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PRIMER NOTE

Isolation and characterization of microsatellite loci in hybridizing California and Gambel's quail (*Callipepla californica* and *C. gambelii*)

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Abstract

***Callipepla californica* and *C. gambelii* are sibling species of quail that hybridize throughout Southern California where their ranges overlap. We developed seven highly polymorphic microsatellite markers that will be used to assess introgression between these species. Numbers of alleles ranged in *C. californica* from seven to 24 and in *C. gambelii* from five to 18, with high expected levels of heterozygosity in both species. Alleles exclusive to each species are present at most loci.**

Keywords: *Callipepla*, cline, hybrid zone, introgression, microsatellites, quail

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California Quail and Gambel's Quail (*Callipepla californica* and *C. gambelii*) are part of the Scaled Quail complex, four species which hybridize in the wild to some degree with each other and with two more distantly related quail, the Northern Bobwhite (*Colinus virginianus*) and Mountain Quails (*Oreortyx pictus*) (Johnsgard 1973). Hybrid zones vary in their size and permanence, and accordingly, the hybridizing parental species become admixed to varying extents. Introgression, the permanent exchange of select genes between genetically distinct populations, may alter the evolutionary trajectories of hybridizing populations (Lewontin & Birch 1966). Microsatellites will be used to examine gene flow between species, population structure within species, and relatedness between individuals within sympatric social groups.

Two genomic libraries were constructed. The first used an equal mixture of total DNA from four individuals two *C. californica* (Seattle, WA and Auberry, CA) and two *C. gambelii* (Tucson, AZ) that was extracted from blood preserved on filter paper (Petren *et al*. 1999). Total DNA was digested with Sau3AI, and 200–500 bp fragments were purified by a 1.75% agarose gel electroelution (Sambrook *et al*. 1989), ligated into a CIP-treated pUC plasmid (PVZ1), and transformed into electroporation competent *E. coli* (SURE cells, Stratagene). A two-stage screening process (Petren 1998) was used to select approximately 300 colonies from a pool of roughly 100 000 insert-bearing colonies by colony lift hybridization with an equimolar mixture of [^{32}P]-ATP end-labelled $(CA)_{15}$ and $(GT)_{15}$. 150 of these were screened a second time and yielded 40 positive colonies, which were sequenced. Sixteen primer pairs were designed for repeats > 12 units long (Rozen & Skaletsky 1998), and of 12 yielding polymerase chain reaction (PCR) products, four were variable. Calkins designed two primer pairs from a genomic library of *C. californica* from San Diego. Of 26 positive colonies that were sequenced, primers were designed for four loci and two loci yielded variable PCR products (Cc1, Cc2). A third locus (Cc3) was designed from PCR amplified *C. californica* DNA using primers designed in other species, chicken *Gallus gallus* (13 total, Cheng & Crittenden 1994; Hanotte *et al*. 1997), and grouse *Lagopus lagopus scoticus* (2 total; Piertney & Dallas 1997). The PCR product was excised, sequenced and used to design primers specific to *C. californica*. For genotyping, blood was collected by brachial vein puncture or toenail clippings and either preserved with EDTA on filter paper (Petren *et al*. 1999) or stored in lysis buffer (Seutin *et al*. 1991) and tissue was stored in 70% Ethanol. DNA was extracted using a combination of salt precipitation (Miller *et al*. 1988) and phenol extraction (Sambrook *et al*. 1989).

