



PROBLEMS IN DIVERSITY IN THE COOING OF HYBRID ZEBRA DOVES AND POSSIBLE SOLUTIONS

¹Suyono, ²Isnawati, ³Ahmad Fudhaili, ⁴Ariij Hady Tsana, ⁵Muhammad Zahrudin Afnan

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, State University of Surabaya

^{2,3,4}Department of Biology, Faculty of Mathematics and Natural Sciences, State University of Surabaya

⁵Magister Programme of Biology Education, Faculty of Mathematics and Natural Sciences, State University of Surabaya

Abstract : This study aimed to assess the degree to which different parent cooing traits could be crossed to generate champion cooing offspring. For several generations, fifty pairs of Zebra Doves were crossed. The cooing quality scores of their progeny were assessed using the cooing standards established by P3SI (Indonesian Zebra Dove Conservation Association). The findings showed that 10% of the 50 pairs of Zebra Doves produced offspring with consistent champion cooing from generation to generation, 12% of the pairs produced offspring with only 50% stability of champion cooing from generation to generation, and 6% of the pairs did not reproduce. Zebra Dove crossings produced a variety of cooing offspring, some of whom did not necessarily transmit their parents' cooing traits. A sample of 15 Zebra Dove infants had low cooing quality, scores ranging from 8 to 8.5 (the minimum needed for an excellent category of 8.75). A difference of 4.05% has been seen in the FoxP2 gene sequence between Zebra Doves of champion, medium, and low quality. It is a known fact that the FoxP2 gene exhibits sequence similarities throughout the three groups of Zebra Doves according to their respective Zebra Doves, specifically between nucleotides 1 through 348 and between nucleotides 249 through the final nucleotide (5829). It should be noted that there is little consistency in the quality of Zebra Doves passed down from parents to their offspring. It might be possible to produce champion Zebra Doves differently by mutating the FoxP2 gene.

IndexTerms - FoxP2, gene mutation, Zebra Dove

I. INTRODUCTION

INTRODUCTION

Zebra dove (*Geopelia striata*) is a species of bird in the Pigeon family. Basic greyish-brown feathers, blue-grey head and neck feathers, black and white horizontal stripes on the chest and neck, and a length of around 25 cm are the common characteristics of the zebra dove. This species' endemic distribution stretches from Singapore, Tenaselim, Peninsular Malaysia, Southern Thailand, and the Indonesian islands of Java and Sumatra. It might also be indigenous to the Philippine, Bali, Lombok, Borneo, and Sumbawa [1,2,3]. Zebra doves were once a popular bird in affluent society, owned by kings, aristocracy, royal officials, and municipal authorities [2]. Zebra dove aficionados are currently widely dispersed throughout society, largely due to their exquisite singing abilities, which endear this bird to people's hearts [3]. Their ability to sing has drawn a lot of admirers who want to possess it in society. Zebra dove enthusiasts compete to produce children with the most beautiful vocals when zebra dove singing competitions are held to determine which bird has the finest voice. Additionally, obtaining a Champion Songbird can raise one's social standing, bring notoriety, and yield significant financial gains [3]. These factors, along with the growing interest in zebra doves, make their cultivation a beautiful commercial possibility. Breeders go to great lengths to obtain these advantageous zebra dove breeds to satisfy consumer demand. Starting with conventional farming methods that involve crossing parents who are fertile to controlling nutrition [4][5]. But because their offspring are inconsistent, traditional growing methods are labor-intensive and costly.

Extensive and intense research has been done on the cultivation and study connected to efforts to achieve champion honors in Zebra Doves. It is demonstrated by the large number of breeders of Zebra Doves in Indonesia, particularly Bangkok Zebra Doves, which are expected to reach 1311 by 2021 and are registered with the P3SI (Indonesian Zebra Dove Conservation Association). If you include breeders who are not registered with P3SI, this figure rises even further. This is because the Zebra Dove industry is one that affects the economy and offers significant profit margins [6]. Zebra Doves range widely in price, with champion honors going up to 3.5 billion or even hundreds of millions of money. As a result, a lot of Zebra Dove breeders use different techniques to carry out breeding to produce Zebra Doves that have won champion status.

