



ESTIMATION OF GENETIC PARAMETERS AND RESPONSE TO SELECTION FOR ORNAMENTATION TRAITS IN GUPPY *Poecilia reticulata* (Peters, 1859)

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M.F.Sc. (Fish Genetics and Breeding)

by

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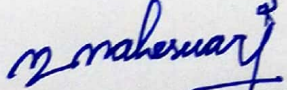
*Dedicated to God
and My Family*

DECLARATION

I hereby declare that the dissertation entitled "**ESTIMATION OF GENETIC PARAMETERS AND RESPONSE TO SELECTION FOR ORNAMENTATION TRAITS IN GUPPY *Poecilia reticulata* (Peters, 1859)**" is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship fellowship or any other similar title.

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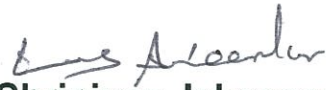
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CERTIFICATE

Certified that the dissertation entitled “**ESTIMATION OF GENETIC PARAMETERS AND RESPONSE TO SELECTION FOR ORNAMENTATION TRAITS IN GUPPY *POECILIA RETICULATA* (PETERS, 1859)**” is a bonafide record of independent research work carried out by **Ms. M. MAHESWARI (FGB-MA7-02)** during the period of study from August, 2018 to June, 2019 under our supervision and guidance for the degree of **Master of Fisheries Science (Fish Genetics and Breeding)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

गप्पी (*Poecilia reticulata*) आर्थिक रूप से एक महत्वपूर्ण सजावटी मछली है। चुनिंदा प्रजनन के माध्यम से नए उपभेदों का विकास किया जा सकता है। नेस्टेड मेटिंग प्रयोग की दो पीढ़ियों को चयनित उद्देश्य के साथ चयनित किया गया ताकि गप्पी में चयनित अलंकरण लक्षणों में सुधार हो सके। पहली पीढ़ी को 29 पूर्ण- सहोदर परिवारों के साथ स्थापित किया गया था, जिसमें 13 प्रजनक आधे- सहोदर शामिल थे और दूसरी पीढ़ी में 25 पूर्ण- सहोदर थे, जिनमें 8 प्रजनक आधे-सहोदर शामिल थे। यह लक्षण दोनों पीढ़ियों में 70 और 90 दिनों की उम्र में दर्ज किए गए थे। 90 दिनों की आयु में औसत कुल लंबाई (mm²) 22.5 ± 0.59 mm था., और दोनों पीढ़ियों में मादा मछली, नर मछली की तुलना में काफी बड़ी थीं। 90 दिनों की उम्र में औसत कुल नारंगी क्षेत्र (mm²) 4.02 ± 0.09 था, और यह पहली पीढ़ी की तुलना से दूसरी पीढ़ी में काफी अधिक था। शरीर के आकार के लक्षणों के लिए आनुवांशिकता का अनुमान मामूली रूप से अधिक था. और यह कुल लंबाई के लिए 0.44 ± 0.11 था। कुल नारंगी क्षेत्र और 90 दिनों की उम्र में नारंगी स्थानों की संख्या के लिए आनुवांशिकता का अनुमान क्रमशः 0.4 ± 0.14 और 0.09 ± 0.19 था। दूसरी पीढ़ी में 90 दिनों की उम्र में कुल नारंगी क्षेत्र और नारंगी स्पॉट की संख्या के बीच आनुवंशिक सहसंबंध 0.53 ± 0.17 था। दूसरी पीढ़ी में अपेक्षित आनुवंशिक लाभ की तुलना में वास्तविक आनुवंशिक लाभ तुलनात्मक रूप से अधिक था। पहली और दूसरी पीढ़ी में 90 दिनों की आयु में कुल नारंगी क्षेत्र के लिए वास्तविक आनुवांशिक लाभ क्रमशः -6.45% और -5.76% था जबकि नारंगी स्थानों की संख्या के लिए आनुवंशिक लाभ क्रमशः 9.16% और 25.7% था। परिणामों ने संकेत दिया कि आनुवांशिक चयन के माध्यम से इस आबादी में नारंगी रंग में और सुधार किया जा सकता है। वर्तमान आनुवंशिक अध्ययन से पता चलता है कि गप्पी आबादी अलंकरण लक्षणों के लिए चयन को अनुकूल जवाब देती है।.

ABSTRACT

The guppy (*Poecilia reticulata*) is an economically important ornamental fish. Developing new strains can be achieved through selective breeding. Genetic selection was carried for two generations with selection objective to improve the selected ornamentation traits in guppy. The first generation was established with 29 full-sib families which included 13 sire half-sib families and the second generation had 25 full-sibs including 8 sire half-sib families. The traits were recorded at 70 and 90 days' of age in both generations. The least squares mean of total length (mm) at 90 days' of age was 22.5 ± 0.59 mm and females were significantly larger than males in both generations. The least squares mean of total orange area at 90 days' of age was 4.02 ± 0.09 mm² and it was significantly higher in second generation compared to first generation. Heritability estimates of body shape traits were moderately high and it was 0.44 ± 0.11 for the total length. The heritability estimate for total orange area and number of orange spots at 90 days' of age was 0.40 ± 0.14 and 0.09 ± 0.19 , respectively. The genetic correlation between total orange area and number of orange spots at 90 days' of age in second generation was 0.53 ± 0.17 . The realized genetic gain was comparatively higher than expected genetic gain in second generation. The realized genetic gain for total orange area at 90 days' of age in first and second generation was -6.45% and -5.76% respectively while for number of orange spots the genetic gain was 9.16% and 25.7% respectively. The results indicated that through genetic selection the orange coloration can be further improved in this population. The present genetic study suggests that the guppy populations respond to selection for ornamentation traits.

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ABBREVIATIONS

Abbreviations	Full-form
TL	Total length
SL	Standard length
DFL	Dorsal fin length
CFL	Caudal fin length
CFA	Caudal fin area
BDA	Body area
TORA	Total orange area
ORAS	Number of orange spots
RORA	Relative orange area
MB	Mean brightness of orange spots
MS	Mean saturation of orange spots
MH	Mean hue of orange spots
TL2	Total length at 90 days
BDA2	Body area at 90 days
TORA2	Total orange area at 90 days

1. INTRODUCTION

Ornamental fishes are visually exciting objects and are nature's awesome creation. Ornamental fish are usually small sized, colorful and often have unique shape and body forms (Basu *et al.*, 2012). Ornamental fish keeping is the second most preferred hobby in the modern world next to photography (ICAR-CIFA, 2016). In 2017, the global Ornamental Fish market size was 8380 million US\$ and is forecast to 13600 million US\$ in 2025, growing @ 6.2% from 2018 (<https://www.reuters.com/brandfeatures/venture-capital/article?id=57410>). In India, the ornamental fisheries are growing at a rate of 20 percent annually (Basu *et al.*, 2012). The guppy alone represents more than 25% of the market by volume and more than 14% by value (Dey, 2014).

The guppy, *Poecilia reticulata*, is a small freshwater fish native to the Central American countries (Magurran, 2005) and are introduced to many habitats and are now found all over the world including India where they were introduced about a century ago. Guppy adapts easily to a variety of environmental conditions and matures and breeds naturally including in captive conditions (Chervinski, 1984; Fujio *et al.*, 1990; Chung, 2001). A number of naturally evolved and genetically developed strains of guppies are available across the globe.

Guppies exhibit a great deal of sexual dimorphism and ornamental color and color patterns are expressed only in male guppy. Majority of the colour inheritance in guppy have been attributed to sex-linked genes. Even though the guppies display a wide range of colors, the orange color stands apart among all others. Orange color has got high economic significance as guppy with large bright orange colour commands high market price, further the orange color plays an important role in reproduction as the female guppies prefer males with more intense hue and saturation of orange color as their mates (Kodric-Brown, 1989; Houde and Torio, 1992; Grether, 2000; Pitcher *et al.*, 2007). The hue and saturation of male orange coloration is highly influenced by the environmental conditions specially the availability of the carotenoids in the diet (Kodric-Brown, 1989; Grether, 2000, Pitcher *et al.*, 2007). In contrast to hue and saturation, the

area of orange coloration does not appear to be affected by carotenoid intake (Kodric-Brown, 1989; Grether, 2000) but instead has a substantial genetic component (Houde, 1992; Pitcher *et al.*, 2007).

Heritable traits are amenable to genetic selection. The color patterns of male guppies can be improved by adopting genetic selection methods and new true breeding strains can be developed within a few generations (Karino and Haijima, 2000). A number of selective breeding programs for enhancing the ornamentation traits have been developed and implemented by geneticists. Selection for high-performance males using traits associated with total orange area and number of orange spots could be an important strategy to develop new strain, assuming that these traits are heritable. The genetic improvement of a trait depends upon the existence of genetic variation in that trait and several factors affect the response to selection (Arcos *et al.*, 2004). Heritability estimates provides the information about the genetic variation in the population and is essential to implement a selective breeding program.

The reviewed literature suggests that even though a number of strains of guppies are developed by breeders but the methods of selection and the genetic parameters of the ornamentation traits are not reported probably due to the economic significance/trade secrecy. The literature review also suggest to the best of my knowledge that there are no genetic selection programs being implemented for improving ornamentation traits in guppy to develop new strain/s in India.

Keeping in view of the above mentioned points, the present study is envisaged with the following objectives:

- To estimate the genetic parameters of ornamentation trait
- To compare the expected and realized genetic gain in selected ornamentation trait

2. REVIEW OF LITERATURE

2.1. Guppy as a model organism

The guppy (*Poecilia reticulata*), is a tropical ornamental fish found all over the world. Guppy is a small benthopelagic and non-migratory fish that inhabits fresh and brackish waters of tropical regions. Male guppies exhibit a high degree of color-polymorphism in body color patterns, and females are uniform olive brown and are usually larger than males (<https://www.cabi.org/isc/datasheet/68208>). Many varieties and strains of guppy have been developed by selectively breeding across the globe. The guppy is routinely used as a model organism for genetic selection experiments because of its small size, short generation interval, ease of breeding and relatively high reproductive capacity (Nakajima and Taniguchi, 2001).

2.2. Taxonomic hierarchy (ITIS, 2016)

Kingdom: Animalia

Phylum: Chordata

Subphylum: Vertebrata

Superclass: Actinopterygii

Class: Teleostei

Order: Cyprinodontiformes

Family: Poeciliidae

Genus: *Poecilia*

Species: *Poecilia reticulata*

2.3. Distribution and habitat

The guppy is native to Venezuela, Trinidad, Antilles, Windward and Leeward Islands of Central America. They inhabit in shallow ditches, canals, streams, lakes, rivers, pools, ponds (Khoo *et al.*, 2007). Guppy was introduced in India in 1910 (Kaira *et al.*, 1967). In non-native areas, guppies are commonly found in heavily polluted water bodies (Barua *et al.*, 2001). Guppies have broad environmental tolerances and can withstand a wide range of salinity and temperature (Travis, 1957).

2.4. Sexual dimorphism and reproduction in guppy

P. reticulata is a live-bearing fish and is a prolific breeder. The females are usually larger than males (Karino and Hajima, 2001). Male guppies are color-polymorphic and have a high degree of variation in color, color pattern and other ornamental traits, and females are uniform olive brown (<https://www.cabi.org/isc/datasheet/68208>). The male and female guppies attain sexual maturation at 60 to 120 days of age; depending upon the environmental and climatic factors. Males can be identified by body shape, the appearance of coloration and the presence of gonopodium, while females are having rounded abdomen and usually carry the dull gray coloration (Houde, 1997).

In guppy, fertilization takes place internally with male transferring the sperms into the oviduct of the female through gonopodium. The gestation period in guppy is 25-35 days (Shahjahan *et al.*, 2013).

2.5. Color and color patterns

Color and color patterns are prominent features of animals and have important functions in communication, such as camouflage, mate choice. Animal color and color patterns are composed of specialized pigment-producing cells, the chromatophores (Singh and Volhard, 2015). Kottler *et al.* (2014) had reported five pigment cell types in the skin of guppy: black melanophores, orange to yellow xanthophores, red erythrophores, blue to green iridophores, white leucophores in which

xanthophores contain light-absorbing pigments colors, namely carotenoid and pteridine. Carotenoids are important types of pigments and are stored in xanthophores (Braasch *et al.*, 2007). The carotenoid pigments indicate a male's genetic quality and physical condition (Kottler *et al.*, 2014).

Grether (2000) conducted a mate choice experiment and observed that females prefer males with larger and brighter orange spots. Cole *et al.* (2016) reported the color and color patterns of males guppies develop faster and brighter in the presence of accessible females under different light environments. Hughes *et al.* (2013) reported that the females prefer males with rare and novel color patterns at the time of courtship.

2.6. Color inheritance in guppy

The guppy is the first species in which a Y-linked inheritance was demonstrated (Winge, 1923, 1938). The color coding genes are usually located close to or within a sex-determining region on the Y-chromosome. The appearance of the color and color patterns in male guppies coincides with sexual maturation that is about 3 months of age (Houde and Torio, 1992). The color patterns are complex, conspicuous and manifested as spots, speckles, and lines of various pigmented colors, including black, white, red-orange, yellow and green (Tezuka *et al.*, 2011). The size, shape, position, and a number of orange spots greatly vary in the male both within and between populations (Brooks and Endler, 2001).

Dinesh (2014) had reported that the post opercular orange spot in guppy as a dominant sex-limited trait. Colihueque and Araneda, (2014) found 49 QTLs for 11 areas of pigmentation traits in which QTL located on linkage group 12, that corresponds to the sex chromosome of the guppy, and indicates that loci responsible for polymorphisms in guppy color patterns are clustered on a sex chromosome. Expression of phenotypic color patterns in cultured strains has been found to be determined by dominant X- and Y-linked genes (Nayudu, 1979; Kirpichnikov, 1981; Fernando and Phang, 1989a, 1989b; Phang *et al.*, 1990; Phang and Fernando, 1991).

The tetra fish (*Gymnocorymbus ternetzi* Characidae) is particularly popular with hobbyists, like guppy; it is attractive in appearance and easily bred. Frankel (2004) conducted an experiment with 11 different crosses for the inheritance of trunk banding (black vertical bands) in tetra and found that these bands are controlled by genes situated on two autosomal loci. In another experiment Frankel (2011) crossed 12 different strain of red base tetra to study the inheritance of shoulder spotting (black spot) and found that this spot is controlled by two autosomal loci.

The anabantoids are quite popular with aquarium hobbyists for their reproductive behavior and phenotypic appearance. Studies had found that the inheritance of humeral spotting in croaking gourami is controlled by single autosomal dominance allele (Frankel *et al.*, 2012).

2.7. Analysis of coloration

Photography is a method to quantify color and is more sensitive than human vision (Vilafuente and Negro, 1998). Advantage of using digital image to analysis coloration is the existence of many powerful software programs, coupled with variety of programming languages (Stevens *et al.*, 2007) viz., Image J. Photoshop, MATLAB etc. Image J permits customized programs written in Java language to accompany it. The MATLAB (The Mathworks Inc.) is also an extremely effective package for writing calibrations and designing sophisticated computational models of vision. A number of 'toolboxes' are available in MATLAB that have numerous functions already written for various tasks, including image processing, statistics, and wavelet transformations (Andrew, 2004).

