Genetic markers for the Oncocyclus irises

Investigators:

Dr. Yuval Sapir, Indiana University

Dr. Amy Bouck, Duke University

Research subject:

Identification and development of molecular genetic markers for *Oncocyclus* irises. **Introduction**

The Royal Irises (*Iris* section *Oncocyclus*) comprise some 30+ species in the Middle-East, and are endangered due to habitat destruction throughout their distribution area. Especially in Israel there are certain threats on the survivorship of *Oncocyclus* populations. For example, a new village planned on the top of mount Gilboa in Northern Israel will damage thousands of plants of *Iris haynei*, and moreover, will half the large and dense population on the mountain (Kintisch, 2005). Another example is the coastal species

Iris atropurpurea, of which a third of the known populations have recently gone extinct, and the remaining populations are highly fragmented. The effect of future population fragmentation on population genetic structure and viability is not known. This may be assessed if genetic and statistical tools were available. We propose to develop a number of genetic markers that will be used to assess population genetic structure and diversity in order to both inform planning for the conservation of the unique *Oncocyclus* irises, and address basic questions concerning their evolution and ecology.

Oncocyclus species in Israel and Jordan have been the subject of taxonomic work based on morphological characters (Dykes, 1913; Mathew, 1989; Rix, 1997). Sapir et al (2002) analyzed floral and vegetative characters in all *Oncocyclus* iris species in Israel and Jordan. Much overlap was found among species in most diagnostic traits. Modern studies on the phylogeny of the Iridaceae family and the genus *Iris* have not investigated taxonomic relationships below the level of sections within the genus (Reeves *et al.*, 2001; Tillie *et al.*, 2001). Moreover, all the *Oncocyclus* species have the same number of chromosomes, and are able to hybridize (Avishai & Zohary, 1977, 1980), which further complicates species delimitation. It is unclear whether the current taxa merit designation as distinct species, and it is possible that the section is comprised of a hybrid swarm 2

without reproductive barriers (M. Arnold, Personal Comm.). In this era of molecular genetics, and given the low-cost methods for sequencing and analyzing DNA sequences, it is the time to resolve the phylogenetic mystery of the *Oncocyclus* irises.

Along the aridity gradient in Israel, flower, stem and leaf sizes were found to decrease towards the south, possibly as an adaptation to aridity (Arafeh *et al.*, 2002), but it is not known whether this adaptation is a plastic reaction of the plants or due to genetically determined local adaptation. A common-garden experiment in the Botanical Garden at the Hebrew University of Jerusalem is currently underway to investigate this (Y. Sapir, unpublished

data). There is a need to conduct a detailed QTL (Quantitative Trait Loci) study

in order to determine the genetic basis of inter-population variation in morphology. This kind of study requires a large number of reliable molecular markers that are scattered throughout the genome (Martin *et al.*, 2005). Such molecular markers currently do not exist for the *Oncocyclus* irises.

There have been few attempts to use molecular markers for the *Oncocyclus* irises in the recent past. One successful study used amplification of random DNA markers (RAPDS) to

study the genetic divergence of *Iris haynei* and *Iris atrofusca* populations along the North-

South aridity gradient in Israel (Arafeh *et al.*, 2002). This study found a fair amount of gene flow between two species, but the results were inconclusive. Other genetic work used

restriction enzymes for digesting chloroplast DNA (RFLPs), but proved unsuccessful in differentiating species. Another method (AFLP) completely failed in both *Oncocyclus* and Louisiana irises (H. P. Comes and O. Fragman, Personal comm.; A. Bouck, un-published data).

Here we propose to develop and test a series of genetic tools in order to establish reliable and repeatable genetic markers for the *Oncocyclus* irises. We will use three types of molecular markers: 1) transposon display markers (TDs); 2) simple sequence repeats (SSR; microsatellites); and 3) chloroplast DNA (cpDNA) sequences. The use of TDs was fruitful in Louisiana irises (Bouck *et al.*, 2005; Kentner *et al.*, 2003; Martin *et al.*, 2005) and used for QTL analyses of hybrids between *Iris fulva* and *Iris brevicaulis* (A. Bouck, *in*

prep). These markers can be used for QTL analyses and population genetics of the *Oncocyclus* irises. SSRs are sequences with a relatively high mutation rate (Hancock, 1999), and are used for many genetic analyses that use the identity of the individual 3

(Arnaud-Haond *et al.*, 2005; Austerlitz *et al.*, 2004) including analyses of hybridization and population genetics. Two SSR marker sets developed for irises in the past (Burke & Arnold, 1999; Meerow *et al.*, 2005), comprise just 15 loci in total. These markers were designed for species of other sections of the genus *Iris* and are not guaranteed to work in the *Oncocyclus* irises. Nonetheless, these markers will be tested as well. SSR markers are a powerful tool in ecological studies, enabling individual identification and analyses such as paternity analyses, population genetic structure and estimation of gene flow.

Chloroplast DNA was used for phylogeny of the sections in the genus (Wilson, 2004), and

we hope it will give an insight on the exact phylogeny of the section. Using the three types

of markers, we will obtain multi-level screening of the *Oncocyclus* genome, from large/medium elements of chromosomes, to short sequences and to polymorphism in a single nucleotide position.

