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An evidence of the missing complete pectoral fins trait in *Clarias gariepinus* reared in pond is Heritable

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ABSTRACT

This research is aimed to evaluate if the missing complete pectoral fins trait in *C.gariepinus* reared in pond is heritable. The trait assessed by the analysis of pectoral fin phenotypes in the first generation (F1) and the second generation (F2) from the parents (P) with missing complete pectoral fins. The methods were descriptive, with the primary variable being the pectoral fin phenotype (individuals). F1 population obtained wide range variation in pectoral fins phenotypes, i.e pectoral fin only one, pectoral fins deformed, and normal pectoral fins while missing complete pectoral fins trait emerged from F2 generation. As many as 15 individuals with missing complete pectoral fins-trait obtained from the cross of B & F1 with normal pectoral fins x @ & F1 with normal pectoral fins, while the crossing between B & F1 with normal pectoral fins and @ & with missing complete pectoral fins, that is obtained 6 Individual with pectoral fins loss- character. Based on F2 phenotype, it concluded that the pectoral fins loss-trait is heritable. The trait shows epistasis and multigenic-character. The full sibling mating inherits more offspring with missing complete pectoral fins. These findings can help the Aquaculturist to manage abnormal traits which emerged in the fish pond, especially pectoral fin anomalies.

***Key words**: Pectoral, Epistasis, Alel, Gene interaction, Heritable trait*

Introduction

Abnormalities in a cultured fish population are common incidents and have long been subject to studies. The fish abnormality bibliographies compiled by Dawson (1964; 1966; 1971) and Dawson and Heal (1976) noted more than 1,498 types of abnormalities suffered by hundreds of species from marine ecosystems in many countries with varied kinds of deformities. Fish abnormalities remain an exciting topic to study for these problems persist today (Boglione *et al.*, 2013), either in the wild fish population or cultured (Tave 2011).

The frequency of abnormalities found in culture enclosures has been higher than it is in the wild, owing to more intense attention. Regular handling through observation of thousands of fish in ponds over a given period makes it easy to detect abnormalities. Aquaculture activities are improving the survival that makes the chance of survival of fish with various deformities relatively higher than wild habitat (Tave and Handwerker, 1998).

Abnormalities in fish culture are often assumed to be caused by genetic factors. Cultivation of fish commonly uses minimum parental populations to yield fish seeds, triggering inbreeding, while a high frequency of abnormalities is a sign of inbreeding

(Tave *et al.,* 2011). Some abnormal characters are heritable, but most are not (Tave, 1993). For an example, saddleback syndromes, the missing of part or all of the dorsal fin due to a gradual loss of dorsal fin spines in Sarotherodonaureus, which is controlled by dominant lethal genes (Tave *et al.,* 1983) and displaying incomplete dominance (Tave, 2011). Caudal Deformities Syndrome commonly accompanied by abnormalities of the operculum of *Oreochromis niloticus*, controlled by recessive lethal genes (Mair, 1992). Vertebral ankylosis in Poecilia reticulata is also heritable (Yamamoto *et al.,* 1963). Meanwhile, some traits not heritable, including anophthalmia (Tave and Handwerker, 1998), operculum anomalies (Handwerker, 1993), stump body, and taillessness (Dunham *et al.,* 1991).

Fin anomalies are often observed to occur from fish farming. The frequency, type of fin experienced, and severity of abnormalities vary greatly depending on species, the technique of maintenance, and condition of the maintenance container (Boglione *et al.,* 2013). Fin anomalies in domesticated *C. gariepinus* is interesting to study because their present have correlation to swim balance and survivaality.

Aluko *et al.* (2011) have examined the genetic basis of pectoral fin anomalies and concluded that there are genetic factors that control the pectoral fin abnormalities in the *C. gariepinus*. He found the character of pectoral fins loss (1-5 individual) from two types crosses (female complete-pectoral fins *C.gariepinus* x female with only has one pectoral fin *C.gariepinus*; female with only has one-left pectoral fin *C.gariepinus* and male with complete-pectoral fins *C.gariepinus*). Male with pectoral fins loss crossed with a complete-pectoral fins'female, yielded an abnormal offspring with a high elevation of pectoral fin asymmetry (Farikhah *et al.,* 2017). Asymmetry as a reflection of developmental instability (Bengtsson, 1985) and low heterozygosity (Mitton and Grant 1984; Blanco *et al.,* 1990). The asymmetrical elevation is an indicator for an individual's condition (Palmer and Storbeck, 1986; Moller and Swaddle, 1997), leading to low resistance to diseases, low energy efficiency, and reduced fitness. Based on the description above, this study was needed to fill the gap in the information about the pectoral fins-loss character of *C. gariepinus*. The purpose of this research was to explain the pectoral fins-loss trait in domesticated *C. gariepinus* by studying the pectoral fin phenotypes of their offspring. This study was expected to lay a foundation in the exploration of pectoral fin abnormalities at a deeper level, namely the molecular aspect of defects in *C. gariepinus*.

