GIS and Path Analysis: Examining Associations Between the Birds, the Bees, and Plant Sex in *Echinocereus coccineus* (Cactaceae)

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Abstract—*We tested hypotheses of how pollinators and water resource gradients influence the evolution of dioecy using* Echinocereus coccineus*, a cactus with both hermaphroditic and dioecious populations growing over wide climatic and biotic gradients in the Madrean Archipelago. A GIS database was compiled from herbarium specimens, rainfall data, and hummingbird abundance records. We used structural equation modeling to assess the relative direct and indirect influence of hummingbird abundance and mean annual rainfall on the presence of dioecy. The best fit models contained a direct influence of hummingbird abundance on dioecy; any direct effect of rainfall was negligible. These results support pollinator selection as the major influence on evolution of gender in* Echinocereus coccineus.

Introduction

Most flowering plants require animal pollinators to carry out mating between individuals. However, very few empirical studies have focused on the effect of the behavioral ecology of pollinators on plant mating system evolution. Dioecy is a mating system consisting of separate male and female plants. When it occurs in animal-pollinated species, dioecy may be brought about by a change in pollinators. In many plant families an association between mating system and pollinator specialization has been reported: Species with more specialized pollinators are mostly hermaphroditic, and species with depauperate or small, generalist pollinator fauna tend to be dioecious (Delph 1990; Bawa 1980; but see Renner and Feil 1993). In his review of the evolution of dioecy in flowering plants, for example, Bawa (1980) found examples from four taxonomic groups where, with one exception, all of the hermaphroditic species were bird-pollinated and all of the dioecious species were pollinated by insects. How a shift in pollinator type within one plant species could select for dioecy is not well understood (Renner and Ricklefs 1995). Many authors have proposed that certain types of pollinators produce higher self-fertilization rates when visiting flowers. This higher self-fertilization rate then in turn may select for dioecy to avoid selfing (Bawa 1994; Lloyd 1982; Schultz and Ganders 1996).

The specific genetic conditions that favor evolution of dioecy in plant populations are outlined by Lloyd's outcrossing advantage hypothesis (Lloyd 1975). This hypothesis states that in a hermaphrodite population, if inbreeding depression (relative loss of fitness due to selfing) and selfing rate are both sufficiently high, mutations that eliminate self-fertilization will be selected. Specifically, any completely dominant nuclear mutation that produces a female plant by causing male sterility will increase in a population where loss of fitness in hermaphrodite plants due to self-fertilization is greater than one-half of seed fitness (Lloyd, 1975). Linked modifier mutations in hermaphrodites that produce male plants by causing female sterility can then spread more easily through the population as female frequency increases. Thus, we would expect that if the pollinator assemblage of an outcrossing hermaphrodite population were to change such that selfing increased, and inbreeding depression was high in the population, then this could result in evolution of dioecy if the requisite mutational variation occurs in the population.

Despite its widespread use as an explanation for the evolution of dioecy in animal-pollinated plants, this hypothesis has not been tested empirically. This is probably due to lack of a suitable model species in which populations vary in mating system from purely hermaphrodite to completely dioecious. Most previous studies of dioecy have compared dioecious species to hermaphroditic relatives, and have looked for both biotic and abiotic correlates of dioecy (Bawa 1980). In these systems, however, it is impossible to estimate how interactions with pollinators may have selected for dioecy because it is unknown what the pollinator community composition was at the time when dioecy was initially evolving.

Both pollinator community composition and plant population dynamics respond to changes in resources (Abrams 1995), and interactions among plants, pollinators, and resources can be complex (Schemske and Horvitz 1988; Iriondo et al. 2003). Pollinator distribution and abundance often follow patterns determined by climatic variables. In a study in the mountains of Mexico, Cruden (1972) found that hummingbirds were more effective pollinators at high elevations due to the high incidence of rainfall in these areas. Rainfall gradients could indirectly select for dioecy through their influence on pollinator distribution and abundance (Weller et al.1995; Sakai et al. 1997; Soltis et al. 1996). The mechanism proposed in these systems is that when plants invade drier areas, conditions are no longer favorable for effective pollinators; plants are left with inferior pollinators that may increase the amount of self-fertilization in these populations, and selection favors separation of the sexual functions. An association between rainfall and dioecy has been noted in several studies, but whether it acts directly upon the fitness of the gender morphs or indirectly through its influence on pollinator distributions is still in question. A competing hypothesis proposed by Freeman (1980) states that lack of rainfall produces resource limitation and directly promotes disruptive selection and the resulting niche segregation of male and female plants. Therefore, dioecy could be selected in the absence of any effects of pollinators. This paper describes the first study to jointly test the outcrossing advantage and resource limitation hypotheses within a geographical context in which evolution of dioecy is incipient.

