ABSTRACT

Quantifying the Genetic Basis of Yellow Pigmentation
in Lake Malawi Cichlid Fish

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As a likely target of sexual selection via female mate choice, male nuptial patterns are thought to have played a role in the extraordinary diversification of Lake Malawi's rock-dwelling (*mbuna*) cichlids. The extent to which male coloration can explain the rapid evolution of the *mbuna* clade, however, is dependent on its underlying genetic architecture. If male nuptial coloration is found to be mediated by few genes of large effect, then this genetic architecture could support rapid phenotypic evolution and offers a potential mechanism explaining the extraordinary diversity of Lake Malawi cichlids. To this end, this study aims to quantify the number of genetic factors and the mode of gene action influencing male xanthophores pigmentation in two closely related sympatric *mbuna* species of Lake Malawi. I estimate that xanthophore pigmentation in both scales and pelvic fins is regulated by few genes of large effect that exhibit epistatic effects. These results provide insight into the genetics of male coloration and add to a body of literature supporting pigmentation patterns' roles in cichlid diversification.

QUANTIFYING THE GENETIC BASIS OF YELLOW PIGMENTATION IN LAKE MALAWI CICHLID FISH

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By

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DEDICATION

I have dedicated this work to my loving and impossibly caring family.

Thank you Mom, Dad, Victoria, and Andrew.

Also, thank you for the soup,

Catherine “Kitty” Sotello

I’m not very good at poetry anyways.

CHAPTER ONE

Introduction

Within the past 10 million years (Genner et al., 2007), over 2000 species of cichlid have evolved in the three East African Great Lakes. This striking example of a rapidly speciating clade offers an excellent opportunity to study species formation. In Lake Malawi alone, over 800 species of haplochromine cichlids (Danley *et al*., 2012) are thought to have radiated from a single ancestor that invaded the lake 700,000 years ago. Put into perspective, this outnumbers all the species of fresh water fish in North America and Europe combined and raises the question of how such extraordinary diversity was generated [Maitland, 1994; Page *et al.,* 2011].

Phylogenetic analyses suggest that the diversification of Lake Malawian cichlids occurred in three distinct stages (Danley and Kocher, 2001). The first of these radiations split the diverging lineages among the major habitats in the lake and produced two large benthic clades: the sand-dwellers and the rock-dwellers (or mbuna). A second radiation characterized by a diversification of trophic morphology followed (Danley and Kocher, 2001). During the ongoing third stage, male secondary sexual characteristics diverged in apparent response to sexual selection via female choice (Holzberg 1978; Ribbink *et al.* 1983; Dominey 1984; Hert 1991; McKaye 1991).

As a trait likely under sexual selection (Deutsch, 1997; Seehausen & van Alphen, 1998; Danley & Kocher, 2001), male pigmentation patterns might have played a

defining role in the evolutionary history of cichlid diversification. The speed with which male pigmentation has evolved, and the resulting rapid evolution of the *mbuna* clade, may have been facilitated by the underlying genetic architecture of this phenotype (Chenoweth and McGuigan, 2010). If male nuptial coloration is mediated by few genes of large effect, this genetic architecture could facilitate rapid phenotypic evolution (Dobzhansky and Dobzhansky 1937, Ayala et al. 1997, Kronforst et al. 2006) and offers a potential mechanism explaining the extraordinary diversity of Lake Malawian cichlids.

In the interest of elucidating male nuptial colorations role in cichlid diversification, the goal of this study was to elucidate the genetic factors that underlie male xanthophore pigmentation. To this end, the density of xanthophore cells, pigmentation cell types responsible for yellow-orange coloration, was measured in two closely related sympatric *mbuna* species and their line-cross derivatives to estimate mode of gene action and the number of factors underlying the trait.

