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Adaptive Divergence Versus Gene Flow in the Wild: Evaluation in Trinidadian Guppy Populations

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Final Report for Period: 04/2007 - 03/2008**Submitted on:** 06/25/2008**Principal Investigator:** Kinnison, Michael T.**Award ID:** 0235605**Organization:** University of Maine**Submitted By:**

Kinnison, Michael - Principal Investigator

Title:

Adaptive Divergence Versus Gene Flow in the Wild: Evaluation in Trinidadian Guppy Populations

Project Participants

Senior Personnel

Name: Kinnison, Michael**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Acted as primary investigator and administrator for this grant. Coordinated and co-supervised all field work, administrated funds, developed research resources and academic collaborations. Spent several months in Trinidad collecting guppies and performing mark-recapture studies of selection in low and high predation sites as well as gene flow manipulations. Continued to develop a guppy rearing facility and microsatellite genotyping capacity at U-Maine. Corresponded with Andrew Hendry, David Reznick and Paul Bentzen. Supervised field work by U-Maine graduate and undergraduate students as well as many of the students from other institutions (particularly McGill graduate students). Collaborated in analyses of clinal life history and microsatellite variation. Supervised professional and student staff on guppy facility maintenance and laboratory data collection. Supervised a professional webdesigner (Ronald Kozlowski) on development of an educational website. Co-authored publications deriving from this research program.

Name: Reznick, David**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Provided important logistical insights and support for research on Trinidadian guppies based on his decades of experience in this system. Helped coordinate and supervise collection of clinal data in Trinidad and develop sites and techniques for gene flow manipulations. Helped performed some mark-recapture studies. Advisor for Martin Turcotte, Ronald Brassar and Sonja Auer. Coordinated and supervised laboratory processing of guppies for life history data. Coauthored three related publications.

Name: Bentzen, Paul**Worked for more than 160 Hours:** No**Contribution to Project:**

Administrated and coordinated development of microsatellite loci for this research program, including cloning, primer development and optimization. Co-authored two publications, including a primer note in *Molecular Ecology* (in press as of January 2005) describing 10 novel tetranucleotide microsatellite loci. Genotyped ca. 2000 guppies for clinal analysis and field experiments. Supervised Ian Paterson, Kristy Brohman, Vicky Yaroshewski, Erika Crispo and Craig Blackie. This research was supported by a subcontract within our NSF budget.

Name: Hendry, Andrew**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Coordinated and supervised (with Mike Kinnison) clinal work described in the first section of the NSF proposal. Coordinated and supervised (with Mike Kinnison) other aspects of field work in Trinidad, including collecting habitat data and guppies, recording trait variation, and implementing mark-recapture studies. Coordinated and supervised the work of Nathan Millar (use of digital photographs to quantify colour pattern variation), Swanne Gordon (use of mark-recapture to quantify selective regimes in natural guppy populations), Amy Schwartz (mate choice in high and low predation guppies), Erika Crispo (use of microsatellites to infer gene flow), and Meghan Kelly (use of digital photographs to quantify body shape variation). Also supervised some undergraduate volunteers (e.g., Laura Easty, Zaki Zahavi). Co-authored related publications.

Post-doc

Graduate Student**Name:** Millar, Nathan**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Nathan Millar (MSc. McGill) Used digital photographs from 2002 to digitize and analyze colour pattern variation from all male guppies collected for the clinal work (N = 34 sites, n = 20 males from each site). His work examined how color variation is related to predation intensity and various habitat features, including spectral properties of the water, substrate size and colour, and distance from the ocean. Participated in field work in Trinidad: including collecting habitat data, collecting guppies, and taking digital pictures of guppies. This grant funded his field work and the collection of digital pictures for color analysis. Gave presentations at professional meetings. Wrote and published a paper for the journal *Oikos*: 'Disentangling the selective factors that act on male coloration in wild guppies.'

Name: Crispo, Erika**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Erika Crispo (MSc. McGill) measured morphological traits (mass, length, width, depth) and extracted DNA from guppies collected in one year of the clinal work (N = 34 sites, 20 males and 20 females per site). Analyzed microsatellite data for the clinal portion of this project. This grant funded her field work and the microsatellite genotyping. Gave a presentations on her research at several professional meetings. Has written and published a paper based on this work in *Molecular Ecology*.

Name: Weese, Dylan**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Dylan Weese (PhD. U-Maine) was responsible for several phases of the experiment in which natural selection was measured in the field and rates of gene flow were manipulated. To this end, he implemented multiple mark-recapture studies across a suite of sites from the two different predation regimes and quantified patterns of selection on size and color in the wild. This is the first time that patterns of selection have actually been directly measured on color in the guppy system. In 2005-2006 he performed our first manipulations of gene flow among a subset of these sites and a mate choice experiment in the wild. This grant funded all of Dylan's field work, tuition and salary as a Ph.D. student at the University of Maine. While in the field he supervised undergraduate and graduate student helpers. Dylan has presented his research at multiple professional meetings and is drafting several manuscripts. One manuscript is current in review at *Evolution*.

Name: Wilke, Nathan**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Nathan Wilke (MSc. U-Maine) Assisted in mark-recapture studies of guppies in Trinidad. He has since moved to Memorial University to compete a PhD. This grant supported his travel and field work in Trinidad.

Name: Lage, Christopher**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Christopher Lage (PhD. U-Maine) assisted in mark-recapture studies of guppies in Trinidad. Chris was a NSF K-12 sponsored PhD student who coordinated biology educational programs at K-12 schools in Maine, in addition to his graduate research. Involving him in our guppy research provided a valuable experience that he used to educate a spectrum of students on principles of evolution and tropical ecology. Chris has since obtained a faculty teaching position at the University of Maine at Augusta. This grant funded his travel and field work in Trinidad.

Name: Gordon, Swanne**Worked for more than 160 Hours:** Yes**Contribution to Project:**

Swanne Gordon (MSc. McGill) performed mark-recapture work to quantify selective environments in high- and low-predation sites on the north slope. Performed translocation experiments in Damier River that are part of contemporary evolution component of gene flow study. This grant funded her field work. Gave presentations on her research at multiple meetings and wrote a manuscript for *American Naturalist*.

Name: Bailey, Michael

Worked for more than 160 Hours: Yes

Contribution to Project:

Michael Bailey (PhD. U-Maine) PIs and Dylan Weese in field work involving manipulation experiments on gene flow. This grant supported his travel and field work in Trinidad.

Name: Schwartz, Amy

Worked for more than 160 Hours: Yes

Contribution to Project:

Amy Schwartz (MSc and PhD McGill) performed extensive laboratory work on guppy mate choice (as a mechanism related to the efficacy of gene flow). She also provided extensive field support during our gene flow manipulations in 2005 and 2006 and for our sexual selection experiment in 2006.

Name: Turcotte, Martin

Worked for more than 160 Hours: Yes

Contribution to Project:

Martin Turcotte (U.C. Riverside) assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006.

Name: Blackie, Craig

Worked for more than 160 Hours: Yes

Contribution to Project:

Craig Blackie (Dalhousie) assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006.

Name: Bassar, Ronald

Worked for more than 160 Hours: Yes

Contribution to Project:

Ronald Bassar (U.C. Riverside) assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006. He is now working on a guppy PhD project within our guppy FIBR grant.

Name: Auer, Sonja

Worked for more than 160 Hours: Yes

Contribution to Project:

Sonja Auer (U.C. Riverside) assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006. She is now working a PhD project within our guppy FIBR project.

Undergraduate Student

Name: Kelly, Meghan

Worked for more than 160 Hours: Yes

Contribution to Project:

Meghan Kelly (McGill) used digital photographs from 2002 and 2003 to digitize and perform geometric morphometric analyses on body shape of all male and female guppies collected for the clinal work ($N = 68$ collections [34 sites in each of 2 years], $n = 20$ males and 20 females from each collection). Analyzed how body shape variation is related to predation intensity and various habitat features, including spectral properties of the water, substrate size and colour, distance from the ocean. This grant supported the collection of photos used in her analyses. Participated in field work in Trinidad. She helped write and publish a paper based on this work in the Journal of Evolutionary Biology.

