

The Bioinformatics Analysis in the Whole Genome mtDNA Sequence of Chickens (*Gallus gallus*)

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ABSTRACT

Chicken is important livestock in the world that is kept for egg and meat productions. This study was aimed to perform a bioinformatics analysis in the whole genome mtDNA (16,979 bp) of many chicken breeds. Total 84 whole-genome mtDNA sequences were used in this study and obtained from the reference sequence (GenBank). Four molecular packages of BioEdit, MEGA-X, DNAsp, and Arlequin were used in this study to analyze the observed sequences. Therefore, the whole genome mtDNA sequence of birds in this study was originated from Asia, the USA, and New Guinea. Research showed that a total of 445 mutation sites and 81 haplotypes were obtained in this study. Thus, the nucleotide diversity in the observed sequences included of high (0.99). In addition, a total of 10 clusters were observed according to the phylogenetic analysis with the UPGMA method. In conclusion, a close genetic relationship was observed among chickens of Asia based on the median-joining network. Meanwhile, the geographical factors were contributed about 9.12% in a sequence variation.

Keywords: Bioinformatics, Chicken, GenBank, Genetic diversity, mtDNA.

1. INTRODUCTION

The physical geography of an area affects the number of species of inhabitants [1]. During the last decade conservation organizations and government agencies have worked to identify, prioritize, manage and restore wild species and habitats. Many countries have begun to commit to emphasizing research on biodiversity mapping [2]. Biodiversity decline is of concern for several reasons. Most immediately, many people depend directly on biodiversity for food, fibre, fuel, and medicines [3]. Chicken is one of the most popular biodiversity varieties in the field of animal husbandry [4].

Chicken is a type of poultry that breeds or varieties that are spread almost all over the world. The chickens were domesticated from a common ancestor, the Red Jungle Fowl (*Gallus gallus*), which originates from Southeast Asia [5, 6]. Moreover, with the massive use of selection and crossbreeding, local breeds and lines in different parts of Europe were developed, and Asian breeds of the Chinese and Malay types were introduced [7]. All of these sources contributed to the modern

biodiversity of chicken populations. Inter-crossing, however, may have partly extinguished differences among groups or breeds, with the result that genetic relationships between chicken populations are not always definitive [8,9,10].

In this world, there are four types of jungle fowl, namely: gray jungle fowl (*Gallus sonneratii Temminck*), orange jungle fowl (*Gallus lafayetii Lesson*), chicken red jungle fowl (*Gallus gallus Linnaeus*), and green jungle fowl (*Gallus varius Shaw and Nodder*) [11]. The four types of jungle fowl are classified into: genus *Gallus*, family Phasianidae, and Order Galliformes [12]. The poultry industry comprises two major elements, the egg industry, and the chicken meat industry.

The science of bioinformatics can be divided into several branches based on the experimental material used for the study [13]. Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data. As an interdisciplinary field of science, bioinformatics combines computer science, statistics, mathematics, and engineering to

analyze and interpret biological data [14]. Bioinformaticians need a solid background in computer science but also a good understanding of biology. Since bioinformaticians work closely with biologists, they need the ability to communicate complex topics in an understandable way and keep up-to-date with new developments in biology [15].

Based on the explanation above, the aim of the research is to perform a bioinformatics analysis in the whole genome mtDNA (16,979 bp) of many chicken breeds. In addition, bioinformatics assignment analysis was also performed to evaluate the genetic structure of the whole genome mtDNA sequence of chickens (*Gallus gallus*).

2. MATERIALS AND METHODS

Total 84 whole genome of mtDNA sequence of chickens (16,797 bp) from Asia, New Guinea and USA were used in this study and obtained from the reference sequence (GenBank) as listed in the Table 1. Therefore, the bioinformatic analysis was performed using four molecular packages of BioEdit [16], MEGA-X [17], DNAsp [18] and Arlequine [19]. Thus, these packages were used to obtain the phylogenetic tree, polymorphism statistical analysis, median joining network and analysis of molecular (AMOVA).

