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Morphometric Diversity and Genetic Relationship of “Bangkok” Chicken (Thai Game Fowl) in East Java, Indonesia

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Abstract

Commercial chickens that were selected for both meat and egg production were domesticated from the descendants of red junglefowl (*Gallus gallus*) species that have been widely spreading in Asia, including Indonesia. There were an estimated 32 strains of local chicken in Indonesia with high morphological diversity. The one of contributors to the diversity of Indonesian local chicken is called “Bangkok” chicken. As a reference to the name of the chicken, it is a suspected descendent of Thai game fowl (*Gallus gallus domesticus* Linnaeus, 1758) which was introduced in Thailand region many years ago. The objective of this study was to explore the existence of this chicken related to the genetic relationship with the Indonesian local chicken. This study was conducted in East Java Province which covered sample areas Banyuwangi, Pasuruan, and Madiun Regencies. The materials were “Bangkok” chicken offspring with a total of 450 birds. The observed variables consisted of qualitative and quantitative morphological characters, both male and female of adult chickens (1 yr to 1.5 yr old). The highest frequency of the comb shape was Single comb (36.9 %) and the lowest was Rose shape (11.8 %). While the highest frequency of shank colour was blackish-yellow (45.6 %) and the white color (3.6 %) was the lowest. The closest genetic distance base on morphometric diversity was between Pasuruan and Banyuwangi chickens (96.04). It was suspected due to the distance area between Pasuruan and Banyuwangi closer than Madiun and Banyuwangi, while Madiun and Pasuruan was the farthest genetic distance (682.03). Hence, the breeding program of Bangkok chicken based on the genetic distance needs further consideration to prevent inbreeding from occurring. Otherwise, the closest distance should be called for to upgrade the purity of the Bangkok chicken ancestor.

Keywords: Cock, Comb, *Gallus gallusdomesticus* (Linnaeus, 1758), Genetic distance, Morphological character, Native chicken, Shank color

1. Introduction

Native chickens are included in the chicken family and there is no comprehensive information on strains or breeds. These local chickens are raised by farmers and generally have a large phenotypic diversity (FAO, 2012). However, this animal diversity is important for the life and food security of small-holder farmers (Widodo *et al*., 2019). The genetic potential of native chicken to meet changing environmental conditions is a potential source of genes related to resistance or tolerance among emerging diseases and is compatible with consumer preferences for meat and egg products (Carbales, 2013); while the ancestor of Indonesia's local chicken was also descendent from *Gallus gallus* species. Hence, chicken has been one of the genetic resources of animal biodiversity in Indonesia. The Indonesian local chickens have different appearance characteristics that were found around 32 types of local chickens in Indonesian, namely Ayunai, Balenggek, Banten, Bangkok, Burgo, Bekisar, Cangehgar (or Cukir or Alas), Cemani, Ciparage, Gaok, Jepun, Kampung, Kasintu, Kedu (Black and White Kedu), Pelung, Lamba, Maleo, Malay, Merawang, Nagrak, Nunukan, Nusa Penida, Olagan, Rintit or Walik, Sedayu, Sentul, Siem, Sumatra, Tolaki, Tukung and Wareng (Nataamijaya, 2010; Iskandar 2012; Widodo *et al.,* 2022). However, Rusdin *et al.* (2011) reported that were found around 31 local chicken strains in Indonesia with different morphological diversity.

The total population of Indonesia's local chickens was about 312 × 10^6 birds in 2019 (BPS, 2020). While, by 2020, the world's chicken population has reached more than 33 ×10^9 birds and about 46 % of them were in the Asian continent (FAO 2022). Therefore, the potency of native chicken is high, which can be important to fulfil protein demand. Sulandari *et al.,* (2008) reported that Indonesia is the one of main centres of chicken domestication in the world. The one contributor to the genetic diversity of Indonesia local chicken is “Bangkok” chicken or Thai game fowl chicken. This chicken was presumably developed genetically in part of Thailand

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region as a reference to the name and then famous as a fighting chicken. While having a variety of colors ranging from solid white to grey, black, and even brown, Thai chicken is recognized by their black feathers with metallic green sheen and blood red back feathers, and neck hackles. This is a stereotypical depiction of Thai game birds, as most of them have been acknowledged based on this particular bird (Duengkae *et al*., 2021; Oocities.org, 2009). Thus, when this chicken was introduced to Indonesia, it was popular as a fighting cock and the popularity is suitable with the culture of cockfighting in some Indonesian communities and areas as well.

