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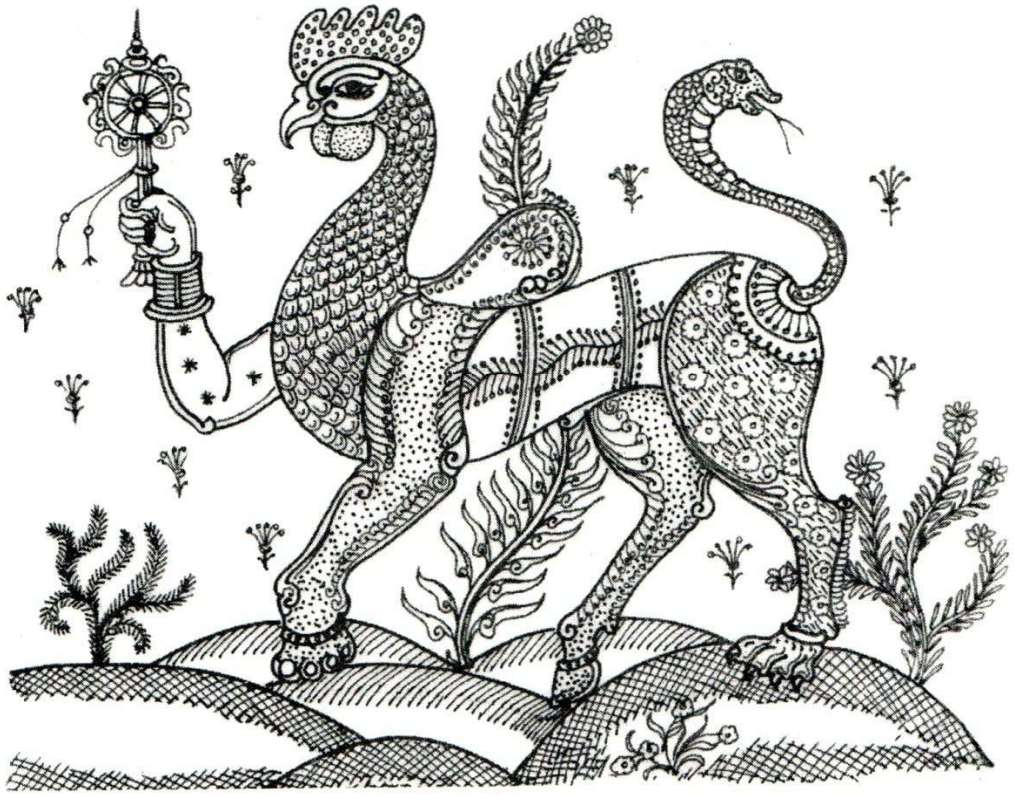

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The emblem of Pranikee

The emblem "***NABAGUNJARA***" is a chimeric animal and a common motif of Odishan art and literature. It literally means "Nine form". This form has been described by poet Sarala Das in the Odia version of the epic Mahabharata. Apparently, Lord Krishna appeared in Nabagunjara form consisting of the body of an elephant, a leg each of a horse, a deer and a tiger respectively; throat of a peacock, tail in the form of a serpent, waist of a lion, hump of a bull and head of a cock, to fool his friend Arjuna. The Chimera was holding a lotus flower in a human hand. Arjuna had never seen such a creature in his life and guessed that this could not be a real animal but a form assumed by Lord Krishna and immediately bowed down at his feet. It is said that the human hand with the lotus provided the clue. In the paintings and sculptures however, the lotus is often replaced by a "Chakra" or the "stylized discus" of Lord Krishna. Chimeric forms are encountered in literature and art all over the world. However, a chimera of nine animals is uniquely Odishan. Therefore, it was considered to be an appropriate emblem for the Journal of Zoological Society of Odisha.

Padma Shri Prof. Priyambada Mohanty-Hejmadi

Former Editor

From the Editor's desk

The study of Zoology often accommodates the rival mundane occurrences of the common place and the marvelous. Everything in nature seems to be natural; one feels no need to explain why a frog leaps or butterflies fly. It appears as if it is their nature to do so – they always have leaped and flown and they always will do so. But, in any case, these simple generalisations of Zoology could only be understood and established by the most extensive and intensive researches on living beings. Keeping this in view, the Zoological Society of Orissa has made a modest attempt to provide effective communication among Zoologists through the publication of the Journal "PRANIKEE", which features the research work carried out by zoologists of the state and the country.

The present edition of the journal (Volume XXIX) is ready for circulation. This volume contains one review article and six research papers. The review article describes human gene polymorphism and susceptibility to diseases. Research articles cover different aspects of zoological sciences including effects of chlorpyrifos on the erythrocytes of Asian toad, butterfly diversity in Nilgiri wildlife range, breeding activities of the common Asian toad, haematological parameters of skink, dactylographic analysis of identical twins and retinoic acid mediated changes in epithelial cells during tail regeneration in the tadpoles of the Indian tree frog.

PK Mahapatra

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HUMAN GENE POLYMORPHISM AND SUSCEPTIBILITY TO DISEASES

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ABSTRACT

Humans share genetic similarity with apes particularly chimpanzee since both diverged 5 million years ago. Gene sequences of humans differ from chimpanzee by 5 to 7%. Whereas prevalence of disease in wild chimpanzee is scant compared to human, incidence of diseases are highly observed in humans which cannot be related to dietary or environmental differences but can also be related to genetic changes that occurred during evolution of human. Identification of the causative factors that make human susceptible to different diseases is essential prerequisite for safe therapeutic intervention to save precious life. Therefore the present review attempts to understand the interaction of factors making humans susceptible to different diseases and option open to find out the cause of diseases. Relevant studies examining the association of human gene polymorphism, their association with diseases, disease resistance, their epigenetic mechanism, role of natural selection, ethnic association in causation of diseases were collected from PubMed, Science Direct. Review of articles reveals that disease susceptibility in humans is influenced by positive selection and in certain circumstances by balancing selection. Epigenetic factors and ethnicity of population play important role in making humans either more susceptible or resistant to particular diseases, depending on the environmental circumstances to which an individual is exposed. Ethnicity, natural selection, epigenetic factors play important roles in making an individual susceptible or resistant to a disease which can be answered through gene expression profile by genomics approaches.

Key words: Disease, epigenetics, gene, human, natural selection, polymorphism

INTRODUCTION

It is an established fact that genome of two individuals show more than 99.5% sequence similarities to each other. However we differ from each other by as much as 1 to 3% sequences. Sequence variations comprises single nucleotide and multi nucleotide variants (SNVs and MNVs respectively, together known as SNP), insertions and deletions (indels) of few nucleotides, structural variants consisting of larger copy number variations (CNVs), similarly sized copy neutral inversions and translocations. Depending on the locations of variable nucleotides in coding and non-coding sequences of transcript, genetic variants bring about differential expression of genes. SNV may result in synonymous and non-synonymous mutations in coding sequence of transcript. Indels produce either frameshift or non-frameshift mutations, CNVs affect transcript by deleting or inserting exonic sequences that may result in frameshift or non-frameshift mutations or splice variants depending on the length or position of the CNV relative to the exons.

These small fractions of genome variations and their interactions regulate gene expressions in such a way that can make each human unique for susceptibility to different diseases whether genetic or infectious. The host gene variants responsible for susceptibility to diseases are influenced by natural selection, host epigenetic factors and early life exposures during immune maturation of fetus. The present review attempts to explain the interaction of these factors and their necessity to understand the

mechanism that makes a person susceptible to diseases in view of recent advances in the field of disease biology (Harakshingh and Synder, 2013; Coleman, 2017; Lewis, 2015).

Gene polymorphism and disease susceptibility

It is generally found that nearly 50% of all deaths among people less than 65 years of age are due to either congenital factors (12%) or other non-communicable disorders (38%) such as liver and heart disease, osteoporosis, diabetes and asthma. This implies that at least 50% of all diseases that cause human mortality may have genetic causes. Genetic differences in the host response to infectious agents are important determinants of both susceptibility to and outcome of disease (Friedmann et al., 2014). Single nucleotide polymorphism (SNP) represents most widespread sequence variant in genome and it acts as genetic marker for revealing the evolutionary history and common genetic polymorphisms that explain the heritable risk for common diseases (Fareed and Afzal, 2013). Genome wide association assay reveals association of SNPs with susceptibility to different diseases (Table 1).

Table 1 List of human gene polymorphisms responsible for susceptibility to different diseases

Type of disease	Disease susceptible genes & gene polymorphism	Clinical outcome	Reference
Diabetes neuropathy	Rs2910164 (G>C) of miR146A, rs11888095 (C>T) of miR128A DD genotype of ACE, null allele of GSTT1 and GSTM1, CC genotype of Glyoxylase1 C936T gene polymorphism in VEGF; Rs1050450, C>T of Glutathione peroxidase	High Risk	Politi et al., 2016
Neuroblastoma	CASC 15/NBAT1(6p22), BARD1(2q35), 1MO1(11p15), LIN 28B(6q16)	Show high risk phenotypes	Tolbert et al., 2017
	DDX4 (5q11), IL31RA(5q11), HSD 17B12(11p11)	Show low risk phenotypes	
Oral Cancer	GSTM1(null allele), CCND1 (G870A), TNFα (-308 rs800629), XPD (codon 751),	High risk phenotypes	Shridhar et al., 2016
Fluorosis	COL1A, CTR, ESR, COMT, GSTP, PRL, VDR genes	Could increase or decrease risk	Pramanik and Saha, 2017

		phenotype	
Psoriasis	IL12B, IL23A, R381Q in IL23R, HLA Cw6allele of PSORS1 gene	High risk phenotypes	Mahil et al., 2015
Lung cancer	Snp rs12333226 of HLA-A, HLA-H	High risk phenotypes	Qin et al., 2017
Cardiovascular disease	CDKN2A, CDKN2b, SH2B3, ALDH2	Late onset of disease	Smith and Cheh, 2015
Familial Gastric cancer	CDH1, CTNNA1, MLH1, BRCA1, BRCA2	High risk phenotypes	Oliveria et al., 2015
Alzheimer's disease	CR1 rs3818361	Leads to early onset of mortality	Patel et al., 2014
Schizophrenia	rs7939644 AA of BBOX1 gene and 'c' allele of rs10767592	High risk phenotype	Lee et al., 2018
Fungal disease	Variant D of MBL2; GT1011A in intron 1 of MBL2; Variant 'B' of MBL2; homozygous mutant Asp472Asn, Asp299Gly of TLR4; Arg80Thr, Asn248Ser of TLR1, Ser249Pro of TLR6 ; IFNγ 874 T>T alone or with 1063 A>G of TLR4	High risk phenotype	Carvalho et al., 2010 and Pana et al., 2014
Malaria	Genetic loci 5q31-33, 6q21-p23, 10p15, 12q22,17p12	Control of falciparum parasite levels in blood	Marquet, 2017
Tuberculosis	IFNGR1, IFNGR2, IL12B, IL12B1, STAT1, ISG15, X-linked NEMO and CYBB genes; rs1524107CT of IL6, rs1544410CT of VDR, rs9373523GT of STXBP5)	High risk phenotype	Abel et al., 2017; Rong et al., 2017; Zheng et al., 2017
	Loss of function in P2X7 (SNP rs7958311 and rs1718119); rs1544410T of VDR gene in age <30 years old but rs9373523 of STXBP5 in females only	Reduced risk	
HCV	VDR variants such as rs7975232-C, rs2239185-T, rs11574129-T; IFNλ4 70P; rs12979860 C/C of IFNL3	Reduced risk	Wu et al., 2016 and Heim et al., 2016
	ΔG variant of rs368234815 of IFNL4;	High risk phenotype	Kenney et al., 2017

Influenza	Homozygosity of SNP rs12252-C of IFITM3;	High risk phenotype	Kenney et al., 2017
Respiratory syncytial virus infection	Gain of function variant (-590T) of IL4	High risk phenotype	Kenney et al., 2017
HIV infection	Homozygous 32bp deletion of CCR5 gene and KIR3DS1 of KIR	Low risk phenotype	Kenney et al., 2017
	Variants of TRIMα, IFITM3	High risk phenotype	Kenney et al., 2017
Human papilloma virus	Rs1800629-A polymorphism of TNF promoter; homozygosity of p53-p72R	High risk phenotype	Kenney et al., 2017
Herpes Simplex Virus (HSV-2)	SNP rs17244587-A of 3'UTR of TBX21	High risk phenotype	Kenney et al., 2017
HBV infection	A NTPC variant (S267F) in Asian population	Low risk phenotype	Kenney et al., 2017
Norovirus and rotavirus infection	Missense mutation in FUT2 (428G>A) and nonsense mutation in FUT2 (385 A>T)	High risk phenotype	Kenney et al., 2017
Epstein Barr virus infection	Missense mutation in FUT2 (428G>A) and nonsense mutation in FUT2 (385A>T)	High risk phenotype	Kenney et al., 2017
Cytomegalovirus infection	SNP rs2910164 of hsa-mir-146a SNP rs11614913 of hsa-mir-196a2 SNP rs3746444 of hsa-mir-499a	Fivefold High risk phenotypes	Mira et al., 2015
Helicobacter pylori infection	Blood group 'O' and 'A' more susceptible blood group 'B' and 'AB'	Differential susceptibility to malaria severity	De Mattos and de Mattos, 2017
Vibrio cholera infection	CC genotype of -4191 of CD14 promoter gene; Expression of functional α(1,2) fucosylated glycan make more susceptible to viral infection but resistant to bacterial infection	36% higher risk for developing infectious diarrhea	Flores and Okhuysen, 2009; Taylor et al., 2017
	Blood group 'O'	Less susceptible	Taylor et al., 2017
Meningococcal infection	Specific haplotype of CECAM Asp299Gly of TLR4; alleles of codon 52,54,57 of MBL gene; 4G/4G of plasminogen activator inhibitor (PAI)	Increased risk and mortality	Wright et al.,2009
	A SNP rs529948 of NFKBIE gene	Risk of	

Pneumococcal infection		pneumococcal infection	Sangil et al., 2018
	A SNP rs3138053 and rs2233406 of NFKBIA gene	Protection from pneumococcal infection	

Increased susceptibility to tuberculosis bacteria (TB) is attributed to germ line mutations in seven autosomal genes such as IFNGR1, IFNGR2, IL12B, IL12B1, STAT1, IRF8, ISG 15 along with two X-linked (NEMO & CYBB) genes (Abel et al., 2017; Rong et al., 2017; Zheng et al., 2017). But susceptibility to TB is less in those having SNP rs9373523 of STXBP5 gene association. There is increased risk for susceptibility to fungal diseases in those having variant D of MBL2 gene, GT1011A polymorphism of MBL2, Asp299Gly polymorphism of TLR4 (Pana et al., 2014; Carvalho et al., 2010). Individuals with specific VDR variants (rs7975232-C, rs2239185-T, and rs11574129-T) show decreased risk for HCV (hepatitis C virus) infection. About 30% of human genome has evolved due to virus-human interaction since divergence from chimpanzee. This has affected various genes responsible for encoding virus receptors, receptor modifying enzymes and a wide variety of innate and adaptive immunity proteins (Heim et al., 2016; Wu et al., 2016). In case of HIV infection resistance to infection arises in those with homozygous deletion of 32bp deletion of CCR5 gene but those having gene variants of TRIMα, IFITM3 gene show accelerated disease progression (Kenney et al., 2017).

