Sex-Role Reversal and "Dominance Genes" in African Cichlid Fish

The broad scope of my research aims to understand the genetic basis of behavior from an evolutionary perspective using a comparative approach by combining behavioral, physiological, and ecological techniques with modern genomic and molecular techniques.

The current focus of my research with African Cichlid Fishes aims to understand the genetic basis of species-specific "sex-role" behaviors, and how these behaviors relate to environmental conditions and thus represent evolutionary adaptation.

I seek support of the Jordan Endowment Award to purchase a "data-recorder" and accompanying software to enhance the accuracy and efficiency of data acquisition for behavioral observations in the field. The requested equipment will streamline the integration of Lake Tanganyika "field data" and experimental "laboratory data" to ensure comparable and consistent analysis in a manner suitable for current state of the art protocols.

My proposed research includes 2 specific aims designed to test the hypothesis that species-specific "sex-role" behaviors, imposed by ecological factors, result from differential gene expression in the brain.

Aim 1 Quantitative description of type, strength, duration, and frequency for "dominant" behaviors within and between sexes for several closely related cichlid species.

1a Field observations of behavior will be correlated with ecological factors

1b Laboratory experiments will accurately quantify specific behaviors

Aim 2 Genomic analysis of genes involved in the neural control of dominance behavior through the use of micro-array technology.

Background and Significance

The recent and explosive radiation of East African Cichlid fish has produced cichlid species which occupy nearly all habitats available within the African Great Lakes (Barlow, 2000). Lake Tanganyika has ca. 200 behaviorally and ecologically diverse species of cichlid fishes, most of which belong to few monophyletic and endemic clades. Reproductive isolation between these closely related species is largely maintained through behavioral mechanisms (Seehausen *et al*., 1998). Therefore, cichlids represent a unique opportunity to study the evolution complex social behaviors. Owing to the ease of cichlid husbandry and the ability to reproduce an extensive behavioral repertoire in the laboratory, captive fish can provide a reliable resource necessary for state of the art molecular analysis of these complex, natural behaviors.

Most animal species exhibit sex-specific behaviors in competition for mates (Darwin, 1871). The males of most species compete more intensely for mates than do the females and display the "dominant sex-role" (Anderson 1994). This inequality is imposed by a variety of physiological and ecological factors which determine the relative reproductive success of males and females. When the females of a species exhibit dominance behaviors that are conventionally associated with males, a species is said to exhibit "sex-role reversal." Species in which the sex-roles are reversed have been used to critically test theories concerning the evolution of mate competition. Examples of sex-role reversed species can be found among amphibians (Verrell and Brown, 1993), birds (Lignon, 1999), insects (Gwynne and Simmons, 1990), and fish (Vincent et al., 1992). Within the range of cichlid characteristics one finds examples of a wide variety of mating patterns and parental care, including species which exhibit sex-role reversal (Balshine-Earn and McAndrew, 1995; Yamagaishi and Kohda, 1996; Barlow and Lee submitted).

Most of the data on sex-role reversal derives from field studies, therefore few physiological and no genetic mechanisms of sex-role reversal are known. The few studies that have tackled the physiological basis of sex role reversal have failed to uncover reversal in the levels of sex steroid hormones (review, Eens and Pinxten, 2000) possibly due to constraints imposed by hormone regulation of gamete production. With the progress of modern genomic capabilities we can now track gene expression levels for thousands of genes in parallel, even in nontraditional model organisms (e.g. Whitfield et al., 2002; Oleksiak et al., 2002; Hofmann

2003). I propose the first study of sex-role reversal to combine traditional behavioral studies with modern genomic analysis. This project is therefore not limited to obvious candidate hormones and their receptors.

To allow a genomic analysis of behavior, the Hofmann lab at the Bauer Center for Genomics Research, has constructed a cDNA micro-array containing >6000 clones (~4,000 genes) from the brain of the cichlid *Astototilapia burtoni* which is thought to represent the ancestral type of Tanganyikan cichlid (Lowe-McConnell, 1991). Because African Cichlid Fishes are recently diverged there is a high degree of genomic conservation among species. Due to that genomic similarity the cDNA clones on the Hofmann lab micro-array will also be useful for identifying gene expression profiles for other African Cichlid Fishes in addition to *A. burtoni* (see appended data).