CHAPTER TWO

Materials and Methods

Study Animals

This study focused on the difference in xanthophore pigmentation in *Maylandia benetos* and *Maylandia zebra.* These species are closely related members of the *mbuna* clade and are sympatric at Mazinzi Reef in the southeast arm of Lake Malawi, Africa (Stauffer *et al.*, 1997). *Maylandia benetos* and *M. zebra* are similar ecologically, morphologically, and behaviorally, yet they can be distinguished by adult pigmentation patterns (Stauffer et al., (1997). Territorial *M. zebra* males possess a bright, blue background interrupted by five to seven dorso-ventral black body bars and prominently black-pigmented pelvic fins. Dominant *M. benetos* share the same blue background, lack the melanistic body bars and pelvic fin pigmentation, and possess yellow-pigmented regions located on the breast and caudal, dorsal, pectoral, and pelvic fins [Fig.1]. Females of both species are drab olive to brown in coloration with less conspicuous vertical bars.

Pigmentation Phenotype

Pigmentation patterns of the *mbuna* cichlids are the product of three cell types: black melanophores, iridescent iridophores, and the yellow-orange xanthophores (Mills and Patterson, 2009). The pigments of the melanophores and xanthophores are under physiological control and are associated with a microtubule system

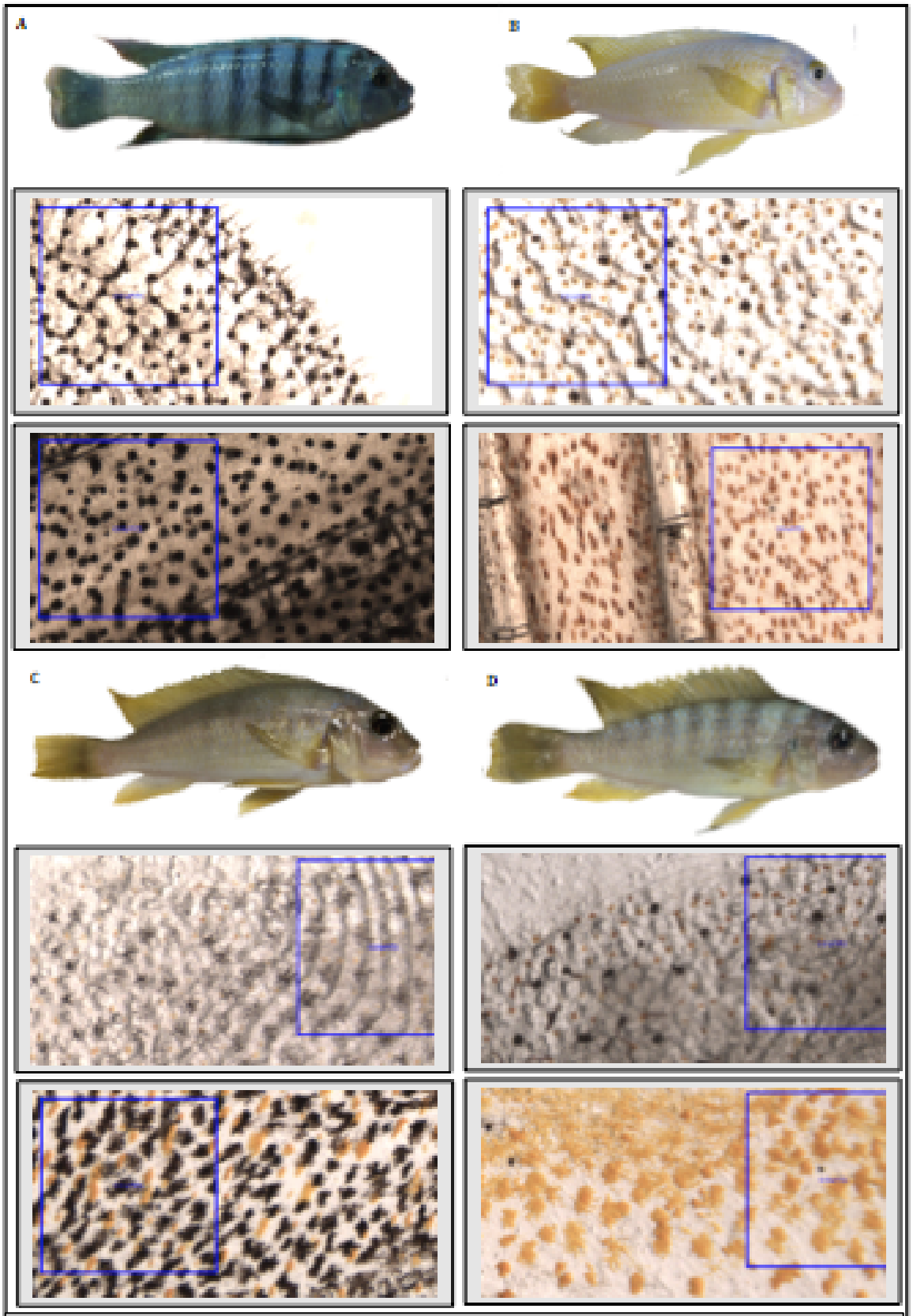

Fig 1: *M. zebra* male (A), female (C) & *M. benetos* male (B) female (D) and their scales and fins.

capable of aggregating and dispersing intracellular pigments to and away from the cell's center (Matsumoto et al., 1977; Obika, 1986). Identifying individual cells is difficult when the pigment is dispersed throughout the cell's cytoplasm. Stimulating pigment contraction with an aggregating solution (Ohta, 1974), however, facilitates the identification and quantification of chromatophore cells. Xanthophores in particular are rendered easily visible and quantifiable under a light microscope due to intracellular pteridine and carotenoid pigments (Matsumoto et al., 1977; Obika, 1986).

Producing Hybrids

To generate hybrids from the two reproductively isolated study species, artificial fertilization was required. The abdomens of male and female fish were gently compressed to release sperm and eggs into a petri dish. The eggs were mixed with the sperm for five minutes and the fertilized eggs were incubated at 28 °C until hatched larvae were capable of swimming independently. Both the *M. zebra* (♀) x *M. benetos* (♂) cross and its reciprocal were performed, however the