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Polymorphic Microsatellite markers with Egg Production Traits in local Chickens: Review

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Abstract

Egg production traits are quantitative trait in nature that control by regions of the genome are termed microsatellites that considered to be association with this trait. This review was aimed to provide information related to polymorphism egg production trait of local chickens and association with microsatellites markers.

Keyword: microsatellite, local chickens, QTL, quantitative trait, Iraqi chickens, marker assisted selection, practical breeding programs, SSR

Introduction

local chickens are valuable genetic resources due to their adaptability to difficult condition when raised in pastoral area or when reared in outer system as free range chickens, these chickens responded well to improve their environment conditions, especially, nutrition and exhibited improvement in body weight at sexual maturity and egg weight and also found that they classified as a layer type especially one type of them (Brown line) showed a good performance for egg production among other lines (Brown, Barred, Black, White, White neck-naked and Brown neck-naked) [1] Egg production features are quantitative in natures whether the birds are high or low performance. The genome regions that control such features are termed as quantitative traits loci (QTL) and the flanking markers can be employed in a marker

assist selection to insert or retain useful QTL allele. However, markers must be very closely related to the causal mutation in the gene of this trait if they are to remain linked with particular QTL alleles through various generations of selection, sothey are helpful in practical breeding programs [2].

Genome scan approach which is one of important approaches for QTLs detection, by which microsatellites can be investigated because they are extremely polymorphic repetitive DNA sequences, randomly dole out throughout the genome, which displaying highly levels of variance, consequently, are ideal for decode genetic variability[3]. Microsatellite markers evince to be more efficient in the studies of genetic diversity, evaluation of pedigree and genetic mapping as compared to other molecular markers such as like restriction fragment length polymorphism (RFLP) and inter simple sequence repeats ISSRs, due to their virtue co dominance and multiple alleles [4] Due to its economic importance, the raising of egg-producing chickens has undergone significant changes since the early 20th century. Some QTLs for eggs number and eggs weight are detected on chromosome number 5, besides chromosome number 1 and 2. So, the aforementioned chromosomes have became the hotspot regions for studying QTLs for traits of production in poultry birds [5] .

Generally, microsatellites provide maximum information about polymorphic content of the genome, thus, the microsatellites located on these chromosomes containing genes that control growth, reproduction and traits of disease resistance and have been believed to explore the correlation of their variability with traits eggs production [6] .The egg production chain has to be short and variable range from one series to another for the same individual [7]

The genetic performance of the birds (such as the Iraqi chicken) has been improved basically over time, in order to be capable to continue to enhance as the laying hen chickens.[8].

Origin and domestication of chicken

The Chickens were originated in Southeast Asia and were presented into the rest of the world by seafarer and traders. Nowadays, the presence of indigenous village chicken as a result of centuries of cross breeding with foreign breeds and randomly breeding within the flock, thus it is impossible to calibrate the characteristics and productive performance for this indigenous chicken [9].

There is no inclusive list of the breeds and assortment of chickens utilized by rural small holders, but there is an enormous information on some indigenous chickens from different regions, which based on color of feather and other easily measured features (genetic traits), and more detailed information are becoming obtainable [10].

Previous molecular study proposed a single domestic origin in the Indian and Southeast Asia [11]. At least, there are six special maternal genetic lineages have been identified [12], suggesting more than one domestication center. Four living species of genus Gallus are known: red jungle fowl (*Gallus gallus*), La Fayette's jungle fowl (*Gallus lafayettei*), gray jungle fowl (*Gallus sonnerati*), and green jungle fowl (*Gallus varius*), that differ by their morphological aspect and geographical distribution in Asia [9].

Gallus gallus is the closest to domestic chicken by its morphological feature and provides a fertile offspring when crossing with domestic chicken, whilst mating between domestic chicken and any of the other three wild species produces extremely poor hatchability and chick survival. This Red jungle fowl showed a vigrous sexually dimorphism with males having a red fleshy wattles. This chicken is most broadly dispersed over the Southeast Asia.[13].

Avian species and chicken breeds

Chickens are the oldest type of poultry. However, their represent a vital category (about 63% of all-out avian breeds) and the oldest kind of the poultry [14].

Chicken breeds have been partitioned according to type and their classes include: layers (utilized solely for eggs generation), broilers (generation of chicken meat), dual-purpose breeds (meat and eggs), battling breeds and ornamental breeds. The most vital breeds were used in the development of modern egg-laying strain including: the White Leghorn, New Hampshire, Plymouth Rock, ISA Brawn and Ross Hen, which were progressed only in the second half of the 19th century [15].