Susceptibility to diseases is also associated with SNPs of host microRNA-tag SNPs such as rs2910164 (of has-miR146a), rs11614913 (of hsa-miR196a2), rs3746444 (of MYH7B gene and has-miR-499a and 499b) show 5 times increased risk for clinical manifestation of symptomatic human cytomegalovirus infection (Mishra et al., 2015). Polymorphism for blood group antigens particularly 'O' and 'A' blood group make individuals more prone to infection by *Helicobacter pylori* causing gastric problems compared to those with 'B' and 'AB' groups (de Mattos and de Mattods, 2017). Similarly resistance to malaria severity is associated with 'O' blood group (Arama et al., 2015). It is observed that glycans (polysaccharide) of mucosal surface act as pathogen receptor. Those individuals having functional FUT2 gene express α(1,2) fucosylated glycans on mucosal cells making them highly susceptible to viral infection such as HIV, influenza, norovirus whereas they are at reduced risk for susceptible to infection by bacteria like *Streptococcus, Pneumonia, Neisseria, Hemophilus* and *Salmonella enterica* etc.(Taylor et al., 2017; Lin et al., 2013). This is one of the important causes of differential susceptibility to enteric pathogens (Flores and Okhuysen, 2009). Host genetics may determine micro biome gut microbe composition and susceptibility to enteric diseases, since existing factors such as diet, environment, medication use explain only 10 to 20% of inter individual gut micro biome variation. This is brought about by polymorphism in innate immunity genes such as TLR, PRP, RIG-1 and CLEC etc. Three polymorphic CLECs (C-type lectin receptors) such as CLEC 4F- CD207 at 2p13.3, CLEC4A- FAM90A1 at 12p13 and CLEC16A at 16p13 in addition to SNP located in IL23R, IL20, CD5/CD6, CD86, CCL2/CCL7/CCL8 are strongly associated with microbiome composition (Kurilsnikov et al., 2017). Host genetic factors determine the susceptibility to *Plasmodium falciparum* malaria severity. It has been found that human chromosomal regions 5q31-q33, 6q21-p23, 10p15,

12q22, 17p12 play important role in genetic control of falciparum parasite levels in blood. In 5q31-q33 loci, IRF1 gene polymorphism (rs2706384) and interleukin3 gene polymorphism (rs40401) protect individuals from malaria severity but ARHGAP26 gene promotes disease progression. The 7p12 region is linked with asymptomatic parasitemia. TNFα gene polymorphism of 6q21-p23 is associated with mild malaria (Marquet, 2017). Different gene loci particularly 5q31-q33, 13q32-q34, 11q13, 16q12 make individuals differentially susceptible to helminthes infection (Quinnel, 2003). Similarly susceptibility to meningococcal infection, schizophrenia and Alzheimer disease are very often linked to host gene polymorphism (Wright et al., 2009; Lee et al., 2018, Patel et al., 2014).

Host gene polymorphism also determines person's susceptibility to many complex disorders like cancer, diabetes, neural disorders. Increased susceptibility to diabetic neuropathy is associated with polymorphism in ACE, MTHFR, GST, Glycosylase, VEGF genes (Politi et al, 2016). High risk phenotypes of neuroblastoma are associated with loci, CASC 15/NBAT-1(6p22), BARD1 (2q35) but low risk phenotypes are observed in case of association with loci DDx4 (5q11), 1L31RA (5q11), HSD 17B12 (11q11) (Tolbert et al., 2017). In majority of population high risk alleles for susceptibility to oral cancer are found to be SNP in GST M1 (null), CCND1 (G870A), MMp3 (-1171, promoter, 5A risk allele), TNFα (-308, rs800629 risk allele 'A') (Shridhar et al, 2016). Genetic variants in some candidate genes like COL1A, CTR, ESR, COMT, GSTP, PRL and VDR could increase or decrease the risk of fluorosis differentially among the exposed individuals living in endemic areas (Pramanik and Saha, 2017). Increased susceptibility to psoriasis is associated with polymorphism in IL12B, IL23A, R381Q, HLA Cw6 of PSORS1 (Mahil et al, 2015). SNP rs12333226 of HLA-A, HLA-H show strong association with lung cancer in young individuals (Qin et al., 2017). Host gene variants influence late onset of cardiovascular diseases (e.g. SNP rs2383207 of CDK N2B, rs10744777 of SH2B3 genes, SNPs of chromosome 7p21, 9p21, pq34) (Smith and Newton-Cheh, 2015), familial gastric cancer by mutation in CDH1, CTNNA1, MLH1, BRCA1, BRCA2 (Oliverian et al., 2015), autoimmune diseases (+49A/G of CTLA4 gene) (Fernandez-mestre et al., 2009). Genome wide association studies reveal that certain SNPs in telomere maintenance genes: TERT, TERC, TNKS, TEP1, CSNK2A2, ACD, TRF1 and TRF2 are associated with type2 diabetes mellitus susceptibility in Han Chinese, Caucasians and Indian Punjabi Sikhs due to telomere attrition (Sethi et al., 2016).

Host gene also determines the successful outcome of drug response to different diseases. Any failure to respond drugs also makes the person susceptible to disease which varies among different ethnic groups. Failure to respond to anti-tuberculosis drugs is influenced by the loss of function allele of genes CYP2B6 (rs3745274, rs28399499), CYP2A6 (rs28399433) (Abel et al., 2017) and polymorphism in VDR, SLC 11A1, HLA, SLCO1B1 genes (Ben-Kahla and Al-Hajoj., 2016). In case of hepatititis C virus infection rs12979860 C/C of IFNL3 gene of a person is more effective than T/T allele of this SNP in decreasing the viral load when anti HCV drugs are given (Schweitzer and Liang, 2013). The IFNL4 S70 variant (rs117648444) of IFNL4 gene is more protective against HCV infection in response to antiviral drugs (Kenney et al., 2017). Better clinical outcome in response to anti-psoriasis drugs are found to be associated with SNPs of ABCC2, ABCG2, TNF AIP3 genes (Mahil et al., 2015). In case of treatment of one type of cancer known as diffuse B-cell lymphoma by multidrug chemotherapy R-CHOP, failure to respond to this therapy in host is influenced by certain polymorphisms of genes encoding cytokines controlling cellular tumour growth (rs2231142 of ABCG2 gene, AA genotype of rs

1056892 of CBR gene, rs1800629A allele of TFF, rs1800871C and rs1800872C of IL10 gene) (Falduto et al., 2017). Failure to chemotherapy by breast cancer patient is associated with specific genotype at 1236 codon of exon1 of MDR1 gene (Alsaif et al, 2013). Similarly drug resistant epilepsy in an individual is influenced by SNP rs57095329 of miR-146a (Cui et al., 2015).

Gene polymorphism and disease susceptibility in ethnic population

Gene polymorphism among ethnic populations influences susceptibility to diseases. In West Africa, Fulani population have higher incidence of IL4-590T allele producing more IL-4 cytokines and higher incidence of 'O' blood group, increased lactase persistence, -286 C/T/A CRP polymorphism making these people more resistant to falciparum malaria severity than that of sympatric population of other ethnic groups of West Africa who are infected with malaria (Arama et al., 2015). African American men have high incidence of prostate cancer than Caucasian American and Asian patients. African-American has high expression of aberrant expression of oncoprotein ERG due to its fusion with transcription factor TMPRSS2 and high expression of SPINK1 than their counterparts (Ateeq et al., 2016). Sometimes it may happen that two ethnic populations susceptible to same disease have different gene polymorphisms. For instance non secretor phenotype makes an individual susceptible to gut infection and is caused by nonsense SNP in African and European descent but by missense mutation in Asian population (Flores and Okhuysen, 2009). There exist racial differences in susceptibility to breast cancer. The GM3 allele of IgG1 (14q32) makes the person susceptible to breast cancer in Brazilian white population but GM allele association with breast cancer is found to be absent in Japanese, Whites or black population (Pandey et al., 2012). It may be that a putative risk converting genes for breast cancer and GM alleles may be in linkage disequilibrium which might be different from other population therefore indicating ethnic differences.

Role of epigenetics in differential susceptibility to disease by ethnic population

Epigenetic mechanisms that include DNA methylation, histones post-translational modifications (HPTMs) and RNA-based mechanisms play important role in making an individual susceptible to a disease. It has been observed that there exist ethnic/racial differences in human DNA methylation pattern making differential susceptibility to cancer in different population. Variation in DNA methylation arises due to association of environmental factors including nutrition and exposure to environmental pollutants and social environment conditions. For instance DNA methylation is altered by the dietary availability of methyl groups. Folate in form of 5-methyl tetra hydrofolate (MTHF) is involved in remethylation of homocysteine (Hcy) to methionine, the precursor of S-adenosylmethionine (SAM) which is the primary methyl donor for majority of biological methylation reaction (Xia et al., 2014). The racial difference in cancer susceptibility and survival arise due to association with obesity, chronic inflammation and immune response besides host genetic polymorphism (Ozdemir and Dotto., 2017). It is found that epigenetic trans generational inheritance of disease make a person susceptible to a disease (Skinner, 2004). Epigenetics play a critical role in tumorigenesis which is evidenced by promoter hypermethylation and heterochromatinization in patients with cancer. For instance INK4 (cyclin dependent kinase inhibitor) and tumour suppressive microRNAs (miR-34a, 34b/c, miR-124, miR-137) are suppressed by DNA hypermethylation. It has been found that expression of epigenetic changes in pancreatic islets and beta cells significantly causes

diabetes risk in individuals if DNA methylation at CpG site located at 182 base pairs upstream of insulin promoter resides (H4 hyperacetylation and H3 dimethylation at lysine 4occurs) leading to insufficient insulin secretion from pancreas. Similarly, occurrence of Prader Willi syndrome and Angelman syndrome – an example of genome imprinting is controlled by hypermethylation of male parent and female parent specific gene loci (Shamsi et al., 2017; Schiattarella et al., 2018).

Role of early life exposures for immune maturation and disease susceptibility

Most of the immune cell types appear during the first trimester and then expand significantly until birth. Placenta that harbors its own microbiota and the environment inside the womb influence prenatal immune development and susceptibility of newborns to diseases. Development of fetal immune system is influenced by maternal exposures. These maternal factors are maternal malnutrition, maternal obesity, maternal stress, maternal smoking, maternal farm exposure (mother's exposure to farm animals during pregnancy) promote prenatal immune interaction of child and susceptibility to disease. Disease susceptibility of the child after birth is influenced by education of neonatal immune system. Labor and vaginal delivery intensifies neutrophil chemotaxis, expression level of TLR2 and TLR4 of monocytes and augments microbicidal capacity of newborn against immediate infection by bacteria. However, this protection is absent when a child is born by caesarian operation. Breast feeding in neonatal stage, constant exposure to environment microbes during neonatal period, consumption of farm milk influence diversity and composition of internal microbiota of child. This is also crucial for induction of T regulatory cells in lung and development of tolerance against aeroallergens later in life (Golwitzer and Marsland, 2015).

Role of natural selection in disease susceptibility

The inter-individual variability for susceptibility to diseases is very often selected by nature. There are many genetic markers of natural selection in human genome. Some of these are adaptation to high altitude in Tibetans than relative Han Chinese, high incidence of lactase persistence in Europeans, enrichment of potassium channel genes in Bangladesh population exposed to *Vibrio cholera*, protection of Andean high altitude population from cardiovascular defects but protection of Tibetan high altitude population from reduced polycythemia indicate that genome variation in human cannot escape the effect of natural selection (Fumagalli and Sironi, 2014; Crawford et al., 2017). Natural selection act simultaneously more than one level of biological organization in which every individual is a fundamental unit of this organization. This multilevel selection theory suggests that the vital rates of survival and reproduction for each individual are influenced by both genetic and stochastic processes. Life history of individuals are marked by two major phases of selection- pre-zygotic and post-zygotic selection. At pre-zygotic selection genetic factors that make variation in susceptibility to diseases are inbreeding, prenatal age related to lasting range (lasting range is defined as age range having higher potential to have children with superior evolutionary fitness), infertility due to aneuploidy, monogenic, polygenic sex linked inheritance, mitochondrial variants, genetic incompatibility and imprinting. On the other hand at post-zygotic selection genetic variants making individuals susceptible to diseases are determined by processes such as accumulation of mutations and epimutations during cell division of zygote, fetal wastages/ embryo wastages from conception to neonatal stages (incidence of neonatal wastages are found to be 30% each during preimplantation, post implantation and live birth, 10%

during miscarriage) where nearly 60%of embryos are lost before six weeks of gestation and an additional 10% are lost prior to 12 weeks of gestation. Besides individual selection such as death before reproductive age (e.g. Huntington disease, Marfan syndrome), kin ship (family selection) in presence of individual selection (e.g. influence of family's unique characteristics also affect fitness of individuals among families) (Tibayrene and Francisco, 2017), presence of liability trait (a trait observed in an individual having exceeded threshold limit) in a person, contextual selection (Where interaction between host gene variants and age, gender, cultural, social and physical environments of the person), positional segments of genomic segments bring about variability in susceptibility to various diseases. Sometimes balancing selection is found to be responsible for variable susceptibility to diseases by individuals after long term exposure to environment pressure. For instance a 32bp deletion of CCR5 gene is found at higher frequency in individuals resistant to HIV infection but these individuals are more susceptible to west Nile Virus infection. In malaria endemic areas of Africa, although high incidence of sickle cell gene, G6PD deficiency alleles are found to give protection against malaria severity, these people are susceptible to sickle cell mediated hemolytic anemia (Hedrick, 2017). If positive selection is stronger than a negative selection, positive selection can fix a linked deleterious mutations in presence of genetic recombination- a phenomenon known as genetic hitchhiking. This has caused association of susceptibility alleles for type 1 diabetes with protective alleles for Cohn's disease (Fay, 2013).

Susceptibility to infection may arise due to human pathogen coevolutions which are influenced by certain events such as high incidence of CCR5-Δ32 allele due to small pox epidemic in Europe, susceptibility to influenza virus due to rapid antigenic drift and agricultural practice adaptation to malaria by sickle cell gene (Muehlenbein, 2015). Similarly depending on the size and structure of host population pathogen driven natural selection has brought about great changes in human genome, evidenced by highly polymorphic MHC, HLA polymorphic innate immune genes causing each individual unique in susceptibility to a disease (Hedrick, 2017).

It is hypothesized that direct effect of population specific natural selection could be the reason for higher frequency of disease risk alleles, since human genome is shaped by adaptation to environmental pressure. This hypothesis is evidenced by existence of MHC (Major Histo Compatibility locus) heterozygote superiority against multiple pathogens. This may be the reason that contributes to evolution of HLA diversity and it can explain the persistence of alleles conferring susceptibility to disease (Ramos, 2017). Similarly predominant sources of divergence of disease gene expression between species may be due to natural selection led genetic variation that involve interaction among transcription factors and their interaction with DNA binding site leading to variable expression of genes and their susceptibility to diseases (Qidwai et al., 2016).

Modern approaches to find out the cause of diseases susceptibility

Identification of disease genes and analysis of differential gene expression making an individual either susceptible or resistant to a disease can help in increasing the survival chances of a patient and improving predictability of course of action of disease. The recent advent of next generation sequencing (NGS) entailing transcriptome profiling, epigenetic profiling, *de novo* sequencing along with the use of modern day bioinformatics algorithms have identified disease causing genes, their

expression profile, protein-protein interaction network along with signatures of natural selection in a population. To cite a few, use of these approaches by Genome wide association study (GWAS) have identified more than 300 immune related genes as putative targets of positive selection where, a total of 61 genomic regions with evidence of natural selection have been associated with rheumatic disease in human (Maji et al., 2016; Ramos, 2017). GWAS has revealed that some polymorphisms in brain derived neurotrophic factor (BDNF) gene including rs925946, rs10501087, rs6265 and rs988712 could be genetic risk factors for obesity (Akbarian et al., 2018). Similarly, whole genome sequencing has also identified signature of natural selection in form of evolution of varieties of *Drosophila* resistant to high altitude hypoxia environment condition (Lian et al., 2017). NGS has identified the mechanism of interaction of microorganism and platelets making differential susceptibility to thrombotic complications during bacterial and viral infection in human (Wen and Chen, 2018).

CONCLUSION

It seems that susceptibility to diseases by human gene polymorphism is influenced by interplay of environmental factors, mainly epigenetic forces, early exposure of life for mutation of immune cells, ethnic differences, natural selection besides host genes themselves. This implies that approach to treatment for a disease may not be universal but should be ethnic specific, environment specific, host specific. The recent arrival of personal genomics by use of next generation sequencing approaches, may predict the susceptible genes of a disease for each individual but clinical management of each disease only depends on finding out the factors, issues, influencing the expression of disease gene in an individual.

