Isolation and characterization of 17 microsatellite loci for the house finch (*Carpodacus mexicanus*)

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Abstract

The house finch (*Carpodacus mexicanus*) has emerged recently as a model species in studies of sexual selection, reproductive physiology, population genetics, and epizootic disease ecology. Here we describe 17 highly polymorphic microsatellite loci for this species. In a sample of 36 individuals, we observed an average of 16 alleles per locus and heterozygosity ranged from 0.61 to 0.97. One locus showed significant deviation from Hardy–Weinberg proportions, but no significant gametic disequilibrium was observed among any of the loci. Amplification by polymerase chain reaction was optimized under similar parameters across loci, thereby facilitating multiplexing and rapid multilocus genotyping.

Keywords: Carduelinae, extra-pair paternity, genetic complementarity, relatedness

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The house finch — a North American cardueline finch — has emerged over the last two decades as a model species for field studies of sexual selection (Brush & Power 1976; Badyaev & Hill 2002; Hill 2002; Oh & Badyaev 2006, 2008). In addition, the unprecedented expansion of this species' range in North America over the last 70 years and associated adaptive radiation have made it a focus of studies in population genetics and evolutionary ecology (Wootton 1987; Veit & Lewis 1996; Badyaev & Hill 2000; Badyaev *et al*. 2002; Wang *et al*. 2003; Hawley *et al*. 2006), emergent epizootic events (Duckworth *et al*. 2003a; Dhondt *et al*. 2006; Hawley *et al*. 2006; Lindstedt *et al*. 2006), and reproductive physiology (Duckworth *et al*. 2003b; Badyaev *et al*. 2008). Here we report the isolation and characterization of 17 highly polymorphic microsatellite loci in the house finch.

A blood sample was collected from a wild house finch captured near Missoula, Montana (46°59′N, 114°5′W), and genomic DNA extracted using the Puregene DNA Purification Kit (QIAGEN Inc.) following standard protocol. An enriched library was made by Ecogenics GmbH from size-selected genomic DNA ligated into TSPAD-linker (Tenzer *et al*. 1999) and enriched by magnetic bead selection with biotin-labelled $(CA)_{13}$ and $(ACAG)_7$ oligonucleotide repeats (Gautschi *et al*. 2000a, b). Of 384 recombinant colonies screened, 147 gave a positive signal after hybridization. Plasmids from 96 positive clones were sequenced and primers were designed for 21 microsatellites, of which 19 were tested for polymorphism. Of these, two loci yielded complicated allelic patterns that were difficult to interpret and were therefore excluded.

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Polymorphism was assayed in a sample of 36 presumably unrelated individuals captured from a wild population in southern Arizona (32°15′N, 110°56′W). Genomic DNA was extracted from blood samples as described above. Microsatellite regions were amplified by polymerase chain reaction (PCR) in 10-μL reactions containing 20–50 ng template DNA, 225 μM dNTPs each, 0.125 μM of each forward and reverse primers, 1 U HotMaster *Taq* polymerase and 1× buffer (5-PRIME, Inc.) resulting in final concentration of 2.5 mM Mg^{2+}. In each primer pair, forward primers were labelled with fluorescent dyes (Table 1; Applied Biosystems). PCR was carried out using a Mastercycler thermal cycler (Eppendorf) under the following thermotreatment conditions: initial denaturation at 94 °C for 2 min, followed by 25 cycles of denaturation at 94 °C for 20 s, annealing at 60 °C or 63 °C (Table 1) for 20 s, extension at 65 °C for 45 s, and ending with a final extension at 65 °C for 45 s. Amplified products were resolved via capillary electrophoresis using an ABI PRISM 3730 DNA Analyser (Applied Biosystems) and discrete alleles were called using GenoTyper software (Applied Biosystems). Exact tests for deviations from Hardy–Weinberg proportions and gametic disequilibrium were carried out using GenePop software (Raymond & Rousset 1995). To account for multiple comparisons in results, alpha was adjusted using a Bonferroni correction.

