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Genetic structure of the populations of *Spilostethus pandurus* in the wadis of the St Katherine Protectorate, South Sinai

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Abstract

The genetic diversity of *Spilostethus pandurus* was studied using RAPD markers in individuals sampled from four wadis within the St Katherine area. A total of 109 different RAPD bands were generated for the whole sample: site-specific bands occurred at low frequency. Even though there were many genetic differences among individuals within sites, the sites were statistically distinct. Thus even in thus fairly long-lived and apparently fairly mobile insect, there is evidence of genetic isolation among the wadis of this highly dissected environment.

Keywords: aposematism, gene flow, genetic diversity, RAPD, Sinai

Introduction

The aposematically colored soldier bug (*Spilostethus pandurus* Scopoli, 1763; Hemiptera, Lygaeidae) is is widely distributed in tropical and subtropical parts of the eastern Old World (Gentry 1965, CAB International 2003). It is polyphagous, feeding upon a wide range of cultivated and wild plants (Kugelberg 1973, Chopra & Yadav 1974), and can be reared on sunflower and *Asclepias syriaca* seeds (Kugelberg 1974, Thangavelu 1978). It is said to feed preferentially on members of the Apocynaceae (Asclepiadaceae) such as *Calotropis* (Sweet 2000). It is found all over Egypt (Priesner & Alfieri 1953) and is very common in South Sinai, living in small groups and feeding on the seeds and tissues of Sinai milkweed, *Asclepias (=Gomphocarpus) sinaica* (Boiss.) Muschl., 1912.

Aposematism is widespread among the specialized herbivores of the Apocynaceae, and many sequester toxins from their host plants for defense against predators. Another lygaeid bug, *Oncopeltus fasciatus,* shows morphological and physiological adaptations for sequestration of cardenolides (Scudder 1986). In Sinai, *S. pandurus* sequesters toxic cardiac glycosides from milkweed plants, presumably for its own protection (El-Banna 2004). The gregariousness shown by *S.pandurus* is common among aposematic species (Järvi *et al*. 1981), probably to amplfy the aposematic signal (Gamberale & Tullberg 1996).

This study forms part of a larger research programme on the possibility of micro-coevolution between insects and their host plants in the highly dissected and fragmented environment of the St Katherine Protectorate of South Sinai (Gilbert et al. 1996, Gilbert 1999). We use random amplified polymorphic DNA via the polymerase chain reaction (RAPD–PCR) as molecular markers to investigate level of genetic variation within and between samples of insects from four wadis partially isolated by high mountain ridges (allopatry), and also within the continuous habitat of one wadi without geographical separation (sympatry). RAPDs are good markers for distinguishing between species (Figueroa *et al*. 1999) and subspecies (Gallusser *et al*. 2004), and in studying the genetic structure of species (Moya *et al.* 2001).

Materials & Methods

During June 2004, *Spilostethus pandurus* were collected by hand in small vials and stored afterwards in –20ºC until use. The bugs were collected from four wadis within the St Katherine

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Protectorate (Fig. 1): wadi El Arbaein is a steep rocky gorge running SW from the town of St Katherine for 3 km (8 individuals sampled); wadi El Dir contains the Monastery of St Katherine, and meets the Plain of el Raha (9); wadi El Tofaha is the shortest, steepest and narrowest wadi, running for 1 km south from the town (10); and El Rasis is a broad flat wadi near the town (9).

We selected wadi El Arbaein for the study of genetic variation within a single wadi. Four quadrats of 50 x 50 m were established 600 m apart in a linear transect along the wadi bed, and 3-4 individual *S. pandurus* were sampled from each quadrat. These samples were treated in the same way as the among-wadi samples.