Crossbreeding is the usual method used in Zebra Dove breeding. Typically, to facilitate crossbreeding, two male and female Zebra Doves are kept together in an 80 x 100 x 90 cm³ cage. In a typical egg-laying season, two eggs are laid and incubated for 14–17 days [7]. Breeders and merchants of Zebra Doves choose male and female parents who have high-quality cooing. Three methods are typically used to pick cooing: first, directly listening to the cooing based on the champion cooing standards established by P3SI. Parents will be chosen based on their completion of the front sound, middle sound, end sound, rhythm, and base sound high score requirements. The goal is to have offspring who inherit their parents' grace. The second method involves choosing the parent based on "KITIR," or a card that has the Zebra Dove's family tree on it. The next approach is to choose the parent by combining the first and second methods, that is, by seeing it cooing and by looking at the crosses' pedigree that are kept in KITIR. The two children that are frequently born from Zebra Dove crossings, neither of which necessarily inherits the cooing of both parents, yield different results for some breeders of Zebra Doves. The degree of variation rises across multiple generations of progeny, and all progeny groups exhibit a highly varied level of grace. Extensive studies on the stability of champion grace inheritance from strip to strip or generation to generation, as well as the efficacy of crossing particular parents to create offspring with champion grace, have not been conducted to date.

By promoting the expression of genes involved in sound and song synthesis, it is possible to improve the quality of the song molecularly. One gene that regulates the production of regulatory proteins, which in turn stimulates the expression of other genes involved in sound production (e.g., singing, chirping) is the FoxP2 gene [8]. The FoxP2 gene's ability to induce the expression of several genes involved in creating champions varies greatly depending on the sequence that produces the protein. Numerous pharmacological substances, including dopamine and its precursors like caffeine, can either trigger gene mutations or activate the expression of the FoxP2 gene. Unsaturated fatty acids, including EPA, AA, and DHA, can both activate and mutate the FoxP2 gene [9][10]. Thus, by giving these substances orally, it is possible to enhance the Zebra Dove's cooing quality.

This research aimed to characterize the grace of the crossing-offspring Zebra Doves and assess how well chosen parent crossings produce champion-graced Zebra Doves that are stable from generation to generation. The objective of this research is to investigate the mechanism of mutation and the activation of the primary gene expression responsible for Zebra Dove vocalization, to develop champion grace Zebra Doves at the molecular level. The study's findings will offer a summary of the two approaches' relative efficacy as well as suggestions for how to innovate a new technique using the mutation mechanism or for the crossover method to continue.

II. RESEARCH METHODOLOGY

There are a number of tools and materials used in this study. The equipment used includes Zebra Dove cages, feeding and drinking equipment, equipment to maintain cage cleanliness, and equipment for bird care [11]. While the materials used are 50 pairs of Zebra Dove with cooing selected by 3 methods, namely listening to the quality of their cooing directly, based on "KITIR" and a combination of both, Zebra Dove food and drink and medicine and supplements. Zebra Dove crossing is done by placing a pair of selected Zebra Doves in one cage so that they mate and produce offspring. The resulting offspring are raised until they are large and their cooing is observed. The offspring observed include several generations to see the stability of the champion cooing of the offspring. The determination of cooing quality is based on the following criteria. The score obtained from a maximum score of 9 in each aspect (**Table 1**)

Examination of the FoxP2 gene sequence requires special DNA extraction KIT materials for animals (Bioflux), PCR buffer, agarose [12]. Meanwhile, the implementation of FoxP2 gene sequencing is carried out by sending the results of FoxP2 gene amplification to PT Genetika Science Indonesia for further processing. The FoxP2 gene amplification process is carried out with various primer pairs as follows. Forward 5'-TTCAGCGATCGCAAAGGGACTTGAGAAACCTCAAATG-3' and reverse 5'-GCTTTCAGAAAGCT TTTGGGTAAGTGGGAAAACCC-3'; forward 5'-TTCCCCCAACCAAGGGACCTCATAACCTG-3' and reverse 5'-ATTGCGGCCGCTTATAAATGGATCAAAAAGAATTATATTTTTTAT-3' [13]. The PCR conditions were as follows: 1 min at 95°C and 35 cycles of 30 s at 95°C, 30 s at 55°C, and 2 min at 72°C, followed by 10 min at 72°C [13].

IV. RESULTS AND DISCUSSION

4.1 Results of Descriptive Statics of Study Variables

The research results presented include (1) Results of Zebra Dove crosses related to offspring cooing criteria, (2) cooing quality profiles of Zebra Dove offspring samples from crosses, and (3) alignment of FoxP2 gene sequences of three Zebra Dove categories based on cooing quality. Each result is presented as follows. The results obtained from Zebra Dove crosses in 50 test cages can be presented in **Table 2** below.

Based on **Table 2**, it can be stated that the results of offspring that have champion cooing from strip to strip (generation to generation) vary greatly (72%), only 10% have stable cooing from generation to generation, there are 12% whose offspring have cooing half following the cooing of their parents, the rest (6%) of the Zebra Dove pairs do not reproduce. Based on the results in **Table 2**, it can be stated that a pair of parents with champion cooing will produce most offspring that do not inherit the characteristics of both parents. The layout of the cage and the results are according to the criteria in **Table 2**, as in **Figure 1** below.

