

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% 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.

Proximity to agriculture may lead to altered evolutionary trajectories for native species. For example, gene flow (e.g., crop-to-native or invasive-to-native) commonly influences the evolution of native species (Ellstrand *et al.*, 1999; Pilson & Prendeville, 2004). However, the homogenization of biotic communities associated with agriculture may also affect evolution of native species via other mechanisms that remain nearly unexplored. Altered natural selection due to anthropogenic homogenization is such a mechanism. In a natural landscape, abiotic and biotic heterogeneity produce spatially divergent patterns of natural selection, contributing to divergent evolutionary paths among populations. However, reduction of this natural heterogeneity could reduce natural geographic variation in evolutionary trajectories. A few recent studies provide suggestive evidence. For example, the presence of an invasive plant and a shared, invasive herbivore jointly altered natural selection on herbivore resistance traits in a native plant (Lau, 2006). Given that many, if not most, native species now occur in human-altered matrices, it is likely that the evolution of many species is affected by anthropogenic homogenization. ***However, no studies have experimentally examined the possible evolutionary consequences of landscape-level homogenization of biotic interactions, which requires experiments in multiple populations and a geographic perspective.***

There are multiple pathways by which anthropogenic alteration of biotic communities may affect natural selection on native species. A common finding in biotic homogenization studies is that rare species are lost, while common species persist (e.g., Olden *et al.*, 2004). Thus, both abundance and community composition are affected by anthropogenic disturbance, and these changes may influence natural selection independently, or in combination. For example, the abundance of insect herbivores on native plants could increase with the flow of crop-associated species from adjacent agricultural fields (e.g., McKone *et al.*, 2001), thereby selecting for increased herbivore resistance in native species. Alternatively, changes in community species composition could drive selection (review: Strauss & Irwin, 2004). For example, proximity to agriculture may reduce the diversity, but not the abundance, of pollinators of nearby native plants, altering selection on floral traits with reduced pollinator trait diversity.

It is important to tease apart the selective effects mediated by plant mutualists versus plant antagonists, as their abundance and community composition may be differentially affected by agriculture. 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). 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., Cariveau *et al.*, 2004). In addition, conflicting selection pressures from mutualists and antagonists enhance phenotypic variation in natural populations relative to selection mediated by only one interaction type (Irwin *et al.*, 2003; Siepielski & Benkman, 2010).

QUESTIONS FOR KEY IMPROVEMENTS

My dissertation research explores the influence of crop proximity on natural selection on floral traits of a related native plant. Specifically, by experimentally manipulating the proximity of a native sunflower species to crop sunflower fields, I am examining how natural selection on floral traits is altered across the landscape. I conducted observational and experimental studies in 2009 and 2010, respectively, and have found that crop proximity does alter natural selection on floral traits in the native species (see *Progress to Date*). The mechanisms underlying altered patterns of selection remain unclear. Recent research highlights the (previously unappreciated) importance of the joint effects of biotic interactions that have opposing ecological and evolutionary impacts on a focal species (i.e., mutualists vs. antagonists) (Morris *et al.*, 2007; Siepielski & Benkman, 2010). Few studies have tested how mutualists and antagonists jointly contribute to selection, and no studies have done so for traits of a native species in an agricultural landscape. ***Thus I propose to examine how both mutualists and antagonists contribute to natural selection on floral traits and thereby identify mechanisms that underlie the evolutionary impacts***

of anthropogenic homogenization in this system.

Q1. How does the proximity to crops affect the abundance and community composition of mutualists and antagonists of native plants?

Some current evidence suggests that plant mutualists, such as pollinators, can decline in abundance near crops (Ricketts *et al.*, 2008; Le Féon *et al.*, 2010). Alternatively, pollinator abundance could increase near crops when the crop requires pollinators for reproduction (Westphal *et al.*, 2003). Evidence for plant antagonists, such as herbivores, suggests that abundances on native plants can increase near crops (Elton, 1958; Chen & Welter, 2007). However, some plant antagonists can increase in abundance farther from crops, e.g. in the cases of specialized antagonists that only attack wild plants or species sensitive to pesticides used near crops (Navntoft *et al.*, 2009). Agriculture often results in fewer and more common species across the landscape, creating spatially homogenized communities (Olden *et al.*, 2004). For the sunflower system, in which both the crop and native species produce showy outcrossing flowers, I predict that pollinators will be more abundant near crops than far from crops, while seed predators will be less abundant near crops than far from crops (Table 1). For both plant mutualists and antagonists, I predict that community composition will be more diverse far from crops and homogenized near crops (Table 1).