Name: Brohman, Kristy

Worked for more than 160 Hours: Yes

Contribution to Project:

Kristy Brohman (Dalhousie) worked on genotyping our guppies. She worked on this project full time May-August 2004, and part-time during the winter of 2005, for a total of ~ 800 hours during the second project year. This work was supported via subcontract to Paul Bentzen.

Name: Lynch, Christopher

Worked for more than 160 Hours: Yes

Contribution to Project:

Assisted in construction and subsequent maintenance of guppy rearing facility at University of Maine. Christopher was hired as an undergraduate technician supported by this NSF grant.

Name: Stevens, Emily

Worked for more than 160 Hours: Yes

Contribution to Project:

Emily Stevens (U-Maine) designed and implemented experiments on environment dependent mate choice in guppies. Mate choice is thought to be an important factor influencing gene flow. Her experiments also helped us to become familiar with guppy laboratory procedures before embarking on more expansive lab studies. This grant supported the laboratory resources required for her work.

Name: Hoffman, Christopher

Worked for more than 160 Hours: No

Contribution to Project:

Christopher Hoffman (U-Maine) assisted in care of guppies and maintenance of guppy facility at University of Maine. Student salary partly supported by this grant.

Name: Holbrook, Christopher

Worked for more than 160 Hours: Yes

Contribution to Project:

Christopher Holbrook (U-Maine) assisted in mark-recapture studies of guppies in Trinidad. This grant funded his travel expenses. He subsequently began and completed his own graduate program at U-Maine.

Name: Litz, Marissa

Worked for more than 160 Hours: No

Contribution to Project:

Merissa was a work-study student supported by this project. She assisted with care of guppies and with digitization of photos to quantify color variation. She has since gone on to graduate school.

Name: Yaroshewski, Vicky

Worked for more than 160 Hours: Yes

Contribution to Project:

Vicky Yaroshewski (Dalhousie) worked part-time (~10 hours/week) during Fall 2004 genotyping guppies, for a total of ~160 hours.

Name: Easty, Laura

Worked for more than 160 Hours: Yes

Contribution to Project:

Laura Easty (McGill) participated in field implementation of our gene flow experiments during the spring of 2005. This field work provided a valuable capstone to prior laboratory guppy work. Laura has now indicated a sincere desire to begin an academic career in research.

Name: Daniel, Moire

Worked for more than 160 Hours: Yes

Contribution to Project:

Moire Daniel (U-Maine) completed an independent (honors) research project looking at a boldness/shyness behavioral syndrome in high and low predation guppies.

Name: Libby, Brandon

Worked for more than 160 Hours: Yes

Contribution to Project:

Brandon Libby (U-Maine), assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006. He also completed an independent (honors) study project measuring selection on morphology of female guppies and presented his work at an undergraduate research expo (where he won second place for best poster).

Name: Gardner, Cory

Worked for more than 160 Hours: Yes

Contribution to Project:

Cory Gardner (U-Maine) assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006.

Name: Zahavi, Zaki

Worked for more than 160 Hours: Yes

Contribution to Project:

Zaki Zahavi (McGill) assisted with our long-term mark-recapture monitoring of the gene flow manipulation experiments in summer of 2006.

Name: Barton, Brittany

Worked for more than 160 Hours: No

Contribution to Project:

Brittany Barton (U-Maine) helped with care and rearing of guppies for quantitative genetic analyses (U-Maine Guppy Facility).

Name: McLellan, James

Worked for more than 160 Hours: Yes

Contribution to Project:

James McLellan (U-Maine) helped with care and rearing of guppies for quantitative genetic analyses (U-Maine Guppy Facility).

Name: McClintock, Maureen

Worked for more than 160 Hours: Yes

Contribution to Project:

Maureen McClintock (U-Maine) helped with care and rearing of guppies for quantitative genetic analyses (U-Maine Guppy Facility).

Name: Kierman, Mellissa

Worked for more than 160 Hours: No

Contribution to Project:

Mellissa Kierman (U-Maine) helped with care and rearing of guppies for quantitative genetic analyses (U-Maine Guppy Facility).

Name: Dacey, Jeffrey

Worked for more than 160 Hours: No

Contribution to Project:

Jeffrey Dacey (U-Maine) helped with care and rearing of guppies for quantitative genetic analyses (U-Maine Guppy Facility).

Technician, Programmer

Name: Paterson, Ian

Worked for more than 160 Hours: Yes

Contribution to Project:

Ian's has had primary responsibility for microsatellite development and optimization, as well as overseeing the activities of student employees at Dalhousie. Ian wrote a paper on guppy microsatellite primers for Molecular Ecology Notes.

Name: Wright, Wesley

Worked for more than 160 Hours: Yes

Contribution to Project:

Wes Wright (U-Maine) assisted in design and construction of a guppy rearing lab at University of Maine. He also developed guppy care protocols. As a professional staff member he also supervised undergraduate students working in our guppy lab facility. Wes Wright wright's salary was paid by the University of Maine, but his lab construction and maintenance activities were supported by NSF.

Name: Kozlowski, Ronald

Worked for more than 160 Hours: No

Contribution to Project:

Ronald Kozlowski (U-Maine) coordinated development of our guppy websites and continues to maintain the servers that support these websites.

Other Participant

Name: LaChapelle, Kevin

Worked for more than 160 Hours: Yes

Contribution to Project:

Kevin LaChapelle (U-Maine) assisted in care of guppies and maintenance of guppy facility at University of Maine. He also assisted in field research activities in Trinidad in 2005 and 2006. His student salary partly was supported by this grant.

Research Experience for Undergraduates**Organizational Partners****McGill University**

McGill University is the home organization of Dr. Andrew Hendry and his students. Dr. Hendry is a senior personnel on this project and close collaborator. His students have also contributed extensively to our field and laboratory investigations.

Dalhousie University

Dalhousie University is Dr. Paul Bentzen's home institution. Through subcontract, Dr. Bentzen is providing much of our microsatellite marker development and subsequent analyses. Furthermore, students working in other labs have visited Dr. Bentzen's facilities for training in molecular approaches.

University of California-Riverside

The University of California, Riverside, is the home institution for Dr. David Reznick. Dr. Reznick is a co-PI on this grant and his collaborations have been essential to establishing our field program. Furthermore, his lab has been important in measuring life history trait data for our clinal analyses.

Other Collaborators or Contacts

I (Dr. Kinnison) maintained two important contacts within the State of Maine. Both of these involved other researchers working with Trinidadian guppies. Dr. Shyril O'Steen, previously of Bates College, and Dr. Jeffrey Walker of the University of Southern Maine. Dr. Walker currently serves on the graduate committee for Dylan Weese. In addition we maintain contacts with an international group of scientists working on evolutionary ecology of guppies and other fishes, including investigators from Canada (Helen Rodd), the UK (Anne Magurran), and US (Douglas Fraser, Jim Gillam, Cameron Galahmbor).

Activities and Findings**Research and Education Activities:**

The overarching goal of this research program was to use empirical data to test hypotheses surrounding the role of gene flow in constraining natural selection and the reciprocal role of natural selection in constraining gene flow. The project met this goal through three primary components originally described in our proposal: 1) a survey of clinal variation in potentially adaptive traits of guppies throughout two entire river systems, 2) a detailed quantification of trait variation and natural selection in focal populations occupying alternate predatory regimes, and 3) experimental manipulations of migration among populations adapted to alternate predatory regimes to infer causal mechanisms controlling the adaptive consequences of gene flow in the wild. In addition to completing these planned components, our research also led us to incorporate consideration of the broader fitness and population dynamic consequences of gene flow-selection interactions. Such eco-evolutionary dynamics have now become an important new research theme for all of the PIs on this original project.