In the developing countries, the commercial synthetic strain overwhelm the production of meat and eggs, whearas the local breeds are marginalized and limited to some hobby sector so, in some developing countries local breeds still play a major imperative role and in some cases, make up about 70-80 % of the national chicken [16,17].

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Global strains of chickens

Strains

Strains are families or breeding populations with a common charasteresics. These strains may be subdivisions of a breed or assortment or may even be systematic crosses. However, a strain exhibite a relation more exacting than that for others of similar guise. Strains are the products of individual or a breeding program of organizations [18].

A few of the conventional breeds are still kept by poultry fanciers, and these are ordinarily classified into the heavy, medium and light birds. Nowadays, The commercial meat birds represented the heavier types, whilst the eggs producers stem from the lighter types. The commercial predominant laying bird is a “brown or white egger”, which derived from the imported strain selected for the light body weight, high eggs production, feed conversion efficacy, eggs weight and the live ability. Egg or day-old chickens may be gained from the breeders’ companies whilst started pullets might be gained from the commercial suppliers. These breeds were developed for the rapid growth and efficacy feed conversion [19].

Egg laying strains

The pattern of egg laying in domestic hen is characterized to the breed of birds. Genetically superior bird's have a fewer pauses as compared with native breed of birds which developed for dual purpose, and its resistant to diseass and reverse climatic variables such as backyard poultry in rural areas. The characteristics of Eggs laying including: age at first ovipositor, sequence length, inter-sequence pause days, reproductive performance in terms of production of eggs and the hormonal profile [14]. This note focuses on the laying hen selection, despite in all cases the commercial poultry breeding follow the same system: within the company a great number of pure lines are chosen for a variety of characteristics. These lines are crossing in a particular combinations to convey the parents of commercial laying hens or commercial broilers, thus a commercial hens or broilers are so called 4-way cross. Different capabilities could be combined as a result of the crossing scheme. Also, there is a clear, positive impact of heterosis (e.g. the fact that a hybrid or cross is superior to both parental lines). Although there is no recent or public information on the comparison of pure-line with cross-line bird’s performance available, from the theory and the experimental comparisons of over

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50 years ago, it still can be supposed that cross-line hens execute nearly 10 to 40 % better than the pure-line hens. The greatest improvements can be visible in the low heritable characteristics , such as live ability and reproductive characteristics (Fig. 2.1)

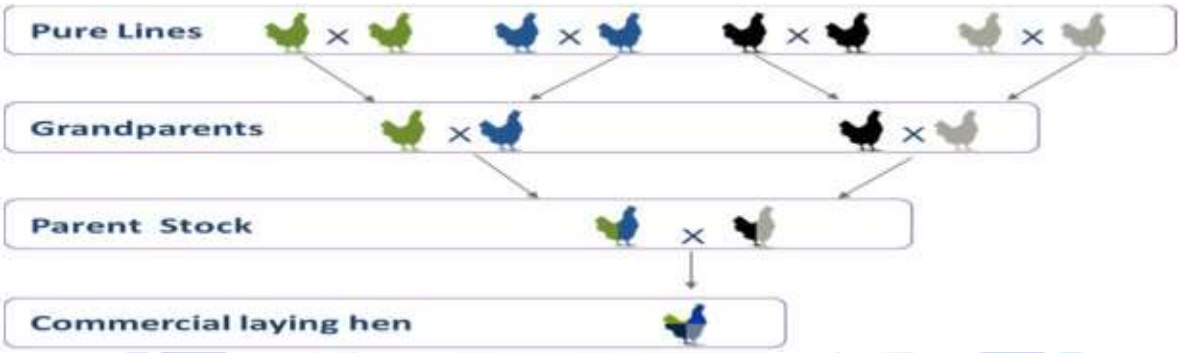

Figure (2-1) Scheme of hybridization or cross breeding of commercial laying hens [20]

Egg production trait

the production of eggs relay on several characters like: age at sexual maturity, the eggs number, the body weight, the eggs weight, thickness of shell, the specific gravity egg and others [21], which affect the eggs production system independently and/ or related with each other. The breed variation in the associations among these characteristics to perform eggs production system was revealed by [22,23].

