*Acta Zoologica Academiae Scientiarum Hungaricae 49 (3), pp. 167–178, 2003*

# TEMPORAL AND SPATIAL PATTERN OF GENETIC DIFFERENTIATION IN ISOPHYA KRAUSSI (ORTHOPTERA: TETTIGONOIDEA) IN NE HUNGARY

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Allozyme polymorphism was studied in four samples of *Isophya kraussi* (BRUNNER VON WATTENWYL, 1878), a brachipterous, sedentary tettigonoid species. Three samples were collected in the Aggtelek Karst region (Haragistya 1998, Mogyoróskuti meadows 1999 and 2001) and one in the Zemplén Mts (Gyertyánkúti meadows 1998–1999). Enzyme polymorphism was investigated by polyacrylamide gel electrophoresis. All 10 scorable loci (*Aldox*, *Est*, *Got*, αGpdh, *Hk, Idh, Mdh, Me, Pgi* and *Pgm*) had alternative alleles in at least one sample. We detected a high level of polymorphism. On average, more than 60% of the investigated loci were polymorphic and the average number of alleles was about 2.1. The average heterozygote frequency was also high approximately 0.19. Large $F_{IT}$ values indicated a considerable level of genetic variation as well. The larger part of the total variation was explained by the within sample component and a relatively smaller portion of it was attributable to the between sample component. Many of the $F_{IS}$ values were positive indicating some heterozygote deficiency. In the analysis of spatial variation, we obtained highly significant $F_{ST}$ values, which suggested strong genetic differentiation between the local populations. In contrast, the $F_{ST}$ value calculated in the temporal analysis of variation was not significant. The average value of Nei's genetic distances between pairs of samples of geographically distinct populations was 0.071 while, that estimated within the Mogyoróskút population was 0.027. Thus, the results of all analyses indicated a higher level of differentiation among the geographically distinct populations than between the two samples collected in a single population in different years. The results of the PCA analyses fully confirmed those obtained in other analyses.

Key words: enzyme polymorphism, genetic differentiation, *Isophya kraussi*, Orthoptera

## INTRODUCTION

*Isophya kraussi* (BRUNNER VON WATTENWYL, 1878) (Orthoptera: Tettigonoidea) is known to be the most abundant among the six *Isophya* species described in Hungary (SZÖVÉNYI *et al*. 2001). It has a Central and South-East European distribution (RÁCZ 1998, RÁCZ *et al*. 1996). The species is widespread in hilly and mountainous areas of Hungary (NAGY & RÁCZ 1996). *I. kraussi* is phytophagous feeding on various dicotyledonous species. It prefers dense grassy vegetation with bushes in the thermic budgets of forest skirts and clearings (VARGA 1997). Like

other *Isophya* species *I. kraussi* is also flightless and consequently has a low dispersal rate and week colonizing ability (SZÖVÉNYI *et al*. 2001). In addition, it has a fairly slow life cycle. The eggs have a diapause for 1–4 years. As a consequence, one adult generation is composed of several cohorts. Although *I. kraussi* is not protected in Hungary it is potentially vulnerable due to the extensive fragmentation of its suitable habitats.

In theory, habitat fragmentation results in isolation and population subdivision. Consequently, it may have grave influence on the genetic structure of populations via decreasing effective population size and loss of genetic variation together with increasing differentiation among local populations (GILPIN 1991, HANSKI & GILPIN 1991). Nevertheless, different species may experience the same fragmented habitat in a different way (ROLSTAD 1991).

The main goal of this study was to analyse the level and structure of genetic variation in Hungarian *I. kraussi* populations. We also aimed at comparing the spatial and temporal components of this variation. The species is expected to have a low migration efficiency, which affects the spatial structure of variation. At the same time, it practically has overlapping generations influencing temporal variation within the population. Allozyme polymorphism was surveyed to estimate the level and structure of genetic variation in the investigated *I. kraussi* populations. Since the level of enzyme polymorphism in insect species is relative higher than in other taxa, we expected sufficient level of variation to analyse the genetic structure of the populations.

