

# A Review on Population Structure, Genetic Diversity Analysis, Genetic Distance between Population and Genetic Singularity in Livestock

Anteneh Worku\*<sup>1</sup> Yosef Tadesse <sup>2</sup>

1. Debre Markos University, Collage of Agriculture and Natural Resource, Po Box 269, Debre Markos, Ethiopia.

2. Harmaya University, College of Agriculture, School of Animal and Range Science P.O.Box; 138, Dire Dawa, Ethiopia

## Abstract

This review was undertaken to clarify importance of genetic diversity and genetic distance of domestic livestock species in the future. Importance of farm animal genetic resources is very diverse, particularly for the poor and under smallholder production systems in the developing world. Uses include subsistence consumption of livestock products, manure, traction, savings, risk management, capital accumulation and socio-cultural functions. The present domestic animal diversity as represented in the multitude of our livestock breeds is the result of thousands of generations of rural communities manipulating their livestock populations according to the requirements of their environment, their subsistence needs and cultural concepts. Genetic characterization has recently been the method of describing and classifying livestock breeds/populations using measures of genetic distances between populations. Genetic markers like microsatellites, and Single Nucleotide Polymorphisms (SNPs) and recently the whole genome are tools used for characterizing genetic diversity between and within populations. Hence, SNP is more preferable to other markers for developing countries due to its simplicity, ability to differentiate variations, useful to identify genes of economically important traits and it is cheap.

**Keywords:** Genetic marker, SNP, Genetic Diversity Analysis, Genetic Distance

## 1. INTRODUCTION

### 1.1 Background

Since the domestication process in the Neolithic age livestock have spread all over the world mainly as a result of human migration exchange among neighboring human population (Diamond And bell wood ,2005; zeder,2008). Domestic animals were multifunctional and they were used for drought work, clothes, manure, fuel and food FAO (2007 ). As domestic animals reached different place they slowly adapted to specific environmental conditions of the area and uses and cultural preferences of their new herdsman giving raise to the livestock genetic diversity (Wallny, 2003). Livestock contributes significantly to food production and economic output in all regions. The relative contribution of livestock to agricultural GDP is higher in the developed regions but the trend has been slightly downwards over the past 30 years, whereas in most developing regions there has been a rise in the importance of livestock (FAO, 2011). Country reports on farm animal genetic resources (FAO, 2007) illustrate that importance of farm animal genetic resources is very diverse, particularly for the poor under smallholder production systems in developing world. Uses include subsistence consumption of livestock products, manure, traction, savings, risk management, capital accumulation and socio-cultural functions. In wild animals, patterns of population genetic structure are usually explained by factors that disrupt gene flow, such as isolation-by-distance (Wright, 1943), historical geological factors (Gübitz *et al.*, 2000) or physical barriers (Nicholls and Austin, 2005). Unlike populations in the wild, dispersal ability and hence gene flow among domestic animal populations is believed to be governed more by human intervention than by physical barriers. Differences in ancestral origins and migration events are important causative factors explaining genetic differences between current populations (Cañón *et al.*, 2001; Rendo *et al.*, 2004). Local management and cultural separation (Rege, 2002) can cause genetic isolation of populations leading to reduced effective population size and further divergence through drift.

## 2. REVIEW

### 2.1 ANIMAL GENETIC RESOURCE

Farm animal genetic resources includes all animal species, breeds/strains and populations used for food and agricultural production and their wild and semi-domesticated relatives. They encompass about 40 species of domesticated animals that have been diversified into more than 7,000 breeds during the 12,000 years since humans started farming and raising livestock and adapted to very diverse and specific challenges. According to the Food and Agriculture Organization (FAO), about one-third of the documented breeds is threatened or has already become extinct (Scherf, 2000). The present domestic animal diversity as represented in the multitude of our livestock breeds is the result of thousands of generations of communities manipulating their livestock populations according to the requirements of their environment, their subsistence needs and cultural concepts. It

is a consequence of cultural diversity and represents a legacy that needs to be stewarded wisely for the future of all humankind (Sanders, 2004).

