unesp

CAMPUS DE SÃO JOSÉ DO RIO PRETO

Effects of selection of morphological traits on aggression and physiology in *Pterophyllum scalare*

Felipe Dorigão Guimarães

MESTRADO

PÓS GRADUAÇÃO
EM BIOLOGIA ANIMAL

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

Felipe Dorigão Guimarães

Effects of selection of morphological traits on aggression and physiology in *Pterophyllum scalare*

São José do Rio Preto
2016

Felipe Dorigão Guimarães

Effects of selection of morphological traits on aggression and physiology in *Pterophyllum scalare*

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

Financiadora: CAPES

Orientador: Prof. Dr. Rui Filipe Nunes Pais de Oliveira

Coorientadora: Profa. Dra. Eliane Gonçalves de Freitas

São José do Rio Preto
2016

Guimarães, Felipe Dorigão.
Effects of selection of morphological traits on aggression and physiology in Pterophyllum scalare / Felipe Dorigão Guimarães. -- São José do Rio Preto, 2016
28 f. : il.

Orientador: Rui Filipe Nunes Pais de Oliveira
Coorientador: Eliane Gonçalves de Freitas
Dissertação (mestrado) – Universidade Estadual Paulista “Júlio de Mesquita Filho”, Instituto de Biociências, Letras e Ciências Exatas

1. Biologia de água doce. 2. Peixe ornamental - Comportamento. 3. Acará (Peixe) 4. Scalare. 5. Domesticação. 6. Animais - Comportamento agressivo. 7. Andrógenos. I. Oliveira, R. II. Freitas, Eliane Gonçalves de. III. Universidade Estadual Paulista "Júlio de Mesquita Filho". Instituto de Biociências, Letras e Ciências Exatas. IV. Título.

CDU – 597

Ficha catalográfica elaborada pela Biblioteca do IBILCE
UNESP - Câmpus de São José do Rio Preto

Felipe Dorigão Guimarães

Effects of selection of morphological traits on aggression and physiology in *Pterophyllum scalare*

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

Financiadora: CAPES

Comissão Examinadora

Prof. Dr. Rui Filipe Nunes Pais de Oliveira
ISPA - Lisboa
Orientador

Prof. Dr. Matias Pandolfi
Facultad de Ciencias Exactas y Naturales – Buenos Aires

Profa. Dra. Ana Célia Silva Barbato
Universidad de la República – Montevidéu

São José do Rio Preto
31 de agosto de 2016

Resumo

Domesticação animal tipicamente seleciona *loci* gênicos que controlam um traço de interesse humano, podendo levar à seleção indireta de outros traços devido aos efeitos pleiotrópicos. Aqui, nós testamos como a seleção artificial pela coloração amarela que ocorreu durante a domesticação da linhagem doméstica *new gold* do ciclídeo acará-bandeira (*Pterophyllum scalare*) afetou a agressão e os níveis de andrógenos. Esta espécie amazônica apresenta várias linhagens domésticas ornamentais. Nós comparamos a coloração, comprimento das nadadeiras, agressão e níveis de andrógenos (testosterona e 11-cetotestosterona) entre as linhagens selvagem e doméstica. Machos selvagens eram mais negros, mais agressivos e apresentaram maior índice de metabolização de 11KT do que as fêmeas selvagens. Machos domésticos tinham nadadeiras dorsais maiores e menor área corporal amarela do que as fêmeas domésticas. Nadadeira anal menor foi indiretamente selecionada na linhagem doméstica. Fêmeas domésticas foram mais agressivas (em ambos os testes do espelho e intruso) e apresentaram maior metabolização de 11KT do que as fêmeas selvagens. Machos domésticos foram mais agressivos contra o espelho, mas exibiram menor agressão contra o intruso e tiveram menores níveis de T quando comparados com os machos selvagens. 11KT e agressividade estavam correlacionados significativamente somente em fêmeas selvagens. Assim, os efeitos da domesticação sobre uma espécie de ciclídeo monomórfica parece ser sexo-específica.

Palavras-chave: domesticação; agressividade; integração fenotípica; andrógenos; 11-cetotestosterona; testosterona; acará-bandeira

Abstract

Animal domestication typically selects genetic loci that control a trait of human interest, potentially leading to indirect selection of other traits, due to pleiotropic effects. Here, we tested how artificial selection for yellow coloration that occurred during the domestication of the new gold domestic strain of the cichlid angelfish (*Pterophyllum scalare*) affects aggression and androgen levels. This Amazonian species has several domesticated strains of ornamental fish. We compared the coloration, fin length, aggression, and androgen levels (testosterone and 11-ketotestosterone) between wild and domestic strains. Wild type males were darker, more aggressive and had a higher 11KT metabolization index than wild type females. Domesticated line males had larger dorsal fins and smaller yellow body surface area than domesticated line females. Smaller anal fins were indirectly selected in domestic strain. Domestic females were more aggressive (in both mirror and intruder tests) and showed higher 11KT metabolization than wild females. Domestic males were more aggressive against the mirror, but exhibit less aggression against the intruder and showed lower levels of T when compared with the wild males. 11KT and aggressiveness were significantly correlated only in wild females. Thus, the effects of domestication in a monomorphic cichlid species seem to be sex-specific.

