

1 Biotic simplification of plant mutualists and  
2 antagonists in agricultural landscapes

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4 March 3, 2012

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7 For submission to *Oecologia* as an Original Research Paper

8 Submitted X February 2012

# 1 Abstract

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## 2 Introduction

Anthropogenic disturbances, such as urbanization, fragmentation, and introduction of invasive species, can homogenize biotic communities by reducing the variation in species composition across locations. For example, urbanization has caused the homogenization of both plant and bird communities relative to natural areas [?]. Global agricultural intensification has also produced biotic homogenization. For example, in Europe, increased pesticide use has led to increased similarities in both bee and hemipteran communities relative to non-agricultural areas [?]. Croplands, pastures, and rangelands constituted 50 percent of the global vegetated land surface as of 2005 [?]. Thus, agriculture has the potential to significantly impact diversity of natural communities.

Given the fact that biotic communities are being altered in agricultural landscapes relative to pristine landscapes, what framework can be used to think about the mechanisms and consequences of this change? [?] provided a framework based loosely around spatial flows [cf ?] for understanding changes in predator communities in agricultural landscapes. Here, we propose a mutualist-antagonist (MA) framework to simultaneously understand the impacts of changes in biotic communities on crop and wild plants (Chamberlain and Burke, unpublished; Fig 1.). The MA framework predicts that abundance and community composition of mutualists and antagonists should be altered in different ways in agricultural landscapes. For example, farmers often manage crop plant antagonists by spraying pesticides and tilling soil, thereby altering abundance of plant antagonists near crops (REF). Whereas, farmers either do not purposefully alter plant mutualists, but do sometimes supplement mutualists in crop fields (REF).

What is the utility of an MA framework? Changing the relative abundances and community composition of mutualists and antagonists can have both ecological and evolutionary consequences. Simultaneous selection on the same trait, or (ecological pleiotropy), should be common for traits that both plant mutualists and antagonists use as cues [?]. For example, selection on floral traits is likely to exhibit greater spatial variability if there are

conflicting pressures from pollinators versus seed predators [e.g., ?]. In addition, conflicting selection pressures from mutualists and antagonists enhance phenotypic variation in natural populations relative to selection mediated by only one interaction type [??].

Here, we investigate how plant mutualists and antagonists are altered in agricultural landscapes using a study system involving crop sunflowers (*Helianthus annuus*), their wild sunflower relatives (*H.a. texanus*), and the mutualists (pollinators) and antagonists (seed predators) of both plant species. We ask the following two specific questions: 1) Do mutualists and antagonists differ in abundance near vs. far from sunflower crops?; and 2) Do mutualists and antagonists differ in community structure/diversity near vs. far from sunflower crops? We show that abundance and community structure of mutualists and antagonists are changed in nearly opposite ways, suggesting that an MA framework can be extremely useful in thinking about biotic homogeneity in agricultural landscapes.

## 3 Methods

### 3.1 Study system

Cultivated *Helianthus annuus* and its native congeners (sunflowers; Asteraceae) provide a highly tractable system for studying how agriculture alters the evolutionary trajectories of native species. First, native *Helianthus* commonly occur along the borders of sunflower crop fields [?]. Second, in sunflower growing regions in the US, crop and wild sunflowers can overlap for 5-6 mo. in flowering phenology (K. Whitney, pers. obs.), leading to high potential for shared pollinators (mutualists) and seed predators (antagonists) among crop and wild sunflowers. Texas hosts 20 native *Helianthus* species, many of which produce viable, hybrid offspring with crop sunflowers [??], a further indication of shared insect pollinators. Third, as Asteraceae have sporophytic self-incompatibility [?], self pollen grains do not germinate pollen tubes allowing for the quantification of outcross pollen grains deposited by pollinators. Finally, my target native species, *Helianthus annuus texanus*, is an annual, which is ideal for measuring lifetime fitness and selection in nature.

A diverse biotic community interacts with native and crop sunflowers. In general, the pollinator communities of both crop and wild sunflowers are

77 dominated by several hundred species of bees Hurd1980, with honey bees  
78 particularly prevalent in crop sunflowers ?]. Seed predators attack both na-  
79 tive and crop sunflowers, and their species-specific damage to sunflower seeds  
80 is easily quantified [?]. These biotic communities influence selection on sun-  
81 flower traits [?].

## 82 3.2 Study sites and design

83 In an observational study in 2009, at three naturally occurring populations  
84 of *H.a. texanus* near to (<10 m), and four populations far from (~ 2.5  
85 km) crop sunflowers, I marked with metal tags approximately 100 plants per  
86 population (Fig 1).