### **2.1.2 Genetic Characterization Tools**

#### **2.1.3 Genetic Markers**

To understand the history and evolution of populations it is usually necessary to study a large number of polymorphisms (Cavalli-Sforza 1998). Through molecular revolution over the last few decades, a lot of techniques have been developed to study population structure using genetic markers. At the first stage of research, almost all markers identified have been protein and blood group polymorphism, and only a few hundred were previously known (Nei and Roychodhury 1988). These markers are also known as 'classical polymorphisms' to distinguish them from those obtained by direct Deoxyribonucleic acid (DNA) analyses. The use of DNA segments to analyze genetic polymorphisms has resulted in the identification of a great number of markers and genetic polymorphisms. A lot of markers were identified to detect population structure and selection signatures within populations, consequently identifying quantitative trait loci (QTL) affecting traits of economic interest (MacNeil & Grosz 2002; Thaller *et al.*, 2003), as well as qualitative trait loci (Drögemüller *et al.*, 2005; Fontanesi *et al.*, 2010). Commonly considered DNA markers are microsatellites and Single Nucleotide Polymorphisms (SNPs or 'snips').

#### **2.1.4 Microsatellites**

Microsatellites are direct tandem repeated sequences of DNA with a repeat size ranging from 1 to 6 base pairs (bp). Hence, a microsatellite is also called a simple sequence repeat (SSR). To identify microsatellite loci suitable for use as genetic markers, different approaches have been developed. One of the prevailing methods to identify microsatellites is the use of primers, which are commonly designed directly from the sequence data. In this context, the primers serve to detect the variation of microsatellites, by flanking the different numbers of repeats provided by Polymerase Chain Reaction (PCR) (Saiki *et al.*, 1988; Newton & Graham 1997). Microsatellites are more useful and highly polymorphic than RAPD, AFLP in many animal and plant species (Morgante & Olivieri 1993; Queller *et al.*, 1993). Especially the high variation of microsatellite DNA marker have led in the past decades for a large number of population structure studies. Before a decade study Baumung *et al.* (2004) explained that within livestock species approximately 90% of all projects were used microsatellite genetic markers. while Single Nucleotide Polymorphism (SNP) markers were only investigated in about 10% of all projects. In contrast to the past decade, recent analyses in human (Hannelius *et al.*, 2008), cattle (The Bovine HapMap Consortium 2009) and sheep Kijas *et al.* (2009) show that currently SNPs regaining favor for uncovering genome-wide population structures.

#### **2.1.5. Single Nucleotide Polymorphisms (SNPs)**

A SNP is a small (single base pair) genetic change, or variation, that can occur within an individual's DNA sequence. This property of SNPs allows to make associations between marker alleles with QTL's affecting important economic traits in livestock. It has been hypothesized that these SNP studies are most powerful method in identifying genes that are responsible for important traits, like Myostatin (MSTN) for double muscling within animals (Hill *et al.*, 2010) as well as for undesirable genes such as bovine leukocyte adhesion deficiency (BLAD), complex vertebral malformation (CVM) and congenital muscular dystonia (CMD) (Charlier *et al.*, 2008). Single Nucleotide Polymorphisms are a good choice of marker for the studies of economically important traits because of their low mutation rate, high incidence throughout the genome and bi-allelic nature making them amenable to automated detection techniques (Dawson 1999). It has been estimated that a well-designed genome-wide SNP map requires as many as 500,000 SNPs in human (Vega & Kreitman 2000) and likely up to 300,000 SNPs in cattle (Eck *et al.*, 2009; Shannon, 2010). Hence, the numbers of available SNP chips are increasing annually. In cattle alone, there are currently 11 commercial SNP chips produced by three major companies (Illumina, Neogen-GeneSeek and Affymetrix) (Nicolazzi, 2015). In addition, there are a constantly growing number of custom SNP chips protected by intellectual property (IP). However, as study (Nicolazzi, 2015) described that a list of currently available commercial SNP chips not completely protected by IP for the six major livestock species (cow, pig, horse, sheep, goat and chicken).