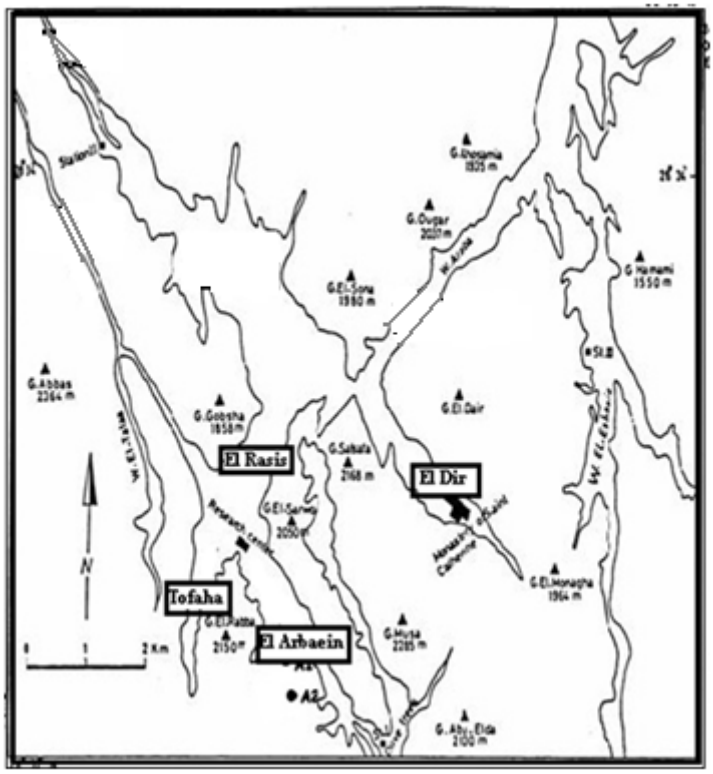

Fig. 1: Map of St Katherine, the wadis in black frame represents the sites from which species under study were collected.

DNA was extracted from the whole insect using a standard CTAB extraction procedure (Wolff *et al*. 1994, modified after Saghai-Maroof *et al*. 1984). DNA samples were cleaned in ammonium acetate solution by adding half the volume of 7.5 M cold ammonium acetate to each DNA sample, then cooled in a fridge for 15 min, followed by spinning for 15 min at 5000 rpm. To the supernatant was added two volumes of cold 96% ethanol, mixed gently and left for 30 min in a freezer. After spinning for 15 min, the precipitate was taken and 500 µl of cold 70% ethanol added for washing; the supernatant was removed, and the precipitate left to air-dry at room temperature for 10-20 min and then dissolved in a suitable volume of TE buffer.

Before further analysis, it is important to determine the concentration and condition of the DNA isolate. This was done by comparison with DNA standards on concentration gels. Twenty primers from the OPH set were screened for polymorphisms among individual plants: five primers (OPH-04, OPH-07, OPH-13, OPH-18, and OPH-19) with clear and reproducible bands were chosen for this study. The genomic DNA extracts were used as templates for PCR experiments. A reaction mixture (master mix) was prepared for each primer sufficient for all samples plus one negative control to which water was added instead of DNA. RAPD analysis was performed in 25-µl volume reactions (according to Wolff & Peters Van Rijn 1993). All

reagents were centrifuged and kept on ice during the preparation of the master mix. Amplifications were carried out in a Mastercycler gradient programmed according to Wolff (1996). After amplifications, the samples were electophoresed on a 1.4% agarose gel: visualization of the DNA fragments was done in a UV cabinet unit and photographed with a Polaroid camera connected to a computer system with software for the analysis of the DNA fragments.

RAPD bands were scored as 1 (present) or 0 (absent) using GelDocuAdvanced software. The resulting presence/absence matrix was analyzed using several computer programs. MVSP was used to construct a Neighbour Joining tree of all individuals using Euclidean distances computed between all pairs of individuals. Shannon's index of diversity ($H = -\sum pi \ln pi$, where pi is the frequency of a given RAPD fragment, and ln is the natural logarithm) was calculated from the frequencies of RAPD bands within and among each site (King & Schaal 1989) to obtain estimates of the within-sample genetic diversity (H_s) and the total genetic diversity across all the sites (H_t). The proportion of diversity within sites was estimated as H_s/H_t, and the proportion of diversity among sites as $(H_t-H_s)/H_t$.

Genetic dissimilarities were calculated among samples using the routine SimPer of the Community Analysis Package 4.1.3 (Pisces Conservation Ltd, Lymington, UK) and significant differences among samples tested using Analysis of Similarity implemented by the same package. Nei's genetic distance was calculated from band frequencies as if they were alleles using the routine gnkdst from the program DISPAN by T.Ota, available as freeware. Since there were only four sites, it was not possible to perform the non-paraemtric Mantel tests to test for relationships between genetic and geographic distances (minimum number of sites = 5).