Echinocereus coccineus is an excellent model system to test hypotheses about the effects of pollinator changes and edaphic conditions on the evolution of dioecy. It possesses contemporaneously both hermaphroditic and dioecious populations and possibly gynodioecious populations as well. *Echinocereus coccineus* flowers appear specialized to hummingbirds but are also pollinated by bees. These two floral visitors vary greatly in their pollinating behavior, and based on previous research (Scobell 1999; Scobell 2002; England et al. 2001) we hypothesize that pollination by bees alone will result in a higher selfing rate than that produced when hummingbirds are also present. Distribution and abundance of these pollinator types varies across the geographic range of the plant: both hummingbirds and bees visit flowers in the center of the geographic range, in the areas along the Rocky Mountain/Sierra Madre migration corridor. In the drier, lower altitude areas to the east and west of this range, hummingbirds become rare or absent. Distribution of dioecious populations appears to be associated with areas of low hummingbird abundance, low elevation, and low rainfall (figure 1). Hypotheses we test in this paper include:

- Evolution of dioecy is pollinator-driven: Hummingbirds produce a higher outcrossing rate than bees; therefore, hermaphrodite populations are maintained only in populations with higher hummingbird abundance. In populations lacking hummingbirds, dioecy is selected for by the higher selfing rates bee pollination produces.
- Evolution of dioecy is resource-driven: In arid areas dioecy is selected for due to improved resource allocation of separate male and female plants.
- Evolution of dioecy is driven by a combination of these factors: In the Southwestern United States, both rainfall and pollinator distributions vary with elevation. Pollinators and resources may both be involved in the selection for dioecy in this species.

We used Structural Equation Modeling (SEM) to test these hypotheses. SEM is a statistical technique for solving simultaneous linear equations that combines traditional path analysis with factor analysis (Joreskog and Sorbom 1982). Causal graphs of hypothesized relationships among the data are produced, and then statistically tested, using a chi-square goodness of fit test to select the hypothesis that best describes the data (Shipley 2000).

Several studies have used structural equation modeling (or its predecessor, path analysis) (Wright 1934) to evaluate the direct and indirect influences of pollinators and/or edaphic factors on plant demographics and fitness (Iriondo et al. 2003; Schemske and Horvitz 1984; Mitchell, 1992). Schemske and Horvitz (1984) used path analysis to unravel the complex interactions among pollinators, herbivores, and ant guards and their effects on the number of mature fruits produced by *Calathea ovandensis* (Marantaceae). Iriondo et al. (2003) used structural equation modeling to determine which factors influenced seed production on two different soil types in an endangered plant *Erodium paularense* (Geraniaceae). Mitchell et al. (1992) used path analysis to determine which factors influenced hummingbird approach rate and probes/flower and how much these variables influenced fruit set in *Ipomopsis aggregata* (Polemoniaceae). Each of these studies was able to explore the interactions among the variables and assess their relative influence on the dependent variable (measures of plant fitness) more thoroughly than through linear or multiple regression techniques.