This rapid development in the SNP chip technology also provides new insights into the population structure of livestock breeds since previous studies have been focused on small sets of microsatellite loci. The increase in the number of SNP chips available, however, was not accompanied by an organized effort to standardize the genomic/genotype data, making comparison and cross-compatibility of SNP arrays difficult.

## **2.2. POPULATION STRUCTURE**

In the population ecology of animals 'population structure' refers to the age distribution of individuals of each sex within the population. Knowledge of the relative proportions of young and old or males and females, often summarized diagrammatically as a 'pyramid of numbers', is regarded as a preliminary step in the quantitative description of that population. This knowledge is an essential prerequisite if reliable predictions of future population trends are to be attempted. Highly informative genetic markers are essential to study the origin,

history and evolution of livestock populations (Troy *et al.*, 2001; Li *et al.*, 2009). After a decade of domination by microsatellite markers, recently Single Nucleotide Polymorphisms (SNPs) are becoming more attractive to population genetic studies (Behar *et al.*, 2010). High throughput sequencing (Kaller *et al.*, 2007), have led that SNPs are the most technologically developed abundant markers in genetic science. Additionally it is becoming increasingly feasible to genotype hundreds or even thousands of individuals for these large numbers of SNPs. These advantages in genetic science provide new insights into complex population structures (Gompert *et al.*, 2010).

### 2.2.1. Genetic Diversity

Genetic diversity allows a population to mount a successful evolutionary response to unpredictable environmental challenges such as changing weather, disturbance events, and variations in resource availability, and population sizes of competitors (Falk *et al.*, 2006). Hoffmann and Willi (2008) showed that the level of genetic variation influences the ability of species to respond to threats and environmental changes. When a population has a high level of genetic variation, it is better able to adapt to new environmental challenges. On the other hand, low genetic variation in a population will limit a species' ability to respond to the changes in the short term and to persist in the long term (Amos and Harwood, 1998). Genetic diversity is affected by several factors such as mating patterns, migration, natural selection, and genetic drift. Mating patterns are important because nonrandom mating can occur when a population interbreeds. When a population interbreeds, nonrandom mating can sometimes occur because one organism chooses to mate with another based on certain traits. In this case, individuals in the population make specific behavioral choices, and these choices shape the genetic combinations that appear in successive generations.

Genetic drift is the change in relative allele frequency, which can either increase or decrease by chance over time. When genetic drift occurs in small populations, it may decrease the genetic diversity of a population or cause extinction through loss of alleles in a population resulting in only one allele present at a particular locus.

### 2.2.2. Cause of changes in Genetic Diversity

Genetic diversity' refers to the variation of genes within species that is the mix of genes contained within individuals. This covers distinct populations of the same species or genetic variation within a population. Ultimately, genetic diversity resides in changes in the sequence of the four base-pairs of the DNA that constitutes the genetic code. Because changes in populations require changes in gene frequencies, The primary causes of changes are mutation, migration, recombination, selection and drift. Despite its cardinal role in evolution and domestication, the origin, nature, dynamics, function, significance, and maintenance of genetic diversity in nature remains controversial. Likewise, questions concerning which evolutionary processes influence natural genetic variation for phenotypic, quantitative-trait (QTL) variation (Mitchell-Olds *et al.*, 2007) or what the genetic basis of ecologically important morphological variation such as diverse color patterns of mammals (Steiner *et al.*, 2007) emerged. The basic problems are how much of molecular diversity in nature, is adaptive and how much of the mutations occurring in natural populations are *nonrandom* rather than random, neutral, nearly neutral, non-adaptive or deleterious (Galhardo *et al.*, 2007).