Q2. How do crop-mediated changes in mutualists or antagonists affect selection on floral traits?

Limited abundance of pollinators leads to pollen limitation (Ashman *et al.*, 2004); pollen limited populations experience stronger selection on floral traits than populations not pollen limited (Table 1; Fishman & Willis, 2008). Unlike mutualists, for which

benefits to host plants saturate with increasing abundance, antagonists should produce stronger selection without saturation as abundances increase, leading to stronger selection on defense traits far from crops (Table 1). A pattern of decreased community heterogeneity in the agricultural landscape could lead to stronger selection near crops, as a simpler community may produce less diffuse selection compared to a more diverse community far from crops (Table 1). Finally, homogenous communities near crops will lead to less variable selection near crops relative to far from crops (Table 1).

Table 1. Hypotheses and predicted effect directions. See Fig. 5 for how Question 2 hypotheses will be examined.	
Hypothesis	Predicted effect near crops
Question 1	
Q1.1 Mutualist abundance	▲
Q1.2 Antagonist abundance	▼
Q1.3 Mutualist and antagonist species diversity	▼
Question 2	
Q2.1 Strength of mutualist-mediated selection	▼
Q2.2 Strength of antagonist-mediated selection	▼
Q2.3 Strength of selection with homogenized community	▲
Q2.4 Spatial variation in selection with homogenized community	▼
Q2.5 Confirming causation between agents and natural selection	na

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 (Burke *et al.*, 2002). Second, in sunflower growing regions in the US, crop and wild sunflowers can overlap for 5-6 mo. in flowering phenology (K. Whitney, pers. comm.), leading to high potential for shared pollinators (mutualists) and seed predators (antagonists). Texas hosts 20 native *Helianthus* species, many of which produce viable, hybrid offspring with crop sunflowers (Whitton *et al.*, 1997; Linder *et al.*, 1998), a further indication of shared insect pollinators. Third, as Asteraceae have sporophytic self-incompatibility (Richards, 1986), self pollen grains do not germinate pollen tubes, allowing for the quantification of outcross pollen grains deposited by pollinators (via germinated pollen tubes). 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 dominated by several hundred species of bees (Hurd *et al.*, 1980), 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) and respond to the presence of crop sunflowers (see *Progress to Date* below).