The properties of color include brightness, saturation, and hue. The brightness is the lightness or darkness of a color and it ranges from dark (0%) to fully illuminated (100%). Saturation refers to the purity of a hue. It ranges from pure color (100%) to gray (0%) (<https://www.premiumbeat.com/blog/basic-properties-color/>)

2.8. Heritability of ornamentation traits

Heritability is the most important genetic parameter in selective breeding program. Heritability is defined as the ratio of the heritable genetic variance to the total phenotypic variance (Falconer and Mackey, 1996; Gjedrem, 2005). The heritability estimates helps to design the genetic selection programs.

Body size as in many other species of fishes is a heritable trait in guppies also (Brooks and Endler, 2001; Karino and Haijima, 2001), and females enhance their offspring growth and reproductive output of their daughters when they mate with male possessing large total length (Reynolds and Gross, 1992). In general it was observed that the heritability estimates of body size and shape traits were moderate to high in guppies (Reynolds and Gross, 1992; Brooks and Endler, 2001; Karino and Haijima, 2001; Smrithi, 2018).

The heritability of total length in guppies ranged from 0.19 to 1.00 (Karino and Hajima, 2001; Evans, 2010; Brooks and Endler, 2001; Amirtha, 2016; Smrithi, 2018). Heritability of standard length ranged from 0.04 to 0.66 (Karino and Hajima, 2001; Evans, 2010; Brooks and Endler, 2000; Smrithi, 2018). The reviewed literature suggests that the heritability estimates of body area ranged from 0.19 to 0.87 (Evans, 2010; Brooks and Endler, 2001; Smrithi, 2018). Heritability of relative dorsal and caudal fin length was 0.78 and 0.48, respectively.

Heritability estimates of orange area ranged from 0.75 to 1.52 while number of orange spots ranged from 0.01 to 0.85 (Evans, 2010; Brook and Endler, 2001; Amirtha, 2016; Lindholm *et al.*, 2005; Smrithi, 2018).

The realized heritability can be estimated by taking a ratio of realized genetic gain to selection differential (Falconer and Mackey, 1989). Smrithi (2018) reported realized heritability for a number of carotenoid and pteridine spots, mean brightness of carotenoid and pteridine spots and they were very high and also exceeded one.

2.9. Correlation

Correlation is a measure of the variation in one trait attributable to the linear relationship with another trait. The correlation between two traits can be estimated at both genetic and phenotypic levels (Cameron, 1997). Genetic correlation caused by common genetic effects when measuring different traits in the same individual (Gjedrem and Baranksi, 2009). Smrithi (2018) reported genetic correlations between the number of orange spots and total orange area as 0.38, and the genetic correlation between total length and standard length as 0.98. Brooks and Endler, (2001) reported genetic correlation between body area and orange spots as -0.086 while for body area and saturation was -0.066.

The phenotypic correlation is correlation between phenotypic observations of different traits in the same individual (Gjedrem and Baranksi, 2009). Brooks and Endler (2001) reported phenotypic correlation between body area and orange area as 0.094 while for body area and saturation was -0.08.

2.10. Breeding value

The breeding value of an individual is judged by the mean value of its progeny (Falconer and Mackay, 1996). The breeding value of individuals can be estimated by sire and animal model. The animal model helps to estimate breeding value of all animals in the pedigree, even for those animals without records (Cameron, 1997).

2.11. Genetic selection

The genetic selection is the selection of parent broodstock from a larger group of candidates based on their breeding value, to produce a new generation with improved performance (Gjedrem and Baranksi, 2009). The different methods of selection are practiced in breeding of animals Viz., Individual selection, repeated measurements, sib selection, within family and between family selections; progeny testing etc. The within family selection is the deviation of each individual from the mean

value of the family. The sib selection is only based on the phenotypic values of relatives (Falconer and Mackay, 1996).

Various selection methods are practiced in ornamental fish species for developing new variety. The ornamental carp varieties apparently have been developed by selective breeding of color mutants (Axelord, 1973). Varieties of gold fish were created by Chinese and Japanese breeders by selective breeding of visible mutations affecting body pigmentation and shape and the structure of fins and eyes (Hulata, 1995).

2.12. Genetic gain

Genetic gain is the change in the value of a trait due to selection and caused by the change in allele frequencies of genes responsible for selected traits (Gjedrem and Baranski, 2009). The expected genetic gain can be estimated as the product of selection differential and heritability (Cameron, 1997). Smrithi (2018) reported an expected genetic gain of 0.08, 3.84, 0.01 number of carotenoids and pteridine spots, mean brightness of carotenoid and pteridine spots, mean saturation of carotenoid and pteridine spots in guppy. The realized genetic gain was estimated as the difference between the mean phenotypic value of the offspring of the selected parents ($\overline{Pop}$) and mean of whole parental population before selection ($\overline{Ppop}$). Smrithi (2018) has reported 20 % realized genetic gain for number of orange spots.

Several factors affect the genetic gain viz., accuracy of selection, selection intensity, genetic variation, generation interval. Accuracy of selection is the accuracy of breeding value predicted and it ranges from zero, when there is no performance information used, to almost one, when there is an abundance of information. The accuracy of selection depends upon the heritability and selection methods (<http://www.luresext.edu/?q=content/factors-affecting-rate-genetic-change-herd-or-breed>). Animals are selected based on the accuracy of predicted breeding values. Selection intensity is expressed as the portion of the breeding candidates that are used as parents for the next generation (Gjedrem and Baranski, 2009). Genetic variation is refers to the variability in breeding values within a population for a trait under selection.

The genetic gain is directly proportional to the genetic variation. The genetic gain will be faster when animals have shorter generation interval (<http://www.luresext.edu/?q=content/factors-affecting-rate-genetic-change-herd-or-breed>).

3. MATERIAL AND METHODS

3.1. Site of Experiment

The genetic selection for ornamentation traits in guppy was started in September 2018 in wet-laboratory of Fish Genetics and Biotechnology Division, Seven Bungalow campus of ICAR-Central Institute of Fisheries Education, Mumbai, India. This experiment was conducted over a period of 263 days.

3.2. Source of animals

The guppy fish stock available in FGB division wet laboratory from previous genetic work and the wild guppy strain obtained from rivers and lakes of Mumbai formed the population for the present study.

3.3. Quarantine of wild stock of guppy

All wild guppy fish were initially kept in large glass aquarium tanks (L 60cm x B 30 cm x H 22 cm) for 48 hours. The aquarium was provided with adequate aeration. For the first 24 hours, the fish were not provided with any feed. After 24 hours 100 percent water exchange was carried. Fish were examined for sign and symptoms of disease, deformity and if found any, were immediately separated. Further, the wild fish were given a KMnO_4 (5ppm/l) dip treatment which helped to remove ecto-parasites if any and treat minor aberrations on the body. After 48 hours of quarantine male and female guppy were separated. Wild caught females were treated by immersing 500 ml of water mixed with 0.1ml of prostaglandin (Cloprostenol®) for 1 hour. The berried females expelled the young one after 24 hours. This treatment was repeated after two weeks to make sure the female fish are devoid of carrying young ones from the previous mating.

3.4. Culture practices

3.4.1. Breeding setup

Initially, glass tanks (30cm x 15cm x 22cm) were disinfected by Immersing the tanks KMnO₄ solution for 1 hour. Then tanks were filled with 8 liters of de-chlorinated tap water and were provided with mild aeration. The lights were placed over the tanks. The fishes were reared at 12 hour light: 12 hour dark period throughout. The lights were automatically switched on at 07.00 hour and switched off at 19.00 hour IST with help of a timer. The aquatic plants like Cabomba (*Cabomba caroliniana*) and Hydrilla (*Hydrilla verticillata*) were placed inside the breeding tanks which served as the hiding sites for young ones. The tanks were labeled appropriately to identify the families.

3.4.2. Water quality management

The water quality is important for the growth and survival of guppies. The water quality parameters Viz., temperature, pH and Dissolved oxygen (DO) were maintained at the optimum level and checked once in every 15 days. During the experiment, the water temperature was 24°-29° C, pH 6.8-7.8 and DO 3-5 mg/l. Water exchange was done at a rate of 25% once in two days and siphoning was done every day to remove fecal matter and feed waste.

3.4.3. Food and Feeding

Brooders were fed ad-libitum thrice a day with live tubifex worms and freeze dried blood worms. Tubifex contained approximately 52% crude protein(<https://www.globalsources.com/si/AS/BeijingNew/6008826464995/pdtl/Tubifex>). Live tubifex worms were obtained from a local shop and were kept in de-chlorinated water with high aeration for 24 hours to empty their gut content. The worms were thoroughly washed with water and chopped with the help of a clean kitchen knife just before feeding. During the grow-out period, fish were fed three times in a day with chopped live tubifex and freeze dried blood worms. The young ones up to 30 days' of age were fed three times in a day with live brine shrimp nauplii (*A. salina*).

3.5. Breeding of animals

3.5.1. Mating design

A nested mating design where one sire was mated to two dams was employed to produce the future generations. In a nested mating design one male guppy was mated to two female guppy fish.

3.5.2. Production of F1 generation

The F1 generation was formed by breeding the selected fish from laboratory and wild stock. To produce F1 generation 30 sires and 45 dams from laboratory stock and 15 dams from wild stock were used. Male and female guppies of laboratory stock were selected by following the within-family selection criterion, while wild female guppy were selected based on individual selection criterion. Laboratory born male guppies were only used as sires to produce offspring of F1 generation. The male fish were selected based on their 90 days breeding values for orange area and orange spots while females were selected based on their family breeding values and wild females were selected based on their phenotypic appearance. Animals were ranked based on their breeding value and high ranking male was paired with high ranking female while taking care to not pair the full and half sibs. To differentiate the two female brooders from one another, caudal fin of one of the female was clipped using scissor. The fin-clipped female was treated with KMnO_4 to prevent infection. Since the fin use to regenerate, repeated clipping was necessary and on every third day clipping was done till the gravid spot was observed. From the day of pairing, females were regularly checked for the appearance of the gravid spot. The male fish were removed from the breeding tank once the dots (indicating the eyes of young ones) on the gravid spot were observed. The female brooders were also separated and placed in separate tanks till the spawning was completed and young ones from the first clutch were used in this experiment. A total of 29 full-sib families were produced which included 12 half-sib families. All young ones were reared full-sib family wise.

3.5.3. Production of F2 generation

The F2 generation was produced using fish selected from F1 generation. Again within family selection criterion was followed for selection of the fish. The male fish were selected based on their 90 days breeding values for orange area while females were selected based on their family breeding values. Animals were ranked based on their breeding value and High ranking male was paired with high ranking female. While pairing care was taken to avoid the full and half-sib mating. A total of 25 full-sib families were produced which included 8 half-sib families. All young ones were reared full-sib family wise.

3.4. Measurement of ornamentation traits

The observation were made on total length, standard length, dorsal fin length, caudal fin length, caudal fin area, body area, total area on both male and female fish, while observation on orange spot, number of orange spots, relative area of orange spots, mean brightness of orange spot, mean saturation of orange spot, mean hue of orange spot were made on male fish. The traits were recorded at 70 and 90 days of age in both F1 and F2 generation. The steps for measuring ornamentation traits include image capturing, digitizing the image and analysis.

3.4.1. Imaging

Photos of fish were taken with a digital camera (Canon EOS 700D fitted with 250 mm macro-lens; (18 megapixels) fitted on a tripod. All photos were taken under uniform distance (5cm) and shutter speed (1/250s). Before taking the photographs fish were anesthetized by immersing them in a beaker containing 500 ml of water mixed with 0.05 ml of clove oil for 5 minutes. To capture the image a single fish was placed in a petri dish containing a small amount of water and this petri dish was placed on a wooden stand with a flat surface covered with black paper. All images were saved in JPEG format.

3.4.2. Analysis of images

All images were analyzed using image J and MATLAB (R 2015a).

3.4.2.1. Image J

Image J is a public domain Java image processing program. It can display, edit, analyze, process, save and print 16-bit images. The traits measured included total length (mm), standard length (mm), caudal fin length (mm), dorsal fin length (mm), body area (mm²), caudal fin area (mm²), and orange color area (mm²). The numbers of orange spots were manually counted on the image. The image J consists of menu bar, toolbar. The menu bar provides options to open the file containing the image and set measurements. Toolbar consists of tools for area selection, line selection tools were used for measuring area and length of fishes respectively.

3.4.2.2. MATLAB

The MATLAB (R 2015a) is a multi-paradigm numerical computing environment and proprietary programming language. The traits measured included mean brightness (pixel intensity), mean saturation (%), mean hue (°) of orange spots.

3.5. Statistical analysis

3.5.1. Normality test

The R 3.5.1 (“Feather spray”) function Shapiro. Test () used to test normality of data. When the p-value is more than 0.05, then the data is considered to be normally distributed. Whenever, data were found to be significantly different from the normal distribution, log transformation was employed to bring the data to normal distribution and was retransformed to the actual value for reporting the mean and their standard errors (www.sthda.com/english/wiki/normality-test-in-r).

3.5.2. Estimation of non-genetic factors

The significant effects of non-genetic factors on ornamentation traits were quantified by employing proc GLM of SAS® 9.3 The following model was employed

$$Y_{ijk} = \mu + \text{Sex}_i + \text{Generation}_j + e_{ijk}$$

Y_{ijk} = observation on k^{th} individual on i^{th} sex belonging to the j^{th} generation

μ = overall mean

Sex = fixed effect of the sex of individual, i = female, male

Generation = fixed effect of the generation, j = 1, 2

e_{ijk} = is assumed to be distributed normally and independently with mean 0 and variance 1, $e \sim (0,1)$

3.5.3. Heritability Estimation

3.5.3.1. Sire model

Heritability of ornamentation traits were estimated using Sire model for F1 and F2 generations and combining the data. A linear mixed model was used, in that sire, and dam were considered as random effects and sex as fixed effect (Eq.1). For estimation of heritability of combined data generation was also added as one of the fixed effects (Eq.2.) The following mixed model was used.

$$Y_{ijkl} = \mu + \text{Sex}_k + S_i + d_{j(i)} + e_{ijkl}$$

$$Y_{ijklm} = \mu + \text{Sex}_k + \text{Generation}_l + S_i + d_{j(i)} + e_{ijklm}$$

$Y_{ijkl/m}$ = observation of l^{th} individual born in (l^{th} generation) of k^{th} sex born to j^{th} dam mated to i^{th} sire

μ = Overall mean

S_i = Random effect of j^{th} sire where $i=1, 2, 3, 4, 5, 6, \dots, 39$

$D_{j(i)}$ = Random effect of j^{th} dam mated with i^{th} sire where $j=1,2, 3, 4,\dots, 58$

Sex_k = Fixed effect of individual sex, where $k=\text{Female, Male}$

Generation_l = Fixed effect of generation, where $l= 1, 2$

$e_{ijkl/m}$ = random error associated with $Y_{ijkl/m}$

Heritability was estimated by the following formula (Saxton, 2004)

$$h^2_{\text{(sire component)}} = 4 (\sigma^2 S) / (\sigma^2 S + \sigma^2 D + \sigma^2 e)$$

$$h^2_{\text{(dam component)}} = 4 (\sigma^2 D) / (\sigma^2 S + \sigma^2 D + \sigma^2 e)$$

$$h^2_{\text{(sire + dam component)}} = 2 (\sigma^2 S + \sigma^2 D) / (\sigma^2 S + \sigma^2 D + \sigma^2 e)$$

where,

h^2 = heritability of the trait

$\sigma^2 S$ = sire variance

$\sigma^2 D$ = dam variance

$\sigma^2 e$ = residual variance

3.5.3.2. Animal model (Hendreson, 1984; Saxton, 2004)

Heritability of ornamentation traits for both F1 and F2 generations was estimated by adopting animal model using univariate mixed model in Echidna (2018). A linear mixed model was adopted and animal was considered as random effect and sex (and generation for combined data) as fixed effect/s.

$$y = X\beta + Za + e$$

Where,

y = vector of phenotypic observations

X = incidence matrix of fixed effects

β = p x 1 vector of fixed effects; p = number of levels in fixed effects

Z = Incidence matrix of random animal effects

a = q x 1 vector of random animal effects; q = number of levels for random effects

e = n x 1 vector of residual effects

Heritability was estimated using the following formula

$$h^2 = (\sigma^2 A) / (\sigma^2 A + \sigma^2 e)$$

where,

h^2 = heritability of the trait

$\sigma^2 A$ = variance of individual

$\sigma^2 e$ = residual variance

3.5.4. Estimation of genetic and phenotypic correlation

Genetic and phenotypic correlations between various traits were estimated by using bivariate animal model in Echidna (2018).