In the first two years of this project we completed our initial surveys of clinal variation in color patterns, morphology and microsatellite variation (component 1). For this work we sampled guppies for color patterns, morphology and life history variation at more than 30 sites in the

Marianne and Paria Rivers (Figure 1). In addition to this trait variation, we also mapped barriers to fish movement and the geographic extent of predators. This data was analyzed to characterize broad geographic patterns of trait diversity as relates to natural patterns of gene flow (inferred from microsatellites), barriers to migration, selective regimes and correlated environmental variables.

In preparation for our experimental manipulations of gene flow, we performed an extensive series of mark-recapture studies in the wild (component 2). These studies were performed primarily to allow us to empirically estimate patterns of selection acting on guppies inhabiting different predator environments. Despite the fact that Trinidadian guppies are often touted as one of the best examples of natural selection in the wild, almost everything we know about selection in this system comes from indirect inferences about selection, such as patterns of population divergence. Hence, there was a considerable need for direct estimates of selection to not only inform our own research on gene flow but also to evaluate existing hypotheses about the nature of divergent selection in this classic study system.

In total we performed 10 separate mark-recapture studies across low- and high-predation habitats. Guppies were captured live at each site and transported to our base of operations. There, guppies were photographed for size, morphology and color before they were individually marked with elastomer (Northwest Marine Technologies) for later identification. During several of these experiments we collected fin tissue or scales to use in refining genetic assignment approaches for later elements of our study. Fish were released back into their sites of origin after 2-10 days. We then resampled sites after a two week period and, in some cases, again after 4-6 weeks. These time scales represent a large fraction of the adult life span of most wild guppies. In the process of collecting this data we also obtained more detailed data on growth rates, color pattern, behavior, morphology, and life history (i.e., maturation schedule and male size at maturity) than was possible in our broader geographic surveys. Mark-recapture data was then combined with information on individual phenotypic trait values to estimate natural selection in high- and low- predation environments for comparison with observed patterns of trait variation.

In spring-summer of 2005 and 2006, we performed our migration manipulations (component 3) by again collecting guppies from our focal study sites and marking them. Scale samples were collected for all guppies to allow genotyping and their phenotypes were recorded with photographs. Low-predation guppies (male and female) were then released alongside high-predation guppies in high predation sites (mimicking the natural direction of gene flow, but exceeding the usual magnitude). We then resampled these sites at approximately two-week intervals for up to four months to account for patterns of mortality and recruitment. Offspring recruiting to the populations were photographed, scale samples were taken for DNA extraction, and fish were marked and released back into the sites. DNA samples were assayed at 6-9 microsatellite loci that we developed to assign offspring recruiting in the wild back to their population of origin (high- or low-predation). Relative contributions of parental types to the offspring cohort were then used to determine the impact of gene flow on the phenotype of the offspring generation and on population demography.

An analogous, but temporally less intensive, study was also conducted in 2005 to assess how much contemporary evolution might similarly contribute to performance differences of high- and low-predation fish and hence incipient limitations on gene flow. For this experiment we contrasted the performance of guppy populations in high- and low-predation environments in the Damier system, where they were introduced from a Yarra low-predation environment in 1996. Yarra high- and low-predation fish were also tested alongside the Damier fish in these experiments to provide a yardstick for comparison with contemporary evolution effects. In this study we only assessed relative survival of the different guppy sources but we did so in both high- and low-predation habitats.

In summer 2006 we performed a set of experiments to assess the relative role of sexual selection as a factor in the relative fitness of migrant and local fish. This experiment involved rearing of virgin female guppies from high and low predation sources to maturity (to avoid possible confounding effects of sperm storage in this species). These females were then placed in confined natural pools with high and low predation males at natural densities. Mating activities were then allowed for two days before females were recaptured and reared for two weeks until their offspring were large enough to be removed by dissection. Offspring were again genotyped to identify paternity for analyses that assesses how sexual selection (driven by mate choice and male coercion) might contribute to the success or failure of migrants, separate from the effects of natural selection on parents and offspring. In combination, this experiment and our migration manipulations have allowed us to assess the evolutionary mechanisms shaping effective gene in the wild.

In addition to data collection activities in the field, we developed a guppy laboratory facility at the University of Maine. We have used this facility to rear guppies captured in the field throughout multiple generations for the purpose of estimating genetic variances and covariances. Although laborious, we now have nearly all of the generations of data we need to estimate the necessary genetic parameters that will ultimately allow us to model gene flow-selection-demography dynamics in the wild for comparison existing theory and empirical data.

Finally, we have been very active in distributing our results to the broader scientific community. Each of the PIs, and several of the graduate students, have presented components of this research at various professional meetings and invited seminars (estimated at more than two dozen venues in the last few years). Despite the fact that many components of this project involved collecting data over several seasons, we have also been active publishing our findings and theoretical extensions (see products section). Six papers have already been published, two papers are currently in review and we have at least two more planned for journal submission within this year. The great depth of our dataset also bodes

well for further analyses and papers over the course of several more years.

Findings: (See PDF version submitted by PI at the end of the report)

Geographic surveys:

Predation was the most important factor for most color pattern elements (it explained between 23 and 64 % of the among-site variation in blue and orange coloration, but only between 10 and 24 % of the variation among individuals). However, canopy openness, spectral properties, and river (Marianne or Paria) also explained some of the variation. We thus hypothesize that differences in coloration between Marianne and Paria low predation populations are due to differences in abundances of *Macrobrachium* and to variation in female preference. We also found a previously unreported strong inverse relationship between blue and orange coloration of guppies (Figure 2).

In the case of morphology, we found that sex had by far the strongest effect on multivariate shape. Environmental gradients had much weaker effects, but significant trends included: (1) females had larger abdomens at sites with more open canopies and without predators, (2) males had deeper caudal regions at sites with more open canopies and with predators, and (3) females and males had deeper caudal regions at sites with higher flow. In each case, sex-specific effects of environmental gradients were substantial, indicating that although the sexes exhibit some parallel patterns of divergence, they also show substantial independent responses (Figure 3). Gene flow, measured with microsatellite variation, was strongly reduced by physical barriers and geographic distance but less so by divergent natural selection (Figure 4). In particular, we found little evidence that differences in predation or physical habitat features reduced effective gene flow. This result is interesting, given that phenotypic differences clearly exist among predation regimes (e.g., color results above). One possible explanation is gene flow or selection are episodically strong and weak, such that high rates of gene flow occasionally overwhelm adaptive divergence enough to contribute to an appreciable signal of gene flow at neutral markers, but subsequently strong selection maintains divergent trait values. However, these interpretations are tentative on the grounds that we had limited ability to distinguish the effects of predation regimes from that of barriers given that the two almost always coincide in this study system.

Natural Selection in the Wild:

Results of our mark-recapture studies provide, for the first time, direct support for general hypotheses regarding patterns of divergent natural selection in this classic study system. Mortality rates are higher in high-predation sites, consistent with a greater opportunity for selection in those habitats. Selection differentials and gradients were moderate to strong relative to literature values (>37-81%), consistent with the perception that strong selection drives divergence in guppies (e.g., Endler's and Reznick's classic experiments). Natural selection was shown to generally disfavor brighter coloration in both high- and low-predation environments consistent with the idea that male coloration is maintained by sexual selection (Figure 5). We also found support for the idea that natural selection against size and some color components is stronger in high-predation sites consistent with the observation that low predation guppies are more colorful and larger than high-predation guppies. Nonetheless, our data also reveal that natural selection in the wild is much more complex than often portrayed for this study system. For example, selection does indeed appear to be quite episodic and variable at any given site and some of our strongest estimates of selection actually came from low-predation locales (Figure 5). Such variable selection would again theoretically allow a pattern of episodic waves of gene flow followed by purging.