Research Program

a. Transposon Display Markers

Transposable element markers (transposon display) have been widely used in plants for genetic mapping and other applications (Bouck *et al.*, 2005; Casa *et al.*, 2000; Ellis *et al.*, 1998; Jiang *et al.*, 2002; Manninen *et al.*, 2000; van den Broeck *et al.*, 1998; Waugh *et al.*, 1997). These markers tag DNA sequence variation around retrotransposon elements that are widely dispersed in plant genomes. Unlike DNA transposable elements, retrotransposon insertions are stable, making them useful as dominant genetic markers. Kentner et al (2003) published a characterization of an LTR retrotransposon family (called

IRRE) that is widely conserved throughout the genus *Iris*. We propose to use the method described in Kentner et al (2003) to clone and sequence *IRRE* retrotransposons from *Oncocyclus* irises, which will allow us to design a maker system for *Oncocylus* irises similar to the one described in Kentner et al (2003) for use in Louisiana Irises. Briefly, this

process will involve sequencing the 5-prime end of *IRRE* retrotranposon LTR motifs from *Oncocyclus* irises, and then using this information to design *Oncocylus*-specific transposon display primers. We will be able to make use of the *Iris* genus–specific degenerate primers published in Kentner at al (2003) to do this by simple PCR and 4

sequencing of *Oncocyclus* genomic DNAs. Sequences obtained will be aligned and clustered, and confirmed by homology to retrotransposon sequences in GenBank. Transposon display primers will be designed to the most conserved subgroups observed and subsequently tested and optimized in 3 species.

b. Microsatellites

In order to save time and to optimize the results, the first step of cloning enriched libraries with microsatellites will be done by external source (Vizon SciTec. Inc.; see appendix). The company supply 192 clones of DNA sequences rich with microsatellites elements. In the next step, we will test these sequences for polymorphism within and between species of Oncocyclus irises. We will sequence the clones in order to design primers for genotyping, and will test these primers for polymorphism on four individuals of each of three Oncocyclus species by genotyping with M13 designed primers (Schuelke,

2000).

c. DNA sequencing

Chloroplast DNA (cpDNA) was used in many biosystematic studies with restrictionsite digestion and gel-electrophoresis techniques (Demesure *et al.*, 1995) that found size differences based on size of the DNA fragments (e.g., Caujape-Castells *et al.*, 1999; Comes & Abbott, 1999; e.g., Jackson *et al.*, 1999). Given that the *Oncocyclus* irises did not show between-species variation (see above), we would like to take this a step ahead and to use the sequence of specific genes on the chloroplast genome (cpDNA), that proved

to bear high rate of mutations allows phylogenetic analyses (Graham *et al.*, 2005; Holderegger & Abbott, 2003; Kenicer *et al.*, 2005). A recent study on the phylogeny of the genus *Iris* that used cpDNA sequence showed that the section *Oncocyclus* is monophyletic, based on four taxa from Turkey and Georgia (Wilson, 2004). We will sequence two regions of the chloroplast genome (*trnL-matK* and *ycf4*) and will look for sequence variation in four individuals of each of three species.

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References

Arafeh, R.M.H., Sapir, Y., Shmida, A., Iraki, N., Fragman, O., & Comes, H.P. (2002) Patterns of

genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. *Oncocyclus* = the Royal Irises) along an environmental gradient in Israel and the West Bank. *Molecular Ecology*, **11**, 39-53.

Arnaud-Haond, S., Alberto, F., Teixeira, S., Procaccini, G., Serrao, E.A., & Duarte, C.M. (2005)

Assessing Genetic Diversity in Clonal Organisms: Low Diversity or Low Resolution? Combining Power and Cost Efficiency in Selecting Markers. *Journal of Heredity*, **96**, 434-440.

Austerlitz, F., Dick, C.W., Dutech, C., Klein, E.K., Oddou-Muratorio, S., Smouse, P.E., & Sork,

V.L. (2004) Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology*, **13**, 937-954.

Avishai, M. & Zohary, D. (1977) Chromosomes in the Oncocyclus Irises. *Botanical Gazette*, **138**,

502-511.

Avishai, M. & Zohary, D. (1980) Genetic affinities among the Oncocyclus Irises. *Botanical Gazette*, **141**, 107-115.

Bouck, A.C., Peeler, R., Arnold, M.L., & Wessler, S.R. (2005) Genetic mapping of species boundaries in Louisiana Irises using IRRE retrotransposon display markers. *Genetics*, **171**, 1289-1303.

Burke, J.M. & Arnold, M.L. (1999) Isolation and characterization of microsatellites in Iris. *Molecular Ecology*, **8**, 1075-1092.

Casa, A.M., Brouwer, C., Nagel, A., Wang, L.J., Zhang, Q., Kresovich, S., & Wessler, S.R. (2000)

The MITE family Heartbreaker (Hbr): Molecular markers in maize. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 10083-10089.

Caujape-Castells, J., Jansen, R.K., Pedrola-Monfort, J., & Membrives, N. (1999) Chloroplast DNA

restriction site phylogeny of the genus *Androcymbium* (Colchicaceae). *Systematic Botany*, **24**, 581-597.

