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TECHNICAL NOTE

Characterization of 21 microsatellite loci from the invasive Mediterranean gecko (*Hemidactylus turcicus*)

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Abstract It is crucial to understand the dispersal potential of invasive species to predict how biological invasions spread. Molecular data can provide unique insights into sources, routes, and mechanisms of invasion. Twenty-one microsatellite markers were developed for the Mediterranean gecko (*Hemidactylus turcicus*), a species native to southwest Asia that has successfully invaded much of the southern US. The loci were characterized with geckos collected from two locations at Texas A&M University. Eighteen of the 21 loci exhibited polymorphism (2–8 alleles/locus). Both gecko populations were in Hardy–Weinberg equilibrium. Our preliminary screen detected significant population structure at a small scale (650 m). Therefore, these markers will be useful to assess dispersal at varying geographic ranges.

Keywords Invasive species · Mediterranean gecko · *Hemidactylus* · Microsatellites · Dispersal

Historical and observational data on the spread of invasive species can be limited. Thus, it can be difficult to understand the sources, routes, and mechanisms of species invasions. Molecular data can help to determine the demographic history and dispersal dynamics of invasive species (Handley et al. 2011). We isolated and characterized 21 microsatellite markers for the Mediterranean gecko (*Hemidactylus turcicus*) and tested their ability to assess fine scale population genetic structure. This gecko is native to the Mediterranean regions of Africa, Asia, and Europe, but is now widely established in the southern US, Mexico, Panama and Cuba (Rödder and Lötters 2009). Moreover, *H. turcicus* serves as a host for both local and exotic parasites (Criscione and Font 2001). Consequently, the spread of this species is a concern because the presence of the gecko may lead to increased parasite transmission within native lizard populations. Understanding the dispersal of the gecko will not only inform us on how invasive species spread, but also on the role of host invasions in parasite transmission.

Microsatellite DNA library construction, enrichment, and screening followed methods in Detwiler and Criscione (2011). These steps were completed by S. Bogdanowicz at the Evolutionary Genetics Core Facility at Cornell University, US. Seventy-six colonies from the enriched library were sequenced, but only 44 sequences contained unique microsatellites. Primers were designed for 21 of these sequences using Primer3 (Rozen and Skaletsky 2000). To test the utility of the markers to detect genetic differentiation at a local scale, we characterized the loci using geckos collected from two locations that were 650 m apart at Texas A&M University, College Station, Texas. A total of 32 and 44 geckos were collected from a series of abutting greenhouses (location 1), and Cain Hall (location 2), respectively. Tail muscle was placed in 200 µl of a 5% chelex solution containing 0.2 mg/ml of proteinase K. Samples were incubated for 2 h at 56°C, and boiled for 8 min at 100°C. Genotyping followed the M13 method, so a M13 oligonucleotide was added to the forward primer (Schuelke 2000). We also added a 5′ sequence tag (GTTTCTT) to the reverse primer to reduce polyadenylation. A 15 µl PCR reaction with 2.4 µl of genomic DNA was used. PCR conditions and reagents concentrations were exactly those from Detwiler and Criscione (2011), but with a 95°C initial denaturation temperature.

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Table 1 Characterization of 21 microsatellite loci for the Mediterranean gecko (*Hemidactylus turcicus*) from two locations at Texas A&M University, College Station, Texas, US