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Microsatellite allele sizes were determined by autoradiography with the forward primer labelled with ^{32}P (Petren 1998) as well as fluorescence-automated detection using a dye-labelled forward primer (FAM, TET, HEX; Integrated DNA Technologies). PCR amplification was performed on a Thermo-Hybaid PCR Express thermocycler under the following conditions: 33 cycles of 94 °C (30 s), T_a °C [(30 s) see Table 1 and 72 °C (30 s)]. We used the following PCR reaction (25 µL volumes): 25 ng DNA, 16.4 µL H2O, 10X Cetus buffer 2.5 µL, $MgCl_2$ (25 mM), 1.0 µL dNTP's (10 mM), 0.65 µL of each primer (12.5% end-labelled with [^{32}P]-ATP), and 0.235 µL Taq polymerase. Amplified products were resolved using 6% acrylamide gels (90/gel) with a pUC derived sequencing ladder (PVZ1). The PCR cocktail for automatic sequencing (15 µL total volume) contained 5.5 µL H_20, 0.5 µL of each flouro-labelled primer, and 7.5 µL Ampli*Taq* Gold™ (Perkin Elmer, Foster City, CA, USA). Gels were analysed with GENESCAN 3.1 software (Applied Biosystems/Perkin Elmer).

We screened genetically admixed individuals and pure parental types from sympatry ($n = 283$) as well as pure parental types originating from the geographical extremities of each species range ($n > 60$). The number of alleles ranged from seven to 24 in *C. californica* and from five to 18 in *C. gambelii*. Expected heterozygosity varied between 0.7 and 0.95 in California Quail and between 0.48 and 0.9 in Gambel's Quail. Significant deficiencies of heterozygotes were observed (Cc25 and Cc93 in *C. californica*, Cc1 and Cc3 in *C. gambelii*). An exact test showed highly significant levels of genic differentiation between California and Gambel's Quail at all loci ($P < 0.0001$, GENEPOP 3.1c Raymond & Rousset 1995). These seven markers both differentiate the two species clearly and are highly variable within each species.

Two levels of analysis will be especially suited to microsatellite analysis in this system. Microsatellite variation will aid in tracing patterns of introgression across their narrow hybrid zones. At the individual level, these loci will allow us to test parent-offspring relatedness in mixed species coveys. Together with field and experimental studies, these genetic markers will help elucidate the mating systems in these species and determine the effect of interspecific pairing on hybridization and introgression.

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Table 1 Characterization of microsatellites in *Callipepla californica* (Cc) and *Callipepla gambelii* (Cg).

	Accession				No. Alleles		Size Range (bp)		H_O		H_E	
	Nos.	Primer set (5'- 3')	Motif	Tm	Cc	Cg	Cc	Cg	Cc	Cg	Cc	Cg
Cc1	AY127559	F: GTGGAACGGGAGTACTGAAGG (21)	(GT)14	62	17	16	198-238	196-228	0.83	0.64	0.86	0.9
		R: CACCTCAAACAGCCTGTGG (19)										
Cc2	AY127560	F: CCTTCCCATATTTGCTTTGC(20)	(CA)12	53	9	6	81-103	81-95	0.64	0.76	0.68	0.69
		R: AGATAAAAATCCTTCCCTTA(20)										
Cc3	AY127561	F: CTGGGACCACTCTAGGCTGA(20)	(CA)10	62	7	5	101-113	101-111	0.71	0.3	0.7	0.48
		R: CTGAGGTCATTAAGCACGCA(20)										
Cc25	AY127563	F: GCCCTTATAAAATCTCCGTGC(21)	(CA)19	53	19	15	174-222	176-212	0.82	0.79	0.9	0.88
		R: GCCATGTGGTTCCTTCATTC(20)										
Cc93	AY127562	F: TCCATTCTGTGACAA(15)	(CA)13	53	25	13	223-273	233-273	0.76	0.76	0.95	0.85
		R: GCTTCTGATTGACTGCTCAGG(21)										
Cc269	AY127564	F: CTGGATTTTAGGGCTCACTTG(21)	(GT)17	58	18	18	121-167	127-181	0.84	0.88	0.86	0.92
		R: ACATCTCTGCTAATTCACAACC(22)										
Cc346	AY127565	F: TGGCTGGAGACACTTGATG(19)	(CA)13	62	14	9	143-175	139-155	0.81	0.63	0.85	0.73

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