1	2	3	4	5	6	7	8	9	10
11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30

31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50

Figure 1. Layout of turtledove crossing cage to determine the quality of offspring cooing from generation to generation.

The numbers of **Figure 1** indicate the individual Zebra Dove number, the black box indicates that the Zebra Dove does not reproduce, the yellow box indicates that half of the offspring population has cooing like its parent, the green box indicates that the offspring cooing is stable from generation to generation and the red box indicates that the offspring cooing varies. The quality of the Zebra Dove cooing is assessed in 5 (five) categories, namely front voice, middle voice, end voice, rhythm, and voice quality. Front voice, with the criteria of length, swinging/bending, and clean. Middle voice, with the criteria of pressure, complete, and clear. End voice, with the criteria of round, long, and swinging. Rhythm, with the criteria of loose, graceful, elegant, and beautiful. Voice quality/basic, with the criteria of thick, dry, clean/clear, and nasal (ng), Scoring for each category consecutively starts from 8, 8.5, 8.75, and 9 if it is truly maximum (Decision of the 2022 P3SI National Conference: 39). The results of the evaluation of the quality of the cooing of 15 Zebra Dove offspring taken randomly showed that the quality of the cooing was still low with a score between 8 and 8.5. The minimum score to be categorized as good is 8.75 or equivalent to 43.75. Figures 2 and 3 show the results of the evaluation of the quality of the cooing of 15 Zebra Dove offspring taken randomly.



Figure 2. Diagram of Cooing Quality of 15 Zebra Doves in Five Categories (1 front voice, 2 middle voice, 3 end voice, 4 Rhythm and 5 Voice Quality).

Explanation of scores 8, 8.5, 8.75, and 9 in **Figure 2**, respectively represent very low, low, good, and very good quality. So based on the data in the diagram, the results of the analysis can be written that the 15 Zebra Doves of the research sample are classified into low-quality birds in the five cooing categories. Zebra Dove sample number 3 (color) even got a very low score in the five cooing categories. Based on **Figure 2**, it can be stated that the lowest score for each category is between 8 and 8.5. This still does not reach the minimum score to enter the good category, which is at a score of 8.75. This means that the offspring produced from Zebra Dove parents with champion cooing does not guarantee that the offspring have the same cooing quality as their parents.

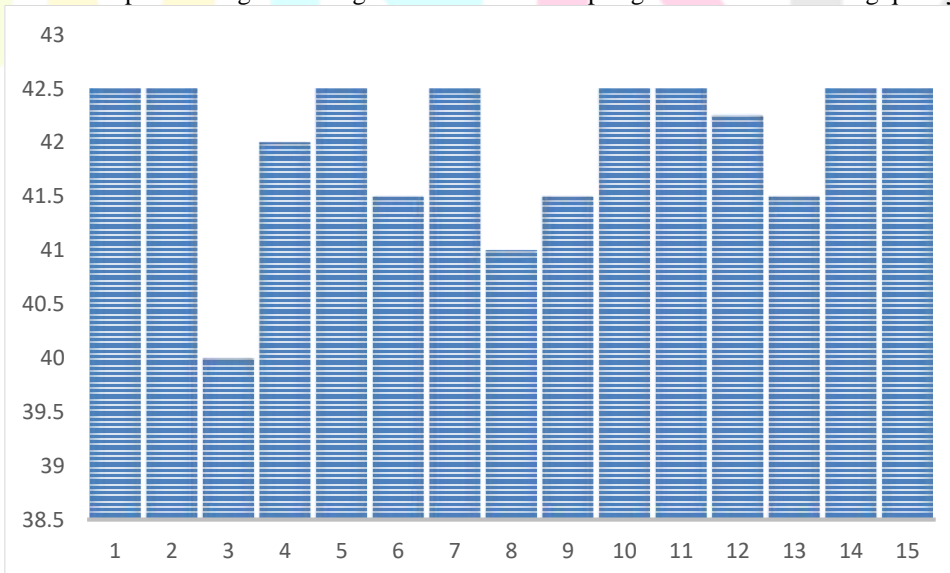


Figure 3. Total Score Diagram of Cooing Quality of 15 Zebra Doves in Research Samples (turtles numbered 1 to 15)

Based on **Figure 3**, it can be stated that in addition to the Zebra Dove sample number 3, there are other Zebra Dove samples that are still of very low qualifications such as birds numbered 6, 8, 9, and 13. To achieve good qualifications, each bird must obtain a total score of 43.75, meaning it gets a score of 8.75 in each assessment category. The fifteen Zebra Doves in the research sample have not achieved good qualifications, they are still of low and very low. The question is, what efforts must be made to improve Zebra Doves with low and very low qualifications.