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MICROSCOPIC STUDIES ON THE EFFECTS OF CHLORPYRIFOS ON THE ERYTHROCYTES OF *DUTTAPHRYNUS MELANOSTICTUS* (SCHNEIDER, 1799) TADPOLES AND ITS PHYSIOLOGICAL SIGNIFICANCE

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ABSTRACT

Amphibians are important components of the ecosystem. Most of them spend a part of their life cycle in the aquatic environment. They were also considered as indicators of environmental change and contamination. In recent years, these are rapidly declining from the diversified habitats in the global scale. Insecticides could be one among the drivers to this observed amphibian decline since their use in agriculture has increased over the years which are then transported to water bodies through surface run off. Keeping this in view, the present investigation aims at understanding the adverse effects of commercial formulations of chlorpyrifos, a commonly used insecticide at sub-lethal concentration on some vital tissues of *Duttaphrynus melanostictus* tadpoles such as blood. The LC_{50} value for *Duttaphrynus melanostictus* tadpoles after 48 hours exposure to chlorpyrifos was found to be 3.691 ppm. The present investigation reveals morphological changes in the RBCs such as lobopodial projection, membrane internalization, vacuolated cell, ruptured erythrocytes and other abnormal shaped erythrocytes of *Duttaphrynus melanostictus* tadpoles after exposure to sub-lethal concentration of chlorpyrifos through light microscopy (LM) and scanning electron microscopy (SEM).

Key words: Chlorpyrifos, *Duttaphrynus melanostictus,* RBC, tadpoles.

INTRODUCTION

In many natural ecosystems, amphibians are one of the significant components as they form an energy efficient trophic link between invertebrates and vertebrates (Sparling et al., 2000). It may be mentioned that in the food web, adult anurans are important as preys and predators but their position changes during development at the tadpole stage as they feed on algae (Murphy et al., 2000). Some biologists also consider amphibians as indicators of environmental change (Blaustein, 1994; Blaustein and Wake 1995) both in the terrestrial and aquatic habitats due to their highly semi-permeable skin and complex life cycle (Alford and Richards, 1999). Among vertebrates, amphibians are the most threatened and rapidly declining from different habitats in the global scale due to the epidemics and global warming

(Pounds et al., 2006). Other potential stressors are competition with alien species, increased ultraviolet radiation, global warming, emerging infectious diseases, and habitat loss due to change in land use and pollution (Blaustein et al., 1994; Collins and Storfer, 2003; Becker et al., 2007; Sodhi et al., 2008). However, it is also believed that pesticides could be one among the driver to this observed amphibian decline due to the increased use of pesticides in the agricultural fields (Sparling et al., 2001; Hayes et al., 2006; Oromi et al., 2008; Mannet al., 2009). It is reported that pesticides have an adverse impact on amphibians as their highly permeable skin allows gas, water, and electrolyte exchange (Bruhlet al., 2013). Similarly, their eggs can also readily absorb chemicals from the environment as they have no shells (Blaustein and Bancroft, 2007).

Chlorpyrifos [*O, O*-diethyl *O*-(3, 5, 6-trichloropyridin-2-yl) phosphorothioate] is a broad spectrum organophosphate insecticide designed to be effective by direct contact, ingestion, and inhalation (Tomlin, 2009). Keeping this in view, the present investigation aims at understanding the effects of chlorpyrifos on some vital tissues such as blood in amphibians which acts as sensitive indicator of aquatic contamination (Cabagna et al., 2005; Raffel et al., 2006; Barni et al., 2007). Light and scanning electron microscopic analyses has been undertaken to access the same. A study was undertaken to investigate the morphological changes if any, in the RBCs of *Duttaphrynus melanostictus tadpoles,* as most of the time, pesticide application coincides with breeding and larval development of anurans.

MATERIALS AND METHODS

Eggs of *Duttaphrynus melanostictus* were collected from a stream in Mawpat, Shillong, Meghalaya (altitude of 500-1800 m ASL) during the breeding season (February-May). The eggs were maintained in plastic trays filled with pond water at room temperature (22°C) in the laboratory (Fig. 1) till they reach the tadpole stage 26-30 (Gosner, 1960) (Fig. 2). Commercially available chlorpyrifos (Tricel, chlorpyrifos, 20% EC, manufactured by Excel Crop Care limited, Gujarat, India) was purchased and diluted with acetone to prepare a stock solution of 1000 ppm. Stock solution was added to glass bowls (150 mm in diameter) containing 500 ml of dechlorinated water to make the sub-lethal concentration (0.1, 0.5 and 1) ppm, as it had been estimated that concentration of chlorpyrifos in small water bodies were in the range of 0.1 ppm to 1 ppm (Moore et al., 1998; Mazanti et al., 2003). The tadpoles (Gosner, stage 26-30) were divided into 4 groups (control and treated) with 10 tadpoles in each group in 3 replicates for 48 hours.

The tadpoles maintained in the laboratory from the control group and those treated with chlorpyrifos were anaesthetized with tricaine methane sulfonate-MS 222 and the blood of tadpoles were collected by tail amputation through the mid of the tail. A thin blood smear was prepared on a clean slide using push slide technique. The slides were air dried overnight in a dust and moisture free environment at room temperature and then fixed by dipping in absolute methanol for 10 min and again air dried for 1 hour. The slides were stained with Giemsa's stain for 20 min and washed in distilled water (Krishna and Hayashi, 2000). The slides were observed under light microscopy (Magnus MLX microscope) and photographed using Nikon camera with wide Optical Zoom.

Blood of tadpoles from control and treated groups was collected and standard procedure for scanning electron microscopy (Postek et al., 1980) was followed. Three drops of blood were fixed by putting in a tube containing 0.1 M 2.5% glutaraldehyde buffered with sodium cacodylate for 30 min. The sample was centrifuged for 5 min at 1,500 rpm, washed and resuspended in distilled water. The process was

repeated three times. The supernatant was decanted and a thin film was applied on a clean cover slip after resuspension in distilled water. The sample was then air dried and coated with gold in a JFC-1100 (JEOL, Tokyo, Japan) ion sputter and observed on JSM-6360 (JEOL) SEM at an accelerating voltage of 15-20 kV using the secondary electron emission mode. The average percentage of morphological change or deformed RBCs in the treated tadpole blood was calculated from the electron micrographs.

RESULTS

The determination of the lethal level (LC_{50}) of chlorpyrifos for tadpoles after 48 hours exposure was recorded to be 3.691 ppm. Light microscopic studies of blood of tadpoles from the control group showed different shapes of RBCs (Fig. 3a). Studies with scanning electron microscopy due to its high resolution further revealed the surface morphology of RBCs (Fig. 4a). Light microscopy and scanning electron microscopy studies (Fig. 3a, 4a) revealed normal shaped erythrocytes which are oval cell with eccentrically placed nucleus (A), rounded cells with centrally placed nucleus (Ro), oval cell with centrally placed nucleus (O), comma-shaped cell (C) and elliptical cell with centrally placed nucleus (E). Scanning electron micrograph of some normal erythrocytes at higher magnification are taken (Figs. 5a, 5b, 5c). The various normal shapes of erythrocytes in *Duttaphrynus melanostictus* tadpole blood were also recorded (Table 1).

Tadpoles treated with chlorpyrifos at sub-lethal concentration (0.1, 0.5 and 1) ppm, revealed some morphological changes in the erythrocytes. This indicates that this pesticide has an effect on the shapes of the RBCs. Some of these abnormal or deformed erythrocytes have been recorded in all the exposed concentration of chlorpyrifos such as lobopodial projections (L) having small foot-like projections from the membrane (Figs. 3d, 4b, 4c, 4d, 5f) while some showed membrane internalization (MI) due to internal infoldings of the membranes (Figs. 3d, 4b, 4c, 4d, 5g 5h). Most of the normal oval, round or elliptical shaped erythrocytes changed to become more rounded and less dense (Sp) in treated groups compared to the normal RBCs (Figs. 4b, 4c, 4d) which are similar to the sperocytes cells observed in the albino mice offspring blood born to females exposed to lead (Dey et al., 1999). Few erythrocytes covered with crenations or spicules (E) projected from the cell surface were observed at 0.1 ppm and 0.5 ppm (Figs. 4b, 4c). In addition to the above mentioned deformed erythrocytes, at 0.5 ppm and 1 ppm, it was observed that some of the membrane of the swollen erythrocytes ruptured (R) **(**Figs.3d, 4c, 4d). Similarly, few erythrocytes with prominent vacuoles or holes (V) located in the juxtaplasmalemmal position were observed (Figs. 3d, 4c, 4d, 5e, 5f) which is similar to the result of Gin et al., (1969) and Ben-Bassat et al., (1972) after treating human blood with a drug, primaquine. The size of the holes in these erythrocytes varies from 0.77 µm to 3.08 µm in diameter. Few cells were found to undergo reticulocytogenesis (Re) at 0.5 ppm characterized by the presence of a network of membranes and their sizes were also reduced (Fig. 4c) which is similar to the reticulocytes observed in albino mice exposed to lead (Dey et al., 1999). In the present study, tadpoles exposed to higher concentration of chlorpyrifos (1 ppm) showed that some of the erythrocytes become dumb-bell shape (D) compared to the normal elliptical shape RBCs (Figs. 3d, 5d) while few cells were found to be contracted (Co) (Fig. 4d) which is similar to the observation in the fish erythrocytes exposed to malathion (Sawhney and Johal, 2000). The presence (+), absence (-) and percentage of various deformed erythrocytes of tadpoles exposed to different concentration (0.1, 0.5 and 1) ppm of

chlorpyrifos were recorded (Table 1). This revealed that the percentage of deformities increased with the increase in the concentration of chlorpyrifos which shows that the deformity is dosage dependent.

Fig. 1 Eggs of Duttaphrynus melanostictus in the laboratory.

Fig. 2 Tadpole of Duttaphrynus melanostictus.

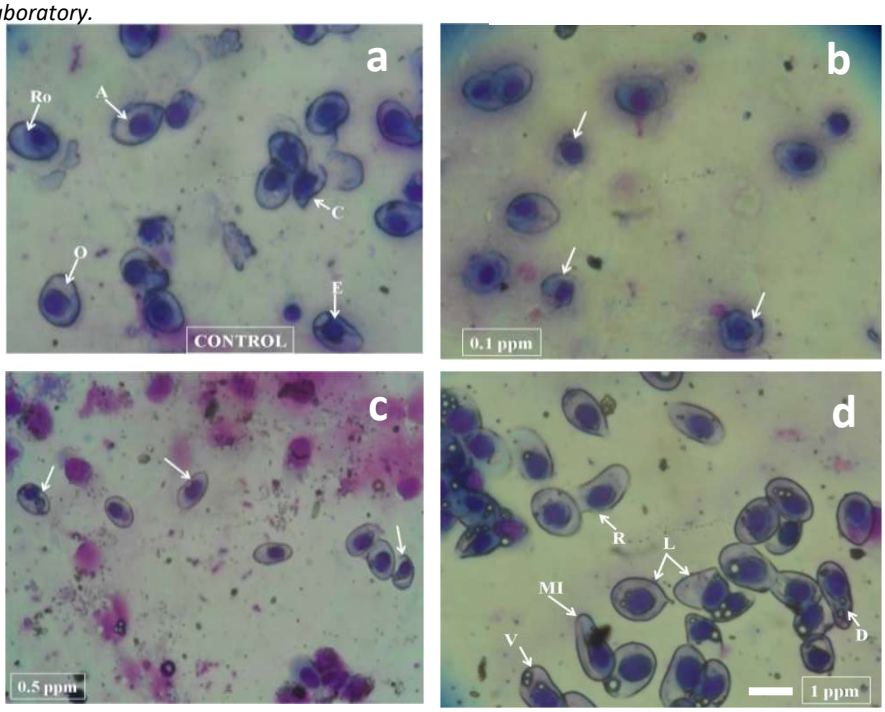

Fig. 3 Light micrograph of different erythrocytes found in the tadpoles of *D. melanostictus*. **(a)** Control group shows normal RBCs. **(b-d)** Treated groups show various deformed RBCs (arrow) in the chlorpyrifos at sub-lethal concentrations (0.1, 0.5 & 1 ppm). (scale bar = 10 µm)

[Oval cell with eccentrically placed nucleus (A), Comma-shaped cell (C), Dumb-bell shape cells (D), Elliptical cell with centrally placed nucleus (E), Lobopodial projection (L), Membrane internalization (MI), Oval cell with centrally placed nucleus (O), Ruptured erythrocytes (R), Rounded cells with centrally placed nuclei (Ro), Erythrocytes with vacuoles or holes (V)]

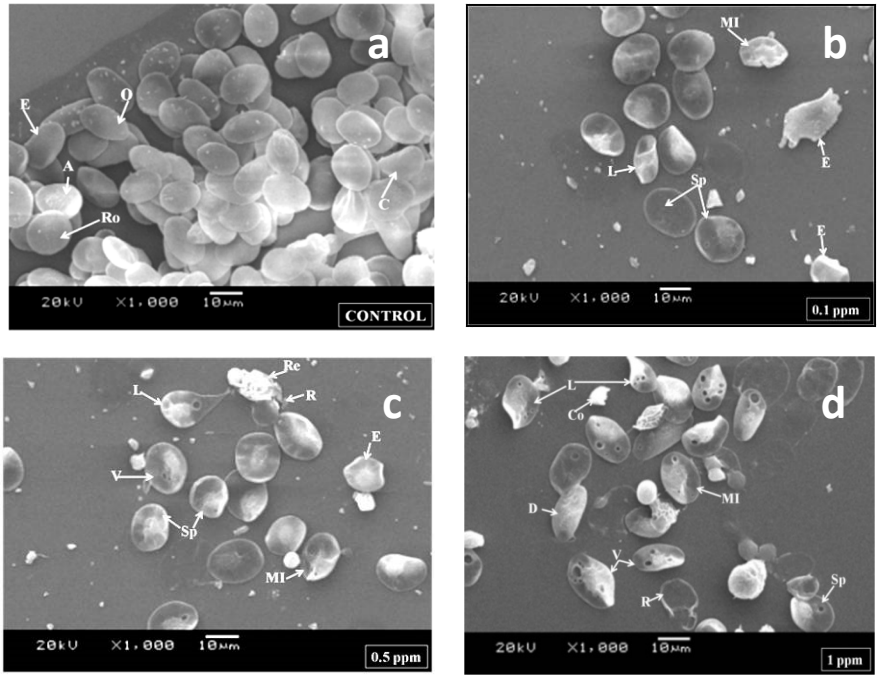

Fig. 4 Scanning electron micrograph of different erythrocytes in the tadpoles of *D. melanostictus* **(a)** Control group shows normal RBCs. **(b-d)** Treated groups show various deformed RBCs in the chlorpyrifos at sub-lethal concentrations (0.1, 0.5 & 1 ppm) (scale bar = 10 µm).

