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MICROSATELLITE LETTERS

Isolation and characterization of polymorphic microsatellites in the specialist grasshopper *Ramburiella hispanica* (Orthoptera: Acrididae)

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Abstract We describe 12 polymorphic microsatellite markers for *Ramburiella hispanica* (Orthoptera: Acrididae), a specialist Mediterranean grasshopper that often forms highly fragmented populations due to extensive clearing of natural vegetation for agriculture. Polymorphism at these loci was evaluated in 20 individuals from La Mancha region, Central Spain. The number of alleles per locus ranged from 7 to 19 and their observed and expected heterozygosities ranged from 0.41 to 0.90 and from 0.76 to 0.91, respectively. These loci will be highly useful for the study of the genetic structure and diversity of this grasshopper species and understanding the demographic and genetic consequences of population fragmentation in Mediterranean terrestrial organisms.

Keywords Genetic diversity · Landscape genetics · Population fragmentation

Introduction

Ramburiella hispanica (Rambur 1838) (Orthoptera: Acrididae) is a Mediterranean grasshopper distributed in east France, Spain, Morocco, Tunisia, Algeria and Libia. It is a specialized organism generally restricted to areas covered with esparto grasses, particularly *Lygeum spartum* and *Stipa* sp. In many regions across its distribution range, this species forms highly fragmented populations due to historical and extensive natural vegetation clearing for agriculture. This is the case of La Mancha region (Central Spain), where we are performing a long-term study aimed to understand the consequences of population fragmentation across a network of microreserves (~4,000 km^2) using as study system several grasshopper species with different dispersal capacities and habitat requirements (see Ortego et al. 2012). The study of population genetic structure and diversity at the landscape scale requires information that can be only provided by highly variable genetic markers. Here, we report the development of twelve polymorphic microsatellite loci from the grasshopper *R. hispanica* that will be useful to estimate effective population sizes and understanding spatial patterns of genetic variation and metapopulation connectivity.

Microsatellite libraries were generated by Genetic Identification Services Inc. (Chatsworth, CA, USA) from an individual *R. hispanica* collected from Lillo (Toledo province, Central Spain, 39°42′06.6″N, 3°18′13.0″W) and using magnetic bead capture technology with CA, AAC, ATG and TAGA microsatellite motif capture molecules (Peacock et al. 2002; see Adams et al. 2013 for more details). Twenty-four primer pairs were designed from microsatellite-containing sequences and tested using 20 individuals collected from the same locality. Primers producing products of expected size were labelled with fluorescent dyes (6-FAM, PET, NED or VIC) to allow analysis on an automated DNA sequencer and determination of levels of polymorphism. Twelve of the twenty-four loci were discarded because they did not amplify, were

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monomorphic or produced non-resolvable electropherograms. Amplifications were conducted in 10-μL reaction volumes containing 5 ng of genomic DNA, 1X reaction buffer (67 mM Tris–HCL, pH 8.3, 16 mM $(NH_4)_2SO_4$, 0.01 % Tween-20, EcoStart Reaction Buffer, Ecogen), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.15 μM of each primer and 0.1 U of Taq DNA EcoStart Polymerase (Ecogen). The PCR programme used was 9 min denaturing at 95 °C followed by 40 cycles of 30 s at 94 °C, 45 s at the annealing temperature (Table S1) and 45 s at 72 °C, ending with a 5 min final elongation stage at 72 °C. Amplification products were run on an ABI 310 Genetic Analyzer (Applied Biosystems) and genotypes were scored using GeneMapper 3.7 (Applied Biosystems).

Tests for departure from Hardy–Weinberg equilibrium (HWE) and pairwise linkage disequilibrium were performed using Genepop 4.2 (Raymond and Rousset 1995). Significance levels were adjusted for multiple tests using the sequential Bonferroni correction for $\alpha = 0.05$. We found no evidence of genotypic linkage disequilibrium at any pair of loci. Two loci deviated significantly from HWE and Micro-Checker (Van Oosterhout et al. 2004) analyses indicated that these two loci showed evidence of null alleles (Table S1). The number of alleles (N_A) per locus ranged from 7 to 19 and their observed (H_O) and expected (H_E) heterozygosities ranged from 0.41 to 0.90 and from 0.76 to 0.91, respectively. Polymorphism characteristics for the twelve microsatellite markers are summarized in Table S1. Overall, these novel polymorphic microsatellites provide a useful genetic tool to study the genetic diversity and structure of *R. hispanica* and address questions on the conservation of highly fragmented Mediterranean landscapes. In combination with mtDNA markers, these microsatellite loci are also a valuable tool to understand the phylogeographic structure and the historical factors structuring genetic variation of this specialist grasshopper species.

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