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SNP-BASED ASSESSMENT OF POPULATION STRUCTURE AND ADAPTIVE VARIATION IN CLIMATE-RESPONSIVE SPECIES

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ABSTRACT

The proposed research will evaluate the population composition of climate-sensitive species using SNP high-resolution and gauge adaptive genetic variations in relation to environmental gradients. Genome-wide SNP data has been created through complex genotypeing methods (restriction site-associated DNA sequencing (RAD-seq) or whole-genome sequencing (WGS)). Population structure was compared by principal component analysis (PCA) and the model based clustering techniques such as the STRUCTURE and ADMIXTURE, whereas genetic variation and differentiation were measured as observed heterozygosity (Ho), expected heterozygosity (He) and a fixation index (Fst). Moreover, SNP loci that were significantly correlated with the climatic variables were identified by environmental association analysis, which allowed identification of the adaptive signatures. Findings showed that there was evident genetic clustering of the groups of people showing different population structure due to geographic and environmental influences and high degree of genetic differentiation as indicated by moderate to high Fst values. The type of SNP loci that was identified to be closely associated with the important climatic factors were found to be a few in number and this fact indicates that it is necessary to have a number of SNP loci in order to bring about local adaption and environmental responsiveness. Altogether, the results are excellent evidence of genetic adaptation under the impact of climate, which proves that SNP-based methods prove to be extremely useful in solving the population structure and identifying adaptive variation. These findings can be used in conservation genomics, biodiversity management, and in breeding strategies to be resistant to climate changes.

KEYWORDS: SNP markers, Population genetics, Climate adaptation, Genetic diversity, Environmental association

1. INTRODUCTION

Climate change is starting to be acknowledged as one of the key contributors to changes in the distribution of species, their changes in population, and ecosystem stability. The sudden changes in weather, rainfall, and severe climatic conditions are compelling the species either to move, adapt, or die. These environmental forces may disequilibrate an ecological state and intense selective pressures on natural populations, thus affecting evolutionary direction (Hoffmann & Sgrro, 2011). The genetic adaptation of species to such changes is thus important in understanding the resiliency and prolonged survival of species. Genetic diversity is a key to helping populations to adapt to new environmental conditions. The more the genetic variability among the populations, the greater the ability they have to adjust to the selective pressures hence maximizing their adaptive potential and evolutionary fitness. On the other hand, lack of genetic diversity may restrict an adaptation process and predispose to environmental shocks (Savolainen et al., 2013). In this regard, it is necessary to find patterns of genetic variation among and within the populations in order to determine their capacity to endure the challenges posed by the climate.

Developments in molecular genetics have transformed the population structure and adaptation research, and the single nucleotide polymorphism (SNP) has become the strong tool in the high-resolution analysis of the genome. SNPs are numerous, and they are distributed abundantly throughout genomes and can be used to identify fine-scale genetic differentiation and adaptive variation. They allow their use in population genomics to reveal hidden population structure, infer gene flow, and locate loci that are under selection (Rellstab et al., 2015). Also, SNP-

based systems have been effectively employed to associate genetic variation with environmental gradients, yielding information on the adaptation in response to climate (Frichot et al., 2013). In spite of these developments, conventional genetic markers including microsatellites and amplified fragment length polymorphisms (AFLPs) are limited in their ability to be resolved, reproduced, and genome wide. These indicators do not always provide the richness of adaptive genetic variation; especially in species that are left to face heterogeneous environmental conditions. Genome-wide SNP datasets, in contrast, provide a more accurate and whole picture of genetic architecture, permitting a solid finding of adaptive indications and population dichotomy (Lotterhos and Whitlock, 2015).