3.5.5. Estimation of selection differential (Cameron, 1997)

The selection differential (SD) was estimated as the difference between mean phenotypic value of individuals selected as parents ($\overline{Psp}$) and the overall population mean before selection ($\overline{Ppop}$) (Cameron, 1997)

$$\text{Selection differential (SD)} = (\overline{Psp} - \overline{Ppop})$$

Where,

$\overline{Psp}$ = mean of the selected parents

$\overline{Ppop}$ = overall population mean

3.5.6. Estimation of expected genetic gain (ΔG)

The expected genetic gain (ΔG) was estimated as a product of the selection differential (SD) and heritability (h^2) (Cameron, 1997)

$$(\Delta G) = SD \times h^2$$

Where,

ΔG = expected genetic gain

SD = selection differential

h^2 = heritability

3.5.7. Estimation of Realized genetic gain (Cameron, 1997)

The realized genetic gain was estimated as the difference between the mean phenotypic value of the offspring of the selected parents ($\bar{P}_{op}$) and mean of whole parental population before selection $\bar{P}_{pop}$.

$$\text{Realized genetic gain} = (\bar{P}_{op} - \bar{P}_{pop})$$

Where,

$\bar{P}_{op}$ = Mean phenotypic value of the offspring of the selected parents

$\bar{P}_{pop}$ = Mean phenotypic value of whole parental population before selection

3.5.8. Estimation of realized heritability (h^2_R)

The realized heritability was estimated as ratio between the observed genetic gain and selection differential (Cameron, 1997)

$$h^2_R = \Delta G / SD$$

Where,

h^2_R = realized heritability

ΔG = Realized genetic gain

SD = selection differential

Note: To estimate the selection differential, expected genetic gain in base generation the means reported by Smrithi (2018) were used (Table A).

Table A: Number of observations and sex wise least squares means and their standard errors and genetic parameters of 90 days' age ornamentation traits of base generation (Source: Smrithi, 2018)

SOURCE	N	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
OVERALL	192	21.80±0.23	16.70±0.19	2.75±0.04	5.43±0.05	15.51±0.38	56.32±1.37	**	**	**	**	**	**
SEX													
MALE	91	21.08±0.33 ^a	16.03±0.27 ^a	2.82±0.06 ^a	5.39±0.08 ^a	14.88±0.55 ^a	50.89±0.92 ^a	4.12±0.27	2.33±0.10	0.08±0.01	149.31±2.59	0.21±0.01	0.35±1.63
FEMALE	101	22.46±0.31 ^a	17.31±0.26 ^b	2.69±0.06 ^a	5.48±0.08 ^a	16.09±0.52 ^a	61.21±1.82 ^b						
h ²		0.08±0.25	0.04±0.25	0.05±0.25	0.48±0.26	0.19±0.24	0.41±0.26	0.31±0.16	0.10±0.08	0.20±0.12	0.46±0.15	0.20±0.15	0.40±0.16

** (91 no.s) – Traits are only expressed in males

Means bearing varies superscript are significantly different from each other

TL- total length, SL – standard length, DFL – dorsal fin length, CFL – caudal fin length, CFA – caudal fin area, BDA - body area,

TORA – total orange area, ORAS – number of orange spots, RORA– relative orange area, MB- mean brightness of orange spots,

MS- mean saturation of orange spots, MH - mean hue of orange spots

4. RESULTS

In the present study least squares means of body shape and size and that of ornamentation traits of guppy at 70 and 90 days' of age of F1 and F2 generation were estimated. The effects of genetic and non-genetic factors affecting these traits were quantified by adopting linear mixed models. Genetic parameters viz., heritability, genetic and phenotypic correlations were also estimated by sire and animal model. Expected and realized genetic gains were estimated for the total orange area and orange spot numbers.

4.1. Least squares means of 70 days' age ornamentation traits of combined generation

The number of observations, least squares means along with their, standard errors of body traits and orange color related traits are presented in Table 1. The overall least squares mean of body shape traits including total length, standard length, dorsal fin length, caudal fin length, caudal fin area and body area was 19.22 ± 0.13 mm, 14.73 ± 0.11 mm, 2.65 ± 0.02 mm, 4.58 ± 0.04 mm, 12.77 ± 0.17 mm² and 39.77 ± 0.50 mm², respectively. The females were significantly longer and broader than males at 70 days' of age (Table3). The overall least squares mean of orange color related traits including total orange area (mm²), number of orange spots, relative orange area (mm²), mean brightness of orange spots (pixel intensity), mean saturation of orange spots (%) and mean hue of orange spots (radian) was 3.48 ± 0.09 , 0.18 ± 0.05 , 0.104 ± 0.01 , 183.65 ± 1.55 , 0.43 ± 0.01 , 0.13 ± 0.0 , respectively. The least squares means of F1 and F2 generation for ornamentation traits were significantly different from each other (Table 4).

4.2. Least squares means of 90 days' age ornamentation traits of combined generation

The number of observations, least squares means along with their, standard errors of body traits and carotenoid and orange color related traits are presented in Table 2. The overall least squares mean of body shape traits including total length (mm), standard length (mm), dorsal fin length (mm), caudal fin length (mm), caudal fin area (mm²), body area (mm²) was 22.5 ± 0.59 , 17.72 ± 0.17 ,

2.95±0.026, 4.91±0.05, 16.67±0.25, 45.13±0.5, respectively. The females were significantly longer and broader than males at 90 days' age (Table 3 and Figure 1 and 2). The overall least squares mean of orange color related traits including total orange area (mm²), number of orange spots, relative orange area (mm²), mean brightness of orange spots (pixel intensity), mean saturation of orange spots (%) and mean hue of orange spots (radian) was 3.99±0.09, 2.47±0.05, 0.101±0.027, 192.36±2.1, 0.489±0.01, 0.174±0.005, respectively. The least squares means of F1 and F2 generation for ornamentation traits were significantly different from each other (Table 4 and Figure 3).

4.3. Effects of non-genetic factors on 70 and 90 days' age ornamentation traits

In the present study sex of the individual and generation were considered as the non-genetic factors with fixed effects and were included in the model for analysis. The sex of the individual had a significant effect on total length, standard length, dorsal fin length, caudal fin length, caudal fin area, body area at both 70 and 90 days' of age (Table 3). Similarly the generation also had a significant effect on total length, standard length, dorsal fin length, caudal fin length, caudal fin area, body area, total orange area. Only male guppy exhibits the orange color, hence only the effect of generation was estimated on number of orange spots, relative orange area, mean brightness of orange spots, mean saturation and mean hue of orange spots at 70 and 90 days of age and it was significant on all measured traits (Table 3 and 4).

4.4. Frequency distribution of total orange area and number of orange spots at 90 days' of age in F1 and F2 generations

The frequency distribution of total orange area and number of orange spots in F1 and F2 generations at 90 days' of age are presented in Table 5 and 6 respectively.

Table 1: Number of observations, overall, sex and generation wise least squares means and their standard errors of 70 days' age ornamentation traits of combined generation

SOURCE	N	TL (mm)	SL (mm)	DFL (mm)	CFL (mm)	CFA (mm ²)	BDA (mm ²)	TORA (mm ²)	ORAS	RORA (%)	MB (pixel intensity)	MS (%)	MH (°)
OVERALL	393	19.2±0.13	14.7±0.11	2.65±0.02	4.58±0.04	12.8±0.17	39.8±0.50	*	*	*	*	*	*
SEX													
M	203	17.8±0.14 ^a	13.5±0.12 ^a	2.57±0.03 ^a	4.42±0.05 ^a	11.3±0.23 ^a	33.6±0.60 ^a	3.53±0.09	2.23±0.04	0.11±0.01	182.63±1.54	0.44±0.01	0.13±0.01
F	190	20.2±0.15 ^b	15.6±0.13 ^b	2.69±0.03 ^b	4.67±0.05 ^b	13.7±0.24 ^b	45.2±0.60 ^b						
GENERATIONS													
F1	243	20.1±0.13 ^a	15.3±0.11 ^a	2.71±0.02 ^a	4.72±0.04 ^a	13.7±0.20 ^a	41.7±0.50 ^a	3.26±0.12 ^a	1.92±0.06 ^a	0.09±0.03 ^a	188.23±2.04 ^a	0.401±0.01 ^a	0.14±0.01 ^a
F2	150	17.9±0.17 ^b	13.8±0.15 ^b	2.55±0.03 ^b	4.37±0.05 ^b	11.3±0.26 ^b	37.1±0.70 ^b	3.81±0.14 ^b	2.54±0.07 ^b	0.12±0.03 ^b	177.03±2.49 ^b	0.47±0.01 ^b	0.12±0.01 ^b
SEX*GENERATIONS													
F1M	120	18.5±0.18	13.9±0.16	2.65±0.03	4.55±0.06	12.4±0.29	35.2±0.77						
F1F	123	21.6±0.18	16.7±0.16	2.78±0.03	4.88±0.06	14.9±0.28	48.1±0.75						
F2M	83	17.7±0.22	14.6±0.22	2.49±0.04	4.29±0.07	10.2±0.35	31.9±0.93						
F2F	67	18.8±0.25	13.1±0.19	2.60±0.05	4.45±0.08	12.5±0.29	42.3±1.03						

*(203 no.s) – Traits are only expressed in males

Means bearing varies superscript are significantly different from each other

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

TORA – TOTAL ORANGE AREA, ORAS – NUMBER OF ORANGE SPOTS, RORA – RELATIVE ORANGE AREA, MB- MEAN BRIGHTNESS OF ORANGE SPOTS,

MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

Table 2: Number of observations, overall, sex and generation wise least squares means and their standard errors of 90 days' age ornamentation traits of combined generation

SOURCE	N	TL (mm)	SL (mm)	DFL (mm)	CFL (mm)	CFA (mm ²)	BDA (mm ²)	TORA (mm ²)	ORAS	RORA (%)	MB (pixel intensity)	MS (%)	MH (°)
OVERALL	349	22.5±0.59	17.72±0.17	2.95±0.026	4.91±0.05	16.67±0.25	45.13±0.5	*	*	*	*	*	*
SEX													
M	171	20.8±0.23 ^a	16.3±0.22 ^a	2.85±0.03 ^a	4.68±0.06 ^a	14.6±0.31 ^a	40.5±1.07 ^a	4.02±0.09	2.53±0.04	0.104±0.002	194.78±2.02	0.49±0.01	0.17±0.01
F	178	23.6±0.24 ^b	18.8±0.23 ^b	2.99±0.03 ^b	4.91±0.06 ^b	17.9±0.33 ^b	56.2±1.11 ^b						
GENERATIONS													
F1	216	23.42±0.21 ^a	18.15±0.21 ^a	3.04±0.03 ^a	5.25±0.05 ^a	17.9±0.28 ^a	55.9±0.98 ^a	3.83±0.13 ^a	2.18±0.06 ^a	0.09±0.01 ^a	204.71±2.69 ^a	0.47±0.01 ^a	0.19±0.06 ^a
F2	133	21.04±0.28 ^b	17.05±0.27 ^b	2.78±0.03 ^b	4.34±0.07 ^b	14.61±0.37 ^b	40.8±1.29 ^b	4.19±0.15 ^b	2.87±0.07 ^b	0.11±0.01 ^b	184.86±3.3 ^b	0.52±0.02 ^b	0.14±0.07 ^b
SEX*GENERATIONS													
F1M	101	21.9±0.31	16.9±0.29	2.98±0.04	5.07±0.08	16.0±0.4	46.1±1.40						
F1F	115	24.8±0.29	19.4±0.28	3.11±0.04	5.43±0.07	19.8±0.39	65.7±1.34						
F2M	70	19.7±0.37	15.7±0.36	2.73±0.05	4.28±0.09	13.3±0.50	34.9±1.72						
F2F	63	22.4±0.40	18.3±0.38	2.85±0.05	4.39±0.10	15.9±0.53	46.7±1.82						

*(176 no.s) – Traits are only expressed in males

Means bearing varies superscript are significantly different from each other

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

TORA – TOTAL ORANGE AREA, ORAS – NUMBER OF ORANGE SPOTS, RORA – RELATIVE ORANGE AREA, MB- MEAN BRIGHTNESS OF ORANGE SPOTS,

MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

Table 3: Mean sum of squares from ANOVA for sex and generation wise for ornamentation traits of combined generation at 70th and 90th day of age

SOURCE	DF	MEAN SUM OF SQUARES					
		TL	SL	DFL	CFL	CFA	BDA
70 days							
Sex	1	714.14*	551.89*	1.82*	7.891*	664.12*	14721.4*
Generation	1	340.74*	140.46*	1.07*	17.453*	343.54*	2001.8*
Error	391	4.99	3.68	0.175	0.5215	10.9	72.4
R-Square (%)		47.8	27.5	2.3	3.5	1.3	34.2
90 days							
Sex	1	741.81*	592.63*	1.95*	8.07*	1120.26*	26539.6*
Generation	1	290.25*	27.975*	4.073*	76.33*	627.96*	163.8*
Error	347	10.54	9.38	0.192	0.858	18.81	243.4
R-Square (%)		16.6	15.1	2.5	2.3	14.4	23.6

*Significant ($p \leq 0.05$)

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

Table 4: Mean sum of squares from ANOVA for Generation wise for ornamentation traits of combined generation at 70th and 90th day of age

SOURCE	DF	MEAN SUM OF SQUARES					
		TORA	ORAS	RORA	MB	MS	MH
70 days							
Generation	1	22.01*	17.47*	0.039*	5655.9*	0.4*	0.023*
Error	201	1.579	0.4186	0.0011	463.7	0.014	0.0021
R-Square (%)		6.2	16.7	13.7	5.2	11.3	4.7
90 days							
Generation	1	10.09*	17.96*	0.04*	14316.7*	0.195*	0.132*
Error	169	1.57	0.358	0.0011	674.1	0.016	0.003
R-Square (%)		3.1	22.4	17.4	10.6	6.3	17.2

*Significant ($p \leq 0.05$)

TORA – TOTAL ORANGE AREA, ORAS – NUMBER OF ORANGE SPOTS, RORA – RELATIVE ORANGE AREA, MB- MEAN BRIGHTNESS OF ORANGE SPOTS,

MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

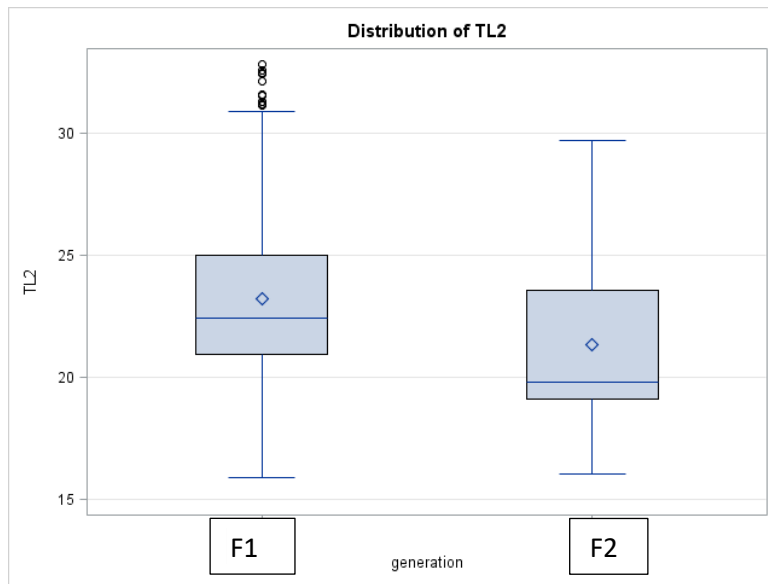


Figure 1: Box plot of F1 and F2 generation total length of 90 days age

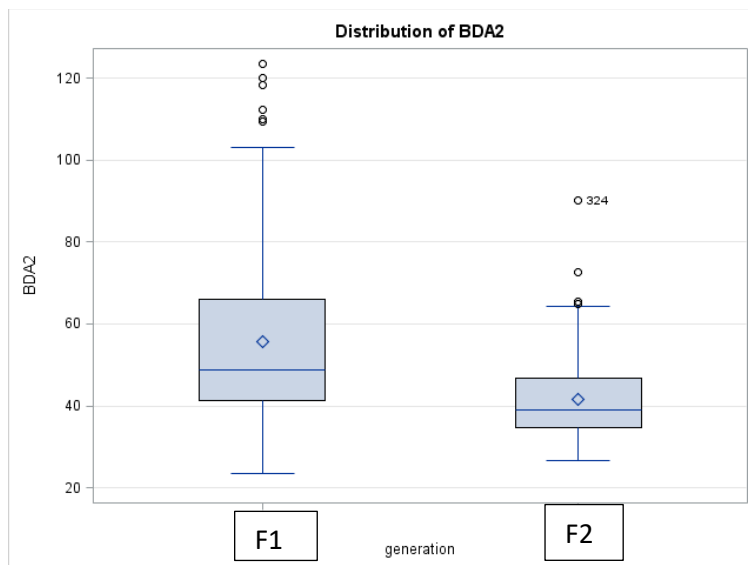


Figure2: Box plot of F1 and F2 generation body area of 90 days age

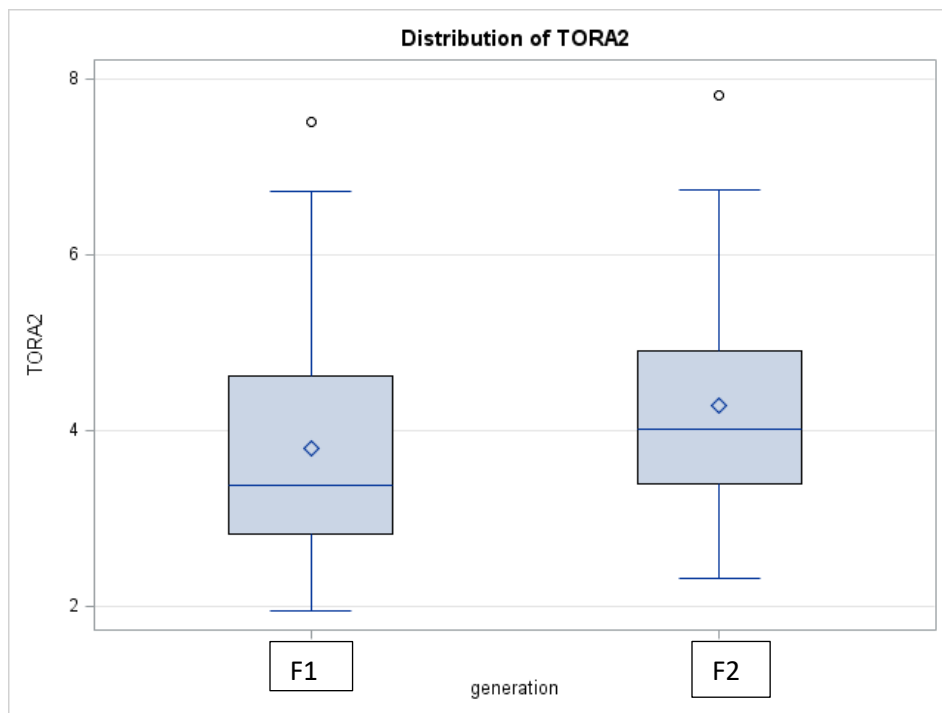


Figure 3: Box plot of F1 and F2 generation total orange area of 90 days age

Table 5: Frequency distribution in percentage (absolute numbers) of total orange area at 90 days' in F1 and F2 generation

CLASS INTERVAL	FREQUENCY OF F1	FREQUENCY OF F2
1.3-2.4	26.73 (27)	34.29 (24)
2.4-3.4	30.69 (31)	41.43 (29)
4.4-5.4	23.76 (24)	11.42 (8)
5.4-6.4	18.81 (19)	12.86 (9)
Total	100 (101)	100 (70)

Table 6: Frequency distribution in percentage (absolute numbers) of number of orange spots at 90 days' in F1 and F2 generation

CLASS INTERVAL	FREQUENCY OF F1	FREQUENCY OF F2
1	8.91 (9)	0
2	64.30 (65)	24.28 (17)
3	24.75 (25)	45.54 (46)
4	1.98 (2)	6.93 (7)
Total	100 (101)	100 (70)

4.5. Heritability Estimates

4.5.1. Heritability Estimates of ornamentation traits of F1 generation at 70 and 90 days' of age

Heritability estimates of ornamentation traits of F1 generation at 70 and 90 days' of age were estimated using sire and animal model and are presented in Table 7. A total of 29 full-sib families which included 12 half-sib families with 243 off-springs were included in the analysis. The heritability of total orange area (mm²) estimated by sire and animal model at 90 days' of age was 0.65 ± 0.49 and 0.60 ± 0.25 , respectively.

4.5.2. Heritability Estimates of ornamentation traits of F2 generation at 70 and 90 days' of age

Heritability of ornamentation traits of F2 generation at 70 and 90 days' of age were estimated using sire and animal model and are presented in Table 7. A total 25 full-sib families which included 8 half-sib families with 150 off-springs were included in the analysis. The heritability estimate of relative orange area (%) was estimated by sire and animal model at 90 days' of age was 0.61 ± 0.92 and 0.66 ± 0.31 respectively.

4.5.3. Heritability Estimates of ornamentation traits of combined generation at 70 and 90 days' of age

Heritability estimates of ornamentation traits at 70 and 90 days' of age of combined generations were estimated using sire and animal model and are presented in Table 7. A total 54 full-sib families which included 20 half-sib families with 393 off-springs were included in the analysis. The heritability of relative orange area (%) was estimated by sire and animal model at 90 days' of age and they were 0.53 ± 0.12 and 0.39 ± 0.12 respectively.

Table 7: Heritability estimates of ornamentation traits of 70 and 90 days' age in F1 & F2 & combined generation

TRAITS	F1 GENERATION						F2 GENERATION						COMBINED GENERATION			
	70 DAYS' AGE			90 DAYS AGE			70 DAYS' AGE			90 DAYS AGE			70 DAYS' AGE		90 DAYS AGE	
	SIRE MODEL		ANIMAL MODEL	SIRE MODEL		ANIMAL MODEL	SIRE MODEL		ANIMAL MODEL		SIRE MODEL	ANIMAL MODEL	SIRE MODEL K= 5.9 & 3.4	ANIMAL MODEL	SIRE MODEL K=4.7& 2.9	ANIMAL MODEL
	K Value	h ² ±SE	h ² ±SE	K value	h ² ±SE	h ² ±SE	K Value	h ² ±SE	h ² ±SE	K value	h ² ±SE	h ² ±SE	h ² ±SE	h ² ±SE	h ² ±SE	h ² ±SE
TL(mm)	4.9	0.65±0.10	0.85±0.17	4.9	0.47±0.20	0.48±0.18	4.4	0.21±0.19	0.19±0.14	3.72	0.37±0.17	0.24±0.15	0.49±0.10	0.47±0.12	0.44±0.11	0.31±0.11
SL(mm)	4.9	0.72±0.09	0.98±0.12	4.9	0.56±0.13	0.65±0.18	4.4	0.11±0.18	0.11±0.12	3.72	0.41±0.17	0.27±0.16	0.52±0.09	0.54±0.12	0.51±0.10	0.38±0.12
CFL(mm)	4.9	0.69±0.10	0.51±0.16	4.9	0.66±0.11	0.53±0.18	4.4	0.13±0.18	0.09±0.11	3.72	0.09±0.17	Non-Est	0.54±0.09	0.38±0.11	0.54±0.10	0.35±0.12
DFL(mm)	4.9	0.51±0.14	0.7±0.18	4.9	0.68±0.10	0.33±0.16	4.4	0.48±0.14	0.47±0.18	3.72	0.58±0.13	0.53±0.20	0.47±0.10	0.37±0.11	0.42±0.11	0.13±0.08
CFA(mm ²)	4.9	0.69±0.09	0.99±0.01	4.9	0.29±0.16	0.26±0.13	4.4	0.62±0.12	0.62±0.20	3.72	0.44±0.17	0.31±0.17	0.62±0.08	0.55±0.10	0.41±0.11	0.17±0.08
BDA(mm ²)	4.9	0.73±0.09	0.89±0.17	5.21	0.44±0.14	0.35±0.16	3.12	0.34±0.17	0.33±0.17	3.72	0.15±0.20	0.14±0.13	0.57±0.09	0.34±0.10	0.39±0.11	0.14±0.08
TORA(mm ²)	2.3	0.53±0.15	0.54±0.21	5.21	0.49±0.14	0.48±0.24	3.12	Non-Est	0.30±0.26	2.11	0.15±0.34	0.17±0.25	0.40±0.13	0.40±0.16	0.40±0.14	0.36±0.17
RORA(%)	2.3	0.49±0.16	0.42±0.20	5.21	0.51±0.17	0.25±0.19	3.12	Non-Est	0.12±0.20	2.11	0.19±0.28	0.65±0.29	0.32±0.13	0.27±0.14	0.31±0.15	0.20±0.13
ORAS	2.3	0.51±0.16	0.52±0.24	5.21	0.37±0.20	0.24±0.2	3.12	0.08±0.28	0.12±0.18	2.11	Non-Est	Non-Est	0.33±0.15	0.32±0.15	0.09±0.19	0.03±0.08
MB(PI)	2.3	0.45±0.19	0.4±0.21	5.21	0.38±0.20	0.53±0.27	3.12	0.2±0.30	0.39±0.25	2.11	Non-Est	0.15±0.23	0.28±0.16	0.12±0.12	0.21±0.18	0.19±0.15
MS(%)	2.3	0.33±0.19	0.91±0.20	5.21	0.29±0.22	0.36±0.23	3.12	0.46±0.20	0.58±0.26	2.11	0.46±0.23	0.5±0.29	0.55±0.11	0.56±0.17	0.41±0.15	0.32±0.17
MH(°)	2.3	0.62±0.13	0.43±0.22	5.21	0.2±0.24	0.37±0.25	3.12	Non-Est	Non-Est	2.11	Non-Est	Non-Est	0.36±0.14	0.24±0.12	0.20±0.20	0.22±0.14

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MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

PI-PIXEL INTENSITY

4.6. Genetic and phenotypic correlations between various ornamentation traits

The genetic and phenotypic correlations along with their standard errors between various ornamentation traits of 70 and 90 days' age in F1 generation are presented in Table 8 and 9 while for F2 generation they are presented in Table 10 and 11. In general the genetic and phenotypic correlations between body shape traits were positive and moderate to high at both the age groups. The genetic and phenotypic correlation between total orange area and number of orange spots in the combined generations at 90 days' of age was 0.54 ± 1.65 , 0.65 ± 0.11 , respectively (Table 13). The genetic and phenotypic correlations between hue and saturation in the combined generations at 90 days' of age were -0.13 ± 0.45 and -0.16 ± 0.14 , respectively.

Table 8: Genetic and phenotypic correlation between ornamentation traits for F1 generation at 70 days' of age

TRAITS	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
TL	-	0.96±0.02	0.57±0.17	0.22±0.25	0.75±0.09	0.78±0.10	0.44±0.25	0.17±0.33	-0.07±0.33	0.43±0.30	0.14±0.27	0.14±0.33
SL	0.99±2.6	-	0.67±0.14	-0.05±0.27	0.78±0.08	0.68±0.13	0.44±0.24	0.13±0.31	-0.09±0.33	0.53±0.27	0.23±0.26	0.13±0.32
DFL	0.76±0.31	0.69±2.08	-	-0.34±0.27	0.83±0.08	0.22±0.24	0.20±0.30	0.48±0.26	0.01±0.35	0.40±0.31	-0.01±0.28	-0.09±0.36
CFL	1.00±0.57	0.99±5.97	0.79±0.34	-	-0.02±0.26	0.38±0.23	0.29±0.31	0.41±0.33	0.31±0.32	0.08±0.35	-0.20±0.28	-0.04±0.36
CFA	0.37±16.1	0.00±0.00	0.00±0.00	0.00±0.71	-	0.56±0.16	0.06±0.30	-0.02±0.31	-0.41±0.27	0.35±0.30	-0.02±0.26	0.01±0.32
BDA	0.42±0.66	0.61±1.95	0.90±0.71	0.32±0.42	0.69±0.46	-	0.46±0.25	-0.35±0.28	-0.29±0.30	0.49±0.27	0.17±0.26	0.39±0.27
TORA	-0.26±0.6	-0.35±2.84	0.26±0.32	-0.35±0.29	-0.20±0.74	-0.08±0.65	-	0.28±0.34	0.93±0.06	0.73±0.33	0.48±0.28	-0.35±0.40
ORAS	-0.50±0.52	-0.71±3.52	-0.2±0.45	-0.52±0.26	-0.02±0.19	0.55±0.91	0.88±0.19	-	0.54±0.28	-0.22±0.39	-0.03±0.34	-0.27±0.40
RORA	-0.30±0.12	-0.56±2.77	0.15±0.29	-0.52±0.26	-0.13±0.23	-0.29±0.48	0.94±0.04	0.66±0.16	-	0.58±0.40	0.44±0.31	-0.33±0.40
MB	-0.22±0.5	-0.43±2.86	-0.12±0.3	-0.21±0.23	-0.70±0.44	-0.33±0.69	-0.25±0.27	0.33±0.31	-0.18±0.22	-	-0.11±0.35	-0.40±0.41
MS	-0.54±1.3	-1.24±3.93	-0.02±0.8	0.16±0.6	-0.49±0.41	-0.16±1.57	-0.93±1.29	0.15±0.72	-0.76±1.21	-0.15±0.61	-	-0.40±0.32
MH	0.18±0.42	0.14±1.3	0.21±0.29	0.17±0.23	0.73±0.29	-0.06±0.59	0.39±0.26	0.38±0.30	0.28±0.24	0.12±0.24	0.00±0.64	-