reciprocal cross failed to generate any male offspring. Thus all subsequent hybrid generations were created using offspring of the *M. zebra* (♀) x *M. benetos (♂)* cross. Upon reaching sexual maturity, F_1 hybrids were randomly selected to produce the F_2 generation as well as backcrosses to *M. benetos* (BCB) and *M. zebra* (BCZ) lines. Water temperature was kept between 26° and 28°C. Light cycles within the lab were kept at diurnal 12-hour light/dark cycles. Fish were fed commercial fish food twice daily.

Phenotyping Color Pattern

Scales and pelvic fins were collected from > 1 year old fish in order to quantify xanthophore density of the parental species and resulting hybrid generations. Because observed sexual dimorphisms in pigmentation at the gross level do not necessarily imply differences at the cellular level, scales and fins were removed from both males and females. Pelvic fins and scales sampled from the first body bar directly below the lateral line were treated with a K^+ rich aggregating fluid (Ohta 1974) to aggregate the chromatophores. After photographing the tissues at 30x magnification with a Nikon SMZ1500 stereomicroscope, xanthophores were quantified by counting individual xanthophores in a $0.25mm^2$ area of highest xanthophore density on each tissue.

Statistical Analyses

In total, 377 fish were phenotyped [Table: 1]. Of these, there were 44 *M. benetos* (25 ♂, 19 ♀), 39 *M. zebra* (20 ♂, 19 ♀), 33 F_1 hybrids (17 ♂, 16 ♀) produced with (♀ *M. zebra* x ♂ *M. benetos*), 218 F_2 hybrids (58 ♂, 160 ♀) produced from three independent crosses, 13 backcrossed progeny (10 ♂, 3 ♀) of an (F_1 hybrid x *M. benetos*) and 30 backcrossed progeny (16 ♂, 14 ♀) of an (F_1 hybrid x *M. zebra*). All xanthophore counts were square root transformed before statistical analysis to increase the normality of the data. A Shapiro-Wilk test of normality indicated that the square-root transformation normalized all data sets except for the following: male *M. zebra* scales ($p < 0.005$) and fins ($p < 0.005$), F_2 hybrid fins ($p < 0.005$),

backcrossed M. zebra scales ($p = 0.046$) and female F_2 hybrid scales ($p < 0.005$) [Fig 2].

A one-way ANOVA was run for both parental lines to determine whether xanthophore counts differed between males and females. An ANOVA and subsequent Tukey Comparison tests were then conducted to determine the differences in counts between the parental species and the various hybrid lines. This and all subsequent statistical tests were performed with R Statistical Software (R Development Core Team, 2012).

