

SUBSPECIFIC DIFFERENTIATION AND CONSERVATION OF SONG SPARROWS (*MELOSPIZA MELODIA*) IN THE SAN FRANCISCO BAY REGION INFERRED BY MICROSATELLITE LOCI ANALYSIS

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ABSTRACT.—We examined genetic population structure of five putative subspecies of Song Sparrows (*Melospiza melodia*) in the San Francisco Bay region (*M. m. samuelis*, *M. m. maxillaris*, *M. m. pusillula*, *M. m. gouldii*, and *M. m. heermanni*) at nine microsatellite loci to assist the development of Song Sparrow conservation and management strategies. We sampled nine populations from five putative subspecies and found low estimates of differentiation between populations within subspecies and between. Despite low estimates of divergence, genetic structure at the subspecies level was indicated by the larger amount of variance accounted for by subspecies than populations. We propose that a management unit encompassing the range of *M. m. pusillula* be given priority for conservation on the basis of the extent of genetic divergence shown by Cavalli-Sforza and Edward's chord distance, and the topology of an unweighted pair group cluster analysis supported by 100% of bootstrap replicates across loci. Although *M. m. samuelis* and *M. m. maxillaris* appear undifferentiated from *M. m. heermanni*, it remains possible that adaptive differences between those types were not identified with neutral loci. Received 14 March 2001, accepted 15 March 2002.

RÉSUMÉ.—Nous avons examiné la structure génétique de populations de cinq sous-espèces putatives de Bruant chanteur (*Melospiza melodia*) dans la région de la Baie de San Francisco (*M. m. samuelis*, *M. m. maxillaris*, *M. m. pusillula*, *M. m. gouldii*, et *M. m. heermanni*) au niveau de neuf loci microsatellites pour venir en aide au développement de stratégies de conservation et d'aménagement pour le Bruant chanteur. Nous avons échantillonné neuf populations à partir de cinq sous-espèces putatives et nous avons trouvé de faibles estimations de différenciation à l'intérieur et entre les populations intra et inter sous-spécifiques. Malgré de faibles estimations de divergence, la structure génétique au niveau sous-spécifique était représentée par une plus grande variance au sein des sous-espèces que dans les populations. Nous proposons qu'une unité d'aménagement contenant la gamme de *M. m. pusillula* puisse être reconnue comme prioritaire sur le plan de la conservation en se basant sur l'étendue de la divergence génétique montrée par la distance d'accord de Cavalli-Sforza et Edward, et la topologie d'une analyse par groupe pairé, non pondérée et supportée par 100% des réplifications de rééchantillonnage à travers les loci. Bien que *M. m. samuelis* et *M. m. maxillaris* apparaissent indifférencier de *M. m. heermanni*, il est encore possible que ces différences adaptatives entre ces types n'aient pas été identifiées par des loci neutres.

THE DETERIORATION, fragmentation, and loss of natural environments have led conservationists to protect areas that remain relatively intact and harbor high biodiversity. Although the importance of ecological and demographic factors in conserving biodiversity have been emphasized (Caughley 1994, Lande 1988), genetic factors also play a role. Surveys of genetic variation allow quantification of the extent and

distribution of genetic variability crucial to recognizing and maintaining biodiversity (Moritz 1994a, Moritz et al. 1996). Genetic surveys also provide insight into the evolutionary processes that generate biodiversity (Smith and Wayne 1996) and aid in identifying unique populations with distinct evolutionary potential (Moritz 1994b). Genetic studies may also complement ecological and demographic studies related to population viability. Loss of genetic variability has been linked to declines in fitness

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(Bouzat et al. 1998), and the preservation of genetic variability may help species adapt to novel conditions (Lande and Shannon 1996).

We examined genetic variation and population structure within and among five subspecies of Song Sparrow (*Melospiza melodia*) in the San Francisco Bay region. That region, 70 × 100 km in size, has several phenotypically distinct, year-round resident subspecies of song sparrow described. Three endemic subspecies reside in tidal salt marshes, each restricted to one of three sub-bays of the greater San Francisco Bay (Fig. 1A; *M. m. samuelis* referred to as "samuelis"; *M. m. maxillaris* as "maxillaris"; *M. m. pusillula* as "pusillula"). The Marin Song Sparrow (*M. m. gouldii* referred to as "gouldii") occupies upland habitats surrounding those bays (Grinnell and Miller 1944) and the Heermann Song Sparrow (*M. m. heermanni* referred to as "heermanni") occupies riparian habitats east of San Francisco Bay (Marshall 1948).