Comes, H.P. & Abbott, R.J. (1999) Population genetic structure and gene flow across arid versus

mesic environments: a comparative study of two parapatric *Senecio* species from the Near East. *Evolution*, **53**, 36-54.

Demesure, B., Sodzi, N., & Petit, R.J. (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **4**, 129-131.

Dykes, W.R. (1913) The Genus Iris Dover Publications, New York.

Ellis, T.H.N., Poyser, S.J., Knox, M.R., Vershinin, A.V., & Ambrose, M.J. (1998) Polymorphism

of insertion sites of Ty1-copia class retrotransposons and its use for linkage and diversity analysis in pea. *Molecular and General Genetics*, **260**, 9-19.

Graham, S.A., Hall, J., Sytsma, K., & Shi, S.-h. (2005) Phylogenetic analysis of the Lythraceae

based on four gene regions and morphology. *International Journal of Plant Sciences*, **166**, 995-1017.

Hancock, J.M. (1999). Microsatellites and other simple sequences: genomic context and mutational mechanisms. In *Microsatellites - Evolution and Applications* (eds D.B. Goldstein & C. Schlötterer), pp. 1-9. Oxford University Press, New-York, NY.

Holderegger, R. & Abbott, R.J. (2003) Phylogeography of the Arctic-Alpine Saxifraga oppositifolia (Saxifragaceae) and some related taxa based on cpDNA and ITS sequence variation. *American Journal of Botany*, **90**, 931-936.

Jackson, H.D., Steane, D.A., Potts, B.M., & Vaillancourt, R.E. (1999) Chloroplast DNA evidence

for reticulate evolution in Eucalyptus (Myrtaceae). Molecular Ecology, 8, 739-751.

Jiang, N., Bao, Z., Temnykh, S., Cheng, Z., Jiang, J., Wing, R.A., McCouch, S.R., & Wessler, S.R.

(2002) Dasheng: A recently amplified nonautonomous long terminal repeat element that is a major component of pericentromeric regions in rice. *Genetics*, **161**, 1293-1305.

Kenicer, G.J., Kajita, T., Pennington, R.T., & Murata, J. (2005) Systematics and biogeography of

Lathyrus (Leguminosae) based on internal transcribed spacer and cpDNA sequence data. *American Journal of Botany*, **92**, 1199-1209.

Kentner, E.K., Arnold, M.L., & Wessler, S.R. (2003) Characterization of high-copy-number retrotransposons from the large genomes of the Louisiana Iris species and their use as molecular markers. *Genetics*, **164**, 685-697.

Kintisch, E. (2005) Israeli Controversy Blossoms Over Protecting Gilboa Iris. *Science*, **308**, 1251-.

Manninen, O., Kalendar, R., Robinson, J., & Schulman, A.H. (2000) Application of BARE-1 retrotransposon markers to the mapping of a major resistance gene for net blotch in barley. *Molecular and General Genetics*, **264**, 325-334.

Martin, N., H., Bouck, A.C., & Arnold, M.L. (2005) Loci affecting long-term hybrid survivorship

in Louisiana irises: implications for reproductive isolation and introgression. *Evolution*, **59**, 2116-2124.

Mathew, B. (1989) The Iris, 2 edn. Batsford, London.

Meerow, A.W., Gideon, M., Kuhn, D.N., & Schnell, R.J. (2005) Isolation and characterization of

10 microsatellite loci from *Iris hexagona* (Iridaceae). *Molecular Ecology Notes*, **5**, 410-412.

Reeves, G., Chase, M.W., Goldblatt, P., Rudall, P., Fay, M.F., Cox, A.V., Lejeune, B., & Souza-

Chies, T. (2001) Molecular systematics of Iridaceae: evidence from four plastid DNA regions. *American Journal of Botany*, **88**, 2074-2087.

Rix, M. (1997). Section Oncocyclus (Siemssen) Baker. In *A Guide to Species Irises* (ed The Species Group of the British Iris Society), pp. 62-90. Cambridge University Press.

Schuelke, M. (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature*

Biotechnology, **18**, 233-234.

Tillie, N., Chase, M.W., & Hall, T. (2001) Molecular studies in the genus *Iris* L.: a preliminary

study. International conference of Irises and Iridaceae: Biodiversity and Systematics, Rome, Italy. Annali Di Botanica Nuova Serie, 1, 105-112.

van den Broeck, D., Maes, T., Sauer, M., Zethof, J., De Keukeleire, P., D'Hauw, M., van Montagu,

M., & Gerats, T. (1998) Transposon display identifies individual transposable elements in high copy number lines. *Plant Journal*, **13**, 121-129.

Waugh, R., McLean, K., Flavell, A.J., Pearce, S.R., Kumar, A., Thomas, B.B.T., & Powell, W.

(1997) Genetic distribution of Bare-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). *Molecular and General Genetics*, **253**, 687-694.

Wilson, C.A. (2004) Phylogeny of Iris based on chloroplast matK gene and trnK intron sequence

data. *Molecular Phylogenetics and Evolution*, **33**, 402-412.