All loci were highly polymorphic and number of alleles per locus ranged from 7 to 23 (Table 1). Only one locus (*Hofi30*) showed significant deviation from Hardy–Weinberg proportions ($P < 0.003$, Bonferroni correction for multiple

Table 1 Characterization of 17 microsatellite loci for *Carpodacus mexicanus* (n = 36 individuals genotyped)

Locus name	Repeat motif based on sequenced clone	Primer sequence (5′–3′)	T_a (°C)	Dye label	N_a	Size-range (bp)	H_O	H_E	Accession no.
Hofi07	$(GT)_{19}$	F: CTGGAAGCACTGGGGTCACT R: CTTGCCTGACAGGGTGGTC	63	NED	16	151–189	0.92	0.91	FJ467625
Hofi10	$(GT)_{19}$	F: TTGGCCCAGATTTCTACCAC R: CAGACCAGATTCCCCAAATC	60	NED	19	182–208	0.97	0.93	FJ467626
Hofi16	$(GT)_{28}$	F: AAGAGGAGCACTGGTATTTGC R: TCATGAGGTGGGTTCCTACG	60	HEX	16	107–155	0.86	0.92	FJ467627
Hofi19	$(GT)_4AC(GT)_{23}$	F: TCAGGCAAGTGTAGCAGGAC R: TTTTAGATGACAGTTATGGCACTATC	60	HEX	15	183–223	0.86	0.86	FJ467628
Hofi26	$(CA)_{23}$	F: GCTCAGACAGCTGGGACTG R: GCTGGTGGGAAGAGCATC	60	NED	21	72–128	0.92	0.94	FJ467629
Hofi29	$(GT)_{27}$	F: AGCCAGGACAGAGCAGATCC R: CATTTTCTCTGGGTGAGAAAGC	60	HEX	23	166–224	0.92	0.95	FJ467630
Hofi30	$(CA)_{21}TGTA(TG)_5$	F: TGTATATCATATGGTGACATGTGTAGG R: CAGTGGTCTATAGAACTTTGTCACC	60	6-FAM	12	109–133	0.61	0.89	FJ467631
Hofi35	$(GT)_{32}$	F: GCCCAGGGACACAGTAAATG R: AACATCCCGTGGCAAAGTC	60	HEX	16	76–130	0.89	0.91	FJ467632
Hofi39	$(GT)_{22}$	F: GCAGATGTGATCATGCTGAAG R: GCAGCCACTCAAGATTTTGTC	63	6-FAM	18	182–228	0.97	0.94	FJ467633
Hofi53	$(TA)_3(GT)_{18}$	F: GTGGGTGTCTGCTAAGATGC R: CTGGTTTTGGTACACGGTTG	60	6-FAM	15	163–207	0.70	0.71	FJ467634
Hofi69	$(CA)_{21}$	F: CAACATGCTGTAATCCCAACTC R: CCTTTTGGTCATTCCACTTCTATC	60	HEX	16	129–170	0.81	0.90	FJ467635
Hofi70	$(CA)_{21}$	F: GCAGGCAACATCCATGAAG R: CCGGATCGTTTTGTTTCATC	60	NED	19	126–159	0.89	0.93	FJ467636
HofiACAG01	$(GTCT)_9$	F: AACTGCATCATGCCTTGGAC R: AAAGGACTGCAGAGCATCGT	60	NED	9	78–112	0.64	0.73	FJ467637
HofiACAG07	$(GACA)_{11}$	F: AGAAGATGGGTTAGCAGCTGAG R: CCAAGGGATCCTCCTGATG	60	6-FAM	14	207–244	0.81	0.88	FJ467638
HofiACAG15	$(CTGT)_8CC(GTCT)_5$	F: CACCTTTCCCCACCGAAG R: AAGTGAGCTCCCGTCAAAGC	60	NED	23	186–264	0.97	0.95	FJ467639
HofiACAG18	$(CAGA)_{14}$	F: TTCATGAAGCCACGCTACAG R: GCAGCCTCCTGGTAAAGAAG	60	6-FAM	13	125–178	0.89	0.90	FJ467640
HofiACAG25	$(CTGT)_{11}$	F: GATTTTTGAACCCCCAGACTC R: TAGCTGCATCCAGCACCAGT	60	6-FAM	7	105–130	0.70	0.66	FJ467641

F, forward primer; R, reverse primer; T_a, optimized annealing temperature; N_a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

comparisons). No significant gametic disequilibrium was observed among any of the loci. Across all loci, the combined exclusion probability (Jamieson & Taylor 1997) was > 0.999, suggesting that these microsatellites will provide robust genetic tools for assessing paternity in wild house finch populations. Moreover, the similarity in annealing temperatures across loci along with the wide range of fragment sizes (Table 1) should greatly facilitate PCR multiplexing, thereby enabling rapid generation of multilocus genotypes necessary for studies of population structure (Pritchard *et al*. 2000) or estimating relatedness among individuals of unknown pedigree (Queller *et al*. 1993). Thus, these markers will be especially useful for future studies aimed at inferring population history and detecting fine-scale population structure across the recently expanded range of this species.

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References

Badyaev AV, Hill GE (2000) The evolution of sexual dimorphism in the house finch. I. Population divergence in morphological covariance structure. *Evolution*, **54**, 1784–1794.