[Oval cell with eccentrically placed nucleus (A), Comma-shaped cell (C), Contracted erythrocytes (Co), Dumb-bell shape cells (D), Elliptical cell with centrally placed nucleus (E) Rounded cells with centrally placed nuclei (Ro), Erythrocytes with crenations or spicules projected from the membrane (Es), Lobopodial projection (L) Membrane internalization (MI) Oval cell with centrally placed nucleus (O) Ruptured erythrocytes (R), Reticulocytes (Re), Rounded and less dense erythrocytes (Sp), Erythrocytes with vacuoles or holes (V)]

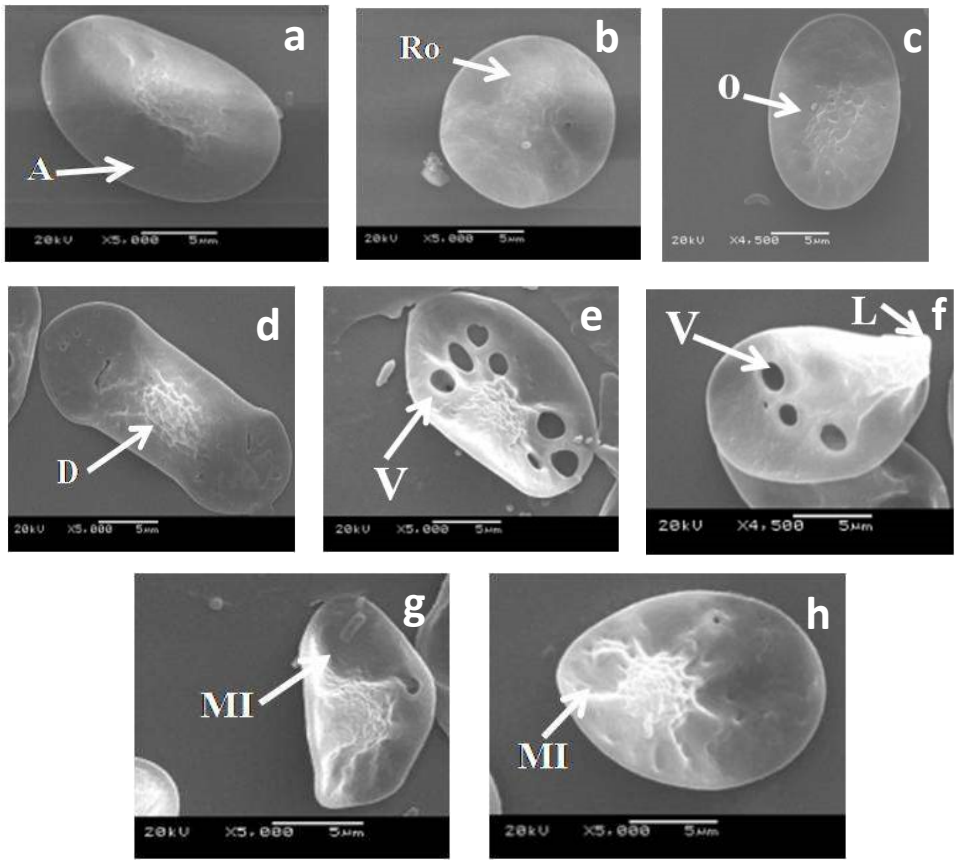

Fig. 5 Scanning electron micrograph of different erythrocytes in the tadpoles of *D. melanostictus***(a)** Oval cell with eccentrically placed nucleus (A) **(b)** Rounded cell with centrally placed nucleus (Ro) **(c)** Oval cell with centrally placed nucleus (O) (**d-h)** Some deformed shape erythrocytes treated with chlorpyrifos at sub-lethal concentrations (0.1, 0.5 & 1 ppm) at magnified view **(d)** Dumb-bell shape cells (D) **(e & f)** Erythrocytes with vacuoles or holes (V) (**f)** Erythrocytes with lobopodial projection (L) (**g & h)** Membrane internalization (MI) (scale bar = 5 µm).

[Oval cell with eccentrically placed nucleus (A), Comma-shaped cell (C), Dumb-bell shape cells (D), Rounded cells with centrally placed nuclei (Ro), Lobopodial projection (L), Membrane internalization (MI), Oval cell with centrally placed nucleus (O), Erythrocytes with vacuoles or holes (V)]

Table 1 The different shapes of RBCs in the blood of *D. melanostictus* tadpoles of the control group and tadpoles exposed to chlorpyrifos at sub-lethal concentration for 48 hours showing deformed RBCs. (+) indicates the presence and (–) indicates the absence of the deformed RBCs. All values are given Mean ± SE; N = 40

Abnormal shape RBCs in chlorpyrifos exposed tadpole at sub-lethal concentration (0.1, 0.5 and1) ppm						
Deformed shape RBCs	**0.1ppm**		**0.5ppm**		**1ppm**	
	(+)/(-)	**%**	**(+)/(-)**	**%**	**(+)/(-)**	**%**
Lobopodial projection (L)	+	4.99 ± 0.94	+	7.02 ± 1.48	+	6.69 ± 1.18
Membrane internalization (MI)	+	10.47 ± 0.87	+	9.77 ± 1.01	+	11.09 ± 0.95
Rounded and less dense erythrocytes (Sp)	+	13.55 ± 0.66	+	11.38 ± 1.33	+	10.38 ± 1.59
Erythrocytes with crenations or spicules projected from the membrane (E)	+	5.99 ± 1.15	+	8.65 ± 0.97	+	3.45 ± 1.01
Ruptured erythrocytes (R)	-	0	+	5.99 ± 1.15	+	5.99 ± 1.15
Erythrocytes with vacuoles or holes (V)	-	0	+	11.19± 1.44	+	22.41 ± 1.49
Reticulocytes (Re)	-	0	+	3.99 ± 1.15	+	1.33 ± 0.89
Dumb-bell shape cells (D)	-	0	-	0	+	11.19 ± 1.44
Contracted erythrocytes (Co)	-	0	-	0	+	3.78 ± 1.08
Total deformed RBCs		37.66 ± 0.59		57.99 ± 1.05		75.32 ± 1.78

DISCUSSION

The results of the present investigation clarify the general nature of blood cells in response to the presence of contamination in the aquatic environment due to chlorpyrifos. The erythrocytes of the tadpoles in the control group observed in the present study were similar to previous studies on other larval amphibians (Das and Mahapatra, 2012; Hota et al., 2013). The changes in the shape of erythrocytes were considered important information on the physiopathological state of the cell (Dey et

al., 1999). Some have reported that chlorpyrifos could induce alteration in the shape of blood cells in fishes (Naskar et al., 2006; Witeska et al., 2011) in which some of the morphological changes were similar in the anuran tadpole of the present investigation, i.e., echinocytes, spherocytes, ruptured cells, lobopodial projections, membrane internalization and contracted cells.

Some opine that abnormal shaped RBCs having cytoplasmic projections might lead to death of the tadpoles during metamorphosis which could be due to anemic conditions (Vankin et al., 1970; anonymous, 2012). These projections hamper the passage of blood cells through the blood vessels thereby affecting cellular functions (Moss and Hathway, 1964). An imbalance in the internal concentration of Ca^{2+}, Mg^{2+} and ATP could caused membrane internalization of the RBCs which have been elucidated in the present investigation on tadpole blood (Ben-Bassat et al.,1972) where Mg^{2+} and ATP could enhance deformities while Ca^{2+} caused membrane rigidity (Weed et al., 1969; Weed and LaCelle, 1969). It was assumed that membrane internalization could continue beyond the stage of reticulocytes with surface membrane lost, thus, erythrocytes become more spheroidal in shape (Ben-Bassat et al., 1972). Damaging of the cell membrane allows free entry of ions and water into the cell leading to swelling, thereby changing normal erythrocytes into spherocyte which then finally ruptures (Dourmashkin and Rosse, 1966). Moreover, change of erythrocytes to echinocytes in blood may be attributed to liver dysfunction caused by increase in the level of cholesterol (Remia et al., 2008). Gin et al. (1969) propose that the mechanism of vacuole formation involves membrane internalization. It is also suggested that lipid peroxidation increases due to toxic chemicals leading to increased porosity and fluidity (Sawhney and Johal, 2000). The erythrocyte membrane permeability to pollutants can alter their shape and size which eventually lead to its destruction (Suwalsky et al., 2004). Some studies also suggest that alterations in the shape of erythrocytes may be due to oxidative stress which effect blood flow, oxygen uptake, and release (Youdim et al., 2000). Dumb-bell shaped erythrocytes observed in the present study might be due to blood viscosity creating stresses that oppose blood flow (Fung, 1984). The shape and size of erythrocytes indicate the availability of surface for gas exchange during cellular respiration (Hartman and Lessler, 1964). It is to be noted that any morphological change affect exchange of gas leading to hypoxaemia, hypercapnia, blood acidosis etc. (Jindal and Batoye, 2015). It is also illustrated that deformities in shape of blood cells could be due to change in the lipid composition of the membrane in response to chemical treatments (Sherman, 1979).

Hence, in the present investigation, the deformities observed in the erythrocytes indirectly suggest that the various vital tissues may be affected by chlorpyrifos in the anuran tadpole. This investigation reveals that chlorpyrifos, an insecticide used in agricultural fields, reached the adjacent aquatic bodies through surface run off and contaminates the water bodies. Hence, this might have an undesirable impact on non-target organisms like anuran tadpoles and many other aquatic organisms.

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DIVERSITY OF BUTTERFLIES IN NILGIRI WILDLIFE RANGE, BALASORE, ODISHA

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ABSTRACT

Inventory of butterfly diversity of a habitat reveals the environmental pollution level as these are the indicators of nature. In the present study, 57 species of butterflies belonging to five different families are reported from Nilgiri Wildlife Range of Odisha which needs attention for conservation.

Key Words: Butterfly, Insecta, Lepidoptera, Nilgiri

INTRODUCTION

The butterflies constitute the second largest group under the order Lepidoptera and the class Insecta having colorful wing pattern. Further, butterflies are biological indicators of habitat quality as well as general health of the surrounding (Kocher and Williams, 2000; Swachik et al., 2005, Padhy et al., 2006). Many species are strictly seasonal and prefer to particular habitats (Kunte, 2000). Lepidoptera is regarded as one of the important components of biodiversity constituting approximately 1, 50,000 species (Scott and Williams, 2000). These insects also react quickly to any kind of disturbance and change in the habitat quality making a good indicator to study changes in the habitat and landscape structure variations. In this study, it is attempted to understand the distribution and variation in butterfly diversity in different habitats of the Nilgiri Wildlife Range, Balasore.

MATERIALS AND METHODS

The Nilgiri Wildlife Range is situated in 21°27'38.1276 N Latitude and 86°46'37.038 E Longitude of the Balasore district of Odisha. The entire division is diversified with various flora and fauna. This hill range is famous for various wild animals like sloath bears, deer, sambars, pythons, cobras, elephants and various birds. Habitat is congenial for the butterflies as this ecosystem provides a wide range of nectar secreting plants and host plants for the caterpillar.

The study site is mostly dominated by Indian jujube (*Ziziphus mauritiana*), Indian banyan (*Ficus benghalensis*), Peepal tree (*Ficus religiosa*), Tamerin (*Tamarindus indica*), Arjun (*Terminalia Arjuna*), Mango tree (*Mangifera indica*), Custard apple *(Annona reticulata*), False Ashoka (*Polyalthia*

longifolia), Guava (*Psidium guajava*), Mexicanoleander (*Thevetia peruviana*), Peacock flower (*Caesalpinia pulcherrima*), China rose (*Hibiscus* sp.) Banana (*Musa* sp.), Prickly poppy (*Argemone mexicana), Ixora Coccinea, Mimosa pudica*, etc.

The survey was carried out from January 2017 to October 2017 during morning from 8 AM to 10 AM and evening from 4 PM to 5 PM with direct visual evidence and also by netting using insect net which are not under the Wildlife (Protection) Act (1972). The photos were taken through Nikon Coolpix S7000 camera. Butterflies were identified by comparing the photographs with identification key by Kehimkar (2008).

RESULTS AND DISCUSSION

During the survey, 57 species of butterflies belonging to five families Papilionidae, Pieridae, Nymphalidae, Lycaenidae, and Hesperidae were collected. The family Nymphalidae topped the list (38%), then Pieridae (23%), Lycaenidae (14%), Papilionidae (23%), and Hesperidae (9%). The IUCN and WPA status of each family were recorded. From the survey, no IUCN threatened status species was identified. Some of the species, not coming under the IUCN status, are included under the Wildlife (Protection) Act, 1972 under different schedules. The maximum number of butterflies were recorded when the temperature was about 28±2°C. The flocks of butterflies were noticed during the morning and afternoon periods (Table 1).

Table 1 Butterflies collected from Nilgiri wildlife range

Common Name	Zoological Name	WPA Status	IUCN Status	Abundance
Family: Papilionidae				
Common Mime	*Papilio clytia*	**I**	**-**	**C**
Lime	*Papilio demoleus*	**-**	**-**	**C**
Common Mormon	*Papilio polytes*	**-**	**-**	**C**
Tailed Jay	*Graphium Agamemnon*	**-**	**-**	**R**
Common Jay	*Graphium doson*	**-**	**-**	**VR**
Crimson rose	*Pachliopta hector*	**I**	**-**	**C**
Common Rose	*Atrophneura aristolochiae*	**-**	**-**	**C**
Blue Mormon	*Papilio polymnestor*	**-**	**-**	**C**
Common Banded Pecock	*Papilio crino*	**-**	**I**	**C**

Family: Pieridae				
Common emigrant	*Catopsilia Pomona*	-	-	**VC**
Mottled emigrant	*Catopsilia pyranthe*	-	-	**VC**
Common albatross	*Appias albino*	-	-	**VC**
Stripped albatross	*Appias libythea*	**I**	-	**VR**
Yellow orange tip	*Ixias pyrene*	-	-	**R**
Common bush brown	*Mycalesis perseus*	-	-	**R**
Common grass yellow	*Eurema hecabe*	-	-	**VC**
Small grass yellow	*Eurema brigitta*	-	-	**VC**
Spotless grass yellow	*Eurema laeta*	-	-	**C**
Pioneer	*Belenois mesentina*	-	-	**R**
Psyche	*Leptosia nina*	-	-	**R**
Common jezebel	*Delias eucharis*	-	-	**C**
Common gull	*Cepora nerissa*	**II**	-	**VR**
Family: Nyphalidae				
Twany coster	*Acraea violae*	-	-	**VC**
Common Coster	*Ariadne merione*	-	-	**C**
Common sergent	*Athyma perius*	-	-	**R**
Indian fritillary	*Argynnis hyperbius*	-	-	**R**
Common crow	*Euploea core*	**IV**	-	**VC**

Plain tiger	*Danaus chrysippus*	-	-	**VC**
Stripped tiger	*Danaus genutia*	-	-	**VC**
Great eggfly	*Hypolimnas bolina*	-	-	**C**
Danaid eggfly	*Hypolimnas misippus*	**I**	-	**VR**
Common leopard	*Phalanta phalantha*	-	-	**VC**
Common three ring	*Ypthima asterope*	-	-	**VC**
Common four ring	*Ypthima huebneri*	-	-	**C**
Common sailer	*Neptis hylas*	-	-	**R**
Common castor	*Ariadne merione*	-	-	**R**
Angeled castor	*Ariadne ariadne*	-	-	**R**
Lemon pansy	*Junonia lemonias*	-	-	**C**
Chocolate pansy	*Junonia iphita*	-	-	**C**
Peacock pansy	*Junonia almana*	-	-	**C**
Yellow pansy	*Junonia hierta*	-	-	**VR**
Grey pansy	*Junonia atlites*	-	-	**VR**
Common Palmfly	*Elymnias hypermnestra*	-	-	**C**
Oakleaf	*Kallima inachus*	-	-	**R**
Family: Lycaenidae				
Common pierrot	*Castalius roimon*	**I**	-	**C**
Striped pierrot	*Tarucus nara*	-	-	**VC**

Zebra blue	*Leptotes plinius*	-	-	**C**
Pale grass blue	*Psuedozizeeria maha*	-	-	**C**
Lesser grass blue	*Zizina otis*	-	-	**VC**
Yam fly	*Loxura atymnus*	-	-	**VR**
Pea blue	*Lampides boeticus*	-	-	
Lime blue	*Chilades laius*	-	-	**VC**
Family: Hesperiidae				
Grass demon	*Udaspes folus*	-	-	**C**
Rice swift	*Borbo cinnara*	-	-	**C**
Common grass dart	*Taractrocera ceramas*	**IV**	-	**C**
Indian skipper	*Spialia galba*	-	-	**C**
Common red eye	*Matapa aria*	-	-	**R**

Abbreviation: I = Wild Life Protection Act (1972) **Schedule I** Species, II = Wild Life Protection Act **Schedule II** Species, IV = Wild Life Protection Act **Schedule IV** Species.
C = Common, VC = Very common, R = Rare, VR = Very Rare

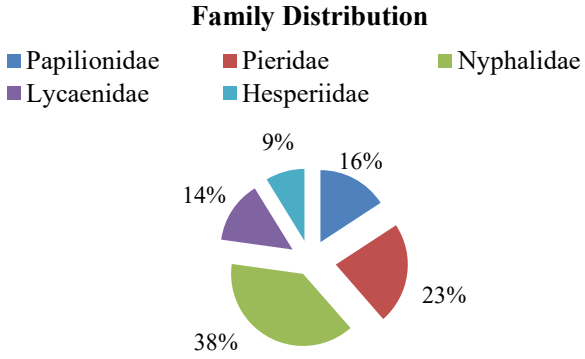

Fig.1 Distribution of butterfly families (in percentage) in the study area.