"Bangkok" chicken was suspected to have been introduced to Indonesia firstly in the city of Tuban, East Java Province of Indonesia around 1980. This chicken was introduced by the people that like this chicken in that area. However, it is not clear who was the first person to introduce and develop this chicken in Indonesia (Tika, 2017). Therefore, a study to determine the potential of "Bangkok" chicken offspring is important since the potential of additive genes introduced to Indonesia local chickens can be utilized to improve the genetic quality.

The objective of this study was to determine the morphometric characteristics of Thai game fowl chicken offspring in East Java related to finding the genetic variation and its relationship among chickens that was spreading in East Java Province. Also, the genetic data and distance will be useful for the genetic selection of filial Bangkok chicken related to meat and egg productivity. The morphometric and meristic characteristics can help in the recognition and classification of the species (Hossain *et al.,* 2016; Nawer *et al.,* 2017). This data is very useful in the preparation of a superior future Bangkok chicken breeding program. This study can also use additional information that the East Java area is the center of Thai game fowl offspring chicken in Indonesia.

2. Materials and methods

2.1. Sampling area

The sampling materials were taken from three areas of East Java Province, consisting of Pasuruan, Madiun, and Banyuwangi Regencies (Figure 1). This province is located in the coordinates of 1110 4' to 1140 4' E and 7012' to 804' S. The total area is 47 963 km^2 which includes two main parts, namely East Java mainland and the Madura Islands. The mainland area is 88.70 % or 42 541 km^2, while Madura Island area is 11.30 % or 5 422 km^2. The total population in 2020 reached 39 886 288 inhabitants (BPS Jatim, 2020), while the total local chicken population in 2019 was estimated 39 291 778 birds (Dispet Jatim, 2020); and East Java Province is the second largest local chicken population in Indonesia with 36 609 094 birds after Central Java by 40 633 383 heads (BPS, 2020).

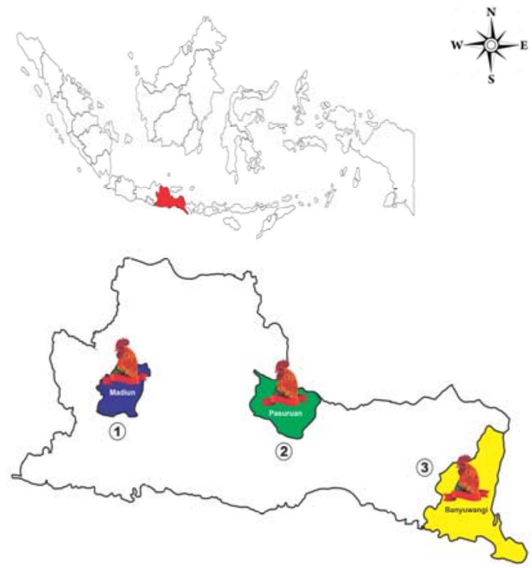


Figure 1. Map of sampling chicken area in East Java Province, Indonesia (red color). The regencies of sampling area are as follows (1) Madiun, (2) Pasuruan and (3) Banyuwangi.

2.2. Sampling materials and equipment

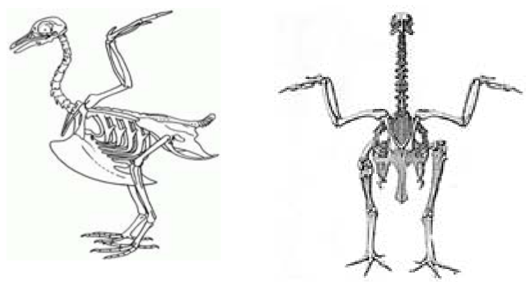
A total of 450 chickens were chosen for analysis in the study area. Each regency area sampled about 150 chickens. The study area was determined by considering based on chicken population number, agroecology, and socioeconomic that supported chicken growth. The geographical map of the study area is illustrated in Figure 1, while the observation and measurement of chicken morphology were applied on adult layer female and male chickens by around 2 yr old.

