

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

3 Scott Chamberlain  
Jennifer Rudgers  
Ken Whitney

4 December 14, 2011

5 Department of Ecology and Evolutionary Biology, Rice University,  
6 Houston, TX USA

7 For submission to Oecologia as an Original Research Paper

8 Submitted X February 2012

# 1 Abstract

write abstract here

## 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 McKinney (2006). 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 Dormann *et al.* (2007). Croplands, pastures, and rangelands constituted 50 percent of the global vegetated land surface as of 2005 (Foley *et al.*, 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? Rand *et al.* (2006) provided a framework based loosely around spatial flows (cf Polis *et al.*, 1997) 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simultaneously understand the impacts of changes in biotic communities on crop and wild plants (Chamberlain and Burke, unpublished; Fig 1.).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 (Strauss & Irwin, 2004).

43 For example, selection on floral traits is likely to exhibit greater spatial vari-  
44 ability if there are conflicting pressures from pollinators versus seed predators  
45 (e.g., Cariveau *et al.*, 2004). In addition, conflicting selection pressures from  
46 mutualists and antagonists enhance phenotypic variation in natural popula-  
47 tions relative to selection mediated by only one interaction type (Irwin *et al.*,  
48 2003; Siepielski & Benkman, 2010).

49 Here, we investigate how plant mutualists and antagonists are altered in  
50 agricultural landscapes using a study system involving crop sunflowers (*He-*  
51 *lianthus annuus*), their wild sunflower relatives (*H.a. texanus*), and the mu-  
52 tualists (pollinators) and antagonists (seed predators) of both plant species.  
53 We ask the following two specific questions: 1) Do mutualists and antag-  
54 onists differ in abundance near vs. far from sunflower crops?; and 2) Do  
55 mutualists and antagonists differ in community structure/diversity near vs.  
56 far from sunflower crops? We show that abundance and community structure  
57 of mutualists and antagonists are changed in nearly opposite ways, suggest-  
58 ing that an MA framework can be extremely useful in thinking about biotic  
59 homogeneity in agricultural landscapes.

## 60 3 Methods

### 61 3.1 Study system

62 Cultivated *Helianthus annuus* and its native congeners (sunflowers; Aster-  
63 aceae) provide a highly tractable system for studying how agriculture alters  
64 the evolutionary trajectories of native species. First, native *Helianthus* com-  
65 monly occur along the borders of sunflower crop fields (Burke *et al.*, 2002).  
66 Second, in sunflower growing regions in the US, crop and wild sunflowers can  
67 overlap for 5-6 mo. in flowering phenology (K. Whitney, pers. obs.), leading  
68 to high potential for shared pollinators (mutualists) and seed predators (an-  
69 tagonists) among crop and wild sunflowers. Texas hosts 20 native *Helianthus*  
70 species, many of which produce viable, hybrid offspring with crop sunflowers  
71 (Whitton *et al.*, 1997; Linder *et al.*, 1998), a further indication of shared in-  
72 sect pollinators. Third, as Asteraceae have sporophytic self-incompatibility  
73 (Linder *et al.*, 1998), self pollen grains do not germinate pollen tubes allow-  
74 ing for the quantification of outcross pollen grains deposited by pollinators.  
75 Finally, my target native species, *Helianthus annuus texanus*, is an annual,  
76 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 dominated by several hundred species of bees Hurd1980, with honey bees particularly prevalent in crop sunflowers Greenleaf & Kremen (2006). Seed predators attack both native and crop sunflowers, and their species-specific damage to sunflower seeds is easily quantified (Whitney *et al.*, 2006). These biotic communities influence selection on sunflower traits (Whitney *et al.*, 2006).

## 3.2 Study sites and design

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

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

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

## 3.3 Pollinators

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

The water bowl trap method is the most common way to estimate pollinator abundance, is the most efficient method to capture as much diversity as possible, and is the least prone to observer bias (Westphal *et al.*, 2008). This method catches the subset of pollinators that visit *H.a. texanus*, as well as pollinators that do not visit this plant species; although, there are few

111 other resources for pollinators besides crop sunflowers or the *H.a. texanus*  
112 plants in our plots. Thus, the direct observations are more relevant from the  
113 plants perspective. We set out three to six bowls, of three different colors  
114 (white, blue, yellow), at each of two dates throughout the flowering period  
115 each year. We filled bowls with water and few drops of soap to break surface  
116 tension. Bowls were collected after 24 hrs, samples placed in 70% ethanol,  
117 and sorted to the lowest possible taxonomic level following Michener (2000).

### 118 **3.4 Seed predators**

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

### 128 **3.5 Data analysis**

#### 129 **3.5.1 Abundance**

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

#### 136 **3.5.2 Richness and evenness**

137 We calculated richness by XXXXX. We calculated evenness using XXXX.  
138 To determine if differences in richness were due to differences in abundance  
139 between treatment levels, we generated sample-based rarefaction curves using  
140 each plot as a replicate with 1000 randomization runs using the vegan package  
141 (Oksanen *et al.*, 2010). We used Chao1 as the estimator of richness (Chao,  
142 1984). All analyses were done in R v.2.14 (Team, 2008).

