ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

SHORT COMMUNICATION doi:10.1111/j.1365-2052.2011.02185.x

# Genetic diversity of ten Egyptian chicken strains using 29 microsatellite markers

**M. Eltanany*, U. Philipp†, S. Weigend‡ and O. Distl†**

*Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University, Moshtohor, Toukh, Egypt. †Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Bünteweg 17p, 30559 Hannover, Germany. ‡Institute of Farm Animal Genetics, Friedrich-Loeffler Institut, 31535 Neustadt, Germany

## Summary

In this study, we assessed the genetic diversity of three Egyptian local chicken strains (Fayoumi, Dandarawi and Sinai) and six synthetic breeds derived from Fayoumi and Sinai by intercrossing with Barren Plymouth Rock, Rhode Island Red or White Cornish. Diversity measures were based on interrogation of 29 microsatellites. We identified three main clusters of chicken populations encompassing selected Fayoumi lines and Doki-4 (cluster-1), native Dandarawi (cluster-2) and Sinai, and all six synthetic breeds (cluster-3). Dandarawi and Fayoumi lines exhibited lower intra-population genetic diversity and allelic privacy than Sinai and synthetic breeds. The global inbreeding ($F_{IT}$) was 0.11, among-population differentiation ($F_{ST}$) was 0.07, and within-population differentiation ($F_{IS}$) was 0.04. The between-population marker-estimated kinship was lower than within-population estimates. The cluster analysis classified the Fayoumi lines, Dandarawi and Gimmizah as clearly separated populations. The other strains were configured in mosaic admixed groups.

**Keywords** chicken, cluster analysis, genetic diversity, microsatellite.

Egypt possesses versatile varieties of chickens including several local types. Two of them, Fayoumi and Dandarawi, are native breeds, while Sinai, a mongrel chicken breed with its origins in the Sinai desert, goes back to crosses of old local strains and exotic types. In particular, Fayoumi chickens were used for the development of synthetic breeds by intercrossing them with high-producing lines from Europe and the United States in order to increase productivity and to maintain their ability to adapt to local conditions. Increasing expansion of the commercial chicken industry and intermixing of commercial hybrids with local strains in rural backyards are eroding the genetic uniqueness of native breeds and their potential to adapt to local conditions (Hosny 2006).

We aimed to assess the genetic diversity and phylogenetic relationships of a wide spectrum of Egyptian chicken strains using 29 microsatellites. The strains studied were the native Dandarawi and Fayoumi (two selected lines), Sinai mongrel chicken, and six synthetic breeds including Doki-4, Inshas, Golden Montazah, Silver Montazah, Gimmizah and Bandara. Doki-4 is most often utilized in the creation of synthetic breeds like Silver and Golden Montazah and Gimmizah (Table S1; Fig. S1).

We randomly collected blood samples from 251 chickens from Al-Azzab station-4. The Al-Azzab integrated poultry project is the largest in Egypt, providing both rural backyards and research centres with pure-bred birds since 1983 (Hosny 2006). Flock sizes for each strain are about 5000 birds, with sex ratios of one rooster per ten hens. DNA was extracted from EDTA-blood using standard methods. We used the marker set of 29 autosomal microsatellites (Table S2) and reference samples as given on the AVIANDIV website (http://aviandiv.tzv.fal.de). Genotyping data were analysed using software as described in detail in Table S3. Within- and between-population marker-estimated kinships (MEK) and MEK-based distances were estimated according to Eding & Meuwissen (2001) and Mateus *et al.* (2004).

The total number of observed alleles at all loci was 213 across populations, with an average of 7.3. The mean effective number of alleles was 3. The mean polymorphism information content was 0.61 (Table S4). There were 35 distinct unique alleles (16.4%), which were mainly observed in the synthetic breeds, especially in the Gimmizah (Table S5).

The intra-population diversity of the studied Egyptian strains inferred from mean expected-heterozygosity ($H_E$) was

Address for correspondence

O. Distl, Institute for Animal Breeding and Genetics, University of Veterinary Medicine Hannover, Bünteweg 17 p, 30559 Hannover, Germany.
E-mail: ottmar.distl@tiho-hannover.de

*Accepted for publication 15 November 2010*

0.60; mean allelic number (MNA) was 4.92; heterozygote-deficit ($F_{IS}$) was 0.04 and mean MEK was 0.25. The global inbreeding-coefficient ($F_{IT}$) was 0.11, and the overall among-population differentiation ($F_{ST}$) was 0.07. The strains could be categorized into a low-diversity class with Dandarawi, the Fayoumi lines and Doki-4 (TNA: 125–135; MNA: 4.31–4.66; $H_E$: 0.49–0.56), and a high-diversity class with Sinai and the other synthetic breeds (TNA: 146–157; MNA: 5.04–5.41; $H_E$: 0.61–0.67) (Table S5).

