United States Department of Agriculture

Research, Education, and Economics
Agricultural Research Service

June 9, 2008

Mr. Gary White, Chair
America Iris Society Scientific Advisory Committee
701 Old Cheney Rd.
Lincoln, NE 68512

Dear Mr. White:

This letter acknowledges agency support for Dr. Alan Meerow who is applying for a research grant from the American Iris Society. As Research Leader and Location Coordinator of this unit, I am the authorized approving officer for grant applications by our scientists.

Thank you for your consideration of Dr. Meerow's proposal.

Sincerely,

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Heterogeneity, ecological specialization and introgression in a large population of *Iris savannarum*

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RESEARCH PROSPECTUS

I. Introduction

Iris savannarum Small is the most common of three iris species that occur natively in Florida. It is a member of the section *Hexagonae*, a small complex of 3-4 species and numerous hybrid populations (Randolph, 1966; Arnold et al. 1990a, b; Arnold, 1993a). *I. savannarum* occurs mostly in open, freshwater swamps in Florida, Georgia and Alabama (Henderson, 2000), but it is in Florida where it achieves its broadest geographic range, occurring throughout the peninsula. While typically included in *I. hexagona*, our previous studies support Henderson's (2000) treatment of *I. hexagona* as a narrowly distributed species that is actually rare in Florida (Meerow et al., in prep.).

One of us (M.G.) has documented much broader variation in morphology and habitat than has been previously associated with this species. In particular, a cluster of unusual populations in Highlands County, Florida occur in much drier habitats than usually associated with the species. We refer to the floral phenotype associated with these populations as the "Highlands" type (Fig. 1), most easily characterized by the more slender and drooping falls. By contrast, more hydrophilic populations of *I. savannarum* have a strikingly different floral phenotype (Fig. 2) that we refer to as "coastal," as it is the dominant type of flower seen in the

wetland populations towards the Gulf coast of the Florida peninsula. The falls are broader and more spreading in the flowers of this phenotype. We hypothesize that where these two ecotypes come into secondary contact, hybridization is possible.

The Jacks Branch population of *I. savannarum* is located in Glades county in a long, broad slough punctuated by sandy, dry uplands, and consists of millions of ramets. It is one of 11 populations in south Florida that we recently investigated (Meerow et al., 2007), and possibly the largest in the Caloosahatchee drainage. Both phenotypes can be observed in Jacks Branch: the highlands type on the sandy uplands, and the coastal type in the wettest portion of the slough. We hypothesize that hybrids inhabit intermediate habitats.

Figure 1. "Highlands" phenotype of *Iris savannarum* from Jacks Branch slough.

Figure 2. "Coastal" phenotype of *Iris savannarum* from Jacks Branch slough.

The *Hexagonae* group of *Iris* have been recognized as a textbook case of introgressive hybridization since the classic work of Anderson (1949) summarized the findings of Viscosa (1935), Foster (1937) and Riley (1938, 1939), which over-turned Small and Alexander's (1931) unprecedented recognition of over 80 species in the Louisiana iris group. Arnold and his students and colleagues (Arnold 1992, 1993a, b; Arnold and Bennett 1993; Arnold et al. 1990a, b; Arnold et al. 1991; Arnold et al. 1993; Burke and Arnold 2001; Burke et

al. 2000a, b; Cruzan and Arnold 1994; Cruzan et al. 1994; Emms and Arnold 1997) have broadened this investigation on various fronts with molecular data and both in- and ex-situ