To explore the genetic architecture of xanthophore pigmentation, quantitative genetic analysis was used to estimate the mode of gene action and the number of genetic factors. Mode of gene action, which describes the influence of additive, dominance, and epistatic effects on a phenotype, was calculated using the joint-scaling test (Cavalli 1952; Hayman 1960). This test evaluates a genetic model's adequacy of fit by estimating the model parameters and then comparing them to the observed line means via least squares regression:

$$\bar{z}_i = \mu_0 + \theta_{Si}\alpha_i^c + \theta_{Hi}\delta_i^c + e_i$$

where $\bar{z}$ is a matrix output of line means' parameter estimates, μ_0 is the matrix of all line means, $\theta_{Si}\alpha_i^c$ and $\theta_{Hi}\delta_1^c$ are the genetic effects of additivity and dominance respectively, and e_i denotes the line means' deviations from the model's prediction. Though insufficient line means were available for analysis of epistatic effects, the above model includes the effects of epistasis in its error calculation.

The number of effective genetic factors required to explain the observed means and variances of the parentals and subsequent line crosses were approximated

using the Castle-Wright estimator as described in Lynch and Walsh (1998). This biometrical approach uses the observed means and variances of the parentals and the calculated segregational variance to produce a biased estimate of genetic factors:

$$\hat{n}_e = \frac{[\overline{z}(\mathrm{P}_1) - \overline{z}(\mathrm{P}_2)]^2 - \mathrm{Var}[\overline{z}(\mathrm{P}_1)] - \mathrm{Var}[\overline{z}(\mathrm{P}_2)]}{8\mathrm{Var}(S)}$$

where $\hat{n}_e$ is the estimated number of factors, $\overline{z}(\mathrm{P}_i)$ and $\mathrm{Var}[\overline{z}(\mathrm{P}_i)]$ denotes the observed means and variances of the parental lines, and $\mathrm{Var}(S)$ represents the estimated segregational variation. The segregational variance was calculated via the weighted least squared method.

Pearson's test of correlation was used to determine whether scales and fins shared a common genetic basis.

CHAPTER THREE

Results:

Sexual Dimorphism in Xanthophores Pigmentation

Significant sexual dimorphism in xanthophore density was observed in many of the lines (Fig 2).