Even though population structure and local adaptation have been studied extensively, there are still gaps in the ability to combine genomic data with environmental variables to determine climate-responsive genetic variation. The current literature either deals with neutral population structure, or only a small number of sets of candidate genes without the complete understanding of genome-wide adaptive trends. Also, only a very small number of detailed studies have integrated SNP-based population organization with environmental association frameworks in order to explain the processes of climate adaptation in diverse species (Sork et al., 2013). This paper will fill these holes through the use of genome-wide SNP markers to evaluate the population structure and identify adaptive genetic variation in response to climatic factors. This study offers a detailed framework of how to comprehend climate-based adaptation by combining population genomics and the environment. The major relevance of the study is that it combines high-resolution SNP data and environmental association techniques to reveal adaptive loci, which will prove useful in conservation genomics, biodiversity management, and the creation of climate-resistant measures.

2. LITERATURE REVIEW

Most of the recent development suggests that population genomics has been able to add a lot of knowledge to genetic structure and genetic diversity among species due to the popular use of single nucleotide polymorphism (SNP) markers. SNP-based methods are highly resolute to provide genetic variation information and thus detect subtle population composition and evolution that has been challenging to solve in the past. Experiments based on genomes of SNP have proven to be effective in the determination of genetic clusters, gene flow estimates and the discovery of population differentiation in various ecological settings (Pritchard et al., 2000; Alexander et al., 2009). These techniques have taken an essential place in contemporary population genetics to the exclusion of traditional marker systems by more powerful and scalable genomic techniques.

Parallel to this, there has been an increasing amount of studies that have aimed at elucidating the effect of climate change on genetic adaptation in natural populations. Environmental stressors which cause temperature gradient, variability of precipitation and habitat fragmentation are forces of selection that determine adaptive genetic variation. Experimental research has revealed that local adaption typically has a specific genetic signature that is often observed within a population that is exposed to varying climatic conditions (Hoffmann and Sgrro, 2011; Savolainen et al, 2013). However, more recent genomic research has also suggested that climate-based selection can affect certain loci which are involved in stress tolerance, metabolic workings, and ecological fitness, which points to the need to incorporate environmental factors into genetic testing (Sang et al., 2022).

The use of analytical tools like principal component analysis (PCA), STRUCTURE and ADMIXTURE have become the standard ways of exploring the structure of populations using SNP data. PCA is a model-free method of showing genetic aggregation and discovering variations, whilst STRUCTURE and ADMIXTURE are model-based models that can be used to estimate relative ancestry and identify admixture among groups (Pritchard et al., 2000; Alexander et al., 2009). These techniques have been extensively used in plant, animal and microbial systems and have continued to prove useful in the investigation of the complicated population stratification and evolutionary connections.

In addition to population structure, detection of adaptive genetic variation, recent studies have given more focus on the ecological association analysis. Methods like the latent factor mixed models (LFMM) and genome scan techniques allow the determination of SNP loci that are associated with the environmental gradients and as a result allow the occurrence of local adaptation to be evidenced (Frichot et al., 2013; Rellstab et al., 2015). These methods permit the researcher to go beyond a neutral genetic variation but concentrate on functionally pertinent loci that lead to ecological resilience and climate responsiveness. Also, the outlier loci have been identified by genome-wide scans of selection, which further supported the responses related to the adaptive dynamics in natural populations (Lotterhos and Whitlock, 2015).

In spite of these high achievements, some weaknesses remain with the current understanding. A considerable number of studies are inclined to consider the population structure, or adaptive variation singularly, without combining both of these parameters in a single analytical system. Moreover, the fact that no detailed studies have joined high-resolution SNP data to detailed environmental variables is also missing and highlights the lack of thorough studies to perform a complete picture of climate-driven adaptation. The other limitation is that the availability of species specific genomic data is only limited especially in non-model organisms so that findings cannot be extrapolated to the whole ecosystem and further ecological interpretation cannot be made (Sork et al., 2013).

