,550}$ = 191.75, p < 0.0001). This relationship was strongest

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Figure 1. Phylogenetically independent contrasts of fruit length vs. flower length for each of three regional floras: Great Plains, Hawaii and St. John. Correlation coefficients are adjusted for constraining the regression through the origin. *n* is the number of independent contrasts for each flora, which equals (1 - the number of taxa in the analysis).

for the St. John flora ($r = 0.67$, p < 0.0001), intermediate for the Great Plains ($r = 0.49$, p < 0.0001), and weakest for the Hawaii flora ($r = 0.43$, p < 0.0001), as indicated by a significant interaction between flora and flower size ($F_{2,550} = 3.58$, p = 0.0286). Mean fruit size did not significantly differ among the three floras ($F_{2,550} = 0.91$, p = 0.4032), but mean flower size was significantly larger in the Hawaiian flora (mean length ± s.e. = 22.9 ± 1.3 mm)

than in either the Great Plains (10.7 ± 1.2 mm) or St. John (10.0 ± 1.3 mm) ($F_{2,550} = 15.39$, p < 0.0001).

Accounting for the evolutionary history of plant taxa via phylogenetically independent contrasts did not eliminate the positive association between fruit size and flower size (**Fig. 1**). However, removing phylogenetic signal did change the rankings of the strength of the fruit/flower relationship among the floras, from St. John > Great Plains > Hawaii to Great Plains > St. John > Hawaii (**Fig. 1**).

Discussion

Using data on floral and fruit investment reported in regional floras, we showed that animal-pollinated and -dispersed plant species that invest in large flowers also produce large fruits. The correlation between fruit length and flower length remained significant and positive even when plant phylogenetic relatedness was taken into account. Overall, this pattern is consistent with the hypothesis that the interguild mutualisms of pollination and seed dispersal may synergistically influence selection on plant investment in signals and rewards. The positive correlation does not support the hypothesis that investment in one mutualist guild constrains plant investment in the other mutualist guild.

This result is consistent with three potential scenarios linking the evolution of flowers and fruits. First, plants may face genetic constraints linking flower and fruit size. For example, genes underlying floral size traits may also affect fruit size traits via pleiotropy. This mechanism could be bi-directional—that is, selection on flower size could influence the fruit size, and vice-versa. To our knowledge, such genetic constraints have yet to be demonstrated. However, positive correlations between flower size and achene size have been detected in a wind-dispersed clade (*Crepis*),[18] suggesting that positive relationships may be common in plants. Furthermore, a gene involved in the control of fruit shape in tomato (*Lycopersicon*) is also expressed during floral development, and its overexpression reduces floral size,[19] consistent with the existence of genetic constraints linking the signals/rewards of fruits and flowers.

Second, plants may express a physiological constraint (mechanical or allocation-based) linking flower and fruit size. Because this mechanism requires changes in plant resource allocation or physical structures during plant development, this effect would be uni-directional; that is, flower size could constrain fruit size, but not the reverse. A mechanical constraint would require that the size of floral structures (e.g., the corolla) would restrict the ability of an ovary to expand into a fruit. Because flowers are modular, with petals, stamens, and pistils arising from ontogenetically distinct pathways,[12] we argue that such a mechanical constraint is unlikely and is probably not driving the pattern in which plants with small flowers also have small fruits. It seems more likely that the constraint could be allocation-based, rather than mechanical. While it is easy to envision scenarios under which high resource investment in floral rewards or signals could limit resource availability to later-developing fruits (an allocation trade-off), it is also possible that plant allocation toward increasing flower size would also divert high levels of resources to fruits,[20] perhaps through source-sink dynamics within the plant.[21] Deciphering whether constraints are

genetically-based or physiological will ultimately require detailed experiments within single species that can control the genetic background of individuals (see similar issues for plant-herbivore interactions in Strauss[22]). However, it would also be interesting to assess whether plant species displaying high investment in both flowers and fruits tend to occur in resource-rich habitats, also suggestive of an allocation-based mechanism.

Third, the positive correlation may reflect the known underlying size-number tradeoff for plant reproduction.[23,24] Given finite resources, plants can either allocate resources to few large structures or many small structures, but cannot provision many large structures. This tradeoff could affect flowers, fruits or both, and would result in natural selection for different size-number balances in different species according to the fitness contributions of differently-sized structures.[25] Once an evolutionary equilibrium is achieved in a given species, such a tradeoff could be expressed in the following way. If the number of structures is small, a plant would have a lot of resources to invest per flower *and* per fruit, whereas if the number of structures is large, a plant would have to spread the resource pool among many flowers *and* many fruits. Given a range of tradeoffs in different species, the observed pattern across species would be a positive correlation between flower and fruit size.

In conclusion, we have shown that plant taxa with large flowers also have large fruits, and that this pattern is robust across three regional floras and after accounting for plant evolutionary history. We found no evidence that investment in signals/rewards to pollinators restricts such investment to seed dispersers, or vice-versa. We hope this work sparks new interest in exploring how interguild mutualisms, such as pollination and seed dispersal, shape the evolution of plant traits involved in signaling and rewards.

Materials and Methods

We collected data on flower and fruit size from three floras: the Caribbean island of St. John, U.S. Virgin Islands,[14] Hawaii[15] and the Great Plains of the U.S.[16] These floras were chosen because their standardized species descriptions allowed trait data to be extracted consistently across species. For each native, animal-pollinated, fleshy-fruited (presumably endozoochorously-dispersed) species, we recorded floral tube length (if present), petal length, fruit length and fruit width. Flower size was then calculated as floral tube length + petal length, and fruit length was the maximum fruit dimension reported in the flora.

If plant investment in the signal/reward to one mutualist constrains investment in the signal/reward to another mutualist, we expect a negative correlation between flower and fruit size. We first examined the relationship between flower and fruit sizes with a general linear model, including flora as a fixed factor and flower size as a continuous predictor variable. A significant flora x flower size interaction indicated that the relationship between flower size and fruit size varied among the three floras. We then calculated Pearson correlation coefficients within each flora. Analyses met assumptions of normality of residuals and homogeneity of variances following log-transformation of both flower and fruit lengths.

To account for the phylogenetic nonindependence of our observations, we revisited the relationship between flower and fruit sizes using phylogenetically independent contrasts[17] (PICs). To accommodate recent changes in phylogenetic hypotheses, we analyzed all floras at the generic level and reassigned those genera and their associated species to families based on Stevens' Angiosperm Phylogeny Website.[26] We employed Phylomatic[27] using the Davies et al. angiosperm supertree[28] to construct base family-level phylogenies for each flora. Given a lack of information about fine-scale phylogenetic relationships, genera and species were represented as arising from polytomies within families and genera, respectively. Phylogenies are available from the lead author on request.

Phylogenetically independent contrasts were generated using the PDAP:PDTREE module in Mesquite.[29-31] Actual branch lengths are unknown, but diagnostics indicated that branch lengths of 1.0 were adequate in all analyses (except for Hawaiian flower lengths, for which Grafen branch lengths performed better); furthermore, the method is generally robust to various branch length assumptions.[32,33] Standardized contrasts were obtained by dividing the raw contrasts by their standard deviations. We used linear regression to compare fruit length contrasts to flower length contrasts for each flora, with the intercept constrained through zero.[34] We report the adjusted correlation coefficients from these analyses. We eliminated one outlier from the St. John analysis because it was 10 times the standard deviation of the mean contrast value for flower length; this did not qualitatively change the results and made the correlation slightly more conservative.

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