Generally, each hen has only one useful ovary, commonly lay on the left side of the body, which containing a mass of ova. Only some of these will ultimately form an egg. The commercial egg layers begin the egg production from 16-22 weeks of age, it can produce 250-300 eggs by 70 weeks of age day length, also light intensity have a critical role in the reproductive system progression. Birds designed for eggs production are preferable reared on a constant 10-12 h day length from 3 weeks of age. However, this is only possible in the light proof housing. If this is not obtainable, birds have been allow to natural day length but, in all cases, boost the day length using lights from about 12-15 weeks of age in weekly steps till it is nearly half an hour longer than the longest day length for that gauge. Once in lay, the lights for birds is held steady at 16-17 h a day to obtain maximum eggs production. The lighting

minimum intensity is 5lux, Under this intensity it is still sensible for a someone to read a newspaper [24].

Chicken genome and linkage mapping

Chicken genome consists of thirty-nine pairs of chromosomes; the first eight large-sized called macro chromosomes thirty pairs are small called micro chromosomes and in addition to the sex chromosome called (w, z). Furthermore, the size of the chicken genome is small (1.2×10^9) bp [25].

In spite of the primary genetic linkage map in chicken was published more than 60 years ago, it was not until the development of huge numbers of molecular markers in the last decade that the generation of linkage maps in chicken expanded. In the chicken, there are three distinctive linkage maps have been developed utilizing three distinctive mapping populations. The first genetic map, depend completely on the DNA markers, have been published by. This map, depend on the Compton (C) reference population, which composed only from the restriction fragment length polymorphism markers (RFLP). The second genetic map to be published has been depend on the East Lansing (EL) reference populations and composed primarily of RFLPs, random amplified polymorphic DNA markers (RAPD), and chicken repeat element 1 markers (CRI). Since at that point, both populations were utilized to map a great number of microsatellite markers and AFLP markers as well. The third map has been depend on a large F2 population and composed only of microsatellite and amplified fragment length polymorphism markers (AFLP) [25]. The polymorphisms of protein were the primary markers utilized for genetic studies in livestock. In fact, the number of polymorphic loci that can be inspected, and the polymorphisms level noticed at the loci are regularly low, which significantly limits their application in the studies of genetic diversity. With the improvement of new technologies, DNA polymorphisms have gotten to be the markers of choice for molecular-based scanning of genetic variations [26].

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DNA markers and genetic diversity:

DNA markers are valuable in both basic (such as phylogenetic analysis and search for profitable genes) and applied research (such as marker assisted selection, paternity testing and food traceability). On around worldwide scale, animal biodiversity is defined as the variability among organisms of distinctive or same species regarding to the environment in which they live, giving particular attentiveness to genetic biodiversity [27]. For the livestock sector, animal genetic diversity is a resource could be drawn upon to choose stocks and evolve new breeds. Generally, diverse livestock populations got to give society with a more extent of option to meet future requests, so the administration of the world's agricultural biodiversity has become a critical aspect to the universal community [14].

Differences among the organisms is a result of variations in the sequences of DNA as well as environmental impacts. Genetic variation is a fundamental, and each individual of a species, with the exception of monozygotic twins, has a unique DNA sequence. DNA variations are mutations coming about from substitution of a single nucleotides (which called single nucleotide polymorphisms – SNPs), insertion or deletion of DNA fragments of various lengths (from a single to several thousand nucleotides), or duplication or inversion of DNA fragments [28]. DNA variations have been classified as "neutral" when they cause no alteration in metabolic or phenotypic traits, hence were not subjected to positive, negative, or balancing selection; else, they are classified as "functional" [29].

Among the isolating populaces, the phylogenetic markers are exceptionally valuable for mapping the polygenic characteristics as well as Mendelian traits. The most prevalent among the polygenic markers are the Variable number of tandem repeats (VNTRs) or called as Microsatellites. The locus specific probe fails to cover it and views the highly poly-allelic fragment length variation, for example the tandem repeat loci related with rRNA genes which are centered at nucleolar organizing regions (NORs) of a particular chromosome of an individual. Likewise, most VNTRs loci concentrated in pro- terminal regions of human chromosomes. So, the desirable density of markers doesn't provide. This difficulty has been overcome in microsatellite or simple sequence repeats loci (SSR). [30].

