Does Genotype Determine Habitat Preferences? – Studies on Forest and Field Roe Deer

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The main habitat of roe deer includes the outer edges of forests and wooded steppes but this species has also adapted itself to living in open agricultural areas all year round. The presence of forest roe deer as well as field roe deer was confirmed at the turn of the 20th and 21st centuries with radio telemetry research projects. Therefore, the question arises as to how there are two roe deer populations with different habitat preferences and behaviour existing in the same areas in Europe for so many decades. Genetic differences between forest and field roe deer were examined by analyzing the polymorphism of eight microsatellite DNA loci of individuals within three regions of Poland. The fact that the individuals studied belonged to specific populations did not result in significant differences in allele and genotype frequencies. The obtained results suggest a lack of genetic differences between the forest and field roe deers. Negligible genetic differentiation is probably a consequence of the matching of individuals from both populations neighbouring each other in many areas. Moreover, some forest roe deer periodically live in agricultural territories in the summer, during the period of rut. A hypothesis was put forward that young roe deer choose their future habitat (forests or fields) by observing the behaviour of adults, especially their mothers whom they accompany in the first year of their lives. In this case, the obtained genetic resemblance for progeny can result from the field origin of their dam(s).

Key words: Behaviour, genetic differences, habitat preferences, microsatellite markers.

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The preferred habitat of roe deer includes forest edges and wooded steppes but this species has also adapted itself to living in agricultural areas (ANDERSEN et al. 1998). Settling in open agricultural landscape and occupying this habitat during the entire year has been observed at least since the beginning of the 20th century (SCHECHTEL 1929). This process progressed in the following decades and found its reflection in large winter groupings of roe deer registered especially in the agricultural fields of central Europe (ZEJDA 1978; BRESIŃSKI 1982). Translocations of roe deer from forests to fields and the other way around confirmed that both populations had strong bonds with the forest environment. This resulted in the conclusion that the choice of habitat, abounding with shelters for roe deer throughout the year, might be conditioned genetically (GRACZYK 1978; PIEŁOWSKI & BRESIŃSKI 1982). A comparison of forest and field roe deer instead revealed differences in indicators of energy metabolism and enzymatic activity which were explained by changes in field roe deer
The differences in body weight and length in favour of the field roe deer population probably resulted from better accessibility of more nutritious food in agricultural areas (FRUZINSKI et al. 1982). A comparative analysis of selected sections of alimentary canals of forest and field roe deer did not show any significant differences (HOFMANN et al. 1988). As a consequence of a limited number or scale of differences between the populations of forest and field roe deer, in the European monograph devoted to this species (ANDERSEN et al. 1998) the question is left open whether there is a field ecotype or whether the occurrence of roe deer in the fields is an extreme case of flexibility of behaviour of this species which is especially exhibited by its specific social organization during winter.

Radio telemetry research projects confirmed the results of previous studies on roe deer from forests (STRANDGAARD 1972; PIELOWSKI & BRESINSKI 1982; AULAK & BABINSKA-WERKA 1990). Alongside animals living solely in forests and using feeding grounds at forest edges, there are individuals (bucks and does) that at the turn of spring and summer migrate to farmlands and then in autumn return to the forest. In the following years some of them were exploiting the same summer and winter home ranges (WASILEWSKI 2001). Observations from agricultural areas (PIELOWSKI 1984; ZEJDA & BAUEROVA 1985) which indicated that certain roe deer prefer only fields, were confirmed as well. The locations of the radiotracked field roe deer were far away from forests – 1.2 km on average (KAMIENIARZ 2013). The question remains open as to why in the case of overlapping of forest and field roe deer home ranges, some still prefer forests and some choose living in open fields.

The answer to this question is important because at the turn of the 20th and 21st centuries the regular occurrence of other species primarily associated with forests was also observed in agroecoses. During the vegetative period, the agricultural landscape becomes the bed site for a part of the wild boar population, among others in Poland (FRUZINSKI & LABUZDZKI 2002) and Germany (KEULING et al. 2009). On the other hand, foxes in the western part of Poland began to more intensively use the agricultural lands throughout the entire year (PANEK & BRESINSKI 2002; KAMIENIARZ et al. 2008). This resulted in changes in interspecies interactions (PANEK 2005; PANEK et al. 2006) and in interactions between animals and habitat (GEISSER & REYER 2004; KAMIENIARZ & PANEK 2008; FRACKOWIACK et al. 2013).