Keywords: domestication; aggressiveness; phenotypic integration; androgens; 11-ketotestosterone; testosterone; angelfish

Sumário

Effects of selection of morphological traits on aggression and physiology in *Pterophyllum scalare*

Felipe D. Guimarães[a], Eliane Gonçalves-de-Freitas[ab], Rui F. Oliveira[cde]

[a] Instituto de Biociências, Letras e Ciências Exatas da UNESP, Rua Cristóvão Colombo 2265, 15054-000 São José do Rio Preto, Brasil

[b] Centro de Aquicultura da UNESP, Via de Acesso Prof. Paulo Donato Castellane, 14884-900 Jaboticabal, Brasil

[c] ISPA — Instituto Universitário, Rua Jardim do Tabaco 34, 1149-041 Lisboa, Portugal

[d] Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 27å80-156 Oeiras, Portugal

[e] Champalimaud Neuroscience Program, Av. Brasília, Doca de Pedrouços, 1400-038 Lisboa, Portugal

Abstract

Animal domestication typically selects genetic loci that control a trait of human interest, potentially leading to indirect selection of other traits, due to pleiotropic effects. Here, we tested how artificial selection for yellow coloration that occurred during the domestication of the new gold domestic strain of the cichlid angelfish (*Pterophyllum scalare*) affects aggression and androgen levels. This Amazonian species has several domesticated strains of ornamental fish. We compared the coloration, fin length, aggression, and androgen levels (testosterone and 11-ketotestosterone) between wild and domestic strains. Wild type males were darker, more aggressive and had a higher 11KT metabolization index than wild type females. Domesticated line males had larger dorsal fins and smaller yellow body surface area than domesticated line females. Smaller anal fins were indirectly selected in domestic strain. Domestic females were more aggressive (in both mirror and intruder tests) and showed higher 11KT metabolization than wild females. Domestic males were more aggressive against the mirror, but exhibit less aggression against the intruder and showed lower levels of T when compared with the wild males. 11KT and aggressiveness were significantly correlated only in wild females. Thus, the effects of domestication in a monomorphic cichlid species seem to be sex-specific.

Keywords: domestication; aggressiveness; phenotypic integration; androgens; 11-ketotestosterone; testosterone; angelfish

Introduction

Domestication occurs through the mutualistic interaction between a domesticator and a domesticated animal that leads to a series of genotypic and phenotypic changes (Zeder, 2015). Although domestication is focused on the selection of certain genetic loci that underlie animal traits of interest to humans (Rubin et al., 2010), domestication may cause a series of secondary unintended changes at multiple levels, such as morphological (Wilkins et al., 2014), hormonal (Künzl and Sachser, 1999) and behavioral (Huntingford, 2004; Price, 1999) that also become fixed in domesticated populations. Therefore an ensemble of traits typically emerges in domesticated organisms that have been described as a domestication syndrome (e.g. plants: Brown et al., 2009; mammals: Wilkins et al., 2014). These domestication syndromes are often caused by a shared mechanisms, which can be genetic (Rubin et al., 2010) or hormonal (Badyaev, 2004; Ketterson et al., 2009), underlying the traits involved, that result in a pleiotropic effect. The potential role of hormones on domestication syndromes is particularly interesting because selection can act at two different levels in endocrine systems, the hormone itself and sensitivity of the target tissue to the hormone, with opposite consequences for phenotypic integration. Selection on signal strength (i.e. hormone levels) will equally affect all traits dependent on that hormone, hence promoting phenotypic correlation among these traits and consequently generating a hormone dependent syndrome (McGlothlin et al., 2010, 2008). In contrast selection on the sensitivity of the target tissue to the hormone (e.g. variation in hormone receptor expression), will allow a compartmentalization of the selected trait from other traits that depend on the same hormone (Rosvall et al., 2012). Thus, depending on the mode of selection on the hormonal mechanism either phenotypic integration or modularization

may evolve, with the former being predicted when all hormone-dependent traits are adaptive and the later when contrasting selective pressures act on different hormone dependent traits (McGlothlin and Ketterson, 2008).

The cichlid angelfish, *Pterophyllum scalare* (Schultze, 1823), is an Amazonian species of great interest for fish hobbyists around the world (Chapman et al., 1997). Several strains have been obtained through artificial selection focused on elaborate ornaments (e.g. bright colors and long fins), yielding strains with different morphological phenotypes (Goldstein, 2001), which provide interesting models to test hypothesis on domestication mechanisms. Although the effects of domestication on feeding, reproductive, aggressive and anti-predatory behavior have already been described in some species (Huntingford, 2004; El Balaa and Blouin-Demers, 2011), little is known on how domestication acts on the mechanisms underlying the integration of behavior, hormones and morphological traits. In one of the ornamental strains of *P. scalare*, the new gold angelfish, individuals have a marked reduction in melanisation presenting a bright silver body with a yellow-pigmented dorsal region (Goldstein, 2001). Since melanin-based dark coloration is known to be an androgen-dependent trait in many vertebrates including cichlid fish (reviews by Hill and McGraw 2003; Jawor and Breitwisch, 2003; McGraw 2006; cichlids: Oliveira and Almada, 1998), and in fish melanin accumulation has been shown to be affected by androgens in a dose-dependent manner (Adachi et al., 2010) , this strain offers an excellent opportunity to test the hypotheses detailed above on the potentially role of hormones (in this case androgens) on domestication syndromes. Thus, in this study we compared the new gold strain (Figure 1A) with wild type fish (Fig. 1B) to test whether the selection for reduced melanisation in this strain has also selected against other androgen-dependent traits, namely fin length and aggression, and androgen levels.

Material and Methods

Animals

P.scalare wild type adults (male: n=10; female: n=20) were collected from Amazon River basin in Brazil in January 2015 by commercial suppliers and transported to Universidade Federal do Amazonas (Manaus). New gold domestic strain adults (male: n=16; female: n=14) were provided by the Ornamental fish lab of the Aquaculture Center of UNESP (Jaboticabal) and transported to UNESP campus (São José do Rio Preto) in February 2016. Thus, both wild type and domestic fish were tested in the summer and care was taken to provide the same conditions both in both labs (Manaus and São José do Rio Preto). Both wild type and domestic strains were acclimated in the lab for at least 20 days in 500L polypropylene water tanks before being tested, and were kept at a temperature of 29º C and a photoperiod of 12L:12D (lights on from from 7:00 to 19:00). Fish were fed twice a day until satiety using a *P. scalare* specific comercial ration (TetraMin, Tetra).

Experimental procedures

Wild type and domestic fish were tested for differences in coloration (relative body surface melanised), morphology (dorsal, anal and caudal fin length), aggressive behavior (directed towards a mirror-image and towards an intruder fish) and androgen levels [Testosterone (T) and 11-Ketotestosterone (11KT)].