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Nuclear DNA markers

A number of markers are now available to detect polymorphisms in nuclear DNA:

1- Single Nucleotide polymorphisms (SNPs)

2-Restriction fragment length polymorphisms (RFLPs)

3- Amplified Fragment Length Polymorphisms (AFLPs)

4-Mitochondrial DNA markers

5- Microsatellite molecular marker

Microsatellite molecular marker

In genetic diversity study, the most frequently used markers are microsatellites. The term Microsatellite was first coined by [31]. These are also known as SSR or Short tandem repeat (STR). These are the stretches of DNA, consist of tandemly repeating mono-, di-, tri-, tetra- and penta- nucleotide units. These are arranged over the most eukaryotic species genomes. For example, (AAAAAAAAAAA) would be referred to as A, (GTGTGTGTGTGT) would be referred to as GT, (ACTCACTCACTCACTC) would be referred to as ACTC [32].

Microsatellites are generally shorter in length than sequenced loci (100–300 vs. 500–1500 base pairs), which could still be amplified with PCR in spite of some DNA degradation [33]. Taking a multiple sample of the genome by combining that comes about many loci gives a more exact and statistically strong way to compare the populations and individuals [34].

Microsatellites have gotten to be so prevalent since they are single locus, co-dominant markers for which numerous loci can be productively combined in the genotyping process to supply a fast and cheap replicated sampling of the genome. Generally, microsatellite markers have a high mutation rate leading to a high-level allelic diversity [33]. Co-dominant transmission and the heterozygotes could be distinguished from homozygotes, in contrast to RAPD and AFLP, which are binary in nature, highly polymorphic and hypervariable, high content information and provides a great pattern, relative multitude with regular genome coverage, higher mutation rate than gauge sequences (up to 0.001 gametes per generation), high chance of back mutation [35].

Microsatellite data are also commonly used to assess genetic relationships between populations and individuals through the estimation of genetic distances. The foremost commonly used gauge of genetic distances is Nei standard genetic distance (DS) [36]. However, for closely concerning populations, where the genetic drift is the major factor of genetic differentiation, like in the case of livestock breeds, especially in the developing world, the modified Cavalli Sforza distance (DA) was recommended [37]. Genetic relevance among breeds is often evoke through the reconstruction of a phylogeny, predominantly utilizing the neighbor joining (N-J) method [38].

However, a master drawback in reconstruction of the phylogenetic tree is that the evolution of lineages is supposed to be a non-reticulate, the lineages could diverge, but could not result from crosses among lineages. This presumption will seldom hold for livestock, where new breeds often evolve from the cross-breeding between two or more ancestral breeds. So, the visualization of the breeds evolution provided by phylogenetic reconstruction should be interpreted carefully. the multivariate analysis and the recently Bayesian clustering approaches have been proposed for mixture analysis of microsatellite data from various populations [39]. possibly the most inclusive study for this type in livestock is a continent -wide study of African cattle [40], which detects the genetic signatures of the origins, secondary movements, and the differentiation pastoralism of African cattle.

The molecular genetic information in conjunction with, and complemented by, other sources such as archaeological proof and written records, provide beneficial data on the origins and next movements and developments of genetic diversity in livestock species. Mapping the origin of current genetic diversity potentially permits inferences to be made about where functional genetic variation might be establish within a species for which a limited data on phenotypic variation exists. joined analysis of microsatellite data gained by a various study is highly eligible, but has seldom been possible. This is due to most genetic studies using DNA markers are limited to small numbers of breeds, overwhelmingly from a single country [41]. Predominating, several subsets of the [14] FAO-recommended markers are utilized, and there is no standard samples are genotyped across projects. The application of various microsatellite genotyping systems leads to a variation among studies in the estimation the size of alleles at the same loci [14].

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Significance of Microsatellites in Genome

As there are often many alleles present at a microsatellite locus, genotypes within pedigrees are often fully informative, in that the progenitor of a particular allele can often be identified. In this way, microsatellites can be used for determining paternity, population genetic studies and recombination mapping. It is also the only molecular marker to provide clues about which alleles are more closely related. Also, these markers often present high levels of inter- and intra-specific polymorphism, particularly when tandem repeats number are 10 or greater. Microsatellites determine the genotype of an individual [42].

