January 2008

Development of polymorphic microsatellite loci for the common vampire bat, *Desmodus rotundus* (Chiroptera: Phylostomidae)

Antoinette J. Piaggio
USDA/APHIS/WS National Wildlife Research Center, Toni.J.Piaggio@aphis.usda.gov

John J. Johnston
USDA-APHIS-Wildlife Services

Susan L. Perkins
American Museum of Natural History

Molecular Ecology Resources (2008) **8**, 440–442 doi: 10.1111/j.1471-8286.2007.01986.x

PERMANENT GENETIC RESOURCES

Development of polymorphic microsatellite loci for the common vampire bat, *Desmodus rotundus* (Chiroptera: Phylostomidae)

ANTOINETTE J. PIAGGIO,* JOHN J. JOHNSTON* and SUSAN L. PERKINS†
**USDA, Wildlife Services, National Wildlife Research Center, Wildlife Genetics Laboratory, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA, †Division of Invertebrates, American Museum of Natural History, Central Park West at 79th Street, New York, NY 10024, USA*

Abstract

The common vampire bat (*Desmodus rotundus*) is one of three haematophagous species of bats and the only species in this genus. These New World bats prey on mammals and create significant economic impacts through transmission of rabies in areas where livestock are prevalent. Furthermore, in some portions of their range, it is not uncommon for them to prey upon humans. It is critical to the management of this species and for understanding the spread of bat rabies that detailed studies of *D. rotundus* population structure be conducted. To further such studies, we have characterized 12 microsatellite loci for this species.

Keywords: *Desmodus rotundus*, microsatellite, rabies, vampire bat

Received 23 June 2007; revision accepted 8 August 2007

The common vampire bat (*Desmodus rotundus*), the sole species in the genus *Desmodus* of the family Phyllostomidae, is a medium-sized, haematophagous bat (Greenhall *et al*. 1983). These bats are broadly distributed from Mexico south to Argentina and Chile. Across their range, they specialize on blood of mammalian prey, including livestock and humans (Greenhall 1970). One study found that 90% of vampire bats captured in various regions of Mexico had fed on domestic livestock (Campos-Vela 1972). Vampire bats are carriers of the rabies virus and their predation habits make them a primary transmitter of this disease to livestock. Bites from vampire bats can also transmit other diseases and parasites (Greenhall *et al*. 1983). Subsequent economic losses from fatality of livestock as a result of pathogen transmission from *D. rotundus* can be measured in the millions of dollars (US; Shwiff *et al*. 2007).

The management of *D. rotundus* has focused on reducing population sizes in areas of bat rabies outbreaks in domestic livestock. It is possible that if detailed biological information was collected, then control measures could be more targeted and less damaging to the genetic diversity of these bats. Traditional mark–recapture methodology suggests that *D. rotundus* occupies multiple roosts but shows strong fidelity to these roosts and their home range resulting in stable colony membership (Wimsatt 1969; Lopez-Forment *et al*. 1971). However, no investigations of *D. rotundus* populations have included the use of molecular makers. Our goal is to use microsatellite markers and population genetic analyses to investigate population structure, connectivity and dispersal patterns of *D. rotundus*. These data will be used to help develop effective management approaches to control the economic impact of the transmission of bat rabies in domestic livestock and will serve to protect the genetic diversity of this unique species. To this end, we have developed 12 polymorphic microsatellite markers.

We developed a microsatellite library following the same methodology as Budinoff *et al*. (2004) who adapted a protocol for enrichment and development (Glenn & Schable 2005). Thirty-one clone sequences containing repeats were chosen for primer design. STADEN PACKAGE (Staden *et al*. 1998), TROLL (Castelo *et al*. 2002; Martins *et al*. 2006), and web-based PRIMER 3 (Rozen & Skaletsky 2000) software packages were used to identify repeat regions within clones and design primers in the flanking regions. Parameters for STADEN PACKAGE software were set according to step-by-step instructions provided with TROLL. PRIMER 3 parameters

Correspondence: Antoinette J. Piaggio, Fax: (970) 266 6063; E-mail: toni.j.piaggio@aphis.usda.gov

Table 1 Characteristics of the 12 microsatellite loci that were developed and optimized from *Desmodus rotundus*. Panel indicates the primers used in a specific multiplex PCR panel