### 2.2.3. Genetic Diversity Analysis

According to FAO recommendations, determining classic genetic distances using neutral, highly polymorphic microsatellite markers is the method of choice for investigating genetic relationships and breed differentiation. This methodology also provides information for establishing preservation priorities for livestock breeds (Barker 1999; Marletta, 2006). Nevertheless, phylogenetic methods based on genetic distances cannot be exhaustive in their quantification of genetic diversity between closely related groups because they ignore within-group genetic variation (Ruane 1999; Marletta, 2006). Genetic diversity is a function of relatedness between individuals that share one or more ancestors. The level of relatedness can be expressed as a coefficient of kinship, which appears to be of central importance in the definition and measurement of genetic diversity (Eding & Meuwissen 2001). A molecular co ancestry-based method has recently been proposed for analyzing the genetic diversity in subdivided populations (Caballero & Toro 2002). This uses molecular data to obtain co ancestry coefficients that integrate the well-known genetic distances DS, DR and FST, taking into account the allelic frequencies and diversity of supposed founder populations. However, only a few authors have explored this approach for analysing actual data sets (Caballero & Toro 2002; Fabuel *et al.*, 2004). A valid alternative is a clustering method that uses individual multilocus genotypes to infer population structure and to assign individuals to theoretical populations (Pritchard *et al.*, 2000).

### 2.2.4. Genetic Distance between Populations

Genetic distance is "that difference between two entities that can be described by allelic variation." (Nei 1973). This definition was later elaborated by Nei (1987 as "the extent of gene differences between populations or species that is measured by some numerical quantity." A more comprehensive definition of genetic distance is "any quantitative measure of genetic difference be it at the sequence level or the allele frequency level it is calculated between individuals population or species" (Beaumont *et al.*, 1998). likewise, Genetic distance is the degree of gene difference (genomic difference ) between species or populations that is measured by some

numerical method thus the average number of codon or nucleotide differences per gene is a measure of genetic distance when the two species to be compared have distantly related data on amino acid or nucleotide sequence. Populations with many similar alleles have small genetic distances. This indicates that they are closely related and have a recent common ancestor. Genetic distance is useful for reconstructing the history of populations. Genetic distance is also used for understanding the origin of breed or species and to determine which breed or species should be protected to maintain genetic diversity Ruane, (1999).

### 2.2.5. Genetic Singularity

Variation provides considerable information on the effects of gene flow and selection. The shape of a cline can be used to estimate the overall strength of the barrier to gene exchange given certain assumptions about selection and dispersal (Vines *et al.*, 2003). Recent analyses using many molecular markers have focused on heterogeneity in patterns of differentiation across markers and what it might say about the genetic basis of reproductive isolation and ecological adaptation (Rieseberg *et al.*, 1999; Turner *et al.*, 2005; Stinchcombe & Hoekstra 2007; Yatabe *et al.*, 2007). The studies support the prediction that genomes can be 'porous', engaging in extensive gene flow while maintaining significant differentiation near loci responsible for divergently selected traits (Mallet, 2005). This is in contrast to the conventional idea of 'the genome' as a delicately co-adapted gene complex that could be severely disrupted by gene exchange (Dobzhansky 1937; Mayr 1963). The distribution of genotypes among individuals within localities is the most direct indication of whether distinct groups can co-exist at the same place and time. 'Genotypic clusters' has even been offered as a phenomenological definition of 'species' (Mallet, 1995). The stable co-existence of distinct clusters requires barriers to gene exchange and is almost always associated with ecological and behavioural differentiation (Jiggins & Mallet 2000; Schluter 2000; Coyne & Orr 2004). An important question is whether ecological, morphological, and behavioural distinctiveness require barriers to gene exchange that have genome-wide effects or whether selection processes affecting only some genomic regions cause and maintain distinctiveness despite high levels of interbreeding and gene exchange across other genomic regions.

## 3. CONCLUSION

The present domestic animal diversity as represented in the multitude of our livestock breeds is the result of thousands of generations of rural communities manipulating their livestock populations according to the requirements of their environment, their subsistence needs and cultural concepts. Genetic characterization has recently been the method of describing and classifying livestock breeds using measures of genetic distances between populations. Genetic Markers, Microsatellites and Single Nucleotide Polymorphisms (SNPs) are tools used to characterize genetic diversity and distance of populations and to study association of SNP with QTL. So that recently SNPs have become markers of choice for diversity and for selection of gene of interest because of availability in large quantity and its neutrality.