Values above diagonal are genetic correlation and values below diagonal are phenotypic correlation

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

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Table 9: Genetic and phenotypic correlation between ornamentation traits for F1 generation at 90 days' of age

TRAITS	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
TL	-	0.96±0.02	0.68±0.21	-0.25±0.3	0.90±0.08	0.63±0.19	0.22±0.39	-0.06±0.47	-0.40±0.37	0.16±0.38	0.23±0.42	-0.79±0.25
SL	0.98±0.03	-	0.68±0.20	-0.5±0.24	0.81±0.13	0.34±0.27	-0.05±0.37	-0.04±0.46	-0.52±0.31	-0.05±0.36	0.34±0.39	-0.81±0.23
DFL	0.51±0.12	0.45±0.16	-	-0.25±0.32	0.74±0.21	0.34±0.33	-0.01±0.43	-0.69±0.38	-0.70±0.36	-0.09±0.44	-0.23±0.44	-1.26±0.11
CFL	0.81±0.27	0.68±0.43	0.58±0.23	-	0.12±0.35	0.72±0.2	0.62±0.26	0.11±0.48	0.18±0.43	0.64±0.31	0.25±0.45	-0.41±0.37
CFA	0.79±0.06	0.83±0.10	0.52±0.09	0.44±0.16	-	0.78±0.15	0.36±0.38	0.08±0.50	-0.11±0.46	0.52±0.34	0.25±0.45	-0.74±0.34
BDA	0.99±0.06	1.16±0.19	0.53±0.12	0.29±0.16	0.82±0.05	-	0.43±0.34	0.33±0.47	-0.30±0.39	0.59±0.32	-0.06±0.44	-0.42±0.41
TORA	-0.10±0.27	-0.01±0.33	0.09±0.23	-0.22±0.35	-0.14±0.23	-0.18±0.27	-	-0.30±0.60	0.80±0.17	0.54±0.32	0.61±0.45	0.17±0.47
ORAS	0.14±0.21	0.16±0.26	0.49±0.22	-0.08±0.23	0.06±0.18	-0.06±0.19	0.73±0.18	-	-0.06±0.64	0.01±0.53	-0.65±0.63	0.61±0.55
RORA	-0.44±0.18	-0.36±0.23	-0.06±0.19	-0.08±0.24	-0.58±0.14	-0.63±0.15	0.95±0.08	0.46±0.15	-	0.11±0.51	0.83±0.46	0.69±0.47
MB	-0.11±0.29	-0.01±0.35	0.25±0.23	-0.29±0.33	-0.48±0.33	-0.28±0.3	0.14±0.33	0.68±0.25	0.32±0.23	-	-0.98±0.19	0.43±0.46
MS	-0.11±0.24	-0.18±0.30	0.10±0.21	0.13±0.27	-0.23±0.19	-0.09±0.21	0.00±0.24	0.35±0.20	0.00±0.19	0.74±0.54	-	0.06±0.52
MH	0.58±0.34	0.79±0.50	0.53±0.21	-0.20±0.28	0.26±0.22	0.31±0.22	-0.03±0.27	-0.15±0.21	-0.19±0.23	-0.18±0.31	-0.24±0.24	-

Values above diagonal are genetic correlation and values below diagonal are phenotypic correlation

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

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Table 10: Genetic and phenotypic correlation between ornamentation traits for F2 generation at 70 days' of age

TRAITS	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
TL	-	0.72±1.39	0.75±0.26	0.57±0.48	0.93±0.13	1.05±0.10	1.10±0.20	-0.06±0.85	0.87±0.39	-0.08±0.52	-0.42±0.57	0.64±0.24
SL	0.94±0.01	-	0.92±0.32	0.55±0.64	0.76±0.33	0.97±0.39	1.14±0.43	-0.29±0.99	0.83±0.64	0.13±0.65	-1.00±1.03	-1.00±1.03
DFL	0.40±0.13	0.34±0.12	-	0.64±0.37	0.55±0.24	0.70±0.22	0.66±0.24	-0.23±0.62	0.59±0.47	0.42±0.40	-0.12±0.38	-0.12±0.38
CFL	0.58±0.08	0.39±0.10	0.40±0.12	-	0.54±0.38	0.74±0.33	0.94±0.21	0.13±1.18	0.95±0.28	-0.63±0.54	0.54±0.44	-0.12±0.90
CFA	0.90±0.07	0.85±0.11	0.37±0.21	0.71±0.11	-	0.96±0.06	0.95±0.15	-0.20±0.76	0.85±0.3	-0.11±0.38	-0.13±0.36	-0.70±0.89
BDA	0.77±0.05	0.70±0.07	0.36±0.15	0.59±0.09	0.78±0.08	-	0.98±0.09	0.21±0.75	0.90±0.33	0.28±0.43	-0.17±0.41	-0.17±0.41
TORA	0.28±0.16	0.22±0.15	0.50±0.19	0.09±0.23	0.18±0.29	0.33±0.21	-	-0.34±1.02	-0.13±1.02	-0.30±2.02	-0.23±1.42	-0.43±1.03
ORAS	-0.05±0.17	-0.05±0.15	0.31±0.19	-0.10±0.14	-0.19±0.23	-0.14±0.18	0.66±0.16	-	-0.43±2.02	-0.96±0.86	0.70±0.46	0.70±0.46
RORA	-0.23±0.20	-0.17±0.17	0.18±0.20	-0.20±0.21	-0.49±0.36	-0.46±0.24	0.94±0.06	0.57±0.13	-	1.31±1.06	-0.22±0.74	-0.22±0.74
MB	0.16±0.22	-0.02±0.19	-0.43±0.27	0.32±0.22	0.18±0.34	0.13±0.22	-0.48±0.17	0.12±0.21	-0.47±0.22	-	-0.05±0.47	1.46±0.54
MS	0.37±0.23	0.33±0.19	0.10±0.30	0.02±0.24	0.07±0.38	0.01±0.27	0.29±0.28	0.14±0.26	0.36±0.25	-0.23±0.31	--	-0.29±0.60
MH	-0.40±0.14	-0.38±0.13	-0.02±0.17	-0.13±0.14	-0.21±0.21	-0.26±0.16	-0.39±0.12	0.00±0.15	-0.17±0.16	-0.81±0.21	-0.28±0.28	-

Values above diagonal are genetic correlation and values below diagonal are phenotypic correlation

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

TORA – TOTAL ORANGE AREA, ORAS – NUMBER OF ORANGE SPOTS, RORA – RELATIVE ORANGE AREA, MB- MEAN BRIGHTNESS OF ORANGE SPOTS,

MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

Table 11: Genetic and phenotypic correlation between ornamentation traits for F2 generation at 90 days' of age

TRAITS	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
TL	-	0.99±0.01	0.36±0.16	0.17±0.46	0.62±0.28	0.68±0.35	0.08±0.71	0.12±0.43	0.12±0.43	0.22±0.63	0.17±0.46	0.17±0.46
SL	0.98±0.01	-	0.32±0.35	1.02±1.91	0.64±0.27	0.72±0.33	0.20±0.55	0.22±0.44	0.16±0.41	0.24±0.59	0.22±0.44	0.22±0.44
DFL	0.31±0.17	0.35±0.17	-	-0.02±0.15	0.04±0.38	0.49±0.39	0.46±0.31	1.02±1.91	0.34±0.32	0.51±0.51	0.31±0.38	1.02±1.91
CFL	-0.21±0.10	-0.33±0.10	-0.02±0.10	-	0.02±1.91	0.02±0.91	0.79±0.10	1.02±1.91	0.91±1.50	0.02±1.91	1.02±1.01	0.12±1.91
CFA	0.64±0.09	0.61±0.10	0.36±0.20	-0.01±0.10	-	0.17±0.57	-0.19±0.63	0.94±0.04	-0.14±0.40	-0.27±0.59	0.15±0.45	0.11±1.09
BDA	0.62±0.08	0.58±0.09	0.30±0.16	0.08±0.11	0.74±0.07	-	0.13±0.62	0.94±0.04	-0.36±0.50	0.28±0.73	0.17±0.57	1.02±1.91
TORA	0.58±0.13	0.61±0.14	0.31±0.30	-0.24±0.18	0.51±0.18	0.64±0.15	-	0.53±0.17	0.99±0.82	1.02±1.91	0.54±0.04	0.91±0.04
ORAS	0.09±0.16	0.07±0.17	0.10±0.21	0.01±0.15	0.01±0.17	0.10±0.17	0.94±0.04	-	0.66±0.16	0.40±0.04	0.90±0.04	0.24±0.04
RORA	0.28±0.30	0.35±0.31	-0.05±0.50	-0.36±0.20	0.15±0.32	-0.23±0.20	1.07±0.19	0.46±0.21	-	0.50±0.77	0.60±0.37	0.90±0.04
MB	-0.01±0.20	0.05±0.19	-0.40±0.26	-0.17±0.10	0.14±0.21	0.17±0.18	-0.08±0.20	0.25±0.17	-0.3±0.37	-	0.78±1.58	0.40±0.04
MS	0.03±0.26	0.06±0.27	-0.10±0.35	-0.30±0.24	0.10±0.27	0.25±0.22	-0.04±0.00	-0.03±0.20	-0.01±0.40	-0.36±0.24	-	0.90±0.07
MH	-0.37±0.2	-0.35±0.50	0.02±0.21	0.08±0.14	-0.11±0.7	-0.05±0.20	-0.2±0.18	-0.01±0.40	-0.24±0.24	-0.30±0.18	-0.05±0.22	-

Values above diagonal are genetic correlation and values below diagonal are phenotypic correlation 0.94±0.04

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

TORA – TOTAL ORANGE AREA, ORAS – NUMBER OF ORANGE SPOTS, RORA – RELATIVE ORANGE AREA, MB- MEAN BRIGHTNESS OF ORANGE SPOTS,

MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

Table 12: Genetic and phenotypic correlation between ornamentation traits for combined generation at 70 days' of age

TRAITS	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
TL	-	0.95±0.02	0.48±0.17	0.24±0.21	0.72±0.10	0.88±0.06	0.59±0.21	0.41±0.30	0.36±0.32	0.44±0.45	0.26±0.26	-0.10±0.32
SL	0.92±0.02	-	0.55±0.16	-0.06±0.23	0.73±0.09	0.79±0.09	0.46±0.24	0.26±0.31	0.19±0.33	0.56±0.42	0.29±0.26	-0.20±0.12
DFL	0.59±0.08	0.52±0.09	-	-0.18±0.26	0.64±0.14	0.43±0.20	0.41±0.26	0.24±0.30	0.36±0.31	0.41±0.46	0.09±0.28	-0.40±0.3
CFL	0.60±0.09	0.32±0.12	0.43±0.09	-	0.01±0.2	0.40±0.22	0.38±0.25	0.45±0.29	0.36±0.30	-0.21±0.48	0.07±0.26	-0.20±0.30
CFA	0.76±0.05	0.66±0.07	0.47±0.08	0.51±0.10	-	0.69±0.11	0.28±0.25	-0.06±0.29	-0.03±0.30	0.00±0.47	0.15±0.24	-0.25±0.28
BDA	0.69±0.05	0.62±0.07	0.39±0.08	0.51±0.10	0.72±0.06	-	0.60±0.22	-0.12±0.31	0.05±0.34	0.65±0.46	0.16±0.27	0.17±0.31
TORA	0.16±0.15	0.20±0.16	0.34±0.12	-0.09±0.16	0.19±0.16	0.35±0.11	-	-0.17±0.37	0.84±0.11	1.01±0.83	0.42±0.27	-0.12±0.37
ORAS	-0.27±0.16	-0.21±0.16	0.18±0.13	-0.22±0.14	-0.13±0.15	-0.05±0.13	0.78±0.12	-	0.24±0.37	-0.93±0.31	0.31±0.30	-0.30±0.40
RORA	-0.35±0.14	-0.25±0.15	0.10±0.13	-0.25±0.14	-0.26±0.15	-0.28±0.12	0.93±0.03	0.62±0.09	-	0.33±0.59	0.47±0.29	-0.17±0.41
MB	0.05±0.13	0.01±0.14	-0.05±0.12	0.02±0.12	0.16±0.13	0.11±0.11	-0.10±0.13	0.31±0.15	-0.06±0.12	-	-0.49±0.49	0.71±0.52
MS	-0.06±0.18	-0.03±0.19	-0.09±0.17	-0.09±0.17	-0.26±0.19	-0.14±0.16	-0.09±0.19	0.04±0.18	-0.06±0.17	-0.01±0.15	-	-0.06±0.33
MH	0.03±0.13	-0.03±0.14	0.17±0.12	0.17±0.13	0.10±0.14	0.00±0.12	0.04±0.13	0.14±0.12	0.05±0.12	-0.14±0.11	-0.37±0.17	-

Values above diagonal are genetic correlation and values below diagonal are phenotypic correlation

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

TORA – TOTAL ORANGE AREA, ORAS – NUMBER OF ORANGE SPOTS, RORA – RELATIVE ORANGE AREA, MB- MEAN BRIGHTNESS OF ORANGE SPOTS,

MS - MEAN SATURATION OF ORANGE SPOTS, MH - MEAN HUE OF ORANGE SPOTS

Table 13: Genetic and phenotypic correlation between ornamentation traits for combined generation at 90 days' of age

TRAITS	TL	SL	DFL	CFL	CFA	BDA	TORA	ORAS	RORA	MB	MS	MH
TL	-	0.97±0.02	0.67±0.22	-0.22±0.26	0.87±0.09	0.80±0.13	0.10±0.35	0.27±0.94	-0.33±0.32	0.00±0.42	0.11±0.37	-0.53±0.29
SL	0.96±0.01	-	0.70±0.21	-0.42±0.22	0.84±0.10	0.64±0.19	-0.10±0.32	0.19±0.87	-0.38±0.30	-0.28±0.38	0.18±0.36	-0.50±0.28
DFL	0.45±0.07	0.41±0.07	-	-0.28±0.35	0.25±0.36	0.06±0.41	0.14±0.40	-0.97±0.58	-0.17±0.46	-0.34±0.54	0.67±0.40	-1.02±0.21
CFL	0.42±0.10	0.18±0.12	0.35±0.09	-	-0.07±0.3	0.60±0.26	0.65±0.24	0.64±1.39	0.36±0.35	0.81±0.27	-0.33±0.34	0.05±0.38
CFA	0.74±0.04	0.69±0.05	0.51±0.06	0.39±0.09	-	0.61±0.22	0.24±0.37	0.26±4.62	0.01±0.41	0.13±0.44	0.21±0.39	-0.54±0.32
BDA	0.80±0.04	0.75±0.05	0.49±0.06	0.82±0.03	0.82±0.03	-	0.29±0.38	0.20±3.33	-0.32±0.37	0.47±0.43	-0.11±0.41	-0.30±0.41
TORA	0.16±0.14	0.19±0.15	0.19±0.12	-0.14±0.17	0.10±0.13	0.14±0.12	-	0.54±1.65	0.80±0.15	0.02±0.49	0.90±0.26	0.90±0.26
ORAS	0.04±0.11	0.05±0.12	0.28±0.10	-0.09±0.12	-0.06±0.1	-0.03±0.10	0.65±0.11	-	-1.97±8.45	-1.04±1.00	-0.35±1.42	0.09±1.06
RORA	-0.30±0.12	-0.25±0.13	-0.08±0.11	-0.16±0.14	-0.44±0.11	-0.51±0.09	0.88±0.04	0.48±0.08	-	-0.46±0.56	0.74±0.35	0.45±0.43
MB	0.03±0.13	0.08±0.14	0.11±0.11	-0.17±0.15	-0.05±0.12	0.06±0.11	0.30±0.14	0.43±0.11	0.29±0.12	-	-1.05±0.15	0.97±0.33
MS	-0.04±0.14	-0.03±0.15	-0.1±0.12	0.02±0.16	-0.12±0.13	-0.02±0.13	0.04±0.16	0.15±0.12	0.11±0.13	0.33±0.21	-	-0.13±0.45
MH	0.10±0.13	0.11±0.14	0.16±0.12	0.00±0.14	0.08±0.12	0.14±0.12	-0.03±0.15	-0.01±0.11	-0.12±0.14	-0.22±0.15	-0.16±0.14	

Values above diagonal are genetic correlation and values below diagonal are phenotypic correlation

TL- TOTAL LENGTH, SL – STANDARD LENGTH, DFL – DORSAL FIN LENGTH, CFL – CAUDAL FIN LENGTH, CFA – CAUDAL FIN AREA, BDA - BODY AREA,

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4.7. The expected and realized genetic gain

The selection differential, expected genetic gain, realized genetic gain and realized heritability are presented in Table 14 and 15. The selection differential for total orange area in the base generation was 0.37 mm² and that for number of orange spots was 0.27 (Table 14). Similarly the selection differential for total orange area in the F1 generation was 0.14 mm² and that for number of orange spots was 0.49 (Table 14). There was a negative genetic gain for the total orange area and number of orange spots in F1 generation. In F2 generation the realized genetic gain for total orange area was 9.16 per cent.