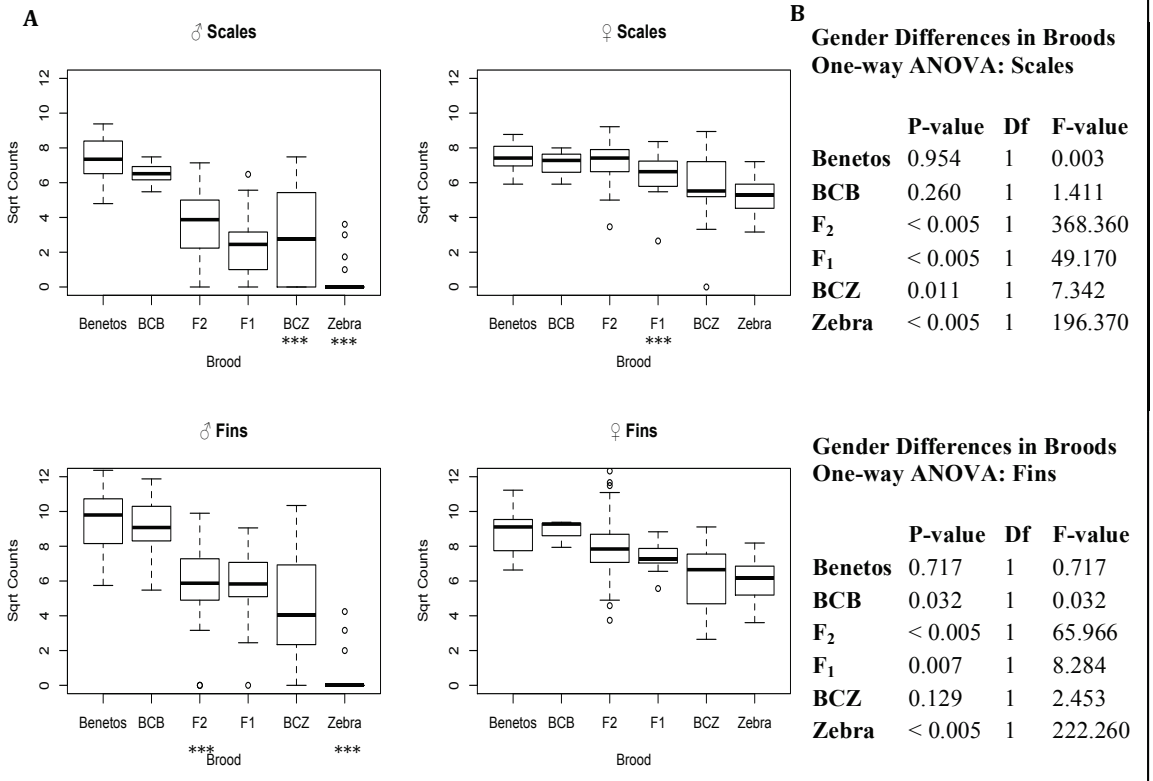

Fig. 2: (A) Boxplots of phenotypic distributions of parentals and hybrids for both genders. Asterisks indicate unnormalized data. (B) One-way ANOVA between broods for scales and fins. $p < 0.05$ indicates significant difference in gender counts.

Maylandia zebra is sexually dimorphic for both scale ($F = 96.370$, df: 1, $p < 0.005$) and fin ($F = 222.260$, df:1, $p < 0.005$) xanthophores. In contrast, *M. benetos* is not

sexually dimorphic for either phenotype (scales: F = 0.003, df = 1, p = 0.954; fins: F = 0.566, df = 1, p = 0.456). Many of the hybrid phenotypes exhibited sexual dimorphism: F_1 scales (F = 49.170, df = 1, p < 0.005) and fins (F = 8.284, df = 1, p = 0.007); F_2 scales (F = 368.360, df = 1, p < 0.005) and fins (F = 65.966, df = 1, p < 0.005); and backcross *M. zebra* scales (F = 7.342, df = 1, p = 0.011). However, sexual dimorphism was not observed in the backcross *M. benetos* scales (F = 1.411, df = 1, p = 0.260) and fins (F = 0.032, df = 1, p = 0.862) and backcross *M. zebra* fins (F = 2.453, df = 1, p = 0.129).

Differences Exist Between Genetic Lines

Table 1: Mean(Var) of broods and genders. P-value < 0.05 indicate significant difference between parental counts.

Male	***M. benetos***	***M. zebra***	**P-value**	**F_1 hybrid**	**F_2 hybrid**	**BCB**	**BCZ**
Scales	56.95(368.39)	1.30(11.91)	< 0.005	9.00(138.88)	16.67(184.29)	42.80(59.29)	17.19(385.10)
Fins	92.68(1209.90)	1.60(20.46)	< 0.005	37.47(433.14)	39.66(535.35)	84.70(932.01)	31.31(1259.30)
Female	***M. benetos***	***M. zebra***	**P-value**	**F_1 hybrid**	**F_2 hybrid**	**BCB**	**BCZ**
Scales	57.05(145.72)	28.47(123.26)	<0.005	42.75(230.73)	53.28(165.98)	50.67(214.33)	36.00(416.15)
Fins	80.47(507.60)	38.05(185.83)	<0.005	55.13(126.38)	64.69(500.36)	79.00(193.00)	42.14(618.75)

A one-way ANOVA's performed on both scales (F = 42.579, df = 5, p < 0.005) and fins (F = 44.344, df = 5, p < 0.005) revealed that significant difference in means existed among the male *M. benetos, M. zebra* and their line-crosses. The Post-hoc Tukey multiple comparisons tests (Table 2) revealed that xanthophore count means were significantly higher *in M. benetos* than *M. zebra* in both scales (p adj < 0.005) and fins (p adj < 0.005).

Table 2: Tukey Multiple Comparison tests comparing differences between broods for both scales and fins.