Fish were anesthetized with benzocaine (55mg/L), photographed from their left side of the body, individually tagged with with Visible Implant Elastomer (Northwest Marine Technology, Inc.) (Goldsmith et al., 2003), and placed in a pre-test aquarium

(45 cm X 80 cm X 30 cm) in groups of six fish for 24 hours. After this period, they were isolated in a test aquarium (40 cm X 30 cm X 40 cm) for 2 hours before the aggression tests begin. After these tests were concluded in the first day, fish returned to the pre-test aquarium for 20 hours, and then the aggression tests were repeated. Then, fish were anesthetized with benzocaine (55mg/L) for blood collection and subsequently killed by benzocaine overdose (110mg/L). Since this species is not sexually dimorphic (Chien, 1973), sex was assessed post-mortem by direct inspection of the gonads.

Coloration and morphological measurements

The software ImageJ was used to measure (from scaled pictures) fish standard length, anal, caudal and dorsal fin length (i.e. distance from insertion to tip of longest ray on each fin), pigmentation area (dark and yellow) and body lateral surface area. In order to correct for body size absolute fin lengths were expressed as relative fin lengths (i.e. divided by standard body length). Pigmentation data was expressed as relative area as percentage of lateral body surface area.

Behavioral measurements

After the 2 hours of isolation, a mirror was placed aside the aquarium and the agonistic response towards the mirror was video recorded for 15 minutes (similar to Cacho et al., 2006). Then, the mirror was removed, and 45 min later a small transparent container (13 cm X 8 cm X 11.5 cm) with an intruder fish inside was placed inside the test aquarium. The agonistic interaction was also video recorded for 15 min (modified from Perreault et al., 2003). The intruder was then removed from the test aquarium and the fish stayed

alone for 45 minutes before being placed again in the group in the pre-test aquarium. These aggression tests were repeated on the next day. Video-recordings were analysed and the following behavioral measures were taken for each test on each day: latency to attack (mirror or intruder); frequency of agonistic behavior (towards mirror or intruder).

Hormone measurements

We collected blood (80 – 150 μL) from the caudal vein using a 0.5 mL syringe and a heparinized needle. Blood samples were centrifuged for 15 minutes at 5,000 rpm, and plasma samples were frozen at -20ºC. T and 11KT levels were measured by using enzyme immunoassay kits (IBL International RE52151, Cayman Chemical #582751, respectively) following the manufacturer's instructions. Intra-assay coefficients of variation were 7.75% for 11KT and 3.14% for T. Due to small volume of blood collected from each fish in some samples it was only possible to assay one hormone and priority was given to T (T was assayed from 9 wild females, 8 wild males, 9 domestic females and 13 domestic males; 11KT were assayed from 5 wild females, 4 wild males, 9 domestic females and 13 domestic males). Since 11KT is a metabolite of T a metabolization index was also computed as 11KT/ (11KT + T) (after Oliveira et al., 2001).

Statistical analysis

Mean of two days behavioral, morphological and hormonal data were tested for outliers by Grubbs' test, and removed from the data. Then, data were transformed by log+1 for comparisons between sex intra-strain and between strains. They were contrasted by

Planned Comparisons, whereas parametric analyses are more robust than nonparametric. Spearman's rank was used to analyze correlations between morphological, hormonal and behavioral variables and to test how these phenotypes are integrated in each sex and strain. Statistical tests were performed using the software STATISTICA v.13 with significance set to p-level of 0.05.

Results

Males vs. females

Wild type male had a larger dark body surface area ($F_{(1,48)}$=12.94, p<0.01) (Fig. 1F), a higher frequency of attacks against the intruder ($F_{(1,48)}$=5.61, p=0.02) (Fig. 2D) and a higher 11KT metabolization index ($F_{(1,48)}$=4.27, p=0.05) (Fig. 3B) than wild type female. Domesticated females showed larger yellow body surface area ($F_{(1,48)}$=5.53, p=0.02) (Fig. 1E) and smaller dorsal fin ($F_{(1,48)}$=3.91, p=0.053) than domesticated males (Fig. 1C).

Fin length

There were no significant differences between strains regarding the relative length of both the dorsal (males: $F_{(1,48)}$<0.01, p=0.93; females: $F_{(1,48)}$=0.13; p=0.71) (Fig. 1C) and caudal fins (males: $F_{(1,48)}$=2.09; p=0.15; females: $F_{(1,48)}$=0.43; p=0.51) (Fig. 1D), and the