The main equipment used in this study consisted of i) measuring tools for quantitative traits, including measuring tape with a scale of 150 cm, digital hanging scales with a capacity of 10 kg, and a metal ruler; and ii) supporting tools for activities in the field such as baskets, gloves, masks, stationery, GPS and documentations kits. The main activity of this study was to collect the existing data, primarily on phenotypic data, including quantitative and qualitative data obtained directly from the study area. The phenotypic data were then tabulated and followed by data analyzed using descriptive analysis, nested design, and discriminant analysis.

The data source was phenotypic variables including quantitative and qualitative characters of chickens that were recorded following the FAO (2012) standard descriptor. A physical descriptors list was determined to record both quantitative and qualitative morphological characters. In each study area, the selected household owner of chicken was identified as having experience in rearing "Bangkok" or Thai game fowl chicken for more or less than one and a half years. Furthermore, the selected chicken owners were depth interviewed to describe the family's historical rearing "Bangkok" chicken and the history of chicken origin.

2.3. Morphological data collection

Quantitative traits of body morphological characters were obtained from chickens aged around one and a half to 2 yr old. Body weight was weighed using a scale, while chicken morphological measures cover back length (taken when the chicken is standing, then taken from the curve of the chicken's neck to the end of the tailbone), beak length (from the tip of the beak to the base of the beak), shank length (from the hock joint to the spur of either leg), chest width (from the tip of the right chest to the tip of the left chest horizontally), chest circumference (circular from the tip of the pectus [hind breast] to the of front chest), wing length (the distance between the tips of right and left wings when fully stretched) and the 3rd finger length (from the tip of the chicken's 3rd toe to its base) (Figure 2). The qualitative variables observed were the shape of the comb and the color of the shank (Figure 3). Body morphological measurements and photography of chickens (front, side, and back) were carried out by placing the chicken on its two legs and placing it on a flat surface withholding it; hence, the chicken did not escape.



a. Left side b. Front side

Fig. 2. The topological morphology measurement on Thai game fowl chicken (picture modified from Bell, 2002; Mustefa *et al*., 2021)

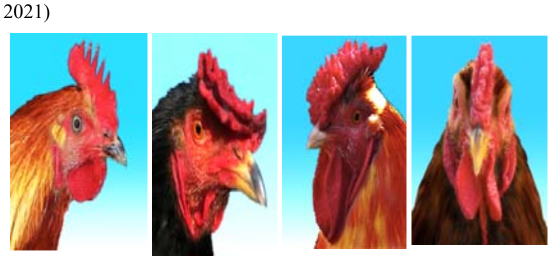


Figure 3. The comb shape found in "Bangkok" chicken (a. Single comb; b. Pea comb; c. Rose comb; and d. Walnut / Strawberry comb) (adapted from Imsland *et al.,* 2012)

2.4. Data analysis

The data measurement from chicken morphometric variables, both quantitative and qualitative data, was analyzed using descriptive analysis, based on frequency and cross-tabulation for comparisons between study areas. Nested analysis was applied to determine the differences between "Bangkok" chickens that were nested in the three sampling areas. The linear mathematical model for the two-staged nested design (Adinurani, 2022; Montgomery, 2008) in Equation (1):

$$Y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \varepsilon_{\kappa(ij)} \quad \begin{cases} i = 1, 2, ..., a \\ j = 1, 2, ..., b \\ k = 1, 2, ..., n \end{cases} \qquad (1)$$

Where;

Y_{ijk} = an observation factor ***a*** at level -i_{th}; factor ***b*** at level -j_{th}; and repetition -k_{th}

