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# Molecular Parentage Analysis in Aquaculture: Principles, Applications, and Challenges: A Review

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***Author's contribution***

*The sole author designed, analysed, interpreted and prepared the manuscript.*

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## ABSTRACT

Molecular parentage analysis is a powerful tool for reconstructing pedigrees and estimating genetic parameters in aquaculture species. It is based on the comparison of DNA marker genotypes between offspring and potential parents. This review provides a concise overview of molecular parentage analysis in aquaculture, covering its principles, methods, applications, challenges, and limitations. It describes common DNA markers, including microsatellites and single nucleotide polymorphisms (SNPs), used in parentage analysis and the criteria for their selection. The software and statistical methods for assigning parentage and evaluating the accuracy and power of the assignment are also discussed. This review demonstrates applications for estimating genetic parameters, investigating inbreeding, evaluating reproductive success, and improving selective breeding programs. In conclusion, while molecular parentage analysis is a valuable tool for improving genetic management in aquaculture, careful planning, implementation, and interpretation are essential.

*Keywords: Molecular parentage; aquaculture; DNA markers; selective breeding; genetic parameters; breeding programs.*

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## 1. INTRODUCTION

Aquaculture, the controlled cultivation of aquatic organisms, plays a pivotal role in meeting the growing global demand for seafood [1] [2]. Enhancing the genetic management of aquaculture species is paramount for achieving sustainable and efficient production. However, the adoption of genetically improved stocks in aquaculture is still limited [2]. One of the challenges in developing optimized selective breeding schemes in aquaculture is the difficulty and high cost associated with obtaining pedigree information, which is vital for estimating genetic parameters and implementing genetic improvement programs in aquaculture species [3]. In other animal and plant species, physical tagging of individuals is a feasible method for obtaining pedigree information. These traditional methods of pedigree recording in aquaculture often face challenges such as misidentification, incomplete records, and difficulties in tracing parentage. Molecular parentage analysis, based on the comparison of DNA marker genotypes, offers a precise and reliable alternative for reconstructing pedigrees and estimating genetic parameters. This technology has witnessed remarkable advancements in recent years, contributing to a deeper understanding of the genetic architecture of aquaculture species [4]. The basic idea behind molecular parentage analysis is that offspring inherit one allele from each parent at every locus. Therefore, the genotype of the offspring matches one allele from the mother and one allele from the father at each locus [5]. The exploration of parentage began to gain momentum when researchers discovered the utility of DNA probes in humans and other organisms for identifying variation at minisatellite loci. This groundbreaking technique, known as DNA fingerprinting, revolutionized the study of genetic relationships [6]. The introduction of microsatellite markers resulted in a complete parentage analysis overhaul, because they were the first easily assayable single-locus, co-dominant, hypervariable markers [6] [7]. The availability of microsatellite markers in the 1990s marked the beginning of parentage assignment studies in fish [5] [8]. Since then, molecular parentage analysis has been widely applied in various aspects of aquaculture, such as estimating genetic parameters, controlling inbreeding, optimizing mating designs, and tracing genetic origin [8]. Microsatellites have been the most commonly used markers for parentage analysis in aquaculture, due to their high polymorphism, co-dominance, and simple genotyping. However, microsatellites have some limitations, such as high mutation rates, genotyping errors, and difficulties in standardization and comparison across laboratories [9]. In recent years, single nucleotide polymorphisms (SNPs) have emerged as alternative markers for parentage analysis in aquaculture, owing to their low mutation rate, high accuracy, and high-throughput genotyping [9]. SNPs have been successfully used for parentage analysis in several aquaculture species, such as Pacific oyster [9], Atlantic salmon [10], and Asian seabass [11]. For instance, molecular parentage analysis is essential in breeding Asian seabass (*Lates calcarifer*), a commercially important marine fish species in Southeast Asia and Australia. Asian seabass is a highly fecund species that spawn in large groups, resulting in high genetic diversity but also high variance in family contribution. Traditional breeding methods, based on mass selection or communal spawning, are inefficient and may lead to genetic erosion and inbreeding. Therefore, molecular parentage analysis using DNA markers is needed to identify the parents and offspring of each family, as well as to implement selective breeding programs based on individual or family performance [11]. [11] suggested that in order to preserve genetic diversity within a breeding population, its crucial to guarantee that every breeding individual produces offspring. However, even if all breeding individuals contribute offspring, variations in their contributions can lead to a decrease in effective population sizes in future generations.