The Fayoumi GG line revealed the lowest $H_E$ (0.49) and MNA (4.31), complete allelic fixation of the *MCW14* locus and the greatest within-population MEK (0.431). Even if $F_{IS}$ (0.036) was moderate, the Fayoumi GG line deviated highly from genetic equilibrium. Inshas and Golden Montazah demonstrated the highest $H_E$ (0.67) and MNA (5.38–5.41), moderate $F_{IS}$ (0.027–0.030), as well the lowest intra-population MEK (0.147–0.087). The Fayoumi PP line, Dandarawi and Gimmizah exhibited the highest degree of inbreeding ($F_{IS}$ = 0.07). Sinai and Doki-4 had the lowest inbreeding estimates ($F_{IS}$ = 0.04–0.02) (Table S5).

Between-population differentiation inferred using AMOVA was 7% of the total genetic variance including synthetic breeds and 8% without synthetic breeds. The mean Nei's genetic distance (Nei-GD) was 0.15, and the mean Reynolds' genetic distance (Rey-GD) was 0.09 among populations. Moderate Nei-GD distinguished Dandarawi from the Fayoumi lines as well as the wide distances that separated them from the other breeds, except for Doki-4. Doki-4 had Fayoumi sires as ancestors, thus explaining its low genetic distances from the Fayoumi lines. Similarly, the low genetic distance among Inshas and Sinai is caused by the existence of common Sinai sires as ancestors. Regarding genetic-drift differences, high Rey-GD and $F_{ST}$ values were detected between Dandarawi and the Fayoumi lines. In agreement with these distance measures, the Fayoumi lines showed distant genetic relationships with all the other breeds except for Doki-4, which exhibited the closest relationships with the Fayoumi lines (Tables S6 and S7).

The between-population MEK values (mean = 0.18) were lower than within-population estimates. The highest between-population MEK was between the Fayoumi GG line and Doki-4, while Gimmizah and Golden Montazah had a between-population MEK of zero. The Fayoumi GG line had higher MEK values with all breeds than the Fayoumi PP line (Table S7). The phylogenetic relationships based on Nei-GD and MEK-based distances are in good agreement (Fig. 1).