A paper on spatio-temporal patterns of natural selection on male guppy size and coloration has been written and submitted to the journal *Evolution* for review.

Migration Manipulations:

Given the considerable variability in selection that we observed in the wild, and given that microsatellite data suggests that gene flow does occur among wild guppy populations, our migration manipulations proved quite striking. By design, we implemented an extremely high level of migration in our experiments to provide us with better statistical power to detect gene flow effects. Indeed, migration was set a rate such that the experimental populations consisted of roughly 50% low-predation migrants. It is fortunate that we did implement such high rates, because the net strength of natural selection against migrants proved to be extreme in both replicates of the experiment. Relative to their high-predation counterparts, low-predation migrants had much lower survival rates (Figure 6) and suffered roughly an order of magnitude lower fitness (Figure 7), well below that needed for demographic replacement (as quantified by the number of adult offspring they contributed to subsequent generations). This result held whether migrants were derived from a more geographically distant and phenotypically divergent source (2005), or a more geographically proximate and phenotypically similar low-predation source (2006). This may limit some of our power for model testing given that fitness was universally bad for migrants. On the other hand, we have uncovered a level of functional divergence much greater than previously suggested for the guppy system. Whereas others have repeatedly noted that isolating mechanisms appear generally weak for guppies (based on laboratory assays or limited field observations), we have found evidence for strong ecological isolating mechanisms driven by natural selection in the wild.

Sexual Selection Experiments:

As it turns out, not all of the processes contributing to isolation of differentially adapted guppies are associated with ecological selection. Natural selection on adult migrants is clearly important, but our sexual selection experiments suggest that a modest to strong mating bias also works against immigrants (Figure 8). High-predation males sired 1.6 to 7.4 times the number of offspring of low-predation males in the relatively natural side channel pools we used for our experimental design. The fact that mating biases reinforce ecologically mediated natural selection would suggest that parallel ecological speciation is indeed underway, if incomplete, in the Trinidadian guppy system.

We have largely completed our analyses for our migration manipulation and sexual selection experiments and are drafting a manuscript that combines these results and our demographic inferences (see below).

Contemporary Evolution Effects:

Our comparison of the performance of high- and low-predation guppies from the Damier system in both habitat types and with ancestral analogs provided two interesting results. First, some, but not all phenotypic aspects of divergence in the Damier system matched comparable patterns from the Yarra, suggesting that nuances of local adaptation prevent contemporary evolution from being perfectly parallel with historical patterns in their closest ancestors. Nonetheless, we did find evidence that after just 8 years Damier high- and low-predation fish now perform better than their ancestors from the Yarra, supporting the theory that local adaptation can quickly contribute to the sort of fitness differences that diminish potential gene flow effects during the earliest stages of population divergence. This experiment has been written up as a manuscript that was recently reviewed at the *American Naturalist*. We are now revising the manuscript for further consideration at that journal.

Demographic Effects:

As noted in prior reports, our streams experienced a severe flooding event in 2005 prior to the initiation of our migration manipulation experiments. This flooding resulted in a severe reduction in the population abundance of high predation guppies in the mainstem rivers, whereas low-predation populations in neighboring tributaries were less affected. This natural disturbance led us to ponder the potential role of population dynamics in affecting the balance between natural selection and gene flow as well as the reciprocal role of gene flow-selection interactions in affecting population dynamics. As a result we wrote two theory/perspective papers on such eco-evolutionary dynamics and their ties to conservation. Moreover, we set out to assess the population ecology consequences of selection on migrants within the context of population recovery in our own migration manipulation experiments. In so doing we uncovered an unusual example of the ecological cost of adaptive contemporary evolution. Namely, we found that contemporary evolution, in the form of selection against migrants, reduced the peak population recovery size by nearly 50% relative to the evolutionary null (pure ecological) expectation.

Directions for Future Study:

In addition to our growing interest eco-evolutionary dynamics, which is now formalized in a separate NSF FIBR funded project (P.I. D.Reznick), a few other insights are worthy of noting for their potential for future research.

Two undergraduate student projects, both implemented within the scope of this project, provided promising results regarding patterns of divergence in female body shape and boldness/shyness behavior in both sexes. Particularly interesting, both of these students not only demonstrated divergence in these traits across predation regimes, but they also measured selection on these characters for individuals released back into the wild. In both cases selection in the wild corresponds with the patterns expected to produce observed divergence. Due to the somewhat limited scope of undergraduate research, we will nonetheless need to replicate or more extensively analyze these findings for ultimate publication.

The relatively large magnitude of selection on migrants among wild populations is interesting, but this limits our ability to consider the interaction of more subtle selection and gene flow. As a result, we are now interested in developing a more definitive experiment to assess the interaction of gene flow and selection across the range of smaller divergence values where gene flow may be more prevalent. Our findings from populations that have diverged in contemporary time suggests that it may be possible to use contemporarily diverging populations of guppies to track the time course of gene-flow selection interactions as populations diverge in contemporary time. An opportunity may exist to do this within the context of our NSF FIBR project or within a new project. We are also considering the use of domestication (artificial selection) of wild guppies as another way to precisely titrate divergence and quantify its effects on gene flow, fitness and population dynamics. Such an approach may be especially useful given that domestication poses a real world problem in conservation, where several studies have provided evidence that it can quickly impair threatened populations either directly or indirectly via gene flow.

Training and Development:

NSF support for travel, field work, and laboratory study has contributed to the training and development of numerous graduate and undergraduate students. Indeed, in just five years this project has afforded various academic experiences for more than 31 students. Admittedly, for some of these students experiences were limited to workstudy employment, but even these students usually obtained some first-hand experience in tropical field work, experimental design, laboratory techniques, data management, or animal husbandry. Graduate students and advanced undergraduates obtained further experience in data analysis, scientific writing and professional presentation.

Field work in Trinidad, construction of a guppy rearing facility, and collaborations with Dr. Reznick (all partly supported by this grant) have significantly contributed to guppy expertise in the Kinnison and Hendry labs that will be of value for many years. Collaborations with Dr. Bentzen have similarly fostered microsatellite analysis expertise in the Kinnison lab. It is worth reiterating that all of the investigators on this project are now participating in a FIBR project that investigates the role of evolution as driver of ecological dynamics. This new research is a logical collaboration building off some of the eco-evolutionary lines of investigation that began in the present project.

Finally, this grant has contributed to equipping a guppy laboratory at the university of Maine through infrastructure such as microscopes, field equipment, and a ventilated work area for handling preserved fish specimens.

Outreach Activities:

Our guppy research has been featured in several outlets available to the public and conservation or management practitioners. For example, Dr. Kinnison and this research program were featured in 'U-Maine Today', a magazine distributed to faculty, students and alumni of the University of Maine system. This article reached a wide audience with a goal of educating readers on the importance of natural selection and gene flow. Dr. Kinnison's research and its applications to conservation biology were featured in a cover story in the magazine 'Conservation in Practice' (published by the Society for Conservation Biology). Dr. Reznick will be interviewed by National Geographic in July for an article on Darwin's legacy.

As a joint effort between this project and our NSF FIBR project we have continued to develop web resources for guppy researches and for the general public. A guppy research coordination website, which includes calendaring, correspondence, data sharing and file transfer tools, is now in full implementation. As a result, guppy researchers are able to coordinate their efforts for larger and more integrative projects (such as our NSF FIBR project). Following a reasonable period of testing within our current research groups we anticipate opening up a comparable webtool to all scientists working in Trinidadian stream systems.

In recognition that our public internet project will continue to develop under FIBR and thus serve a broader educational goal, and to keep up with the ever evolving media experience that internet users expect, we have also refined the infrastructure for what will ultimately be our public website. Recent revisions will allow for a more immersive educational experience, including access to video and audio recordings and an interactive glossary of scientific terms to allow users with different background levels (i.e., second school to college) to access the same materials. Text for several sections of the site has been written and other sections are under solicitation from experts in the field.