Badyaev AV, Hill GE (2002) Paternal care as a conditional strategy: distinct reproductive tactics associated with elaboration of plumage ornamentation in the house finch. *Behavioral Ecology*, **13**, 591–597.

Badyaev AV, Hill GE, Beck ML *et al*. (2002) Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science*, **295**, 316–318.

Badyaev AV, Young RL, Hill GE, Duckworth RA (2008) Evolution of sex-biased maternal effects in birds. IV. Intra-ovarian growth dynamics can link sex-determination and sex-specific acquisition of resources. *Journal of Evolutionary Biology*, **21**, 449–460.

Brush AH, Power DM (1976) House finch pigmentation: carotenoid metabolism and the effect of diet. *Auk*, **93**, 725–739.

Dhondt AA, Badyaev AV, Dobson AP *et al*. (2006) Mycoplasmal conjunctivitis spreads more slowly in native than in introduced range of the host. *Ecohealth*, **3**, 95–102.

Duckworth RA, Badyaev AV, Farmer KL, Hill GE, Roberts SC (2003a) First case of *Mycoplasma gallisepticum* infection in the western range of the House Finch (*Carpodacus mexicanus*). *Auk*, **120**, 528–530.

Duckworth RA, Badyaev AV, Parlow AF (2003b) Elaborately ornamented males avoid costly parental care in the house finch (*Carpodacus mexicanus*): a proximate perspective. *Behavioral Ecology and Sociobiology*, **55**, 176–183.

Gautschi B, Tenzer I, Müller JP, Schmid B (2000a) Isolation and characterization of microsatellite loci in the bearded vulture (*Gypaetus barbatus*) and cross-amplification in three Old World vulture species. *Molecular Ecology*, **9**, 2193–2195.

Gautschi B, Widmer A, Koella J (2000b) Isolation and characterization of microsatellite loci in the dice snake (*Natrix tessellata*). *Molecular Ecology*, **9**, 2191–2193.

Hawley DM, Hanley D, Dhondt AA, Lovette IJ (2006) Molecular evidence for a founder effect in invasive house finch (*Carpodacus mexicanus*) populations experiencing an emergent disease epidemic. *Molecular Ecology*, **15**, 263–275.

Hill GE (2002) *A Red Bird in a Brown Bag: The Function and Evolution of Colorful Plumage in the House Finch*. Oxford University Press, Oxford, UK.

Jamieson A, Taylor SCS (1997) Comparisons of three probability formulae for parentage exclusion. *Animal Genetics*, **28**, 397–400.

Lindstedt E, Oh KP, Badyaev AV (2006) Ecological, social, and genetic contingency of extrapair behavior in a socially monogmous bird. *Journal of Avian Biology*, **38**, 214–238.

Oh KP, Badyaev AV (2006) Adaptive genetic complementarity in mate choice coexists with preference for elaborate sexual traits. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 1913–1919.

Oh KP, Badyaev AV (2008) Evolution of adaptation and mate choice: parental relatedness affects expression of phenotypic variance in a natural population. *Evolutionary Biology*, **35**, 111–124.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Queller DC, Strassmann JE, Hughes CR (1993) Microsatellites and kinship. *Trends in Ecology & Evolution*, **8**, 285–288.

Raymond M, Rousset F (1995) GenePop (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Tenzer I, degli Ivanissevich S, Morgante M, Gessler C (1999) Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. *Phytopathology*, **89**, 748–753.

Veit RR, Lewis MA (1996) Dispersal, population growth, and the Allee effect: dynamics of the house finch invasion of eastern North America. *American Naturalist*, **148**, 255–274.

Wang Z, Baker AJ, Hill GE, Edwards SV (2003) Reconciling actual and inferred population histories in the house finch (*Carpodacus mexicanus*) by AFLP analysis. *Evolution*, **57**, 2852–2864.

Wootton JT (1987) Interspecific competition between introduced house finch populations and two associated passerine species. *Oecologia*, **71**, 325–331.

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Isolation and characterization of new microsatellite markers for rose bitterlings, *Rhodeus ocellatus*

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Abstract

The Japanese rose bitterling (*Rhodeus ocellatus kurumeus*) is facing imminent extinction because of hybridization and competition from an invasive alien subspecies (*Rhodeus ocellatus ocellatus*). Eleven new microsatellite markers for the two subspecies were developed using dinucleotide repeat specific polymerase chain reaction. The number of alleles per locus and the heterozygosity in *R. o. kurumeus* were lower than those in *R. o. ocellatus*. Most of these microsatellite markers were successfully cross-amplified in three Acheilognathinae species.

Keywords: Acheilognathinae, cross-species amplification, microsatellite markers, *Rhodeus ocellatus*, rose bitterling

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