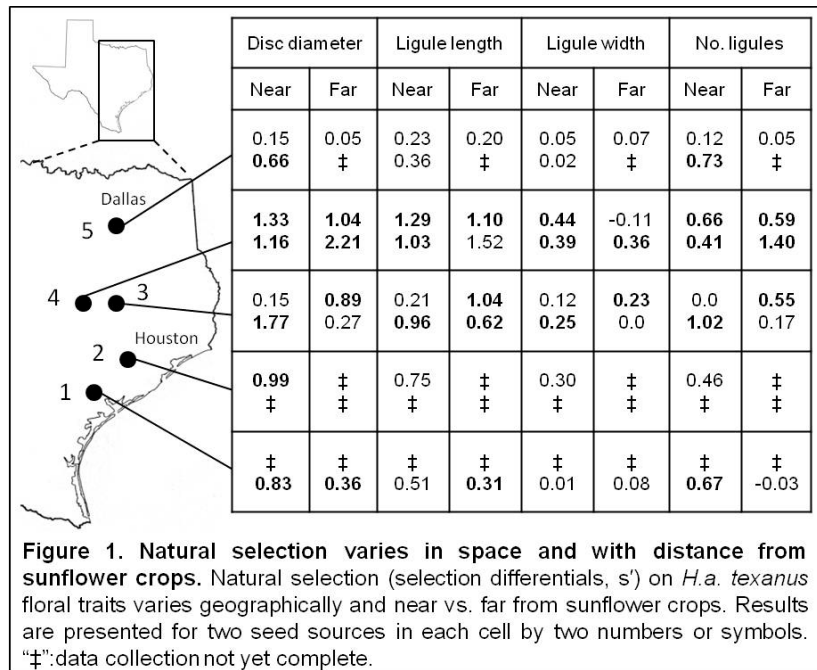
PROGRESS TO DATE

Natural selection on *H. a. texanus* floral traits varies in space and with distance to sunflower crops. 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 measured four floral traits (disc diameter, ligule length, ligule width, and number of ligules) and fitness for 100 plants/population. I looked at two metrics of natural selection: selection differentials, which represent total selection on a trait (regardless of correlations with other traits), and selection gradients, which represent direct selection on a trait (accounting for correlations with other traits). In phenotypic selection analyses for each site, some traits showed selection differentials that differed significantly from zero (s; e.g., disc diameter), and several traits showed significant selection gradients (β ; e.g., disc diameter, number of ligules). In a model combining all sites, selection gradients were spatially variable for number of ligules (Site*Trait, $P < 0.05$).

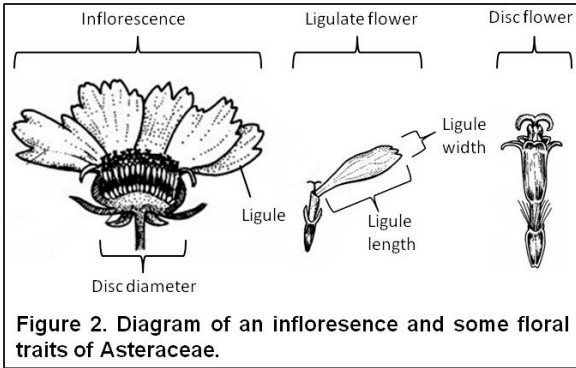
In 2010, I conducted an experiment manipulating the proximity of *H. a. texanus* to crop sunflowers: Near [array of *H. a. texanus* ≤ 15 m from the crop] vs. far [array ~2.5 km from any sunflower crop, and near natural habitat]. Plots were replicated at each of five farms in TX to examine spatial heterogeneity, with the prediction of greater spatial variation in selection among far plots than among near plots. The proximity treatment

was crossed factorially with a seed origin treatment (seeds from two wild populations) to enhance the generality of results. At Sites 1, 2 and 5, selection gradients did not differ by distance from crop, although many data are still being collected for these sites. At Site 3, selection gradients for disc diameter and number of ligules differed by distance from crop and seed source (ANCOVA; distance*seed source*trait, both $P < 0.02$), with consistently weaker selection on traits at far sites for one seed source, and the opposite pattern for the other seed source. This result suggests that effects of proximity to crops depend on the genetic composition of the native plant population. At Site 4, selection

gradients differed by distance from crop (ANCOVA; distance*trait, all $P < 0.03$) for ligule width and number of ligules, and showed an interaction between distance from crop and seed source (distance*seed source*trait, $F = 7.18$, $P = 0.008$) for ligule width. Analyzing each of the 15 plots separately, I found many statistically significant selection differentials and gradients, both near and far from crops (selection differentials presented in Fig. 1.). Thirty out of 56 (54%) selection differentials were statistically significant. Nine out of 56 (16%) selection gradients were statistically significant (data not presented). All significant differentials were positive, meaning floral traits are selected for larger trait values, regardless of site or distance from crop. Seventeen out of 32 (53%) and 13 out of 24 (54%) selection differentials were significant near and far from crops, respectively. Selection differentials were more variable far from crops (CV% = 107%) than near crops (80%), and selection gradients were also more variable far from crops (178%) than near crops (164%). Thus, preliminary studies indicate that **proximity to agriculture can alter natural selection on floral traits, selection on floral traits appears to vary in space, and selection may be more variable far from crops relative to near crops.**



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Floral traits are variable and heritable in a common garden. In 2010, I planted a common garden at one site to estimate variation in floral traits and broad-sense heritability. Heritability is important because response to selection (R) is a function of selection strength (s) and heritability (H^2) – strongly heritable traits increase response to selection. I planted 20-148 plants from each of nine populations of *H. a. texanus* ($N = 775$ plants). I measured floral traits (described above; Fig. 2.), stem diameter, and height on 1-4 inflorescences per plant. Floral traits varied significantly among source populations (MANOVA; Pillai's trace = 0.36, $F = 8.08$, $P < 0.001$),