Methods

Study System

Echinocereus coccineus has both hermaphroditic populations (Scobell 1999) and dimorphic populations (Hoffman 1992; Powell 1995) (figure 1). All dimorphic populations that have been tested with hand-pollination crosses between morphs are functionally dioecious (one by Hoffman (1992), one by Powell (1995), and three by Scobell (unpublished data—TCNM, VFNM, and ALTX—figure 1)). We found anecdotal evidence of gynodioecy in three other dimorphic populations observed before and after seed set (Hualupai Mt, AZ (HUAZ), Zion National Park (ZNUT), and Flagstaff, AZ (FLAZ) (figure 1)). In these populations, females are present and some of the hermaphrodite morphs produced seed (1/10 of hermaphrodites in HUAZ, 7/10 in ZNUT, and 9/10 in FLAZ; Scobell, unpublished data). Herein, all dimorphic populations that have been determined experimentally to be dioecious will be termed "dioecious." All other untested dimorphic populations will be termed "dimorphic." Further breeding system experiments on northwestern populations of this species are needed to determine the functional mating system of these populations.

Echinocereus coccineus is part of the Triglochidiatus group (Taylor 1985). Members of this group are characterized as cacti that produce large, red, tubular flowers with abundant sucrose-rich nectar. These floral traits are termed hummingbird-syndrome traits (Grant 1967). In previous research, we observed four species of hummingbirds visiting this cactus in the Chiricahua Mountains of southern Arizona (Scobell 2002). The broad-tailed hummingbird (*Selasphorus platycercus*) was the predominant pollinator at all other sites observed, except in the Huachuca Mountains (HUAZ; figure 1) where Anna's hummingbird (*Calypte anna*) was the sole pollinator (Scobell, unpublished data). However, in a survey of two dioecious populations in central New Mexico, Hoffman (1992)

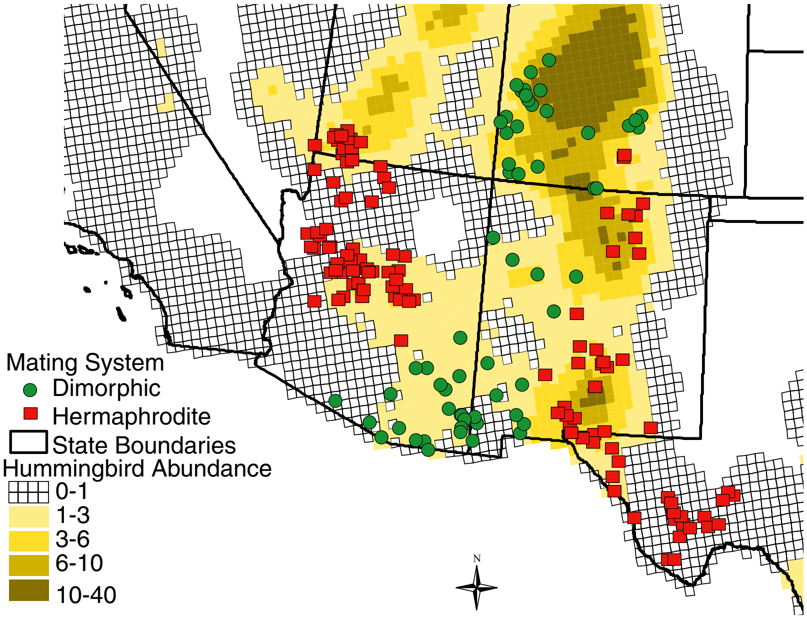

Figure 1—Distribution of dimorphic (dioecious or gynodioecious-red squares) and hermaphroditic (green circles) *E. coccineus* populations in the Southwestern United States superimposed upon a map of hummingbird (broad-tail *Selasphorus platycercus* and black-chinned *Archilochus alexandri*) distribution and abundance obtained from the Audubon Breeding Bird Survey website (http://www.mbr-pwrc.usgs.gov/bbs/bbs.html). Abundance values indicate the average number of hummingbirds seen in 2.5 hours of observation.

found only small halictid bees visiting flowers. Dr. Michael Powell observed that hummingbirds were rare in populations he knew to be dioecious in Texas (A. M. Powell, personal communication).