Multiplex/loci	Primer sequence (5′–3′) F, forward; R, reverse	Primer quantity for PCR using 1 μM primer	Repeat motif	Size of cloned allele (bp)	N_A	H_O	H_E
Panel 1: Dero_B03F_B03R	F: NED-CTAGGCAAGTTGGGAGAGTTC R: ATACGTACTTTGGGACTGAGCTT	0.7 μL	$(AC)_{11}$	1111	5	0.64	0.66
Dero_B10F_E01R	F: HEX-GAAGTTGGGGTGTCTATGG R: GGAGTTCTTTTAGCCTGTGC	0.6 μL	$(GA)_{20}$	660	6	0.79	0.74
Dero_B11F_B11R	F: HEX-TAAATGATGAGAACAGGACAGG R: GTGTGTGGTTAGCATGTTGC	0.6 μL	$(AC)_8$	291	3	0.51	0.51
Dero_C12F_B02R	F: NED-GCTGGGTCACCTAAGTATGG R: CAAATCAGATATACAAAGAAGCAAG	0.8 μL	$(CA)_{17}$	625	6	0.81	0.77
Dero_D06F_D06R	F: 6-FAM-CCTCCCTAGTTGTTCCATCC R: TTTGGGCAACATTAAAATAGC	0.6 μL	$(TC)_6$	545	2	0.55	0.48
Panel 2: Dero_C07F_A02R	F: 6-FAM-CCTAGGGCAAGAATGAGTATCC R: ACAGTATGGCACACAAACACG	0.4 μL	$(TG)_9$	807	5	0.67	0.64
Dero_D12F_D12R	F: NED-ACATGCAAATCCATCTTGAT R: CCCAAATCCAAAACCTCAT	0.4 μL	$(CA)_{11}(AC)_8$	696	7	0.76	0.78
Dero_G10F_B03R	F: HEX-AAAGAACTTTAATTCCCCATCG R: CTCTTGTCAGTTCACATTTAGCC	0.5 μL	$(GA)_{18}$	889	6	0.49	0.70*
Dero_H02F_C03R	F: 6-FAM-GACTGCCTGAGATGAAAAACC R: GCCTCTTTTCCTGGTTACTCC	0.4 μL	$(GT)_{15}$	461	4	0.16	0.59*
Panel 3: Dero_A08F_B01R	F: NED-CTACATTCATCATTAAGACATATGC R: GCAACTTCTAATTCACTCTAGAGG	1.2 μL	$(TC)_{27}(CA)_{23}$	486	13	0.91	0.90
Dero_C11F_C11R	F: 6-FAM-GTTAATAAGCCTTCAGGAAAAGC R: TCCTTCTGCACTCAAGAATTTTA	0.9 μL	$(AG)_9$	1101	6	0.41	0.46
Dero_D02F_D02R	F: NED-GCCAATAGATTGAGAACATGC R: TTAGTGATGAGGTTGTGTGTGC	0.6 μL	$(GT)_7(GA)_{13}(AG)_8$	533	13	0.76	0.78

N_A, mean number of alleles per locus; H_O, observed and H_E, expected heterozygosities; *, indicates significant deviation from Hardy–Weinberg equilibrium after Bonferroni corrections (Rice 1989).

were set with product size range of 50–400, optimal primer size at 20 bp, primer melting temperature at 58°, and remaining parameters set to default settings. Primer pairs designed in PRIMER 3 were double-checked for self-annealing, loops, and pair annealing in NETPRIMER (Premier Biosoft International; www.premierbiosoft.com/netprimer). Primers were ordered with a 5′ end label of NED, FAM, or HEX (Table 1) from Applied Biosystems. Polymerase chain reactions (PCR) were carried out using 0.4–1.2 μL each of 1 μM primer (Table 1) and panel-specific chemistry listed in Table 2. The thermal profile for all loci was an initial denaturation at 94 °C for 4 or 5 min (Table 2) followed by 35–45 cycles of 94 °C (Table 2) for 30 s, 52 °C for 30 s, and 72 °C for 45 s. Cycling was followed with a 30-min extension at 60 °C. There were initially 31 primer pairs designed and optimized. Of these 31, 16 pairs were monomorphic, 3 pairs did not amplify, and 12 pairs amplified and were variable (GenBank Accession nos EF591569–EF591580). All reactions were carried out on a Mastercycler ep Gradient (Eppendorf).