and each trait independently varied significantly among source populations (ANOVAs: all $P < 0.001$). Broad-sense heritability (H^2) estimates the genotypic contribution to the phenotype (Falconer, 1989), i.e., the genotypic variance over the phenotypic variance (V_G/V_P), estimated here by a linear random-effects model. Heritabilities were disc diameter (0.22), ligule length (0.23), ligule width (0.14), no. of ligules (0.20), stem diameter (0.19), and height (0.40; for $H_0 = 0$, all $P < 0.0001$), suggesting substantial potential for evolution of these traits. As common garden studies cannot rule out maternal effects on phenotypic traits (Roach & Wulff, 1987), in 2011, I will use sire-offspring regression to calculate narrow-sense heritability (Lynch & Walsh, 1998). Nonzero heritabilities for floral traits confirm that the altered patterns of natural selection I am documenting will have evolutionary consequences.

IMPROVEMENTS

Q1.1-1.3. How does the proximity to crops affect the abundance and community composition of mutualists and antagonists of native plants?

Preliminary data.

Pollinator abundance can be greater near sunflower crops. In each of the 2009 naturally occurring populations, I recorded pollinator visitation (no. visits/plant/minute; 6 min/plant), and used water bowl traps (bowls with water that attract bees and other pollinators). I recorded a total of 57 pollinator morphospecies, most of which were bees. For bowl trap data, pollinator abundance was greater near (individuals per bowl; 2.5 ± 0.16) relative to far (0.6 ± 0.19) from crops (t -test: $P = 0.003$). Visitation rates were also greater near (visits per plant per minute; 0.24 ± 0.04) versus far (0.16 ± 0.04) from crops, but not significantly, and statistical power was limited (t -test; $P = 0.193$). In 2010, I observed pollinator visitation (no. visits/plant; 5 min/plant). Visitation rates were higher for near plots than far plots at some sites, but not at others (site*distance; $F = 2.91$, $P = 0.035$; Fig. 3).

Seed predator damage is greater far from sunflower crops. In 2010, I quantified seed predator damage for one seed predator species (*Isophrictis* sp.). Damage was ~5 times greater far from (± 1 s.e.; 0.89 ± 0.10 holes/receptacle) than near to sunflower crops (0.18 ± 0.03 ; randomization equivalent of two-way ANOVA, 10,000 iterations; $P < 0.001$). Damage also significantly differed among sites ($P < 0.001$), and the difference between far and near differed among sites ($P < 0.001$). I will examine abundances of additional species and community composition after additional data collection.

Pollinator community composition was more homogenous near crops. In observational data from

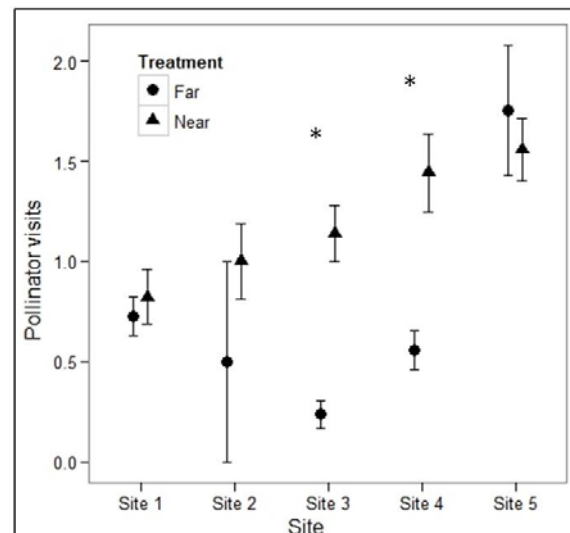


Figure 3. Pollinator visitation can be greater near sunflower crops. Visitation varied among sites ($F = 2.65$, $P = 0.049$), was greater near vs. far from crops ($F = 7.61$, $P = 0.009$), and the near vs. far effect differed among sites (Site x distance; $F = 2.91$, $P = 0.035$). Asterisks indicate that pollinator visitation differed Near vs Far from crops (paired t -tests).

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2009, pollinator communities visiting *H. a. texanus* significantly differed near versus far from sunflower crops (MRPP: $A = 0.12$, $P = 0.005$), but only marginally varied among the three geographic locations (MRPP: $A = 0.08$, $P = 0.074$). However, near versus far was not completely independent of region. In experimental data from 2010, pollinator communities diverged both among sites (ANOSIM [similar to MRPP]; $R = 0.71$, $P = 0.0007$) and near vs. far from sunflower crops ($R = 0.57$, $P = 0.012$). In accordance with expectation, pollinator communities were more homogenous near crops (PERMDISP, 9,999 iterations; mean distance to centroid: 0.41) relative to far from crops (0.52, $F = 7.55$, $P = 0.016$; Fig. 4).