Over the course of this project the guppy facility at U-Maine has hosted tours by grade school students as part an NSF K-12 teaching initiative.

Journal Publications

Nathan P. Millar, David N. Reznick, Michael T. Kinnison and Andrew P. Hendry, "Disentangling selective factors that act on male colour in wild guppies", *Oikos*, p. 1-12, vol. 113, (2006). Published,

Patterson, I. G., E. Crispo, A. P. Hendry, M. T. Kinnison, and P. Bentzen, "Characterization of tetranucleotide microsatellite markers in the guppy (*Poecilia reticulata*)", *Molecular Ecology Notes*, p. 269, vol. 5, (2005). Published,

Crispo, E., A.P. Hendry, P. Bentzen, D.N. Reznick, and M.T. Kinnison, "The relative influence of natural selection and geography on gene flow in guppies", *Molecular Ecology*, p. 49, vol. 15, (2006). Published,

Hendry, A.P., M.L. Kelly, M.T. Kinnison, and D.N. Reznick, "Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies", *Journal of Evolutionary Biology*, p. 741-754, vol. 19, (2006). Published,

Michael T. Kinnison and Nelson Hairston Jr., "Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence", *Functional Ecology*, p. 4, vol. 21, (2007). Published,

Kinnison, M.T.; Hendry, A.P.; Stockwell, C.A., "Contemporary evolution meets conservation biology II: Impediments to integration and application", *ECOLOGICAL RESEARCH*, p. 947, vol. 22, (2007). Published, 10.1007/s11284-007-0416-

Gordon, S.P., Reznick, D.N., Kinnison, M.T., Bryant, M.J., Rasanen, K., Weese, D.J., Millar, N.P. and Hendry, A.P., "Does adaptive change improve survival in altered environments? An experimental test using introduced guppies", *American Naturalist*, p. , vol. , (2008). In revision following review,

Weese, D.J., Gordon, S.P., Hendry, A.P. and Kinnison, M.T., "Spatio-temporal variation in natural selection on body color in wild guppies (*Poecilia reticulata*)", *Evolution*, p. , vol. , (2008). Submitted,

Books or Other One-time Publications

Web/Internet Site

Other Specific Products

Product Type:

Data or databases

Product Description:

We have continued to compile an extensive dataset mapping guppy coloration, life history, morphology and microsatellite variation throughout two major drainages on the North Slope of Trinidad's Northern Mountains (34 collection sites throughout these rivers). For each of these sites we also have physical and biological parameters. We have earmarked a subset of these sites for long-term study for all of the above data types. We have also measured selection on guppy phenotypes for the first time at several of these sites. This represents a uniquely comprehensive dataset for understanding drainage wide patterns of guppy trait variation, selection regimes and gene flow.

Sharing Information:

We will share this dataset with other investigators after reasonable initial analyses and publication, consistent with ethical and fair academic tradition.

Product Type:

Audio or video products

Product Description:

We have collected photos, video and audio of different high- and low-predation research sites.

Sharing Information:

We plan to use photos and audio-visual clips to further enhance the experience for people using our guppy websites. Ultimately visitors will be able experience the sights and sounds of our actual jungle stream research. This will require some modifications to the current site design, and we hope to have this completed by the end of summer 2008.

Contributions

Contributions within Discipline:

This work contributes to insights in the discipline of population biology, in particular evolutionary ecology and its interaction with migration and population demography. Current knowledge of the role of gene flow in adaptive evolution in the wild is largely based on theoretical models and indirect assessments. Our study sought to provide empirical insights into the role of gene flow in the diversification of populations subject to different adaptive environments by actually manipulating this evolutionary process. The results of these manipulations in turn form a basis for evaluating theories on the reciprocal influences of gene flow and natural selection and their ties to important demographic processes, such as population rebound after some disturbance.

Specific contributions include:

- 1) Developed new Microsatellite loci that will be of wide utility to many other researchers working on guppies (and possibly related species as well).
- 2) Described patterns of adaptive trait variation and gene flow throughout two entire drainages, thus providing a landscape perspective on gene flow - selection interactions.
- 3) Provided the first formal selection analyses on size and color variation in high and low predation environments, thus offering a critical assessment of existing hypotheses central to this important study system.
- 4) Performed some of the first actual experimental manipulations of gene flow in the wild and quantified the degree of selection acting on migrants as well as its consequences for population dynamics.
- 5) Assessed the strength of sexual selection acting on migrants independent and showed that mating bias reinforces natural selection, providing support for replicated ecological speciation (albeit partial) in guppies.

Contributions to Other Disciplines:

We anticipate our findings will have substantial implications for conservation biology. Gene flow is often considered an indicator of population distinctness for protection and many cases of population conservation are fraught with decisions about whether to limit or increase gene flow. Without empirical data, decisions regarding the distinctness of populations for conservation and their subsequent genetic management are currently based on largely untested theory. Our findings may thus assist in determining appropriate gene flow models for the critical management of biodiversity.

Also, due to a chance set of events our work also provides some rare empirical insight into how local adaptation and gene flow affect population recovery following disturbance. The extension from contemporary evolutionary interactions to population dynamic processes represents an important empirical demonstration of eco-evolutionary dynamics. Eco-evolutionary dynamics shows great promise as a theoretical bridge between evolution and both basic and applied areas of ecology. For example, interactions between contemporary natural selection, gene flow, population growth and community/ecosystem interactions will be critical to the persistence of biodiversity in the face of rapid climate change.

Contributions to Human Resource Development:

This research program involved 31 students, with nearly equal representation by men and women. Some of these students represent minorities in the sciences and many have participated in our field studies or constructed independent (honors) projects out of components of our program. Several undergraduate students have since gone on to graduate programs and two students (an undergraduate and a graduate) have adapted their research background to teaching at pre-college and college levels. Many of the student participants in this research program come from the state of Maine, an EPSCOR state that has traditionally been underrepresented in science funding and education.

School groups have visited our guppy laboratory facility which has afforded us the opportunity to talk about guppies, evolution and research.

Contributions to Resources for Research and Education:

We have made significant strides in the development of guppy related websites that contribute to both coordinate research and public education. At present these websites are being carried forward and integrated with our broader impacts mission of an NSF-FIBR award that shares many of the same PIs.

Our guppy facility is now heavily in use for both graduate research as well as for undergraduate projects. Hence, it is proving a valuable resource for both research and education.

Contributions Beyond Science and Engineering:

Management of wild populations for harvest or conservation is a very challenging and underfunded endeavor. Hence, it is not surprising that managers often seek simplifying assumptions about where to focus their time and resources. One of the most pervasive simplifications in population management is that contemporary evolutionary processes, such as the interaction between gene flow and natural selection, are minimal, or at least secondary to immediate demographic concerns. Unfortunately this simplifying assumption obviates the fact that evolutionary processes and population demography are often implicitly linked. Some members of our research team (Dr. Kinnison and Dr. Bentzen) commonly serve as advisors for groups challenged with managing endangered fish species (e.g., Maine Atlantic Salmon Technical Advisory Committee, NCEAS panel on salmon responses to climate change). We often draw from the results of our guppy research to help managers appreciate the potentially large role that contemporary evolutionary processes (and not just historic evolution) may play in population growth or decline.