A history of diking, filling, and conversion of marsh to salt evaporation ponds (Walton 1978) has eliminated 85% of the original tidal salt marsh in San Francisco Bay (Marshall and Dredrick 1994) and fragmented what remains (Walton 1978). That loss and fragmentation of habitat threatens the long-term persistence of the tidal marsh Song Sparrow populations in San Francisco Bay and has led to the recognition of *samuelis*, *maxillaris*, and *pusillula* in the California Natural Diversity Database (CNDDB) as endangered subspecies. These subspecies are also listed federally and by the state of California as special concern species because of their history of decline, limited range, and other threats that may make them vulnerable to extinction.

Previous research on Song Sparrows has found marked patterns of geographic structure in plumage and morphology but detected little structured variation in other traits. Marshall (1948) studied over 2,000 study skins of Song Sparrows from the San Francisco Bay region to describe a well-ordered pattern of geographic structure in plumage and morphology. However, Mulligan (1963), who studied song variation in tidal marsh Song Sparrows, suggested that variation among birds was so great that differences between subspecies could not be resolved. Ferrell (1966) studied variation in erythrocyte antigen frequencies and found that variation among populations was about twice that

observed among subspecies, and that variation between subspecies was explained largely by variation between populations (Ferrell 1966). Molecular genetic studies of Song Sparrows across North America have also had difficulty finding concordance between genetic variation and subspecific designations based on phenotype. In particular, much work employing mitochondrial DNA (mtDNA) found no clear pattern of geographic variation in haplotype (Hare and Shields 1992, Zink and Dittmann 1993, Fry and Zink 1998).

To further investigate the potential links between subspecific designation and population genetic structure in Song Sparrows in the San Francisco Bay Region, we used nine hypervariable tandem repeat nuclear loci (microsatellites) under the assumption that their high mutation rates and large numbers of alleles (Goldstein et al. 1995) might provide more sensitive estimates of divergence than mtDNA. Microsatellites have been used to detect fine-scale genetic structure in polar bears (*Ursus maritimus*; Paetkau et al. 1995), brown trout (*Salmo trutta*; Estoup et al. 1998), and fire ants (*Solenopsis invicta*; Ross et al. 1999). In birds, hypervariable markers have also been used to detect fine-scale population genetic structure in Willow Ptarmigan (*Lagopus lagopus*; Piertney et al. 1998) and Savannah Sparrows (*Passerculus sandwichensis*; Freeman-Gallant 1996).

Estimating amount of differentiation and degree of genetic structure among Song Sparrow populations and subspecies could aid the development of appropriate conservation units and management actions. In particular, because upland subspecies are considered safe whereas the tidal marsh subspecies are threatened, we designed this study to compare two replicate populations within the range of each tidal marsh subspecies to an upland population nearby (Fig. 1B). We then asked, first, if upland Song Sparrow populations within the range of *gouldii* and *heermanni* differed genetically from tidal marsh Song Sparrow populations (*samuelis*, *maxillaris*, and *pusillula*). Second, we asked if differences in allele frequency also occurred between tidal marsh populations within the range of each putative subspecies. Third, to clarify the existing subspecific designations on which current conservation measures are based, we examined the differentiation and extent of genetic structure that was accounted for