Table 14: Expected genetic gain and realized genetic gain for F1 generation ornamentation traits

TRAITS	MEAN OF THE SELECTED PARENTS ($\overline{Psp}$)	OVERALL POPULATION MEAN BEFORE SELECTION ($\overline{Ppop}$)	SELECTION DIFFERENTIAL (SD)	$h^2 \pm SE$	EXPECTED GENETIC GAIN (ΔG)	PROGENY MEAN	REALIZED GENETIC GAIN (%)	REALIZED HERITABILITY(h^2_R)
Total orange spot area (mm ²)	4.49±1.07	4.12±0.27	0.37	0.31±0.16	0.11	3.83±0.13	-0.29 (-6.45%)	-0.78
Number of orange spots	2.60±1.05	2.33±0.10	0.27	0.10±0.08	0.027	2.18±0.06	-0.15(-5.76%)	-0.55

Table 15: Expected genetic gain and realized genetic gain for F2 generation ornamentation traits

TRAITS	MEAN OF THE SELECTED PARENTS ($\overline{P_{sp}}$)	OVERALL POPULATION MEAN BEFORE SELECTION ($\overline{P_{pop}}$)	SELECTION DIFFERENTIAL (SD)	$h^2 \pm SE$	EXPECTED GENETIC GAIN(ΔG)	PROGENY MEAN	REALIZED GENETIC GAIN (%)	REALIZED HERITABILITY(h^2_R)
Total orange spot area (mm ²)	3.93±1.07	3.83±0.13	0.1	0.49±0.14	0.05	4.19±0.15	0.36 (9.16%)	3.60
Number of orange spots	2.68±1.05	2.18±0.06	0.5	0.37±0.20	0.18	2.87±0.07	0.69 (25.7%)	1.38

Figure 4 : Orange color spots and their distribution in F1 generation male guppies at 90 days'of age

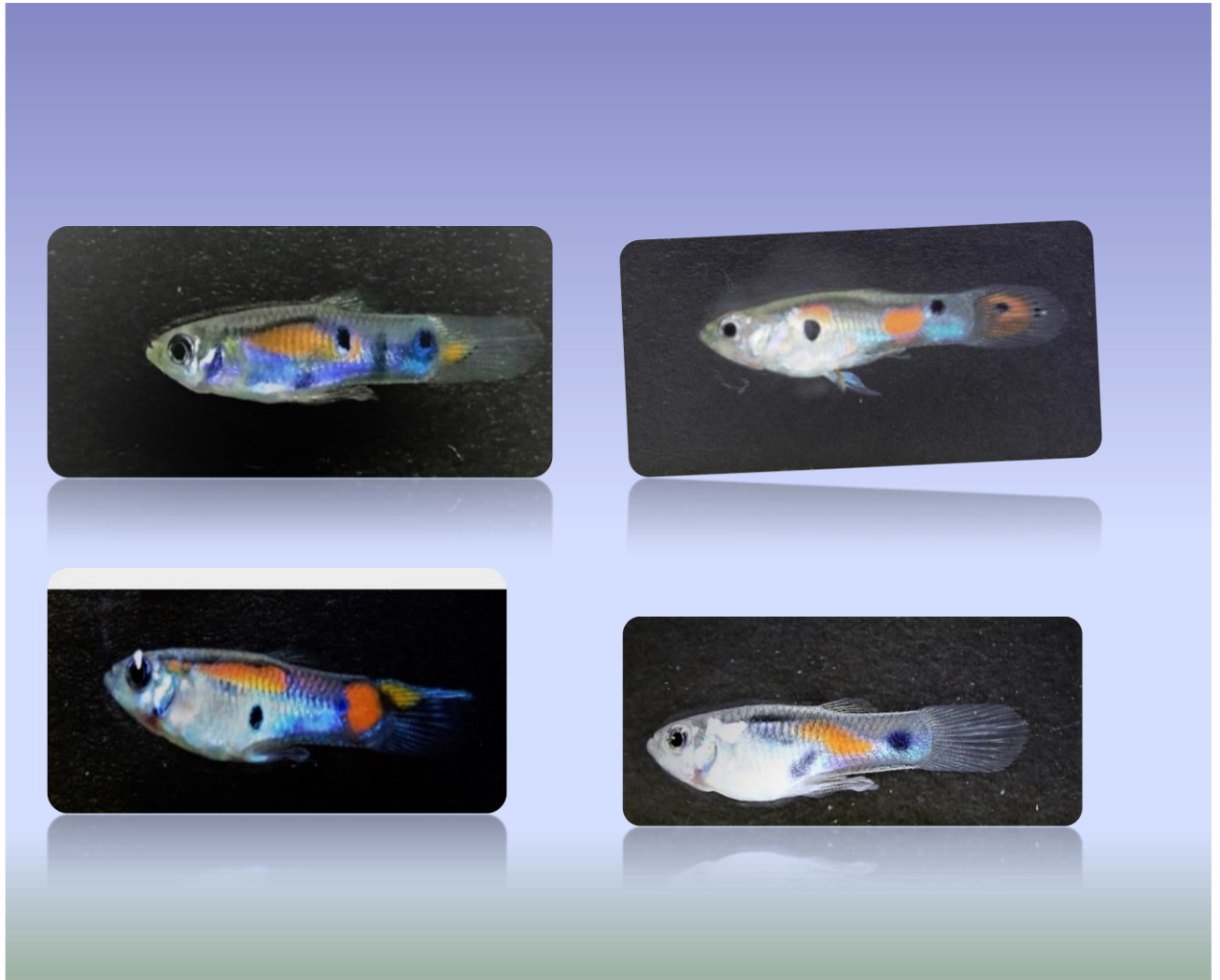


Figure 5: Orange color spots and their distribution in F1 generation male guppies at 90 days' of age

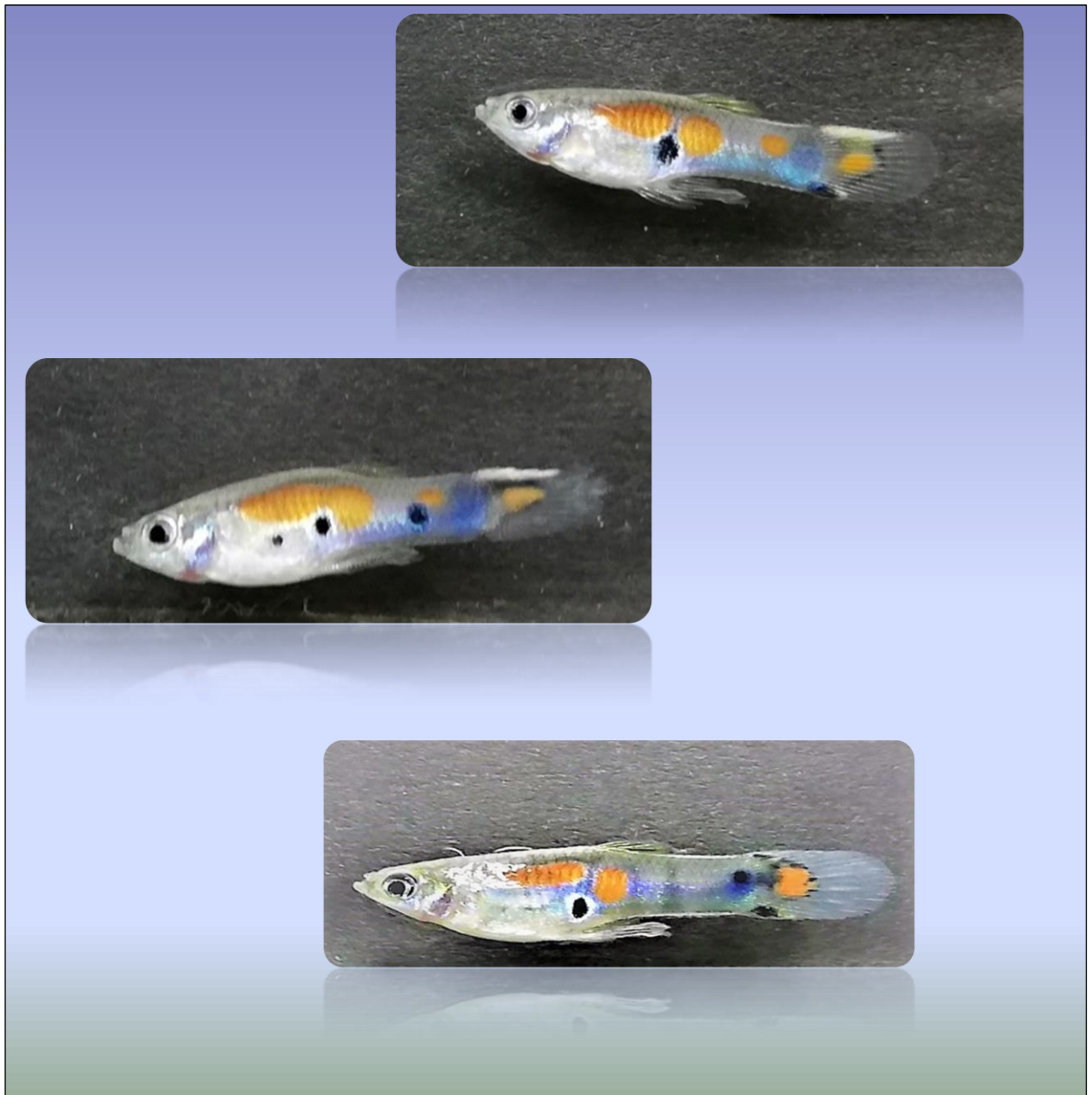


Figure 6: Orange color spots and their distribution in F1 generation male guppies at 90 days' of age (Sire with its offspring)

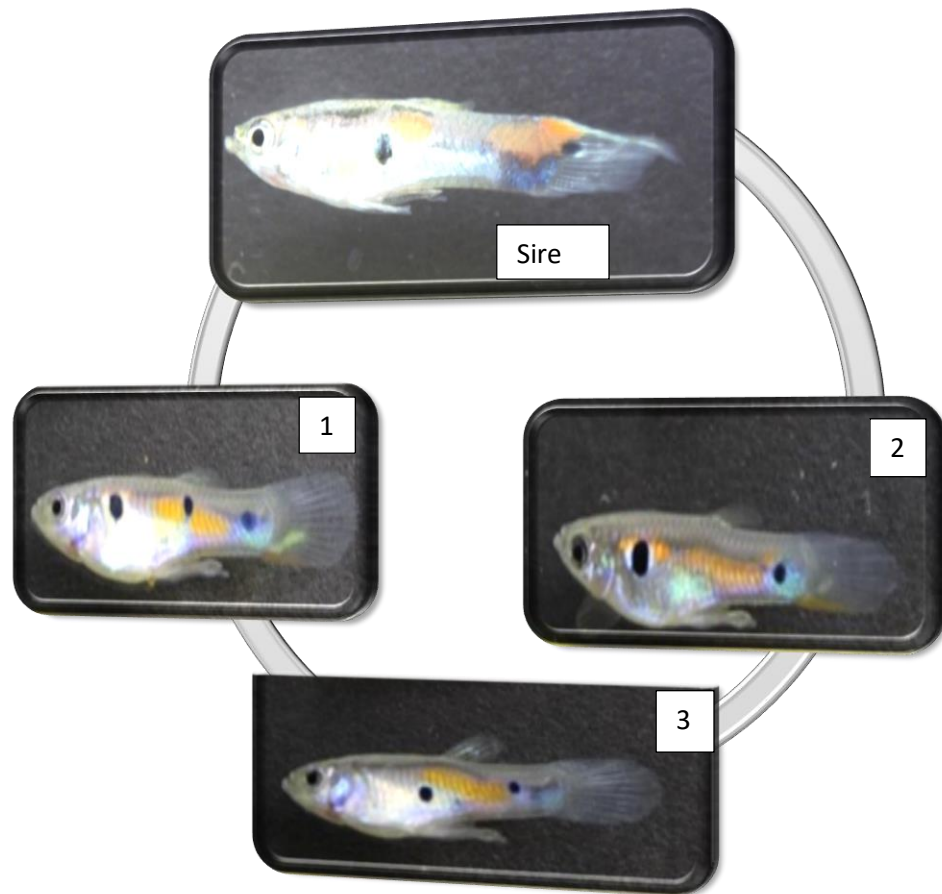
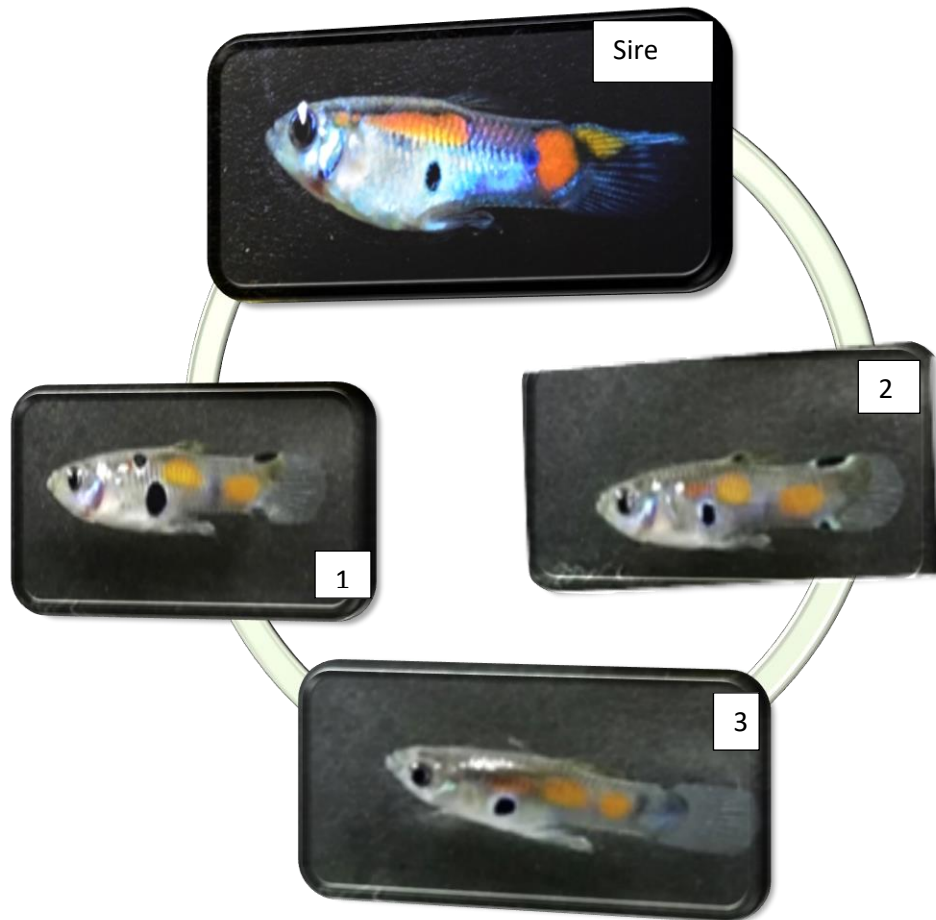