Proposed methods. This experiment extends a similar 2010 experiment, allowing examination of temporal, in addition to spatial, variation in mutualists and antagonists. This additional year will allow more detailed species identifications. Furthermore, with funding, I will be able to collect two new sets of data (pollen deposition and bowl trap data, see below), adding essential components to the examination of pollinator visitation (Q1) and pollinator contribution to natural selection (Q2).

Experimental design. I will use a 2-way factorial experiment to manipulate distance from sunflower crops (near and far), and variation among sites ($n = 3$ of the same sites used in 2010). I will plant *H. a. texanus* both near (array of *H. a. texanus* ≤ 15 m from the crop) and far (array ~ 2.5 km from any sunflower crop, and near natural habitat) from sunflower crops. The far treatment is at 2.5 km because a recent review of foraging distances of bees states that 92.32% of bee foraging flights are less than 2.5 km ($n = 66$ species; Zurbuchen *et al.*, 2010); whereas far arrays placed beyond the maximum bee flight distance (14 km) would likely confound treatment and abiotic conditions. In each array, I will use 100 plants ($n = 600$ plants total). I will attempt to control for environmental heterogeneity within each site by placing near and far plant arrays in similar abiotic conditions. It is essential that this experiment be conducted at sites across the landscape to estimate the generality of the effects. I will install arrays in mid-April to overlap with sunflower crop phenology. Importantly, using the same 2010 sites in 2011 will allow me to determine temporal variation in mutualist and antagonist communities.

Pollinators. I will sample pollinators using two methods. First, during four visits throughout the flowering season I will identify all pollinator species, and quantify their per inflorescence visitation rates (5 min/plant) to 30 plants in all arrays. Second, I will sample pollinators with water bowl traps, using five water bowl traps in each array, and will trap for 24 h three times during the flowering period to avoid depleting pollinators. In addition, funding will allow me to process bowl trap data from 2010.

Pollen deposition. As pollen deposition is often correlated with visitation rate (Engel & Irwin, 2003), pollen deposition is included here as an additional estimate of pollinator visitation, but will also be used in analysis of pollinators' contribution to selection on floral traits (Q2.1-2.3). Self pollen grains do not germinate pollen tubes, thus a count of germinated pollen tubes estimates of the number of outcross pollen grains deposited by pollinators. Stigmas will be collected in the field, stained with aniline blue, squashed under a microscope slide, photographed with a digital camera, and pollen grains counted with Image J software (Rasband, 2009). I will collect ~ 10 stigmas per inflorescence on each of 10 inflorescences per plant, collected throughout the season. I will estimate pollen deposition per plant (avg. no. pollen grains/stigma * avg. no. flowers/inflorescence * no. inflorescences/plant).

Seed predators. I will quantify seed predator damage and species composition using species-specific damage marks on seeds following Whitney *et al.* (2006). These estimates will include the sunflower seed midge (*Neolasioptera helianthus*; Diptera: Cecidomyiidae), *Isophrictis* sp. (Lepidoptera: Gelechiidae), and the gray seed weevil (*Smicronyx soidus*; Coleoptera: Curculionidae).

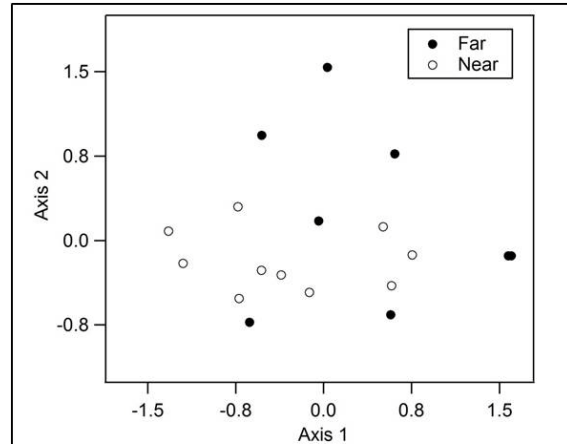


Figure 4. Pollinator communities are more homogenous Near relative to Far from crops (compare wider scatter of filled vs empty circles; PERMDISP: $F = 7.55$, $P = 0.016$). Two-dimensional non-metric multi-dimensional scaling results for pollinator visitation in 18 plots at five sites, both far from and near to sunflower crops. Stress = 0.13. Pollinator communities diverged among sites (ANOSIM: $R = 0.71$, $P = 0.0007$) and far vs. near from sunflower crops ($R = 0.57$, $P = 0.012$).