Results

Among wadis

There were one hundred and nine DNA fragments recorded from the whole set of sampled individuals. The total number of bands scored per primer ranged from 17 (OPH-13) to 25 (OPH-4 & OPH-19). The size of the amplified fragments ranged from 100 to 2107 base pairs. Twenty-one bands were common in all samples (Fig. 2). Of the 109 bands analyzed, nine DNA fragments were exclusive to wadi El Rasis; eight to El Arbaein; seven to El Dir and six to wadi El Tofaha.

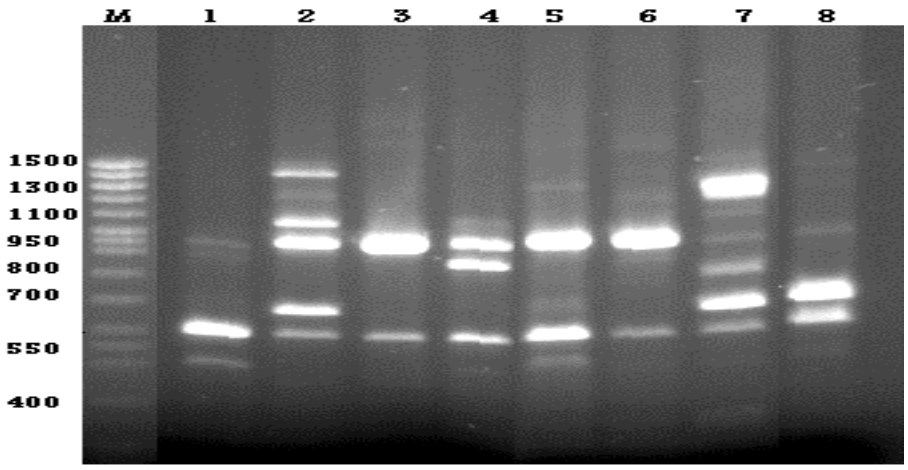

Fig. 2: RAPD pattern from genomic DNA of *Spilostethus pandurus* collected from wadi El Arbaein using primer 7. M refers to the marker; 1-8 represents the individual insects.

The neighbour-joining tree of the 36 sampled individuals (Fig. 3) showed that individuals from the same site clustered together, and are therefore more genetically similar than individuals

from other sites. Individuals from El Dir and El Arbaein are slightly more related to each other than they are the other two sites.

