Feasibility study on the FAO chicken microsatellite panel to assess genetic variability in the turkey (Meleagris gallopavo)

Elena Colombo, Maria G. Strillacci, Maria C. Cozzi, Manuela Madeddu, Maria G. Mangiagalli, Fabio Mosca, Luisa Zamboni, Alessandro Bagnato, Silvia Cerolini
Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università di Milano, Italy

Abstract

The aim of this work was to study the feasibility of the Food and Agriculture Organization (FAO) microsatellite panel developed for chickens to assess genetic variability in turkeys. Genomic DNA was extracted from a total of 37 blood samples collected from turkey of different breeds (15 Brianzolo (BR); 12 Colli Euganei (EU); 10 Nero d’Italia (NI)), and all 31 chicken microsatellite markers recommended by the FAO were tested. The results show that 22 chicken markers out of 31 suggested by FAO guidelines can be applied to turkey populations. In particular, the multiplex groups confirmed in the turkey were the Multiplex Master Mix 1 (ADL0268, ADL0278, LEI0094, MCW0216, MCW0248) and the Master Mix 2 (MCW0034, MCW0069, MCW0081, MCW0222, MCW0295), whereas 13 microsatellites were amplified only under single polymerase chain reaction (PCR) conditions. No PCR products were obtained for 9 markers (LEI0166, MCW0020, MCW0078, MCW0080, MCW0104, MCW0123, MCW0248, MCW0284 and MCW0339), which is 29% of the total markers used. A panel of 22 markers was used to assess genetic diversity in three turkey breeds and a total number of 63 alleles were found. Observed (Ho) and expected (He) heterozygosity and polymorphism information content (PIC) values for each microsatellite and the relative mean values were also calculated. The mean values were 0.210, 0.250, 0.203 for Ho; 0.301, 0.348, 0.228 for He; and 0.265, 0.313, 0.199 for PIC in NI, BR and EU, respectively.

Introduction

It is well recognized at a global level that animal genetic resources have to be optimized in order to preserve endangered animal genomes in a world of decreasing biodiversity and rationalization of agriculture by environmentally sustainable productions. The global use of highly productive animals has led to the gradual erosion of genetic variability in most species, and among them poultry species are the most endangered (Pilling and Rischkowsky, 2007).

Knowledge of the genetic structure and diversity of the breeds, information about the genetic variability, productive characteristics and relationship between their genetic make-up and performance, adaptability and resistance to diseases, play an important role in the conservation of poultry local breeds (Wilkinson et al., 2011). Particularly, the maintenance of genetic variability is necessary for genetic improvement strategies – present and future – to allow a response to changes in farming systems, for better adapting to environmental changes (response to stress/illness) and, more importantly, for understanding phenotypic variability.

Since the 1990s, the characterization of genetic diversity has focused on molecular data (Groeneveld et al., 2010) and has long been based on the use of neutral genetic markers. Informative DNA markers help the measurement of diversity and they are particularly useful in two ways. First, to overcome the problem of observing pedigree directly in the same species, which can be very common in poultry and fishes. The second involves the extensive genotyping across the genome in order to estimate precisely the actual proportion of DNA shared by sibs or other relatives.

Studies on molecular diversity relied for many years on microsatellite genotyping and the Food and Agriculture Organization of the United Nations (FAO) Committee developed a specific microsatellite panel for 9 species, including the chicken Gallus gallus (FAO, 2011). The first non-official chicken DNA genotyping comparison test was established in 2009 and discussed in the 2010 at the International Society Animal Genetics meeting. On the contrary, genetic studies on turkey populations or breeds still are very limited. Although a great number of turkey microsatellites were identified and mapped on specific linkage maps (Reed et al., 2000, 2002; Burt et al., 2003; Chaves et al., 2005), at present, a FAO specific panel for turkey species Meleagris gallopavo is not available.

Materials and methods

The study considered the following turkey breeds: Nero d’Italia (NI), Brianzolo (BR) and Colli Euganei (EU). The NI is a turkey breed characterized by light weight, black feathers and dark reddish shanks. The BR is characterized by medium body size, good disease resistance and brooding behaviour in females. Different plumage colours are present and the most common is bronze with bars on the tail. The EU typically has small body size, high rusticity and a very good brooding behaviour in females. Both males and females have good metallic-bronze plumage.