Schedule

March-April 2006 – collecting samples; DNA extractions

June-August 2006 - identification of TDs; sequencing cpDNA; design SSR primers;

August - October 2006 – testing SSR markers for polymorphism

October-December 2006 - preliminary phylogenetic analyses; report

Budget requested

DNA extractions \$150 Microsatellite clone libraries by Vizon

SciTec Inc. (see appendix)

\$5500

Genotyping with SSR primers \$600

cpDNA sequencing \$350

Transposon display marker development \$1500 Total \$8100

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Appendix – quote for microsatellite clone libraries

Microsatellite Clone Libraries

Vizon SciTec (formerly BC Research Inc.) has been providing enriched libraries and marker sets to academic, gov't and private organizations for the last 10 years. *Microsatellite clone libraries* are sets (n=192) of microsatellite genomic clones from your organism of interest. Each clone is provided as a purified glycerol culture in two 96 well format plates. Protocols for clone work-up are provided. Options include: larger clones set

libraries (e.g. for mapping), microsatellite cloning from cDNA libraries, from BAC clones or pools, custom simple sequence motifs, and organelle microsatellites.

Protocol

 Client provides genomic DNA (>0.5 μg) or tissue.
 Digestion of genomic DNA with multiple 4 cutter restriction enzymes and linker addition.
 Enrichment with biotinylated TCn, TGn, and GATAn simple sequence motifs (or specified).
 Cloning into Eco RI site of plasmid pUC19 and colony hybridisation with 32P labelled TCn, ACn and GATAn simple sequence motifs.
 Pick 192 primary positives and suspend in 96 well format plates for cryogenic storage (0.2 ml LB plus 15% glycerol).

Deliverables and Quality Control

Two 96 well microtitre plates (-80°C) containing single positive bacterial colonies in 0.2 ml LB glycerol.
Agarose gel analysis of 48 random clones after amplification with M13 primers to check insert size diversity.

• Enriched DNA preps, ligation reactions, (if requested) and protocols for clone workup.

Notes

Markers have been made successfully from low amounts (<0.5ug) of degraded genomic DNA. Depending on the micrcosatellite motif and species, clone insert size diversity ranges from 50-75%. We guarantee that >95% of the clones contain a microsatellite motif.

Costs: \$5,000 (US) plus applicable taxes and shipping

Contact: Dr. C. H. Newton Molecular Diagnostics, Vizon SciTec Inc. Tel: 604 224-4331, Fax 604 224 0540 Email: cnewton@vizonscitec.com

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Information on researchers

Yuval Sapir is a post-doctoral researcher at Indiana University. He received his PhD from the

Hebrew University of Jerusalem. His Masters and PhD research were on the taxonomy, distribution, ecology and conservation of Oncocyclus irises in Israel and Jordan. Especially his

research emphasized the pollination and reproduction system of the Oncocyclus irises. He found

that night-sheltering male bees are the sole pollinators of the Oncocyclus irises in the nature.

These insects are rewarded by the heat gained in the flowers at the first hour after sunrise. His

work on irises has been published in scientific journals, as well as in American Iris Society Bulletin,

and Aril Society International Yearbook. He is also active in conservation efforts concerning the

irises in Israel. Recently he has launched a genetic study on *Iris atrofusca* in order to determine

populations that are important for conservation. His hobbies are hiking with his children and flowers photography.

Amy Bouck's previous work used genetic mapping to investigate species boundaries and the

genetics of traits involved in speciation in the Louisiana Irises. Her work focused on the species

İris fulva and *I. brevicaulis*, wild irises native to the Mississippi river drainage which are known to

form hybrid zones in the southernmost extent of their range, often in conjunction with habitat

disturbance. This work investigated how the outcome of hybridization between these species

might be influenced by the genetic underpinnings of reproductive traits that distinguish them.

Genetic and QTL (Quantitative Trait Locus) mapping was used to 1.) investigate the extent to

which intrinsic genetic incompatibilities isolate these two species, and 2.) characterize the genetic

underpinnings of floral traits that confer specialization to different pollinators. Amy used retrotransposon-based genetic markers to construct separate genetic linkage maps of *I. fulva* and

I. brevicaulis, incorporating over 600 markers in total across both maps. Completion of genetic

maps of *I. fulva* and *I. brevicaulis* provided a means of examining the genomic distribution of

genetic incompatibilities isolating these species. Amy Bouck's work has also used genetic approaches to address how the formation of hybrid zones in natural plant populations may be

influenced by patterns of pollinator visitation. *Iris fulva* and *I. brevicaulis* possess distinctly different

suites of floral traits adapting them to hummingbird and bumblebee pollinators, respectively. Amy

genetically mapped QTLs (quantitative trait loci) underlying 10 divergent floral traits of *I. fulva* and

I. brevicaulis that are involved in specialization to these different pollinators. Some traits appeared

to have a simple genetic basis, as indicated by the presence of major QTLs. Overall, QTLs for

floral traits were more co-localized than would be expected by chance, indicating a genetic basis

for complex trait correlations. These correlations would help to facilitate the maintenance of *I*.

fulva's and *I. brevicaulis*' distinct floral characteristics, even in the face of gene flow due to hybridization.

Amy Bouck is currently a postdoctoral associate working in the field of plant genomics and bioinformatics, focusing on the plant genus *Mimulus*. She is interested in developing methods to

apply genomic data to research questions involving diverse wild species and plant evolutionary

biology and ecology.