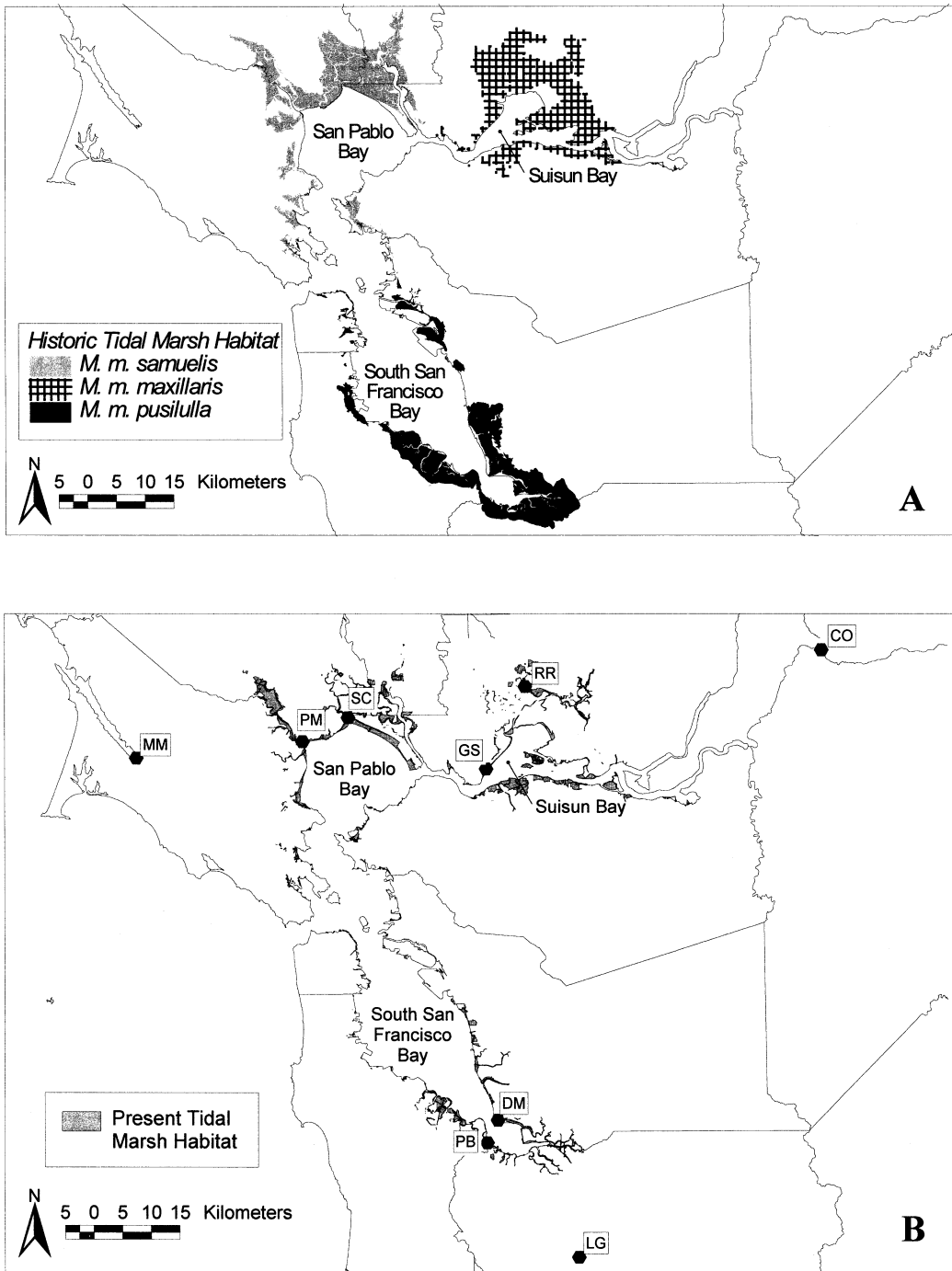


FIG. 1. (A) Extent of historical tidal marsh habitat surrounding San Francisco Bay. The purported range of *samuelis* is San Pablo Bay, *maxillaris* is Suisun Bay, and *pusillula* is San Francisco Bay. (B) Present tidal marsh habitat with sampling sites marked (PM and SC = *samuelis*, GS and RR = *maxillaris*, PB and DM = *pusillula*, MM and LG = *gouldii*, and CO = *heermanni*). (Bay Area EcoAtlas version 1.50b4, San Francisco Estuary Institute, 1998).

TABLE 1. Sampling locality for microsatellite analysis.

Subspecies	Population locality	Acronym	County, state
<i>Melospiza melodia gouldii</i>	Mark's Marsh, Tomales Bay, Audubon Canyon Ranch	MM	Marin County, California
<i>M. m. pusillula</i>	Los Gatos Creek County Park	LG	Santa Clara County, California
	Palo Alto Baylands Nature Preserve	PB	San Mateo County, California
<i>M. m. samuelis</i>	Dumbarton Marsh, Don Edwards San Francisco Bay National Wildlife Refuge	DM	Alameda County, California
	Petaluma River Mouth, California Department Fish and Game	PM	Sonoma County, California
<i>M. m. maxillaris</i>	Sonoma Creek, San Pablo Bay National Wildlife Refuge	SC	Solano County, California
	Goodyear Slough Unit, Grizzly Island Wildlife Area	GS	Solano County, California
<i>M. m. heermanni</i>	Rush Ranch Open Space, Solano County Farmlands and Open Space Foundation	RR	Solano County, California
	The Nature Conservancy Cosumnes River Preserve	CO	Sacramento County, California