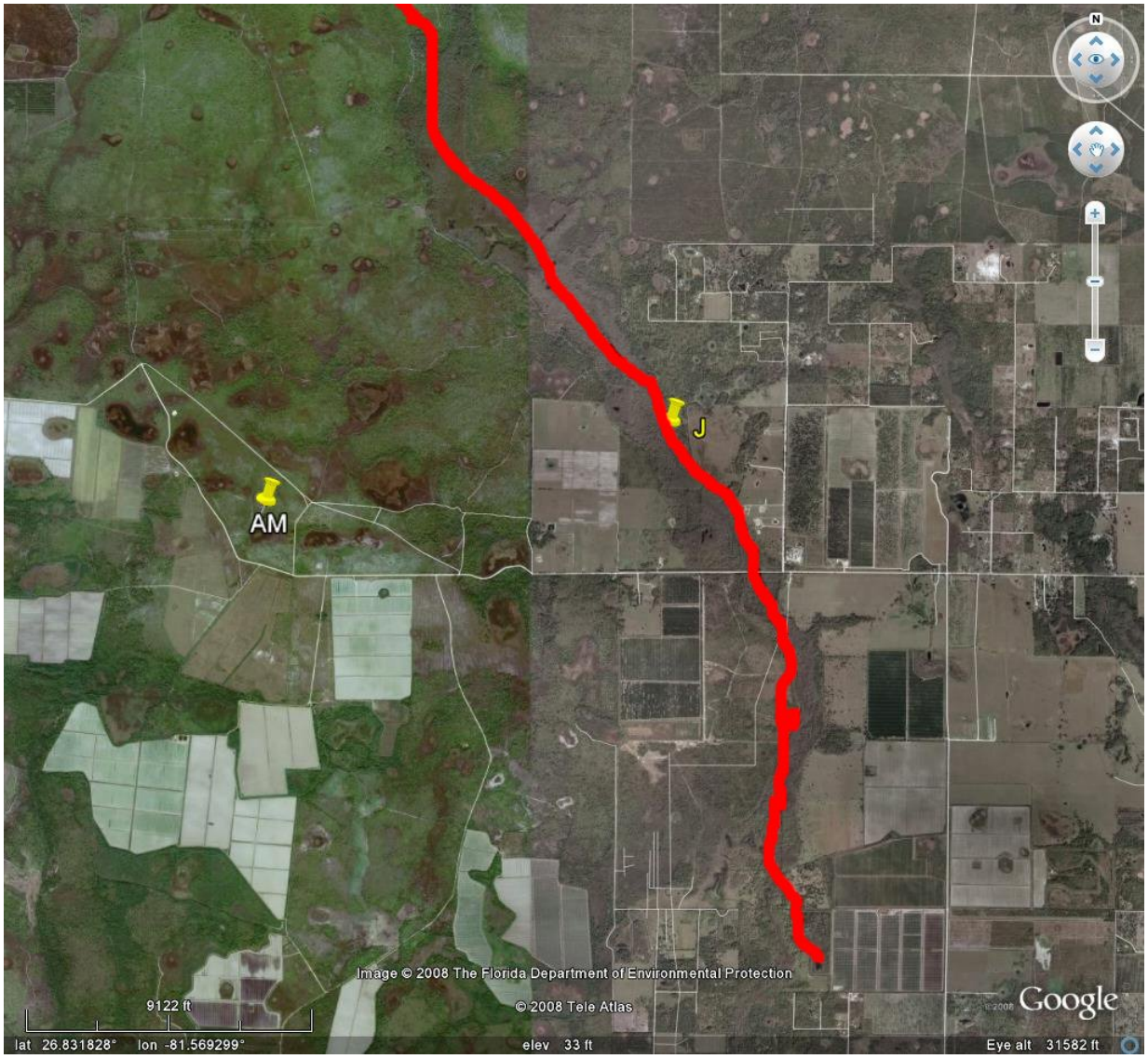

Figure 3. Jack Branch slough (in red), Glades Co., Florida.

experiments, not only confirming hypotheses concerning introgressive hybridization among Louisiana iris species, but using the group as a model of the processes involved in natural hybridization and evolution (Arnold 2000; Arnold et al. 2003).

These studies uncovered the unexpected phenomenon of low frequency of F_1 hybrid formation in nature when two Louisiana iris species are sympatric (Cruzan and Arnold 1993;

Arnold 2000) due to a number of reproductive barriers including phenology (Cruzan and Arnold, 1994, 1999), pollinator behavior (Burke et al., 2000b; Wesselingh and Arnold, 2000a, b; Emms and Arnold, 2000), assortative mating due to clonal reproduction (Burke et al., 2000a), and gamete competition Carney et al., 1994; Emms et al., 1996; Carney and Arnold, 1997). However, once the rare F_1 generation is formed, there occurs a great deal of introgressive hybridization between the hybrids and one or both of the parents (Arnold, 1994, 2000).

While the ecotypes in the Jacks Branch population do not represent different species, our hyothesis is that a similar pattern should be discernable. We propose to test these hypotheses using microsatellite DNA markers that we developed from the genomic DNA of *I. savannarum* (Meerow et al., 2005, 2007).

II. Materials and Methods

A. Sampling

Genomic DNA from 20 to 30 individuals from each ecotype in the Jacks Branch slough population (Fig 3) will be amplified with the 19 SSR primer pairs that have yielded allele polymorphisms in our previous studies.

B. Microsatellite isolation; DNA extraction, amplification, and visualization

SSRs were isolated and primers designed as described by Meerow et al. (2005), using a method modified from Edwards et al. (1996) with streptavidin coated beads (Dynal, Oslo, Norway) in conjunction with a Dynal Magnetic Particle Concentrator. DNA will be extracted and amplified with SSR primer pairs (the forward primer fluorescently labeled) as described in Meerow et al. (2005). Differences in allele size will be detected on an ABI 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA) using capillary gel electrophoresis as described

in Meerow et al. (2007). Preliminary analysis of raw microsatellite data was performed using Genemapper 4.0 (Applied Biosystems).