Butterflies belonging to family nyphalidae were found to be the maximum in number (Fig.1). This was followed by the butterflies belonging to the family pieridae where as family hesperiidae represented the minimum types of butterflies.

Fig. 2 Common rose

Fig. 3 Spottless grass yellow

Fig. 4 Common leopard

Fig. 5 Rounded pierrot

Fig. 6 Peacock pansy

CONCLUSION

The current study has shown diversed butterfly fauna. Diversity is proposed to be related to plenty availability of host plants, suitable roosting ground and water streams. This rich faunal and floral diversity is indicative of an environment not disturbed by anthropogenic pollutants.

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BREEDING ACTIVITIES OF ASIAN COMMON TOAD (*DUTTAPHRYNUS MELANOSTICTUS*) IN JAGATSINGHPUR DISTRICT, ODISHA, INDIA

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ABSTRACT

Amphibians require specific habitats for spawning. Loss or degradation of such habitats can hamper reproductive success. Selection of breeding sites is an important attribute for amphibians that breed in diverse habitats. Anuran breeding is inclined by environmental conditions. The breeding activities of *Duttaphrynus melanostictus* were observed under natural conditions at different study sites of Jagatsinghpur district of Odisha for a period of 3 consecutive rainy seasons (2015-2017). The microhabitat parameters (pH, water depth, air temperature, water temperature, relative humidity, canopy cover, organic matter, water colour, turbidity, and submerged vegetation) were analyzed using Pearson's correlation. Our observation suggests that oviposition sites of *D. melanostictus* tend to have slightly acidic pH, shallower water depth, and lower temperature implying that those microhabitat variables are important for the successful reproduction of this species.

Keywords: Amphibians, breeding sites, conservation, *Duttaphrynus melanostictus*, environmental parameters, microhabitat.

INTRODUCTION

The ecological success of organisms largely depends on their ability to adjust to their environmental conditions. Amphibians, being biphasic, are mostly susceptible to changes in their environment (Wells, 2007). Loss of habitat and degradation pose a major threat to biodiversity conservation and in recent years this has been attributed to be a major cause for global amphibian crisis (Wilson, 1992; Sodhiet al., 2008). Oviposition site selection within a habitat is also important as site quality is linked to larval survivability and metamorphic success. Knowledge on the ecology and life history characteristics of amphibians are needed in order to conserve these species but such information is currently limited. Studies on habitat usage of a species would provide valuable information to ensure successful conservation efforts.

An essential attribute of any surviving species or population is the ability to produce offspring (Duellman and Trueb, 1994; Sarah et al., 2009). In amphibians, successful reproduction depends on the availability and stimulation of potential mates (Wells, 2007; Mellovet al., 2010), and selection of breeding site (Kaeferet al., 2007). In nature, amphibian breeding activity is accelerated by several environmental factors such as rainfall (Lynch and Wilczynski, 2005), photoperiod (Saidapur, 1989), pool desiccation (Lind et al., 2008), food supply (Girish and Saidapur, 2000), and pond hydrology (Ryan and Winne, 2001; Hagman and Shine, 2006). Amphibian populations are exposed to constantly changing abiotic and biotic environment which plays a major role in influencing their persistence and

wellbeing (Licht, 1996; Alford and Richards, 1999). A detailed knowledge of a species' habitat relationships is essential for understanding that organism's fundamental strategies for persistence (Holt, 1987). The purpose of this study was to examine the breeding activities of Asian common toad, *Duttaphrynus melanostictus* under natural conditions in Jagatsinghpur district of Odisha.

MATERIAL AND METHODS

Tadpoles were collected from different study sites of Jagatsinghpur district of Odisha during three consecutive rainy season (2015-2017), using a variety of large and small nets, adjusted to the specific conditions of each water body. Aquatic habitats of the study area were searched visually (Heyer et al., 1994) to locate tadpoles during morning hours (6:00–10:00 AM). We examined different ecological variables such as air and water temperature, pH, canopy cover, depth of water, relative humidity, water colour, submerged vegetation at breeding as well as at non-breeding water bodies twice during each breeding season. Geographical coordinates were obtained using a Garmin™ 76 CSX GPS. The air temperature and water temperature were recorded with a mercury bulb thermometer and percent relative humidity by using a thermo-hygrometer. Using a waterproof digital pH tester (pH Tester 10, Ecotester, Eutech Instruments) pH was measured. We used a spherical densitometer to measure canopy cover and a ruler to measure water depth. Water colour and submerged vegetation were classified through visual inspection. Data on rainfall of the study sites were obtained from the Indian Meteorology Department, India.

Data for environmental parameters of breeding sites of *D. melanostictus* were presented as mean values and analyzed using descriptive analysis. We used Pearson correlation for describing the temporal variations of the observed environmental parameters. All analyses were carried out using the software IBM SPSS Statistics v. 20 on a window platform.

RESULTS

Out of the 60 water bodies surveyed, we found two hundred thirty six *D. melanostictus* egg clutches (Table 1). The clutches were at different stages of development with some being only one to two days old while others were in advanced stages of development along with newly hatched tadpoles. Breeding sites had significantly higher pH, shallower depth of water, lower water temperature than non-breeding habitats. We found *D. melanostictus* eggs on a variety of substrates including sand, silt, gravel and organic matter such as leaves (dead or alive), thin roots, rocks and submerged vegetation. The highest number of egg clutches was recorded in August, 2016 (40 clutches, high rainfall, *and* $p > 0.05$, Table 1).

The mean differences among various ecological variables at breeding (pH - 6.66 ± 0.42, water depth - 0.73 ± 0.52 ft, and water temperature - 30^0C ± 1.38); and at non-breeding sites (pH - 6.20 ± 0.62, depth - 2.54 ± 0.74 ft and temperature – 31.89^0C ± 1.41) were found to be statistically significant (pH $p< 0.05$;water depth $p < 0.001$ and water temperature $p< 0.05$, Table 2). Other variables like organic matter surrounding the clutches, water colour and turbidity of water as well as submerged vegetation

were not significant ($p > 0.05$) in breeding site selection. A summary of these values can be found in Table 2.

Table 1 Monthly rainfall, relative humidity and atmospheric temperature (range, mean ± SD) of the aquatic bodies studied in Jagatsinghpur district, Odisha for occurrence of *D. melanostictus* tadpoles

Month and year	Rainfall (mm)	Humidity (%)	Atmospheric temperature (°C)		No. of egg clutches
			Minimum	Maximum	
May 2015-17	0.1-138.5 88.36 ± 93.22	81.2-85.9 84.9 ± 2.91	24.4-29.8 26.17 ± 2.52	31.0-39.6 37.23 ± 2.75	1-4 2.04 ± 1.90
June 2015-17	0.1-43.4 107.56 ± 20.31	71.4-84.8 78.96 ± 3.68	20.6-29.3 25.73 ± 2.27	30.9-38.7 35.27 ± 2.30	14-16 15.6 ± 1.10
July 2015-17	0.4-50.9 326.8 ± 67.11	82.1-89.8 86.0 ± 1.77	22-28.6 24.82 ± 2.56	30.5-33.4 32.43 ± 1.00	20-35 33.8 ± 6.84
Aug 2015-17	0.4-84.5 358.95 ± 52.15	82.5-90.1 86.4 ± 1.82	21.6-28.8 24.25 ± 2.70	28.5-33.7 31.60 ± 1.53	26-40 32.9 ± 5.60
Sept 2015-17	0.3-79.4 268.25± 101.25	81.5-89.5 81.18 ± 1.80	20.8-28.02 24.75 ± 2.45	30.0-34.2 32.56 ± 1.38	8-14 12.8 ± 1.38

Table 2 Microhabitat features of *Duttaphrynus melanostictus* oviposition sites in Jagatsinghpur district (N = 60)

Water parameters	Range		Mean±SD		SE of Mean		Co-rrelation	*p* value
	Breeding	Non-Breeding	Breeding	Non-Breeding	Breeding	Non-Breeding		
pH*	5.6-7.9	4.8-7.2	6.66± 0.42	6.20 ± 0.62	0.07101	0.13067	0.45538	0.01668
Depth (ft)*	0.4-1.8	0.6-3.5	0.73 ± 0.52	2.54 ± 0.74	0.08367	0.13820	-0.52545	0.00056
Air temperature (^{0}C)	28.0-38.0	29.0-36.0	31.45 ± 1.60	31.58± 1.62	0.30302	0.27880	0.17521	0.31235
Water temperature (^{0}C)*	27.0-33.4	29.0-35.0	30.00±1.38	31.89 ±1.41	0.22723	0.28172	0.46123	0.01526
Relative humidity (%)*	77.0-88.5	50.0-87.9	78.25 ± 6.20	72.90 ± 7.23	1.14946	1.44238	0.18269	0.45760
Canopy cover (%)	0-80	0-90	31.65 ± 24.26	40.6 ± 24.15	4.97773	4.76950	-0.22152	0.22654
Organic matter	1-3	1-3	1.80 ± 0.71	1.65 ± 0.52	0.12309	0.13259	-0.28245	0.12129
Turbidity	1-3	1-3	1.79 ± 0.62	2.34 ± 0.75	0.15601	0.11173	0.06090	0.77863
Water colour	1-3	1-3	1.83 ± 0.88	1.98 ± 0.82	0.14526	0.14975	-0.01052	0.9056
Submerged vegetation	0-1	0-1	0.48 ± 0.23	0.45 ± 0.12	0.08304	0.09504	-0.17558	0.33540

N.B.: * significant at 5% level ($P< 0.05$)

DISCUSSION

The present study has shown that breeding activity of *D. melanostictus* is influenced by temperature (air and water), rainfall, relative humidity, pH, and depth of water. The environmental factors those responsible for breeding site selection of anuran were monitored in different breeding habitats such as permanent pond, ephemeral pond, canal, rice fields etc. with non-breeding habitats where no signs of tadpoles were found. It was observed that some ponds were not selected by the gravid females for egg laying though all the parameters recorded were more or less similar, an observation similar to that of *Hylaannectans* (Ao and Bordoloi, 2001). Tadpoles occur in countless aquatic habitats, feeding at many sites (benthic, mid-water, surface) throughout the water column and have characteristic morphologies and behaviour (McDiarmid and Altig, 1999). Present findings indicate that tadpoles breed both in the lotic as well as lentic ecosystems. A similar condition was also reported for *Bufo celebensis* which breed in streams in south-east Sulawesi (Gillespie et al., 2004) as well as pond habitats in northern Sulawesi (Leong and Chou, 2000). Bufonid tadpoles have features like thick black body, lack of well-developed tail for swimming and weak tail musculature to survive in shallow water habitats. From the present investigation, it is observed that Jagatsinghpur district provides a good habitat for breeding of *D. melanostictus*. During the non-breeding season, most pond-breeding amphibians live in the terrestrial habitat surrounding a pond. Adults migrate to the pond during favourable weather conditions to breed and oviposition usually occurs in the pond, and then adults return to their terrestrial habitat. After hatching, the aquatic larvae feed, grow, and develop until metamorphosis, after which they immigrate as juveniles to terrestrial habitats. The terrestrial habitat adjacent to the breeding site provides food and shelter throughout the prolonged breeding season. The breeding activity seems to be initiated by heavy rainfall, a factor always associated with the amphibian breeding activity (Aichinger, 1987). It was found that most of the species emerge and starts to breed from late May to September where the rainfall ranges from 117.35 mm to 373.81 mm (Table 1) during the study periods. Rain is the primary extrinsic factor affecting the onset of breeding activity for most tropical and subtropical anuran species (Duellman and Trueb, 1994).

During the breeding season from 2015 to 2017, the air temperature (Table 2) and water temperature (Table 2) of breeding sites were relatively low than non-breeding sites. As observed for anuran species, climatic factors, especially rainfall and temperature exert a strong influence on the reproductive activity (Silverin and Andrin, 1992) and conditions of stress can even inhibit ovarian development. Also environmental variables such as humidity, temperature and photoperiod may determine anuran breeding period (Navas and Bevier, 2001, Hatano et al., 2002). According to Beebee (1995), anuran breeding biology is influenced by climatic factors like temperature and rainfall or a combination of these, both for tropical and temperate species.

Amphibians prefer to oviposit in the areas where abiotic and biotic variables suit the continued existence and development of their eggs and larvae (Kats and Sih, 1992; Spieler and Linsenmair, 1997; Rudolf and Rodel, 2005). Environmental variables such as water depth (Goldberg et al., 2006; Pearl et al., 2007); water temperature (Doody, 1996; Goldberg et al., 2006; Snider and Janzen, 2010), and vegetation (Nyman, 1991; Pearl et al., 2007) influence the selection of breeding sites because of their

distinctive effects on survivality of offspring. A strong correlation was observed between specific microhabitat features and spatial distribution of *D. melanostictus* tadpoles. This relationship might suggest the selection of the breeding sites on the basis of important microhabitat variables of the offspring. Exclusively, it choose to oviposit in areas which are characterized by slightly acidic pH, lower water depth, lower temperature and moderate submerged vegetation that can act as a substrate for attachment of egg strings.

Knowing the present condition of environmental degradations, it is essential to understand the importance of habitat variables of anurans and other species that might be helpful in choosing the breeding habitats. Fundamental information like the present one is essential to predict the probable impact of the above on the population abundance and on the ecosystem.

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A STUDY ON HAEMATOLOGICAL PARAMETERS OF *MABUYA CARINATA* SCHNEIDER (1801) WITH REFERENCE TO SEX

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ABSTRACT

The objective of the present study is to find out the correlation between different haematological parameters of adult healthy skink, *Mabuya carinata*. This is useful in understanding the physiological features of skinks. The study was carried out to analyse the haematological parameters like Haemoglobin Concentration (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leucocyte Count (TLC), Total Platelet Count (TPC) and Differential Leucocyte Count (DLC) following standard procedures. The significant difference was determined. The study revealed percentage of eosinophils to be significantly different ($p<0.05$) between both sexes. The mean values of all other parameters were different between male and female individuals. The correlation coefficient also differed in male and female individuals with respect to the parameters analysed. Some parameters were positively correlated with each other while others were found to be negatively correlated. The baseline data obtained could be a useful indicator for monitoring and managing the general health status of the species.

KEYWORDS: Haematological parameters, *Mabuya carinata*.

INTRODUCTION

The lizard, *Mabuya carinata* is found in warm climatic conditions throughout the world. The body is dorsoventrally flattened and covered with small smooth shiny scales. These are mainly ground dwellers and also live on leaf litters. They are insectivorous (Daniel, 2002) in nature. It is known that haemocytological parameters are useful tools in the diagnosis and monitoring of animal health (Christophore et al., 1999). Moreover, the physiological conditions and clinical evaluation in reptiles can be detected by the combinations of different haematological parameters (Campbell and Ellis, 2007; Vasaruchapong et al., 2013). The external and internal factors also affect the haematology of non-mammalian vertebrates (Frye, 1991; Anderson, 1992). Haematolgy of some saurians are widely studied with reference to sexes and also impact of environmental factors on it (Hartman and Lessler, 1964;

Hutchinson and Szarski, 1965; Duguy, 1970). According to Cuadrado et al. (2002), Ponsen et al. (2008) and Troiano et al. (2008), the haemoglobin concentration, haematocrit value, mean cell volume, mean cell haemoglobin and mean cell haemoglobin concentration are studied in several reptiles. Haematological variation throughout life is variable in reptiles and highly dependent on age as well as sex (Parida et al., 2012; 2013). The blood cells of many lizards have been identified and different parameters have also been studied (Mateo et al., 1984; Canfield and Shea 1988; Cannon et al., 1996; Alleman et al., 1999; Sevnic et al., 2000; Sevnic and Uguratus, 2001).