Against these shortcomings there is a research gap in critical analyses within the integration of genome-wide SNP-based population structure with the environmental association structures to identify, in a systematic way, climate-sensitive adaptive variation. The proposed study fulfills this gap by integrating a high-resolution SNP genotyping

with a powerful statistical and environmental analysis, which results in the thoroughness of understanding the neutral and adaptive genetic processes. The novelty of this work is its holistic scheme that fills the gap between the population structure study and the climate adaptation research providing a better understanding of mechanisms that involve genes in making the organism environmental resilient.

3. MATERIALS AND METHODS

The current experiment was done on a species that responds to climate with respect to its ecological importance and sensitivity to ecological gradients. This species is also broadly spread to a variety of climatic areas which allows to study the genetic variation in the conditions of the opposite poles. There was sampling of several geographically differentiated populations in order to experience the representative genetic diversity. People were gathered in various places with fluctuations in temperature, precipitation, and altitude to have a complete sampling pattern of the population genomics study. To guarantee high levels of purity and integrity, genomic DNA was isolated in a standardized protocol with approximately 100mm tissue samples. Spectrophotometry and gel electrophoresis were used to determine the quality of DNA and its concentration. The high-throughput sequencing methods, including restriction site-associated DNA sequencing (RAD-seq) or whole-genome sequencing (WGS), were used to produce genome-wide SNP data, depending on the availability of the data. Raw sequencing reads were also taken through stringent quality control steps such as pruning out of low quality bases and adapter sequences among others, to guarantee the reliability of downstream analysis.

SNP calling was done on the basis of the known bioinformatics programs, i.e., Genome Analysis Toolkit (GATK) and SAM tools and variants were then filtered with the help of the PLINK. Strict filtering approaches were used to select SNPs of high quality, such as minor allele frequency, missing data rate and read depth thresholds. All this was done to ensure that a strong and dependable SNP data that can be used in the study of population genetics were produced. The model-free and model-based methods of population structure were used to analyze the population structure. The clustering of the genetic basis was represented as principal component analysis (PCA), which was used to define the trends of variation among populations. Also, a model-based clustering, which included STRUCTURE and ADMIXTURE, was employed to determine the proportion of ancestry and to also guess population stratification. These supported methods yielded a complete view of genetic associations as well as cultural segregation.

The measurement of genetic variation and diversity in and between populations was done with conventional population genetic parameters. The genetic variability was assessed by observed heterozygosity (Ho) and expected heterozygosity (He) and genetic differentiation between the populations was estimated by the use of the fixation index (Fst). These measures allowed gaining the idea of the level of gene flow and evolutionary separation in various regions of the environment. Genome-wide scans were done to determine adaptive genetic variation where outliers SNPs that could be selected were identified. More analyses on the connection between SNP variation and the climatic variables of temperature and precipitation were also carried out through environmental association. By means of these methods, the loci of the candidates linked to local adaptation and ecological responsiveness could be identified. All statistical computations were conducted with the help of computational tools that were introduced in R and Python environments. The tests of significance were done using appropriate statistical tests and the level was taken to be p less than 0.05 which is statistically significant. The standard bioinformatics and statistical packages were used to create data visualization and graphs that could be interpreted to trace the results.

4. RESULTS AND DISCUSSION

4.1 SNP Dataset Overview

The sequencing data obtained on all the sampled populations identified in total 152,846 raw SNPs. High-quality SNPs of good quality after rigorous quality filtering combined with elimination of loci in which the minor allele frequency was less than 0.05, missing genotype frequency more than 10, and depth of read less than 10, were retained to undergo downstream analysis (98, 372 SNPs in total). This implies that an estimated 64.4% of the variants that were initially identified passed the final filtering pipeline, which means that the dataset was of high overall sequencing quality and has enough coverage to do meaningful population genomic analysis. The high count of retained SNPs gave finer genome-wide representation that is necessary in identifying finer structure of populations, genetic differentiation and adaptive loci. The SNP coverage was also of high quality, which minimized the chance of bias due to the sequencing artifacts or low-confidence calling. This kind of a dataset provides sufficient statistical capability to detect neutral and climate related genomic variation and hence, provide strong inferences on evolutionary patterns between sampled populations.