μ = mean value

τ_i = effect of group -i_{th}

$\beta_{j(i)}$ = effect of sub-group -j_{th} in group -i_{th}

$\varepsilon_{\kappa(ij)}$ = error of sub group -j_{th} in group -i_{th} with repetition -k_{th}

Furthermore, to calculate the genetic distance and the morphometric genetic similarity relationship, the discriminant function was applied by calculating the genetic distance from Mahalanobis and the quadratic minimum distance function is calculated using the Nei formula (Fatmarischa*et al.,* 2014), in Equation (2):

$$D^2 = (m - x)^T . C^{-1}(m - x) \qquad (2)$$

Where,

D^2 is the square of the Mahalanobis distancefor genetic distance between Bangkok chicken

x is the vector of the observation (row in data set for quantitative and qualitative variables of chicken)

m is the vector of the mean values of independent variables (mean of each column for quantitative and qualitative variables of chicken)

$C^{(-1)}$ is the inverse covariance matrix of independent variables

Several procedure analyses were performed by SAS platform. From the analysis, it is expected that the morphological variations and genetic relationship among Thai game fowl offspring chickens will identify in the selected sampling area. This research was conducted with the Description of Ethical Approval No.5.a/048.a/KEPK-UMM/III/2022 issued by the Faculty of Medicine, University of Muhammadiyah Malang.

3. Results and discussions

3.1. The qualitative traits of "Bangkok" chicken offspring

The qualitative traits determined in this study consisted of the comb shape and shank color of chickens. The comb shape that was mostly found in "Bangkok" chicken offspring was a Single (36.9 %) (Fig. 3.a, Fig. 4 c and Table 1), then followed by Walnut or Strawberry comb (32.7 %), Pea (18.7 %) and Rose (11.8 %). This result corresponds to Rafian *et al.,* (2017) study on the local chicken "Burgo" from Bengkulu Province, Sumatra, and Arlina *et al.,* (2015) on Kokok Balenggek, a local chicken from West Sumatra Province, Indonesia with a single comb was the dominant comb. The major single comb of Indonesian native chicken was also found by Maharani *et al.* (2018; 2021) with a dominantly black color on the chest feather, whereas Permadi *et al.* (2020) study showed that people of Tirtomulyo Village, Kendal Regency of Central Java preferred raising single comb (53.3 %) for their backyard chicken. Another study by Asmara *et al.* (2019) stated that Pelung chicken, a local chicken from

West Java, has single comb dominantly. The majority of single-combed local chicken was also found in Saral village of Chhajjian valley, Haripur district in Khyber Pakhtunkhwa, a province in Pakistan, by 92.50 % (Bibi *et al.,* 2021), in "Zoar" – chicken native to Mizoram, India – by 74.63 % (Lalhlimpuia *et al.*, 2021), and even in Black-bone chicken – a native to Thailand – by 100 % (Buranawit *et al.,* 2016). The type of combs in chicken is supposedly related to climate adaptability; for instance, single comb complies with warm temperature. Single comb is the wild type of native chicken genetic with recessive genotype (*rr pp*) (Imsland *et al.,* 2012), and its existence may be the result of genetic selection activities correlated with certain cultural and religious practices. For example, in some areas of the Muslim religion in Ethiopia single comb type was commonly found, while the pea and walnut type was commonly found in non-Muslim religious areas, even in Vietnam (Desta *et al.,* 2013). Nevertheless, the consistency of this finding should be followed by exploring the correlation between comb-type with religion and traditional activities.

a. Pea b. Rose c. Single d. Walnut/Strawberry

Figure 4. The comb shape of "Bangkok" chicken from sample area of East Java Province, Indonesia.