## MATERIAL AND METHODS

### *Samples*

Four samples were taken from two distinct geographical regions in Hungary (Fig. 1). In the Aggtelek Karst region, three samples were collected in Haragistya (near Aggtelek, 1998) and in the Mogyoróskuti meadows (near Jósvafő, 1999 and 2001). In the Zemplén Mts, one small sample was only collected in the Gyertyánkúti meadows (near Telkibánya, 1998 and 1999). In this way, we could determine the level of genetic differentiation in space and time.

### *Study of enzyme polymorphism*

Imagines were collected and stored at –80 °C until electrophoresis. Muscles dissected from the thorax and abdomen of the specimens were homogenised separately in approx. 4 µl/mg muscle of the extraction buffer (0.01M Tris, pH = 7.5). Enzyme polymorphism was investigated by polyacrylamide gel electrophoresis. Thorax samples were used to detect glutamate-oxalacetate transaminase (GOT), α-glycerophosphate dehydrogenase (αGPDH), hexokinase (HK), isocitrate dehydrogenase

(IDH), lactate dehydrogenase, (LDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and superoxid dismutase (SOD). The other four enzymes (aconitase (ACON), aldehyde oxidase (AOX), esterase (EST) and 6-phophogluconate dehydrogenase (6PGDH)) were detected from the abdomen of the specimens. Out of the total 14 loci, 10 (*Aox*, *Est*, *Got*, αGpdh, *Hk*, *Mdh*, *Me*, *Pgi*, *Pgm*) were scored consistently in each sample.

## *Statistical analyses*

Genotype and allele frequencies were calculated on the basis of banding patterns. Measures of genetic variation (average number of alleles, proportion of polymorphic loci, average observed and expected heterozygosity) were calculated for each population. Markov chain method was used to test the Hardy-Weinberg equilibrium (GUO & THOMPSON 1992). An exact test for population differentiation (RAYMOND & ROUSSET 1995*a*) was conducted to test for independence of the allelic composition of the populations. Genepop, version 1.0 (RAYMMOND & ROUSSET 1995*b*) was used to perform the