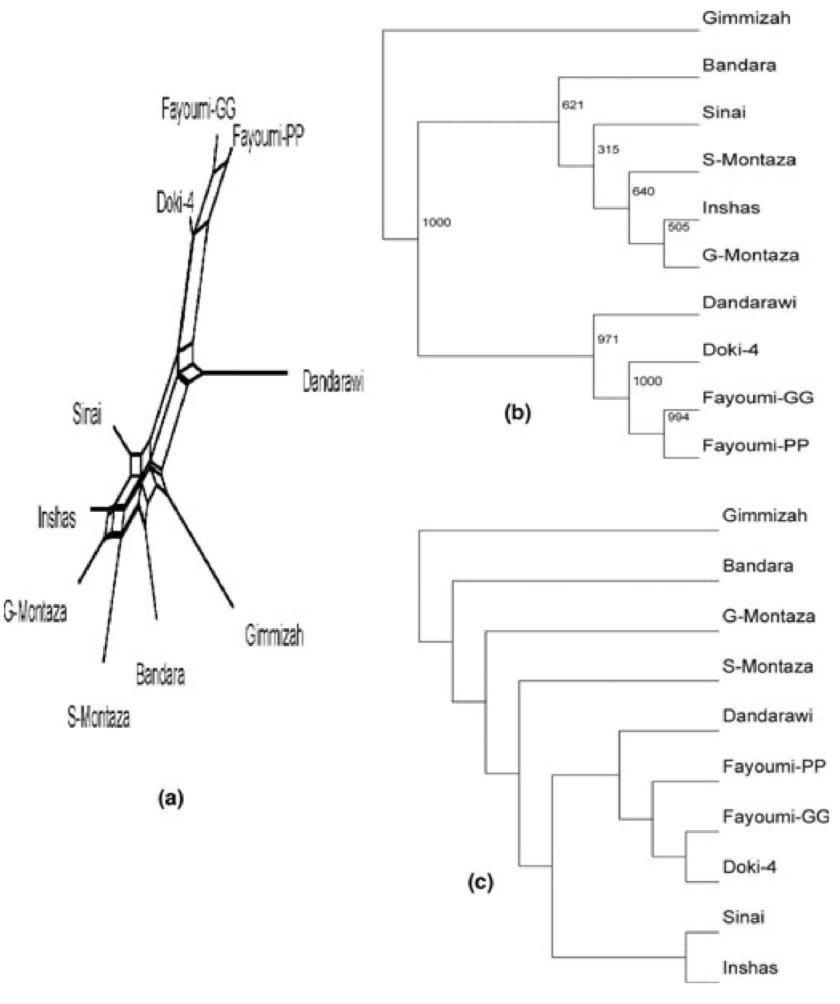

**Figure 1** (a) Neighbour-Net network; (b) Neighbour-Joining cladogram derived from Nei's genetic distance matrix. The numbers within the nodes are bootstrapping values from 1000 replicates across the set of loci; and (c) Neighbour-Joining cladogram derived from a marker estimated kinship (MEK) based distance matrix.

The Fayoumi lines and Doki-4 showed close phylogenetic relationships in the neighbour-joining cladograms and the neighbour-net network. Gimmizah exhibited the highest distance from all other strains for Nei-GD and MEK-based distances. The neighbour-net network displayed two main clusters, with the Fayoumi lines and Doki-4 in one cluster and all other strains in a further cluster. The Dandarawi was located between these two clusters.

The best solution for STRUCTURE (Pritchard *et al.* 2000) from $2 \le K \le 10$ was achieved at $K = 5$. At $K = 2$, the Fayoumi lines, Dandarawi and Doki-4 were separated from Sinai and the other synthetic breeds. At $K = 4$, Gimmizah, harbouring the highest allelic privacy, was distinguished as a separate population. At $K = 5$, Dandarawi was distinguished from the Fayoumi cluster, with mean membership coefficient of 0.81. Doki-4 still clustered with the Fayoumi lines. Notably, the Fayoumi lines clustered as one population until a *K*-value of 10 was reached. The other breeds showed genetic admixture (Fig. S2).

According to Maudet *et al.* (2002), the microsatellites used delivered reliable information through their high frequencies of common alleles and high polymorphism across populations. The loci *MCW103*, *MCW222* and *MCW14* showed the highest values for $F_{ST}$ and $F_{IT}$. In agreement with Berthouly *et al.* (2008), *MCW14* seems to have high discriminative power in differentiating Fayoumi from other strains. It is possible that this marker is hitchhiking with a genomic region associated with a Fayoumi-specific trait. The observed scant allelic privacy in native types could foreshadow the eventual melding of their genetic entities within those of the other crosses. The Egyptian strains studied exhibited higher intra-population genetic diversity than European fancy and purebred commercial lines, but lower diversity than local chicken populations from Vietnam, Malawi and Zimbabwe (Granevitze *et al.* 2007).

The lowest genetic diversity, moderate $F_{IS}$ and highest MEK that were detected within the Fayoumi GG line may be a result of intensive selection since 1970, which was employed based on individual performance for high growth rate. Five generations of full-sib family selection were applied to generate the Fayoumi PP line, and then selection was based on individual performance (El-Hossari 1970). The high $F_{IS}$ within the Fayoumi PP line, Dandarawi and Gimmizah may indicate non-random-mating and substructures in these strains. The Fayoumi lines in our study displayed higher genetic diversity than a purebred Fayoumi line studied by Granevitze *et al.* (2007).

Golden Montazah showed the largest intra-population variation, the lowest intra-population MEK, and moderate inter-population MEK. This indicates that its large population size is experiencing unexpected gene flow, as confirmed by its mosaic clustering.

The Egyptian strains investigated here showed lower between-population variation than that estimated between Finnish chickens ($F_{ST} = 0.29$; Vanhala *et al.* 1998) and 52 distant chicken populations (Rey-GD = 0.29; Nei-GD = 0.44; Hillel *et al.* 2003). However, the between-population variation found here is higher than that previously found between Zimbabwe chickens (Muchadeyi *et al.* 2007). The observed between-population MEK was higher in comparison with other similar studies including Zimbabwe, Sudanese, Malawi and Hungarian chickens, as well as commercial lines (Muchadeyi *et al.* 2007; Bodzsar *et al.* 2009). This might indicate migrations among Egyptian chickens at the rural level and also verifies the mosaic admixture pattern found in the cluster analysis, especially in the Sinai and most synthetic breeds. The higher between-population MEK value between Doki-4 and the Fayoumi GG line compared to the Fayoumi PP line suggests a closer genetic relationship between the Fayoumi GG line and the Fayoumi founders of Doki-4.