In the context of contemporary ecological knowledge, changes of species range may be the outcome of favourable modifications in a thus far unutilised habitat. However if there is at least one limiting factor, the inhabitancy of a particular habitat is a consequence of the appearance of individuals with genotypes which allow them to live in environmental conditions not accepted by other individuals of this species. As a result of natural selection, which favors such individuals, the appearance of populations functioning in new habitats is then possible (KREBS 2011). In the case of roe deer living in forests or wooded steppes, the limiting factor of its occurrence in farmlands may be the nearly total lack of cover after harvest of crops. This corresponds with the hypothesis presented by ANDERSEN et al. (1998). The evolutionary process probably does take place but proceeds slowly. Individuals with different habitat preferences may mate with each other because the mating season of this species occurs mainly during summer when there is a portion of forest roe deer living in farmlands.

The aim of the paper was to assess the differences between forest and field roe deer on the basis of polymorphism of microsatellite DNA loci. The results extend our knowledge of the process of development of new environmental forms within one species which may help in the management of wild populations occupying new habitats.

Material and Methods

Records of 105 roe deer hunted within planned game management (49 males and 56 females) were analysed. Individuals from the following three regions were included: Czempin (N 52°14', E 16°76', Wielkopolska province – an area devoted to the study of roe deer, including field roe deer, since the mid-20th century), Gierloz (N 53°61', E 19°76', Warmia province) and Rudnik by the San river (N 50°45', E 22°25', Podkarpacze province). The vicinities of Czempin are predominantly agricultural areas with small forest complexes (10-285 ha). The share of farmlands in Gierloz was similar to the share of forest areas (composed mainly of two fragments of large forest complexes). Whereas the surroundings of Rudnik comprise expansive primeval forest and adjacent agricultural fields in the valley of the San river.

Samples from 68 individuals (Table 1) were used to analyze the DNA polymorphism between forest and field roe deer. Some samples, mainly from the vicinities of Czempin (forest interspersed with fields), were excluded from the analysis because they could not be unambiguously classified to one of the habitat groups. The genotyped animals (both bucks and does) were classified as forest roe deer (FoRD) if they were hunted in forests or in their close vicinity. The latter included animals which left forests to feed in the
fields (usually in the evening) or returned from such feeding places (usually in the morning). Individual animals were classified as field roe deer (FiRD) if they were hunted in late autumn and winter in fields at least 500 m from the forests. Territories closer to forests are used during this period by forest roe deer (AULAK & BABIŃSKA-WERKA 1990) so in this area it was possible for both populations of roe deer to occur. However, it should be mentioned that hunting females and young animals in Poland is permitted only in late autumn and winter. Males may be hunted from late spring till early autumn, and in this case individuals shot at least 2 km from the forests were included. That was the border distance of summer migrations of forest roe deer into the fields which were observed in areas of co-existence of both populations in the surroundings of Czempin (PIELOWSKI & BRESINSKI 1982).

From each animal a blood sample was taken from the chest, stored in a tube with K₂EDTA and frozen. Only eight (out of twelve) chosen bovine microsatellite markers were used in further analysis. A detailed description of molecular analysis of the data has been given by KAMIENIARZ et al. (2011). Since the number of genotypes for analysed microsatellite markers differs significantly, Figure 1 presents the number of genotypes for FoRD, common and FiRD genotypes.

From a statistical point of view, these two eco-types of roe deer may be treated as populations having at least one common trait. Therefore, by analogy, in order to find differences between them we may use the methodology used for the purpose of assessing genetic distance which is based on the comparison of frequencies of alleles and genotypes.

In the first step of the statistical analysis the hypothesis about the independence of distributions of genotype frequencies for the analyzed eight loci for FoRD and FiRD individuals, based on Pearson’s $\chi^2$ test was verified. Additionally, for each locus mutual information, being another measure of dependence, was calculated based on the formula

$$I(A_i, \text{Ecotype}) = H(A_i) + H(\text{Ecotype}) - H(A_i, \text{Ecotype})$$

where $A_i$ denotes the locus, $i = 1, 2, \ldots, 8$ and $H(\cdot)$ is the entropy.

The significance of mutual information was examined by applying the likelihood ratio (LR) test (see KANG et al. 2008). $P$-values <0.05 were considered statistically significant.