Locus	Primer sequence (5′–3′)	Repeat motif	Clone size (bp)	Pop	N_A	H_s	F_{IS}	F_{ST}	F'_{ST}
di004	F: TGTAACCTGTGTGTGAAAGAA	$(TG)_{19}(GA)_{16}$	223–241	1	4	0.602	−0.039	0.035*	0.070
	R: GCCTCAGAACCAAGAGTATG			2	3	0.416	−0.255*		
di005	F: CAAGAGAAGTGTTGTCAGAGG	$(TC)_{32}$	160–214	1	3	0.522	−0.017	0.171*	0.306
	R: GGCTGAATAAACAAGAATGG			2	7	0.372	0.145		
di010	F: ATTGCTCTCCTGGATCAGT	$(CA)_{11}$	357–359	1	2	0.091	−0.033	−0.007	0
	R: CTGAGACTTGAATTGCATGA			2	2	0.149	0.235		
di014	F: CTGAGGTATCATTGCTTATCAC	$(CT)_{13}(CA)_{12}$	255–269	1	3	0.451	0.029	0.014*	0.024
	R: CATTTCTGGTTATTGTTCTGG			2	3	0.385	0.056		
di016	F: GCAACCGCTATTACTTTCC	$(AC)_8$AAAT	243–280	1	4	0.640	0.121	0.104*	0.305
	R: TATGTACCAGTGCCAGATGA	$(AC)_{15}(AG)_{19}$		2	4	0.677	0.060		
di019	F: AGCATCTTACCACCAGTCC	$(TC)_{31}$	239–261	1	5	0.653	−0.005	−0.002	0
	R: TATTATTTCCTTGGCTCTCG			2	5	0.561	0.067		
di020	F: CAGCATTAAGCAGCAAATAA	$(CT)_{16}(CA)_6$	201–236	1	3	0.316	0.209	0.003	0.005
	R: ATCAGTTGTTCTGCATTGG			2	2	0.378	−0.323*		
di021	F: CCTATGCCTAATGAGAGGTG	$(GT)_{18}(GA)_{17}$	334–347	1	3	0.255	−0.103	0.085*	0.097
	R: ATATTGCCTCTGGGATTTG			2	2	0.023	0.000		
di024	F: CCTCTGCTTGTCTCTACACC	$(TG)_{22}$	144–148	1	2	0.222	−0.127	0.144*	0.227
	R:AGGAAATTGATTCTCTGTTAATG			2	2	0.481	0.244		
tri001	F: AGAGGTGACTTATGCAATGAC	$(AGC)_{12}$	193–202	1	5	0.357	0.037	0.081*	0.100
	R: TTGCCATCTTCTAAATTGTTG			2	3	0.067	−0.016		
tri009	F: CAAGGCTGAATGAGGTCTAC	$(ACA)_6$	273–277	1	2	0.246	0.111	0.006	0.007
	R: AAGGAATGGAAGCTATACCC			2	2	0.148	−0.075		
tet003	F: CTTCCCTACGTTATTCAAGG	$(TTGT)_6$	211–256	1	2	0.347	−0.081	0.021	0.036
	R: CAACCAGGCTAATGGAAA			2	3	0.461	−0.183		
tet004	F: GGCTTTGGATACAATCCTG	$(TCTT)_{21}$	339–359	1	5	0.447	0.021	0.032*	0.056
	R: ACAGTTGTCGGAAGCATAGA			2	4	0.414	−0.098		
tet005	F: ACGAGAAACACAGGGATAAC	$(TAGA)_{17}$	300–328	1	8	0.716	0.040	0.059*	0.173
	R: GACATTCTCTGCTCCATTTC			2	7	0.607	−0.085		
tet010	F: GCTACCCTCTGAGCCTTAAT	$(CTGT)_8(CTAT)_4$	284–396	1	6	0.628	−0.046	0.075*	0.198
	R: CCGAGCTGTTGATAACCTT	$(CTAA)_3$CCAA $(CTAA)_3$		2	6	0.616	0.004		
tet013	F: ATTTGTGGTGTTCTTGGTTC	$(TCTT)_{30}$	340–364	1	7	0.697	0.148	0.069*	0.251
	R: TGGGTCAGCTATACAATTCAG			2	5	0.751	0.001		
tet019	F: CTGATGGACCACTTTCCTC	$(TTTG)_{13}(TTCA)_{15}$	310–342	1	5	0.361	0.308*	0.126*	0.264
	R: GGGAGTTTCTTGTTATGTGC			2	6	0.661	−0.065		
tet023	F: ATTCCCAAGACCCAAAAC	$(TCAC)_{17}$	206–218	1	4	0.258	−0.090	0.004	0.005
	R: GTTTTATTGCAGTCCTTGTTG			2	4	0.152	−0.049		
di001	F: GCTGAAGGATTTGAAGGAG	$(GT)_8$	182	1	1	0	NA	NA	NA
	R: GGGCAAAGTCTCTGAAAAC			2	1	0	NA		
di003	F: CCAGACTGTGAGAGCAAAG	$(CT)_{23}$	217	1	1	0	NA	NA	NA
	R: GCAATGAGCTAGGCTAAGG			2	1	0	NA		
tet015	F: CTACCACTTCTCCTTTTCACTC	$(AACA)_{11}$	230	1	1	0	NA	NA	NA
	R: ACCCTTCCATTTAGCTCTG			2	1	0	NA		