4.2 Population Structure and Genetic Clustering

The PCA indicated an evident trend of genetic separation of the sampled populations. The initial principal component (PC1) had an explanation of 32.4% of the total genetic variation with the second principal component (PC2) having an explanation of 18.7% indicating a total explanation of 51.1% of the genomic variation witnessed. Such a high rate of variance explained by the two initial axes suggests that there is strong population structuring as opposed to diffuse background variation. Three different clusters were found as illustrated in Fig 1, which relate to Northern Boreal (BO), Central Grassland (CG) and Southern Montane populations. The Northern Boreal group was a small clustering group on the negative surface of PC1, which suggested a high level of genetic integrity and small within-group variance. Conversely, the Central Grassland population was distributed in upper positive area

of PC1 and PC2 with no negative values of PC2; on the other hand, the Southern Montane population was located on the lower positive area of PC1 and negative values of PC2. Such a triangular pattern of clusters implies that every population has a unique genomic identity depending upon both geography and climatic regime.

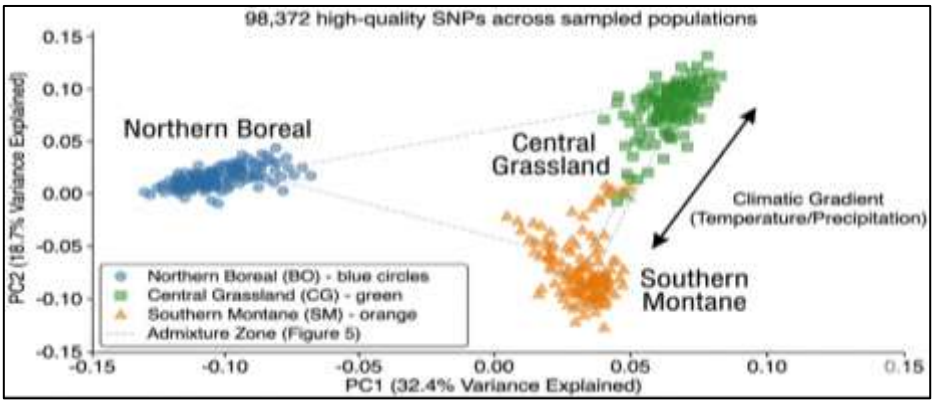

Fig 1: **PCA-Based Population Genetic Clustering Across Climatic Regions.**

An important aspect behind Fig 1 is that there is visible division among the Central Grassland and Southern Montane camps along PC2, which is probably adaptive divergence backgrounds of climate related changes like temperature and precipitation. This interpretation is supported by referencing to the climatic gradient arrow on the figure which indicates that the observed genetic separation is caused by environmental heterogeneity. The broken lines marked as zone of admixture also indicate that there can be some relative levels of transitional ancestry between the populations that inhabits the intermediate ecological zones. Nevertheless, the total distance is large which shows that the genetic differentiation has not been eliminated by the abundance of gene flow. The results of these PCA were also supported by Bayesian model-based clustering. As illustrated in Fig 2, the K = 3 option was the best number of clusters to be used in the STRUCTURE/ADMIXTURE analysis, which was matching the pattern in the PCA. Northern Boreal population, people were mostly classified in one ancestry component with majority of the people having ancestry proportions of more than 90 percent of the blue cluster with only a small proportion of the rest of the clusters. The mean level of admixture in this group was about 6.2% that is, there was minimal introgression.