The hypothesis that variation in pollinator type produces variation in selfing rate rests on the assumption that pollinator types vary in amount of self-pollen they bring to each flower. There are several reasons why we believe this to be the case in this study. Bees require fewer floral visits per foraging bout at *E. coccineus* flowers due to the abundant pollen (approximately 600 stamens; Hoffman 1992) and nectar rewards (35 mg sugar/flower/day, 10x the average hummingbird syndrome flower; Scobell 1999). Most visits by bees recorded in >500 hours of observing pollination of *E. coccineus* are visits within 1 flower or between a few flowers on one plant (Scobell, unpublished data). Only under conditions of severe pollen depletion at the end of flowering do small bees make more trips between plants. Conversely, since the daily energy expenditure of broad-tailed hummingbirds is approximately 23.3 kJ/day, they have to visit >40 flowers per day to meet their energy requirements (Montgomerie and Gass 1981). In his observations on pollination of *Delphinium nelsonii* in Colorado, Waser (1982) found that broad-tailed hummingbirds carried pollen 50-150% longer distances between plants than halictid bees. He estimated that number of plants in the genetic neighborhood (Wright 1969) would increase nearly eight-fold if broad-tailed hummingbirds were the sole pollinator compared to halictid bees (Waser 1982).

Geographic Information System Mapping and Database Compilation

Data on hermaphroditic and dimorphic populations were compiled into a database of over 300 herbarium records. Plants were considered to be from the same population if they were within 1 Km of each other, producing a sample size of 108 populations. All populations were then mapped into a GIS data layer in ArcView 3.3. Populations represented by a herbarium specimen containing female flowers were considered dimorphic. If a flower containing pollen (male or hermaphrodite) was collected within a 30 km radius of a female flower the population of origin of this flower was considered dimorphic as well. These assumptions about the mating system of populations may be overestimating dioecy, but they at least represent populations where selection for dioecy has begun. All herbarium specimen flowers containing pollen that were outside of this 30 km range were considered to come from hermaphroditic populations. These data were coded as 0 = hermaphroditic and 1 = dimorphic. The data layer created by this process was overlain with a data layer of maps of distribution and abundance of broad-tailed and black-chinned hummingbirds. These are the two main species that are present when *E. coccineus* populations are in bloom. These maps are available from the Audubon Society Breeding Bird Survey Database (http://www.mbr-pwrc.usgs.gov/bbs/bbs.html). Mean annual rainfall, in cm, was obtained for each population from the USGS National Atlas Database (http://www.nationalatlas.gov/prismm.html).

Structural Equation Modeling

In order to thoroughly test the hypotheses, associations among the data obtained from the GIS were analyzed using structural equation modeling (SEM). SEM allows for comparison of different causal models (figure 2) using chi-square goodness of fit tests as well as indices such as CFI (Bentler's comparative fit index) and TLI (Tucker-Lewis index). A non-significant chi-square result indicates that the hypothesized model is a good fit to the data (Hayduk 1987). The CFI and TLI fit indices should be over 0.9 as a "rule of thumb" to prevent Type II errors (Bentler and Bonnett 1980). Analyses were carried out with Mplus (Muthen 2002), a free software package that is capable of analyzing a Structural Equation Model that contains categorical dependent variables such as dioecy versus hermaphroditism.

