PROJECT SUMMARY

Intellectual Merit: Outcrossing sexual reproduction has intrigued evolutionary biologists for decades, for the individual that can produce offspring by self-fertilization or asexually has an immediate advantage over outcrossers. Mixed mating systems (with both self-fertilization and outcrossing) provide an excellent way to identify the selective advantage of both modes of reproduction. One form of mixed mating, androdioecy (males + hermaphrodites), is particularly interesting because theoretical treatments suggest such a mixture should be evolutionarily unstable. A recently discovered crustacean (*Eulimnadia texana*) exhibits a persistent form of androdioecy. In this branchiopod shrimp, hermaphrodites can choose between self-fertilizing vs. outcrossing with males. Males are in low proportions but are maintained in most populations.

Previous models of androdioecy have assumed that hermaphrodites can outcross with one another, which is not true in either *E. texana* or *Caenorhabditis elegans* (the two best known examples of androdioecy in animals). In response to this, Otto et al. (1993) modeled the mixed mating system of *E. texana* and found certain combinations of the model's four behavioral and life history parameters allow stable coexistence of both males and hermaphrodites.

In conducting a previous grant, we implemented an integrative approach to the study of androdioecy, using genetic, cellular, physiological, behavioral, and ecological studies to quantify the parameters of the Otto et al. (1993) model in two populations. Although these projects proved quite fruitful, many questions remain, prime among which are estimating these parameters from field populations and testing the model in the field. The current proposal is designed to address these questions. The proposed work is divided into two distinct sets of projects: (a) **Laboratory Tests**, in which a combination of behavioral and genetic studies will be used to further explore two of the model's parameters. The previous estimates of male mating success revealed two alternate mating tactics, which we plan to delineate with a series of lab experiments. Additionally, a recently discovered aspect of *E. texana*'s sex determining system suggests that our previous estimate of relative male longevity must be reevaluated to compare outcrossed males to hermaphrodites. (b) **Field Collections**, in which we will estimate relative male to hermaphrodite survival, inbreeding depression (relative fitness of shrimp of differing heterozygosity classes), and male mating success (paternity analyses using microsatellite DNA techniques) to estimate three of the four parameters of the Otto et al. (1993) model in natural habitats. Two sets of field data will be collected: data from naturally-filled ponds and from artificially-filled ponds which are filled using freshwater from water trucks. Both field sites are in the Jornada LTER site in New Mexico, and will be monitored over a 3-year period. Data collected from both the field and laboratory studies will be used to estimate the four model parameters of the Otto et al. (1993) model, which will in turn be used to test the ability of the model to predict the proportions of the sex types found in each pond. The results of these tests will provide a thoroughly integrative understanding of the maintenance of outcrossing in this system, which will further our understanding of mating system evolution in general.

Broader Impacts: Both PI's have strong commitments to student training. SCW has trained over 40 undergraduates (27 female, 1 minority), 2 high school students (1 female, 1 minority) and 11 graduate students (5 female) in 8 years. RJD has trained 7 undergraduates (2 female) and 1 graduate student (female) in 2 years. Both PI's are on projects with the College of Education at U of A, designed to bring "inquiry-based" science experiences to high school and undergraduate students in the Akron area.

The proposed research will fund 3 graduate and 2 undergraduate students. These students will gain training in modern genetic techniques, and 3 students per year will be enriched by field experiences in the deserts of the Southwest. All will experience the full range of scientific inquiry, from the inception of the ideas through the data collection, analysis, and writing phases. SCW has good success including undergraduates on publications (6 students on 7 papers in 5 years). The results of the previous NSF grants generated 11 publications in international journals, and we expect to continue this success with the currently proposed work.