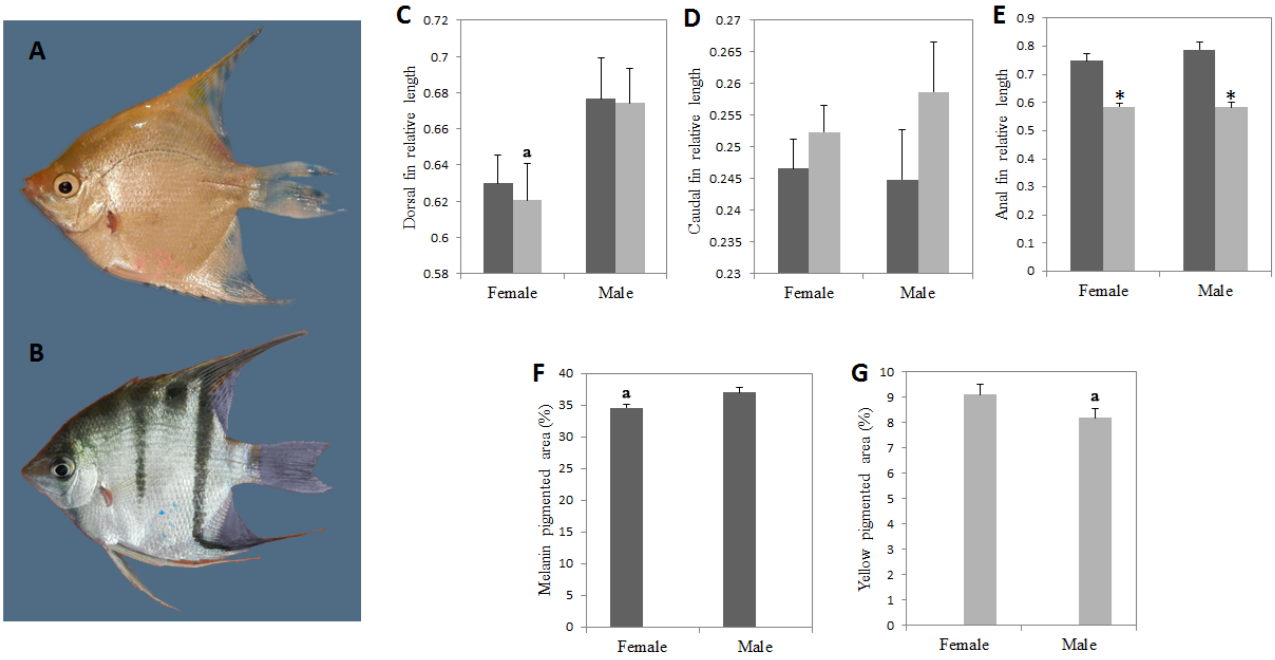

Figure 1. Comparison of morphologic traits (mean ± standard error) between domesticated (A; gray bars in C-G) and wild type (B; black bars in C-G) strains: (A) lateral view of a gold domesticated strain specimen; (B) lateral view of a wild type specimen; (C) Dorsal fin length; (D) caudal fin length; (E) anal fin length; (F) Melanin pigmented body surface area; (G) Yellow body surface area. Asterisks indicate a significant difference between strains. [a] indicates a significant intra-strain difference between sex. Planned comparisons, $p \leq 0.05$.

domestic strain had a smaller anal fin than wild types (males: $F_{(1,48)}=42.89$, $p<0.001$; females: $F_{(1,48)}=31.19$, $p<0.001$) (Fig. 1E).

Aggressiveness

The domestic strain had a shorter latency to attack the mirror (males: $F_{(1,48)}=11.34$, $p<0.01$; females: $F_{(1,48)}=19.97$, $p<0.01$) (Fig. 2A) and the intruder (females: $F_{(1,48)}=14.54$, $p<0.01$; males: $F_{(1,48)}=5.32$, $p=0.03$) (Fig. 2C). Additionally, when compared to wild types, domestic females, but not males, exhibited a higher frequency of attacks towards the mirror (females: $F_{(1,48)}=7.06$; $p=0.01$; males: $F_{(1,48)}=0.72$, $p=0.40$)

(Fig. 2B). On the other hand, domestic males, but not females, showed a lower frequency of attacks towards the intruder than wild types (females: $F_{(1,48)}$=0.26, p=0.61; males: $F_{(1,56)}$=7.58, p=0.01) (Fig. 2D).

Androgens

Wild type males, but not females, had higher T levels (males: $F_{(1,30)}$=5.59, p=0.04; females: $F_{(1,30)}$=3.02, p=0.09) (Fig. 3A) than domesticated line males. Domesticated line females, but not males, had higher 11KT metabolization index (males: $F_{(1,22)}$=0.83, p=0.37; females: $F_{(1,22)}$=4.87, p=0.04) (Fig. 3C). There was no difference in 11KT levels between strains (males: $F_{(1,23)}$=0.74, p=0.40; females: $F_{(1,23)}$=1.79, p=0.19) (Fig. 3B).

Phenotypic Correlations

Wild type females presented negative correlations between Frequency of attacks against the intruder and Latency to attack the intruder, Frequency of attacks against the mirror and Latency to attack the mirror, Frequency of attacks against the mirror and Latency to attack the intruder, Frequency of attacks against the intruder and Latency to attack the mirror, 11KT and Latency to attack the intruder, 11KT/T and Latency to attack the mirror (Fig 4A). They also had positive correlations between Latency to attack the mirror and Latency to attack the intruder, Frequency of attacks against the mirror and Frequency of attacks against the intruder, 11KT and Frequency of attacks against the intruder, 11KT/T and Frequency of attacks against the mirror (Fig. 4A). Wild type males presented the following significant positive correlations: Frequency of attacks

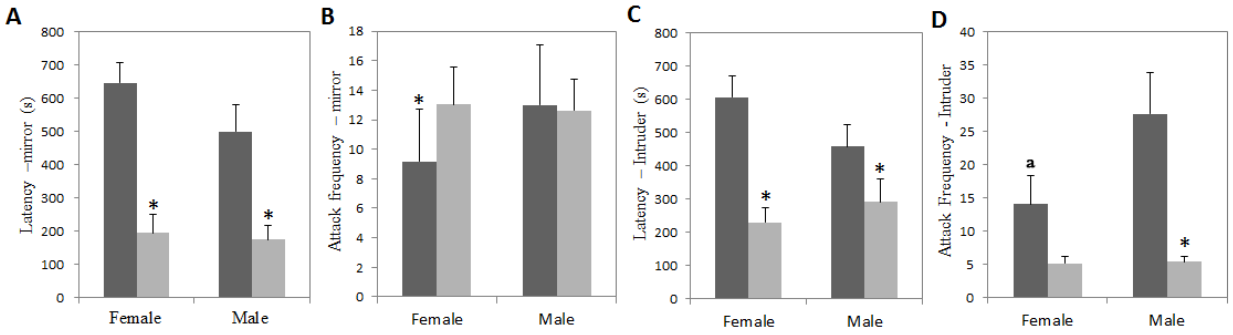

Figure 2. Comparison of aggression (mean ± standard error) between wild type (black bars) and domesticated gold strain (gray bars): (A) latency to attack mirror; (B) Frequency of attacks towards mirror; (C) latency to attack intruder; (D) Frequency of attacks towards intruder. Asterisks indicate a significant difference between strains. [a] indicates a significant intra-strain difference between sex. Planned comparisons, $p \leq 0.05$.