Table 1. The frequency of comb shape on Bangkok chicken offspring

Comb shape	Frequency	Percentage (%)	Total cumulative
Pea	84	18.7	18.7
Rose	53	11.8	30.4
Walnut	147	32.7	63.1
Single	166	36.9	100.0
Total	450	100.0	

The analysis of the shank color of "Bangkok" chicken offspring from 450 samples showed that the highest frequency was blackish yellow by 205 chickens (45 %) and the lowest was blackish white by seven chickens 1.6 %). This finding was in line with Arlina *et al*. (2015); Maharani *et al.* (2018); and Tamsil *et al.* (2020) that yellow and white or a mixture of these color were the dominant shank color found in Indonesia native chicken. Similar studies found that white and yellow were dominant in native chicken shanks in Bangladesh (Sharker *et al.,* 2014), in South-Western Ethiopia (Bayou *et al.,* 2022; Tadele *et al*., 2018), in Eastern and Western Samar of the Philippines (Godinez *at al.,* 2020), and in transboundary area of Jammu and Kashmir in India (Singh *et al.,* 2022).

From an economic viewpoint, the shank color is a very important trait due to the different consumer preferences prevalent in different areas of Korea (Jin *et al.,* 2014). The pigmentation of non-feathered or plumage tissue, in this case, is body skin and shank involving carotenoids and melanin which are responsible for yellow and black color respectively (Gowda *et al.,* 2020). The white color of chicken skin is carried by the dominant allele while the yellow color is homozygous for the recessive allele (Lalhlimpuia *et al.,* 2021). The finding of a low proportion of white skin color in this recent study indicated that there was a small frequency for the dominant allele. In addition, skin color is a genetic trait related to carotenoid pigments and is also related to the type of nutrition, adaptive fitness, and health conditions (Lalhlimpuia *et al.,* 2021). The high frequency of yellow color in this study suggested that the availability of feed sources was almost uniform in the rearing system besides breeder selection practiced towards specific characters related to fighting cocks. Skin pigmentation in chicken such as shank color is related to the levels of carotenoid and melanin (Jin *et al.,* 2014). In addition, an SNP study of Korean native chicken stated that the strong candidate for pigmentation was *MC1R* gene or equivalent with *E* locus.

Table 2. Frequency of shank color on "Bangkok" chicken offspring

Shank color	Frequencies	Percentage (%)	Comulatif
Black	13	2.9	2.9
Blackish white	7	1.6	4.4
Yellow	190	42.2	46.7
Blackish yellow	205	45.6	92.2
Yellowish white	19	4.2	96.4
White	16	3.6	100
Total	450	100	

Figure 5. The variation color shank of "Bangkok" chicken offspring in East Java, Indonesia

3.2. Morphometric measurements of "Bangkok" chicken offspring

In the rearing of "Bangkok" chicken offspring, farmers' practices vary from free range to semi-intensive system. However, the coefficient of variation (CV) of body measurements was still acceptable. Aronhime *et al.* (2014) defined reproducibility parameters as excellent when CV is ≤ 10 %, good when CV is between 10 % to 20 %, acceptable when CV is between 20 % to 30 %, and poor when CV is 30 %. The coefficient of the diversity of body measurements in Banyuwangi Regency was the highest score by 7 % to 25 % (Table 3). This was presumably due to the rearing chicken practical gap between breeders. The field observations showed that some breeders applied intensive rearing for selected superior male chickens to fight cock beside the free-range system. This different rearing system also affected the diversity of body measurements, like body weight in each regency. However, feeding and health management could also impact chicken performance. According to Prasetyo (2017) and Tonda *et al.* (2022, 2023) the variation in body weight could be influenced by several factors, like rearing management, feeding, and health management. The coefficient of variance (CV) of body measurements between sexes was ranged from 7 % to 24 %. This score also allowed for data analysis since it was in the range of normal distribution. The body weight of the female showed was higher than male chicken as shown in Table 4. According to Daikwo *et al.* (2011), male body weight is higher than females due to the dimorphism sexual of chickens that are regulated by genetic mechanisms. Body weight in male chickens can grow faster than in females because of their genetic capability (Kalita *et al.,* 2017). According to Petkov *et al.* (2020), the existence of male chickens in a group begins to accumulate negative effects from the age of 28 d after hormonal changes, sex competition, and sexual dimorphism which began to appear at the age of 35 d. Thus, in this period females will experience male-dominated stage which could affect stress and decreasing feed consumption.