Based on **Figure 4**, the length of the FoxP2 gene nucleotides in Zebra Doves with good chirping sounds is around 5800 bp with a slight difference in length between Zebra Doves with champion chirping (Zebra Dove A) with a length of 5821bp, Zebra

Doves with average chirping (Zebra Dove B) with a length of 5802bp and Zebra Doves with less good chirping (Zebra Dove C) with a length of 5805bp. The percentage of missing sequences is 0.532%, while the dissimilarity between the three sequences is 4.05%. What is interesting is that the dissimilarity of the three FoxP2 sequences is found in the initial sequence to sequence 348, the next sequence 349 to the last sequence (5829) has similar nucleotides [12,13,14,15].

Based on the above study findings, the quality of children's cooing varies significantly from generation to generation, with many not inheriting the caliber of their parents' cooing. This is because qualities emerging from genetic recombination from their ancestors are always inherited in the pattern of traits inherited through crossbreeding (vertically genetic transfer) [17]. It's very easy to understand why the cooing of Zebra Dove chicks produced by crossbreeding varies greatly from generation to generation. The Zebra Dove chicks inherit 50% of the cooing of the female parent and 50% of the cooing of the male parent, 25% of the cooing of their grandfather and grandmother each, 12.5% of the cooing of their great-grandfather each, and so on [18].

The frequency of crossing over events during oogenesis and spermatogenesis also contributes to generational variations in the cooing of Zebra Dove offspring. In meiosis, crossing over typically takes place during prophase I of cell division [14]. The oogenesis processes that take place in the ovaries of female birds frequently involve this crossing-over event, which causes periodic variations in the content of the egg cells (ovum) that these birds generate. Similar to this, spermatogenesis, which takes place in the male bird's testes, occasionally results in spermatozoa with very variable genetic compositions. It will create zygotes during fertilization, including ovules with genetically regulated quality, whose genetic makeup also varies. It was discovered that a variety of genetic traits, including body size, gender, and morphology, affect bird sounds [19, 20]. In eukaryotic animals, cytoplasmic gene transfer occurs in addition to the inheritance of features based on the central genome. This involves genes originating from mitochondria, which are primarily transmitted from the female parent [15].

FoxP2 and Reelin are the genes that govern the caliber of bird calls and chirps. The FoxP2 gene regulates the fundamental characteristics of sound, whereas the Reelin gene expedites the rate at which birds acquire the ability to sing [16]. Since the combined ovum and spermatozoa have a healthy gene composition governing the chirp, there are cross-breeding results where the progeny are champion class from generation to generation (10%). In the same way, in the cases where half of the offspring are in the champion grades.

The study's findings demonstrated that certain Zebra Dove pairings were sterile; in this instance, the female parent failed to lay eggs. According to [5], the causes of this reproductive failure include stress experienced by the birds during mating, weather changes (such as temperature and humidity) that the pair cannot tolerate, and pollution from specific chemical compounds. Another reason why a married pair of birds might not produce eggs is if the fertilization process fails [5].

The outcomes of multiple academics' work aimed at enhancing the quality of Zebra Dove chicks' cooing have been widely disseminated. There are benefits to providing several plant extracts to enhance the quality of cooing [18]. Different cooing outcomes in Zebra Dove chicks have been seen in several earlier studies. Research findings and the experience of several Zebra Dove breeders indicate that different approaches to crossbreeding with different cooing traits of the parents do not ensure champion cooing in the offspring.