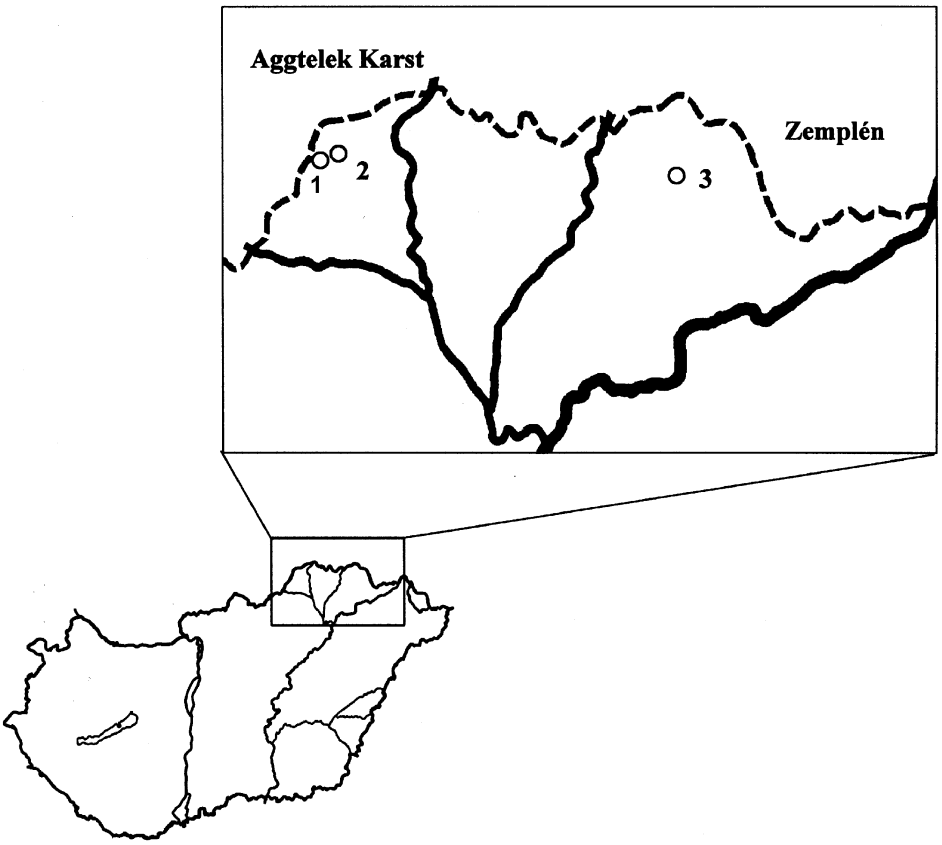


**Fig. 1.** Sample sites. Aggtelek Karst region: Haragistya near Aggtelek (1), Mogyoróskuti meadows near Jósvafő (2); Zemplén Mts.: Gyertyánkúti meadows near Telkibánya (3)

Hardy-Weinberg test, and the exact test of population differentiation. Genetic differentiation among the populations was also analysed by Wright's F-statistics (WRIGHT 1978, WEIR 1990). In this analysis, the total genetic variation of the samples ($F_{IT}$) was partitioned into within ($F_{IS}$) and between population components ($F_{ST}$). $F_{ST}$ values were also calculated using two different restricted data sets. First, F-statistics was computed within the Mogyoróskút population between the two samples collected in different years. As a next step, $F_{ST}$ values were also calculated among the three geographically distinct populations. All analyses were conducted by FSTAT version 1.2 (GOUDET 1995). Allele frequencies were used to estimate Nei's genetic distances (NEI 1975) and an UPGMA dendrogram (SNEATH & SOKAL 1973) was constructed on the basis of these data. The computation was performed by Biosys-1, Release 1.7 (SWOFFORD & SELANDER 1981). In the last part of the study, we carried out a principal component analysis (PCA) using the genotypic composition of the individuals to show the size of overlap in the genetic variation of the populations in a reduced space of variables. PCA was also carried out in two steps. In the first analysis, the two samples collected in Mogyoróskút in different years were only involved. The second analysis was performed using the samples collected in the three geographically distinct populations. Both PCA analyses were performed running R Package Version 4.0 (CASGRAIN & LEGENDRE 2001).

## RESULTS

Although the sample sizes were rather low we detected a high level of polymorphism in the *I. kraussi* samples. Nine out of the total ten scorable loci was found to be polymorphic in at least one sample using the 0.95 criterions. On average, over 60% of the investigated loci were polymorphic and the average number of alleles was about 2.1 (Table 1: P and n). As a consequence, the average heterozygote frequency was also, high approximately 0.19 (Table 1: Ho). The observed frequency of heterozygotes was consistently lower than expected in all

**Table 1.** The most important parameters of genetic variability estimated for the four samples of I. kraussi at the 10 investigated loci. N = average sample size, n = average number of alleles, P = proportion of polymorphic loci; $H_o$ = observed frequency of heterozygotes; $H_e$ = expected frequency of heterozygotes; $F_{IS}$ = the average size of the departure of heterozygote frequency from the Hardy-Weinberg expectation

| | Karst | | | Zemplén |
|---|---|---|---|---|
| | Haragistya | Mogyoróskút | | Gyertyánkút |
| | 1998 | 1999 | 2001 | 1998–99 |
| N | 15.9 | 10.9 | 9.8 | 7.4 |
| n | 1.8 | 2.0 | 2.4 | 2.1 |
| P | 0.50 | 0.50 | 0.90 | 0.80 |
| $H_o$ | 0.184 | 0.194 | 0.229 | 0.168 |
| $H_e$ | 0.231 | 0.282 | 0.343 | 0.331 |
| $F_{IS}$ | 0.208 | 0.317 | 0.349 | 0.524 |