Although the Fayoumi lines and Dandarawi were genetically distinct, they exhibited high kinship relationships. This could reflect their ancient common ancestry, as confirmed by their common clustering up to $K = 4$. In addition, gene flow may have occurred among Fayoumi and Dandarawi because of their geographical origins in Upper Egypt.

In conclusion, the Fayoumi lines clustered as the clearest distinct populations, as well as Dandarawi and Gimmizah, which also demonstrated low admixture with the other studied populations. The Egyptian chickens showed a high intra- and between-population genetic diversity and thus are the subjects of an urgent conservation appeal. Cluster analysis indicates that Fayoumi, Dandarawi, Gimmizah and Sinai are the highest priorities for conservation because of their genetic distinctiveness and national importance.

## Acknowledgements

M. Eltanany was supported by an internal scientific mission (2005/2006) from the High Educational Ministry, Cairo, Egypt.

## Conflict of interests

The authors declare no conflicts of interest.

## References

Berthouly C., Bed'Hom B., Tixier-Boichard M. *et al.* (2008) Using molecular markers and multivariate methods to study the genetic diversity of local European and Asian chicken breeds. *Animal Genetics* **39**, 121–9.

Bodzsar N., Eding H., Revay T. *et al.* (2009) Genetic diversity of Hungarian indigenous chicken breeds based on microsatellite markers. *Animal Genetics* **40**, 516–23.

Eding H. & Meuwissen T.H.E. (2001) Marker-based estimate of between and within population kinships for the conservation of genetic diversity. *Journal of Animal Breeding and Genetics* **118**, 141–59.

El-Hossari M.A. (1970) Effects of natural selection on selection for high body weight at 8 genetic and phenotypic variations in two strains of Fayoumi chickens. *U.A.R. Journal of Animal Production* **10**, 55–63.

Granevitze Z., Hillel J., Chen G.H. *et al.* (2007) Genetic diversity within chicken populations from different continents and management histories. *Animal Genetics* **38**, 576–83.

Hillel J., Groenen M.A., Tixier-Biochard M. *et al.* (2003) Biodiversity of 52 chicken populations assessed by microsatellite typing of DNA pools. *Genetics Selection Evolution* **35**, 533–57.

Hosny F.A. (2006) The structure and importance of the commercial and village based poultry system in Egypt. In: *Poultry Sector Country Review* (Ed. by Food and Agricultural Organization of The United Nations), pp. 1–39. FAO, Rome.

Mateus J.C., Eding H., Penedo M.C.T. *et al.* (2004) Contributions of Portuguese cattle breeds to genetic diversity using marker-estimated kinships. *Animal Genetics* **35**, 305–13.

Maudet C., Miller C., Bassano B. *et al.* (2002) Microsatellite DNA and recent statistical methods in wild conservation management: application in alpine *Ibex capra ibex* (ibex). *Molecular Ecology* **11**, 421–36.

Muchadeyi F.C., Eding H., Wollny C.B.A. *et al.* (2007) Absence of population substructuring in Zimbabwe chicken ecotypes inferred using microsatellite analysis. *Animal Genetics* **38**, 332–9.

Pritchard J.K., Stephens M. & Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–59.

Vanhala T., Tuiskula-Haavisto M., Elo K. *et al.* (1998) Evaluation of genetic variability and genetic distances between eight chicken lines using microsatellite markers. *Poultry Science* **77**, 783–90.

## Supporting information

Additional supporting information may be found in the online version of this article.

**Figure S1** Morphological appearance of male, female and chicks of nine Egyptian local strains.

**Figure S2** STRUCTURE clustering of the studied ten Egyptian chicken strains.

**Table S1** Some features of the Egyptian chicken strains used in the current study according to records of Integrated Fowl-Project, Fayoum.

**Table S2** Some properties of the used microsatellite markers in the current study.

**Table S3** Software used in the present study.

**Table S4** Marker polymorphisms across the studied Egyptian chicken strains.

**Table S5** Genetic diversity within the ten Egyptian chicken strains studied.

**Table S6** Nei's genetic distance (below diagonal, with mean of 0.15 and SD of 0.05) and Reynold's genetic distance (above diagonal, with mean of 0.09 and SD of 0.04) matrices between ten Egyptian chicken strains.

**Table S7** Pairwise $F_{ST}$ estimates and Marker Estimated Kinship values between and within ten Egyptian chicken strains.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.