Table 1

<table>
<thead>
<tr>
<th>Research area</th>
<th>Forest roe deer (FoRD, n=44)</th>
<th>Field roe deer (FiRD, n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma$</td>
<td>$\varphi$</td>
</tr>
<tr>
<td>Czempin</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Gierlo³</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Rudnik</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>
In the second part of the analysis the Gower coefficient of dissimilarity (GOWER 1971) was calculated for the data. The Gower coefficient of dissimilarity equals:

$$d_{ij} = 1 - \frac{1}{\sum_{k=1}^{s_{ij}}}$$

Where

$$s_{ij} = \begin{cases} 0 & \text{if individual } i \text{ and } j \text{ have no common allele in locus } k, \\ 1 & \text{if individual } i \text{ and } j \text{ have one common allele in locus } k, \\ 2 & \text{if individual } i \text{ and } j \text{ have two common alleles in locus } k. \end{cases}$$

Based on these values, a dendrogram grouping the individuals was constructed. The calculations were done in the Statistica v.10 package (2002).

Results

The results of the $\chi^2$ tests are listed in Table 2. They show that based on Pearson’s test for all the analyzed loci the distributions of genotype frequencies were the same in both populations, in other words the populations did not differ in the frequencies of genotypes. Different conclusions were obtained when testing the significance of mutual information. Two markers, NVHRT21 and RT13 (with the p-values 0.038 and 0.056, respectively), can be perceived as the most informative for both populations studied.

It should be noted that a high level of polymorphism was registered for all the analyzed loci, especially for two specific ones (NVHRT21 and RT13) differentiating forest and field roe deer groups. The observed heterozygosity coefficients of both (above mentioned) loci were 0.819 (RT13) and 0.878 (NVHRT21). Moreover, relatively high polymorphism of other studied loci was noted as well. Detailed characteristics of molecular parameters was given by KAMIENIARZ et al. 2011. Hence, the inference about differences between both roe deer groups should be reliable.

The dendrogram grouping observations for the analyzed set is shown in Figure 2. In the dendrogram in Figure 2, the 68 individuals are grouped in two clusters. There are 38 FoRD and 17 FiRD in the first cluster and 6 FoRD with 7 FiRD in the second cluster. This proves that there are no genetic differences between these two groups with regard to the eight analyzed loci.

Discussion

The results of studies on the genetic differentiation of roe deer populations unambiguously indicate that microsatellite markers are very useful. The molecular analysis of wild animals in comparison to domestic ones was carried out on a smaller sample of individual animals and consequently the number of markers accounted for was also smaller, thus it may affect the statistical conclusions. On the other hand, the revealed differentiation between the populations of roe deer, regardless of their geographic localities, gives us ground to search for genetic distinctness between forest and field roe deer (MILOSEVIC-ZLATANOVIC et al. 2005; KAMIENIARZ et al. 2011; BAKER & HOELZEL 2012; PLAKHINA et al. 2014).

Generally, the results did not reveal considerable differences in the distributions of the frequencies of analyzed loci of forest and field roe deer and thus cannot be used to determine whether there is genetic differentiation between these populations. On the other hand, as already mentioned, for one locus (NVHRT21) the difference between forest and field populations was statistically significant. This may be perceived as a sign of potential intergenetic variability of both populations. The current study is based on eight polymorphic DNA markers. By contrast to livestock species, the number of analysed loci in wild animal populations is relatively small. Moreover, sample sizes for wild animals are considerably smaller than for domestic populations. Also, no pedigree information is available. In consequence, the statistical inferences for wild animal populations are limited.

Table 2

<table>
<thead>
<tr>
<th>Test</th>
<th>Microsatellite loci</th>
<th>NVHRT16</th>
<th>NVHRT21</th>
<th>NVHRT48</th>
<th>NVHRT73</th>
<th>RT1</th>
<th>RT7</th>
<th>RT13</th>
<th>RT27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson’s $\chi^2$</td>
<td></td>
<td>0.417</td>
<td>0.260</td>
<td>0.393</td>
<td>0.377</td>
<td>0.526</td>
<td>0.554</td>
<td>0.251</td>
<td>0.396</td>
</tr>
<tr>
<td>LR $\chi^2$</td>
<td></td>
<td>0.226</td>
<td>0.038</td>
<td>0.325</td>
<td>0.218</td>
<td>0.175</td>
<td>0.233</td>
<td>0.056</td>
<td>0.291</td>
</tr>
</tbody>
</table>