Table 3. The body measurements parameter of "Bangkok" chicken between sample areas

Body measurement	Region			Total
	Banyuwangi	Madiun	Pasuruan	
	x̄±sd (% CV)	x̄±sd (% CV)	x̄±sd (% CV)	x̄±sd (% CV)
Sample number (birds)	150	150	150	450
Body weight (g)	1 781.46±536.47 (25)	2 366.74 ± 749.46 (19)	2 463.19±549.65 (18)	2 203.60±667.36 (10.03)
Shank length (cm)	9.74±1.35 (12)	11.21±1.75 (10)	11.33±1.46 (10)	10.760±1.69 (5.32)
Beak length (cm)	1.99±0.12 (7)	2.00±0.15 (7)	2.04±0.19 (7)	2.03±0.15 (3.56)
Breast depth (cm)	8.69±1.09 (12)	9.42±1.30 (11)	10.965±1.20 (19)	9.69±1.53 (5.17)
Brisket length (cm)	21.24±2.36 (10)	23.54±3.12 (9)	23.42±2.06 (9)	22.73±2.76 (4.76)
Breast circumference (cm)	29.67±4.51 (11)	34.20±4.16 (9)	34.17±3.19 (9)	32.68±4,52 (4.90)
Wing length (cm)	20.16±2.33 (8)	21.44±2.60 (8)	18.88±2.89 (9)	20.16±2,81 (4.22)
Third toe length (cm)	6.59±1.04 (14)	8.46±1.37 (11)	8.54±1.01 (11)	7.86±1.46 (5.70)

Table 4. The variation of body measurement between "Bangkok" chicken offspring based on sex

Body measurements	Sex	
	Male x̄±sd (% CV)	Female x̄±sd (% CV)
Sample number (chickens)	225	225
Body weight (g)	2 580.71±695.75 (17)	1 826.88±423.37 (24)
Shank length (cm)	11.68±1.72 (10)	9.84±1.03 (12)
Beak length (cm)	2.05±0.18 (7)	1.98±0.11 (7)
Breast depth (cm)	10.21±1.59 (10)	9.18±1.27 (11)
Brisket length (cm)	23.66±2.59 (9)	21.81±2.26 (10)
Breast circumference (cm)	34.56±4.37 (9)	30.796±3.83 (10)
Wing length (cm)	21.54±2.62 (8)	18.786±2.28 (9)
Third toe length (cm)	8.52±1.20 (11)	7.2±1.41 (12)

3.3. Genetic distance between "Bangkok" chicken offspring

The analysis of similarity and admixture within and between regency of local chickens (Table 5) showed similarity rates ranging from 80.7 % to 86 %. The highest percentage of similarities among area was Pasuruan (86 %), followed by Banyuwangi (80.7%) and the lowest was Madiun regency (72%).

Table 5. Percentage of similarity and admixture within and between sampling area

Area (Regency)		Area (Regency)			Total
		Banyuwangi	Madiun	Pasuruan	
Banyuwangi	N	121	26	3	150
	%	80.7	17.3	2	100
Madiun	N	25	108	17	150
	%	16.7	72	11.3	100
Pasuruan	N	6	15	129	150
	%	4	10	86	100

Notes: N = number of chicken

The diversity of chicken species in the population can be caused by many reasons, but mostly by genetic factors. Segregation of genetic traits in native chickens was influenced by uncontrolled genetic selection practices for a long time resulting in unique traits and providing materials for genetic map study (Wragg *et al.,* 2012). Hence, the genetic structure provides an opportunity to study the effects of artificial selection and ecological influences on the morphological structure of domestic chickens (Desta *et al.,* 2013).

Native chicken populations are greatly genetic variation due to their long-term adaptation in response to varying agroecological zones. Native chickens have unique adaptive traits that enable them to survive and reproduce under harsh conditions, like climate, nutrition, and management which are usually associated with low input–output production systems (Maw *et al.,* 2015). Thus, the difference between the area of "Bangkok" chicken offspring in this recent study can lead to the variation of chicken traits.