Materials and Methods

Provision of *C.gariepinus* with pectoral fins loss as a Parent (P)

The fish making up the Parental population (P) used in this research were non-pectoral-finned with no fin growing on both sides of the bodies. The fish were collected manually from a fish culture business in Lamongan, East Java Province during the harvest time (April, 2016). The fish collected were directly sent alive to the Fish Reproduction Laboratory of the Faculty of Fisheries and Marine Sciences of Brawijaya Malang University, in a particular container. At the laboratory, the fish were adapted and reared in a concrete pond (300*600*80 cm^3). The maintenance is continuing until fish reach sexual maturity — water circulatory system using groundwater, salinity 0 gl^{-1}, ± 25 ^oC, DO ± 3.75 mgL^{-1}, pH ±6.5. The fish fed with formulated feed with 33% protein content.

Obtaining F1

F1 obtained by mating parental (P) with missing complete pectoral fins. The F1 pectoral fin morphology analyzed manually and reared until reach sexual maturation. Some of them taken randomly to be decided as the parent. The chosen fish were separated and analyzed for their morphometric and meristic character. The fish were subjected to hormonal induction with Ovaprim at a dosage of 1.5 $mL.kg^{-1}$ of the bodyweight for the females and 0.75 $mL.kg^{-1}$ for the males then placed in a spawning container (concrete, 200*500*80 cm^3) to spawn naturally. A conditioned container was sterilized, dried, and filled with water to a 30 cm level — the container equipped with an electric heater for regulating the water temperature stability (T±27 ^oC). The fertilized eggs incubated until they developed become embryos. At the ±36 th hour, post-fertilization (PF), the embryos hatched into fish larvae. The larvae started to eat Artemia nauplii in 3 days PF and continued to eat *Tubifex* sp in the fifth days PF. On day 15 PF the *Tubifex sp*. feeding was gradually stopped and replaced by pellet feeding (protein content ± 40%). Powdered pellets replaced by grained

pellets, and the rearing was resumed until the fish grew more prominent. Periodical monitoring was conducted on the water quality concerning the temperature, pH, and dissolved oxygen, all of which fell within the optimal range for fish life. During the incubation, the rearing pond water was monitored continuously (temperature ±27ºC, pH 6.5, DO 3.75 ppm, groundwater salinity $0gl^{-1}$) using a circulatory basis ($v=2lmin^{-1}$).

Obtaining F2

F2 obtained from crossing of two separated population. First population was the P population which did not have any pectoral fins, and the second population was F1 with regular pectoral fins because all of F1 were normal pectoral fins, which all of them have reached sexual maturity. The sexual maturity decided according to Ndimele & Owodeinde (2012). The choses fishes were analyzed for their morphometric and meristic character then subjected to a hormonal induction per prior procedure (in the first cross). Then, every fish incubated in a glass aquarium (70*40*40 cm^3) filled with freshwater and equipped with an aeration system (T ±27 ºC). Twelve hours after the hormonal induction, the females came into the final-stage gonadal maturity. The females were lifted carefully from the aquarium with their head and body covered in soft cloth then subjected to dry stripping (Chattopadhyay, 2017). Some egg cells from two different females, including female F1 fish with typical pectoral fins and female without pectoral fins, were obtained. The males were anesthetized, killed, and the testes removed. The testes chopped by a sectio-set to extract the milt then added with a 0.9% NaCl preservative solution. Two different sorts of milt from two different males, male F1 fish with typical pectoral fins and male with missing complete pectoral fins, were obtained. Every 2 grams of stripped eggs was fertilized with 1 mL of milt to make sure that the fertilization rate was high following the fertilization procedure proposed by Viveen *et al.* (1985). The fertilization design was as follows:

1. ♂ and ♀ F1 *C.gariepinus* with normal pectoral fins
2. ♂F1 *C. gariepinus* with complete pectoral fins x ♀ complete pectoral fins loss
3. ♂*C. gariepinus* complete-pectoral fins loss x ♀F1 *C. gariepinus* F1 complete pectoral fins
4. ♂*C.gariepinus* and ♀ *C.gariepinus* complete-pectoral fins loss.