Since literature available on haematology of *Mabuya carinata* is scanty (Parida et al., 2013), the purpose of this study was to determine the various haematological parameters of normal and apparently healthy skinks.

MATERIALS AND METHODS

Animals

Ten skinks of each sex were collected from the coastal area of Rajnagar block of Kendrapara district, Odisha, located in 20° 20' N to 20° 37' N latitude and 86° 14' E to 87° 01' E longitude. They were caught at day time from the crevices of trees and stones. Skinks were healthy and in good condition and transferred to the cages of animal house. The investigation of haematological profiles on *Mabuya carinata* Schneider (1801) was carried out from the year 2014 to 2017.

Collection of blood

The venipuncture site was prepared aseptically prior to blood collection. Blood was collected from the ventral tail vein of lizards by inserting an insulin syringe (BD Ultra – Fine ^{TM} Needle 12.7 mm × 30G) at an angle of 45-60° between the scales on ventral midline (Esra et al., 1975; Brown 2007). Once blood appeared in the needle hub, it was held steady and a gentle negative pressure was applied to the syringe. The blood was kept in an EDTA vial and then put in icebox for further analysis. The skinks were released to their natural habitat after collection of blood. Whole blood smear was obtained by push slide technique, air dried, fixed with methanol and stained with Giemsa as protocol cited by Lillie (1977).

Haematological analysis

The blood parameters were studied using the procedure (Frye, 1991; Campbell, 2004; Strik et al., 2007; Saggese, 2009).The concentration of haemoglobin was estimated by cyanomethemoglobin method and expressed in g/dl. Packed cell volume (PCV) was determined by microhaematocrit method with a spun of microhaematocrit tube at 2500 rpm for 15 minutes. The quantification of RBCs and WBCs was performed by manual methods using haemocytometer, with Hayem's diluting fluid for RBCs and Turk's diluting fluid for WBCs. Erythrocyte indices like MCV, MCH and MCHC were calculated using standard formulae (Samour, 2006). The percentage of different leucocytes as well as total platelet count was determined following standard procedure (Campbell et al., 2010; Thrall et al., 2012).

Statistical analysis

MS office Excel 2007 was used for statistical analyses

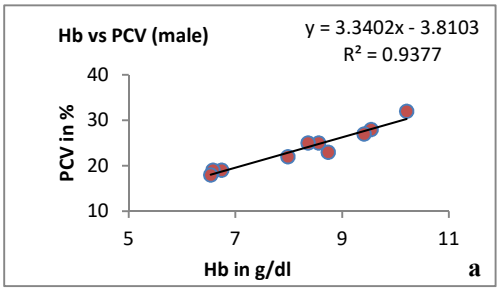
RESULTS

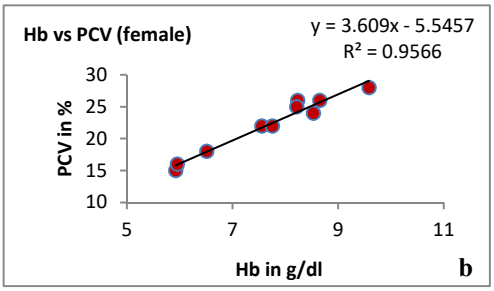
The haematological parameters are presented in Table 1. The percentage of eosinophils showed a significant difference ($p<0.05$) between sexes. The lymphocytes show the highest percentage in number followed by the heterophils. In case of male individuals, the heterophil percentage and eosinophil percentage were found to be higher. In females, the lymphocyte percentage and monocyte percentage were found to be higher. No basophils were observed in this study. The haemoglobin concentration, the haematocrit value, the total erythrocyte count, average volume of individual red blood cell, the average haemoglobin content of a single red blood cell and the total platelet count were higher in male individuals. The average percentage of saturation of an erythrocyte with haemoglobin and the total leucocyte count was the higher in female individuals of the species.

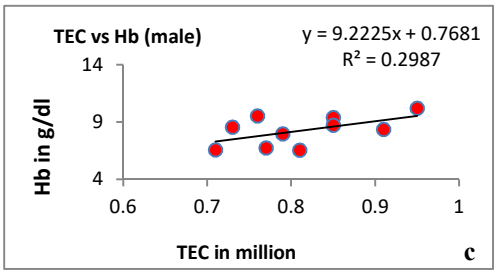
The correlation between different haematological parameters are presented with their regression line and R^2 value in Figs. 1.a-n. The correlation between haemoglobin concentration and packed cell volume, total erythrocyte count and Hb, Hb and MCV, and MCV and MCH was found to be positive both in males and females (Figs.1. a-g, k, l). The MCV and TEC, MCH and TEC, and MCV and MCHC were negatively correlated in males as well as females (Figs.1.h, i, j, m, n).

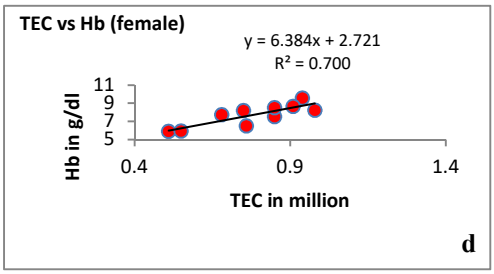
DISCUSSION

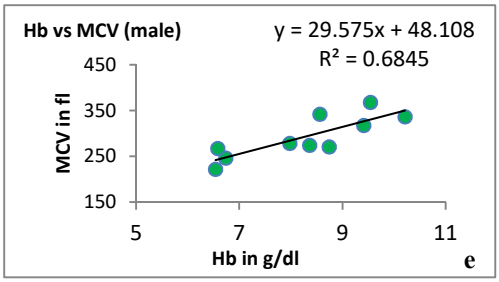
Haematological data are essential to correlate the health status of reptiles with their habitat. Haematology of reptiles provides a way to diagnose the animals (Campbell, 1996). No difference in total haemoglobin concentration in male and female lizards have been reported by Engbreston and Hutchinson (1976). However, the result of our investigation shows a difference in haemoglobin concentration. The difference in the value may be due to some seasonal factors in combination with age and sex of the individuals. According to Hidalgo-Vila et al. (2007), the PCV and TEC are higher in males of free living Mediterranean pond turtle and TEC in other reptiles. Our data regarding PCV and TEC corroborate with this. The PCV and TEC found in this study are lower than the report by Wright (1993) for prehensile tailed skink. The TLC was higher in males and MCV was higher in females in Mediterranean Pond Turtle (Hidalgo-Vila et al., 2007). But in case of *Mabuya carinata,* TLC and MCV were found to be higher in females only.

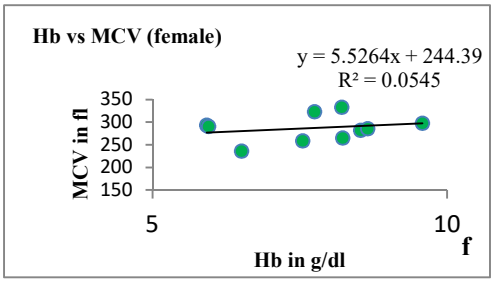

Hb vs PCV (male)
y = 3.3402x - 3.8103
R² = 0.9377
PCV in %
Hb in g/dl
a

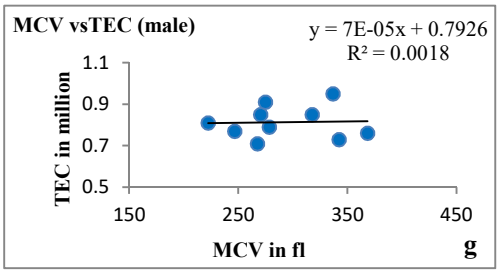

Hb vs PCV (female)
y = 3.609x - 5.5457
R² = 0.9566
PCV in %
Hb in g/dl
b

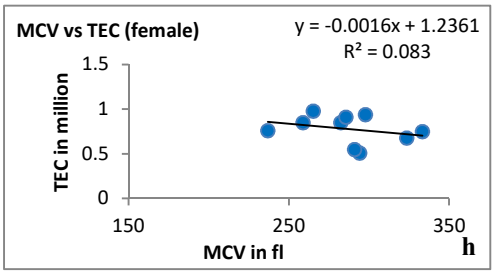

TEC vs Hb (male)
y = 9.2225x + 0.7681
R² = 0.2987
Hb in g/dl
TEC in million
c


TEC vs Hb (female)
y = 6.384x + 2.721
R² = 0.700
Hb in g/dl
TEC in million
d


Hb vs MCV (male)
y = 29.575x + 48.108
R² = 0.6845
MCV in fl
Hb in g/dl
e


Hb vs MCV (female)
y = 5.5264x + 244.39
R² = 0.0545
MCV in fl
Hb in g/dl
f


MCV vsTEC (male)
y = 7E-05x + 0.7926
R² = 0.0018
TEC in million
MCV in fl
g


MCV vs TEC (female)
y = -0.0016x + 1.2361
R² = 0.083
TEC in million
MCV in fl
h

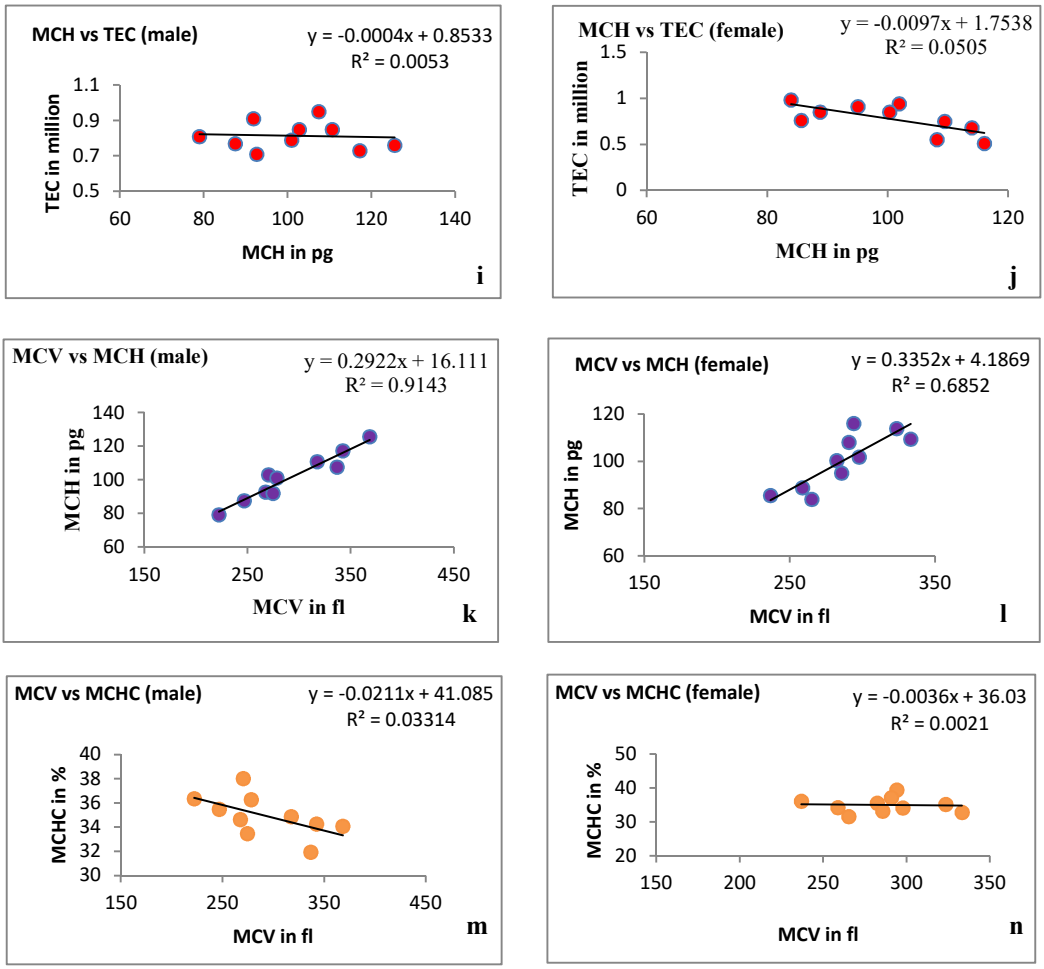


Fig. 1 Correlation between different haematological parameters (a-n) of *Mabuya carinata*.

Table 1 Haematological parameters of *Mabuya carinata* Schneider (1801)

PARAMETERS	UNIT	MALE			FEMALE		
		RANGE	MEAN	SE	RANGE	MEAN	SE
Haemoglobin	g/dl	6.54 – 10.21	8.27	0.41	5.92 – 9.58	7.68	0.38
PCV	%	0.71 – 0.95	23.8	1.42	0.51 – 0.98	22.2	1.42
TEC	$\times 10^6 m\ m^{-3}$	18 - 32	0.81	0.24	15 - 28	0.78	0.15
MCV	fl	222.22 – 368.42	292.57	14.72	236.84 – 333.33	286.87	9.11
MCH	pg	79.01 – 125.55	101.59	4.49	83.97 – 116.67	100.34	3.68
MCHC	g %	31.91 - 38	34.92	0.53	31.65 – 39.46	34.98	0.71
TLC	$10^3 mm^{-3}$	12600 - 18500	15430	625.39	12300 – 18400	16010	649.17
TPC	$\times 10^3 m\ m^{-3}$	20.58 – 49.5	36.79	2.89	16.5 – 42.35	29.85	2.85
Heterophils	%	30 - 40	36.8	1.11	30 - 45	36.6	1.46
Lymphocytes	%	50 - 65	52.2	1.85	50 - 63	57.4	1.37
Eosinophils*	%	1 - 9	5.4	0.76	1 - 6	3.3	0.59
Monocytes	%	1 - 5	2.6	0.49	1 - 6	2.7	0.49

*significantly different, SE – standard error

There was difference in mean values of haematological parameters between sexes of the studied gecko which is also seen in some Agamidae (Mateo et al., 1984) geckos, *Hemidactylus frenatus* (Olayemi, 2011). Our findings of TEC and TLC show closeness with the finding of some Agamidae lizards (de Pienaar, 1962; Efrati et al., 1970; Pal et al., 2008).The result obtained in this study regarding the haematological parameters also fall within the range as studied in iguana (Wagner and Wetzel, 1999). Haematological parameters in reptiles vary with age, sex and seasons (Campbell, 1996; Wilkinson, 2003). The TEC, in both males and females is lower in comparison to *Psammodromus algirus* (Parida et al., 2012). The MCV, MCH and MCHC in both males and females *Naja naja* were found to be the higher (Parida et al., 2014) in comparison to *Mabuya carinata*. The heterophil count, the lymphocyte

count is higher in both males and females in this study in comparison to *Psammophilus blanfordans* and eosinophil and monocyte percentage falls within the range (Ponsen et al., 2008). The monocyte, eosinophil and lymphocyte percentage is higher and heterophil percentage is lower in *Trapelus lessonae* (Gul and Tosunoglu, 2011). Generally the heterophils and lymphocytes are higher followed by eosinophils, monocytes and basophils which are rare in occurrence (Saint Girons, 1970; Campbell, 1996; Mader, 2000).

In *Mabuya carinata*, the PCV shows a positive correlation with increase in haemoglobin concentration in both males and females. This indicates that TEC is also directly proportional to concentration of haemoglobin. With increase in TEC, the PCV and concentration of haemoglobin, both increased in different sexes and were correlated positively. With the increase in MCV, the MCH increases in males and females. MCV increases with increase in concentration of haemoglobin in both the sexes. TEC shows a negative correlation with MCV and MCH in case of both males and females except in case of MCV in males. The MCV is also negatively correlated with MCHC in both sexes. The relation between all the haematological parameters may be due to the influences of different external factors like seasonal variations, diurnal variations, temperature and intrinsic factors including age, sex and body physiology (Nayak and Mohanty, 2018; Acharya and Mohanty, 2018)

CONCLUSION

Haematology is highly dependent on body physiology of lizards with respect to their surrounding. However, it provides the fact that the skinks showed different haemoprofile with respect to their sex. The present study provides a base line reference value for the haematological parameters of *Mabuya carinata*. This data may be useful for further study relating to impact of climate, environmental conditions, use of microhabitat and seasonal fluctuations on haematology of skinks.