3. Data Analysis

Descriptive statistics (number of alleles per locus, A; number of alleles with frequency < 5%, A*f*; number of least common alleles, A*lc*; number of private alleles; total (H_t), observed (H_o), and expected heterozygosity (H_e) were generated with GenAlEx (Peakall and Smouse 2006). STRUCTURE 2.2 (Pritchard et al. 2000) which uses Monte Carlo Monaco Chain (Bayesian) methods to test the goodness of fit of each individual to their assigned population based on allele frequencies, will be used to identify admixture among ecotypes. Our strategy for the use of STRUCTURE is the Δk method of Evanno et al. (2005). Short runs of 50,000 iterations with a 10,000 iteration burn-in (these numbers are set after determining in preliminary runs how many iterations were necessary for stabilization of the log likelihood scores) will be replicated 20 times with k (the number of genetic populations) set from 1-5 using the admixture model with default settings and correlated allele frequencies (default values for alpha and lambda). Δk is defined as the mean of the absolute values of the second order rate of change of the log likelihood scores (LnP(D) in STRUCTURE; L(k) here) at each value of k (averaged over the 20 runs), divided by the standard deviation of the log likelihood scores, and is plotted in a spreadsheet program from the STRUCTURE output using the formula $\Delta k = m(|L(k+1)-2(L(k) + L(k-1)|)/s[L(k)]$. The optimal k value will be determined by plotting the values of Δk and observing at which value of k the highest modal value of Δk is observed. The optimal value of k will then used in replicated (5) final runs of 1,000,000 iterations (after a burn-in of 100,000). A histogram will then be generated using data from the STUCTURE output with DISTRUCT (Rosenberg 2004).

Genetic distance measures and unrooted neighbor-joining (Saitou and Nei 1994) trees with bootstrapping by locus will be generated with the program POPULATIONS v. 1.30.2 (Langella 2002). Four genetic distance measures that incorporate the IAM will be generated: Da (Nei et al. 1983), DAS (Jin and Chakraborty 1993), Dc (Cavalli-Sforza and Edwards (1967), and Cp (Prevosti 1974). The distance coefficient matrices will be used in principal coordinate analyses with GenAlEx. PCA plots will be generated in MS Excel (Microsoft Corp., Bellingham, WA). If our hypotheses are correct, we should see separation of the two ecotypes on the PCA plots with the introgressed hybrids in an intermediate position, but closer to one (or both) putative parental clusters.

IV. Facilities

All accessory equipment for DNA extraction, quantification, visualization, PCR amplification and successful cycle sequencing are available at the performance site, including temperature-controlled centrifuges. The SHRS has a high throughput molecular biology laboratory. Three Applied Biosystems 3100 16 capillary, and one 3730 96 capillary automatic DNA analyzers are fully available for use by this project. Four MJ Research tetrad gradient block thermocyclers are available on site. Two -80 degree C ultra freezers are on site for long-term DNA storage. A fulltime biologist with a MS in molecular biology is assigned to the PI's position. He is able to devote as much of his time to this project as required.

IV. Benefits to the American Iris Society

This research will further the understanding of the evolutionary processes at work in wild populations of Iris. This will have valuable application to conservation strategies for the species and for *Iris* breeders seeking genetically diverse germplasm. Results of our research will be reported in refereed scientific journals and in publishable reports provided to AIS. Primer

sequences will be deposited in the public database GenBank, maintained by the National Center for Biotechnology Information. AIS will be acknowledged in our scientific papers for its support.

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BUDGET

The cost of sample collection, DNA extraction, and genotyping of the Jacks Branch population is estimated at $10,000. Due to flat agency budgets, nearly this full amount is requested to complete the work.

Year One Budget

BIO 101 FastDNA preps, 100 @ $3.00 per sample ……..…….. $300

10 fluorescently-labeled primers for SSR visualization @ $70.00 each ……..……..... $700

Consumables for reactions (tubes, pipette tips, polymerase, other reagents) ……... $2900

Travel for sample collection …….. $500

USDA-ARS Indirect Costs (Overhead), 10% of total ……...……. $440

TOTAL $4840

Year Two Budget

Consumables for reactions (tubes, pipette tips, polymerase, other reagents) ……... $2700

Publication costs ……..…….…… $1050

USDA-ARS Indirect Costs (Overhead), 10% of total ……..…….……. $375

TOTAL $4125

TOTAL REQUEST $8965