Scales	**Zebra**	**Benetos**	**BCB**	**F_2**	**F_1**	**Fins**	**Zebra**	**Benetos**	**BCB**	**F_2**	**F_1**
Benetos	< 0.005					**Benetos**	< 0.005				
BCB	< 0.005	0.692				**BCB**	< 0.005	0.999			
F_2	< 0.005	< 0.005	< 0.005			**F_2**	< 0.005	< 0.005	< 0.005		
F_1	0.022	< 0.005	< 0.005	0.116		**F_1**	< 0.005	< 0.005	< 0.005	0.999	
BCZ	< 0.005	< 0.005	< 0.005	0.945	0.791	**BCZ**	< 0.005	< 0.005	< 0.005	0.177	0.548

Quantitative Genetic Analysis for Fins and Scales

Likelihood ratio statistics (Λ) calculated for both groups indicated that while the additive-dominance model did not improve the fit of the model in the scales (p = 0.375), the model fit was significantly improved in fins (p = 0.001) upon switching to the additive-dominance mode (Table 3). Genetic factor analysis using the Castle-Wright equation estimated that 4.474 (s.d. = 6.380) and 2.970 (s.d. = 2.327) genetic factors influence scale and fin xanthophore phenotypes, respectively.

Table 3: Results of the joint scaling test displaying the observed and expected line means for each model for both scales and fins. χ^2 and p values as well as the Likelihood Ratio statistic are reported directly below.

Scales	**Observed**	**$\hat{z}$ additive**	**$\hat{z}$ additive-dominance**	**Fins**	**Observed**	**$\hat{z}$ additive**	**$\hat{z}$ additive-dominance**
M. benetos	7.43 (0.31)	7.85 (0.19)	8.08 (0.27)	***M. benetos***	9.45 (0.43)	10.43 (0.31)	9.78 (0.40)
M. zebra	0.47 (0.24)	0.33 (0.21)	0.44 (0.23)	***M. zebra***	0.47 (0.27)	0.80 (0.24)	0.54 (0.26)
F_1	2.35 (0.47)	4.09 (0.11)	3.76 (0.29)	**F_1**	5.76 (0.52)	5.62 (0.16)	6.51 (0.37)
F_2	3.61 (0.25)	4.09 (0.11)	4.01 (0.13)	**F_2**	5.94 (0.28)	5.62 (0.16)	5.84 (0.27)
BCB	6.52 (0.19)	5.97 (0.13)	5.92 (0.14)	**BCB**	9.05 (0.55)	8.03 (0.22)	8.15 (0.27)
BCZ	3.15 (0.70)	2.21 (0.15)	2.10 (0.17)	**BCZ**	4.53 (0.85)	3.21 (0.17)	3.53 (0.21)
	χ^2	30.38	29.60		**χ^2**	18.50	7.87
	p	4.09E-06	1.68E-06		p	0.001	0.049
			Λ – 0.375				**Λ** – 0.001

Correlation Between Scales and Fins

A positive Pearson's product-moment correlation was observed between both the scales and fins of the F_2 males (r = 0.339, df = 56, p = 0.009).

CHAPTER FOUR

Discussion and Conclusion

Salient differences in pigmentation patterns within dimorphic species and between sympatric congeners are hallmark characteristic of the rock-dwelling cichlids of Lake Malawi. Coloration at the gross level is mediated by physiological color change, however, so differences in pigmentation may not be marginally different between groups cytologically. After controlling for the effects of physiological color change, sexual dimorphisms in xanthophore counts were observed in *M. zebra* but not *M. benetos*. Sexual dimorphism is a clear evidence of epistasis with sex determining genes. To reduce the effect of epistasis in the data set, only male counts were used to estimate genetic factors.

Consideration of coloration at the cytological rather than the gross level can increase a study's sample size. As seen in *M. benetos* counts, differences between the two genders may be a reflection of variation in physiological control rather than differences in the number of pigment cells. If no significant differences exist between genders at the cellular level for both lines, pooling male and female counts together would be appropriate. Gender dimorphisms in our study, however, precluded the use of female data.

Multiple Comparisons tests reported that significant differences do exist between the parentals and line derivatives at the cytological level. This evidence confirms a genetic basis for differences between lines and legitimizes the use of the

Castle-Wright estimator to elucidate the number of genetic factors that differ between the groups.