Genetic diversity allows a population to mount a successful evolutionary response to unpredictable environmental challenges such as changing weather, disturbance events, and variations in resource availability, and population sizes of competitors. When a population has a high level of genetic variation, it is better able to adapt to new environmental challenges. Determining classic genetic distances using neutral, highly polymorphic microsatellite markers is the method of choice for investigating genetic relationships and breed differentiation. It is generally accepted that genetic variation is the raw material of evolution, without which populations cannot evolve in response to changing environments.

## 4. RECOMMENDATIONS

SNP analysis for diversity and Gene or marker-assisted selection (MAS) is a promising strategy for genetic improvement of economically important quantitative or functional traits in developing countries which have huge number of livestock species with large genetic diversities.

## 5. REFERENCE

- Amos W and Harwood, J. 1998. Factors affecting levels of genetic diversity in natural populations Phil. Trans. R. Soc. B, 353,177-86.
- Barker, J., S.F, 1999. Conservation of livestock breeds diversity. Anim. Genet. Res. Inf., 25, 33–43.
- Barton, NH. 1979. The dynamics of hybrid zones. Heredity, 43, 341–359.
- Beaumont, M.A., K.M. Ibrahim, P.Boursot and M.W.Bruford, 1998. Measuring genetic distance p. 315–325 In A. Karp (ed) Molecular tools for screening biodiversity. Chapman and Hall, London.
- Behar, D. Yunusbayev B, and Metspalu M, 2010. The genome-wide structure of the livestock.
- Brehem, A., Khadem, M., Jesus, J., Andrade, P., Vicente, L., 2001. Lack of congruence between morphometric evolution and genetic differentiation suggests a recent dispersal and local habitat adaptation of the Madeiran lizard *Lacerta dugesii*. Gen. Selec. Evol. 33, 671-85.
- Brown, P.B., Thorpe, R.S., 1991. Within-island microgeographic variation in the colour pattern of the skink,