3. RESULT AND DISCUSSION

This study was intended to observe the mate-pairs both having positions in the same chromosome were scanned against the reference genome by analyzing the deviations in insert size across the genome of chicken. Arrangements of chicken orthologous protein-coding genes illustrate the anticipated design of grouping conservation, with the most elevated character in protein-coding exons and negligible identity in introns [20]. A Certainty interim of 2.5 standard deviations absent from the normal embed estimate at both the tails was utilized to control the scattering of the information after calling the insertion and deletion. The certainty interim was calculated for each line independently. The haplotype of some chicken breeds based on the whole genome mtDNA sequence can be seen in Table 1.

The certainty interim was too utilized to form clusters of insertions or deletions utilizing various leveled clustering, where the deviations of mate-pair remove from the normal embed estimate was utilized to develop a similarity index. This approach might recognize tall number of cancellations as compared to additions. Considering combined rate of recognized indels in High growth line (HL), the low growth line (LL). Research showed that a total of 445 mutation sites and 81 haplotypes were obtained in the 84 whole genome of mtDNA sequence of chickens (Figure 1). The chicken reference genome has a substantial number of incorrectly duplicated sequences, according to analysis of the single

read data previously generated (artificial duplications) [20]. This study were formed analysis molecular variance (AMOVA) in the whole genome of mtDNA in chicken from India (18 sequences), Southeast Asia/New Guinea (13 sequences), USA (5 sequences) and China/Japan (43 sequences). Overlaps between this study recognized indels and these counterfeit duplications, considering that the counterfeit duplications secured as it were 105 Mbp, an cover of 30 % of indels is to a much higher division of covers than would be anticipated by chance. In this way, it is likely that the mistakes within the chicken genome gathering, such as the manufactured duplications bestow an vital source of false-positive indels recognized within the current investigation.

The majority of deletions (96%) were discovered in the 5kbp to 12kbp size range, while the majority of insertions (95%) were located in the 1.8kbp to 3.3kbp size range. In comparison to HL, large insertions of greater than 4kb were more common in LL. Most paired-reads (96%) deviating from typical insert size that supported evidence of insertion ranged from 9 to 310 for insertions, whereas most deletions (98%) were supported by paired-reads ranging from ten to 300 in HL and LL, with 88 percent of deletions seen in the range from 12 to 298 (Figure 1). The standard deviation is determined by the size of the insertion/deletion and the number of paired-reads that differ from the normal insert size. The majority of standard deviations for insertions and deletions were in the range of six to twenty for insertions and 7 to 47 for deletions.

The deletions and insertions were used to identify the deletions and insertions, although the coverage of reads from the matching pipeline on each base pair position in the deletion area did not support all of the regions to be deleted, as many sequence reads were mapped within the allegedly deleted region [21]. In HL, a sub-analysis was carried out on chromosome 15. On chromosome 14, which has 358 areas diagnosed as deletions using mate-pair reads, the read coverage from the matching pipeline was determined. For chromosome 15 in the HL, the areas overlapping with the duplicated regions in the chicken genome were deleted in a sub-analysis. In putative deleted areas found using paired reads, a median coverage of zero (of reads mapped by the matching pipeline) resulted in 95 deleted regions.

The chicken genome contains 1.1 gigabytes [22]. The chromosome 1 is the biggest, at 200 Mbp. The pipeline takes a long time to conduct the analysis, and this chromosome uses a lot of memory. In order to finish the study of larger chromosomes with smaller chromosomes, memory and time resources for scanning the entire chicken genome for insertions and deletions must be optimized. When compared to the methodology of calling deletions based on the coverage of reads over sections of the genome, our method found a substantial number of deletions. In domestic hens, only 1300

deletions bigger than 100 base pairs were discovered [23]. 140,484 polymorphisms in unique DNA were discovered after analyzing small insertions and deletions [24].