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Species community composition. Species community composition will be estimated using non metric multi-dimensional scaling (NMS) for each guild (pollinators, seed predators). Using abundances (also presence/absence) of species in each community, I will run NMS to generate two or three axes that describe pollinator and seed predator communities (Clark & Gorley, 2007). Axes from the ordination will also be used in structural equation models (described in Q2.1-2.3).

Data analyses. Abundance. I will test the hypothesis that the abundance of pollinators (visitation, bowl traps, pollen deposition) increases and seed predator attack decreases for *H.a. texanus* near crops (see Table 1; for those species occurring at all sites via ANOVAs, and for all species combined via MANOVA), with site (random effect) and proximity to sunflower crops (near vs. far, fixed effect) as explanatory factors.

Species community composition. I will ask if species composition of pollinator and seed predator communities significantly differs near versus far from crops (ANOSIM/ADONIS). I will determine which species contribute most to the difference (SIMPER analysis; Clark & Gorley, 2007). Finally, I will test for homogenization using permutational analysis of multivariate dispersions (Anderson, 2006), and a null model approach (Chase, 2007).

This experiment, together with 2010 data, will allow for spatial and temporal examination of variation in abundance and community composition of mutualists and antagonists near and far from sunflower crops (see Table 1). If we continue to find in 2011 that pollinators are more abundant, and seed predators less abundant, near crops, we will be relatively confident of the generality of the pattern in this system. Contrasting 2011 results from 2010 results will be equally interesting, suggesting spatial and/or temporal variation in mutualist and antagonist abundance. If we continue to find decreased diversity near crops, we will have increased confidence in our conclusion that sunflower crops decrease diversity of mutualists and antagonists in nearby wild populations.

Q2. How do crop-mediated changes in mutualists or antagonists affect selection on floral traits?

Preliminary data.

Pollinators. In 2010, when data from each plot were analyzed separately, pollinator visitation was significantly related to number of inflorescences in four plots, disc diameter in one plot, ligule width in three plots, and number of ligules in two plots (multiple regressions; all $P < 0.05$). This suggests that pollinator visits to flowers are not random, and that pollinators have potential to drive selection on floral traits in this system.

Seed predators. Whitney *et al.* (2006) found significant selection for resistance to *Isophrictis* (Lepidoptera) in *H. annuus*, showing that this species is an important antagonist in the system. Furthermore, in an *H. annuus* common garden in TX in 2003, *Isophrictis* damage was significantly negatively related to disc diameter (multiple regression; $t = -2.79$, $P = 0.024$), and significantly positively related to ligule length ($t = 2.96$, $P = 0.018$) and the number of ligules ($t = 3.01$, $P = 0.017$; K. Whitney, unpubl.data). Thus, seed predators may be important agents of selection on floral traits in *H. annuus*.

Q2.1-2.3. Mutualist- and antagonist-mediated selection effects.

Mutualists and antagonists are expected to have positive and negative effects, respectively, on their interaction partners. However, presumed mutualistic and antagonistic interactions can both be negative, neutral or positive in outcome in some sites or years,

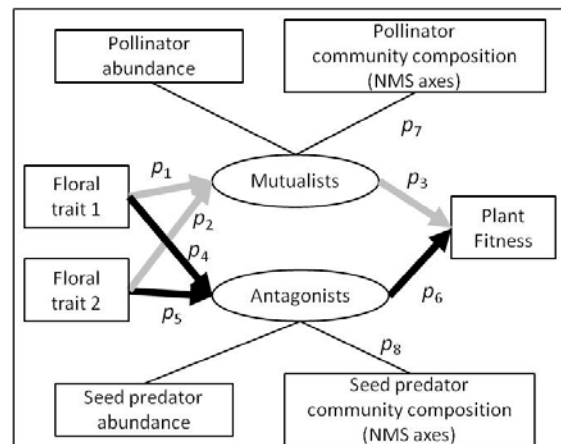


Figure 5. Relative strength of mutualist-mediated selection (grey arrows) vs. antagonist-mediated selection (black arrows) will be tested using structural equation models. I will construct the best model for each plot, both near and far from sunflower crops using data from 2010 and 2011, then compare paths between near and far plots within each site using multigroup analysis (Grace 2003, 2006). Rectangles are measured variables, and ovals are latent variables combining multiple measured variables. Path coefficients are represented by (p_n). Path coefficients are standardized partial regression coefficients, and are used to calculate direct and total selection. Although many floral traits will be measured, only two are shown.