In this review, the exploration extends to the principles, methods, and applications of molecular parentage analysis in aquaculture. The focus includes an examination of the types of DNA markers frequently utilized, the criteria influencing their selection, and the software tools utilized for effective assignment. Furthermore, the review highlights the advantages and results derived from molecular parentage analysis in various aquaculture species, addressing the challenges and limitations inherent in its implementation.

## 2. MOLECULAR PARENTAGE ANALYSIS: PRINCIPLES AND BASIC STEPS

“The principle of molecular parentage analysis is based on the concept that offspring inherit one allele at each locus from each of their parents. By comparing the genotypes of offspring with those of potential parents at DNA marker loci,

paternity can be determined. If the offspring's genotype contains alleles that the presumed parents do not possess, paternity can be excluded with 100% certainty. Conversely, if there is a perfect match between the genotypes of the offspring and potential parents at multiple marker loci, paternity can be confirmed with a high degree of certainty. The use of multiple DNA markers in parentage analysis increases the confidence level of paternity confirmation" [5]. In the recent decades, significant progress has been made in parentage analysis in aquaculture species. These studies have included estimating genetic parameters of traits of interest [5] [12] investigating inbreeding in hatcheries [13], estimating the number of brooders in breeding stocks [14], and evaluating variance in reproductive success among individuals [15]. "Molecular parentage analysis typically involves several key steps: tissue sample collection, DNA extraction, selection of DNA markers, polymerase chain reaction (PCR) amplification of DNA samples, genotyping of PCR products, and data analysis to determine parent-offspring relationships" [16]. "The advent of DNA markers, such as microsatellites, and advanced computer software has facilitated the analysis of relationships among individuals in parentage analysis across various taxonomic groups" [17]. "However, traditional methods for DNA extraction, PCR, and genotyping can be costly and labor-intensive, limiting the large-scale application of parentage analysis in aquaculture species". [91]

## 2.1 Sampling Tissues and DNA Extraction Methods

"DNA can be extracted from any tissue sample since it is present in every cell of fish species. In breeding programs and hatchery management, it is essential to choose tissue sampling methods that allow the fish to be used for growth and reproduction. Fin clips are an ideal tissue sample due to their minimally invasive nature. For parentage analysis, a relatively small amount of DNA is required. Typically, 500 ng of DNA is sufficient for more than 30 PCR amplifications. A small piece of fin clip measuring 3×3 mm can provide more than enough DNA" [5]. "In some cases, mucus samples can also be used for DNA extraction" [18]. "After sample collection, it is crucial to store tissue samples in suitable solutions. Storing them in 75-95% ethanol is a simple and effective method" [19]. "To analyze DNA polymorphisms, it is necessary to disrupt the cell nucleus to release the DNA and remove interfering structural proteins and enzymes. The extracted DNA must be of sufficient quality for PCR amplification" [91].

## 2.2 Marker Selection

The polymorphisms and robustness of DNA markers need to be assessed when they are amplified by PCR, after they are developed. Microsatellites are the marker of choice for parentage analysis [20], because they are easily scorable, codominant, and highly polymorphic markers [21]. Microsatellites have been used for the parentage analysis in more than 20 aquaculture species, such as salmon [22], Asian seabass [12] [23], common carp [24], rainbow trout [13], European sea bass [25], and sea bream [26]. Tri- and tetranucleotide microsatellites are easier to genotype and less likely to cause genotype errors than dinucleotide microsatellites, because they have less stuttering effects during PCR. However, it may take longer to find highly polymorphic tri- and tetranucleotide microsatellites. SNPs have been recently applied to parentage analysis in salmon, and [27] found that 80 SNPs had higher power than 11 microsatellites. The cost-effectiveness of different types of markers is still unclear. With the fast progress of SNP genotyping methods, SNPs are expected to become the main marker system for regular parentage analysis in aquaculture species, such as salmon [28] and catfish [29] [30]. One way to measure the effectiveness of a single marker for parentage assignment is to use the parameter: exclusion probability [17]. This parameter indicates the likelihood that a random parent-pair will not match the genotype of a random offspring at that marker, if they are not the true parents of that offspring [31]. The probability of excluding a marker can be determined by using the allele frequencies at the locus of the marker in a population. By combining the exclusion probabilities of different markers, the combined probability of exclusion of all markers used can be estimated. Some software, such as CERVUS [32], FAP [33], GIMLET [34], and VITASSIGN [35], can do simulations to predict the assignment power of markers. The number of DNA markers needed for parentage analysis varies depending on several factors, such as the variability of markers, their positions on the genome, the size of the parent and offspring groups, and the breeding systems [22]. Usually, in aquaculture species for a cross between 25 males and 25 females, using 8-10 highly polymorphic microsatellites, over 90% of offspring can be assigned to specific parent pairs