It is conceivable that most of the cancellations are untrue positives and assists refinement within the approach is required to extend the likelihood of finding especially genuine erasures and insertions. One of the

Table 1. The haplotype of some chicken breeds based on the whole genome mtDNA sequence 1

No.	Breed / Species	Origin	GenBank	Hap.	No.	Breed / Species	Origin	GenBank	Hap.
1	Gallus spadiceus	-	AP003321	1	43	Jabouillei	China	GU261696	43
2	Gallus gallus	-	AP003322	2	44	Hainan	China	KY039436	44
3	Murghi	India	GU261709	3	45	Gallus spadiceus	China	GU261690	45
4	Murghi	India	GU261707	4	46	Gallus spadiceus	China	GU261693	46
5	Haringhata Black	India	KP211420	5	47	Gallus spadiceus	China	GU261692	47
6	Kadakhath	India	KP211425	6	48	Tibetian	China	MK163562	48
7	Ghagus	India	KP211419	7	59	Huaiyang	China	GU261701	49
8	Murghi	India	GU261708	8	50	Hengshan	China	KP244335	50
9	Gallus gallus	India	KP211423	9	51	Hunan	China	GU261681	51
10	Aseel	India	KP211418	10	52	Huang Lang	China	KF954727	52
11	Nicobari Brown	India	KP211422	11	53	Jiangbian	China	GU261714	53
12	Gallus gallus	India	GU261685	12	54	Jiangbian	China	GU261713	54
13	Gallus gallus	India	GU261698	13	55	Jinhu Wufeng	China	KR347464	55
14	Gallus gallus	India	HQ857212	14	56	Nandan	China	KP269069	56
15	Gallus gallus	India	HQ857209	15	57	Shouguang	China	MK163560	57
16	Gallus galus	India	HQ857210	16	58	Taoyuan	China	KF981434	58
17	Gallus gallus	India	HQ857211	17	59	Tibetian	China	DQ648776	59
18	Gallus gallus	India	GU261680	18	60	Xuefeng	China	KF826490	36
19	Gallus gallus	India	GU261697	19	61	Xuefeng	China	GU261675	60
20	Tellichery	India	KP211424	20	62	Wuding	China	GU261676	61
21	Gallus gallus	Philippine	KY039399	21	63	Xianju	China	GU261677	62
22	Gallus gallus	Indonesia	KY039425	22	64	Yunnan	China	GU261702	63
23	Gallus gallus	Vietnam	KY039430	23	65	Gallus spadiceus	China	GU261706	64
24	Gallus gallus	Laos	AP003319	24	66	Gushi	China	GU261678	65
25	Gallus gallus	Laos	GU261687	25	67	Yunnan	China	GU261702	66
25	Gallus gallus	Laos	GU261682	26	68	Tengchongxue	China	GU261689	67
27	Gallus gallus	Thailand	MG605671	27	69	Tengchongxue	China	GU261688	68
28	Gallus gallus	Myanmar	GU261700	28	70	Chigulu	China	GU261705	69
29	Gallus gallus	Myanmar	GU261691	29	71	Chigulu	China	GU261684	70
30	Gallus gallus	Myanmar	GU261704	30	72	Chigulu	China	GU261719	71
31	Gallus spadiceus	Myanmar	GU261716	31	73	Chigulu	China	GU261715	72
32	Gallus spadiceus	Myanmar	GU261703	32	74	Chigulu	China	GU261717	73
33	Silky	Japan	AB086102	33	75	Wenshanshandi	China	GU261699	74
34	Gallus gallus	New Guinea	KY039394	34	76	Nixi	China	GU261711	75

conceivable perplexing variables within the data set of indels recognized utilizing mapping distances between mate pairs may well be the holes and copied locales within the chicken genome, as such blunders are not considered within the calculation of huge indel scanning.

exclude those with coverage in the suspected deletion region, the regions with a median coverage of zero were chosen, and 73 on chromosome 13 in HL were determined to be among them. Further research can be done to determine the precise breakpoints of the

Table 2. Analysis molecular variance (AMOVA) in the whole genome of mtDNA in chicken from India (18 sequences), Southeast Asia/New Guinea (13 sequences), USA (5 sequences) and China/Japan (43 sequences)