REFERENCES

- [1] del Hoyo, J., Collar, N.J., Christie, D.A., Elliott, A. and Fishpool, L.D.C. 2014. HBW and BirdLife International Illustrated Checklist of the Birds of the World. Lynx Edicions BirdLife International, Barcelona, Spain and Cambridge, UK.
- [2] Tang, L. and Schwarzkopf, L. 2013. Foraging behaviour of the Peaceful Dove (*Geopelia striata*) in relation to predation risk: group size and predator cues in a natural environment. *Emu - Austral Ornithology*, 113(1): 1–7. doi:10.1071/MU12023.
- [3] Harrison, C. J. O. 1969. Some Comparative Notes on the Peaceful and Zebra Doves (*Geopelia striata* ssp.) with Reference to their Taxonomic Status. *Emu - Austral Ornithology*, 69(2): 66–71. doi:10.1071/MU969066.
- [4] Baptista, L.F., Trail, P.W., Horblit, H.M. and Kirwan, G.M. 2016. Zebra Dove (*Geopelia striata*). In del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. and de Juana, E. (eds.). *Handbook of the Birds of the World Alive*. Lynx Edicions, Barcelona.
- [5] Assersohn, K., Marshall, F., Morland, F., Brekke, P. and Hemmings, N. 2021. Why do eggs fail? Causes of hatching failure in threatened populations and consequences for conservation. *Animal Conservation*, 24(4): 540–551. doi:10.1111/acv.12684.
- [6] Flanders Today. 2013. Finch-singing contests recognised as cultural heritage. Retrieved from: <http://www.flanderstoday.eu/current-affairs/finch-singing-contests-recognised-cultural-heritage>
- [7] Surapanpitak, Y. and Vanigasumbun, S. 1995. Breeding of nicobar pigeon (*Caloenas nicobarica*) in Captivity.
- [8] Xiao, L., Merullo, D.P., Koch, T.M.I. et al. 2021. Expression of FoxP2 in the basal ganglia regulates vocal motor sequences in the adult songbird. *Nature Communications*, 12: 2617. doi:10.1038/s41467-021-22918-2.
- [9] Gadagkar, V., Puzerey, P.A., Chen, R., Baird-Daniel, E., Farhang, A.R. and Goldberg, J.H. 2016. Dopamine neurons encode performance error in singing birds. *Science*, 354: 1278–1282. doi:10.1126/science.aah6837.
- [10] Kousparou, C., Fyrylla, M., Stephanou, A. and Patrikios, I. 2023. DHA/EPA (Omega-3) and LA/GLA (Omega-6) as Bioactive Molecules in Neurodegenerative Diseases. *International Journal of Molecular Sciences*, 24(13): 10717. doi:10.3390/ijms241310717.
- [11] He, S., Lin, J., Jin, Q., Ma, X., Liu, Z., Chen, H., Ma, J., Zhang, H., Descovich, K., Phillips, C.J.C., Hartcher, K. and Wu, Z. 2022. The Relationship between Animal Welfare and Farm Profitability in Cage and Free-Range Housing Systems for Laying Hens in China. *Animals*, 12(16): 2090. doi:10.3390/ani12162090.
- [12] Surzycki, S. 2000. General Aspects of DNA Isolation and Purification. In: *Basic Techniques in Molecular Biology*. Springer Lab Manuals. Springer, Berlin, Heidelberg. doi:10.1007/978-3-642-56968-5_1.
- [13] Shi, Z., Luo, G., Fu, L., Fang, Z., Wang, X. and Li, X. 2013. miR-9 and miR-140-5p target FoxP2 and are regulated as a function of the social context of singing behavior in zebra finches. *Journal of Neuroscience*, 33(42): 16510–16521. doi:10.1523/JNEUROSCI.0838-13.2013.
- [14] Wang, S., Zickler, D., Kleckner, N. and Zhang, L. 2015. Meiotic crossover patterns: obligatory crossover, interference and homeostasis in a single process. *Cell Cycle*, 14(3): 305–314. doi:10.4161/15384101.2014.991185.
- [15] Camus, M.F., Lawrie, B.A., Sharbrough, J. and Hurst, G.D.D. 2022. Inheritance Through The Cytoplasm. *Heredity*. doi:10.1038/s41437-022-00540-2.

- [16] Linville, S. 2017. Bird Song Genes, FoxP2 and Reelin. Indiana Public Media. Retrieved from: <https://indianapublicmedia.org/amomentofscience/bird-song-genes-foxp2-reelin.php>
- [17] Lorenzo-Díaz, F., Fernández-López, C., Lurz, R., Bravo, A. and Espinosa, M. 2017. Crosstalk between vertical and horizontal gene transfer: plasmid replication control by a conjugative relaxase. *Nucleic Acids Research*, 45(13): 7774–7785. doi:10.1093/nar/gkx450.
- [18] Purnamasari, D. K. 2006. Effects of leaf extracts of saga, sambiloto and wild pare on song's quality of zebra dove (*Geopelia striata*). Scientific Repository. Retrieved from: <http://repository.ipb.ac.id/handle/123456789/9023>
- [19] Surapanpitak, Y. and Vanigasumbun, S. 1995. Breeding of Zebra Dove (*Geopelia striata*) in Captivity. *FAO*, 4(2): 7–17.
- [20] Wolfgang, F., Claudia, B., Katja, T. and Derégnacourt, S. 2009. The genetic basis of zebra finch vocalizations. *Evolution*, 63(8): 2114–2130. doi:10.1111/j.1558-5646.2009.00688.x.