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DACTYLOGRAPHIC ANALYSIS OF SOME IDENTICAL TWINS OF BHUBANESWAR, ODISHA

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ABSTRACT

The fingerprints of 15 pairs of identical twins belonging to Bhubaneswar city were analysed. A total of 300 fingerprints were taken, in which prints of 10 fingers of both right and left hand were included. Utmost care was taken to ensure best fingerprints at primary level which are divided into categories (ulnar loop, radial loop, plain whorl, double whorl, central pocket loop, plain arch and tented arch) on the basis of visual inspection. Our study revealed 100% fingerprint matching in four cases, 90% matching in one case, 80% matching in three cases, 70% matching in four cases and 60% matching in rest three identical twins cases.

Keywords: Fingerprinting, Identical twins, Non-identical twins.

INTRODUCTION

The study of twins has been important in various physiological and behavioral settings. There are mainly two types of twins, monozygotic and dizygotic twins. Dizygotic twins result from two different fertilized eggs. Monozygotic twins, also called identical twins, are the result of a single fertilized egg splitting into two individual cells and developing into two individuals. Thus, identical twins have similar DNA. The frequency of identical twin birth is about 0.4% across different population all over the world (Nora et al*.,* 1994). DNA contains all the genetic information required to generate an organ of a species but genetic information is not the only factor affecting the organ. It can be influenced by various other factors. As a result, identical twins who share the same genetic expression have many different biometrics including fingerprint, iris and retina (Fernando et al., 2009; Liu and Srihari, 2009). Because of the lack of sufficient data, few studies on twins have been carried out in forensics and biometrics (Sun et al*.,* 2008). This paper demonstrates that, fingerprints of identical twins are similar to a great extent but can be differentiated.

Formation of fingerprints

Fingerprints are fully formed at about seven months of fetal development and finger ridge configurations do not change throughout the life (Galton, 1892). Fingerprint formation is similar to angiogenesis and genes determine the general characteristics of the pattern. The general characteristics of the fingerprint emerge as the skin on the fingertip begins to differentiate. The flow of amniotic fluid around the foetus and its position in the uterus changes during the process of differentiation. Thus, the

cells on the fingertips grow in a microenvironment that differs from hand to hand and finger to finger. The finer details of the fingerprints are determined by this changing microenvironment (Adamu and Taura, 2017). A small difference in micro-environment is amplified by the differentiation process of the cells. There are so many variations during the formation of fingerprints that it would be virtually impossible for two prints to be alike.

Characteristics of fingerprints

A fingerprint is a smoothly flowing pattern of alternating valleys and ridges. Ridges are the impressions left on a piece of paper when printed with the traditional ink pad method. Valleys, on the other hand, are the white spaces that separate consecutive ridges (Anonymous, 2017). A set of distinctive features render a fingerprint unique. These features are unique to every finger and to every person. Fingerprint representations are generally classified into two categories: global representations and local representations (Jinwei et al., 2006). A global representation is an overall attribute of the finger such as the presence of ridges and valleys. Global representations can contain a wide range of features available on fingerprints such as ridge orientations, ridge thickness, ridge separation or locations of singular points, namely core and delta points (Dass, 2013) (Fig. 1). Core and delta points are available on every fingerprint and are used for classification of the overall fingerprint pattern (Dagher et al., 2009). A local representation mainly consists of minutiae and pore based matching of fingerprints. Typically, global representations are used for fingerprint indexing and local representations are used for fingerprint matching (Dagher et al., 2009).

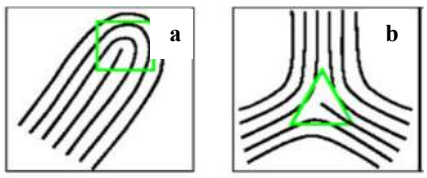

Fig. 1 Singular points in a fingerprint (a) core (b) delta.

Fig. 2 Basic patterns of fingerprints (a) whorl (b) arch (c) loop.

A loop is that type of fingerprint pattern in which the ridges enter on one side of the impression; re-curve (loop around), touch or pass through an imaginary line drawn from the delta to the core and terminate on the same side of the impression from which ridges are entered. Loops occur in about 60-70 per cent of fingerprint patterns in human population. A loop pattern of fingerprint has only one delta. There are two types of loop patterns, i.e., radial loop and ulnar loop. Radial loops flow in the direction of the thumb whereas ulnar loops, flow in the direction of the little finger (Hawthorne, 2009). Whorls form circular or spiral patterns, like tiny whirlpools. Whorls make up about 35 per cent of pattern types. A whorl pattern has two deltas. There are four types of whorl patterns such as plain whorl, central pocket loop, double loop whorl and accidental whorl.

A plain whorl possesses two deltas and at least one ridge making a complete circuit, which may be spiral, oval, circular, or any variant of a circle. Central pocket loop whorl combines the features of both

loops and whorls where the pattern looks like a loop but has a small whorl inside the loop ridges. It has two deltas, one at the edge of the pattern area, and the other the inside the pattern area just below the centermost. Double loop whorl is a pattern that consists of two separate loop formation ridges (inner delta) with two separate and distinct sets of shoulders and two deltas. An accidental whorl consists of a combination of two different types of patterns (with the exception of the plain arch) with two or more deltas or patterns (Hawthorne, 2009).

Arches formed when the ridges enter on one side of the impression and flow or tend to flow out the other with a rise or wave in the center. Arches make up about 5 per cent of all pattern types. Arches are of two types such as plain arch and tented arch. The plain arch has no delta and no real core as in a loop. In case of tented arch, most of the ridges enter on one side of the impression and flow out towards the other side of the finger (Fig. 3). Rastogi and Pillai (2010) have recorded fingerprints in relation to gender and blood group.

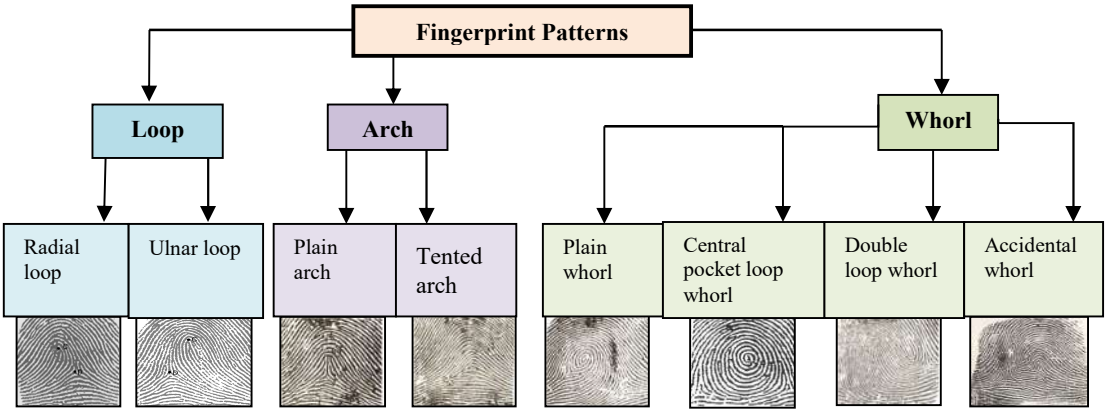

Fig. 3 Different types of fingerprints.

MATERIALS AND METHODS

Fingerprints can be analyzed at three levels. In course level (level 1), a pattern class similarity depends upon categorization of the overall fingerprint pattern into a small number of classes (Jain et al., 2002). Final level (level 2) ridge count feature measures the number of ridges between two salient points (core and delta). If the relative configuration (e.g., placement and orientation) of ridge anomalies (endings and bifurcations) of two fingers is similar, then their minutiae - based similarity is high (Jain et al., 2002). Advanced level (Level 3) analysis includes dimensional, edge shape and pore details within a specific ridge. Sweat pores are regularly spaced along the ridges and whose specific locations and shape can be used as distinctive features for identification (Sun et al., 2008).

For this investigation, the fingerprints of identical twins were collected, after their informed consent. The fingerprint samples of identical twin pairs have been collected in and around Bhubaneswar city. The collection of samples were undertaken from January 2016 to June 2017. A total of 22 twin pairs were investigated during the survey, out of which four were of non-identical twins and rest were

identical twins. Non-identical prints were not considered. Out of remaining 18 samples, 15 were selected for the level 1 analysis. Rest 03 were rejected due to poor quality of fingerprint as their fingerprints were not fully developed (Children of age group 2-3 years). A total of 300 simple prints were taken by the help of inkless fingerprint pad (Fingerprint Inkless Pad Round 1.5 inch, Code: JKC 230014, J K Consultancy Forensic Products, New Delhi), which included prints of 10 fingers of both right and left hand. The samples were then arranged on fingerprint slips. They were studied for level 1 analysis which was performed manually by visual inspection under the guidance of the fingerprint expert of State Crime Records Bureau Bhubaneswar, Odisha. First, the fingerprint samples were analysed to know the pattern by the help of hand lens and Linen ridge counter. The delta and core were located by using pointers. After this, the patterns of 10 fingers of both right and left hand of one member of the twin pair were compared with the other member of the same twin pair (Thai and Tam, 2010). Age, gender and occupation of parents of identical twins studied were recorded. The difference in time of birth between the identical twins was also documented.

RESULTS

The fingerprints were analysed based on percentage of matching at level 1 and pattern of fingerprint which occurred maximum times in the identical twin pairs. From the study, it was found that the percentage of matching of fingerprint patterns of identical twins is very high. Very few fingers of identical twins showed noticeable differences. In case of four twin pairs, the patterns of 10 fingers of one hand of one member of the twin pair matched 100% with the other member of the same twin pair (out of 15 twin pairs). The fingerprint pattern matched 90%, in the case of one twin pair. There was 80% matching of patterns found in case of three twin pairs. There was 70% and 60% matching in four and three twin pairs, respectively (Fig.4). The percentage of level 1 matching of fingerprint patterns of identical twins was found to be 79.33%. Thus, the similarity of patterns at level 1 is 79.33%.

The fingerprint patterns of Group 3A and 3B (3A and 3B refers to the individuals of twin pair group 3) was the rarest of the rare type. The pattern of left thumb of 3A was double loop. The pattern of left thumb of 3B was ulnar loop. The pattern of left index of 3B was radial loop, which was a rare occurrence. When ulnar loop and radial loop patterns 3B come together they form double loop 3A, similar to the above case. The different fingerprint pattern types which had occurred in the identical twin population were as follows. Ulnar loop, radial loop, plain whorl, double whorl, central pocket loop whorl, plain arch, tented arch. The maximum occurrence of ulnar loop was observed with 52.57%. The second highest occurrence was of plain whorl with 37.67%. The lowest occurrence was seen in case of plain arch with 0.33% (Fig.4). The second lowest was radial loop and tented arch with 1% occurrence. Double whorl had 4.67 % and central pocket loop whorl had 2.67% occurrence (Fig.5).

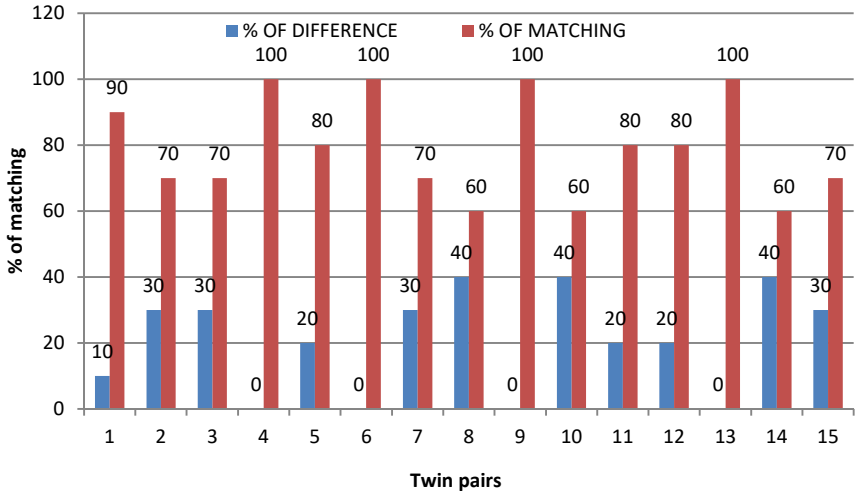

Fig. 4 Percentage of matching and difference of fingerprint among identical twins.

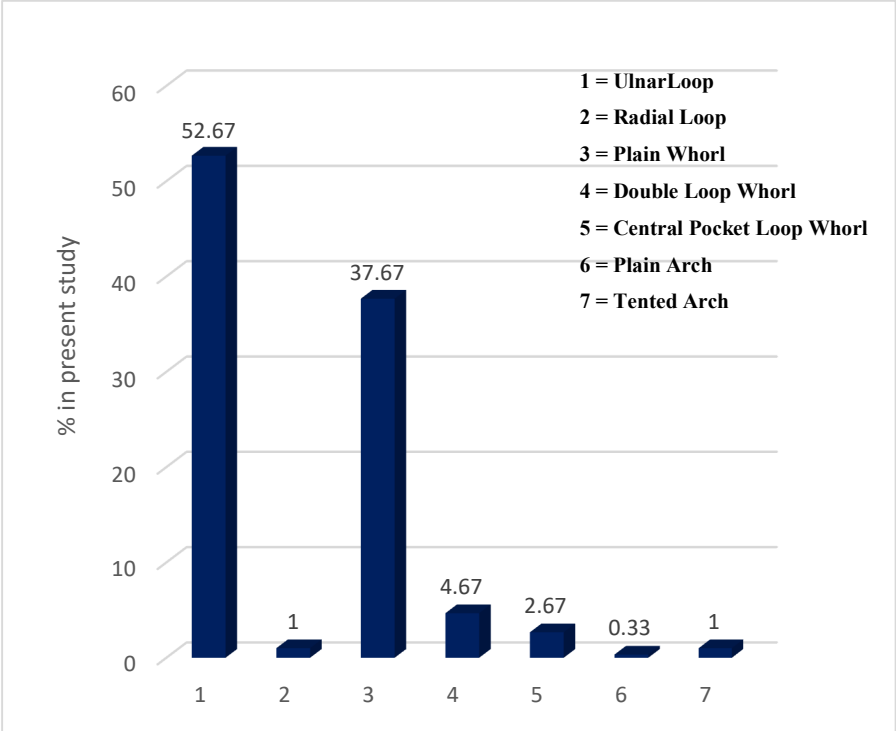

Fig. 5 Distribution of fingerprint pattern in identical twin population.

DISCUSSIONS

Identical twins develop in the same uterine environment, but they have different fingerprint patterns. However, identical twins are situated in different parts of the womb during development and each fetus encounters slightly different intrauterine forces from their siblings. As a result, fingerprints of identical twins have different micro details. Even the fingerprints of both the hands of the same individual differ for the same reason. But, since the fingerprints are differentiated from same genes, so the patterns will not be totally different. The level 1 results, obtained by visual comparison, show that fingers of twin have 79.33% similarity. The implications of the study show high similarity between fingers of twin, with very few but noticeable differences. Twins can be successfully discriminated using advance methods like minutiae extraction or by using fingerprint enhancement methods (Tao et al*.,* 2012; Redhu and Balkishan, 2013). Ulnar loop pattern was found to be the highest frequency of fingerprint pattern during the study. In another study, it is observed that the similarity of fingerprints of identical twins is the same as the fingerprints between fraternal twins (Srihari et al*.,* 2008). Like fingerprints, palm prints are also found to be efficient in distinguishing different identical twins (Kong and Zhang, 2006). Gender based fingerprint analysis can be carried out in case of identical twins which has already been attempted in normal population (Sam et al*.,* 2015). Another fingerprint analysis shows "Arch" type as the most common type of fingerprint pattern present in both identical (42.04%) and non-identical twins (53.10%) whereas the Loop pattern was 26.59% and 22.24% found both in identical and non-identical twins (Panchmal et al*.,* 2017). They also compared fingerprint data with blood group types and report that the individuals in a same identical twin group share same blood group respectively. Out of the eight types of fingerprint patterns found, seven types were observed in the present study. The accidental whorl, which is a rare type of fingerprint pattern was not evident in the present study.