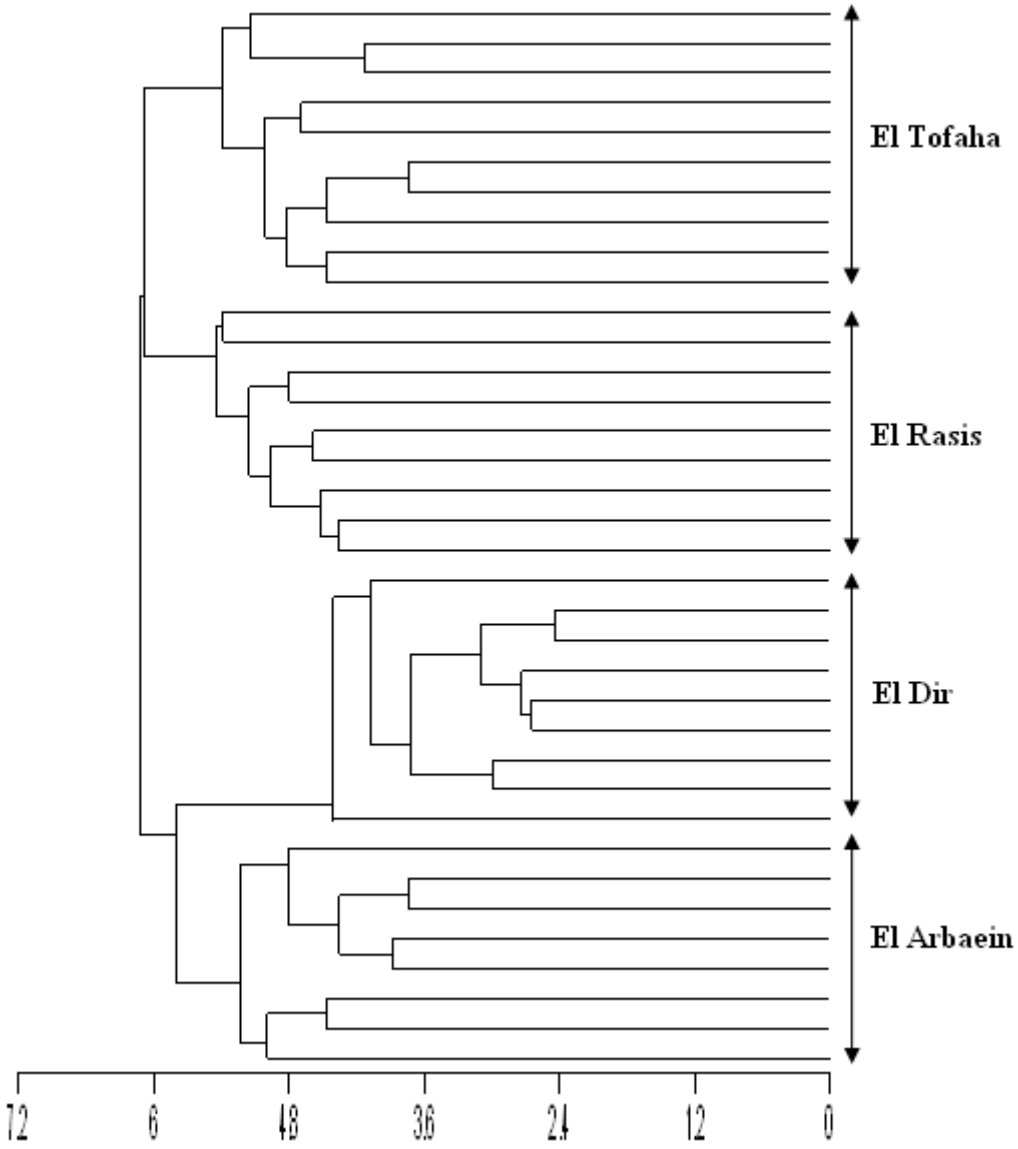

Fig. 3: Neighbour-joining dendogram based on the RAPD pattern of *Spilostethus pandurus* collected from four sites. The scale of the distances in the dendogram is shown as E/n, where E is the Euclidean distance and n is the number of polymorphic fragments.

From the frequencies of the RAPD fragments, estimates of individual genetic diversity, within-sample genetic diversity (H_s) and total genetic diversity across samples (H_t) were obtained (Table 1). The mean band diversity within individuals was between 0.84 and 0.91 of the pooled site diversity. The proportion of total diversity distributed within samples (0.91) was greater than that among samples (0.09). Analysis of similarity (Anosim) showed that sites were significantly distinct genetically (overall test statistic = 0.84, $p<0.001$), and pairwise tests showed that each was significantly different from all the others.

Sample	N	bands	H	mean H
Arbaein	8	314	4.079	3.662
Dir	9	288	3.785	3.462
Rasis	9	312	4.071	3.539
Tofaha	10	314	4.032	3.401
		mean	3.992	3.516
Total	36	1228	4.377	± 0.039

Table 1: Summary of RAPD band diversity among the sampled sites. N=number of individuals sampled; bands = total number of bands registered; H = Shannon-Wiener diversity of bands (data pooled across individuals); mean H = band diversity averaged for the individuals sampled. The s.e. of the total diversity results from a jackknife procedure.

Since the variances in the Shannon genetic diversities among sites were not homogeneous, a Welch test was used: this showed there were significant differences among the band diversities of the four sampled sites (Welch $F_{3,16.9} = 4.23$, $p<0.05$), differences that were still probably present even if a non-parametric test was used (Kruskal-Wallis H = 7.17, df=3, p=0.067) (Fig. 4).