Source of Variation	Degree of Freedom	Sum of Squares	Variance Components	Percentage of Variation (%)
Among populations	3	140.74	1.74	9.12
Within populations	78	1354.86	17.37	90.88
Total	81	1495.60	19.11	100.00

The pairing pipeline discards the majority of the reads because one of the pairings does not align in the genome. This could influence insertion and deletion scans because most of the reads of typical insert size that are lost will reduce coverage in the putative indel region, causing the heterozygous indel to be mistaken for a fixed putative indel [25]. Indel sizes can potentially be miscalculated as a result of this. Because some people may be segregating for the non-inserted/deleted allele, using a pooled sample can lead to the identification of some indels as heterozygous. Because the sequencing was done on a pooled sample, it's probable that some heterozygous indels were mistaken for homozygous indels. The indel's zygosity is also influenced by the confidence interval of the entire mate-pair library and the significance threshold.

This will necessitate more research to refine the settings, as well as some extra processes to identify real homozygous indels. In addition to the studies performed with the big indel tool, mate-pairs from the pairing pipeline were used to check the coverage of reads from the matching pipeline in the regions indicated as probable deletions by the mtDNA lite large indel tool. Some of them received a lot of attention. As a result, if some of them are found to be false positive deletions, more investigation is required to rule them out. Because some mate-pairs match incorrectly in the pairing pipeline and are considered significantly deviated from the normal insert size by the large-indel tool, one of the possible reasons for detecting a high number of false positives in regions along duplications in the genome is because some of the mate-pairs match incorrectly in the pairing pipeline and are considered significantly deviated from the normal insert size by the large-indel tool. Analysis of short insertions and deletions revealed the presence of high numbers of tandem duplications.

There is also some extra quality-based filtering of the reads that is required [26]. Further analysis was carried out to delete duplicated areas in chromosome 13, which resulted in the elimination of 83 deletions while reads from the matching pipeline were still present in the putative deletions discovered by the large-indel tool. To

deletions. We propose aligning the unmapped reads in the genome by separating them into two portions and allowing a space between them that is likely present in the genome assembly due to pertinent indels. In the artificial genome, there are over 8000 indels of chicken genome [27]. Furthermore, the high standard deviation of the insert size in the three chicken lines also limit the possibility of finding true indels.

The reference genome assembly mapping, the algorithm used for matching the reads to the reference genome, and the algorithm and parameters used to scan for the genome for identifying probable insertions and deletions all play a role in indel detection utilizing mate-pairs. DNAsp Lite's matching algorithm and large-indel-tool take into account the chicken genome's gaps and duplications while searching for insertions and deletions. In the chicken genome, artificial duplications have been discovered as an artifact [28]. It's probable that the gap and duplication regions will make true indel detection more difficult. Splitting the readings into equal sections of 23 bp each and realigning them with the reference genome to determine the indels' breakpoints is one method for identifying actual indels [29].

Due to not all reads are created equal, it's possible that only a small percentage of them will be identified. Because all reads are not uniformly separated after splitting from one other to cover the deletion or insertion, this may only identify a small number of reads. The resequencing for each line provided about 7x coverage, which is insufficient to align a significant number of reads to detect breakpoints using the split sequence technique. Following the discovery of breakpoints, multiple new fake reference sequences might be generated, each corresponding to the sequence surrounding a putative indel breakpoint discovered using the splitseq method. These artificial references might then be used to map any unmapped reads that have a read pair that was mapped in the matching pipeline using an alignment approach that allows gaps in the alignments to be inserted. This will make it possible to calculate the percentage of reads in the pool that have been deleted. The evidence is backed up by the reads.

American are descended from the most common haplotype in chickens found throughout the world, which may have its origins on the Indian subcontinent.

In the mtDNA of the chicken populations, there was very little polymorphism. The chicken breeds of northeast India and Niooabri brown India are distantly

linked to red jungle fowl. All of the Asia chickens were grouped together in one region, suggesting that they came from the Asia subcontinent. Our findings will be useful in determining the mtDNA haplotypes of Asian, American, and New Guinea chickens, as well as in researching the origins of domestic chickens.