A total number of 37 turkey blood samples from the 3 Italian turkey breeds (BR=15; EU=12; NI=10) were collected from 2 farms. Genomic DNA was extracted by Nucleospin blood kit (Macherey Nagel, Düren, Germany) using 3 µL of blood sample following manufacturer procedure and whole blood samples were stored at -20°C until further analyses.

Chicken samples were used as positive control for the turkey DNA amplifications. DNA samples were quantified using NanoQuant...
Infinite m200 (TECAN, Männedorf, Germany), and were diluted to 20 ng/µL. Genotyping was carried out using 31 chicken microsatellite markers recommended by FAO. The single polymerase chain reaction (PCR) amplifications were carried out in a final volume of 10 µL using two different methods: the first protocol used a mix composed by 10 Taq Buffer with MgCl2, 50 mM of each dNTP, 5 TaqMaster PCR Enhancer, 1 mM of each primer, Taq DNA polymerase (5 U/µL) MasterTaq Kit (5PRIME, Gaithersburg, MD, USA); the second protocol used a mix composed by 2mM MgCl2, 50 mM of each dNTP, 1 mM of each primer and 1 unit of AmpliTaq Gold® (Life Technologies, Carlsbad, CA, USA). Amplifications were carried out on MJ PTC 100 Thermal Cycler under the following conditions: initial step of 1 min at 94°C for AmpliTaq Gold® activation, followed by 36 cycles of denaturation for 1 min at 94°C; annealing for 1 min at optimized temperature (Table 1); extension for 1 min at 72°C; and final extension for 46 min at 72°C.

Markers giving PCR products were assigned to and tested in multiplex groups according to the FAO Guidelines (2011).

A quality control of the amplified products was performed by electrophoresis on FlashGel™ System Lonza 2.2% Agarose Gel. Polymerase chain reaction products were separated using the ABI 3730 DNA analyzer (Life Technologies) and the corresponding size (bp) was assigned using GeneMapper® software. Number of alleles, effective allele number, polymorphism information content (PIC), observed (Ho) and expected heterozygosity (He) were estimated using the Microsatellite Tool-kit software (Park, 2001).

**Results and discussion**

Polymerase chain reaction conditions, number and allele size of chicken FAO microsatellites identified in turkey populations are reported in Table 1. The results show that 2 out of the 7 multiplex groups proposed by FAO (2011) for the chicken can also be applied to turkey populations (Table 1). In particular, the multiplex groups confirmed in the turkey were the Multiplex Master Mix 1 (ADL0268, ADL0278, LEI0094, MCW0216, MCW0248) and the Master Mix 2 (MCW0034, MCW0069, MCW0111, MCW0123, MCW0183, MCW0206).

### Table 1. Polymerase chain reaction conditions, multiplex groups, number and allele size of chicken Food and Agriculture Organization microsatellites identified in the turkey.