Methods

Lake Tanganyika offers an excellent study site because of its exceptional water clarity and the diversity of cichlid social systems. The proposed field and lab work will focus on the behavior Tanganyikan cichlid species of the genus *Julidochromis*. The genus *Julidochromis* is composed of five species; *J. marlieri, J. dickfeldi, J. ornatus, J. transcriptus* and *J. reganni* (e.g. Stack 1975; Brichard 1989). In general, *Julidochromis* species tend to alternate around the lake shores rather than existing in sympatry (Brichard 1989). Unpublished observations (Barlow per. comm.) and hobbyist reports (see for example www.gca.net; Roth, 1971) suggest that most *Julidochromis* species exhibit conventional sex-role behavior. Rigorous scientific, analysis of behavior is available only for *J. marlieri* (Yamagaishi and Kohda, 1996; Barlow and Lee submitted) revealing that *J. marlieri* exhibits sex-role reversed behavior. I aim to examine the behavior of all five species, however gene expression experiments will focus on lab reared populations of the two species which are found to exhibit the most distinct behaviors.

Aim 1: Quantification of natural sex-role behavior among *Julidochromis* species

Field observations will include both behavioral and ecological data. Field data is necessary in order to formalize a theory explaining the selective forces which have led to and maintain the evolution of different sex-role behaviors. At the TAFIRI Kigoma Station(Tanzania), I will establish study sites containing 10 to 15 breeding pairs. Within these areas (ca. 5m x 5m) territories will be mapped for each individual, identified according to distinct body pattern.

The social complexity of these quadrats will be characterized according to the number of species, the number of individuals, both conspecifics and heterospecifics, with regard to sex ratio and age structure. Field observations will focus on characteristics which are pertinent to several theories which have been developed to explain observed inequality of mating competition. These characteristics include measurements of factors such as the number of partners with which an individual mates (Bateman gradient (Bateman 1948)), the availability of suitable mates (Operational Sex Ratio, OSR (Emlen and Oring 1977). The quadrant map will be produced on a hand held computer (Psion Workabout) which will be used for territory mapping. This technology can be used to track multiple individuals simultaneously (Observer Mobile, Noldus). In addition to spatial tracking the use of a computer for data recording will allow temporal tracking of movements within the territory and also precise timing behaviors and interactions. Important social interactions and behavioral factors such as parental investment which influence reproductive strategies (Potential Reproductive Rate PRR (Clutton-Brock and Vincent 1991)) will be quantified according to strength, duration. Other factors such as the cost of gamete production and mate preference are better quantified in a laboratory setting (see below).

Laboratory observations will include both behavioral and physiological data. Controlled laboratory experimental analysis is important for the quantification of the complex behaviors that have evolved in the different species.

Although mirror experiments have been useful in the quantification of behavior in other cichlids (Holder *et al*., 1991) these do not work in *Julidochromis* (Barlow per. comm.). Therefore I intend to develop synthetic animations as a precise tool for manipulating visual signals involved in social interactions (Rosenthal 1999, 2000; http://people.bu.edu/gil/rosenthal_anims.htm). The behavioral response to simulations will be quantified using Observer digital analysis software (Noldus). This analysis is sensitive to minor movements and precise temporal resolution not detectable by simple human observation. My experiments will include measurements for inter- and intra-species aggression as well as inter and intra-sex aggression.

Physiological measurements of factors important in defining mate competition and sexual selection (e.g. hormone levels and total gamete production) and will be recorded.

Aim 2: Identification of Genes Underlying Sex-Roles and Dominance in Cichlid Fish

The micro-array identification of "dominance genes" expressed in the brain, will focus on *J. marlieri* (sex-role reversed) and one species exhibiting conventional sex-role behavior. In each experiment I will use the Hofmann lab cDNA micro-array and competitively hybridize RNA from the brains of two adult fish which have been rigorously tested in behavioral paradigms (see above).