**Table 2.** Results of F-statistics and the exact test of population differentiation computed on the data of all four samples. $F_{IT}$ measures of total genetic variation; $F_{IS}$ measures of the genetic variability within the populations; $F_{ST}$ measures of the genetic variation among the populations. * significant at 0.05 level; ** significant at 0.01 level; *** significant at 0.001 level

| | F-statistics | | | Exact |
|---|---|---|---|---|
| Loci | FIT | FST | FIS | probability |
| Aox | 0.380** | 0.086* | 0.322** | *** |
| Est | 0.386** | 0.050 | 0.354** | ns |
| Got | 0.499** | 0.058* | 0.468 | * |
| Gpdh | 0.110 | 0.098** | 0.013 | ** |
| Hk | 0 | 0 | 0 | ns |
| Idh | 0.248* | –0.007 | 0.253 | ns |
| Mdh | 0.041 | 0.020 | 0.022 | ns |
| Me | 0.620** | 0.173** | 0.541* | *** |
| Pgi | 1.000** | 0.020 | 1.000* | ** |
| Pgm | 0.651** | 0.083* | 0.619** | * |
| Total | 0.374** | 0.065** | 0.331** | *** |

samples (Table 1: Ho vs He). All further statistical analyses were based on the 10 investigated loci.

The results of the F-statistics also showed a high level of genetic variation (Table 2: $F_{IT}$). The larger part of the total variation was explained by the within sample component (Table 2: $F_{IS}$) and a relatively smaller portion of it was attributable to the between sample component (Table 2: $F_{ST}$). Most of the $F_{IS}$ values calculated for the individual loci were high and positive and 5 of them were significant (Table 2: *Aox*, *Est*, *Me*, *Pgi* and *Pgm*). Consequently, the overall $F_{IS}$ value averaged for the 10 loci was highly significant suggesting considerable heterozygote deficiency in the samples. Similarly to the $F_{IS}$ values, significant Hardy-Weinberg disequilibrium was detected at some of the individual loci in the four samples (Table 3). That is, the *I. kraussi* populations exhibited a general tendency of heterozygote deficiency. An interesting result of the Hardy-Weinberg tests was that consistent deviation from the equilibrium across all samples was observed at those five loci (Table 3: *Aox*, *Est*, *Me*, *Pgi* and *Pgm*), for which the $F_{IS}$ values proved to be significant.

The highly significant overall $F_{ST}$ value indicated relatively high genetic differentiation among the samples (Table 2). In the spatial analysis of variation, the $F_{ST}$ values were calculated in two steps. First, all three populations, which belonged to two different regions were involved. In this analysis, significant differ-

**Table 3.** Results of Hardy-Weinberg tests. E = heterozygote excess; D = heterozygote deficiency; – = no alternative allele was detected in the sample. * significant at 0.05 level; ** significant at 0.01 level; *** significant at 0.001 level. Bold characters indicate that the exact probability is lower than 0.1

| | Karst | | | Zemplén | Total |
|---|---|---|---|---|---|
| Loci | 1998 | 1999 | 2001 | | |
| Aox | **D*** | **D** | D | D | **D*** |
| Est | D | **D*** | D | **D*** | **D*** |
| Got | – | – | – | – | – |
| Gpdh | – | D | E | E | D |
| Hk | – | – | – | – | – |
| Idh | **D** | – | E | – | D |
| Mdh | E | D | **D*** | D | D |
| Me | – | – | D | **D** | **D** |
| Pgi | – | – | **D** | **D** | **D*** |
| Pgm | D | D | **D**** | **D** | **D**** |
| Total | **D*** | **D** | **D**** | **D*** | **D***** |

**Table 4.** $F_{ST}$ values and the results of the exact test of population differentiation calculated on restricted data sets. Time: values estimated for the two samples collected in the Jósvafő population in different years; Space: values estimated for the distinct populations; Karst and Zemplén: the data of all three populations were involved; Karst: the data of the two Karst populations were only included. * significant at 0.05 level; ** significant at 0.01 level; *** significant at 0.001 level

| | Time | | Space | | | |
|---|---|---|---|---|---|---|
| Loci | Mogyoróskút | | Karst and Zemplén | | Karst | |
| | $F_{ST}$ | Exact prob. | $F_{ST}$ | Exact prob. | $F_{ST}$ | Exact prob. |
| Aox | –0.035 | ns | 0.150** | *** | 0.163* | ** |
| Est | 0.054 | ns | –0.029 | ns | –0.013 | ns |
| Got | 0.005 | ns | 0.106* | * | – | – |
| Gpdh | –0.006 | ns | 0.166** | *** | 0.285** | ** |
| Hk | –0.005 | ns | 0.002 | ns | 0.018 | ns |
| Idh | 0.013 | ns | –0.028 | ns | –0.043 | ns |
| Mdh | –0.055 | ns | 0.049 | ns | –0.034 | ns |
| Me | 0.064 | ns | 0.301* | *** | – | – |
| Pgi | 0.010 | ns | 0.064 | ns | – | – |
| Pgm | –0.017 | ns | 0.161* | ** | –0.008 | ns |
| Total | –0.004 | ns | 0.096*** | *** | 0.056* | ** |