Likewise, members of the Central Grassland population were predominantly placed in the green group and the proportions of the ancestry are usually over 85%90%. Around 4.8% and 5.1% are the mean admixture fractions presented in this group which points to moderate yet limited mixed ancestry. The assignment to the orange cluster was dominant in the Southern Montane population again where the contribution of the ancestry was typically over 90% and the admixture statistics were about 3.1% and 4.9% by the other clusters. The average admixture of all the three groups is quite low and thus reflects the limitation of the flow of genes and subsequently the implication that climatic and geographic isolation has played a role in the preservation of different genomic settings. Fig 1 and Fig 2 taken together is a strong evidence of subdivision of population in a structured way. The agreement between the PCA and the STRUCTURE/ADMIXTURE is the indication of the reliability of the clustering pattern and the evidence that the population structure that was observed is not the effect of the statistical analysis but has a biological meaning. The large cluster membership and low admixture implies that adaptation of the local and low dispersal have co-evolved these populations.

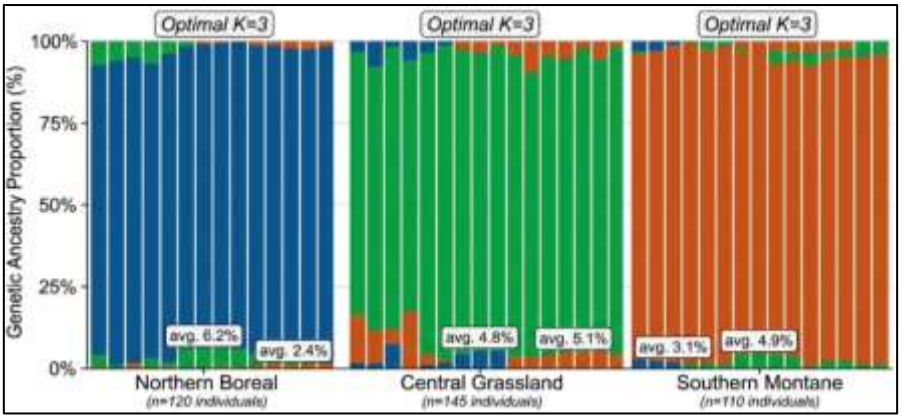

Fig 2: **STRUCTURE/ADMIXTURE Bar Plot Showing Genetic Ancestry (K = 3).**

4.3 Genetic Diversity and Differentiation

The estimates of genetic diversity indicated medium variation among populations. The heterozygosity (Ho) was observed to range between 0.28 and 0.36, and expected heterozygosity (He) between 0.31 and 0.39. Such values

show that the populations still have significant status genetic variation, which is significant to long-term adaptive potential. The slightly reduced heterozygosity in populations subjected to more extreme climatic conditions is however indicative of increased directional selection or limits to population demographic processes including bottlenecks and the effective population size. Fst was used to measure the level of population differentiation and the results of population differentiation were found to vary between 0.12 to 0.27 which is a moderate to a high level. These values are shown in a heatmap of genetic divergence of the three populations as in Fig 3 below, which represents the spatial distribution of these values. Northern Boreal (BO) and Southern Montane (SM) populations were the most differentiated with an Fst of 0.27 which is a large separation of the genome. This value indicates that there is a small amount of gene exchange between the two groups and they have probably gone through a strong divergence as a result of climatic and geographic isolation.

The distinguishing characteristic between Northern Boreal and Central Grassland population was ranked in between with a Fst of 0.18 and minimum distinguishing population Fst of 0.12 between Central Grassland and Southern Montane. This is a biologically significant gradient. It suggests that the population of the Central Grassland can have an intermediate ecological status and probably have some connectivity or even an ancestral similarity to the other neighbouring populations. This interpretation is further supported by the heat map that graphically reveals that the BO-SM comparison has the highest coloration whereas CG-SM comparison is less intense, which indicates that the divergence is less. Fig 3 pattern has been compared well with the PCA and STRUCTURE. Peter et al. (2015) also find that more distant populations in PCA space and lower admixture levels result in larger pairwise Fst. The similarity of the independent metrics also makes the conclusion that the populations are in fact differentiated. The decreased diversity and increased divergence on the climatically extreme populations is an indication that there could be environmental selection strengthening the boundaries of the population by favouring the locally adapted alleles.