| Locus   | PCR condition         | Multiplex group° | Alleles, n | Allele size          |
|---------|-----------------------|------------------|------------|---------------------|
|         | T, °C                 | Taq protocol     |            |                     |
| ADL0112 | 58                    | MasterTaq Kit    | -          | 5                   | 127, 131, 139 | 127, 129, 131 |
| ADL0268 | 48                    | AmpliTaq Gold®   | Mix 1      | 6                   | 97, 101, 109, 111 | 97, 109, 111, 113 |
| ADL0278 | 48                    | AmpliTaq Gold®   | Mix 1      | 5                   | 113, 119 | 113, 119, 121 |
| LEI0094 | 60                    | MasterTaq Kit    | Mix 1      | 3                   | 267          | 261, 281|
| LEI0166 | 60                    | MasterTaq Kit    | No amplification | -            |              |
| LEI0192 | 58                    | MasterTaq Kit    | -          | 1                   | 257          | 257 |
| LEI0234 | 58                    | MasterTaq Kit    | -          | 3                   | 220, 304 | 220, 304, 308 |
| MCV0014 | 55                    | AmpliTaq Gold®   | Mix 1      | 3                   | 193          | 179, 193 |
| MCV0016 | 58                    | MasterTaq Kit    | Mix 2      | 2                   | 132, 144 | 132 |
| MCV0020 | 60                    | MasterTaq Kit    | No amplification | -          |              |
| MCV0034 | 58                    | MasterTaq Kit    | Mix 2      | 3                   | 222, 230, 234 | 234 |
| MCV0037 | 58                    | MasterTaq Kit    | -          | 3                   | 155          | 151, 155, 157 |
| MCV0067 | 58                    | MasterTaq Kit    | -          | 5                   | 173, 175, 177, 181, 183 | 175, 177, 181 |
| MCV0069 | 55                    | AmpliTaq Gold®   | Mix 2      | 2                   | 159          | 159, 165 |
| MCV0078 | 55                    | AmpliTaq Gold®   | No amplification | -         |              |
| MCV0080 | 60                    | MasterTaq Kit    | No amplification | -         |              |
| MCV0081 | 58                    | MasterTaq Kit    | Mix 2      | 3                   | 110, 114 | 114, 118 |
| MCV0098 | 55                    | AmpliTaq Gold®   | -          | 2                   | 227          | 227 |
| MCV0103 | 58                    | MasterTaq Kit    | -          | 3                   | 268          | 268, 282 |
| MCV0104 | 52                    | AmpliTaq Gold®   | No amplification | -         |              |
| MCV0111 | 55                    | AmpliTaq Gold®   | Mix 2      | 2                   | 97, 99 | 97, 99 |
| MCV0123 | 53                    | AmpliTaq Gold®   | No amplification | -         |              |
| MCV0165 | 58                    | MasterTaq Kit    | Mix 2      | 1                   | 116          | 116 |
| MCV0183 | 60                    | MasterTaq Kit    | Mix 1      | 2                   | 287          | 283, 287 |
| MCV0206 | 55                    | AmpliTaq Gold®   | Mix 2      | 2                   | 223          | 223 |
| MCV0216 | 58                    | MasterTaq Kit    | Mix 1      | 2                   | 145, 155 | 145, 155 |
| MCV0222 | 55                    | AmpliTaq Gold®   | Mix 2      | 3                   | 222, 224, 226 | 222 |
| MCV0248 | 60                    | MasterTaq Kit    | No amplification | -         |              |
| MCV0284 | 58                    | MasterTaq Kit    | No amplification | -         |              |
| MCV0295 | 55                    | AmpliTaq Gold®   | Mix 2      | 3                   | 90, 92, 102 | 90, 102 |
| MCV0303 | 58                    | MasterTaq Kit    | No amplification | -         |              |

PCR, polymerase chain reaction; NL, Nero d’Italia; BR, Brianzolo; EU, Colli Euganei. Information about the size of the fragments and PCR primers in the chicken are reported in FAO Guidelines (2011). Genomic DNA obtained from different breeds was analysed with two protocols (MasterTaq Kit: PRIME, Gaithersbug, MD, USA; AmpliTaq Gold®: Life Technologies, Carlsbad, CA, USA). °Column shows the multiplex groups identified in turkeys out of the 7 proposed by FAO for the chicken, the microsatellites amplified in single reaction (-) and microsatellites with no amplification.
Table 2. Observed and expected heterozygosity and polymorphism information content values calculated by locus in different turkey breeds.