entiation was detected at the same five loci as in the analysis of all four samples. Moreover, the overall $F_{ST}$ value calculated for these three populations was slightly higher than the one estimated for all four samples (Table 2 vs Table 4: Space – Karst and Zemplén). When the F-statistics was computed for the two Karst populations only (Haragistya and Mogyoróskút), the overall $F_{ST}$ value was still significant. Nevertheless, significant differentiation was only observed at two loci in this analysis (Table 4: Space – Karst). That is, the level of genetic differentiation between two local populations within a geographic region was still sizeable. In contrast, the overall $F_{ST}$ value was practically zero in the temporal analysis of variation (i.e. between the Mogyoróskút samples collected in two different years (Table 4: Time). Genetic differentiation was also analysed by Fisher's exact test. In these analyses, we obtained similar results to that of the F-statistics (Tables 2 and 4).

Nei's genetic distances calculated on the basis of allele frequencies revealed a similar pattern of variation to that found in other analyses. The D value calculated between the two populations in the Karst region was 0.037 (Fig. 2). Furthermore, the average D value estimated between pairs of populations originating from the two different regions was 0.088. The genetic distance between the two samples collected in Mogyoróskút in 1999 and 2001, however, was only 0.027 (Fig. 2). Thus, genetic distances also suggested a higher level of differentiation among the three distinct populations than between the two samples collected in a single population. Furthermore, the dendrogram also indicated a geographic pattern of spatial variation. In other words, the two populations of the Karst region seemed to be more similar to each other than to the one in the Zemplén Mountains.

The outcome of the PCA analyses confirmed the results we obtained in the other analyses. When the three populations were compared the individuals of the

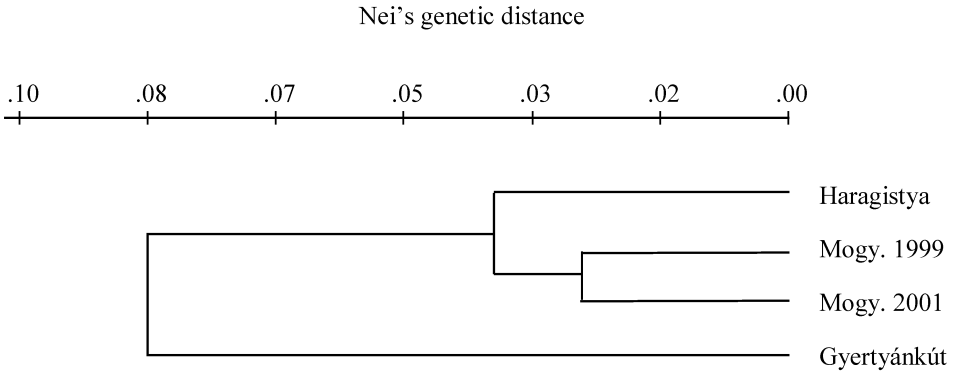


**Fig. 2.** UPGMA dendrogram constructed on the basis of Nei's genetic distances

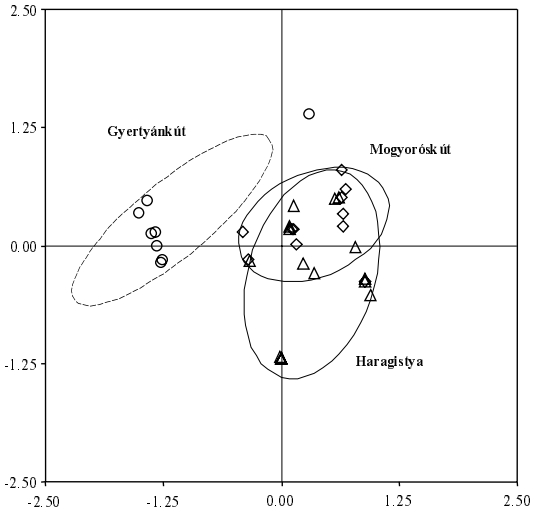


**Fig. 3.** Results of the PCA analysis for the three distinct populations; i.e. spatial variation (Karst region: Mogyoróskuti meadows, Haragistya; Zemplén Mts.: Gyertyánkúti meadows). The points represent the genotypic composition of the individuals along the first two axes in a reduced space of variables