[12]. The power of assigning offspring increases with more markers, but so does the cost. The number of SNPs needed for parent assignment in aquaculture species is unclear. [28] found that 80 SNPs had higher power than 11 microsatellites for parentage analysis. The location of the markers on the genome also affects the power of parentage analysis, and unlinked markers have a higher power than linked markers [27]. Therefore, choosing markers from different linkage groups is preferable if a linkage map is available for a species, to ensure high power of parentage analysis.

## 2.3 Single-Locus PCR and Multiplex PCR

"Single-locus PCR and electrophoresis on an automated DNA sequencer are used to genotype microsatellites" [36]. However, this method can be costly for genotyping many individuals with 8-10 microsatellites. To reduce the cost for genotyping, it is essential to develop multiplex PCR for co-amplification of 8-10 microsatellites in one PCR [36] and co-electrophoresis of the PCR products on automated DNA sequencers, such as ABI3130 and ABI3730xl (Applied Biosystems, South San Francisco, CA, USA). Multiplex PCR [37] coamplifying several loci simultaneously in one PCR is a technique that considerably reduces the time and costs associated with microsatellite genetic analyses. "Automatic genotyping of multiplexing PCR products amplified with fluorescently labeled primers and DNA sequencers considerably increases efficiency and precision of genotyping microsatellites [36]. One important issue of multiplexing is compatibility of microsatellites" [36]. "Primer pairs should have similar annealing temperature range. Some species in aquaculture have optimized multiplex PCRs" [38]. "Although multiplex PCRs have been optimized for some aquaculture species, most multiplex PCRs currently available contain only a few microsatellites, which may not provide enough statistical confidence for parentage assignment" [39]. Therefore, further development of multiplex PCRs to amplify at least nine microsatellites in one reaction is necessary for aquaculture species.

## 2.4 Electrophoresis and Scoring of PCR Products to Obtain Genotypes

After PCR amplification of microsatellites, the next step is to separate the PCR products using electrophoresis and to size PCR products against size standards [40]. For parentage assignment, it is essential to accurately measure the size of alleles at microsatellite loci and keep the genotyping consistent. Microsatellite markers were initially used by separating the alleles in agarose gels [41], but this method is not good at distinguishing alleles that have a small difference in base pairs [42]. Higher resolution and accuracy in separating and sizing the fragments are needed for parentage analysis [17], because most microsatellite alleles differ by only two base pairs. Therefore, electrophoresis using 8-12% polyacrylamide gels, in combination with silver staining, was used to achieve better resolution of fragment separation [43]. Capillary electrophoresis technology enhances the precision and speed of measuring and genotyping microsatellite alleles [44]. The scoring of genotypes is more efficient with the software that each semi-automated DNA sequencer has. Setting bins is critically important in scoring genotypes. Steps of setting bins can be found in user's manuals of different types of sequencers. The same bins must be used for genotyping offspring as for genotyping parental generations, once the bins are established. Capillary electrophoresis, in combination with fragment analysis software, significantly reduced sizing errors and increased genotyping efficiency. This technique has been widely used in genotyping microsatellites in animals and plants [45]. While traditional polyacrylamide gels are still used in some aquaculture laboratories, capillary electrophoresis has become the preferred method [46].