They usually don't have any measurable effect on phenotype, and when they do mutate, may cause a change in the genotype of an individual, Microsatellites may act as a marker for some genetic diseases. They were previously considered to be as the "Junk" DNA which is generally found on the Non-coding regions of DNA and the variation is mostly neutral. In humans, 90% of known microsatellites are found in non-coding regions of the genome [43]. When found in human coding regions, microsatellites are known to cause disease. Interestingly, when found in coding regions, microsatellites are usually trinucleotide repeats. One possible explanation is that any other type of nucleotide repeat would be too detrimental to the coding region, because it would cause a frame-shift mutation; Microsatellites provide a necessary source of genetic variation [44].

The variation in microsatellite alleles in coding regions is thought to be the cause of adaptation in different environments. In other words, a short allele may be adaptive in one environment, and a long allele with many repeats may be adaptive in a different environment, Microsatellite variation may be a way to compensate for loss of genetic variability due to genetic drift and selection. Thus, having variation within the population would ensure the survival of the population in varying environment, Microsatellites may help regulate gene expression and protein function [45].

[46] cited by [47] have suggested that microsatellites may have regulatory roles in gene expression. They are systematically found near coding regions.

Variation in microsatellite alleles have been shown to be associated with quantitative variation in protein function and gene activity the regions surrounding the microsatellite locus, called the flanking regions, may still have the same sequence. This is important because the

flanking regions can therefore be used as PCR primers when amplifying microsatellite loci, and can be conserved across genera or sometimes even families [48].

The flanking regions are critical because they allow us to develop locus-specific primers to amplify the microsatellites with PCR (polymerase chain reaction). That is, given a stretch of unordered DNA 30-50 base pairs (bp) long, the probability of finding that particular stretch more than once in the genome becomes vanishingly small [49]. This combination of widely occurring repeat units and locus-specific flanking regions are a part of strategy for finding and developing microsatellite primers. The primers for PCR will be sequences from these unique flanking regions. By having a forward and a reverse primer on each side of the microsatellite, it would be able to amplify a fairly short (100 to 500 bp, where bp means base pairs) locus-specific microsatellite region. There are two hypotheses that explain how microsatellites mutate [47]. Since microsatellites are widely dispersed in eukaryotic genomes, are highly variable, and are PCR based (requiring only minute amounts of starting template) they have been used in many different areas of research such as:

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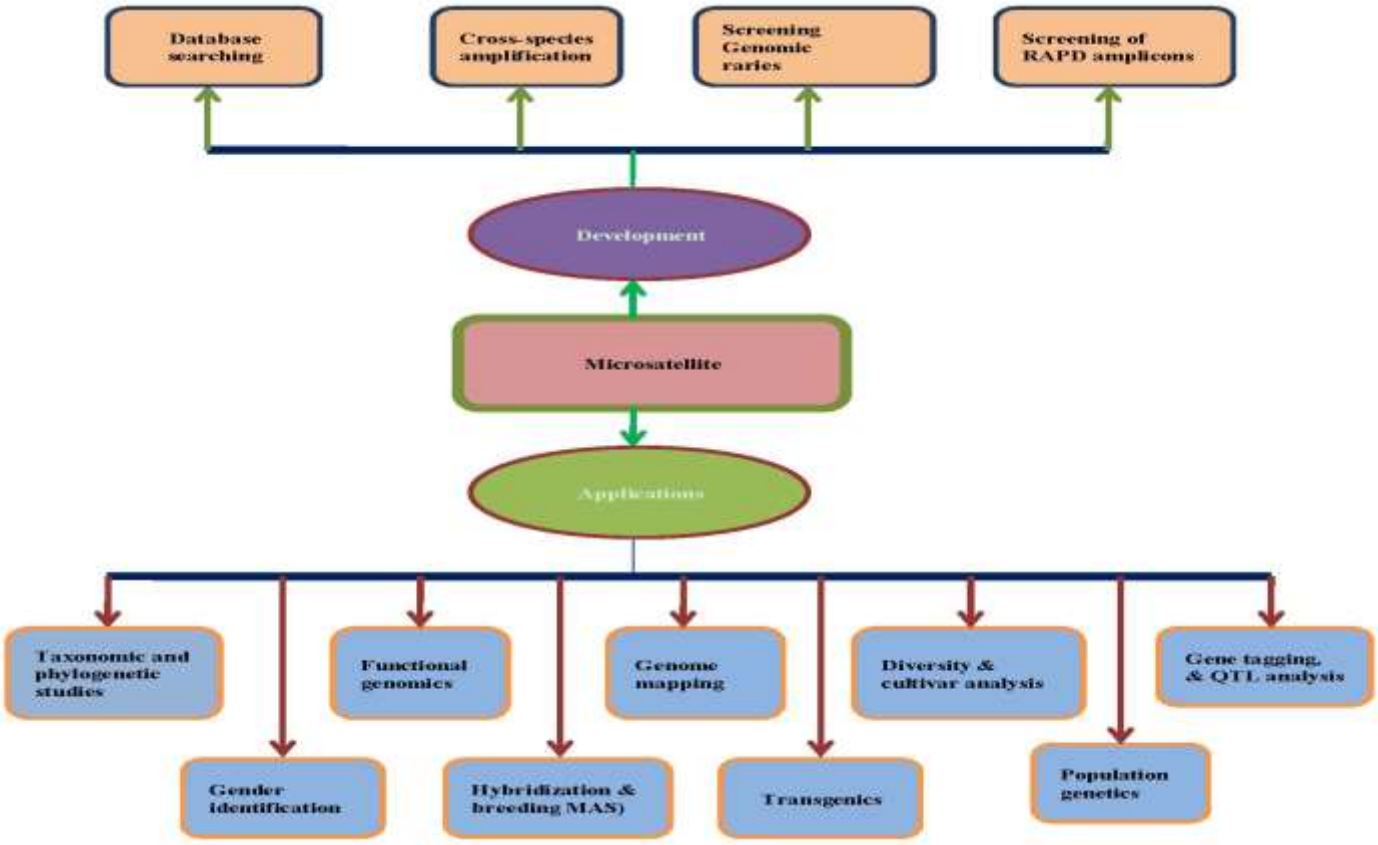