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RETINOIC ACID MEDIATED CHANGES IN THE EPIDERMAL CELLS DURING TAIL REGENERATION IN THE TADPOLES OF THE INDIAN TREE FROG, *POLYPEDATES MACULATUS*

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ABSTRACT

Tail of the anuran tadpole is covered by a continuous epidermal layer. This layer consisting of epithelial cells get regenerated when injury is inflicted. However, tail amputated tadpoles when exposed to exogenous retinoic acid, a potent morphogen, inducing trans differentiation of cells shows various morphological abnormalities in the epidermal layer such as thickening of the layer, loosening and hypertrophy of cells. Some cells exhibited apoptotic morphology.

Keywords: Retinoic acid, tadpole, tail regeneration.

INTRODUCTION

The tadpoles of the Indian tree frog, *Polypedates maculatus* are known to regenerate their tail upon amputation. The regenerated tail is similar to the original tail in terms of its structure and function. However, when the tail amputated tadpoles are exposed to vitamin A palmitate, the regeneration has been documented to be abnormal and stunted in several anuran tadpoles (Mohanty-Hejmadi et al., 1992; Mahapatra and Mohanty-Hejmadi, 1994). The most surprising result of it has been the development of multiple ectopic hind limbs (Mohanty-Hejmadi et al., 1992; Mahapatra and Mohanty-Hejmadi, 1994) along with establishment of a pelvic region at the regenerated tail (Pati et al., 2003). Hence, teratogenicity of vitamin A metabolite such as vitamin A palmitate is established during tail regeneration in the tadpoles of *P. maculatus*. The occurrence of such an incidence of homeotic transformation in a vertebrate animal model has made the regeneration research more puzzling and equally challenging. Hence, it necessitates an evaluation of the changes with respect to the various tissue types present in the tadpole tail undergoing regeneration and also on exposure to vitamin A metabolites. The anuran tail consists of different tissue types such as the epithelial, connective, muscular and the nervous tissue. Hence, an injury to the tail provides an excellent platform to carefully trace the regeneration pattern. Further, treatment with vitamin A provides an opportunity to understand how the abnormalities are manifested when vitamin A metabolite disrupts normal tail regeneration and rearrange the cells to develop into a pelvic zone thereby developing hind limbs along with girdles at an ectopic site.

Rapid re-epithelialization of the wound has been described earlier as a notable feature of regenerating systems (Tanaka and Galliot, 2009). The surface of the amputated stump is covered with an epidermis within 24 h after half of the tail is removed (Mochii et al., 2007). The most distal epidermis thickens to form a multi-cell layer (Sugiura et al., 2004), which may be equivalent to the apical ectodermal cap reported in urodele limb regeneration (Mochii et al., 2007). Development of ectopic limbs from cut end of the tail is known to be initiated with the accumulation of a thick layer of the epithelial cells. An interaction between the epithelial cells with the underlying mesenchymal cells has been hypothesized to be instrumental in development of ectopic limbs (Mahapatra et al., 2004; 2017). Hence, considering re-epithelialization of the overlying epidermis to be an integral and foremost act of the regenerating structure, we in the present study have focussed on the changes with respect to the epidermis during the course of regeneration and retinoic acid (RA) mediated abnormal regeneration in the tail of tadpoles of *P. maculatus*.

MATERIALS AND METHODS

The egg nests of the Indian tree frog, *P. maculatus* were collected from stagnant water bodies in Bhubaneswar during monsoon. The eggs were allowed to hatch in the laboratory and upon development the tadpoles were reared following the standardized protocol (Mohanty-Hejmadi, 1977). They were fed with boiled *Amaranthus ad libitum* and the conditioned tap water was changed regularly. The tadpoles in their hind limb bud stages, Taylor and Kollros (TK) stage I and II were screened (Taylor and Kollros, 1946) for the experimental procedures. Surgical transection of the tail from the middle was carried out using a sharp and sterilized blade. The tadpoles were exposed to MS222 prior to tail amputation to minimize stress to the animals. Tadpoles were exposed to RA at the dose of 250ng/ml for 24 h under low light conditions. All the experiments were approved by the Animal Ethical Committee, Utkal University, Vani Vihar, Odisha, India. Transverse sections (T.S.) of the tissues of the original tail, regenerated tail both control and treated tissues of day 5, 10 and 15 post amputation (pa) were obtained for histological analysis. The sections were stained by Mallory's triple stain and photos were obtained using a Leica DFC450 C camera fitted to Leica DM 3000 LED microscope. The photos were assembled onto plates using Adobe Photoshop software.

RESULTS

The transverse section of the original tail revealed an intact epidermis (e) consisting of two to three layers of epithelial cells (ep) with distinct nucleus (Figs.1 A and B). A thin and continuous basement membrane (bm) was visualized underneath the epidermis separating the underlying mesenchyme (m) (Fig.1 B). Epidermis (e) consisting of epithelial cells (ep) with a distinct basement membrane (bm) beneath was evident from histological analysis (Figs. 1 C - H) in the control tissue sections of day 5, 10 and 15 post amputation (pa). Epidermis in day 5 regenerate comprised of one to two layers of epithelial cells (Fig. D). This layer gradually became thickened with the advancement of regeneration (Figs. 1 F and H). In the day 15 pa tail, this layer was comparable with that of the original one (Fig. H). Moreover, in the day 10 and day 15 pa, melanocytes (ml) were accumulated beneath the regenerated epidermis (Figs. 1 F and H).

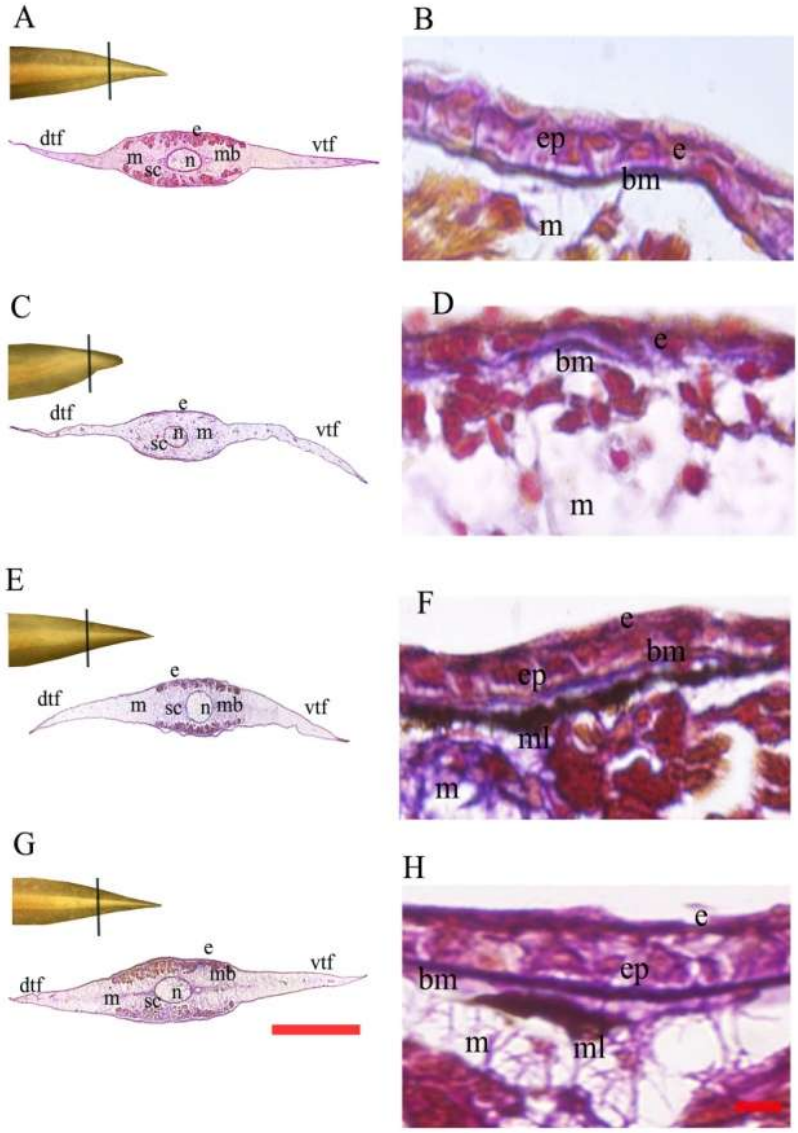

Fig. 1 (A) T.S. of original tadpole tail of *P. maculatus* (Scale bar = 500µm); (B) T.S. of original tadpole tail of *P. maculatus* (Scale bar = 25 µm); (C) T.S. of tadpole tail of *P. maculatus* (Scale bar = 500µm); (D) T.S. of tadpole tail of *P. maculatus* of day 5 post amputation (Scale bar = 25 µm); (E) T.S. of tadpole tail of *P. maculatus* (Scale bar = 500µm); (F) T.S. of tadpole tail of *P. maculatus* (Scale bar = 25 µm); (G) T.S. of tadpole tail of *P. maculatus* (Scale bar = 500µm); (H) T.S. of tadpole tail of *P. maculatus.* (Scale bar = 25 µm).basement membrane (bm), epidermis (e),epithelial cells (ep), dorsal tail fin (dtf), melanocytes (ml) mesenchyme (m), muscle bundles (mb), notochord (n), spinal cord (sc), ventral tail fin (vtf).

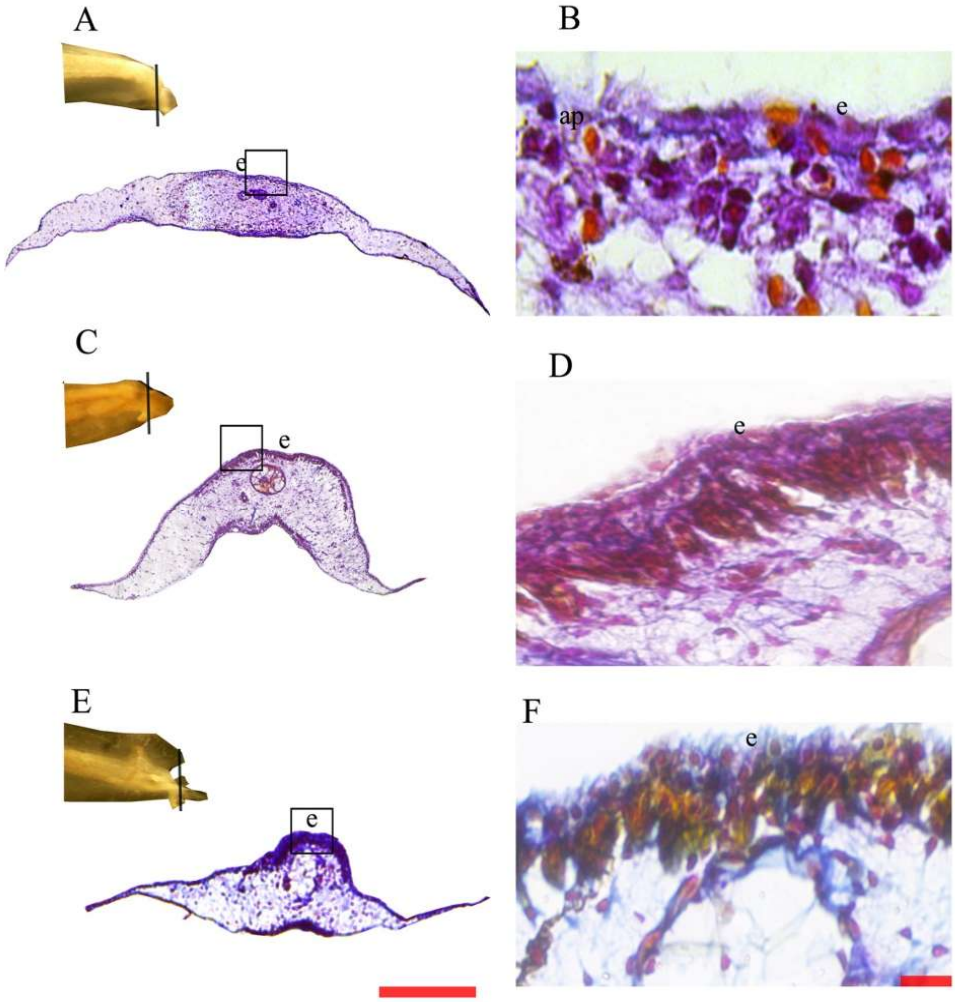

Fig. 2 (A): T.S. of treated tail tissue regenerates of day 5 post amputation showing epidermis (e) (Scale bar = 500µm); (B): T.S. of treated tail tissue regenerates of day 5 post amputation showing epidermis (e) and cell with apoptotic morphology (ap). (Scale bar = 25µm); (C): T.S. of treated tail tissue regenerates of day 10 post amputation showing epidermis (e) (Scale bar = 500µm); (D): T.S. of treated tail tissue regenerates of day 10 post amputation showing epidermis (e) (Scale bar = 25µm); (E): T.S. of treated tail tissue regenerates of day 15 post amputation showing epidermis (e) (Scale bar = 500µm); (F): T.S. of treated tail tissue regenerates of day 15 post amputation showing epidermis (e) (Scale bar = 25µm).

A significant difference however, was witnessed in terms of re-epithelialization in RA treated tissue sections. A proper epidermis (e) consisting of epithelial cells was lacking in the treated tissue regenerates (Figs. 2 A - F). In 5 days regenerated tails, the epidermis (e) showed accumulation of three to four layers of epithelial cells (Fig. 2 B) and some epithelial cells exhibited apoptotic (ap) morphology (Fig. 2 B). The epithelial cells remained multi-layered in the 10 days regenerated tail (Figs. 2 C and D). In the regenerated tissue sections of 15 days pa, epidermis became thicker with four to six layers of epithelial cells (Figs. 2 E and F). However, these epithelial cells were loosely associated and lacked their typical characteristics of cell adhesion (Fig. 2 F). In the outer layer, hypertrophy of the cells was visualized (Fig. 2 F).

DISCUSSION

Regeneration is triggered in anuran tadpoles upon injury. The re-constitution of the tissues in the original plan and manner is a coordinated event and follow an array of events. The foremost being covering of the cut site with an epidermis. It is already reported that upon limb amputation in amphibians, epidermal cells from the remaining stump migrate to cover the wound surface, forming the wound epidermis (Gilbert, 2000). Similarly, Tseng and Levin, (2008) have suggested the wound healing process to be rapidly completed within 6-12 hours post amputation (hpa) as the apical wound epithelium is formed in *Xenopus laevis*, an anuran amphibian. According to Chen et al. (2014) the tadpole tail regeneration proceeds through three phases such as the early, intermediate and late phase where the early phase is associated with scar-less wound healing at the site of the injury. Likewise, it has been reported that the process of regeneration begins with the closure of the wound by epidermal cells (Das and Mohanty- Hejmadi, 1999). Under the influence of RA, the tail abnormalities were however, visualized to a great extent in the *P. maculatus* tadpoles. Similar reports of tail abnormalities have earlier been documented in *P. maculatus* (Das and Mohanty- Hejmadi, 1999; Mahapatra et al., 2017). They have suggested the epidermis to be single-layered in the control tissue sections while multi-layered in the treated tail sections within 48 hpa (Das and Mohanty- Hejmadi, 1999; Mohanty- Hejmadi and Crawford, 2003). In the present study, we have also described multi-layered epithelial cells in the treated regenerated tails. Besides being multi-layered, these cells lacked adhesion and some cells demonstrated apoptotic morphology. Thus, the abnormalities observed in the epidermis with respect to its shape, constituting cells and appearance are confirmatory of an inducing effect of RA in course of abnormal tail regeneration, a pre-requisite for homeotic transformation.

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