| Locus      | Ho | He   | PIC  |
|------------|----|------|------|
|            | NI | BR   | EU   | NI | BR | EU   | NI | BR | EU   |
| ADL0112    | 0.333 | 0.143 | 0.200 | 0.733 | 0.385 | 0.467 | 0.535 | 0.325 | 0.332 |
| ADL0268    | 0.500 | 0.444 | 0.667 | 0.742 | 0.614 | 0.561 | 0.622 | 0.544 | 0.476 |
| ADL0278    | 0.500 | 0.667 | 0.500 | 0.571 | 0.621 | 0.767 | 0.375 | 0.477 | 0.667 |
| LEI0094    | 0.500 | 0.500 | 0.750 | 0.500 | 0.714 | 0.536 | 0.305 | 0.555 | 0.359 |
| LEI0234    | 0.500 | 0.500 | 0.750 | 0.500 | 0.714 | 0.536 | 0.305 | 0.555 | 0.359 |
| MCW0014    | 0   | 0   | 0.333 | 0   | 0.303 | 0.333 | 0   | 0.239 | 0.239 |
| MCW0016    | 0.500 | 0   | 0   | 0.429 | 0   | 0   | 0.305 | 0   | 0   |
| MCW0034    | 0.250 | 0   | 0   | 0.750 | 0   | 0   | 0.582 | 0   | 0   |
| MCW0037    | 0.222 | 0   | 0   | 0   | 0.627 | 0   | 0   | 0.527 | 0   |
| MCW0067    | 0.714 | 0.625 | 0.667 | 0.791 | 0.700 | 0.621 | 0.689 | 0.582 | 0.477 |
| MCW0069    | 0   | 0   | 0.167 | 0   | 0.167 | 0   | 0   | 0.141 | 0   |
| MCW0081    | 0   | 0.125 | 0   | 0.533 | 0.325 | 0.264 | 0.346 | 0.258 | 0.215 |
| MCW0098    | 0   | 0.750 | 0   | 0   | 0.536 | 0   | 0   | 0.359 | 0   |
| MCW1003    | 0   | 0.500 | 1.000 | 0   | 0.500 | 0.571 | 0   | 0.305 | 0.375 |
| MCW111     | 0.571 | 0.308 | 0   | 0.440 | 0.492 | 0   | 0.325 | 0.361 | 0   |
| MCW133     | 0   | 0.200 | 0   | 0   | 0.189 | 0   | 0   | 0.164 | 0   |
| MCW216     | 0.100 | 0.214 | 0.091 | 0.100 | 0.198 | 0.368 | 0.090 | 0.173 | 0.290 |
| MCW222     | 0.333 | 0.500 | 0   | 0.545 | 0.607 | 0   | 0.449 | 0.468 | 0   |
| MCW295     | 0.429 | 0.143 | 0   | 0.484 | 0.143 | 0   | 0.496 | 0.124 | 0   |
| Mean       | 0.210 | 0.250 | 0.203 | 0.301 | 0.348 | 0.228 | 0.265 | 0.313 | 0.199 |

Ho, observed heterozygosity; He, expected heterozygosity; PIC, polymorphism information content; NI, Nero d’Italia; BR, Brianzolo; EU, Colli Euganei.

MCW0081, MCW0222, MCW0295), whereas 13 microsatellite were amplified only in single PCR condition. MCW0248 microsatellite present in Master Mix 1 did not produce PCR product, therefore it was excluded. The markers with 3 or more alleles could be assigned to different multiplex PCR based of PCR conditions and products length.

Table 1 shows the allele size identified for all microsatellite markers in each breed. ADL0268 had the highest number of alleles (6); 3 microsatellites (ADL0278, ADL112, MCW0067) had 5 alleles; 9 microsatellites were tri-allelic; 6 markers had 2 alleles and 3 markers were monomorphic (LEI0192, MCW0165 and MCW0206). No PCR products were obtained for 9 markers (LEI0166, MCW0020, MCW0078, MCW0080, MCW0104, MCW0123, MCW0248, MCW0284 and MCW0330), which represent the 29% of the total markers used. A similar proportion was found by Liu et al. (1996) in commercial turkey lines using a total of 88 chicken microsatellites.

The Ho and He and PIC values for each microsatellite, excluding the three monomorphic, and the relative mean values calculated in different turkey breeds are reported in Table 2. The EU population showed 37 allelic variants and a very low polymorphism. In this breed, 12 microsatellites were monomorphic and all the PIC values for the polymorphic markers, but ADL0278 ranged from 0.215 to 0.477 (Table 2). The lowest mean PIC value (0.199) reflects the poor polymorphism found in the EU breed. The number of allelic variants identified in NI turkeys was 43, and 10 microsatellites were monomorphic. The PIC values were informative (>0.5) for 4 markers (Table 2). Nevertheless, the mean PIC value (0.265) was low.