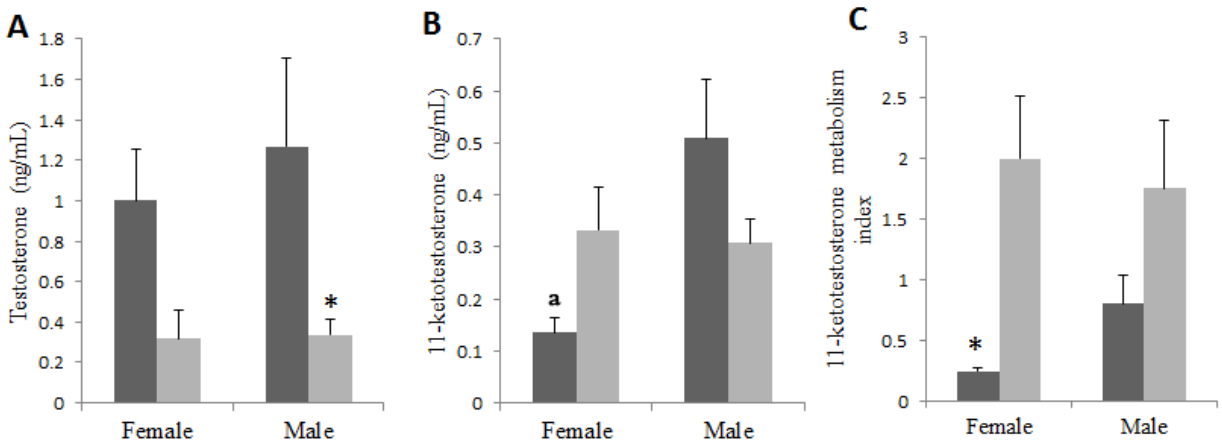

Figure 3. Comparison of androgen levels (mean ± standard error) between wild type (black bars) and domesticated strains (gray bars): (A) testosterone; (B) 11-ketotestosterone; (C) 11-ketotestosterone metabolization index. Asterisks indicate a significant difference. [a] indicates a significant intra-strain difference between sex. Planned comparisons, $p \leq 0.05$.

against the intruder had positive correlations with Dorsal fin and Latency to attack the mirror (Fig. 4B).

Domestic strain females had significant positive correlations between Dorsal fin and Frequency of attacks against the intruder; Dorsal fin and T; Anal fin and %Y; 11KT

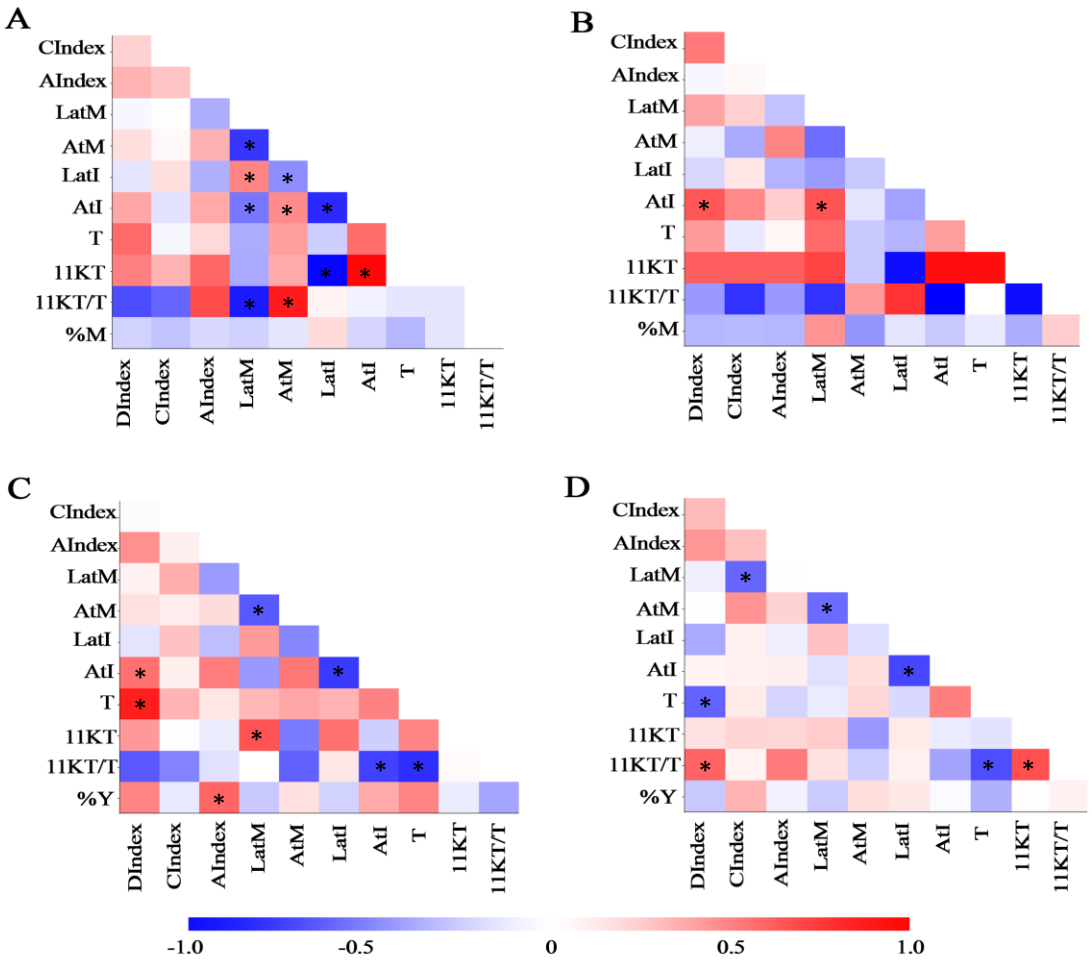

Figure 4. Correlations between measured variables in (A) wild type females, (B) wild type males, (C) domesticated line females and (D) domesticated line males. Dorsal fin relative length (DIndex), caudal fin relative length (CIndex), anal fin relative length (AIndex), latency to attack the mirror (LatM), frequency of attacks against mirror (AtM), Latency to attack the intruder (LatI), frequency of attacks against intruder (AtI), testosterone levels (T), 11-ketotestosterone levels (11KT), 11KT metabolism index (11KT/T), melanin pigmented area (%M) and yellow pigmented area (%Y). Asterisks indicates significant correlations, $p \leq 0.05$. Spearman rank correlation test.