## 2.5 Statistical Methods and Software for Parentage Analysis

Several statistical methods are available for parentage analysis, including exclusion, categorical allocation, fractional allocation, full probability parentage analysis, parental reconstruction, and sib ship reconstruction. In aquaculture species, parentage analysis is commonly used for reconstructing pedigrees and estimating genetic parameters for selective breeding and hatchery management. Therefore, exclusion and categorical allocation methods have been commonly used [12], while other methods were rarely applied. The exclusion method is very simple. For dominant markers, such as microsatellites and SNPs, the inheritance of alleles follows the rule of Mendelian inheritance, namely the offspring inherits one allele from the father and another allele at the same locus from the mother. A potential parent can be ruled out as a true parent

if they do not have an allele in common with the offspring in question. This is a simple method, but it often faces some challenges [17] [9]. The main difficulties come from the errors and problems in measuring the alleles. Some genotypes may be scored incorrectly in potential parents or offspring, due to the presence of null alleles at some loci, strong stuttering bands, allele shifts during electrophoresis, and preferential amplification of some alleles at some marker loci. This makes it impossible to establish the real parent-offspring relationships, using the exclusion method. However, complete exclusion remains the best practice of parentage studies. Every laboratory doing parentage analysis should aim for this goal. If complete exclusion is not feasible, then a parentage allocation method can be used to examine the remaining candidate parents and offspring that are not excluded. The categorical assignment method allocates all offspring to the candidate parent with the highest likelihood or posterior probability of being the actual parent. The categorical assignment methods have the advantage of dealing with scoring errors or mutations and providing methods to determine confidence in parentage assignment. Therefore, categorical allocation is the most widely used method of parentage analysis in aquaculture species. [47] have developed a parentage analysis software using Microsoft Excel. The software PAPA [48], CLONY [49], and CERVUS [32] have been effective for parentage assignment of Asian sea bass over the last 20 years.

## 3. CHALLENGES OF MOLECULAR PARENTAGE ANALYSIS IN AQUACULTURE SPECIES

While molecular markers appear to offer a straightforward method for parentage analysis, there are some challenges to consider:

### 3.1 DNA Quality and Quantity

Some samples may not be amplified by multiplex PCR because of low quality and quantity of DNA, which results in some offspring not being assigned to their parents. A large number of samples are usually analyzed at the same time in parentage analysis. Measuring the quality and quantity of each DNA sample is almost impossible. Therefore, a reliable, cost-effective, and high-throughput DNA extraction method [19] needs to be developed or used.

### 3.2 Preferential Amplification, Null Alleles and New Alleles Due to Mutation

Preferential PCR amplification occurs when two alleles in a heterozygous sample are amplified unequally, potentially leading to incorrect or ambiguous genotyping [50]. The shorter allele at a locus is usually amplified more, while the longer allele product is much weaker. Several factors can cause this preferential amplification of the shorter allele at a given microsatellite locus [50]. First, preferential amplification can happen when the GC (guanine-cytosine) content of the two alleles is very different and the PCR conditions allow only one allele to denature. Second, if there is not enough Taq DNA polymerase in a PCR, the shorter allelic product will be amplified more. Third, if the target DNA is very degraded, the shorter alleles will also be amplified more. Fourth, when the temperate DNA amount is very small, random variation in the number of copies of each allele will cause preferential amplification. Fifth, different efficiency of DNA synthesis priming of one allele versus another can lead to preferential amplification of the other allele. There are some methods to reduce the preferential amplification [51], but they are usually complex and time-consuming and not suitable for genotyping many samples. Preferential amplification is common in microsatellites, and it varies among loci. By selecting carefully, it is possible to find some microsatellites that have weaker preferential amplification, if there are enough polymorphic microsatellites in a given species. In the case of microsatellite markers, null alleles may arise due to mutations in the sequences adjacent to the repeat region, thereby hindering the binding of one or both primers [52]. Null alleles are more common in shellfish than in teleost's, probably because of high variation in the sequences next to microsatellites. Using the strict exclusion method for parentage analysis, null alleles could prevent offspring assignment. Luckily, null alleles can usually be inferred as a significant deviation from Hardy-Weinberg equilibrium using software such as Micro Checker [53]. In aquaculture species, where potential parents are sampled with groups of offspring, null alleles are easier to detect as they cause mismatches between known parents and offspring, especially involving homozygous genotypes. While some software, such as PAPA [48], can infer null alleles, only a few can handle them effectively in the analysis. In parentage analysis, a simple way to deal with loci with null alleles is to check for homozygous genotypes and recode them as heterozygotes

that include the detected null allele. This helps avoid incorrect exclusions. New alleles from mutations can make parentage assignment difficult since offspring with these new mutation alleles cannot be assigned to their parents using the exclusion method. However, the mutation rates of microsatellites are generally low, typically ranging from 10^-3 to 10^-5. Therefore, mutation alleles do not usually cause significant problems in parentage analysis. Several software tools, such as PAPA [48], CLONY [49], and CERVUS [32], can handle mutation alleles as genotyping errors and still assign offspring to their parents.