I will compare male versus female individuals both within and between these two closely related species. This experiment will distinguish genes that are "sex-role" specific (dominant vs. subordinate) from those that are simply sex-specific (i.e. coordinately regulated within a sex regardless of species). (Fig. 1) The expected outcome will be a list of coordinately regulated genes showing increased or decreased expression. These results will be confirmed by independent molecular techniques (*e.g. in situ* hybridization, and quantitative RT-PCR) and will require rigorous analysis in order to identify molecular pathways, modules, and mechanism which mediate these behaviors.

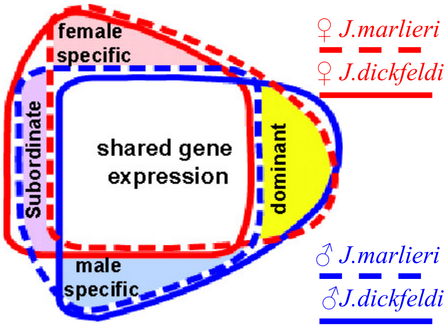

Figure 1: Schematized representation of one possible hypothesis: each outline represents the set of genes coordinately regulated within an individual. My hypothesis is that there is a set of dominance genes (yellow shape) coordinately regulated in both the aggressive females of the sex role reversed species (red dashed line) and the aggressive males of the conventional sex role species (blue solid line) regardless of species (dashed versus solid lines) or sex (red versus blue lines).

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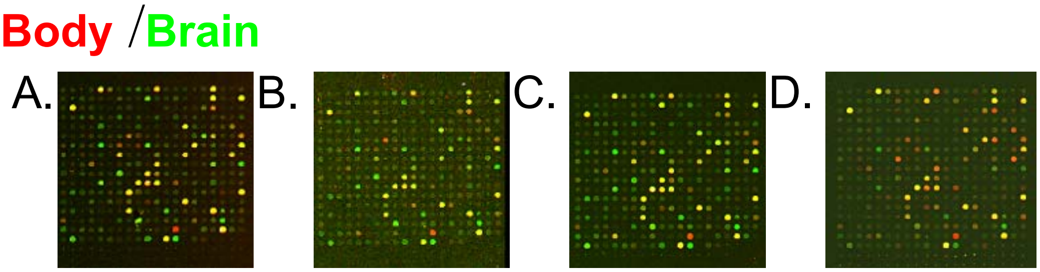
Appended Data

The purpose of this appended data is to demonstrate the use of micro-array technology toward gene expression profiling of closely related cichlid species.

This technique allows the researcher to measure the relative levels of messenger RNA in two different RNA samples in order to determine "gene activity" under specific conditions.

Methods

Each "spot" on our micro-array represents a single un-annotated cDNA from the *A. burtoni* cDNA brain library that has been spotted in picolitre quantity on to a poly-lysine coated glass slide.
In each experiment:
  total RNA (expressed genes) from sample #1 is labeled with red fluorophore and
  total RNA from sample #2 is labeled with a green fluorophore
These two labeled RNA pools are competitively hybridized to the micro-array.
Red spots represent genes highly expressed sample #1, green spots represent genes highly expressed sample #2, yellow spots represent genes expressed in both tissues.



A. burtoni ***E. melanogenys*** ***N. brichardi*** ***Oreochromis niloticus***

Fig 1.The figure shows expression profiles of brain RNA extracted from four different cichlid species hybridized to the Hofmann Lab cichlid array. The similar red/green/yellow pattern for each species reveals that the different "gene expression profiles" of the two tissues is largely reproducible. The reproducible hybridization pattern demonstrates that genomic conservation among cichlid species is sufficient for cross hybridization (one block of the total 16 on the array is shown; each block contains ~ 400 features). This example accentuates the strength of the micro-array technique. I expect fewer and less dramatic differences between the "gene expression profiles" of brains of the different behavioral phenotypes.