and Latency to attack the mirror (Fig. 4C). They also exhibited negative correlations between Latency to attack the mirror and Frequency of attacks against the mirror; Latency to attack the intruder and Frequency of attacks against the intruder; 11KT/T and Frequency of attacks against the intruder; 11KT/T and T (Fig. 4C). In domestic males there were significant positive correlations between Dorsal fin and 11KT/T;

11KT/T and 11KT. They also presented negative correlations between Dorsal fin and T; T and 11KT/T, Caudal fin and Latency to attack the mirror (Fig. 4D).

Discussion

Indirect selection on fin length during domestication process (shorter anal fin in *P. scalare* domesticated line) has already been observed in other species (Pulcini et al., 2013). In *P. scalare*, females prefer males being bigger, more aggressive and more careful to the offspring (Cacho et al., 2006) and, as there is no apparent sexual dimorphism (wild type: males ~2.5% darker than females; domesticated line: females ~1.0% yellower and with dorsal fin ~5.4% shorter than males), the selection on coloration and fins length was the same in both sexes.

Furthermore, the differences between sex in aggressiveness (Fig. 2E) and 11KT levels (Fig. 3B) in wild type strain do not occur in domesticated line. Under milder environmental pressure conditions, traits that generate costs for the individuals and are no longer needed start to be less expressed. This situation is known as "relaxed selection" (Lahti et al., 2009). Thus, higher T levels in wild type males compared to wild type females that were necessary before for the maintenance of some traits cease to be advantageous, since pressures in artificial environments are softer.

Domestic individuals had higher motivation for aggression, which is indicated by lower Latency to attack the mirror and the intruder and, in domestic females, also higher Frequency of attacks against the mirror. Aggression increasing in domestic individuals has already been observed in other species, but only in males (*Ameca splendens* - Campbell et al., 2015; rainbow trout - Kelley et al., 2006). However, there was an exception for this pattern, since domestic males had lower Frequency of attacks

against the intruder. In this case, the different color pattern (lack of dark coloration) could have acted as an aggression reducer (Beeching, 1995; Reddon and Hurd, 2009). The increase in aggression motivation in both sexes from domestic strain can be related to the captive environment where there are usually high population densities. *P. scalare* has biparental care and both parents attack the intruders (Yamamoto et al., 1999). Thus, both more aggressive male and female would present higher fitness for offspring care. In addition, the lack of predators in an artificial environment makes those individuals reduce their expression of some antipredatory behaviors, diminishing energetic costs (Huntingford, 2004). Thereby, domestic individuals express lower Latency to attack the mirror because they are probably less cautious than wild individuals.

We also can note on wild females that besides the expected relationships between aggressiveness variables (Latency to attack the mirror x Frequency of attacks against the mirror: negative; Latency to attack the intruder x Frequency of attacks against the intruder: negative; Latency to attack the intruder x Latency to attack the mirror: positive; Frequency of attacks against the intruder x Frequency of attacks against the mirror: positive; Latency to attack the mirror x Frequency of attacks against the intruder: negative; Latency to attack the intruder x Frequency of attacks against the mirror: negative), which show the higher aggression motivation, the higher attack frequency, they also presented a strong relationship between aggressiveness and endocrinology parameters (11KT x Frequency of attacks against the intruder: positive; 11KT x Latency to attack the intruder: negative; 11KT/T x Frequency of attacks against the mirror: positive; 11KT/T x Latency to attack the mirror: negative). So, on wild females, 11KT probably plays an important role on aggressive behavior maintenance, once it has a positive relationship with aggressiveness in both tests. Wild males showed an unexpected relationship (Latency to attack the mirror x Frequency of attacks against

the intruder: positive) in which individuals with less motivation to start a fight against the mirror had a higher attack frequency against the intruder. Possibly, it happened because the previous "presence" of a virtual opponent for 15 minutes followed by its absence could have prepared the wild male for the next fight.

We observed in domestic strain that some variables are equally correlated in both sexes, such as the aggressiveness ones (Latency to attack the mirror x Frequency of attacks against the mirror: negative; Latency to attack the intruder x Frequency of attacks against the intruder: negative); which shows that individuals with higher motivation to start a fight also had a higher attack frequency against the opponent, what is expected. However, there were also some differences, such as the relationship between Dorsal fin and T, which are positively correlated in females and negatively correlated in males.

As we stated previously, coloration was the trait selected in this domesticated strain and it was equally expressed in both sexes. Nonetheless, some relationships were modified during the domestication process in *P. scalare*. In females, some of the relationships between the aggressiveness variable remained (Latency to attack the mirror x Frequency of attacks against the mirror: negative; Latency to attack the intruder x Frequency of attacks against the intruder: negative) in the domestic strain. Whereas the positive relationships between aggressiveness and androgens (11KT x Frequency of attacks against the intruder: positive; 11KT x Latency to attack the intruder: negative; 11KT/T x Frequency of attacks against the mirror: positive; 11KT/T x Latency to attack the mirror: negative) became the inverse (11KT x Frequency of attacks against the mirror: positive; 11KT/T x At.I: negative) in domestic females. Probably, the mechanisms related to aggression selected on the domestic females are different, because even having smaller T levels, higher 11KT/T and positive correlation

between 11KT and Latency to attack the mirror domestic females are more aggressive than wild ones. In addition, along with the appearance of yellow pigmentation during the domestication process there was the occurrence of a positive correlation between Anal fin and %Y, which only exists in domestic females. They also presented a positive correlation between Dorsal fin and Frequency of attacks against the intruder, similarly to wild males.