by subspecific designation. We did that by partitioning genetic variance among subspecies, among populations within subspecies, and within populations. Finally, we examined genetic relationships between populations to identify populations that are genetically divergent and of unique conservation value, and to identify groups of populations that may be considered conservation units.

METHODS

All birds were sampled during the main breeding season (March to May in 1999) from tidal salt marshes and surrounding freshwater riparian areas in the San Francisco Bay region (Table 1, Fig. 1B). Adults were captured in mist nets, measured, blood sampled, and released. Samples were also taken from one nestling in each of 12 nests at Petaluma Marsh (PM, see Table 1 for acronyms). Overall, we sampled 215 birds from nine populations, two populations from each of three tidal marsh subspecies, and three from two upland subspecies.

The tidal marsh subspecies sampled included the Samuel's Song Sparrow (*M. m. samuelis*) found in San Pablo Bay, the Suisun Song Sparrow (*M. m. maxillaris*) in Suisun Bay, and the Alameda Song Sparrow (*M. m. pusillula*) in South San Francisco Bay. The corresponding freshwater upland populations sampled for each tidal marsh subspecies were the Marin Song Sparrow (*M. m. gouldii*) from the uplands surrounding San Francisco Bay and the Heermann Song Sparrow (*M. m. heermanni*) whose range borders *maxillar-*

is to the east and inhabits freshwater riparian areas of California's Central Valley (Marshall 1948).

DNA EXTRACTION AND MICROSATELLITE AMPLIFICATION

Blood was collected from the brachial vein in heparinized capillary tubes, then transferred to 1 mL of 1× lysis buffer (ABI; Applied Biosystems Division of Perkin Elmer Inc., Foster City, California) and stored at 4°C. DNA was extracted using standard phenol-chloroform extraction methods. Twenty-five microliters of blood was added to 233 µL of extraction buffer (1× TNE, 1 mol Tris-HCl, proteinase K, 25% SDS) and incubated overnight at 37°C. The next day, 150 µL of 6 mol NaCl was added and the solution was centrifuged at 3,000 revolutions per minute. The supernatant was collected and remaining pellet discarded. That was followed by a phenol-chloroform isoamylalcohol (1:1) extraction and spin at 3000 revolutions per minute and 4°C. The second extraction consisted of just chloroform followed by a spin at 3000 revolutions per minute at 4°C. The DNA was then precipitated using 700 µL of 100% ethanol, the DNA pellet was washed with -20°C 70% ethanol, dried, and resuspended in 50 µL of TE buffer (10 mmol Tris, 1 mmol EDTA, pH 7.4).

Nine microsatellite loci were amplified with the primers listed in Table 2. The MME microsatellites were isolated for song sparrows following Hammond et al. (1998) using (CA)₂₂ and (GA)₂₂ repeat nucleotides as probes (see Jeffery et al. 2001 for more details; MME 1, MME 2, MME 3, MME 7, MME 8, and MME 12 can be accessed under GenBank acces-

TABLE 2. Song Sparrow microsatellite loci and PCR conditions.