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Yuval Sapir - Curriculum Vitae

Address: Dept. of Biology, Indiana University, Bloomington, IN 47405, USA. e-mail: ysapir@indiana.edu

Academic education:

2004 PhD, the Hebrew University of Jerusalem.

1998 MSc, the Hebrew University of Jerusalem.

1997 BSc, Inst. of Life Sciences, the Hebrew University of Jerusalem.

Positions:

Sept. 2004 – present: BARD Postdoctoral fellow, Dept. of Biology, Indiana University, Bloomington, USA.

Mar. – Sept. 2004: Postdoctoral research, Inst. of Plant Sciences, Faculty of Agricultural Sciences, The Hebrew University – Rehovot, Israel.

1996 - 2004: Teaching Assistant in the Hebrew University of Jerusalem, Israel

1996 – 2004: Coordinator of the Royal Irises Project, Rotem - Israel Plant Information Center.

1993 – 1998: Research assistant, Rotem – Israel Plant Information Center.

1991 – 1993: Nature guide and guidance instructor in field school, SPNI (Society for Protection

the Nature in Israel).

Awards:

1. 2005: AAAS membership, awarded by Science Program for Excellence in Science 2. 2003: Travel award, the New Phytologist symposium: Plant Speciation (\$600).

3. 1998: Henry Ford European Conservation Awards. 2nd prize in Israel for the Royal Irises Project.

Grants:

1. 2006: Israel Nature-Parks Authority. Research title: Creation of *in situ* gene bank and study of

population biology of existing populations of *Iris atrofusca*. (80,000回). With S. Volis (Ben-Gurion University) and M. Blecher (Nature-Parks Authority)

2. 2004 – 2006: Vaadia-BARD Postdoctoral Fellowship. Research title: Fitness and movement of

domestication traits across a sunflower wild-crop hybrid zone (\$75000).

3. 2002: American Iris Society Foundation. Research title: Speciation in the Oncocyclus Irises

(\$5260).

4. 1998: Israel Ecological Foundation, Israel. Research title: a. Inter and intra population variation

in the Royal Irises (section *Oncocyclus*) in Israel. b. Pollination ecology and reproduction biology of the Royal Irises (25000回).

Memberships: Aril Society International; the Society for the Study of Evolution; AAAS (membership awarded by Science Program for Excellence in Science) 11

Publications

Chapters in books

1. **Sapir**, **Y**., S. Mazer and C. Holzapfel. (in press) Population dynamics – Sex ratio. In: Encyclopedia of Ecology (ed. S. E. Jørgensen). Elsevier, Oxford.

Papers in peer-review journals

1. **Sapir, Y.**, A. Shmida and G. Ne'eman. 2006. Morning floral heat as reward to the pollinators

of the Oncocyclus irises. Oecologia. 147:53-59

Sapir, Y., Á. Shmida and G. Ne'eman. 2005. Pollination of the Oncocyclus irises (*Iris*: Iridaceae) by night-sheltering male bees. *Plant Biology*. 7(4):417-424 (+cover picture)
 Sapir, Y., A. Shmida and O. Fragman. 2003. Constructing Red Numbers for endangered plant species - Israeli flora as a test case. *Journal for Nature conservation* 11(2):91-108
 Shmida, A., O. Fragman, R. Nathan, Z. Shamir and Y. Sapir. 2002. The Red Plants of Israel:

a proposal of updated and revised list of plant species protected by the law. *Ecologia Mediterranea*. 28(1):55-64

5. **Sapir, Y**. and A. Shmida. 2002. Species concepts and ecogeographical divergence of *Oncocyclus* irises. *Israel Journal of Plant Sciences* 50:S119-S127

6. **Sapir, Y**., A. Shmida, O. Fragman and H. P. Comes. 2002. Morphological variation of the

Oncocyclus Irises in the southern Levant. *Botanical Journal of the Linnean Society*. 139: 369-

382

7. Arafeh, R. M. H., **Y. Sapir**, A. Shmida, N. Iraki, O. Fragman and H. P. Comes. 2002. Patterns

of genetic and phenotypic variation in *Iris haynei* and *I. atrofusca* (*Iris* sect. *Oncocyclus* = the

Royal Irises) along an environmental gradient in Israel and the West Bank. *Molecular Ecology*. 11: 39-53

8. **Sapir, Y.**, A. Shmida and H. P. Comes. 2001 *Iris bismarckiana* in Israel and Jordan - new findings and taxonomic remarks. *Israel Journal of Plant Sciences* 49(3):229-235 (+ cover picture)

Papers submitted or in preparation

1. **Sapir, Y.**, A. Shmida and G. Ne'eman. Size doesn't matter: night-sheltering preferences of solitary male bees and their possible role in iris floral evolution. Naturwissenschaften (accepted)

Conference Proceedings

1. **Sapir, Y.**, A. Shmida and U. Ritte. 2001. Population variation in the *Oncocyclus* irises along a

geographic gradient in Israel. *Proceedings of the International conference of Irises and Iridaceae: Biodiversity and Systematics. Rome, Italy. Annali Di Botanica* 1(2): 135-144 **Papers in un-refereed journals**

1. Frumkin, R., A. Shmida, **Y. Sapir**, O. Fragman-Sapir and N. Levin. 2004. Extinct plant species

of Israel. pp. 41-43 in: Frumkin, R., Khenin, D. and A. Idelman (eds.) Vital Signs 2003 – Israel

(WorldWatch Institute). Heschel Center, Tel-Aviv.