### 3.3 Scoring Errors

Allelic binning is very important in the semi-automatic scoring of genotypes using software with genotyping machines. Binning decisions, which are often arbitrary, can cause differences in scoring di-nucleotide alleles, as shown by a comparison study where binning decisions caused 83% of differences [54]. Another study indicated that 21-40% of all errors were attributed to binning errors, underscoring the significance of properly established reading rules. Manual checking of genotypes is essential in parentage analysis after automatic scoring [55]. Stuttering patterns, characterized by changes in DNA amplification resulting in additional bands, vary significantly among different markers. Dinucleotide microsatellites typically exhibit stronger stuttering compared to tri- and tetra-nucleotide microsatellites [21]. It can be hard to score correctly at loci with strong stuttering bands, especially when heterozygotes with adjacent alleles at dinucleotide repeat loci are involved. Stuttering can cause the wrong scoring of heterozygotes as homozygotes, resulting in wrong exclusions in parentage analysis. On the other hand, true homozygotes can be mistyped as heterozygotes because of stuttering bands. However, the chance of this type of mistyping is generally low because microsatellites used in parentage analysis are usually well-characterized, and the shape of a single allele is well-documented. Size-shift errors can also occur, wherein the observed size difference between two alleles after electrophoresis separation does not precisely correspond to the difference in repeat unit length between them [56]. For example, the observed size difference between two adjacent alleles at a dinucleotide locus may not exactly reflect a two-base pair difference.

## 4. AQUACULTURE PARENTAGE ASSIG-NMENT

### 4.1 Genetic Control of Inbreeding

Genetic control of inbreeding, is a critical aspect of aquaculture breeding programs aimed at maintaining genetic diversity and ensuring the long-term sustainability of farmed fish populations. Inbreeding, the mating of closely related individuals, can lead to the expression of deleterious genetic traits, reduced fitness, and increased susceptibility to diseases. Therefore, implementing effective strategies to mitigate inbreeding is essential for the health and productivity of aquaculture stocks. One common approach to inbreeding control in aquaculture is the implementation of selective breeding programs [57]. These programs involve the careful selection of breeding individuals based on desirable traits such as growth rate, disease resistance, and reproductive performance, while simultaneously considering their genetic relatedness. By incorporating information on pedigree and genetic markers, breeders can make informed decisions to minimize the risk of inbreeding within breeding populations. In addition to selective breeding and genetic technologies, the adoption of management practices such as rotational mating, outcrossing, and maintaining large breeding populations can also help mitigate inbreeding depression in aquaculture stocks. Moreover, international collaborations and genetic exchange programs between different hatcheries and research institutions, contribute to the exchange of genetic material and the preservation of genetic diversity within farmed fish populations. Effective inbreeding control is essential for the sustainability and resilience of aquaculture production systems. By integrating selective breeding approaches, molecular genetic tools, and sound management practices, aquaculture stakeholders can mitigate the risks associated with inbreeding and ensure the continued improvement and viability of farmed fish stocks [58,59,60].

### 4.2 Genetic Parameter Assessment

By estimating heritability and genetic correlations, we can assess the expected genetic gains and plan the breeding programs. This is perhaps where genotyping's ability to access pedigree information has made the most significant and fruitful impact on aquaculture genetics so far. Mixed family designs were

optimized for estimating genetic parameters by [61] for strain effects, [62] for heritability, and [63] for genotype by environment (G × E) interaction. After a few feasibility studies with few families [64], heritability's were estimated for a growing number of fish species for growth [65], processing traits [66], flesh color [67], muscle fiber diameter [68], deformities [69], disease resistance [70] or sex ratio [71]), and for growth [72] or meat yield in mussel [72] in shrimps and mollusks. Heritability from mixed family rearing are often higher than those from separate rearing, which may be related to the lack of between family environmental variance due to family mixing, although non-genetic maternal effects may still exist in mixed family rearing [73], which may cause possible overestimation of heritability. Comparisons of the same families in mixed or separate rearing design showed that separate family rearing caused much higher levels of between-families' environmental effects [74]. A drawback of such studies is that individual performances are not available before physical tagging, as fish are usually tagged to collect individual information, which limits genetic studies in the early stages. However, recent advances allow individual tagging [75], which should change this situation.