- Chalcides sexlineatus: Pattern and cause. *J. Evol. Biol.* 4, 557-74.
- Caballero, A., Toro M.A, 2002. Analysis of genetic diversity for the management of conserved subdivided populations. *Conserv. Genet.*, 3, 289-299.
- Cañón, J., Alexanderino, P., Bessa, I., Carleos, C., Carretero, Y., Dunner, S., Ferran, N., Garcia, D., Jordana, J., Laloe, D., Pereira, A., Sanchez, A, and Moazami-Goudarzi, K., 2001.
- Cavalli-Sforza, LL., 1998. The DNA revolution in population genetics *Science* 14, 60-65
- Charlier C, Coppieeters. W, Rollin F., 2008. Highly effective SNP-based association mapping and management of recessive defects in livestock *Nature* 40, 449-454.
- Coyne, JA. And Orr HA, 2004. *Speciation* Sinauer & Associates, Sunderland, Massachusetts.
- Dawson, E., 1999. SNP maps: more markers needed? *Molecular Medicine Today* 4, 419-420.
- Dobzhansky, T , 1937. *Genetics and the Origin of Species*. Columbia University Press, New York.
- Drögemüller C, Wöhlke A, Mömke S, Distl. O, 2005. Fine mapping of the polled locus to a 1-Mb region on bovine chromosome 1q12. *Mammalian Genome* 16, 613-620.
- Eck S, Benet-Pages A., and Flisikowski, K, 2009. Whole genome sequencing of a single *Bos taurus* animal for single nucleotide polymorphism discovery. *Genome Biology* 10, R82.
- Eding H., and Meuwissen T.H.E. 2001. Marker-based estimates of between and within population kinships for the conservation of genetic diversity. *J. Anim. Breed. Genet.*, 118, 141-159.
- Fabuel E., Barraga ´n C., Sillio ´, L., Rodríguez M.C., and Toro M.A., 2004. Analysis of genetic diversity and conservation priorities in Iberian pigs based on microsatellite markers *Heredity*, 93, 104-113.
- Falk DA, Richards CM, Montalvo AM, and Knapp EE. 2006. *Population and Ecological Genetics in Restoration Ecology*. Foundations of Restoration Ecology.14-41. Washington, D.C.: Island Press.
- Fontanesi L, Tazzoli M, Russo V, Beever, J., 2010. Genetic heterogeneity at the bovine KIT gene in cattle breeds carrying different putative alleles at the spotting locus. *Animal Genetics* 41, 295-303.
- Food and Agriculture organization of united nation, 2001. *The states of the world's animal genetic resource food and agriculture* FAO, Rome Italy.
- Food and Agriculture organization of united nation, 2007. *The states of the world's animal genetic resource food and agriculture* FAO, Rome Italy.
- Genetic diversity measures of local European cattle breeds for conservation purposes. *Gen. Selec. Evol.* 33, 311-32.
- Gompert Z, Forister M, and Fordyce, J. 2010. Bayesian analysis of molecular variance in pyrosequence quantifies population genetic structure across the genome of *Lycaeides* butterflies. *Molecular Ecology* 19, 2455-2473.
- Gübitz, T., Thorpe, R.S., and Malhotra, A., 2000. Phylogeographic and natural selection in the Tenerife gecko *Tarentola delalandii*: testing historical and adaptive hypotheses. *Molecular Ecology* 9, 1213-21.
- Hannelius U, Salmela E, Lappalainen T, 2008. Population substructure in Finland and Sweden revealed by the use of spatial coordinates and a small number of unlinked autosomal SNPs. *BMC Genetics* 9, 1471-2156.
- Hill E, McGivney B, Gu J, Whiston R, and MacHugh. D ,2010. A genome-wide SNP association study confirms a sequence variant (g.66493737C>T) in the equine myostatin (MSTN) gene as the most powerful predictor of optimum racing distance for Thoroughbred racehorses. *BMC Genomics*, doi:10.1186/1471-2164 1111-1552.
- Jiggins, CD, Mallet .J, 2000. Bimodal hybrid zones and speciation *Trends in Ecology & Evolution*, 15, 250-255.
- Källner M, Lundeberg, J., and Ahmadian. A, 2007. Arrayed identification of DNA signatures. *Expert Review of Molecular Diagnostics* 7, 65-76.
- Kijas J, Townley, D. and Dalrymple, B, 2009. A Genome Wide Survey of SNP Variation reveals the Genetic Structure of Sheep Breeds. *PLOS One* 4, e4668 doi:4610.1371/journal.pone.0004668.
- Li H, Han, W and Zhu Y, 2009. Analysis of genetic structure and relationship among nine indigenous Chinese chicken populations by the Structure program. *Journal of Genetics* 88, 197 203.
- M.C. devicente IPGRI, 2003. genetic diversity analysis with molecular marker data learning module
- MacNeil MD, Grosz. MD, 2002. Genome-wide scans for QTL affecting carcass traits in Hereford x composite double backcross populations. *J. Anim Sci.* 80, 2316-2324.
- Macneil, M.D., M. A. Cronin, H.D. and Blackburn, L.J. Alexander. 2006. Genetic relationships among breeds of beef cattle in the United States that originated from the British isles, Iberian Peninsula, or west-central europe. *World Congress of Genetics Applied in Livestock Production* 32:1-4.
- Mallet., J, 2005. Hybridization as an invasion of the genome *Trends in Ecology & Evolution*, 20, 229-237.
- Mayr.E , 1963. *Animal Species and Evolution* Belknap, Cambridge, Massachusetts.
- Morgante M, Olivieri, A. 1993. PCR-amplified microsatellites as markers in plant genetics *the Plant Journal* 3, 175-182.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations *Proc. Natl. Acad. Sci. (USA)* 70:3321-3323.