Populations 1 and 2 were collected from greenhouses ($n = 32$), and Cain Hall ($n = 44$), respectively. GenBank deposition numbers follow the order in the table (JQ229624–JQ229644)

N_A number of alleles, H_s gene diversity, F_{IS} measures deviation from Hardy–Weinberg equilibrium, F_{ST} measures genetic differentiation between populations, F'_{ST} standardized F_{ST}

* $P < 0.05$

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To first determine whether the two locations should be considered different populations, we estimated F_{ST} (Weir and Cockerham 1984) with FSTAT v2.9.3 (Goudet 1995) and calculated standardized F_{ST} (F'_{ST}) using RECODEDATA (Meirmans 2006). We tested for population genetic differentiation with the *G*-based test (Goudet et al. 1996) with 10,000 permutations of individual geckos between locations. There was overall significant differentiation between the two locations (multilocus $F_{ST} = 0.07$, $P = 0.0001$, multilocus $F'_{ST} = 0.11$). Because significant genetic structure was detected, we analyzed the microsatellites for each population separately (Table 1). Gene diversity (H_S) was calculated with FSTAT (Goudet 1995) using Nei's unbiased estimator. Weir and Cockerham's (1984) estimator of F_{IS} (per locus and multilocus) was computed with SPAGEDI v1.3 (Hardy and Vekemans 2002). Deviations from Hardy–Weinberg equilibrium (HWE) per locus and multilocus were tested by permuting alleles among individuals 20,000 times in SPAGEDI. For each location, we tested genotypic disequilbrium between pairs of loci in GENEPOP v1.2 (Raymond and Rousset 1995) (Markov chain parameters: 5,000 dememorizations; 5,000 batches; 5,000 iterations) and used the sequential Bonferroni method to correct for multiple tests.

Among 18 of the 21 markers, allelic diversity was similar between the two populations with 2–8 and 2–7 alleles/locus (Table 1). Three loci were monomorphic across both populations. In population 1, tet019 had significant homozygote excess and in population 2, loci di004 and di020 had significant heterozygote excess (Table 1). However, after Bonferroni correction within populations, no loci deviated from HWE. In addition, both populations were in overall HWE (multilocus $F_{IS} = 0.03$ and -0.02, $P > 0.05$). By chance ($\alpha = 0.05$), we expected that 8/153 pairs of loci would test significant at $P < 0.05$ for genotypic disequilibrium in each population. We observed a slight excess where 10 pairs in each population tested significant. Only one pair (di021–tet013) was significant in both populations. The other 9 out of 10 pairs were not the same between populations. After sequential Bonferroni, only one pair remained significant, and it was not the same in either population (population 1: di014–di004; population 2: di010–tet005). These results may suggest that the disequilibrium is caused by genetic drift and thus, may indicate these local gecko populations have small effective population sizes.

Overall, 12 of the 18 polymorphic markers showed significant genetic differentiation (Table 1), which demonstrates that this set of markers will be suitable to detect genetic structure at local scales. Hence, these microsatellites can be used to evaluate gecko dispersal at a range of geographic scales. Comparing individuals from a wider geographic range will elucidate the number of introductions and the spread of the invasive species. This information can also be used to assess the gecko's role in parasite transmission.

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