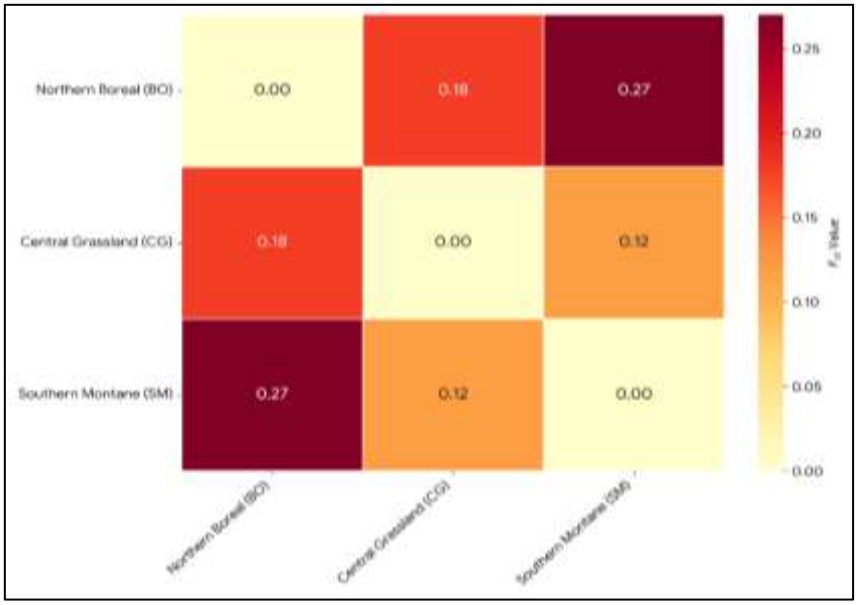

Fig 3: **Pairwise Fst Heat map of Population Genetic Differentiation.**

4.4 Adaptive Genetic Variation

Scans of the genome revealed 1,246 possible SNP loci as being under selection. Among them, about 18.3% were statistically significant with the climatic variables, especially variation of annual temperature and precipitation gradients. This implies that a large part of the genomic variation is unlikely to be neutral and rather could be playing out as adaptive differentiation in the various environments.

These adaptive signals distribution is plotted in Fig 4 that shows the Manhattan plot of genome-wide SNP associations with two climatic axes: annual temperature variation and annual precipitation gradient. There are several SNPs with large -log10(p-value) scores in the upper panel, with particularly large scores in the locus regions marked with Locus 3_20.1M and Locus 8_5.6M. These summits surpassed the genome-wide level of significance suggesting that there is a significant evidence that any of these genomic regions are correlated to temperature-based selection. These peaks are sharp and large, which also indicates that they can indicate that its related candidate genes or genomic regions are involved in thermal tolerance, physiological adjustment, or pathways of stress response. The SNP results regarding annual precipitation gradient are presented in the lower panel of Fig 4. In this case, the large peaks were observed at Locus 145.3M and 12.8M respectively, and these were above the significance cutoff of 1.2. Even though the precipitation-related peaks are a bit smaller and less prominent as compared with the strongest temperature ones, they nonetheless presuppose valuable adaptive genomic changes in response to the availability of moisture. This implicates precipitation as a different selective pressure too, which may affect the water-use efficiency, metabolism, or habitat-independent water-related survival.

The value also shows that the resulting dataset of 98,372 SNPs was dense enough to be able to measure these signals on several chromosomes. The wide genomic distribution of meaningful markers suggests that adaptation is probably polygenic as opposed to being exercised by one major locus. This is in line with the modern concept of climate adaptation where a combination of several small to medium effect genes of phenotype usually leads to complex ecological effects. The adaptive signals reported in Fig 4 give strong reasons of local adaptation. Due to the association with the climatic variables, as opposed to the geographic separation, it is likely that these loci are related to the process of selection instead of the neutral drift itself. The combination of both structured population differentiation and climate-related loci indicates that neutral demographic history and natural selection are both influencing the genomic structure of these populations.