Figure 7: Orange color spots and their distribution in F1 generation male guppies at 90 days' of age (Sire with offspring)



5. DISCUSSION

5.1. Ornamentation traits analysis

5.1.1. Body size-related traits in the guppy

The least squares mean total length of male and female guppy was 20.8 ± 0.23 and 23.6 ± 0.24 mm at 90 days' of age (Table 2). The least squares mean body area of male and female in combined generation was 40.5 ± 1.07 and 56.2 ± 1.11 mm² at 90 days' of age (Table 2). The females were significantly longer and larger than males at 90 days' of age. The means of all measured body shape traits were significantly higher in female guppies as compared to male guppies (Table 3). Female guppies were found to be significantly larger than male guppies in both F1 and F2 generations.

Guppies are live bearers and large body size of female helps them raise large number of young ones and probably the natural selection also favors a large sized female guppy. The body size traits are important economic traits in ornamental fish species as they are also one major component which influences the market price. The body size traits are also important from the point of reproduction as the rate of reproduction is directly related to body traits (Phang and Doyle, 1989).

Body size and shape traits are also of importance in guppies as they play an important role along with the orange coloration in mate selection in the natural environments. Mate selection studies have shown that female guppies prefer larger male as a mate, which helps to improve the breeding rate. The earlier reports reveal the presence of large variation in body size traits in male guppies within the population (Siva, 2013; Amritha, 2016; Smrithi, 2018). In the present study variation was observed in total length, dorsal and caudal fin length etc. These traits helps male guppy to show off and aid in attracting females as their mate (Karino and Hajima, 2001). In the present study a large variation was observed in both between and within male and female guppies in their body size traits (Figure 1 and 2). Brown and Nicoletto (2001) suggested the female guppies having a mean standard length of 17.6 ± 0.54 mm are virgin and readily accept males.

The least squares means of total length, body area, total orange area and other ornamentation traits of F1 and F2 generations are presented in Table 1 and 2, respectively. The means of body shape and size traits were significantly lower in second generation as compared to means of first generation fish (Table 3). One probable reason for lower means in second generation may be due to the more number of male guppies than number of females which may have pulled down the averages of these traits in this generation. The means of sex by generation reveals that there was no difference in the body size traits of females of first and second generation, however, the average size of males of second generation was significantly lower than the averages of first generation males, hence it can be concluded that there was overall reduction in the means of males and this had led to the reduction in the overall means in second generation.

5.1.2. Carotenoid and pteridine pigmentation traits in guppy

The carotenoid and pteridine pigmentation traits are exhibited only in males because these traits are either sex-linked and/or sex-limited traits. The male color patterns are complex, conspicuous and manifested as spots and speckles of orange, black and red color (Tezuka *et al.*, 2011). The size, shape, position and number of orange spots vary greatly both between and within families (Brooks and Endler, 2001). The studies reveal that female guppies mainly prefer male with brighter orange spots as mate (Bischoff *et al.*, 1985) but this behavior of females is also not universal as in areas of high predation the orange color attracts the predators hence, males show reduced orange coloration and probably females also prefer males with less number of orange spots and area in high predation environment (Endler, 1980; Houde, 1997). In the present study the least squares mean of total orange area and number of orange spots at 90 days' of age was $4.02 \pm 0.09 \text{ mm}^2$ and 2.53 ± 0.04 numbers, respectively (Table 2). The number of orange spots ranged from 1 to 4 in the present study (Table 6). The relative orange area, mean brightness of orange spots, mean saturation and mean hue of orange spots at 90 days' of age are presented in the Table 2. In the present study the average total orange area at 90 days' of age in F1 and F2 generations were significantly different from each other, in that the second generation male showed significantly larger orange colored area (Figure 3). In the present study the average numbers of orange spots at 90 days of age were higher than the average number of

orange spots at 70 days of age (Table 1 and 2). It was observed that the orange spots started to appear when male guppies reached 45 days of age that is even before they attain sexual maturity and these numbers kept increasing with age. These results indicate that the number of orange spots may increase with age and probably even after 90 days of age that is the guppies may attain a final sexual maturity beyond 90 days of age and there is a need to determine the final sexual maturity age and size in guppies for a better genetic selection criterion. The average total orange color area and also the relative orange color area were higher at 90 days of age as compared to the average of 70 days of age. Pitcher *et al.* (2007) reported that some of the orange spots reached only 60% of final size at sexual maturity, and the area of orange does not attain its full size until 22–25 days after full maturity in the laboratory. Evans *et al.* (2002) reported that the area of orange coloration increased with age in males.

In the present study a high variation in number of orange spots was observed both within and between families. It was observed that the males with more number of orange spots sired more offspring as compared to the males with less number of spots (Results not shown). Smrithi (2018) reported the least squares mean for total orange area and number of orange spots in guppy as $3.98 \pm 0.31 \text{ mm}^2$ and 2.17 ± 0.10 numbers.

An individual is considered more ornamented if its orange colour had higher hue, is brighter and more saturated (Pitcher *et al.*, 2007). The properties of color such as brightness, saturation, hue are condition dependent pigmentation traits (Karino and Hajima, 2001). Hue is scored within 0° – 360° range and brightness ranges from 0–255 pixel intensity and saturation ranges from 0% (grey)–100 % (pure color) (Viethen *et al.*, 2012). The studies showed that the hue and saturation of orange color in guppy is dependent on the availability of carotenoids in the diet as the guppies like many other species depend on the dietary carotenoids for formation of carotenoid pigments (Kodric-Brown, 1989; Grether, 2000; Pitcher, *et al.*, 2007). In the present study the guppies were fed with tubifex and blood worms which contain the astaxanthin in high concentration and provided enough carotenoids to the fishes. As all fish in the present study were having access to the enough carotenoid, hence any variation in the hue and saturation may be attributed to foraging ability, digest the same and convert it in to the carotenoid pigments. The variation in the

hue, saturation and brightness between the families managed under similar environment and culture practices indicates a genetic basis for these traits albeit indirectly may be, which further needs to be confirmed by conducting the genetic selection studies. The variation in these traits within family indicates non-additive genetic and environmental control.

In contrast to hue and saturation, the number of orange spots and area of orange coloration found on male guppies does not appear to be affected by carotenoid intake (Kodric Brown, 1989; Grether, 2000; Pitcher *et al.*, 2007) but instead has a substantial genetic component (Houde, 1992; Pitcher *et al.*, 2007). In the present study there was a significant difference in the average number of orange spots, orange area and relative orange area of first and second generations at 90 days of age (Table 4). In the present study the fish were selected for higher orange area and higher orange spot numbers and a favorable genetic gain may have been realized leading to improvement in these traits in second generation.

In the present study sex and generation are considered as non-genetic factors. The sex and generation had significant effect on body size related traits (Table 5). Sexual dimorphism is a well-known fact in the guppies where the female guppy tends to be substantially larger and longer than the male guppy (Endler, 1983; Amritha, 2016; Smrithi, 2018) and the present study confirms this further (Table No 2).

5.2. Heritability estimates of ornamentation traits

Heritability is the important genetic parameter in selective breeding program. The estimate helps to decide the method of selection to be adopted and also aids to estimate the accuracy of selection. The heritability estimates are sensitive to sample size, the type of family information and method of estimations. In the present study the heritability of all the traits were estimated by adopting the sire and animal model for F1, F2 and combining the information of both the generations and are presented in Table 7. The value of heritability indicates the inheritance level of traits from parents to offspring and also genetic difference between traits. A high heritability indicates that a substantial proportion of the phenotypic variance is due to additive genetic variance (Cameron, 1997). In the

present study the heritability estimates of body size and shape traits of 90 days' of age were moderate in nature (Table 7) and for orange color related traits they were low to moderate in nature. The heritability values estimated from sire model in general were higher as compared to the heritability values estimated from animal model. In the present very few half-sibs were available and the sire variance component may have contained along with the additive genetic variance a portion of dominance genetic effect, maternal effect and interaction effects among them which may have inflated the heritability values estimated from sire model (Becker, 1984; Falconer and Mackey, 1981). Also in the present study the animals were maintained full-sib family wise and the tank effects like variation in stocking density, etc., may have confounded with the sire variance component and it may have inflated the heritability estimates. The animal model is considered to be superior as compared to sire, sire-dam model (Lynch and Walsh, 1996) as the animal model takes into account the genetic correlation among all the individuals considered in the analysis.

The heritability estimates reported for total length in guppies ranged from 0.19 to 1.00 (Karino and Hajima, 2001; Evans, 2010; Brooks and Endler, 2001; Amirtha, 2016; Smrithi, 2018). Heritability of standard length (mm) ranges from 0.04 to 0.66 (Karino and Hajima, 2001; Evans, 2010; Brooks and Endler, 2000; Smrithi, 2018). Heritability of body area (mm²) ranged from 0.19 to 0.87 (Evans, 2010; Brooks and Endler, 2001; Smrithi, 2018). These results indicate that the heritability estimates for body size and shape are moderate to high in nature and these traits are amenable to genetic improvement.

The heritability estimate of total orange area from combined generation was 0.36 ± 0.17 and that of orange spot numbers was 0.20 ± 0.13 (Table 7). The moderate heritability estimates for these studies indicate that these two traits can be improved by genetic selection method. The reported heritability estimates of orange area ranged from 0.75 to 1.52 and for number of orange spots it ranged from 0.01 to 0.85 (Evans, 2010; Brook and Endler, 2001; Amirtha, 2016; Lindholm *et al.*, 2005; Smrithi, 2018). A number of reasons have been attributed to variation in the heritability estimates for orange color related traits viz., the expression of the trait only in males thus reducing the sample size, sex-linked inheritance, the age at which the measurements are made as these traits are

influenced by sexual maturity stage and mate availability and competition for the mate.

5.3. Genetic and phenotypic correlation between ornamentation traits

The genetic and phenotypic correlation between ornamentation traits at 70 and 90 days' of age in first generation are presented in Table 8 and 9 while for second generation they are presented in Table 10 and 11. The genetic and phenotypic correlations between ornamentation traits at 70 and 90 days' of age in combined generations are presented in Table 12 and 13. In the present study genetic correlation between body traits were high and positive. Smrithi (2018) had reported genetic correlation between body size traits in the range of 0.06 to 0.98. The phenotypic correlations between body size traits were positive and high. Smrithi (2018) had reported phenotypic correlations between body size traits in the range of 0.33 to 0.98.

The genetic and phenotypic correlation between total orange area and number of orange spots at 90 days' of age in F2 generation was 0.53 ± 0.17 (Table 11); indicates traits are moderately correlated and selection for one trait will improve another trait. The genetic correlation between total orange area and mean hue of orange spots was negatively correlated and it may be due to the color pigments getting dispersed during enlargement of orange area. Smrithi (2018) has reported genetic correlation between pigmentation traits in the range of – 0.01 to 0.89.

In the present study phenotypic correlation between pigmentation traits (total orange area, number of orange spots, relative orange area, mean brightness and mean saturation of orange spots) are positively correlated. Smrithi (2018) has reported phenotypic correlation between pigmentation traits are ranges from -0.14 to 0.90.

Knowledge of genetic correlation is important because it allows us to explain expected direction of correlated responses that can be expected for traits other than one under selection. Lynch and Walsh (1996) stated that a simple knowledge of sign of genetic correlation could be very revealing. Considering these factors the positive correlations between various traits related to body shape and

size and the orange color spots and orange color area indicate that the improvement in one of these traits will bring improvement in others also.

5.4. Genetic gain

Genetic gain is the change in the value of a trait due to selection and caused by the change in allele frequencies of genes responsible for selected traits (Gjedrem and Baranski, 2009). In the present study, expected and realized genetic gain for both F1 and F2 generation are presented in Table 14 and 15, respectively.

The expected genetic gain for total orange area and number of orange spots in F2 generation was 1.27% and 6.71%. Smrithi (2018) has reported expected genetic gain of number of orange spot was 3.96%. In the present study realized genetic gain of total orange area and number of orange spots was 9.16% and 25.7%; This value indicates selection can be further improve in this population.

5.5. Response to selection

Response to selection can be estimated as the difference between the mean of phenotypic value of the offspring of the selected parents and the mean of the whole parental generation before selection. In the present study selection response obtained for total orange area and number of orange spots of guppy in second generation was 9.16 and 25.7 percent respectively (Table 15). In first generation there was a negative gain for both the traits (Table 14). The varying response to genetic selection in these two traits in F1 and F2 needs further studies. Even though the heritability estimates for orange area and orange spot in base generation were moderate and accompanied with low standard errors, the negative genetic gain obtained in the present study indicates that these heritability estimates needs to be reworked. The high genetic gain in second generation may be due to the presence in genetic variation in both between and within families for total orange area and number of orange spots. The genetic variation in the population results from change in gene frequencies at loci that influence the selected character because artificial selection produces a change in gene frequency due to non-random mating. Lugo *et al* (2004) reported red color proportion of *Oreochromis niloticus* increased from 5.6% in the first generation to 100% in fifth generation. In

the mass-selected Sydney rock oyster *Saccostrea commercialis*, the average improvements (increase in live weight) were 4 and 18% after one and two generations of selection, respectively (Nell et al., 1996). Zheng et al. (2006) reported 17.56% genetic gain in adult shell length after two successive generation selection for faster growth in a stock of bay scallop *A. irradians irradians* Lamarck (1819). Deng et al. (2009) reported the first generation selected line of pearl oyster *P. martensii* exhibited a 16.6% genetic gain in adult shell length growth.