Categories for which nothing is reported:

Any Book

Any Web/Internet Site

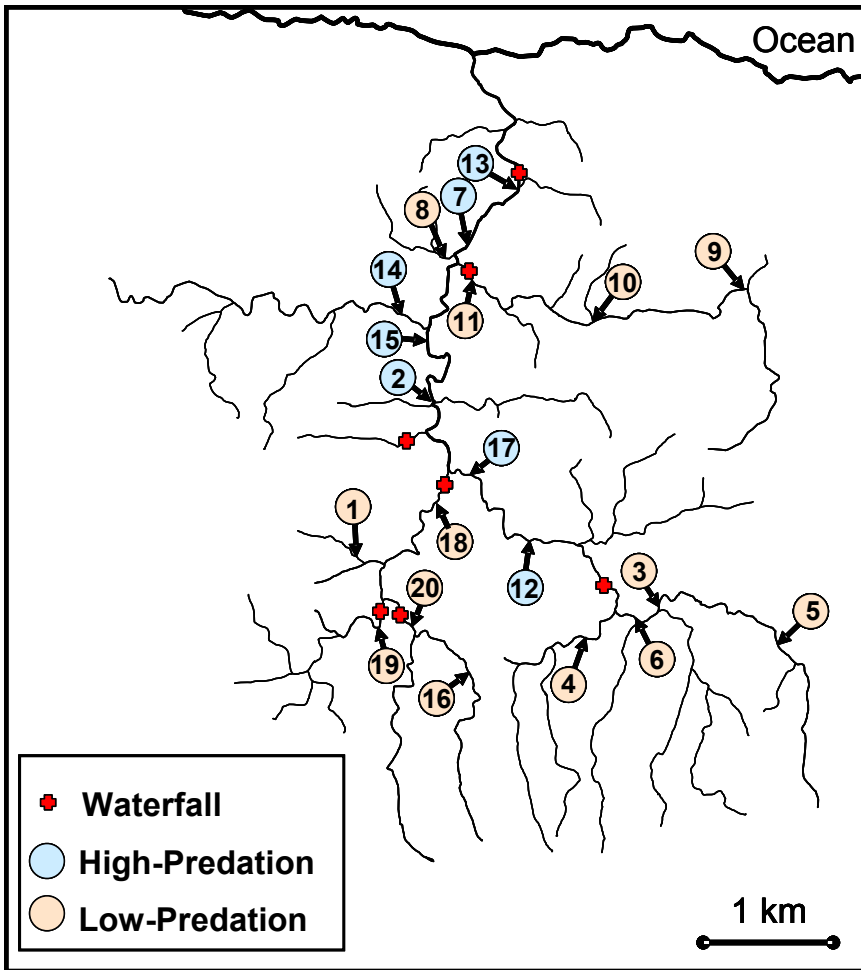


Figure 1: Sites in the Marianne River (Trinidad) sampled for drainage-wide survey of geographic variation in adaptive traits (e.g., color, morphology) and gene flow. A smaller array of sites was similarly distributed throughout the neighboring Paria drainage.

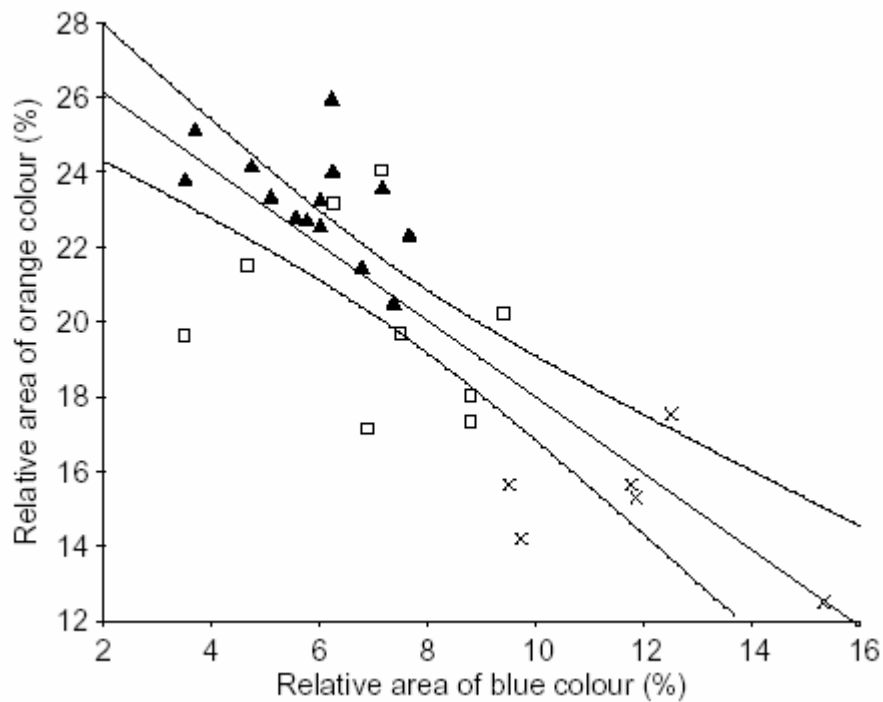


Figure 2. Negative correlation between the relative area (total area of colour divided by the body area) of blue and orange colour across all sites (n_29). Sample sites on the Paria are marked with solid triangles, low predation sites on the Marianne with open squares, and high predation sites on the Marianne with crosses. 95% confidence intervals are indicated. Clearly, high predation populations fall further along this color spectrum toward more blue area and less orange area.

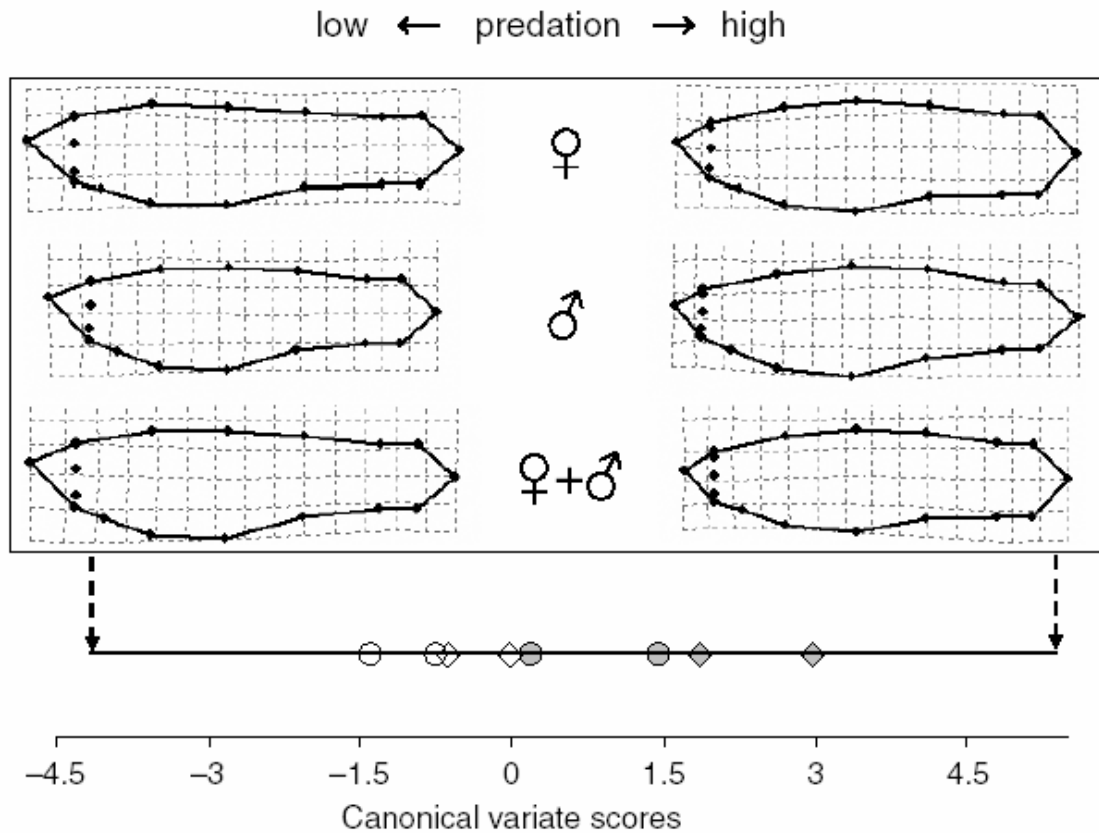


Figure 3. Effects of predation on body shape. The axis at the bottom shows the canonical variate optimized with respect to predation from the MANCOVA with both sexes included. Points above that axis are averages for each combination of sex (open symbols for females; grey symbols for males), predation (circles for low-predation; diamonds for high-predation), and year (specific years not labelled). The ends of the line passing through these averages correspond to the score for the individual whose shape was most low predation (left terminus) or whose shape was most high predation (right terminus). Above this line, the bottom pair of images shows thin-plate spline transformations depicting body shapes at the observed extremes of the canonical variate. The top two pairs of images show the corresponding extremes when males and females are analysed separately.