- Nei, M. 1987. *Molecular evolutionary genetics* Columbia University Press, New York.
- Nei, M. and Roychodhury, A. 1988. *Human Polymorphic Genes: World Distribution* Oxford University Press.
- Newton, C., Graham, A. 1997. *PCR, Introduction to Scientific Techniques* BIOS Scientific Publisher Ltd. 2.
- Nicholls, J.A., and Austin, J.J., 2005. Phylogeography of an east Australian wet-forest bird, the satin bowerbird (*Ptilonorhynchus violaceus*) derived from mtDNA, and its relationship to morphology. *Molecular Ecology* 14, 1485-96.
- Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 55, 945–959.
- Queller D, Strassmann J, and Hughes, C. 1993. Microsatellites and Kinship Trends in Ecology and Evolution 8, 285-288.
- Rege, J.E.O., and Lipner, M.E., 1992. African animal genetic resources: Their characterisation, conservation and utilisation. Proceedings of the Research Planning Workshop, 19–21 February 1992, Addis Ababa, Ethiopia, International Livestock Centre for Africa, Addis Ababa, Ethiopia. 172 pp.
- Rieseberg LH, Whitton J, Gardner K (1999) Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics*, 152, 713–727.
- Ruane, J., 1999. A critical review of the value of genetic distance studies in conservation of animal genetic resources. *J. Anim. Breed. Genet.* 116, 317–323.
- Ruane, J., 1999. A critical review of the value of genetic distance studies in conservation of animal genetic resources. *J. Anim. Breed. Genet.* 116, 317–323.
- Saiki R, Gelfand, D. and Stoffel S, 1988. Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA polymerase *Science* 239, 487-491.
- Sanders, K.L., Malhotra, A., and Thorpe, R.S., 2004. Ecological diversification in a group of Indomalayan pitvipers (*Trimeresurus*): Convergence in taxonomically important traits has implications for species identification. *J. Evol. Biol.* 17, 721-731.
- Schluter ,D .2000. *The Ecology of Adaptive Radiation* Oxford University Press, Oxford, UK.
- Schluter ,D .2000. *The Ecology of Adaptive Radiation* Oxford University Press, Oxford, UK.
- Stinchcombe JR, Hoekstra HE, 2007. Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity*, 1–13.
- Thaller G, Kramer, W. winter, A. 2003. Effects of DGAT variants on milk production traits in German cattle breeds *J. Anim Sci.* 81, 1911-1918
- Troy C, MacHugh D, Bailey J. 2001. Genetic evidence for Near-Eastern origins
- Turner, TL, Hahn MW and Nuzhdin, SV. 2005. Genomic islands of speciation in *Anopheles gambiae*. *PLoS Biology*, 3, 1572–1578. USFWS 1996 Endangered and threatened wildlife and plants: proposed policy and proposed rule on the treatment of intercrosses and intercross progeny (the issue of ‘hybridization’): Request for public comment. *Federal Register*, 61, 4710–4713.
- Vega, FDL, Kreitman, M. 2000. Human Genome Variation: Analysis, Management and Application of SNP Data. *Pacific Symposium on Biocomputing* 5, 633-635.
- Vines TH, Köhler SC, and Thiel, M. 2003. The maintenance of reproductive isolation in a mosaic hybrid zone between the fire-bellied toads *Bombina bombina* B, *variegata* *Evolution*, 57, 1876–1888.
- Wollny, C.B.A., 2003. The need to conserve farm animal genetic resources in Africa: should policy makers be concerned? *Ecological Economics* 45, 341-351.
- Wright, S. 1943. Isolation by distance *genetics* 28, 139-56.
- Yatabe Y, Kane NC, Scotti-Saintagne C, Rieseberg LH , 2007. Rampant gene exchange across a strong reproductive barrier between the annual sunflowers *Helianthus annuus* and *H. petiolaris*. *Genetics*, 175, 1883–1893.