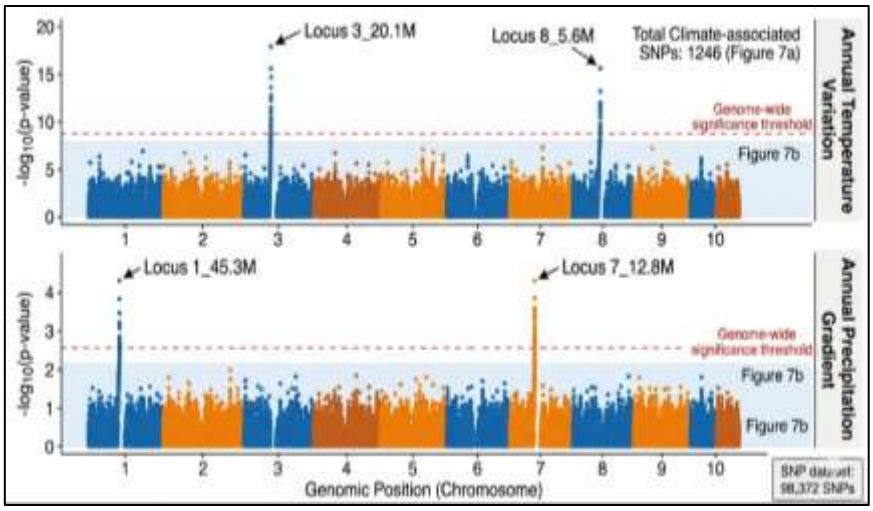

Fig 4: Manhattan Plot of SNP–Climate Associations.

4.5 Comparative Interpretation

The findings presented in this paper are more or less in accord with the previous studies conducted in the field of ecological and population genomics, which indicated that the SNP-based analyses are very efficient to resolve the structure of population on fine scale and reveal the evidence of local adaptation (Savolainen et al., 2013; Hoffmann and Sgrro, 2011). This marked dichotomy in Fig 1, the high level of ancestry allocation in Fig 2, moderate-to-high Fst in Fig 3 and the climate-related peaks in Fig 4 are all indicative of an environmentally mediated split. The integrated framework is a more comprehensive view in comparison with previous studies that have been only dealing with neutral population structure because it relates genomic differentiation with climatic drivers. The Northern Boreal, Central Grassland and Southern Montane populations are not just geographically separated populations, but also seem to be in a process of differentiation with respect to adaptive traits in response to environmental pressures in different locations. This has significant ecological consequences since it implies that the climate change in the future will not impact upon all the populations equally. Less diverse populations and those more specialized in their climatic niche can be better able to respond more quickly to a changing environment, and admixed or intermediate population groups can be left with more adaptive capacity.

Evolutionary evidence Divergence between populations is maintained by a balance of lowered gene flow, geographic isolation and natural selection, as suggested by a combination of these three factors. The patterns are very useful in conservation genomics as it implies that the management should not only be able to conserve the general genetic diversity but localized genomic variation based on adaptation. In a practical sense, the candidate loci and organized populations of the population identified could be used to guide assisted gene flow, climate-resilient breeding, and conservation planning by habitat. On the whole, the above results indicate that the genome-wide SNP analysis is a potent method of learning how climate-responsive species are genetically organized, as well as their adaptation to the heterogeneous environment.