Figure (2-4). Development and applications of microsatellite markers at a glance [44]

1) Forensics

Microsatellite loci, generally known in forensic applications as Short Tandem Repeat (STR) loci, are widely used for forensic identification and relatedness testing, and are a predominant genetic marker in this area of application. They have also become the primary marker for DNA testing in forensics (court) contexts, both for human and wildlife cases. The reason for this prevalence as a forensic marker is their high specificity [13].

Match identities for microsatellite profiles can be very high and the probability that the evidence from the crime scene is not a match with that of the suspect is less than one in many millions in some cases. In forensic identification cases, the goal is typically to link a suspect with a sample of blood, semen or hair taken from a crime. Alternatively, the goal may be to

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link a sample found on a suspect's clothing with a victim. Relatedness testing in criminal work may involve investigating paternity in order to establish rape or incest [50] .

Another application involves linking DNA samples with relatives of a missing person. Because the lengths of microsatellites may vary from one person to the next, scientists have begun to use them to identify criminals and to determine paternity, a procedure known as DNA profiling or "fingerprinting". The features that have made use of microsatellites attractive are due to their relative ease of use, accuracy of typing and high levels of polymorphism. The ability to employ PCR to amplify small samples is particularly valuable in this setting, since in criminal casework only minute samples of DNA may be available [47].

2) Population Studies

By looking at the variation of microsatellites in populations, inferences can be made about population structures and differences, genetic drift, genetic bottlenecks and even the date of a last common ancestor. It can also be very helpful in studying the inheritance of the natural characters of an individual from its ancestor. It also reveals significant information on the correlation of the individual's genetic constitution with respect to its ancestor from which he has been descended [51].

3) Conservation Biology

Microsatellites can be used to detect sudden changes in population, effects of population fragmentation and interaction of different populations. Microsatellites are useful in identification of new and incipient populations. It can be useful in identifying the adaptation of an individual to the ever-changing environment and also its survival for the years in the same environment [52].

4) In mapping genomes

They are very much helpful in mapping of genome and finding or locating the significant portion in the genome of an individual. They have found wide applications in areas such as the widely publicized mapping of the human genome [53].

5) In a Biological/Evolutionary context

They can also be used to address questions concerning degree of relatedness of individuals or groups. For captive or endangered species, microsatellites can serve as tools to evaluate inbreeding levels. From there we can move up to the genetic structure of

subpopulations and populations. They can be used to assess demographic history, to assess effective population size and to assess the magnitude and directionality of gene flow between populations [41]

Microsatellites provide data suitable for phylogeographic studies that seek to explain the concordant biogeographic and genetic histories of the floras and faunas of large-scale regions. They are also useful for fine-scale phylogenies up to the level of closely related species[15].