Utilizing a modified version of the Castle-Wright equation, results of this study suggest that few genetic factors underlie xanthophore pigmentation between the closely related sympatric species pair *M. benetos* and *M. zebra*. This conclusion, that few genetic factors underlie male pigmentation, is corroborated by other studies reporting similar trends. Using the same analysis, Magalhaes and Seehausen (2010) reported that red and yellow coloration in Lake Victorian cichlids are controlled by a minimum of 2-4 loci and a single gene respectively. Working within the *mbuna* clade of Lake Malawi, Barson et al. (2007) reported that 4 – 7 loci underlie blue and yellow body pigmentation and O'Quin (2011) calculated that approximately 1 and 3 genetic factors underlie dorsal and caudal fin yellow pigmentation. However, the simple genetic basis to cichlid pigmentation has not been universally observed (see for example, Ding et al. in prep). Furthermore, each of these studies, including our own, rejected the additive model and additive-dominance model for most pigmentation phenotypes studied. This observation is consistent with the notion that epistatic effects are almost always involved in differentiation of divergent lines (Lynch and Walsh, 1998).

However, several of the assumptions of the Castle-Wright analysis were violated. Specifically, the Castle-Wright estimator assumes additive gene action, a model inconsistent with results of the joint scaling test for both scales and fins. In addition, not all of the data was normally distributed. Most notable of these non-normalized groups was the *M. zebra* counts for both scales and fins due to the counts proximity

to a value of zero. Since violation of the estimator's assumptions can lead to considerable underestimates of the true number of loci (Zeng et al., 1990), the estimates of this study should be considered lower bounds on the total number of actual genetic factors.

Results of the Pearson's product-moment correlation suggest that developmental and regulatory pathways are shared between the scale and pelvic fin phenotypes. This result is consistent with the findings of previous work on covariation in pigmentation. O'Quinn et al. (2011) reported strong positive correlations between xanthophore and melanophore scores at various phenotypes. Barson *et al.* (2007) reported linkage in yellow pigmentation between the belly and dorsal fin. In the context of producing diversity, covariation can constrain the independent evolution of traits and imposes restrictions on phenotypic evolution (Chenoweth and McGuigan, 2010).

Conclusion

We estimate that xanthophore pigmentation in both scales and pelvic fins is regulated by few genes of large effect in a model through genes with epistatic effects. These results provide insight into the genetics of male coloration and add to a body of literature supporting pigmentation patterns' roles in cichlid diversification.

REFERENCES

Ayala FJ, Fitch WM, and N. A. o. Sciences, 1997. Genetics and the origin of species: from Darwin to molecular biology, 60 years after Dobzhansky. *National Academy of Sciences*

Barson NJ, Knight ME, Turner GF., 2007. The genetic architecture of male color differences between a sympatric Lake Malawi cichlid species pair. J Evol Biol 20:45-53

Cavalli L, 1952. An analysis of linkage in quantitative inheritance, pp. 135-144 in *Quantitative Inheritance,* edited by E.C. R. Reeve and C. H. Waddington. Her Majesty's Stationery Office, London.

Chenoweth SF, K. McGuigan, 2010. The Genetic Basis of Sexually Selected Variation. Annual Review of Ecology, Evolution, and Systematics 41(1):81-101.

Danley PD, TD Kocher, 2001. Speciation in rapidly diverging systems: lessons from Lake Malawi. Molecular Ecology 10(5):1075-1086.

Danley PD, M. Husemann, J. Chetta, 2012. Acoustic diversity in Lake Malawi's rock-dwelling cichlids. *Environmental biology of fishes* 93(1):23-30

Deutsch JC, 1997. Colour diversification in Lake Malawi cichlids, evidence for adaptation, reinforcement or sexual selection. *Biological Journal of the Linnaean Society* 62:1-14

Dobzhansky TG, and T. Dobzhansky, 1937. Genetics and the origin of species. Columbia University Press.

Dominey WJ, 1984. Effects of sexual selection and life history on speciation, Species flocks in African cichlids and awaiian Drosophilia. *Evolution of Fish Species Flocks* (eds Echelle AA, Kornfield I), pp. 231-249. University of Maine at Orono Press, Orono, USA.