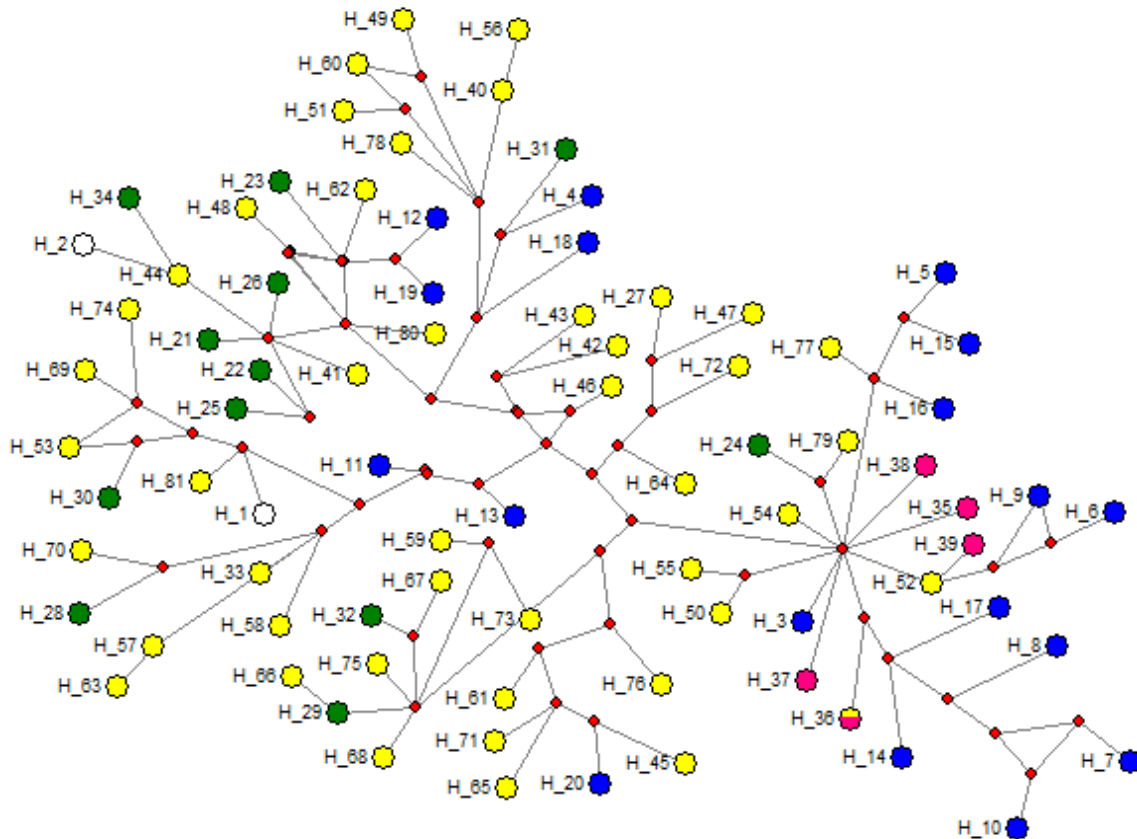


Figure 2. Median joining network of chicken breeds from some regions of India (blue), Southeast Asia/New Guinea (green), China/Japan (yellow) and USA (pink). White circle is other population

4. CONCLUSION

The results of this study were shown that the close genetic relationship was observed among chickens of Asia based on the median-joining network. Meanwhile, the geographical factors were contributed about 9.12% in a sequence variation.

AUTHORS' CONTRIBUTIONS

Suhardi conceptualization of the study, acquisition, analysis and interpretation of data. Widya Pintaka Bayu Putra formulation of proposed strategies, manuscript writing, as well as finalization of edits and revisions

necessary. Rofik provided substantial contribution to theory development and organization of data. Servis Simanjuntak and Khoiru Indana provided material discussion on theory development and critical evaluation of research data and content. Ari Wibowo critical evaluation of research data and content, and facility sourcing as well as checking for manuscript revisions necessary.

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