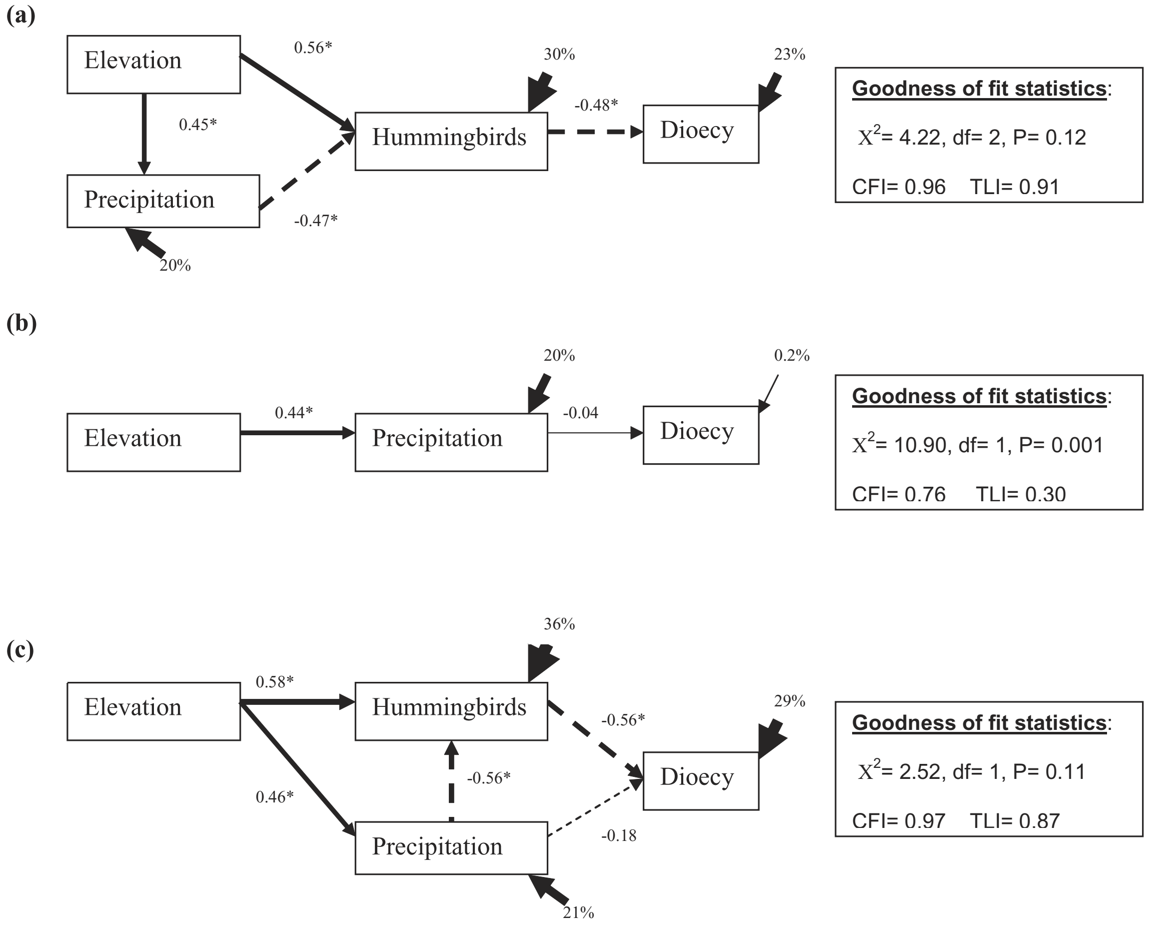

Figure 2—Alternative Structural Models for factors affecting the evolution of dioecy in *E. coccineus*. Numbers indicate value of path coefficients which are the standardized partial regression coefficient for the variables connected by the arrows. Each value represents the independent effect of the variable at the base of the arrow on the variable at the tip of the arrow when all other variables are held constant. Positive paths are shown with solid arrows, negative paths with dashed arrows. Arrow widths are proportional to magnitude of the path coefficients. Asterisks denote paths that are significantly different from zero. Arrows not originating in a variable indicate the amount of variation not explained by the variables in the model. (a) Hypothesis 1: Evolution of dioecy is pollinator-driven. (b) Hypothesis 2: Evolution of dioecy is resource-driven. (c) Hypothesis 3: Evolution of dioecy is driven by both pollinators and resources.

Results

The alternative causal models presented below (figure 2) represent three possible scenarios for how elevation, precipitation, and hummingbird abundance may be directly and indirectly influencing the evolution of dioecy in *E. coccineus*. In the first model (figure 2a) we are testing the hypothesis that only hummingbird abundance is directly affecting the evolution of dioecy in this species. Hummingbird abundance is directly affected by elevation and precipitation, which only indirectly influence the evolution of dioecy. This model is consistent with our data, and has the strongest support out of the three models ($\chi^2 = 4.22$, df = 2, P = 0.11, CFI = 0.962, TLI = 0.906). The negative value for the effect of hummingbirds on dioecy is consistent with the hypothesis that dioecy is associated with areas of lower hummingbird abundance. Elevation is only weakly influencing precipitation and hummingbird abundance. Surprisingly, precipitation is negatively affecting hummingbird abundance, which seems to contrast with the results of Cruden (1972). Possible reasons for this negative correlation will be covered in the discussion.