In domestic males, there was the extinction of the correlations present in wild males (Frequency of attacks against the intruder x Dorsal fin: positive; Frequency of attacks against the intruder x Latency to attack the mirror: positive) and the appearance of correlations between the aggressiveness variables similarly to domestic female (Latency to attack the mirror x Frequency of attacks against the mirror: negative; Latency to attack the intruder x Frequency of attacks against the intruder: negative). In addition, there was a surge of some correlations between morphology and behavior (Caudal fin x Latency to attack the mirror: negative), morphology and endocrine parameters (Dorsal fin x T: negative; Dorsal fin x 11KT/T: positive) and between physiological variables (11KT/T x 11KT: positive; 11KT/T x T: negative).

Unlike the mammals that usually become more docile after the domestication process (Wilkins et al., 2014) and, in some species, an arise on androgen levels are observed (Blottner et al., 2000; Künzl and Sachser, 1999), it is common to observe in fishes an aggression increase as a domestication product (Campbell et al., 2015; Kelley et al., 2006), as we found in this study. Furthermore, the aggression increasing in domesticated line females with no hormonal level changes by domestication gathered to the increasing in motivation to attack the mirror and the intruder with a decrease in T levels in domesticated line males suggest that other physiological mechanisms, such as

the tissue sensibility to androgens (Rosvall et al., 2012; Vullioud et al., 2013), were selected during the domestication process.

Several correlations between morphological traits, behaviors and physiology were modified in the process of selection for individuals without melanin and with yellow pigmentation in *P. scalare*. However, neither wild nor domestic strains showed a direct correlation between coloration and endocrine parameters. Therefore, we cannot state that selection on coloration had a direct effect on androgen levels in domestic strain. For this, the quantitative trait locus (QTL) analysis would be a great tool to elucidate which genetic mechanisms were selected during domestication (Christensen et al., 2014; Jensen, 2014).

Conclusion

We conclude that the selection on pigmentation pattern in *P. scalare* had effects on morphology, aggressiveness and androgen levels in both sexes. In addition, even the same coloration pattern had been selected in both males and females, because it is a species with no clear sexual dimorphism, the correlations between the quantified variables were affected in different ways. This kind of study helps us to understand how traits naturally or artificially selected can affect other phenotypes that also influence the individual's fitness. QTL analysis should be used in future studies for a better understanding of the effects from directional selection on other phenotypes.

References

Adachi, K., Wakamatsu, K., Ito, S., Matsubara, H., Nomura, K., Tanaka, H., Kato, K., 2010. A close relationship between androgen levels and eumelanogenesis in the teleost red seabream (Pagrus major): Quantitative analysis of its seasonal variation and effects of oral treatment with methyl-testosterone. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 156, 184–189. doi:10.1016/j.cbpa.2010.01.021

Badyaev, A. V, 2004. Integration and Modularity in the Evolution of Sexual Ornaments. Phenotypic Integr. Stud. Ecol. Evol. Complex Phenotypes 6, 50–79. doi:10.1046/j.1461-0248.2003.00428.x

Beeching, S.C., 1995. Colour pattern and inhibition of aggression in the cichlid fish Astronotus ocellatus. J. Fish Biol. 47, 50–58. doi:10.1111/j.1095-8649.1995.tb01872.x

Blottner, S., Franz, C., Rohleder, M., Zinke, O., Stuermer, I.W., 2000. Higher testicular activity in laboratory gerbils compared to wild Mongolian gerbils (Meriones unguiculatus). J. Zool., Lond. 250, 461±466. doi:10.1111/j.1469-7998.2000.tb00789.x

Brown, T.A., Jones, M.K., Powell, W., Allaby, R.G., 2009. The complex origins of domesticated crops in the Fertile Crescent. Trends Ecol. Evol. 24, 103–109. doi:10.1016/j.tree.2008.09.008

Cacho, M.D.S.R.F., Chellappa, S., Yamamoto, M.E., 2006. Reproductive success and female preference in the amazonian cichlid angel fish, Pterophyllum scalare

(Lichtenstein, 1823). Neotrop. Ichthyol. 4, 87–91. doi:10.1590/S1679-62252006000100009

Campbell, J.M., Carter, P.A., Wheeler, P.A., Thorgaard, G.H., 2015. Aggressive Behavior, Brain Size and Domestication in Clonal Rainbow Trout Lines. Behav. Genet. 45, 245–254. doi:10.1007/s10519-014-9696-0

Chapman, F. a., Fitz-Coy, S. a., Thunberg, E.M., Adams, C.M., 1997. United States of America Trade in Ornamental Fish. J. World Aquac. Soc. 28, 1–10. doi:10.1111/j.1749-7345.1997.tb00955.x

Chien, A.K., 1973. Reproductive behaviour of the angelfish Pterophyllum scalare (Pisces: Cichilidae) II. Influence of male stimuli upon the spawning rate of females. Anim. Behav. 21, 457–463. doi:10.1016/S0003-3472(73)80005-3

Christensen, K. a, Brunelli, J.P., Wheeler, P. a, Thorgaard, G.H., 2014. Antipredator behavior QTL: differences in rainbow trout clonal lines derived from wild and hatchery populations. Behav. Genet. 44, 535–46. doi:10.1007/s10519-014-9663-9

Goldsmith, R.J., Closs, G.P., Steen, H., 2003. Evaluation of visible implant elastomer for individual marking of small perch and common bully. J. Fish Biol. 63, 631–636. doi:10.1046/j.1095-8649.2003.00176.x

Goldstein, R.J., 2001. Angelfish: Everything about Purchase, Care, Nutrition, Handling, and Behavior. Barron's Educational Series, New York.

Hill, G.E., McGraw, K.J., 2003. Melanin, nutrition, and the lion's mane. Science (Lett) 299:660.