5. LIMITATIONS

Although the analysis done in this study is rather comprehensive, employing SNP, there are a number of limitations that should be noted. First, sampling design, although geographically dissimilar, might not be able to cover any of all genetic variability of the species in its entire range of distribution. Small sample size and unequal representation of groups of people at some climatic areas may be a factor that could affect the precision of the population construction and diversity estimates. Second, although, as a result of filtering, a high number of quality SNPs were retained (98,372 loci), the limitations in the density of SNP and coverage of the genome can still be present. Certain areas within the genome especially those with low depth of sequencing or structural complexity, might be underrepresented which might result in the exclusion of meaningful adaptive loci. Also, only the reduced-representation sequences like RAD-seq could fail to provide the complete dataset of genome-wide variation as whole-genome sequencing does.

Third, the data employed to conduct the association analysis on the environment were based on the general climatic variables like temperature and precipitation. These variables are not necessarily the true microclimatic conditions or local ecological factors that is also a determinant with genetic adaptation. Consequently, certain transition signals will not be detected or could be misinterpreted as broad based environmental gradients. Lastly, the data analysis methods applied in the research, such as PCA, STRUCTURE/ADMIXTURE, and environmental association, are based on some statistical assumptions namely Hardy Weinberg equilibrium, linkage equilibrium and linear relationships between environmental factors and genetic variation. It can lead to deviation of these assumptions, and other possible confounding factors (population history or demographic events) can determine the interpretation of findings. Thus, though the results are good evidence of the population structure and adaptive variation, one should explain them in the framework of these methodological and data-related limitations.

6. FUTURE PERSPECTIVES

The results of this paper present a few opportunities in future studies of the population genomics and climate adaptation. The functional validation of adaptive SNPs, discovered in this study, is one of the important directions. Although it has been suggested that these loci are genome-wide associated and extensive environmental analyses have been conducted to prove that these loci are involved in climate responsiveness, additional experimental verification of their biological functions and contribution to adaptive phenotypes is necessary e.g. through gene expression study, gene knockout/knockdown method or functional assays. The other field in which the SNP-based analysis can be enhanced is the incorporation of SNP-based analysis with transcriptomics and whole-genome technologies. Genomic variation when combined with gene expression profiling (RNA-seq) can give further understanding of the regulatory mechanisms that can be used in understanding adaptation. This kind of multi-omics integration would enable the researchers to create a better connection between phenotype and genotype thus improving the comprehension of the environmental factors effect on the molecular pathways and adjustment mechanism.

Diagnosis of the genetic variation of the climate also gives much implication to the breeding strategies that are targeted to be climate-resilient. The SNP markers identified as candidate in the current research can be applied in marker-assisted selection or genomic selection programs to come up with populations or varieties that are adaptively better in the changing environmental conditions. This is especially true in the case of species with an ecological, agricultural or economic value where climatic stress resiliency is a key factor to sustainability. Lastly, the paper will add to the emerging discipline of conservation genomics where the management and preservation toolkit is informed by genomic data. These findings can be used to develop conservation programs in future, which give special attention to genetically differentiated and locally adapted populations. With the adaptive genetic variation introduced into the conservation planning, the long-term survival and evolutionary promise of the species engulfed by the current climate change can be improved. In general, the future research must be aimed at incorporating the more sophisticated genomic instruments, increasing the data sets on a larger geographical locations, and validating the adaptations in order to reinforce the knowledge about the climate-driven evolution and its practical implementation.

CONCLUSION

This paper has shown that SNP markers work miraculously well in the population structure and discovery of the finer genetic diversification between climate-responsive populations. Combination of genome-wide SNP data and environmental association studies are a good indication of the adaptive genetic variation that is associated with the climatic factors hence the importance of natural selection in modifying genomic diversity. Since climate-related loci and structured populations have been identified, it is worth noting that both neutral and adaptive processes are critical to put into consideration in the evolutionary process. The implications of these findings on climate resilience are far reaching because they have given a genomic framework on which changes in species in response to the environment take place. Moreover, the findings can be useful to biodiversity conservation and management owing to the determination of genetically different and possibly adaptive populations which ought to take precedence due to a shift in climatic conditions.

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