The genetic distance matrix between areas ranged from 96.04 to 682.03 (Table 6), and the closest genetic distance was between Pasuruan and Banyuwangi (96.04). This was presumably because the distance area between Pasuruan and Banyuwangi is close compared with Madiun and Banyuwangi. On the other side, the distance between Madiun and Pasuruan is far, thus the genetic distance was farther. According to Fatmarischa *et al.* (2014), the estimating of genetic distance through body measurement can be useful for an initial step in determining of genetic relationship between geographical areas, and the length of genetic distance was suspected caused by the slow deployment of chickens from one site to another of geographic location.

Since domestication, chickens have been distributed across countries, continents and cultures. Breeds originating from the same geographical area are spread across a spectrum of genetic diversity, especially breeds of European and Asian types. When based on sampling area, genetic diversity can be correlated with geographic distance to the wild type fowl of chicken, *Gallus gallus* within Asian breeds. The pattern of genetic diversity in a population can be better explained by the geographic expansion of its ancestor groups, which correlates with genetic differentiation. Genetic distance between domesticated chicken populations and their wild relatives can predict the genetic diversity of domesticated chicken populations.

Table 6. The genetic distance matrix between sampling area

Sampling Area	Sampling Area		
	Banyuwangi	Madiun	Pasuruan
Banyuwangi	0.000		
Madiun	586.03	0.000	
Pasuruan	96.04	682.03	0.000

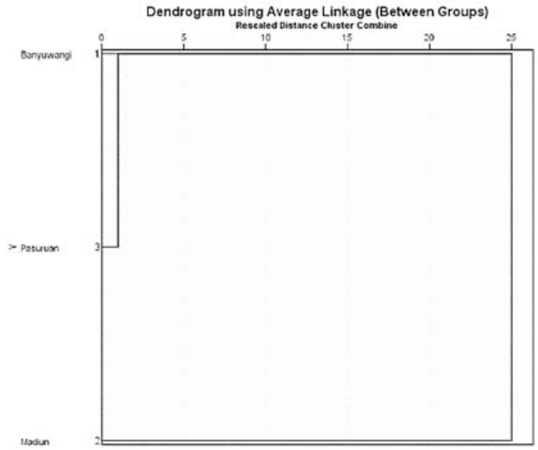


Figure 6. The dendogram of genetic distance of "Bangkok" chicken between sampling area

The dendrogram which confirms genetic distance matrix of "Bangkok" chicken offspring between areas (Table 6) is shown in Figure 6. The genetic distance between Banyuwangi and Madiun was farther than between Banyuwangi and Pasuruan regency. The overall genetic diversity might be the result of population-specific events, such as mutation, natural selection that favors adaption to the current environment, and/or artificial selection (for example for certain production traits) and population genetic drift as well (Malomane *et al.,* 2021). Many local breeds were developed due to the diversity of geographical conditions and the lack of gene flow. For poultry, the genetic flow should be made possible by carrying eggs from one area to another (Bao *et al.,* 2009).

In the case of the "Bangkok" offspring chicken which assumed that the ancestor was originated from Thailand's native chicken was related to the practice of crossbreeding between captured Jungle fowl and native chicken for cockfighting and sports game bird in Thailand and Philippines (Maw *et al.,* 2015). Native chickens of Thailand, Myanmar and Laos were low in genetic diversity when compared to other Asia chickens (Indonesia, China, Nepal, and Vietnam), leading to low diversity based on egg white protein polymorphism. From the center(s) of domestication in Asia, chickens colonized the world through human migration and terrestrial and maritime trading routes (Lawal and Hannote, 2021).

4. Conclusions

The study of "Bangkok" offspring chickens in Indonesia that spread over the East Java region has generally high similarity (72 % to 80 %) based on morphological variables. Thus, the genetic variation is low. Although geographically separated, the similarity is still high. This correlated with the previous studies that local chickens in the ASEAN region, including Indonesia, generally tend to have high similarity although they are separated geographically. Genetic diversity can change more rapidly within genes associated with protein transport and lipid metabolic processes. Thus, these genes are thought to be flexible or elastic to changes according to the needs of the population. For further studies, it is necessary to identify gene-based traits that are relevant to the environmental adaptation.

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