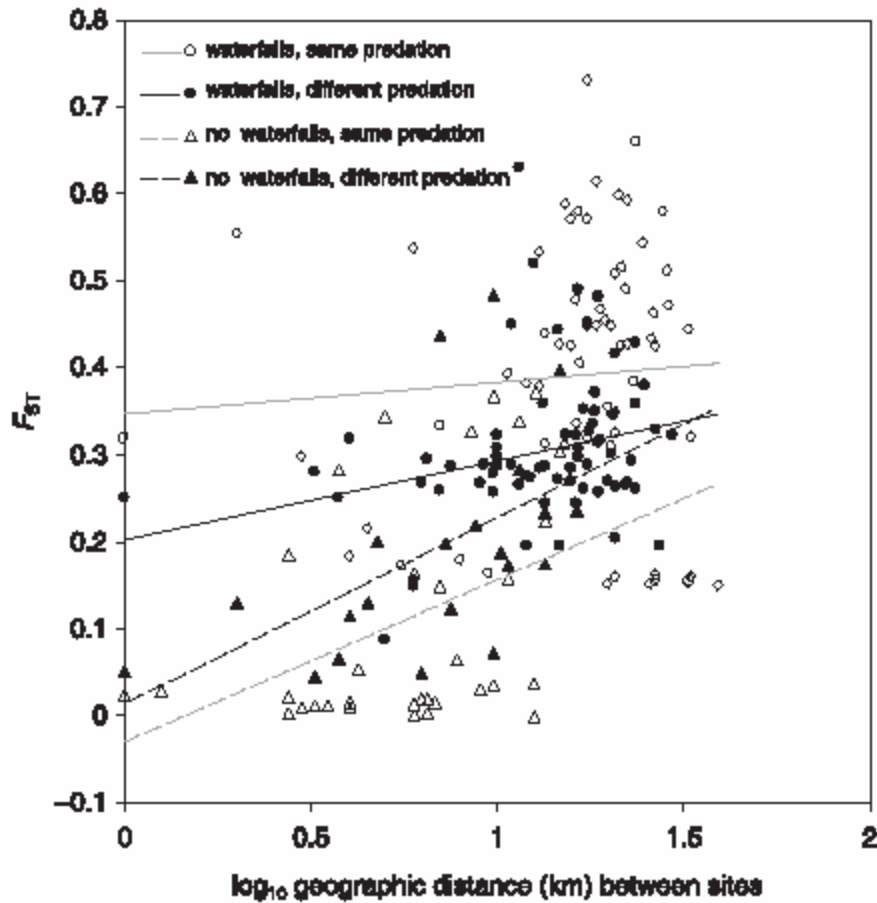


Figure 4. Isolation-by-distance relative to barriers and predation regime. Pairwise F_{ST} values between all possible site pairs in relation to \log_{10} transformed geographic distance (km) between sites along the length of the stream. Circles and solid lines represent site pairs separated by one or more waterfalls, triangles and dashed lines represent site pairs not separated by a waterfall. Open points and grey lines represent site pairs experiencing the same predation regime; closed points and black lines represent site pairs experiencing different predation regimes.

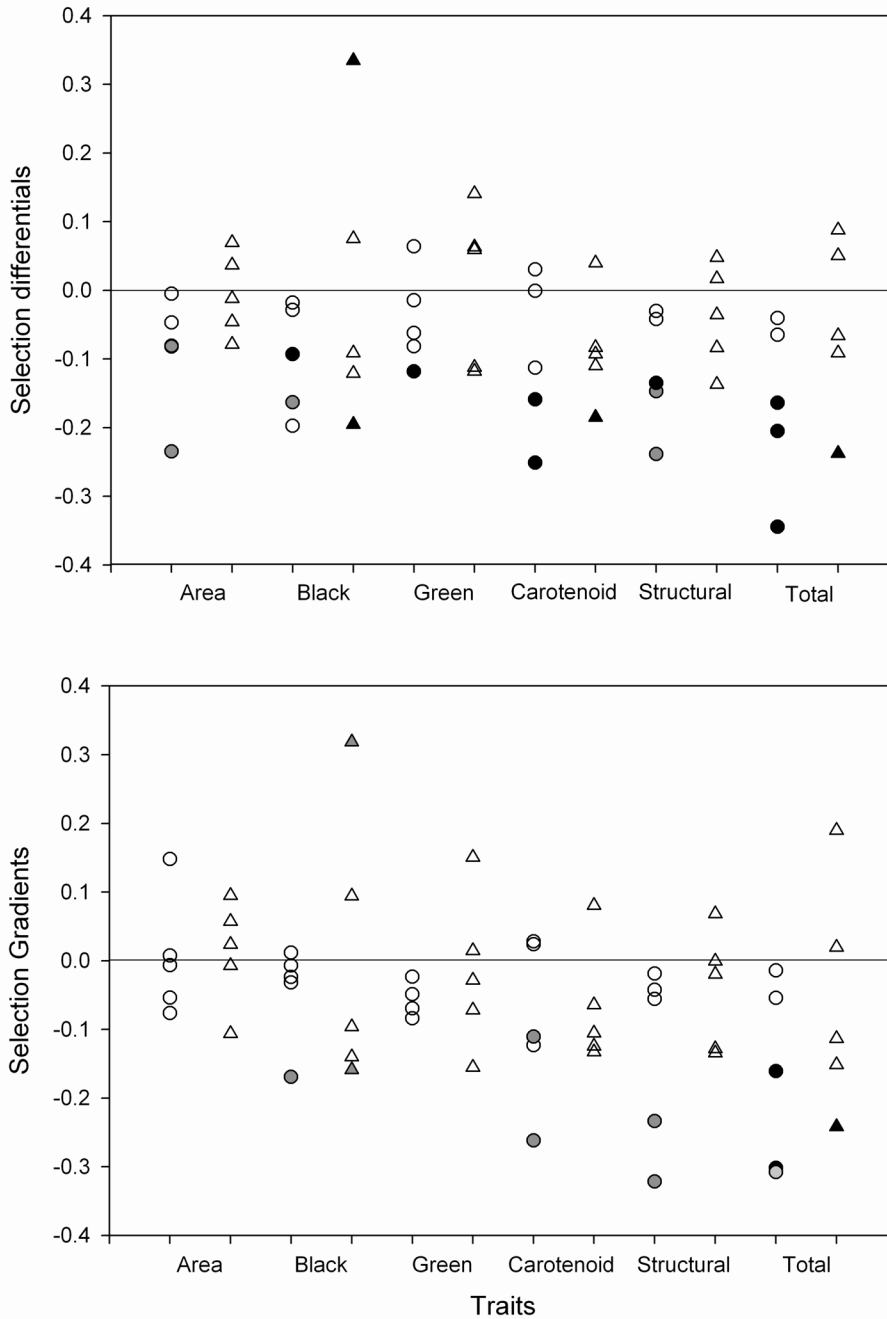


Figure 5. Values for selection coefficients (differential and gradients) for all site/trait combinations considered in this study. Circles are the coefficients associated with low-predation sites, triangles are coefficients associated with high-predation sites. Statistical support is represented by the shading in the symbols interiors (grey fill $p < 0.1$; black fill $p < 0.05$). Figure demonstrates substantial range of selection values within and among predation regimes, but also an overall trend toward selection against male body color.