When this model is compared to the second model (figure 2b), it can be seen that a model with precipitation as the only direct cause of dioecy has a much poorer fit to the data ($\chi^2 = 10.90$, df = 1, P = 0.001, CFI = 0.832, TLI = 0.159). The significant chi-square value makes it clear that this model does not fit the data. The value of the path coefficient from precipitation to dioecy is also not significantly different from zero. This indicates that precipitation is a poor predictor of dioecy in this species.

The third possibility, that rainfall and hummingbirds are both directly influencing the evolution of dioecy, is supported by the data ($\chi^2 = 2.52$, df = 1, P = 0.11, CFI = 0.974, TLI = 0.871), but the path from precipitation to dioecy is not significantly different from zero (95% CI = –0.11 to 0.069). This indicates that the direct effect of precipitation on the occurrence of dioecy is negligible if it exists at all.

All three models have large amounts of the variation in dioecy left unexplained. Future work on this system will use exploratory SEM (Shipley 1997) to determine if including variables such as aspect, temperature, and geologic substrate would improve the explanatory power of these models.

Discussion

There are many advantages to using structural equation models to answer questions about causal relationships between interacting variables. If properly applied, this technique can be used to choose the model that best fits the data by comparing the differences in the model's chi-square statistics (Hayduk 1987). This is not the same thing as proving causality or stating that the selected model is the only causal model that explains the data, but causal models that do not explain the data can be rejected. This is an improvement over conventional path analysis. Using this technique, models can not be statistically tested but can be compared based only on the amount of the variation left unexplained by each model (Sokal and Rolf 1995).

In our case, we can reject model (b) (figure 2b), the hypothesis that rainfall is directly affecting the evolution of dioecy, based on the lack of fit of the model to the data. In this system it appears that a model that includes only direct influence of rainfall as a predictor of dioecy does not adequately explain the distribution of dioecious populations. The effect of rainfall on the presence of dioecious populations is not significantly different from zero. Rejection of this model suggests that Freeman's resource allocation hypothesis for the evolution of dioecy is incorrect for *E. coccineus* (Freeman et al. 1980).

It is interesting to compare models (a) and (c) which were both found to be consistent with the patterns in our data. In both models there is a strong negative effect of hummingbird abundance on the presence of dioecious populations. In other words, dioecious populations are more likely to be found in areas with low hummingbird abundance. When precipitation is included in the model as a direct effect, it has a very small effect (that is not significantly different from zero) in relation to hummingbird abundance. Even though the trend is as predicted by Freeman et al. (1980) with a negative association between precipitation and dioecy, the effect is negligible compared to the effect of hummingbirds.

The strong negative relationship between annual precipitation and hummingbird abundance is at first confusing, considering that both hummingbird abundance and precipitation are shown to increase slightly with elevation, and the GIS shows the highest abundance of hummingbirds at the highest elevations. However, since the data for rainfall and hummingbird abundance were gathered only in places where the cactus populations exist, the natural history of the cactus changes the distribution of the data. These cacti prefer to grow on the drier southeastern slopes of the Sky Islands. Therefore data were not gathered in this study for areas with precipitation levels higher than the tolerance level of this cactus. This leaves out areas that have both high precipitation and high hummingbird abundance.

The findings of these models support the hypothesis that pollinator behavior may be selecting for the evolution of dioecy in this system. To test this hypothesis further, experiments where plants were caged to produce bee pollinated and hummingbird + bee pollinated treatment groups were carried out in six populations in 2000-2003. Microsatellite data will be used to determine if bees produce higher selfing rates than hummingbirds.

The combination of GIS and SEM in this paper in conjunction with the incipient evolution of dioecy has allowed the first combined test of both the inbreeding avoidance hypothesis and the resource allocation hypothesis for the evolution of dioecy. Future work will focus on the effects of hummingbird pollination on gene flow within and between populations of *E. coccineus* across the Madrean Archipelago. The results of this research may have implications for the conservation of many plant species that depend on hummingbirds for pollination.

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