Locus	Primer sequence	Repeat motif	Anneal temp. (mmol)	Mg (mmol)	Product size	Number of alleles	Source
MME 1	F1: AGGAAAAGGGAGGAGGGTG R1: GGGAGTGCAGAAATGTGCAAATG	(TG)7TC(TG)15	50	2.0	140–164	13	Jeffery et al. 2001
MME 3	F1: CCTCAGATTGGCATTGAAAGTTG R1: GGTCCAGTTTCTTGGGTGTTTTTC	(TG)14	53	1.5	162–182	10	Jeffery et al. 2001
MME 7	F1: TGGCAGCCTTCCAAAGTTTG R1: AACCCACATGAAACAGGTCAC	(CA)2TA(CA)18	54	1.5	110–140	16	Jeffery et al. 2001
MME 8	F1: TCATGGAGATGGTGAATGCC R1: TGAATCAGCAGCACACACACC	(TG)3TC(TG)13	56	1.5	208–234	20	Jeffery et al. 2001
MME 12	F1: AGGACTFTCACTGTGGACTGAAG R1: TGGCTTTATGGAAACAAGGCATC	(CCCACA)13	60	1.5	178–250	12	Jeffery et al. 2001
ESCU1	F1: TTCTTTGGTCTATGGAAAGGTG R1: GCTTGAAAGACAGTCACCCAGG	(CA)18	50	2.0	132–170	17	Hanotte et al. 1994
GF2.35	F1: AAACACTGGGAGTGAAGTCT R1: AACTATTCTGTGATCCTGTTACAC	(CA)15	50	2.0	184–240	27	Petren 1998
PSAP335	F1: not published	(TG)13	55	1.5	97–119	12	M. J. Temple, J. M. Wright, and M. Leonard pers. comm.
MME 2	F1: ATCAGAGATTCCTGTACACACC R1: GAAATTGTATCCGCCACCTCATTTC	(TG)30	53	1.0	118–168	23	Jeffery et al. 2001

sion numbers AF127376, AF127377, AF127378, AF127381, AF127382, and AF127385, respectively). ESCU 1 (GenBank accession number X77077), GF2.35 (GenBank accession number AF081929) and PSAP 335 (Table 2) were developed for other species but were screened and found to be polymorphic in Song Sparrows.

MME 1, MME 3, MME 7, MME 12, ESCU 1, GF 2.35, and PSAP 335 were amplified and sized at the University of Wisconsin, Madison. PCR reactions were carried out separately for each locus and consisted of ~25 ng of template DNA combined with 1× PCR reaction buffer (500 mmol KCl, 100 mmol Tris-HCl [pH 9.0], 1.0% Triton X-100), 0.2 mmol dNTPs, 1.5–2.0 mmol MgCl₂, 0.2 μmol forward and reverse primer, 0.75–1.5 units of Promega *Taq* DNA polymerase, and water to a total reaction volume of 25–50 μL. Thermocycling conditions were 94°C for 1 min, annealing temperature for 1 min, 72°C for 1 min, repeated 35×. Annealing temperatures and magnesium concentrations for each locus are listed in Table 2. The forward primers were fluoro-labeled at the 5' end with various fluorescent labels (HEX, 6-FAM, or TAMRA) depending on the loci. PCR products were analyzed on an Applied Biosystems model 373A automated sequencer. PCR products from multiple loci were combined in each lane with an internal sized ROX standard (ABI) and resolved on a 6% polyacrylamide gel, 0.4 mm thick. Up to four loci (total of 2 μL PCR products) were combined in each lane so PCR products were separated on the basis of color and size. Results were analyzed using the GENESCAN (version 1.1) software and viewed using GENOTYPER (version 2.0; ABI). The ROX standard was used to accurately size the length of the alleles; therefore the genotype of the two alleles reflects the size in base pairs.

MME 2 was amplified and sized in the Genetic Data Center, Department of Forest Sciences, University of British Columbia, Canada. PCR reactions consisted of ~20 ng of template DNA combined with 10× PCR reaction buffer (500 mmol KCl, 100 mmol Tris-HCl, 15 mmol MgCl₂, pH 8.3), 0.2 mmol dNTP, 0.05 μmol forward and reverse primer, 0.03 μmol M13 dye-labeled primer, and 1.0 units of Roche *Taq* DNA polymerase. The forward primer was synthesized with an M13 tail for product labeling during the PCR reaction with the M13 dye-labeled primer. Thermocycling conditions for MME 2 consisted of touchdown PCR with 5 cycles each of an annealing temperature of 58, 57, and 56°C, and 20 cycles of 53°C. PCR products were run on a Li-Cor 4200 DNA analyzer, 7% polyacrylamide gel, 0.4 mm thick. Known alleles that were sized and run in Madison were combined into an "allelic ladder" which was run every 10 sample lanes to facilitate sizing of alleles on the Li-Cor. Therefore, allele length represents the number of base pairs comparable to the Madison genotypes.

MME 3 and MME 7 are Z-linked, therefore females are hemizygous and appear as homozygotes. To include MME 3 and MME 7 in the analyses, females were coded as "missing data" for the second allele and included with the remaining loci in all subsequent analyses. The remaining loci are inherited in Mendelian fashion (Jeffery et al. 2001).