2. **Sapir, Y.** 2004. Mating system and pollination by night-sheltering male bees in *Oncocyclus*

irises. Aril Society International Yearbook 2003. pp. 11-17.

3. Sapir, Y. 2003. Evolutionary trends among Oncocyclus irises. Bulletin of the American Iris

Society October 2003. pp. 58-65.

4. **Sapir, Y.**, A. Shmida, O. Fragman, R. Nathan and N. Levin. 2002. The Red Plants of Israel.

Teva-Hadvarim November 2002 (in Hebrew).

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5. **Sapir, Y.** 2001. Pollination ecology of *Oncocyclus* Irises. *Aril Society International Yearbook*

2001. pp. 94-96.

6. **Sapir**, **Y.** and A. Shmida. 2001. *Oncocyclus* Irises of Israel: The Royal Irises Project. *Aril Society International Yearbook 2001*. pp. 69-72.

7. Shmida, A., **Y. Sapir**, H. Ben-David and G. Peli. 1999. *Iris bismarckiana* found in Jordan. *Teva-Hadvarim* 43 - May 1999 (in Hebrew).

Reports to the Israeli Nature Reserve Authority (in Hebrew)

1. Sapir, Y., M. Walzcak and M. Blecher. 2004. Results of damage to *Iris atrofusca* populations

in Goral Hills, Northern Negev.

2. Sapir, Y. and M. Walzcak. 2002. Distribution of Iris bismarckiana in the Galilee.

3. **Sapir, Y.**, M. Walzcak and Z. Kuler. 2001. Distribution of *Iris atropurpurea* in the Coastal Plain.

4. **Sapir, Y.** and D. Kaplan. 2000. Distribution of *Iris lortetii* in the Upper Galilee. **Published abstracts**

1. **Sapir, Y**., A. Shmida, Zalutsky M. and H. Dean. 2002. Criteria for evaluation of open areas for

conservation based on floristic data. *Proceeding of the 32nd annual meeting of the Israeli society for Ecology and Environmental Quality Sciences*. Tel-Aviv, Israel (In Hebrew).

2. **Sapir**, **Y**. 2001. Thermal energy as a reward to pollinators in nectarless *Oncocyclus* irises. *Israel Journal of Zoology* 47(2): 193

3. Katz M. and Y. Sapir. 1998. Diversity and distribution of molluscs in the coral reserve, Eilat.

Israel Journal of Zoology 44(1): 77

4. Danin, A. and **Y. Sapir**. 1997. The impact of bulbs digging by porcupine in east Judean desert

on Urginea maritima population dynamics. Proceeding of the 28th annual meeting of the Israeli

society for Ecology and Environmental Quality Sciences. Haifa, Israel (In Hebrew).

Services:

2000 – 2001: Rare Plants Survey in the Palestinian Authority – advisory committee.

2000 – 2004: Special advisor to Nature Parks Authority for Iris conservation.

2002: Red Data Book of the rare and endangered plant species of Israel – advisory committee.

Review for scientific journals: Israel Journal of Plant Sciences; New Phytologist; Plant Ecology;

International Journal of Plant Sciences; Canadian Journal of Botany.

Review for funding agencies: International Arid Lands Consortium; National Science Foundation

(USA) – population and evolutionary process cluster.

Teaching experience:

1996 – 2004 Teaching Assistant in the Hebrew University of Jerusalem.

2000 – 2004 Botany courses for guides of the SPNI.

Supervision of students:

2006 – Meghal Patel, undergraduate project: Conservation genetics of *Iris atrofusca*. 13

2005-2006 – Alan Richardson, undergraduate project: Pollinators' response to manipulations of

floral display size and reward.

2005-2006 – Amanda Spivey, undergraduate project: The genetic basis of nectar secretion in

sunflowers.

2002 – 2003 – Revital Kashi, M.Sc., Co-advisor: Prof. Avi Shmida: The role of floral color change

in pollination.

2001 -2002 – Noam Stern, 'Etgar' honored undergraduate project: *Iris* seeds dispersal by ants.

2000 - Revital Kashi, undergraduate project: Effect of slope aspect on floral and vegetative traits.

1998 – 1999 – Itamar Shahar and Adi Leist, high-school ecological project: Pollination ecology of

Iris bismarckiana.

Conferences presentations:

Y. Sapir and L. Hadany. 2005. Safe shelter, but no breakfast: Pollinator-mediated selection on

floral size of the Oncocyclus irises. (poster). Evolution 2005, University of Alaska, Fairbanks, USA.

Y. Sapir and L. H. Rieseberg. 2005. Selective sweeps in ecological speciation of a hybrid sunflower, *Helianthus anomalus*. (talk). Evolution 2005, University of Alaska, Fairbanks, USA.

Y. Sapir. 2004. Floral evolution of irises: pollinator-mediated selection for color and size. (talk). Evolution 2004, Colorado State University, Fort Collins, USA.