Incubation of embryos and rearing of the F2 offspring

The fertilized egg cells were spread evenly in a careful, slow manner in a glass aquarium (70*40*40 cm^3) filled with freshwater that came with a heater and an aeration system (T ±28 ºC; pH ± 6.45, DO±3.75 mgL^{-1}). Qualitative observation of the development of egg cells from every test cross was carried out. The inspection covered fertilized egg cell color, the period in which the embryos first hatched, embryonic development, the period in which the hatchlings started to swim actively, and appetite. The natural feed Naupliiartemiahad first given when the hatchlings were three days PF. On the fifth day PF, the natural feed was replaced by young Tubifex sp. It gave in the same portion in every test cross, and it ensured that natural feed was always available in the aquarium to suppress the larvae predation opportunity. *Tubifex* sp. was stopped gradually and replaced by powdered pellets (protein content 40%) when the fries were 15 days old PF. With the fries' growth, the powdered pellets were then substituted with grained pellets gradually, and the fries rearing went on, with 33% protein pellet feeding, until they reached adulthood.

The water monitored daily. The water temperature of every aquarium was measured every morning and late afternoon by an alcohol-based thermometer, the water pH by pHpen, and the dissolved oxygen by DOmeter. On day ten post-fertilization, water siphoning was performed on every aquarium to dispose of the metabolite residue depositing on the bottom of the aquarium — the circulatory system run on day 10 PF.

Analysis of the pectoral fin phenotypes F1 as wll as F2

The pectoral fins were analyzed manually on day 75 PF in F1 population and on day 20 PF in F2. The identification and classification of pectoral fins based on the number of fins the individuals had, including missing complete pectoral fins, missing one pectoral fin, deformed fins, and normal pectoral fins.

$$\text{Phenotype (individual)} = \frac{\text{number of individual of a certain type of pectoral fins}}{\text{total individuals within a population}} \times 100$$

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Data analysis

The variety in the phenotypes of pectoral fins of F1 and F2 Progeny ratio in all test crosses was analyzed descriptively according to Tave & Handwerker (1998).

Results

C.gariepinus without pectoral fins taken as a Parental (P) in Fig. 1. After the parent with missing complete pectoral finsthey mated each other, the offspring produced. Phenotype composition in F1 based on Table 1. There were four groups of phenotype but themissing- pectoral fins was not emerged. According to Table 1, F1 consists of 635 individuals with the ratio between of them, missing CL: missing OP: DP: CP is 0:4:2:96, unfortunately F1 not inherit pectoral fins loss-trait.

The most common phenotype of F1 are normal pectoral fins. It would suggest that the pectoral fins loss-trait is not "Y linked' trait-based on sex determination system of C.gariepinus is ZZ-ZW sex (Ozouf-Costaz *et al.*, 1990). Based on Tave and Werker (1998), when the trait observed less than 50% of offspring population, it indicated that the pectoral fins loss-trait did not control by dominant autosomal alleles. Some variation observed in the F1 offspring described in Figure 2. It shows that regular fish has two pectoral fins (C). There is a trait that is carried by fish, having only one pectoral fin on the left side of the body while the right pectoral fin is lost (A). There are observed also fish with only one pectoral fin on the right side of the body while the left pectoral fin is lost (B).

Analysis of F2

The pectoral fin phenotypes of F2 described in Table 2. Variation of pectoral fin phenotypes is identical to those of F1, but missing-CP-trait obtained in the F2 population. They inherited in two crossings, i.e., between female and male F1 CP (0.9%; 15 individuals) and, between CP male F1 and missing-CP female (0.33%; 6 individuals). The hybridization of full siblings in F1 appears to produce more defect offspring with complete pectoral fins-loss. On the contrary, two remaining combinations (♂missing-CP xF1♀CP and ♂missing-CP x ♀missing CL) did not inherit missing complete-pectoral fins missing-trait in their generation. It seems that the missing-CP 'trait regulated by more than one gene. It also indicated the phenotype that appears correlates with the frequency of the alleles which control pectoral fin phenotype genes in each pair of parent fish. The percentage of missing-CP in F2 is relatively small- less than 1% from each crossing.