HARDY-WEINBERG EQUILIBRIUM AND LINKAGE DISEQUILIBRIUM

Nei's (1978) unbiased estimate for expected heterozygosity and observed heterozygosity were calculated using BIOSYS-2 (Swofford et al. 1997), step VARIAB. The program GENEPOP version 3.1d (updated from version 1.2; Raymond and Rousset 1995a) was used to estimate allele frequencies, test for departures from Hardy-Weinberg equilibrium (HWE), and test for linkage disequilibrium. Testing for HWE in microsatellite data sets aids in detecting nonamplifying alleles (Paetkau et al. 1997) and internal genetic structure, which would result in a Wahlund effect (Hartl and Clark 1997). In a previous study Jeffery et al. (2001) observed allelic dropout at MME 2 and MME 12 related to (1) DNA concentration; (2) DNA extraction; and (3) unknown reasons. GENEPOP version 3.1d employs a Markov chain method to estimate *P*-values for departure from HWE using the method of Guo and Thompson (1992) and Fisher's exact test to test the null hypothesis that genotypes at two different loci are independent of one another (Raymond and Rousset 1995a). Exact tests are most appropriate for hypervariable markers because they accommodate even large numbers of rare alleles (Rousset and Raymond 1995 and references therein). Statistical significance of multiple *P*-values was estimated using Fisher's combined probability method (Raymond and Rousset 1995a).

GENETIC POPULATION AND SUBSPECIFIC STRUCTURE

Heterogeneity of allele frequencies between population pairs was examined using Fisher's exact test following Raymond and Rousset (1995b; GENEPOP version 3.1d) with significance levels adjusted by sequential Bonferroni correction (Rice 1989). The null hypothesis tested was that the distribution of alleles across populations was homogeneous. The degree of differentiation between subspecies and populations was assessed by partitioning of the genetic variance in an analysis of variance framework (Weir and Cockerham 1984), using ARLEQUIN version 2.000 (Schneider et al. 2000). Two sets of statistics were calculated, Φ -statistics that employed differences in allele frequencies only (analogous to *F*-statistics) and Φ -statistics (analogous to *R*-statistics; Slatkin 1995) that used an analysis of molecular variance (AMOVA) and accounted for variance in size between pairs of alleles (Excoffier et al. 1992). Estimation of overall

TABLE 3. Mean sample size per locus, mean number of alleles per locus, and mean heterozygosity for Song Sparrow populations (SE). Heterozygosity measured as direct counts or unbiased estimate (Nei 1978).

Subspecies	Site	Mean sample size per locus	Mean number alleles per locus	Mean heterozygosity	
				Direct count	Unbiased estimate
<i>M. m. gouldii</i>	MM	19.8 (0.6)	9 (1.0)	0.77 (0.049)	0.814 (0.025)
	LG	17.8 (0.2)	10 (1.1)	0.797 (0.036)	0.81 (0.031)
<i>M. m. pusillula</i>	PB	24.6 (1.3)	9.8 (1.6)	0.803 (0.055)	0.8 (0.048)
	DM	25.5 (1.1)	8.6 (0.9)	0.755 (0.041)	0.762 (0.046)
<i>M. m. samuelis</i>	PM	17.1 (1.4)	9.1 (1.2)	0.792 (0.055)	0.831 (0.032)
	SC	25.4 (1.4)	10.6 (1.5)	0.807 (0.028)	0.817 (0.039)
<i>M. m. maxillaris</i>	GS	19 (1.3)	10 (1.2)	0.796 (0.049)	0.815 (0.041)
	RR	28 (0.9)	10.3 (1.1)	0.776 (0.044)	0.813 (0.035)
<i>M. m. heermanni</i>	CO	19.3 (1.0)	10.9 (1.4)	0.796 (0.031)	0.846 (0.025)
<i>M. m. morphna</i>	BB	23.1 (1.1)	9.6 (1.2)	0.651 (0.070)	0.777 (0.037)

population differentiation was calculated as F_{st} and R_{st} . A hierarchical model was then used to partition variation into three components: "within populations" (Φ_{st}), "among populations and within groups" (Φ_{sc}), and "among groups" (Φ_{cr} ; Excoffier et al. 1992). A permutation approach was used to test significance of the variance components and Φ -statistics (Excoffier et al. 1992). In this study, groups represented the putative subspecies (localities in parentheses, abbreviations listed in Table 1), *gouldii* (MM, LG), *pusillula* (DM, PB), *samuelis* (PM, SC), and *maxillaris* (GS, RR).