5.6. Realized heritability

The realized heritability can be estimated by taking a ratio of expected genetic gain to selection differential (Cameron, 1997). In the present study realized heritability of total orange area and number of orange spots in F2 generation was 3.60 and 1.38, respectively, however these values needs to be taken with caution as they are beyond the range of the heritability limits. Smrithi (2018) has reported realized heritability of number of orange spots was 0.20.

5.7. Conclusion

The present study indicated that female guppies are significantly longer and larger than males. A large variation was observed both between and within families for ornamentation traits. In the present study heritability estimates of body size and shape traits of 90 days' of age were moderate in nature and for orange color related traits they were low to moderate in nature. The genetic correlation between total orange area and number of orange spots are moderately correlated. In the present study, realized genetic gain in F2 generation was 9.16 percent and was higher as compared than expected genetic gain. This study suggests that guppies respond to selection for ornamentation traits.

6. SUMMARY

The guppy (*Poecilia reticulata*), is a tropical ornamental fish which exhibit a high degree of color-polymorphism in body color patterns. The orange color and its pattern in guppies stand apart among all others colors and patterns. The hue and saturation of male orange coloration is influenced by the environmental conditions while the area of orange coloration has a substantial genetic component. Many strains of guppy have been developed by selective breeding. The genetic selection methods can be used to enhance overall ornamentation quality of the animals and also to develop a new true-breeding variety. The present study was aimed to estimate genetic parameters of ornamentation traits and compare the expected and realized genetic gain in selected ornamentation traits.

In the present study guppies were collected from Fish Genetics and Biotechnology Division wet- laboratory and from lakes and rivers of Mumbai. The genetic selection experiment for ornamentation traits in guppy was conducted in wet laboratory of CIFE Mumbai. The first generation was formed by breeding the selected fish from laboratory and wild stock. A nested mating design was used for producing the F1 generation and it consisted of 29 full-sib families which included 12 half-sib families. The F2 generation was produced using fish selected from first generation and consisted of 25 full-sib families including 8 half-sib families. All the traits were measured by taking the photographs of all fishes at 70 and 90 days' of age. The images were analyzed using Image J and MATLAB (R 2015a). The traits measured included body shape traits and orange color related traits. Heritability of the traits was estimated by adopting sire and animal models. Male guppies were selected based on their breeding values and female guppy based on their family breeding values.

The least squares mean of total length of male and female were 20.8 ± 0.23 and 23.6 ± 0.24 mm at 90 days' of age respectively. The least squares means of body area of male and female was 40.5 ± 1.07 and 56.2 ± 1.11 mm² at 90 days' of age. The females were significantly longer and larger than males at 90 days' of age. A large variation was observed in both between and within male and female guppies in their

body size traits. The second generation fish were found to be smaller and shorter than first generation fish. The least squares mean of total orange area and number of orange spots was $4.02 \pm 0.09 \text{ mm}^2$ and 2.53 ± 0.04 numbers, respectively. The number of orange spots ranged from 1 to 4. The average total orange area at 90 days' of age in F1 and F2 generations were significantly different from each other, in that the second generation male showed significantly larger orange colored area.

The heritability estimate of the total length using sire and animal model at 90 days' of age was 0.50 ± 0.39 and 0.25 ± 0.15 , respectively. The heritability of total orange area (mm^2) estimated by sire and animal model at 90 days' of age was 0.40 ± 0.14 and 0.36 ± 0.17 , respectively. The heritability estimates of body size and shape traits of 90 days' of age were moderate in nature and for orange color related traits they were low to moderate in nature. The heritability values estimated from sire model in general were higher as compared to the heritability values estimated from animal model. The genetic and phenotypic correlation between total orange area and number of orange spots at 90 days' of age was 0.53 ± 0.17 , 0.94 ± 0.04 , respectively. The genetic and phenotypic correlation between body size traits and pigmentation traits were negatively correlated. The realized genetic gain was comparatively higher than expected genetic gain in second generation. The realized genetic gain for total orange area at 90 days' of age in first and second generation was -6.45% and -5.76% respectively while for number of orange spots the genetic gain was 9.16% and 25.7% , respectively. The results indicated that through genetic selection the orange coloration can be further improved in this population.

The present genetic study suggests that the guppy populations respond to selection for ornamentation traits.

7. REFERENCES

- Amritha, J., 2016. A genetic study on orange type colouration in various strains of guppy. MFSc thesis submitted to ICAR-Central Institute of Fisheries Education, Mumbai.
- Axelord, H. R., 1973. Koi of the World, Japanese colored carp. T. F. H. publication, Hong Kong.
- Barua, S.P., Khan, M.M.H. and Reza, A.H.M.A., 2001, October. The status of alien invasive species in Bangladesh and their impact on the ecosystems. In *Report of the workshop on alien invasive species, GBF-SSEA, Colombo, Sri Lanka. IUCN Biodiversity Programme* (pp. 1-8).
- Basu, A., Dutta, D. and Banerjee, S., 2012. Indigenous ornamental fishes of west Bengal. *Recent research in science and technology*, 4(11).
- Becker, W. A., 1984. Manual of quantitative genetics (No. Ed. 4). Academic Enterprises.
- Braasch, I., Scharlt, M. and Volff, J.N., 2007. Evolution of pigment synthesis pathways by gene and genome duplication in fish. *BMC Evolutionary Biology*, 7(1), p.74.
- Brooks, R. and Endler, J.A., 2001. Direct and indirect sexual selection and quantitative genetics of male traits in guppies (*Poecilia reticulata*). *Evolution*, 55(5), pp.1002-1015.
- Cameron, N.D., 1997. Selection indices and prediction of genetic merit in animal breeding. CAB international.
- Cole, G.L. and Endler, J.A., 2016. Male courtship decisions are influenced by light environment and female receptivity. *Proceedings of the Royal Society B: Biological Sciences*, 283(1839), p.20160861.
- Colihueque, N. and Araneda, C., 2014. Appearance traits in fish farming: progress from classical genetics to genomics, providing insight into current and potential genetic improvement. *Frontiers in genetics*, 5, p.251.

- Deng, Y., Fu, S., Du, X. and Wang, Q., 2009. Realized heritability and genetic gain estimates of larval shell length in the Chinese pearl oyster *Pinctada martensii* at three different salinities. *North American Journal of Aquaculture*, 71(4), pp.302-306.
- Dey, V.K., 2014. The global trade in ornamental fish.
- Dinesh, S., 2014. A genetic study on body colour and fin length inheritance in guppy *Poecilia reticulata* (peters, 1859). MFSc thesis submitted to ICAR- Central Institute of Fisheries Education, Mumbai.
- Endler, J.A., 1978. A predator's view of animal color patterns. In *Evolutionary biology* (pp. 319-364). Springer, Boston, MA.
- Evans, J.P., 2010. Quantitative genetic evidence that males trade attractiveness for ejaculate quality in guppies. *Proceedings of the Royal Society B: Biological Sciences*, 277(1697), pp.3195-3201.
- Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman, New York.
- Fernando, A.A. and Phang, V.P., 1989. X-linked inheritance of red and blue tail colourations of domesticated varieties of guppy, *Poecilia reticulata* and its implications to the farmer. *Singapore Journal of Primary Industries*.
- Fernando, A.A., 1990. Inheritance of the tuxedo and blond tuxedo color pattern phenotypes of the guppy, *Poecilia reticulata*. In *Proceedings of the Second Asian Fisheries Forum* (pp. 487-490). Asian Fisheries Soc.
- Frankel, J.S., 2004. Inheritance of trunk banding in the tetra (*Gymnocorymbus ternetzi* Characidae). *Journal of Heredity*, 95(3), pp.262-264.
- Frankel, J.S., 2011. Inheritance of Shoulder Spotting in the Red-base Tetra (Characidae: *Hemigrammus stictus*). *Atlas Journal of Biology*, 1(3), pp.62-65.

- Frankel, J.S., Adler-Golden, L. and Porter, A.N., 2012. Inheritance of Humeral Spotting in the Croaking Gourami (Osphronemidae: Trichopsis vittatus). *Atlas Journal of Biology*, 2(1), pp.84-87.
- Garduño-Lugo, M., Muñoz-Córdova, G. and Olvera-Novoa, M.Á., 2004. Mass selection for red colour in *Oreochromis niloticus* (Linnaeus 1758). *Aquaculture research*, 35(4), pp.340-344.
- Grether, G.F., 2000. Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution*, 54(5), pp.1712-1724.
- Haskins, C.P., Haskins, E.F., McLaughlin, J.J.A. and Hewitt, R.E., 1961. Polymorphism and population structure in *Lebistes reticulatus*, an ecological study. *Vertebrate speciation*, pp.320-395.
- Henderson, C.R., 1984. Applications of Linear models in Animal Breeding. University of Guelph press, Guelph, Canada
- Houde, A., 1997. *Sex, color, and mate choice in guppies*. Princeton University Press.
- Houde, A.E. and Torio, A.J., 1992. Effect of parasitic infection on male color pattern and female choice in guppies. *Behavioral Ecology*, 3(4), pp.346-351.
- <http://www.luresext.edu/?q=content/factors-affecting-rate-genetic-change-herd-or-breed>
- <https://www.cabi.org/isc/datasheet/68208>
- <https://www.globalsources.com/si/AS/Beijing-New/6008826464995/pdtl/Tubifex-Worm/1063855479.htm>
- <https://www.itis.gov/>
- <https://www.premiumbeat.com/blog/basic-properties-color/>
- Hughes, K.A., Houde, A.E., Price, A.C. and Rodd, F.H., 2013. Mating advantage for rare males in wild guppy populations. *Nature*, 503(7474), p.108.

- Hughes, K.A., Rodd, F.H. and Reznick, D.N., 2005. Genetic and environmental effects on secondary sex traits in guppies (*Poecilia reticulata*). *Journal of Evolutionary Biology*, 18(1), pp.35-45.
- Hulata, G., 1995. A review of genetic improvement of the common carp (*Cyprinus carpio* L.) and other cyprinids by crossbreeding, hybridization and selection. *Aquaculture*, 129(1-4), pp.143-155.
- Kalra, N.L., Wattal, B.L. and Raghavan, N.G.S., 1967. Occurrence of larvivorous fish *Lebistes reticulatus* (Peters) breeding in sullage water at Nagpur-India. *Bulletin of the Indian Society for Malaria & Other Communicable Diseases*, 4(3), pp.253-4.
- Karino, K. and Haijima, Y., 2001. Heritability of male secondary sexual traits in feral guppies in Japan. *Journal of Ethology*, 19(1), pp.33-37.
- Khoo, G., Meng, L.T. and Phang, V.P., 2007. Colour genes of the ornamental guppies of Singapore. *Journal of Science and Technology in the Tropics*, 3(2), pp.75-80.
- Kirpichnikov, V.S., 1981. *Genetic bases of fish selection*. Springer-Verlag.
- Kodric-Brown, A., 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. *Behavioral Ecology and Sociobiology*, 25(6), pp.393-401.
- Kottler, V.A., Koch, I., Flötenmeyer, M., Hashimoto, H., Weigel, D. and Dreyer, C., 2014. Multiple pigment cell types contribute to the black, blue, and orange ornaments of male guppies (*Poecilia reticulata*). *PloS one*, 9(1), p.e85647.
- Li, Q., Wang, Q., Liu, S. and Kong, L., 2011. Selection response and realized heritability for growth in three stocks of the Pacific oyster *Crassostrea gigas*. *Fisheries Science*, 77(4), pp.643-648.
- Lindholm, A.K., Breden, F., Alexander, H.J., CHAN, W.K., Thakurta, S.G. and Brooks, R., 2005. Invasion success and genetic diversity of introduced populations of

- guppies *Poecilia reticulata* in Australia. *Molecular Ecology*, 14(12), pp.3671-3682.
- Lynch, M. Walsh, B., 1998. Genetics and analysis of quantitative traits (Vol. 1: 535-557). Sunderland, MA: Sinauer.
- Nakajima, M. and Taniguchi, N., 2001. Genetics of the guppy as a model for experiment in aquaculture. *Genetica*, 111(1-3), pp.279-289.
- Nayudu, P.L., 1979. Genetic studies of melanic color patterns and atypical sex determination in the guppy, *Poecilia reticulata*. *Copeia*, 1979(2), pp.225-231.
- Phang, V.P.E. and Doyle, R.W., 1989. Analysis of early growth of guppy strains, *Poecilia reticulata*, with different color patterns. *Theoretical and Applied Genetics*, 77(5), pp.645-650.
- Phang, V.P.E. and Fernando, A.A., 1991. Linkage Analysis of the X-linked Green Tail and Blue Tail Color Genes in the Guppy, *Poecilia reticulata*. *Zoological Science*, 8(5), pp.975-981.
- Pitcher, T.E., Rodd, F.H. and Rowe, L., 2007. Sexual colouration and sperm traits in guppies. *Journal of Fish Biology*, 70(1): 165-177.
- Qingheng, W., Yuewen, D., Xiaodong, D., Shao, F. and Yunzhao, L., 2011. Realized heritability and genetic gains of three generation for superior growth in the pearl oyster *Pinctada martensii*. *Acta Ecologica Sinica*, 31(2), pp.108-111.
- Saxton, A.M. ed., 2004. Genetic analysis of complex traits using SAS. Cary, NC, USA: SAS Institute.
- Shahjahan, R.M., Ahmed, M.J., Begum, R.A. and Rashid, M.A., 2013. Breeding biology of guppy fish, *Poecilia reticulata* (Peters, 1859) in the laboratory. *Journal of the Asiatic Society of Bangladesh, Science*, 39(2), pp.259-267.
- Singh, A.P. and Nüsslein-Volhard, C., 2015. Zebrafish stripes as a model for vertebrate colour pattern formation. *Current Biology*, 25(2), pp.R81-R92.

- Smrithi, K., 2018. A genetic study on ornamentation in guppy *Poecilia reticulata* (peters, 1859) MFSc thesis submitted to ICAR-Central Institute of Fisheries Education, Mumbai.
- Stevens, M., Párraga, C.A., Cuthill, I.C., Partridge, J.C. and Troscianko, T.S., 2007. Using digital photography to study animal coloration. *Biological Journal of the Linnean society*, 90(2), pp.211-237.
- Tezuka, A., Yamamoto, H., Yokoyama, J., van Oosterhout, C. and Kawata, M., 2011. The MC1R gene in the guppy (*Poecilia reticulata*): Genotypic and phenotypic polymorphisms. *BMC research notes*, 4(1), p.31.
- Travis, B.V., 1957. Present status and future possibilities of biological control of mosquitoes. *Mosquito News*, 17(3), pp.143-147.
- Viethen, J., Goudbeek, M. and Krahmer, E., 2012. The impact of colour difference and colour codability on reference production. In *Proceedings of the Annual Meeting of the Cognitive Science Society* (Vol. 34, No. 34).
- Villafuerte, R. and Negro, J.J., 1998. Digital imaging for colour measurement in ecological research.
- Winge, Ö., 1923. Crossing-over between the X-and the Y-chromosome in *Lebistes*. *Journal of Genetics*, 13(2), pp.201-217.
- Winge, Ö., 1938. A lethal gene in the Y-chromosome of *Lebistes*. *CR Trav Lab Carlsberg, Ser Physiol*, 22, pp.203-210.
- www.sthda.com/english/wiki/normality-test-in-r