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making their study non-trivial. In addition, a recent meta-analysis suggests that mutualists and antagonists together lead to drastically reduced performance of plants relative to mutualists alone (Morris *et al.*, 2007). This experiment examines interactive effects of both mutualists and antagonists on selection on wild sunflower traits, both near and far from sunflower crops.

Response variables. Using the experimental design described in Q1 at the three sites, I will measure four inflorescence traits (disc diameter, ligule width, ligule length, number of ligules; see Fig. 2.) and several individual floral traits (e.g., flower depth, flower width) on six inflorescences per plant throughout the flowering period. I will collect eight mature inflorescences per plant to estimate seed set. Lifetime fitness will be estimated as mean seeds per inflorescence * number inflorescences per plant. To correct for allometric relationships between number of inflorescences and plant size, I will measure plant volume (height * diameter at base) at the end of the season (Whitney *et al.*, 2006).

Data analyses. I will combine data from 2010 and 2011 on floral traits and plant fitness, and data describing abundance and community composition of pollinators and seed predators (collected in Q1), to examine relationships among putative biotic selective agents, floral traits, and plant fitness within structural equation models (SEM). I will examine the relative contributions of mutualists and antagonists to selection on floral traits using SEM (see Fig. 5.). First, I will construct saturated SE models (with all possible pathways) for each plot for each site including floral traits, the abundances of pollinators and seed predators, and NMS axes describing the community composition of pollinators and seed predators. Units of observation are individual plants. I will specify latent variables that combine abundance and community composition for each of mutualists (pollinators) and antagonists (seed predators). I will estimate the best-fitting SE models for each plot using the Akaike Information Criterion (AIC), then compare pathways between near and far plots within each site using multi-group analysis (Grace, 2003, 2006).

These analyses will test the relative strengths of mutualist- vs. antagonist-mediated selection, through both abundance and community composition pathways (see Table 1). Specifically, significantly weaker selection through mutualists (Fig. 5., path coefficients $p_1*p_3 + p_2*p_3$) near relative to far from crops would support the hypothesis that mutualist-mediated selection strength decreases near crops. In addition, weaker selection through antagonists (Fig. 5., $p_4*p_6 + p_5*p_6$) near relative to far from crops would support the hypothesis that antagonist-mediated selection strength decreases near crops. Significantly stronger selection through community composition (Fig. 5., p_7*p_3 or p_8*p_6) near relative to far from crops would support my hypothesis that homogenized communities lead to increased selection strength near crops.

Q2.4. Decreased variation in selection near crops.

In many studies (Dormann *et al.*, 2007), including this one (see Fig. 4.), biotic communities are more homogenous near relative to far from crops. Thus, spatial variation in natural selection on wild species within agricultural landscapes should diminish compared to wild landscapes (Table 1).

I will use phenotypic selection analysis (Lande & Arnold, 1983) to calculate selection gradients and selection coefficients to combine with 2010 results (see *Progress To Date*). Using general linear mixed models, I will test whether the relationship between floral traits and plant relative fitness differ by distance treatment (near or far, fixed), farm (random), and year (2010 or 2011, fixed). I will include all floral traits in the model (standardized to ± 1 SD). Using these selection gradients (β 's) and selection differentials (s 's), I will ask if gradients (β) or differentials (s) are more heterogeneous far from crops relative to near crops using F -tests (Table 1); I expect homogeneity to be greater near crops. If the distribution of gradients or differentials is not normal, I will use a distribution-free randomization test with 10,000 iterations (Edgington, 1987; Manly, 1991). I will explore weighting β 's with their error estimates to account for differences in final sample size among the experimental plots.

If selection gradients are more similar across near treatment replicates than across far treatment replicates, the hypothesis that agriculturally-mediated biotic homogenization leads to reduced spatial variation in selection on wild species would be supported (Table 1).