Because of their high polymorphism, microsatellites can give a downward-biased estimate of F_{st} (Hedrick 1999). Measures that are not biased by polymorphism include the rare alleles method of estimating effective number of migrants (N_m ; Barton and Slatkin 1986) and genetic assignment tests (Cornuet et al. 1999). Therefore, differentiation between subspecies was also estimated by calculating N_m by the private alleles method (GENEPOP version 3.1d; Barton and Slatkin 1986). Additionally, an assignment test using GENECLASS (Piry and Cornuet 1999) was used to assign individuals to the subspecies where their genotype is most likely to occur following the likelihood approach of Paetkau et al. (1995).

GENETIC DIVERGENCE

Genetic divergence among populations was estimated using Dce, Cavalli-Sforza and Edward's (1967) chord distance. To provide a context for genetic divergence in the San Francisco Bay region, the Pacific Northwest subspecies *M. m. morphna* was included in this analysis. Dce was calculated using step SIMDIST in BIOSYS-2 (Swofford et al. 1997). Takezaki and Nei (1996) advised that correct tree topology was more likely to be obtained using a distance measure independent of mutation models. In addition, Dce has a lower sampling error and makes no assumptions

about constant population size or mutation rates among loci (Takezaki and Nei 1996). The magnitude of that distance is not proportional to evolutionary time, but it has been found to resolve close relationships accurately (Angers and Bernatchez 1998, Paetkau et al. 1997, Takezaki and Nei 1996). Unweighted pair-group cluster analysis (UPGMA) and neighbor-joining (program NEIGHBOR) were used to construct trees of relationships between populations for comparison to morphological relationships. Loci were bootstrapped using BIOSYS-2 (Swofford et al. 1997) to yield a consensus tree from 100 distance matrices, using NEIGHBOR and CONSENSE in PHYLIP version 3.57c (Felsenstein 1995). The bootstrapped consensus trees were rooted with the Pacific Northwest subspecies *morphna* (site Burn's Bog [BB], British Columbia, Canada) and using eight loci (i.e. minus Psp 335).

RESULTS

San Francisco Bay populations of Song Sparrows showed substantial variation at all loci, with the number of alleles ranging from 10 (MME 3) to 27 (GF 235) alleles per locus (Table 3). Average heterozygosity was high and ranged from 0.762 to 0.846 (Table 3). Allelic drop-out was observed in a previous study at MME 2 and MME 12. Significant deviations from HWE were detected in this study at GF 235 (combined probability over all populations using Fisher's method $\chi^2 = 38.6$, $df = 18$, $P = 0.0032$), due to a significant deficiency of heterozygotes (score test, $P < 0.0001$, $SD = 0.0000$), the cause of which is unknown. GF 235 did show some differential amplification of alleles, with larger alleles amplifying less well, which could have caused misscoring of heterozygotes as homozygotes. However, the inclu-

TABLE 4. Results of AMOVA for Song Sparrow microsatellite loci.

Locus		Among subspecies		Among populations within subspecies		Within populations	
		Φ_{CT}	Percentage variance	Φ_{SC}	Percentage variance	Φ_{ST}	Percentage variance
Mme 1	F_{ST}	0.0109	1.09	0.0022	0.22	0.0131	98.69
Mme 3		0.0457*	4.57	0.0146**	1.39	0.0596**	94.04
Mme 7		0.0196	1.96	0.0084**	0.83	0.0278**	97.22
Mme 8		0.017*	1.70	0.0068**	0.67	0.0237**	97.63
Mme 12		0.0078	0.78	-0.0019	-0.18	0.0060	99.41
Escu 1		0.0094	0.94	0.0257**	2.55	0.0349**	96.51
GF 235		0.0072	0.71	0.0130**	1.29	0.0200**	98.00
Psap 335		-0.0077	-0.77	0.0326**	3.29	0.0252**	97.49
Mme 2		0.0200	1.99	0.0098**	0.96	0.0295**	97.05
Total		0.0137*	1.38	0.0122**	1.18	0.0257**	97.44
Mme 1	R_{ST}	0.0111	1.11	-0.0111	-1.10	0.0001	99.99
Mme 3		0.1838**	18.38	-0.0238**	-1.94	0.16433**	83.57
Mme 7		0.0900	9.00	0.0494**	4.49	0.1350**	86.50
Mme 8		0.0288*	2.88	-0.0053	-0.51	0.0237	97.63
Mme 12		0.0020	0.20	-0.0078	-0.78	-0.0058	100.58
Escu 1		-0.0065	-0.65	0.0159	1.60	0.0095	99.05
GF 235		-0.0163	-1.63	0.0116	1.17	-0.0046	100.46
Psap 335		0.0069	0.69	-0.0121	-1.21	-0.0051	100.51
Mme 2		0.1128*	11.28	0.0293**	2.60	0.1388	86.12
Total		0.0174	1.88	0.00433	0.28	0.0217**	97.84