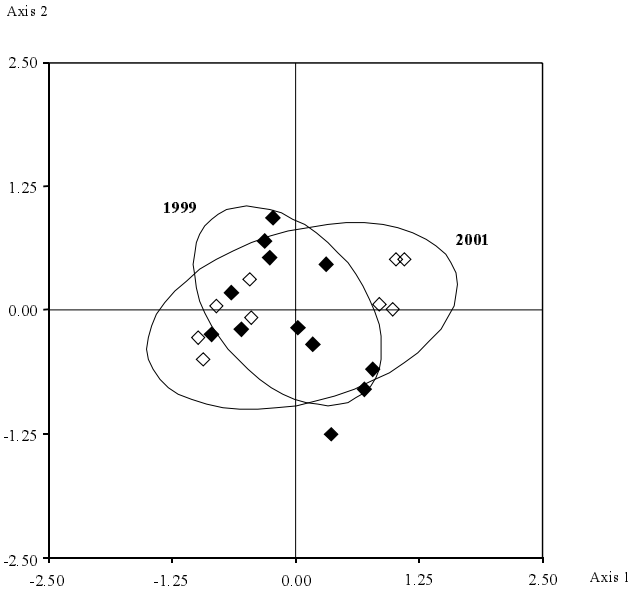


**Fig. 4.** Results of the PCA analysis for the two samples collected in the Mogyoróskuti meadows in 1999 and in 2001; i.e. temporal variation within a population. The points represent the genotypic composition of the individuals along the first two axes in a reduced space of variables

Gyertyánkút (Zemplén Mts) population comprised a distinct cloud of points without any overlap with the two Karst populations (Fig. 3). The first two axes explained approx. 50% of the total variation present at the 10 loci. The ellipses indicated that the differentiation between the populations of the two regions was most pronounced along the first axis (Fig. 3). This component of variation was predominantly determined by the genotypic composition at the *Pgm* locus. Although there was a considerable overlap between the clouds of points representing the two Karst populations some outstanding individuals of the Haragitya population also indicated a sizeable level of differentiation. This was most clear along the second axis (Fig. 3), which was primarily affected by the *Mdh* and *Me* loci. On the contrary, when the PCA analysis was carried out on the samples collected in two different years in the Mogyoróskút population most of the individuals were involved in one large cloud of points along the first two axes resulting in great overlap between the two samples (Fig. 4). These two axes explained about 50% of the total variation.

## DISCUSSION

We observed a high level of polymorphism in the *I. kraussi* samples at the 10 investigated enzyme loci. This level is slightly higher than that described in other Orthoptera species. In the *Ephippiger* species complex, OUDMAN and his co-workers (1990) found that the portion of polymorphic loci was slightly higher than 20 per cent with the heterozygote frequency ranging from 0.03 to 0.14. In the South American populations of a grasshopper (*Trimerotropis pallidipennis* BURMEISTER, 1838), the portion of polymorphic loci was about 0.35 and the average heterozygosity was over 0.15 (MATRAJT *et al.* 1996). Similar level of polymorphism was detected in the Italian populations of a cave cricket (*Dolichopoda schiavazzii* CAPRA, 1934) by ALLEGRUCCI *et al.* (1997). It thus appears that the high level of genetic variation detected by enzyme electrophoresis is a general feature of various orthopteroid insects. It is interesting as the above species belong to different taxa within Orthoptera with various life history and demographic characteristics. Consequently, we expect different levels of polymorphism in their populations especially according to the neutral theory of population genetics (KIMURA 1983).