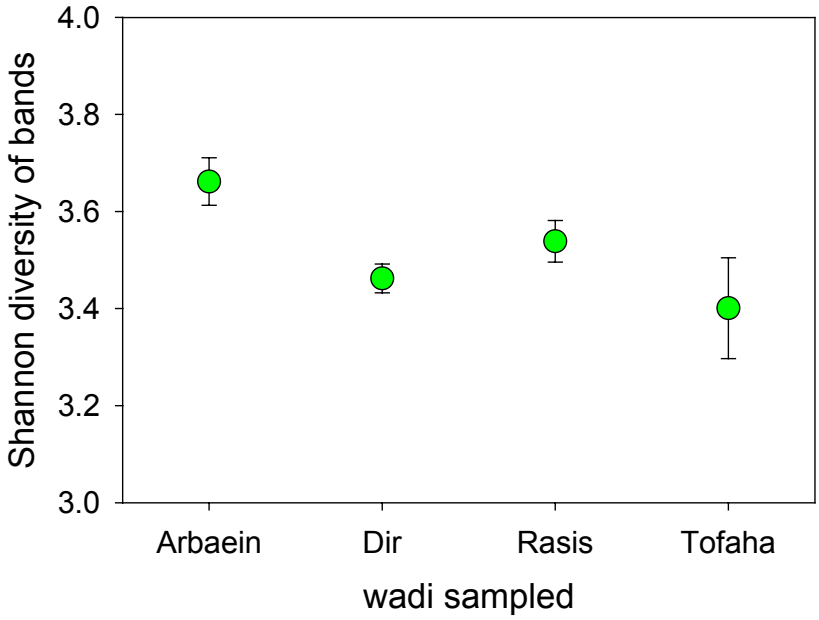

Fig. 4: The difference in mean individual diversity of bands based on RAPD pattern data of *S. pandurus* populations from the four sites

Estimates of genetic dissimilarities based upon the 109 bands showed that the lowest average value is between individuals from wadi El Arbaein and those from wadi El Dir; the greatest average value is between individuals from wadi El Dir and wadi El Tofaha. A scatterplot of the pairwise genetic dissimilarity and geographical distance between sampled sites is shown in Fig. 5. Nei's genetic distances was lowest between El Rasis and El Tofaha, and highest between El Dir and El Tofaha.

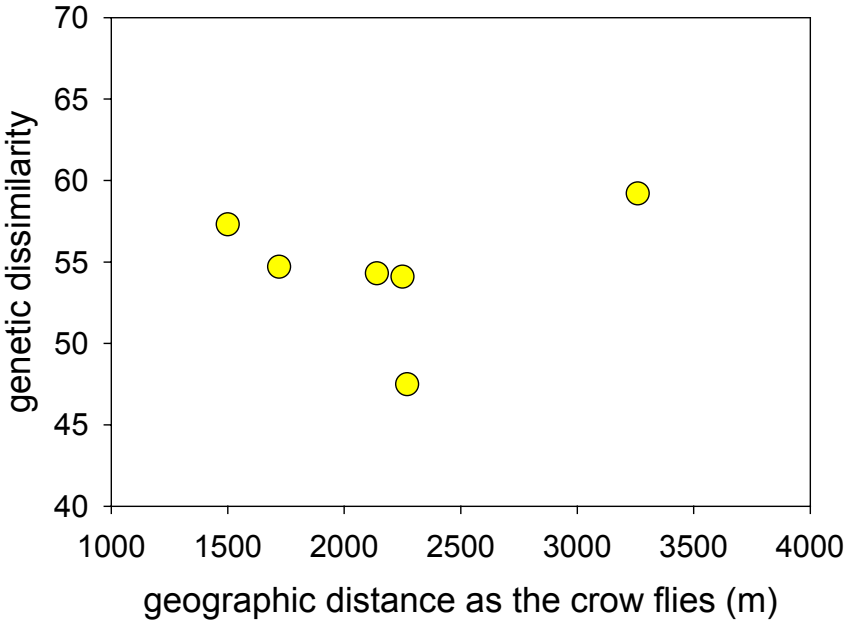

Fig. 5: Relationship between genetic distances and geographical distance between sites where *Spilostethus pandurus* was sampled. There are too few sites for a reliable statistical test of a relationship between these variables.

Within wadi El Arbaein

Individuals from the linear transect up wadi El Arbaein also revealed high levels of genetic variation. Sixty-one DNA fragments were generated from all individuals combined. The total number of bands scored per primer ranged from 11 (OPH-7) to 14 (OPH-4). The size of the amplified fragments ranged from 108 to 1334 base pairs (bp). Twenty-five bands were common in individuals of all quadrats. Of the 61 bands analyzed, seven, two, five and two DNA fragments were exclusive to each of the quadrats from the base to the top of the wadi.

The neighbour-joining tree constructed from the matrix of Euclidean distances between the 15 sampled individuals showed that all individuals from one quadrat clustered together, whereas those from the others were more mixed. There were no differences among quadrats in Shannon's diversity index ($F_{3,11} = 0.84$, n.s.), and only 0.06 of the variation in diversity was estimated to be among-quadrat variation. Despite this, Anosim still was easily able to distinguish the quadrats from the bands of the sampled individuals (test statistic = 0.37, $p<0.01$).