Y. Sapir and A. Shmida. 2003. In the course of speciation: ecogeographical divergence of *Oncocyclus* Irises. (poster). The 11th New Phytologist Symposium Plant Speciation, St. Francis Xavier University, Antigonish, Nova Scotia, Canada.

Y. Sapir and A. Shmida. 2003. In the course of speciation: ecogeographical divergence of *Oncocyclus* Irises. (talk). Evolution 2003, California State University, Chico, CA, USA.
 Y. Sapir, A. Shmida, M. Zalutsky and H. Dean. 2002. Criteria for evaluation of open areas

for conservation based on floristic data (talk). The 32nd annual meeting of the Israeli Society.

conservation based on floristic data. (talk). The 32nd annual meeting of the Israeli Society for

Ecology and Environmental Quality Sciences. Tel-Aviv, Israel.

Y. Sapir. 2000. Thermal energy as an optional reward in *Oncocyclus* Irises (poster). The Annual

Conference of the Israel Zoological Society, Beer-Sheva, Israel.

Y. Sapir, A. Shmida, H. P. Comes and N. Iraki. 1999. Dark-coloured *Oncocyclus* Irises in Israel:

Five species or one? (talk). Symposium Biodiversitat und Evolutionsbiologie. Jena, Germany.

 Y. Sapir, A. Shmida, H. P. Comes and N. Iraki. 1999. Royal Irises Project - Iris Section Oncocyclus (poster). Symposium Biodiversitat und Evolutionsbiologie. Jena, Germany.
 Y. Sapir and A. Shmida. 1999. Dark-Coloured Oncocyclus Irises in Israel: Five Species or One?

(poster). XVI International Botanical Congress, St. Louis, Missouri, USA.

A. Shmida and **Y. Sapir**. 1998. The *Oncocyclus* irises: lodging for male bees (poster) International

conference of Irises and Iridaceae: Biodiversity and Systematics. Rome, Italy.

Y. Sapir, A. Shmida and U. Ritte. 1998. Inter and intra population variation in the *Oncocyclus*

irises along a geographical gradient in Israel (talk). International conference of Irises and Iridaceae: Biodiversity and Systematics. Rome, Italy.

M. Katz and **Y. Sapir**. 1997. Diversity and distribution of molluscs in the corals reserve, Eilat (poster)The 19th Annual Conference of the Israel Zoological Society, Tel-Aviv, Israel.

AMY BOUCK, PHD

Postdoctoral Associate bouck@duke.edu Departments of Biology 919 660 7333 (Duke) Duke University and UNC Chapel Hill 919 962 4253 (UNC)

EDUCATION

2004 PhD, Genetics. The University of Georgia 1994 BS, Communication. Cornell University

PROFESSIONAL EXPERIENCE

Postdoctoral Scholar, laboratories of Todd Vision and John Willis. UNC Chapel Hill and Duke

University Biology Departments. 2/2005 - present

Genetics Research Associate, NOAA/National Marine Fisheries Service Southwest Center, Santa

Cruz Laboratory. 8/2004-1/2005

Graduate Student, laboratories of Susan R. Wessler and Michael L. Arnold (coadvisors), The

University of Georgia Department of Genetics. 1998-2004

Research Technician, laboratory of Sara Via, Cornell University Department of Entomology and

The University of Maryland Department of Biology. 1995-1996, 1997-1998

FELLOWSHIPS AND GRANTS

National Science Foundation Postdoctoral Fellowship in Biological Informatics. 2005-2007

The Graduate School of the University of Georgia Dissertation Completion Fellowship. 2003-2004

National Institute of Health Training Grant to the UGA Department of Genetics. Predoctoral

Research Assistantship. 1998-1999 and 2002-2003

National Science Foundation. QTL mapping of reproductive isolation in Louisiana Irises. 2000-2004.

Assisted in writing of grant under the supervision of principal investigator Michael L. Arnold

National Science Foundation Predoctoral Fellowship. 1999-2002

PUBLICATIONS

Bouck, A, R Peeler, ML Arnold and SR Wessler. 2005. Genetic mapping of species boundaries in

Louisiana irises using *IRRE* retrotransposon display markers. Genetics 171(3):1289-1303 Martin, NH, AC Bouck, ML Arnold. 2005. Loci affecting long-term hybrid survivorship in Louisiana

Irises: Implications for reproductive isolation and introgression. Evolution 59(10): 2116-2124

Martin, NH, A Bouck, ML Arnold. 2005. Detecting adaptive trait introgression between Iris fulva

and Iris brevicaulis in highly-selective field conditions. Genetics: in press.

Villesen, P, UG Mueller, TR Schultz, RMM Adams, AC Bouck. 2004. Evolution of antcultivar

switching in Apterostigma fungus-growing ants. Evolution 58(10): 2252-2265 Jones, FA, MF Poelchau, AC Bouck, SP Hubbell. 2004. Eight microsatellite markers for the

neotropical tree Luehea seemannii (Tiliaceae). Molecular Ecology Notes 4(1): 5-8

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(Publications, continued)

Arnold, ML, AC Bouck, RS Cornman. 2004. Verne Grant and Louisiana Irises: Is there anything new

under the sun? New Phytologist 161(1): 143-149

Arnold, ML, EK Kenter, JA Johnston, S Cornman, and AC Bouck. 2001. Natural hybridization and

fitness. TAXON 50(1): 93-104

Johnston, J, R Wesselingh, AC Bouck, LA Donovan, and ML Arnold. 2001. Intimately linked or

hardly speaking? The relationship between genotype and environmental gradients in a

Louisiana Iris hybrid population. Molecular Ecology 10(3): 673-681

Via, S, AC Bouck, and S Skillman. 2000. Reproductive isolation between divergent races of pea

aphids on two hosts. Il Selection against migrants and hybrids in the parental environment.