The BR population was the most polymorphic and 48 allelic variants were identified. This breed showed 4 PIC values >0.5, highly informative, and 9 PIC values between 0.25 and 0.5, medium informative (Table 2). However, the mean PIC value was 0.313, which is considered rather low.

The low heterozygosity level found in the populations is a consequence of the low number of breed lines. The mean number of alleles per locus was 1.68, 1.95 and 2.18 in EU, NI and BR populations, respectively. Furthermore, most of the bi-allelic markers were in a homozygote state. The low mean number of alleles may be due to inbreeding and founder effects, common factors in rare breeds due to the low number of breeders. Higher number of alleles was observed using turkey microsatellites on the Roslin backcross families (Burt et al., 2003) and on commercial line (Reed et al., 2000, 2002). In contrast, Kamara et al. (2007) observed a low number of alleles in five heritage domestic populations using 10 polymorphic turkey microsatellites chosen from those identified by Burt et al. (2003).

Conclusions

The potential use of chicken microsatellites to analyse genetic variability in turkey populations was studied. Despite the low genetic variability assessed within breed and the low number of birds available per breed, a panel of 22 markers was identified and used to assess genetic diversity in different turkey breeds. Until a specific panel is indicated by FAO as for chickens, the 22 genetic markers can be useful for screening turkey populations in order to evaluate their effective genetic variability and plan a mating programme, thus reducing the risk of inbreeding depression present in these local breeds, as shown by Wilkinson et al. (2011) in chickens.

References

Burt, D.W., Morrice, D.R., Sewalem, A., Smith, J., Paton, I.R., Smith, E.J., Bentley, J., Hocking, P.M., 2003. Preliminary linkage map of the turkey (Meleagris gallopavo) based on microsatellite markers. Anim. Genet. 34:399-409.

Chaves, L.D., Knutson, T.P., Krueth, S.B., Reed, K.M., 2005. Using the chicken genome...
sequence in the development and mapping of genetic markers in the turkey (Meleagris gallopavo). Anim. Genet. 37:130-138.
FAO, 2011. Molecular genetic characterization of animal genetic resources. Food and Agriculture Organization of the United Nations Publ., Rome, Italy. Available from: http://www.fao.org/docrep/014/i2413e/i2413e00.pdf
Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, D., Negrini, R., Finlay, E.K., Jianlin, H., Groeneveld, E., Weigend, S., The GLOBALDIV Consortium, 2010. Genetic diversity in farm animals: a review. Anim. Genet. 41(Suppl.1):6-31.
Kamara, D., Gyenai, K.B., Geng, T., Hammade, H., Smith, E.J., 2007. Microsatellite marker-based genetic analysis of relatedness between commercial and heritage turkeys (Meleagris gallopavo). Poultry Sci. 86:46-49.
Liu, Z., Crooijmans, R.P.M.A., van der Poel, J.J., Groenen, M.A.M., 1996. Use of chicken microsatellite markers in turkey: a pessimistic view. Anim. Genet. 27:191-193.
Park, S., 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. Degree Diss., Trinity College Dublin, Ireland.
Pilling, D., Rischkowsky, B., 2007. The state of the world’s animal genetic resources for food and agriculture. Food and Agriculture Organization of the United Nations Publ., Rome, Italy. Available from: http://www.fao.org/docrep/010/a1250e/a1250e00.htm
Reed, K.M., Chaves, L.D. Rowe, J.A., 2002. Twelve new turkey microsatellite loci. Poultry Sci. 81:1789-1791.
Reed, K.M., Roberts, M.C., Murtaugh, J., Beattie, C.W., Alexander, L.J., 2000. Eight new dinucleotide microsatellite loci in turkey (Meleagris gallopavo). Anim. Genet. 31:140.
Wilkinson, S., Wiener, P., Teverson, D., Haley, C.S., Hocking, P.M., 2011. Characterization of the genetic diversity, structure and admixture of British chicken breeds. Anim. Genet. 43:552-563.