Tissue samples were obtained from 41 individuals of *D. rotundus* captured near Tamosopo, San Luis Potosi, Mexico. These individuals were collected within a 12-km area to maximize the probability that a single population was represented. These samples were genotyped on an Applied Biosystems (ABI) 3130 automated genetic analyser and analysed with ABI GENEMAPPER software. Genotypic disequilibrium between pairs of loci was tested using FSTAT 2.9.3 (Goudet 2001). Other relevant parameters were estimated in ARLEQUIN (Schneider *et al*. 2000) and each locus was tested for null alleles using MICRO-CHECKER (van Oosterhout *et al*. 2004). We found no evidence of linkage disequilibrium between loci. The number of alleles ranged from two to 13 per locus (Table 1). Two loci, Dero_G10F_B03R and Dero_H02F_C03R, demonstrated significant deviations from Hardy–Weinberg Equilibrium (Table 1) after sequential Bonferroni corrections (Rice 1989). These same two loci also showed evidence of null alleles with a 95% CI. With a suite of 12 variable microsatellite loci that amplify reliably in *D. rotundus*, it is now possible to undertake studies investigating

Table 2 PCR chemistry and conditions for each panel

Panel	H_2O (μL)	Invitrogen 5× buffer C (μL)	10 mM dNTP (μL)	5 mg/mL BSA (μL)	Promega *Taq* DNA polymerase (μL)	Genomic DNA (μL)	Initial denaturation time (min)	Cycles
1	0.9	3.3	1.0	0.4	0.2	1.0	5	40
2	1.8	1.0	1.0	0.4	0.3	1.0	5	35
3	2.0	3.2	1.0	0.4	0.2	2.0	4	45

vampire bat population structure, connectivity and dispersal. Data from such studies can be used to help target management and control efforts of *D. rotundus* that will serve to protect human and livestock health while simultaneously protecting the genetic diversity of this bat species.

Acknowledgements

We are indebted to Ignacio Amezcua Osorio, Elizabeth Pérez Torres, Raúl Clímaco Fernández, Alejandro Jiménez Ramírez, Ana Lilia Sandoval-Sanchez, Luis Lecuona, Allison Kerwin, Melissa Neubaum and Dennis Kohler. Additional support was provided by Wildlife Services Deputy Administrator's Office. A.J.P. is supported by an Animal Plant Health Inspection Services Science Fellowship.

References

Budinoff RB, Siddall AM, Siddall ME (2004) Twelve variable microsatellite loci for the North American medicinal leech, *Macrobdella decora*. *Molecular Ecology Notes*, **4**, 491–493.

Campos-Vela J (1972) *Identificación de la ingesta gástrica pare determinar los huéspedes del murciélago Desmodus rotundus como contribución a la epizootiología de la rabia en México*. DVM Dissertation Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad de México, Mexico.

Castelo AT, Martins WS, Gao GR (2002) Tandem repeat occurrence locator. *Bioinformatics*, **8**, 634–636.

Glenn TC, Schable NA (2005) Isolating microsatellite DNA loci. *Methods in Enzymology*, **395**, 202–222.

Goudet J (2001) FSTAT *ver. 2.9.3, A Program to Estimate and Test Gene Diversities and Fixation Indices (updated from Goudet 1995)*. University of Lausanne, Switzerland. http://www.unil.ch/izea/softwares/fstat.html.

Greenhall AM (1970) The use of a precipitin test to determine host preferences of the vampire bats, *Desmodus rotundus* and *Diaemus youngi*. *Bijdragen Dierkunde*, **40**, 36–39.

Greenhall AM, Joermann G, Schmidt U, Seidel MR (1983) *Desmodus rotundus*. *Mammalian Species*, **202**, 1–6.

Lopez-Forment W, Schmidt U, Greenhall AM (1971) Movement and population studies of the vampire bat (*Desmodus rotundus*) in Mexico. *Journal of Mammalogy*, **52**, 227.

Martins W, de Sousa D, Proite K, Guimarães P, Moretzsohn M, Bertoli D (2006) New softwares for automated microsatellite marker development. *Nucleic Acids Research*, **34**, E31.

van Oosterhout C, Hutchison WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, **4**, 535.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rozen S, Skaletsky H (2000) PRIMER 3 on the WWW for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. The Humana Press Inc., Totowa, New Jersey.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN: *Software for Population Genetics Data Analysis*, Version 2.000. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Switzerland.

Shwiff SA, Sterner RT, Jay MT, Parikh S, Bellomy A, Meltzer MI, Rupprecht CE, Slate D (2007) Direct and indirect costs of rabies exposure: a retrospective study in southern California (1998–2002). *Journal of Wildlife Diseases*, **43**, 251–257.

Staden R, Beal KF, Bonfield JK (1998) The STADEN PACKAGE. In: *Computer Methods in Molecular Biology, Bioinformatics Methods and Protocols* (eds Misener S, Krawetz S), Vol. 132, pp. 115–130. The Humana Press Inc., Totowa, New Jersey.

Wimsatt WA (1969) Transient behavior, nocturnal activity patterns, and feeding efficiency of vampire bats (*Desmodus rotundus*) under natural conditions. *Journal of Mammalogy*, **50**, 233–244.