87 In experimental studies in 2010, we manipulated the proximity of *H. a.*  
88 *texanus* to crop sunflowers: Near [array of *H.a. texanus* <10 m from the crop]  
89 vs. far [array ~ 2.5 km from any sunflower crop, and near natural habitat].  
90 Plots were replicated at each of five farms in TX (Fig 1.). The proximity  
91 treatment was crossed factorially with a seed origin treatment (seeds from  
92 two wild populations collected in 2009; indicated in Fig. 1) to enhance the  
93 generality of results.

94 In 2011, we used the exact same design as 2010, but only used two of the  
95 five sites used in 2010 (Marek, Beakley).

## 96 3.3 Pollinators

97 We sampled pollinators using two methods: direct observations on our study  
98 sunflowers, and water bowl traps. In direct observations we observed up  
99 to 30 plants in each plot for 2-5 min per plant, over 4-6 observation periods  
100 during the flowering period (May-September). A pollinator visit was recorded  
101 when we observed a visitor making contact with anthers, stigmas, or both.  
102 Pollinators that could not be identified in the field were collected.

103 The water bowl trap method is the most common way to estimate polli-  
104 nator abundance, is the most efficient method to capture as much diversity  
105 as possible, and is the least prone to observer bias [?]. This method catches  
106 the subset of pollinators that visit *H.a. texanus*, as well as pollinators that  
107 do not visit this plant species; although, there are few other resources for  
108 pollinators besides crop sunflowers or the *H.a. texanus* plants in our plots.  
109 Thus, the direct observations are more relevant from the plants perspective.  
110 We set out three to six bowls, of three different colors (white, blue, yellow),

111 at each of two dates throughout the flowering period each year. We filled  
112 bowls with water and few drops of soap to break surface tension. Bowls  
113 were collected after 24 hrs, samples placed in 70% ethanol, and sorted to the  
114 lowest possible taxonomic level following [?]. Morphospecies were identified  
115 to the lowest taxonomic ranking following [??].

## 116 **3.4 Seed predators**

117 We quantified abundance of seed predators on all plants in each sunflower plot  
118 by putting net bags on three to six inflorescences per plant after pollination,  
119 but before shattering (seed drop) occurred, to allow ample time for seed  
120 predators to interact with the inflorescence. We collected these bagged heads  
121 at the end of the season, after seeds in inflorescences had matured, and  
122 plants had senesced. We pooled all inflorescences, and then sub-sampled  $\sim$   
123 80 seeds with x10 dissecting microscope to quantify species-specific damage.  
124 One damaged seed is assumed equivalent to one individual seed predator; so  
125 we take damaged seeds as equivalent to number of individual seed predators.

## 126 **3.5 Data analysis**

### 127 **3.5.1 Abundance**

128 We compared abundance of pollinators and seed predators separately using  
129 either ANOVAs (normality assumption met), or GLMs (normality not met).  
130 In all these models, we included as explanatory factors site, seed source,  
131 and proximity (near of far), and their interactions. We followed significant  
132 terms in the model with Tukey post-hoc tests (family-wise  $\alpha = 0.05$ ). Seed  
133 predators were modeled as XXX with a XXX error distribution.

### 134 **3.5.2 Richness and evenness**

135 We calculated richness by XXXXX. We calculated evenness using XXXX.  
136 To determine if differences in richness were due to differences in abundance  
137 between treatment levels, we generated sample-based rarefaction curves using  
138 each plot as a replicate with 1000 randomization runs using the vegan package  
139 [?]. We used Chao1 as the estimator of richness [?]. All analyses were done  
140 in R v.2.14 [?].

### 141 **3.5.3 Community structure**

142 We conducted non-linear multidimensional scaling analyses to collapse down  
143 pollinator species into a few axes to better visualize potential differences in  
144 pollinator communities among factors. We DETAILS OF NMS.

145 Furthermore, we tested for differences in pollinator community structure  
146 in a few different ways. First, we

## 147 **4 Results**

148 **4.1 Do mutualists and antagonists differ in abundance**  
149 **near vs. far from sunflower crops?**

150 **4.2 Do mutualists and antagonists differ in community**  
151 **structure/diversity near vs. far from sunflower**  
152 **crops?**

## 153 **5 Discussion**

## 154 **6 Conclusion**

## 155 **7 Acknowledgements**

## 156 References

157 **Figure captions**

158 Fig. 1. Diagram of the mutualist-antagonist framework for understanding  
159 evolutionary change for native plants in agricultural landscapes.

160 Fig. 2. Map of study sites in 2009, 2010, and 2011.

161 Fig. 3. Rarefaction curves for (a) pollinators and (b) seed predators.

162 Fig. 4. Abundance across three years for (a-c) pollinators and (d-f) seed  
163 predators.

164 Fig. 5. Non-metric multidimensional scaling ordination plots across three  
165 years for (a-c) pollinators and (d-f) seed predators.