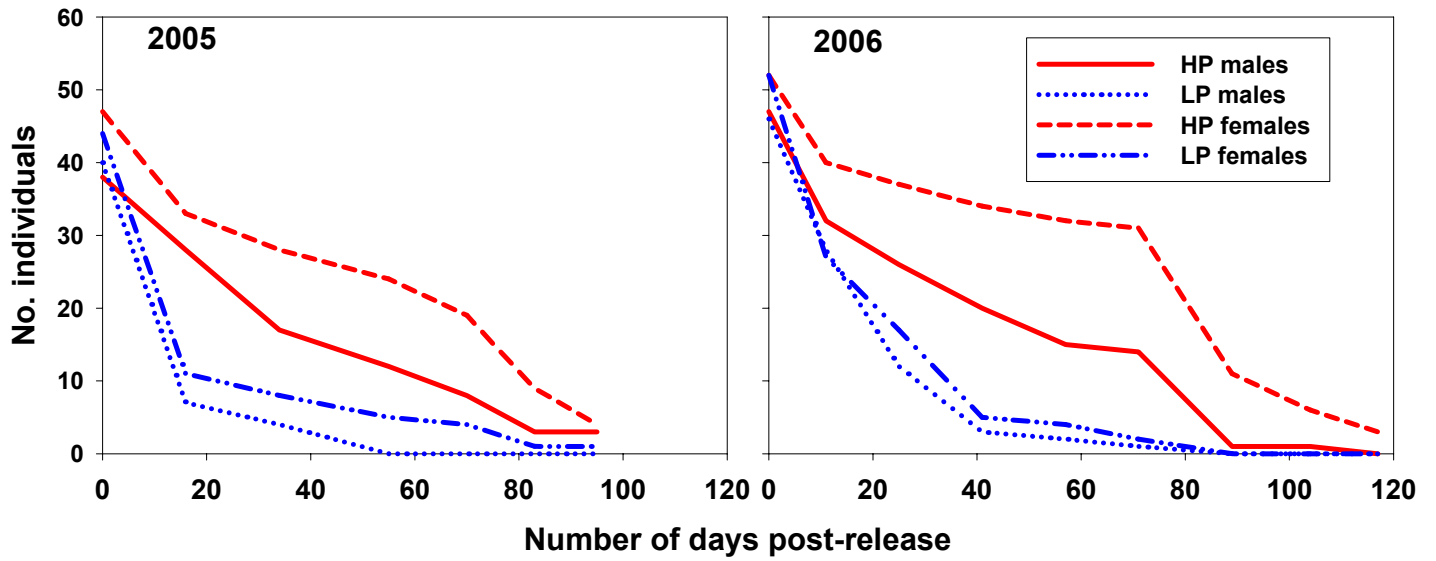
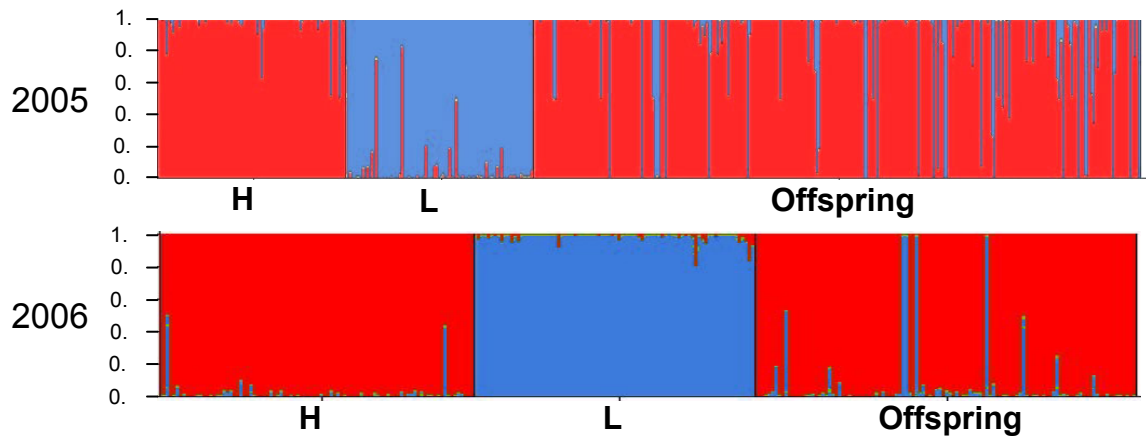


Figure 6. Strong selection on migrants results in higher mortality rates of low-predation guppies when both high- and low-predation guppies are released in a high-predation site. The negative trend for all guppies is expected as the released cohort ages and because this figure does not include recruitment.



	2005	2006
Total N offspring	254	99
N offspring assigned to HP	207	95
N offspring assigned to LP	47	4
HP offspring production	207/81 = 2.55	99/95 = 0.95
LP offspring production	47/83 = 0.566	4/97 = 0.04

Figure 7. Structure analysis of offspring identity for 2005 and 2006 migration manipulation experiments. Table quantifies relative reproductive success of high predation (HP) and low predation (LP) parents. Results show that HP parents come close to or exceed replacement rates of offspring production, whereas the fitness of LP parents falls far short of replacement.

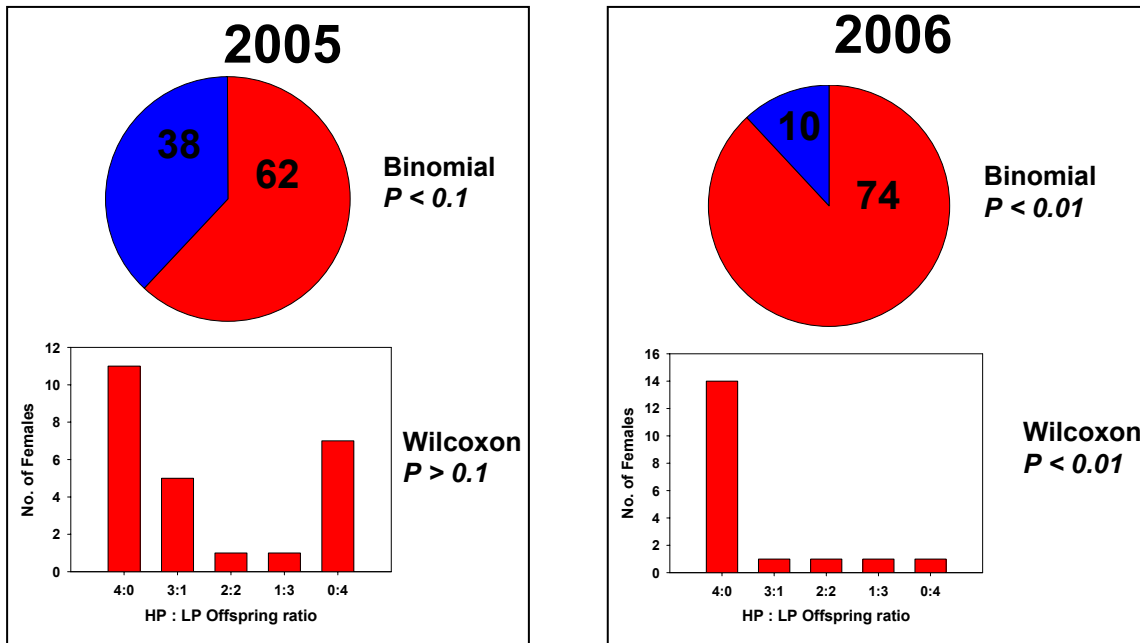


Figure 8. Results of sexual selection experiments. Pies show total proportion of offspring of high-predation females sired by high (red) and low (blue) predation males. Histograms depict the number of females carrying different ratios of offspring sired by high predation or low predation males (four offspring randomly chosen per female). Both years of data support modest to strong mating bias against migrant low-predation males.