## 5. BREEDING PROGRAM PERFORMANCES

The breeding program performance in aquaculture involves a multifaceted approach, integrating strategic planning, rigorous selection, and continuous monitoring to achieve desired genetic improvements in farmed fish populations. These programs are designed to enhance economically important traits such as growth rate, disease resistance, and feed efficiency, all while carefully managing genetic diversity and minimizing the risks associated with inbreeding. Central to the success of aquaculture breeding programs is the careful selection of breeding stock. Breeders meticulously evaluate individuals based on pedigree records, phenotypic performance, and genetic evaluations to identify those with the most desirable traits. This selection process optimizes the genetic merit of the breeding population, ensuring the transmission of favorable genetic characteristics to future generations. Moreover, the generation of genetic information plays a crucial role in guiding breeding decisions. Pedigree and performance data are collected and analyzed to estimate key genetic parameters such as heritability and genetic correlations. In implementing selection strategies, breeders utilize a variety of approaches, including family-based selection, mass selection, and marker-assisted selection. These strategies aim to improve target traits while also maintaining genetic diversity within the population. Selection indices and genomic prediction models help prioritize breeding candidates based on their genetic potential, further optimizing the breeding process. As aquaculture plays an increasingly vital role in global food production, the execution of breeding programs with molecular parentage analysis offers a strategic approach to sustainable and genetically optimized aquatic species production [2]. When developing breeding programs using parentage assignment, key considerations include the cost of genotyping, the ability to produce a large number of families simultaneously to mitigate tank effects, the efficiency of assignment, rapid mass genotyping capabilities (especially for species with short generation intervals), individual tagging for traceability and data collection, automated database systems for performance data storage and linkage, and optimized genetic software for candidate ranking and mating to maximize genetic progress while minimizing inbreeding. Implementation of parentage assignment requires a comprehensive re-optimization of breeding programs, going beyond mere genetic tagging [76,77,78,79].

## 6. BREEDING IMPROVEMENT

Breeding Improvement schemes through parentage assignment are a crucial aspect of aquaculture genetics, aimed at maximizing genetic gain, minimizing inbreeding, and enhancing the efficiency of selection programs. The strategic application of parentage assignment, rooted in genomic technologies and statistical methodologies, has opened new avenues for sustainable genetic improvement. In order to optimize breeding programs using parentage assignment, several strategies have been employed. One approach is to limit the number of individuals genotyped by using two-way nested models for partial pedigrees or extreme phenotypes with family effects considered as fixed effects [80,81]. However, it is important to note that the efficiency of selection may be reduced compared to traditional BLUP (Best Linear Unbiased Prediction) selection when pedigree information is not available for all candidates [82]. Issues related to the mixing of families have also been investigated. Methods to limit non-genetic maternal effects in salmonids

have been explored [73], as well as the impact of grading practices on family contributions to reduce cannibalism in barramundi [83]. Additionally, considering male maturation status has been found to improve the accuracy of estimating the heritability of growth [84]. In the context of aquaculture, knowledge of parentage relationships allows breeders to design selection strategies that maximize genetic diversity while targeting specific traits of interest. This enables more efficient selection of breeding candidates and the realization of breeding goals. This approach proves particularly advantageous in scenarios involving mass selection and family-based selection, where high selection pressure can be applied without compromising genetic diversity [85]. Furthermore, ultrasound tomography has been proposed as a means to predict processing yields in live candidates, reducing the need for slaughtering siblings [86]. Overall, optimizing breeding schemes through parentage assignment is essential for the success and sustainability of aquaculture breeding programs, contributing to the continued genetic improvement of farmed fish populations.