Discussion

Here we have shown that, even on very small spatial scales, there is significant spatial genetic structuring within the population of *S. pandurus*. We were unable to test for a distance effect, so we are unsure of the source of this variation. Among studies of herbivorous insects, studies of isolation by distance are available for large spatial scales, whereas studies over small geographic distances are relatively rare, in particular for species where population turnover is high (Massonnet & Weisser 2004).

Adult *S. pandurus* are active in April, increasing in abundance through early summer months and peaking in July; they have a bimodal activity pattern during the day, avoiding high midday temperatures (El-Banna 2004). Individuals have been observed to fly more than 30 m, visiting different *Asclepias* plants if these are reasonably close together, but individuals tended to return to the same plant for shelter overnight (El-Banna 2004). The only adult longevity

estimates are from captivity: one reported 24-32 days in summer, and 24-48 days in winter (Bhattacherjee 1959), whereas Kugelberg (1973) found that adults live for about three months, with some individuals surviving for up to seven months. *S. pandurus* is capable of completing 6-7 overlapping generations in the warm conditions of southern India (Thangavelu 1979); when reared in field cages at Giza in Egypt, it completed six overlapping generations in one year (El-Shazly 1995).

This insect therefore has the potential to form large populations, and certainly lives long enough to move fairly long distances. It is therefore rather unexpected to find genetic differences among geographically very close sites (whose extent is only 3.5 km^2, and lie only 1.5-4.0 km apart). Leslie & Dingle (1983) and Leslie (1990) found that quantitative molecular-genetic differentiation of *Oncopeltus fasciatus* only existed at the scale of thousands of kilometers. It is possible that the highly dissected and fragmented habitat of South Sinai promotes genetic differentiation among all organisms living there, which makes a detailed study of this area even more interesting.

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الملخص العربيU

التركيب الوراثى لعشائر حشرة بق النبات "سبلوستيثيس باندوروس" فى الوديان المختلفة لمحمية سانت كاترين

منى على محمودP[1] – سامى زلطP[2] – سمية العقادP[3] – فرانسيس جلبرتP[4]

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4- قسم علوم الحياة والبيئة – جامعة نوتنجهام – المملكة المتحدة

يتناول هذا البحث دراسة التركيب الوراثى لأفراد وعشائر أحد أنواع بق النبات المنتشر فى محمية سانت كاترين **" سبلوستيثيس باندوروس"** ، تم جمع العينات من الوديان التالية: وادى الأربعين، وادى الدير، منطقة الرصيص، وادى التفاحة، وتم التعرف على التركيب الجينى لها بإستخدام تقنية الرابد "RAPD" وذلك لبيان مدى التطابق او الاختلاف فى التركيب الوراثى للافراد داخل نفس العشيرة فى الوادى الواحد، وأيضا بين العشائر المختلفة فى الوديان المختلفة.
ولقد أثبتت النتائج:-

1- أن هناك تباين بسيط (غير معنوى) فى التركيب الوراثى لأفراد النوع داخل الوادى الواحد

2- تتشابه أفراد وادى التفاحة مع منطقة الرصيص ويرجع ذلك لقرب المنطقتين من بعضهما البعض بالإضافة إلى الطبيعة الجغرافية للمنطقتين حيث أنهما أراضى منبسطة ومفتوحة ومتصلة مع بعضها البعض

3- أوضح التحليل الإحصائى وجود اختلاف معنوى واضح فى التركيب الوراثى لأفراد العشائر فى الوديان المختلفة بمعنى أن أفراد كل منطقة لا تتشابه وراثياً مع مثيلاتها فى الوديان الآخرى، مما يعنى وجود عزل وراثي بين عشائر حشرة **سبلوستيثيس باندوروس** نتيجة للتباين الموجود في القطع الجينية **(DNA fragments)** بين الوديان المختلفة بالرغم من قدرة هذه الحشرة علي الانتشار والانتقال بين الوديان لكونها من الانواع الواسعة الانتشار وما تتميز به من قدرتها على التغذية على عوائل نباتية متباينة الإ أن الطبيعة الجغرافية ووجود الجبال الشاهقة فى منطقة المحمية حالت دون حدوث إنسياب للجينات بين أفراد العشائر المختلفة فى الوديان المختلفة لتؤكد مدى التميز الوراثى للأنواع الحيوانية فى تلك المنطقة