Fig. 1. Comparison between the phenotypes of complete pectoral fins and missing complete pectoral fins in the domesticated African catfish *Clarias gariepinus*

Table 1. The pectoral fin phenotypes of the F1 yielded from the cross between *C. gariepinus* males with complete-pectoral fins loss and *C. gariepinus* females with complete-pectoral fins (75 days PF culture periods)

No	Pectoral fin phenotype of the offspring (F1)	% (individuals)
1.	Missing complete-pectoral fins (missing CP)	0 (0)
2.	Missing one pectoral fins (missing OP)	4.09(26)
3.	Deformed pectoral fins (DP)	1.89 (12)
4.	Complete-pectoral fins (CP)	95.91(609)
	Total individuals in F1 population	100 (635)

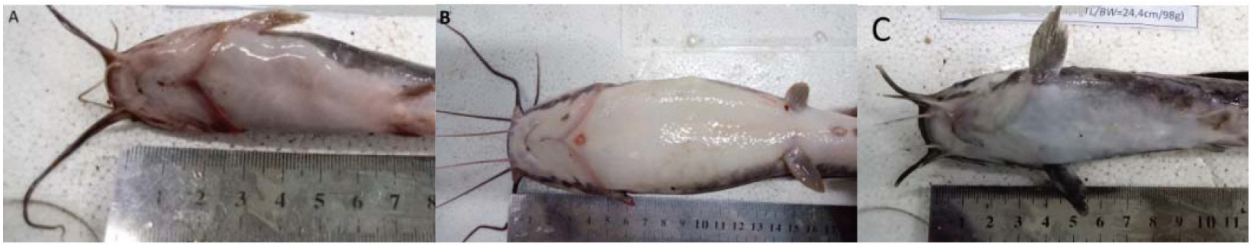

Fig. 2. Pectoral fin variation of the F1 generation on the 75-day post fertilization (A. only one pectoral fin; B. only one pectoral fin and deformed fin; C. complete-pectoral fins)

Table 2. Pectoral fin phenotypes of F2 from the genetic test cross between four crossing combinations

Pectoral fin phenotype of F2	% phenotype of pectoral fins from the crossing combination			
	F1♂&CP x F1♀CP	F1♂CP x♀missing CP	♂missing CP xF1♀CP	♂missing CP x ♀missingCP
1. Missing CP	0.91 (15)*	0.33 (6)*	0 (0)	0 (0)
2. Missing OP	2.08 (34)	1.92 (35)	0.85 (13)	2.83 (55)
3. Deformed pectoral fins (DP)	9.87 (162)	3.91 (72)	5.43 (81)	3.54 (69)
4. Complete-pectoral fins (CP)	87.4 (1430)	93.87(1729)	93.80(1422)	93.63(1823)
Total individuals of F2	100 (1641)	100 (1842)	100(1516)	100 (1947)

Description: *= Significantly inherited; CP= complete pectoral fins; OP= only one pectoral fin

Fig. 4. Some individuals from F2 that inherit missing-complete pectoral fins trait (**red arrow**).

Discussion

According to Tave and Handwerker (1998), if a trait does not appear at the first cross but appears at the second cross, the character indicates the phenomenon of epistasis. Epistasis is a trait inheritance where the effect of allelic substitution on a gene depends on the state of the other gene alleles (Churchill, 2001). Epistasis shows interactions between genes that control a trait in which its complexity depends on the number of loci involved, the susceptibility of alleles and environmental factors which contributed (Cordell, 2002).

This finding confirms the genetic basis of the *C.*

gariepinus catfish pectoral anomaly character, and explains the type of inheritance. The loss of pectoral fins is inherited and the type of inheritance is epistasis. That is, the existence of these traits is determined by other gene alleles, the number of loci involved, and the environmental conditions experienced. This finding supports the statement of Aluko *et al.* (2010) that fin abnormalities seem to be inherited and controlled by genetic factors.

Epistasis in the missing pectoral fin informs us that there are notices about the mechanisms and pathways seen in the character of pectoral fin lost away from individual, especially those related to biological interactions between the proteins involved (Cordell, 2002). Epistasis has long been recognized as a crucial factor in understanding genetic pathways in both structure and function and plays a role in the evolutionary dynamics of complex genetic systems (Philips, 2008)

Conclusion

Missing pectoral fins is is a trait that is often suffered by *C. gariepinus* fish originating from fish farming in ponds. This character is inherited and is epistasis, thus, where the expression depends on the number of genes involved, the condition of other genes, the number of loci affected, and the condition of external factors when the trait is to be inherited. Epistasis means that the trait is controlled by many genes or multiple genes and the loss of an individual's pectoral fins caused by complex gene interaction mechanisms.

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