## 7. PARENTAGE ASSIGNMENT IN ORNAMENTAL FISH BREEDING

There has been a growing emphasis in recent years on breeding ornamental fish, along with the cultivation of edible fish, and parallel to aquaculture activities around the world, their economic importance is increasing, and they seem to be rivaling edible fish in importance [87,88]. Further, the parentage assignment using microsatellite markers has emerged as a powerful tool for genetic management in ornamental fish breeding programs. This approach allows breeders to accurately determine parentage relationships, track genetic lineages, and optimize breeding strategies to achieve desired genetic outcomes. The microsatellite-based parentage assignment has been successfully applied to various ornamental fish species, including cichlids, tetras, goldfish and guppies. By integrating molecular genetics techniques with traditional breeding practices, ornamental fish breeders can make informed decisions regarding mate selection, breeding pair formation, and population management, ultimately leading to the sustainable development of ornamental fish populations. For example, [89] utilized microsatellite markers to confirm parentage relationships and assess genetic diversity in a population of ornamental guppies (*Poecilia reticulata*). Their findings revealed significant genetic variation within the population and highlighted the importance of genetic management in maintaining diverse and healthy stocks of ornamental fish. Overall, microsatellite-based parentage assignment represents a valuable tool for genetic management in ornamental fish breeding programs, offering breeders the ability to optimize breeding strategies, maintain genetic diversity, and preserve desirable traits within captive populations.

## 8. THE FUTURE OF PARENTAGE ANALYSIS: CHALLENGES AND OPPORTUNITIES

Parentage analysis is a powerful tool for studying the genetic and ecological factors that influence reproductive success and population dynamics. Despite the challenges posed by complex mating systems, incomplete sampling, and genotyping errors, the current methods of parentage analysis are robust and reliable in most situations. However, there is still room for improvement and innovation in this field, especially as new types of data and analytical frameworks emerge. In this review, we highlight some of the recent advances and future directions for parentage analysis, focusing on the following aspects:

- Full probability parentage analysis and sibship reconstruction: These methods use likelihood or Bayesian approaches to assign parentage and infer sibship relationships based on multilocus genotypes, without requiring prior information on candidate parents or population allele frequencies. They have been shown to perform well in various scenarios, such as when parents are missing, genotypes are incomplete, or populations are structured. They also allow for the estimation of parameters of interest, such as mating system, dispersal, and relatedness.

- Comparison and evaluation of parentage analysis methods: There is a need for more systematic and comprehensive studies to compare the performance and accuracy of different parentage analysis methods, using both simulated and empirical data sets. Such studies should not only assess the ability of the methods to correctly assign parentage, but also their impact on downstream analyses, such as estimating sexual selection, quantitative

genetics, or population dynamics. Ideally, the methods should be compared under realistic conditions, such as varying levels of marker polymorphism, genotyping error, sampling intensity, and population structure.

- User friendliness and accessibility of parentage analysis software: Moreover, as parentage analysis methods become more sophisticated and complex, it is important to ensure that they are user friendly and accessible to a wide range of researchers. This requires clear and detailed documentation, intuitive and flexible interfaces, and compatibility with different data formats and platforms. One of the most popular programs for parentage analysis, CERVUS [90], owes its success partly to its ease of use and implementation of a sound approach. However, other programs may offer advantages in terms of speed, accuracy, or functionality, depending on the specific research question and data set.

- Design and implementation of parentage analysis studies: The quality and reliability of parentage analysis results depend largely on the design and implementation of the study, especially the sampling strategy and the choice of molecular markers. It is essential to obtain adequate and representative samples from the population of interest, as well as from potential sources of immigrants or migrants. It is also crucial to select a set of molecular markers that are sufficiently polymorphic, informative, and error-free for the purpose of parentage analysis. With careful planning and execution, parentage analysis can provide valuable insights into the evolutionary and ecological processes that shape natural populations.

## 9. CONCLUSION

Molecular parentage analysis presents a valuable tool for enhancing breeding programs and genetic management in aquaculture. Through the examination of principles, methods, and applications discussed in this review, it's evident that parentage analysis aids in improving breeding efficiency, maintaining genetic diversity, and mitigating risks associated with inbreeding. Despite challenges such as DNA quality issues and scoring errors, advancements in marker selection, the PCR techniques, and statistical methods continue to enhance the accuracy and efficiency of parentage analysis. Looking ahead, future research should focus on refining analytical approaches, addressing practical constraints, and exploring emerging technologies to further optimize parentage analysis in aquaculture. By leveraging the insights gained from molecular parentage analysis, aquaculture stakeholders can advance towards sustainable and genetically robust fish populations, contributing to global food security and economic prosperity.

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## COMPETING INTERESTS

Author has declared that no competing interests exist.

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