Huntingford, F., 2004. Implications of domestication and rearing conditions for the

behaviour of cultivated fishes. J. Fish Biol. 65, 122–142. doi:10.1111/j.1095-8649.2004.00562.x

Jawor, J.M., Breitwisch, R., 2003. Melanin ornaments, honesty, and sexual selection. Auk 120, 249–265.

Jensen, P., 2014. Behavior genetics and the domestication of animals. Annu. Rev. Anim. Biosci. 2, 85–104. doi:10.1146/annurev-animal-022513-114135

Kelley, J.L., Magurran, A.E., Mac??as Garc??a, C., 2006. Captive breeding promotes aggression in an endangered Mexican fish. Biol. Conserv. 133, 169–177. doi:10.1016/j.biocon.2006.06.002

Ketterson, E.D., Atwell, J.W., McGlothlin, J.W., 2009. Phenotypic integration and independence: Hormones, performance, and response to environmental change. Integr. Comp. Biol. 49, 365–79. doi:10.1093/icb/icp057

Künzl, C., Sachser, N., 1999. The behavioral endocrinology of domestication: A comparison between the domestic guinea pig (Cavia aperea f. porcellus) and its wild ancestor, the cavy (Cavia aperea). Horm. Behav. 35, 28–37. doi:10.1006/hbeh.1998.1493

Lahti, D.C., Johnson, N.A., Ajie, B.C., Otto, S.P., Hendry, A.P., Blumstein, D.T., Coss, R.G., Donohue, K., Foster, S.A., 2009. Relaxed selection in the wild. Trends Ecol. Evol. 24, 487–496. doi:10.1016/j.tree.2009.03.010

McGlothlin, J.W., Jawor, J.M., Greives, T.J., Casto, J.M., Phillips, J.L., Ketterson, E.D., 2008. Hormones and honest signals: males with larger ornaments elevate testosterone more when challenged. J. Evol. Biol. 21, 39–48. doi:10.1111/j.1420-9101.2007.01471.x

McGlothlin, J.W., Ketterson, E.D., 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 1611–20. doi:10.1098/rstb.2007.0002

McGlothlin, J.W., Whittaker, D.J., Schrock, S.E., Gerlach, N.M., Jawor, J.M., Snajdr, E. a, Ketterson, E.D., 2010. Natural selection on testosterone production in a wild songbird population. Am. Nat. 175, 687–701. doi:10.1086/652469

McGraw, K.J., 2006. Mechanics of melanin-based coloration. In: Hill, G.E., McGraw, K.J. (eds) Bird coloration I. Mechanisms and measurements. Harvard University Press, Cambridge, pp 243–294.

Oliveira, R.F., Almada, V.C., 1998. Androgenization of Dominant Males in a Cichlid Fish : Androgens Mediate the Social Modulation of Sexually Dimorphic Traits 858, 841–858.

Oliveira, R.F., Almada, V.C., Goncalves, E.J., Forsgren, E., Canario, a V.M., 2001. Androgen levels and social interactions in breeding males of the peacock blenny. J. Fish Biol. 58, 897–908. doi:10.1006/jfbi.2000.1501

Perreault, H., Semsar, K., Godwin, J., 2003. Fluoxetine treatment decreases territorial aggression in a coral reef fish. Physiol. Behav. 79, 719–724. doi:10.1016/S0031-9384(03)00211-7

Price, E., 1999. Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 65, 245–271.

Pulcini, D., Wheeler, P.A., Cataudella, S., Russo, T., Thorgaard, G.H., 2013. Domestication shapes morphology in rainbow trout Oncorhynchus mykiss. J. Fish Biol. 82, 390–407. doi:10.1111/jfb.12002

Reddon, A.R., Hurd, P.L., 2009. Differences in aggressive behavior between convict cichlid color morphs: Amelanistic convicts lose even with a size advantage. Acta Ethol. 12, 49–53. doi:10.1007/s10211-009-0054-9

Rosvall, K. a, Bergeon Burns, C.M., Barske, J., Goodson, J.L., Schlinger, B. a, Sengelaub, D.R., Ketterson, E.D., 2012. Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioural evolution. Proc. Biol. Sci. 279, 3547–55. doi:10.1098/rspb.2012.0442

Rubin, C.-J., Zody, M.C., Eriksson, J., Meadows, J.R.S., Sherwood, E., Webster, M.T., Jiang, L., Ingman, M., Sharpe, T., Ka, S., Hallböök, F., Besnier, F., Carlborg, O., Bed'hom, B., Tixier-Boichard, M., Jensen, P., Siegel, P., Lindblad-Toh, K., Andersson, L., 2010. Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464, 587–91. doi:10.1038/nature08832

Vullioud, P., Bshary, R., Ros, A.F.H., 2013. Intra- and interspecific aggression do not modulate androgen levels in dusky gregories, yet male aggression is reduced by an androgen blocker. Horm. Behav. 64, 430–438. doi:10.1016/j.yhbeh.2013.06.007

Wilkins, a. S., Wrangham, R.W., Fitch, W.T., 2014. The "Domestication Syndrome" in Mammals: A Unified Explanation Based on Neural Crest Cell Behavior and Genetics. Genetics 197, 795–808. doi:10.1534/genetics.114.165423

Yamamoto, M.E., Chellappa, S., Cacho, M.S.R.F., Huntingford, F. a, 1999. Mate guarding in an Amazonian cichlid, Pterophyllum scalare. J. Fish Biol. 55, 888–891. doi:10.1111/j.1095-8649.1999.tb00727.x

Zeder, M.A., 2015. Core Questions in Domestication Research. Proc. Natl. Acad. Sci. 112, 3191–3198. doi:10.1073/pnas.1501711112

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José do Rio Preto

TERMO DE REPRODUÇÃO XEROGRÁFICA

Autorizo a reprodução xerográfica do presente Trabalho de Conclusão, na íntegra ou em partes, para fins de pesquisa.

São José do Rio Preto, _____/_____/____

_____

Assinatura do autor