Evolution 54 (5): 1626-1637

MANUSCRIPTS IN PREPARATION

Bouck, A, and TJ Vision. ESTs in Molecular Ecology. *Invited review in prep*, Molecular Ecology

Bouck, A, R Peeler, N Schell, SR Wessler and ML Arnold. The genetic architecture of floral traits

associated with prezygotic reproductive isolation in Louisiana Irises. *In prep,* Genetics Bouck, A, SR Wessler and ML Arnold. QTL mapping reveals a case of heterospecific alleles

relieving inbreeding depression in Louisiana Irises. In prep, Heredity

Bouck, A, TJ Vision and J Willis. *Mimulus* microsatellite markers mined out of EST sequences. *In*

prep, Molecular Ecology Notes

Bouck, A, J Willis and TJ Vision. Past genome duplication events detected in *Mimulus* guttatus

using ESTs. In prep

PRESENTATIONS AT PROFESSIONAL MEETINGS

Bouck, A, J Willis, F Dietrich, TJ Vision. 2005. Using ESTs to detect past genome duplication events

in *Mimulus*. Talk presented at the Meeting of the Society for the Study of Evolution, Fairbanks, AK

Bouck, A, R Peeler, N Schell, SR Wessler and ML Arnold. 2004. QTL mapping of floral traits in

Louisiana Irises. Talk presented at the Meeting of the Society for the Study of Evolution, Fort

Collins, CO

Bouck, AC, R Peeler, SR Wessler and ML Arnold. 2004. Genetic interactions contribute to both

reproductive isolation and introgression in Louisiana Irises. Talk presented at the Southeastern Ecology and Evolution Conference, Atlanta, GA

Bouck, AC, E Kentner, R Peeler, N Schell, ML Arnold and SR Wessler. 2003. Genetic and QTL

mapping of reproductive isolation in Louisiana Irises. Talk presented at the Meeting of the

Society for the Study of Evolution, Chico, CA

Bouck, AC, E Kentner, R Peeler, ML Arnold and SR Wessler. 2003. Genetic mapping reveals both

positive and negative genetic interactions between hybridizing Louisiana Iris genomes.

Poster presented at the Meeting of the Society for the Study of Evolution, Chico, CA

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(Presentations, continued)

Bouck, AC, E Kentner, R Peeler, ML Arnold and SR Wessler. 2002. Genetic mapping of reproductive isolation in Louisiana Irises: segregation distortion due to heterospecific interactions. Talk presented at the Molecular Genetics and Ecology of Plant Adaptation

Conference, The University of British Columbia, Vancouver, BC

Bouck, AC, E Kentner, R Peeler, ML Arnold, and SR Wessler. 2002. Genetic mapping of reproductive isolation in Louisiana Irises. Poster presentation, SEEPAGE Meeting, Beaufort,

NC

Bouck, AC, R Koopman, E Morgan, R Peeler, and ML Arnold. 2001. Phenotypic diversity in traits

relevant to reproductive isolation in Louisiana Iris hybrids. Talk presented at the Meeting of

the Society for the Study of Evolution, Knoxville, TN

TEACHING AND **MENTORING EXPERIENCE**

Guest Lecturer for Evolutionary Biology (undergraduate level). Fall 2003, supervised by Dr. Rodney

Mauricio. Prepared and presented three lectures to a 200-student undergraduate class.

Guest Lecturer for Flowers (undergraduate level). Spring 2003, supervised by Dr. ShuMei Chang.

Prepared and presented lecture on plant hybridization, facilitated in-class discussion of genetic engineering and gene flow between crops and wild plants.

Teaching Assistant for Evolutionary Biology (undergraduate level). Fall 2000. Developed tests,

quizzes and homework problems, led a weekly discussion section of 50 students. Research supervisor. Provided training and supervision of research activities.

Spring 2001 University of Georgia undergraduate independent research students Ryan Peeler, Erinn Morgan and Rebecca Koopman

Spring 2003 Cedar Shoals High School intern Nicole Schell

PROFESSIONAL SERVICE

Reviewer for the National Science Foundation, Evolution and Evolutionary Ecology

OTHER TRAINING AND SKILLS

International Microarray Workshop. Laboratory of David Galbraith, the University of Arizona, January 2006

Introduction to Perl Programming for Biologists Workshop. Duke University Program in Genetics and Genomics, July 2005

BioPerl Programming Workshop. Duke University Program in Genetics and Genomics, August 2005

Courses in Quantitative Genetics and QTL Mapping. NC State Summer Institute In Statistical Genetics. June 1999

Basic competence in Perl, BioPerl, Unix, MySQL and R

Botanical photography published in Nature Reviews Genetics and Natural History