All samples exhibited an overall heterozygote deficiency, which resulted in significant Hardy-Weinberg disequilibrium at some loci. Heterozygote deficiency was also found in e.g. *Dolichopoda* populations by ALLEGRUCCI and his coworkers (1997). They suggested that this phenomenon was due to the presence of two-yearly cohorts in the populations. Since the *I. kraussi* have 1–4 years diapause

in the egg stage its populations are comprised of different cohorts as well. Nevertheless, heterozygote deficiency can be the consequence of several other evolutionary forces in natural populations (e.g. inbreeding, selection, etc.). At present, our data are not sufficient to explain the heterozygote deficiency detected in the investigated populations. Further genetic surveys combined with demographic studies are required to test the hypothesis on the dominating evolutionary force in the *I. kraussi* populations.

Since *I. kraussi* has poor dispersal ability we expected a high level of differentiation among its local populations. Both the $F_{ST}$ values and the results of the exact test of population differentiation fulfilled our expectation. The genetic differences between the two Karst populations were significant and the differentiation was even more pronounced between the two geographic regions. Other studies have described various $F_{ST}$ values for different Orthoptera species. MATRAJT *et al.* (1996) have reported a similar level of differentiation in *Trimerotropis pallidipennis*. At the same time, ORR *et al*. (1994) have found an exceptionally high level of differentiation ($F_{ST}$ = 0.449) in *Melanoplus sanguipes* (FABRICIUS, 1798). They detected, however, a much lower level of differentiation ($F_{ST}$ = 0.084) in *M. devastator* (SCUDDER, 1878), which is a close relative to *M. sanguipes* (ORR *et al*. 1994). Hard to explain the great differences in the level of genetic differentiation among the local populations of these species since the sampling areas were similarly large and the species had comparable dispersal ability. In a flightless bush cricket *Ephippiger ephippiger* (FIEBIG, 1784), OUDMAN *et al*. (1990) have described a relatively high level of differentiation among 20 local populations. They found that Nei's genetic distances ranged from about 0.01 to 0.1. The *D* value we calculated for the three investigated populations of *I. kraussi* falls within this range. That is, the level of genetic differentiation was similarly high in these flightless species, which are predicted to have low migration efficiency. Although in general, Orthoptera species seem to have a relatively high level of differentiation among local populations there are sizeable differences as well in their fine population structure. It is, however, remarkable that these differences among the species are not in apparent correlation with the variation in their vagility or habitat requirements.

We observed that both the $F_{ST}$ value and Nei's genetic distance were much higher in the spatial than in the temporal analysis of variation. These results fulfilled our expectations as they indicated that spatial variation was higher than temporal one in the investigated *I. kraussi* populations. On the one hand, *I. kraussi* is a flightless bush cricket and therefore we can assume low dispersal ability for this species. As a consequence, migration can hardly counterbalance the effect of genetic drift. We, therefore, predicted relatively strong differentiation among local populations that is a high level of spatial variation. On the other hand, we expected

a low variation in the genetic composition of consecutive generations. *I. kraussi* may have long and variable diapause in the egg stage, which results in a similar situation to overlapping generations. That is, all adult generations consist of various cohorts. Since in every particular generation, the offspring of different adult generations may interbreed randomly, there is low chance of great genetic variation among consecutive generations.

In summary, we can conclude that *I. kraussi* populations exhibited a high level of genetic variation. Genetic variation among the three distinct populations was higher than that among different yearly samples within a population. The high level of genetic differentiation among the populations suggests low migration efficiency. Since *I. kraussi* has poor colonising ability there is a very low chance of recolonisation after a population goes extinct. As a consequence, it is of great conservation interest to have their habitats (dense grassy vegetation of forest skirts and clearings) undisturbed.

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*Acknowledgements* – The authors are especially grateful to the Hungarian Nature Conservation Authorities for the permissions of sample collections. The authors are also obliged to Dr. ISTVÁN RÁCZ who collected some of the samples. The technical assistance of VALÉRIA MESTER is highly appreciated. The project was supported by FKFP 0473/200 grant of the Ministry of Education.

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Revised version received September 25, 2003, accepted October 22, 2003, published November 30, 2003