### 143 **3.5.3 Community structure**

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

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

## 149 **4 Results**

150 **4.1 Do mutualists and antagonists differ in abundance**  
151 **near vs. far from sunflower crops?**

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

## 155 **5 Discussion**

## 156 **6 Conclusion**

## 157 **7 Acknowledgements**

## References

- Burke, J. M., Gardner, K. A. & Rieseberg, L. H. (2002). The potential for gene flow between cultivated and wild sunflower (*Helianthus annuus*) in the United States. *American journal of botany*, 89, 1550–1552.
- Cariveau, D., Irwin, R. E., Brody, A. K., Garcia-mayeya, L. S., Ohe, A. V. D. & Ohe, V. D. (2004). Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos*, 1, 15–26.
- Chao, A. (1984). Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics*, 11, 265–270.
- Dormann, C. F., Schweiger, O., Augenstein, I., Bailey, D., Billeter, R., de Blust, G., DeFilippi, R., Frenzel, M., Hendrickx, F. & Herzog, F. (2007). Effects of landscape structure and land-use intensity on similarity of plant and animal communities. *Global Ecology and Biogeography*, 16, 774–787.
- Foley, J. a., Defries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. a., Kucharik, C. J., Monfreda, C., Patz, J. a., Prentice, I. C., Ramankutty, N. & Snyder, P. K. (2005). Global consequences of land use. *Science (New York, N.Y.)*, 309, 570–4.
- Greenleaf, S. S. & Kremen, C. (2006). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 13890–13895.
- Irwin, R. E., Strauss, S. Y., Storz, S., Emerson, A. & Guibert, G. (2003). The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology*, 84, 1733–1743.
- Linder, C. R., Taha, I., Seiler, G. J., Snow, A. A. & Rieseberg, L. H. (1998). Long-term introgression of crop genes into wild sunflower populations. *Theoretical and Applied Genetics*, 96, 339–347.
- McKinney, M. L. (2006). Urbanization as a major cause of biotic homogenization. *Biological Conservation*, 127, 247–260.
- Michener, C. D. (2000). *The bees of the world*. Johns Hopkins Univ Pr, Baltimore, Maryland.

- 189 Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., O'Hara,  
190 R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H. & Wag-  
191 ner, H. (2010). `vegan`: Community Ecology Package. URL  
192 <http://cran.r-project.org/package=vegan>.
- 193 Polis, G. A., Anderson, W. B. & Holt, R. D. (1997). Toward an integration of  
194 landscape and food web ecology: the dynamics of spatially subsidized food  
195 webs. *Annual Review of Ecology and Systematics*, 28, 289–316.
- 196 Rand, T. A., Tylianakis, J. & Tscharntke, T. (2006). Spillover edge effects:  
197 the dispersal of agriculturally subsidized insect natural enemies into adja-  
198 cent natural habitats. *Ecology Letters*, 9, 603–614.
- 199 Siepielski, A. M. & Benkman, C. W. (2010). Conflicting selection from an  
200 antagonist and a mutualist enhances phenotypic variation in a plant. *Evo-*  
201 *lution*, 64, 1120–1128.
- 202 Strauss, S. Y. & Irwin, R. E. (2004). Ecological and evolutionary conse-  
203 quences of multispecies plant-animal interactions. *Annual Review of Ecol-*  
204 *ogy Evolution and Systematics*, 35, 435–466.
- 205 Team, R. D. C. (2008). R: A language and environment for statistical com-  
206 puting.
- 207 Westphal, C., Bommarco, R., Carré, G., Lamborn, E., Morison, N.,  
208 Petanidou, T., Potts, S. G., Roberts, S. P. M., Szentgyörgyi, H. &  
209 Tscheulin, T. (2008). Measuring bee diversity in different European habi-  
210 tats and biogeographical regions. *Ecological Monographs*, 78, 653–671.
- 211 Whitney, K. D., Randell, R. A. & Rieseberg, L. H. (2006). Adaptive intro-  
212 gression of herbivore resistance traits in the weedy sunflower *Helianthus*  
213 *annuus*. *The American Naturalist*, 167, 794–807.
- 214 Whitton, J., Wolf, D. E., Arias, D. M., Snow, A. A. & Rieseberg, L. H.  
215 (1997). The persistence of cultivar alleles in wild populations of sunflowers  
216 five generations after hybridization. *Theoretical and Applied Genetics*, 95,  
217 33–40.



218     **Figure captions** Fig. 1. Diagram of the mutualist-antagonist frame-  
219 work for understanding evolutionary change for native plants in agricultural  
220 landscapes.  
221     Fig. 2. Map of study sites in 2009, 2010, and 2011.  
222     Fig. 3. Rarefaction curves for (a) pollinators and (b) seed predators.  
223     Fig. 4. Abundance across three years for (a-c) pollinators and (d-f) seed  
224 predators.  
225     Fig. 5. Non-metric multidimensional scaling ordination plots across three  
226 years for (a-c) pollinators and (d-f) seed predators.