Note on symbol: LR $\chi^2$ - Likelihood ratio $\chi^2$ test.
Fig. 2. Clustering of forest- and field roe deer from three study areas in Poland.
On the other hand, in recent years, some reports on backgrounds of genetic diversity of roe deer across ecotypes have become available. For instance, KARPINSKI (2013) compared roe deer from two regions in eastern Poland located less than 100 km away. The first included mainly forested areas – habitats of forest roe deer, and the other included agricultural fields with a forest mosaic – habitats typical of field roe deer. His research revealed significant differentiation of biometric traits of roe deer from the two populations, but only insignificant genetic differentiation despite the fact that he took into account 18 microsatellite markers and the analysis of mitochondrial DNA polymorphism.

In the first stage of the analysis of genetic material of roe deer from three regions of Poland inhabited by both field and forest roe deer, the level of genetic variability was not strongly connected to population genetic structure.

Genetic distances between the populations assessed on the basis of Nei’s (1972) method gave results ranging from 0.01 to 0.07 (KAMIENIARZ et al. 2011). The Polish population did not show signs of strong population genetic structure. The results obtained for three genetic I class markers turned out to be especially important as only about 2% of the analyzed population was polymorphic (KAMIENIARZ et al. 2011). That made the assessment of differences between forest and field roe deer especially difficult.

As already mentioned, for applied statistical measures the distribution of microsatellite genotypes across the two ecotypes are very similar. Consequently, caution is advised when a relatively small number of microsatellite markers for a complex clustering of individuals is studied.

The telemetric research in the surroundings of Czemiń (KAMIENIARZ 2013) confirmed the fact that field roe deer populations are sustainable in the agricultural landscape so it remains open how they can accomplish this despite limited genetic differences between field and forest roe deer. Probably the choice of habitat by young roe deer is primarily a result of observation of the mother’s behaviour. A similar process was described for the peregrine falcon. This species also features two populations (eco-types) – cliff- or tree-nesting – with different habitat preferences during the breeding season (RATCLIFE 1993). According to KIRMS (2001), the tendency to nest on cliffs is innate for the peregrine falcon. However, thanks to the experience gained by nestlings raised in nests on trees (in forested areas), such falcons are able to utilize arboreal nests in the future. In the case of field roe deer, their offspring remain under the care of mothers at least until the spring months of the following year (KAMIENIARZ 2013). If during winter the female remains in the fields, which do not provide much cover but are rich in easily digestible food, its offspring acquire life experience there as well. As a consequence they may live in the fields self-sufficiently in the future, which was observed in marked young individuals from a field roe deer population after leaving their mothers (KAMIENIARZ 2013). If fawns are separated from females in winter, which took place in the experiment carried out by GRACZYK (1978), fawns – no matter where they were born and which habitat was preferred by their mothers and the environment where they were resettled, finally chose forest – which was in all likelihood genetically conditioned.

The role of the mother sometimes may be taken over by a herd, a phenomenon which was comprehensively presented by DROŠCHER (1982). Probably, this happens in field roe deer populations characterized by living in herds during the winter period. PIEŁOWSKI & BRESINSKI (1982) claimed that sometimes fawns of forest roe deer remained in the fields where they were resettled without mothers during winter. This took place in agricultural landscape already inhabited by field roe deer. This was probably a consequence of the fact that the young forest roe deer were allowed to join the winter groups of field roe deer and therefore gained experience essential for living in an open farmland in the winter. GRACZYK and BERE-SZYNSKI (1978) observed that a group of roe deer residing in a field enclosure during winter was quickly joined by individuals resettled there from a forest roe deer population.

Further experiments confirming the above hypothesis – which supplements the description of the process of occupying new habitats (KREBS 2011) – would be useful for institutions or persons managing wild populations. In the case of species increasing their range of occurrence, such processes result in changes in interspecies interactions and in the influence of the animals on their environment. One example of such a situation is the increased pressure of foxes on partridges (PANEK 2005) and hares (PANEK et al. 2006). Another problem is the progressively larger damage caused by wild boars in crop fields (GEISSER & REYER 2004; FRĄCKOWIAK et al. 2013) and in urbanized areas (KAMIENIARZ & PANEK 2008). Recognizing the genesis of the process of occupying new habitats could more effectively help to prevent the occurrence of animals in places where their presence is unwelcome.

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