Q2.5. Confirming causal links between selective agents and natural selection.

Preliminary evidence in this study thus far suggests mutualists and antagonists are agents of natural selection on sunflower floral traits. However, experimental manipulation is key to determining if pollinators and seed predators are the agents of natural selection in this system. In addition, this experiment will

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allow me to tease apart the separate causal effects of mutualists vs. antagonists on selection, as well as their interactive effects on selection (whereas, the above study at farm sites cannot infer causation).

Experimental design. At the Katy Prairie Conservancy (NE of Houston, TX), I will manipulate both pollinators (HP: hand pollination; PC: pollination control, or open pollination), and seed predators (B: buds bagged to prevent oviposition; SC: seed predation control, bags attached to stems to control for bag effects). Six times during the season, I will hand pollinate up to 15 inflorescences per plant (using pollen from 30 extra plants planted 10 m from each array), and tag 15 unmanipulated inflorescences on controls. As the timing of hand pollinations can influence pollination success due to stigma phenology (Neff & Simpson, 1990), all hand pollinations will occur in late morning following Rieseberg *et al.* (1995). As some *H. annuus* seed predators oviposit on buds prior to anthesis (Schneider, 1997), I will place net bags on all buds on each B treatment plant to reduce oviposition by seed predators. Bags will be removed at anthesis, either open- (PC) or hand-pollinated (HP), and then replaced to prevent seed predator access. Importantly, bags will be removed for the same period of time for each of B and SC treatments to reduce potential confounding effects of period of access to plants. I will use 200 plants for each of the four treatment combinations (HP*B, HP*SC, PC*B, PC*SC). Plants will be arranged in five blocks of 160 plants each ($n = 800$ plants). Plants will be randomly allocated to one of the four treatment combinations.

Response variables. I will measure the same responses above in Q2.1-2.3 on six inflorescences throughout the flowering period (see Fig. 2.). These traits are correlated with pollinator and seed predator visitation in this study (see Q2 *Preliminary Data* above).

Data analyses. I will test if selection gradients are affected in this experiment by using general linear mixed effects models, including the factors of pollinators (fixed), seed predators (fixed), and floral traits (continuous). Importantly, interactions between floral traits and pollinators or seed predators, or their three-way interaction (pollinators*seed predators*trait), will be consistent with pollinators, seed predators, or pollinators and seed predators acting as agents of selection on floral traits. I will calculate selection differentials as the covariance between relative fitness and each trait, and compare selection differentials among treatments.

INTELLECTUAL MERIT

Agriculture is one of the largest land uses globally, yet we have little understanding of how evolution of native species proceeds in agricultural landscapes. The proposed work is novel because no research has yet examined how natural selection on native plants is altered by the homogenization of biotic interactions at the landscape scale. The proposed multi-population approach will significantly advance our understanding of evolution in anthropogenically-disturbed landscapes. Importantly, the proposed research will determine whether changes in the abundance and community composition of mutualists and antagonists are the mechanisms influencing differential patterns of selection in natural versus agricultural landscapes. This research has potential to yield new insights into factors shaping the evolution of floral traits; such evolution is arguably one of the most important drivers of plant biodiversity and speciation (Johnson *et al.*, 1998) and a key influence on higher trophic level diversity (Michez *et al.*, 2008).

BROADER IMPACTS

I am using my dissertation to mentor a diverse set of young scientists. I have mentored nine students thus far (3 women, 1 Indian, 4 Asian, 2 Middle Eastern), and one high school student. I am also collaborating with an agricultural researcher from Texas A&M University to improve the transfer of knowledge to growers. Understanding the evolutionary consequences of agricultural intensification has the potential to influence land management practices, for example, by altering where and when crops are planted. Furthermore, my work will reveal how agriculture alters biotic interactions between native plants and their mutualists and antagonists, with potential applications in conservation.

Anticipated publications from the proposed research:

- 1) "Natural selection on traits of wild plants in agriculture landscapes"–(in progress)
- 2) "Agriculture-driven homogenization of pollinator and seed predator communities?"–Q1
- 3) "Mechanisms of altered natural selection on a wild plant in agricultural landscapes"–Q2
- 4) "Importance of pollinators vs. seed predators as agents of selection on wild sunflowers"–Q2

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