Genner MJ, MI Taylor, DFR Cleary, SJ Hawkins, ME Knight, and GF Turner, 2004. Beta diversity of rock-restricted cichlid fishes in Lake Malawi: importance of environmental and spatial factors. Ecography **27**:601-610.

Hayman BI, 1960a. The separation of epistatic from additive and dominance variation in generation means 11. *Genetica* 31:133-146.

Hert E, 1991. Female choice based on egg-spots in Pseudotropheus aurora Burgess 1976, a rock-dwelling cichlid of Lake Malawi, Africa. *Journal of Fish Biology* 38(6):951-953.

Holzberg S, 1978. A field and laboratory study of the behavior and ecology of *Pseudotropheus zebra* (Boulenger), an endemic cichlid of Lake Malawi (Pisces; Cichlidae). *Zeitschrift Fuer Zoologische Systematik und Evolutionsforschung* 16:171-187

Kronforst MR, LG Young, DD Kapan, McNeely C, O'Neill RJ, Gilbert LE, 2006. Linkage of butterfly mate preference and wing color preference cue at the genomic location of wingless. *Proceedings of the National Academy of Sciences* 103(17):6575-6580

Lynch M, Walsh B, 1998. Genetics and Analysis of Quantitative Traits. Sinauer.

Magalhaes I, Seehausen O, 2010. Genetics of male nuptial colour divergence between sympatric sister species of a Lake Victoria cichlid fish. *Journal of Evolutionary Biology* 23(5):914-924.

Maitland P, 1994. Conservation of Freshwater Fish in Europe. *Nature and environment series* Issues 18-66

Matsumoto J, Watanabe Y, Obika M, Hadley ME, 1978. Mechanisms controlling pigment movements within swordtail Xiphophorus helleri erythrophores in primary cell culture. *Comparative Biochemistry and Physiology A*. 613:509-517.

McKaye KR, 1991. Sexual selection and the evolution of the cichlid fishes of Lake Malawi, Africa. In: *Cichlid Fishes Behavior*, *Ecology and Evolution* (ed. Keenleyside MHA), 241-257.Chapman & Hall, New York.

Mills MG, Patterson LB, 2009. Not just black and white: Pigment pattern development and evolution in vertebrates. *Semin Cell Dev Biol* 20:72-81

Page L, Brooks M, Burr M, Page LM, 2011. Peterson Field Guide to Freshwater Fishes of North America North of Mexico. *Boston: Houghton Mifflin Harcourt, 2011*. rint.

O'Quin CT, Drilea AC, Roberts RB, Kocher TD, 2012. A Small Number of Genes Underlie Male Pigmentation Traits in Lake Malawi Cichlid Fishes. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 318(3):199-208

Obika M, 1986. Intracellular transport of pigment granules in fish chromatophores. *Zoological science*, 3:1-11

Ohta T, 1974. Movement of Pigment Granules within Melanophores of an Isolated Fish Scale. Effects of Cytochalasin B on Melanophores. *Biological Bulletin* 146(2):258-266.

R Development Core Team, 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, Retrieved from http://www.R-project.org.

Ribbink AJ, Marsh AC, Marsh BC, Ribbink AC, Sharp BJ, 1983. A preliminary survey of the cichlid fishes of the rocky habitats of Lake Malawi. *South African Journal of Zoology* 18(3):149-310.

Seehausen O, van Alphen JJM, 1998. The Effect of Male Coloration on Female Mate Choice in Closely Related Lake Victoria Cichlids (Haplochromis nyererei Complex). *Behavioral Ecology and Sociobiology* 42(1):1-8

Stauffer JR, Bowers NJ, Kellogg KA, McKaye KR, 1997. A revision of the blue-black *Pseudotropheus zebra* (Teleostei, Cichlidae) complex from Lake Malawi, Africa, with a description of a new genus and ten new species. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 148:189-230.

Zeng ZB, Houle D, Cockerham CC, 1990. How Informative is Wright's Estimator of the Number of Genes Affecting a Quantitative Character? *Genetics* 126: 235-247