6) Diagnosis and Identification of Human Diseases

They serve a role in biomedical diagnosis as markers for certain disease conditions. That is, certain microsatellite alleles are associated (through genetic linkage) with certain mutations in coding regions of the DNA that can cause a variety of medical disorders. Because microsatellites change in length early in the development of some cancers, they are useful markers for early cancer detection. Because they are polymorphic, they are useful in linkage studies which attempt to locate genes responsible for various genetic disorders. For example, in one part of chromosome number 4, CAG nucleotides are repeated many times over. They look like this CAGCAGCAGCAG....If the tri-nucleotides are repeated too many times this would cause the person to get Huntington's disease in adult life [54]. Other diseases that involve repeats of three nucleotides are also known to cause neurological diseases. At this time, 14 neurological disorders have been shown to result from the expansion of tri-nucleotide repeats, establishing an expanding class of diseases. Tri-nucleotide repeat diseases can be categorized into two subclasses based on the location of the trinucleotide repeats: diseases involving noncoding repeats (untranslated sequences) and diseases involving coding sequences (exonic). In general tri-nucleotide repeat disorders are either dominantly inherited or X-linked, the one exception being Friedrich's ataxia, which is Autosomal recessive [55].

Not all diseases are caused by a mistake in one gene. Sometimes many genes may be involved in a disease, for example, in schizophrenia. For these diseases' microsatellite sequences have been used as a marker for locating the diseased region of the chromosome. This method is called positional cloning. Microsatellite markers close to the disease gene correlate with the heredity of the disease, and by analysis of these markers within family's scientists can predict how the disease will be inherited [56].

7) Detecting Cancer

The rate of microsatellite expansion (that is, increase in the number of repeats) or contraction (decrease in number of repeats) in cells is increased in some types of cancers, due to defects in enzymes that correct copying mistakes in DNA. Early clinical detection of some types of

Genetic Similarity

Genetic Similarity is important for the population to be able to face future environmental changes and to ensure a long-term response to selection [57]. Concern over the conservation of many threatened species has highlighted the importance of genetic data and the maintenance of genetic diversity is the major goal for some biodiversity conservation programs [58;57].

Neutral genetic markers are assumed to reflect adaptive genetic variation that is important to the evolutionary potential of the species [59;57] and consequently, the selection of useful molecular markers is necessary to conduct these studies.

Among the molecular techniques available, DNA fingerprinting, developed by [60], has been widely utilized in studies of various groups of animals including threatened species of birds [61;62;63]. This technique is based on the detection of many loci minisatellites (i.e., variable number of tandem repeats (VNTRs) [60].

Microsatellites are also VNTRs, but are less frequent in the avian genome than in other organisms [64]. This kind of marker has the advantage of being able to be amplified by PCR (and thus, it does not require large amounts of DNA), is usually highly polymorphic in the number of repeat units and shows a single locus pattern which allows the comparison of populations based on their allele frequencies [65]. However, the development of microsatellite markers also requires a large amount of work, but, fortunately, many primers developed for one species can be used in related species [66].

Microsatellites have been useful in many animal conservations studies, including Komodo dragons [67], turtles [68], whales [69], wolves [70], snakes [71], bears [72], butterflies [73] and birds [74;75;76]

Another approach for estimating genetic variability is to use primers of short repeated sequences (inter simple sequence repeats, ISSRs) to amplify anonymous genomic regions between two microsatellite loci. The principal advantage of this kind of approach is that it is not necessary to construct genomic libraries [66].

The method for estimating genetic diversity according to the polymorphism of genetic productions, as isozyme, blood type and leukocyte antigens, is increasingly replaced by DNA polymorphism. The most common methods for testing the DNA polymorphism include restrictive fragment length polymorphism (RFLP), variable number of tandem repeats (VNTR) and random amplified polymorphic DNA (RAPD), etc[77]. It has been verified in many experiences that the genetic purity in breed and genetic diversity between breeds can be determined effectively by proper statistical method according to the fingerprinting atlas of individual DNA [78]. At present, the genetic similarity between individuals is scaled by Nei's formula, and the individuals' relationship is estimated from it [79; 80].

One can get an electrophoretic atlas by using present molecular mark, and comparing them binately. Using 1-1 denotes having no polymorphism, namely, monomorphism, if both two parallel samples with the same molecular weight have all bands, and 1-0 denotes having polymorphism if only one sample [81; 82].

Conflict of Interests.

There are non-conflicts of interest .

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