* $P < 0.05$, ** $P < 0.001$.

sion of GF 235 had little effect on our overall results. Two populations also showed a deficiency of heterozygotes at all loci, RR ($P = 0.0233$, SD 0.0061) and SC (Fisher's exact test, $P = 0.0276$, SD = 0.0064). Significant linkage disequilibrium was detected in 3 of 36 pairwise comparisons across loci (MME 1 and MME 12, $\chi^2 = 33.895$, $P = 0.01298$; MME 1 and GF 235, $\chi^2 = \text{infinity}$, $P < 0.01$; MME 12 and GF 235, $\chi^2 = \text{infinity}$, $P < 0.01$).

GENETIC SUBSPECIFIC AND POPULATION STRUCTURE

Heterogeneity in allele frequencies across all loci was detected among all but five population comparisons after sequential Bonferroni correction. Population comparisons not significantly differentiated from each other included pairwise comparisons of populations of *samuelis*, *maxillaris*, and *heermanni*, which constitute collectively northern San Francisco Bay and CO from California's Central Valley (specifically CO and PM, CO and SC, GS and RR, PM and SC, RR and SC).

Differentiation was statistically significant ($F_{ST} = 0.02288$, $p < 0.0001$; $R_{ST} = 0.02757$, $p <$

0.0001) but weak between populations, based on estimates of population variance to the total variance (Table 4), and this was also true for differentiation among subspecies (Table 4). However, differences among subspecies accounted for a larger percentage of the total variance in allele frequency than differences between populations within subspecies, especially for the R_{ST} analog statistics (R_{ST} : among subspecies = 1.88%, $P = 0.10$, between populations within subspecies = 0.28%, $P = 0.000$; F_{ST} : among subspecies = 1.38%, $P = 0.0499$, between populations within subspecies = 1.18%, $P = 0.0596$). ARLEQUIN also calculates Φ -statistics, analogous to Cockerham's F -statistics (Cockerham 1969, 1973). Values for Φ_{CT} , the among-subspecies measurement of differentiation, were 0.0137 for the F_{ST} analog (significant at $P < 0.05$) and 0.0174 for the R_{ST} analog. Φ_{SC} , the between-populations, within-subspecies measurement of differentiation, was 0.0122 for the F_{ST} analog and 0.00433 for the R_{ST} analog.

We also calculated two estimates of differentiation unbiased by high polymorphism using the private alleles estimate of N_m and a genetic assignment test. Those estimates indicated high gene flow despite evidence of

TABLE 5. Genetic assignment of individuals to subspecies, percentage of total in parentheses.

Subspecies	Predicted subspecies					Total
	<i>M. m. samuelis</i>	<i>M. m. maxillaris</i>	<i>M. m. pusillula</i>	<i>M. m. gouldii</i>	<i>M. m. heermanni</i>	
<i>M. m. samuelis</i>	29 (61.7)	5 (10.6)	4 (8.5)	5 (10.6)	4 (8.5)	47
<i>M. m. maxillaris</i>	10 (19.2)	27 (51.9)	4 (7.7)	7 (13.5)	4 (7.7)	52
<i>M. m. pusillula</i>	4 (7.1)	3 (5.3)	47 (83.9)	0 (0)	2 (3.6)	56
<i>M. m. gouldii</i>	4 (10.3)	11 (28.2)	0 (0)	20 (51.28)	4 (10.3)	39
<i>M. m. heermanni</i>	7 (33.3)	3 (14.3)	0 (0)	4 (19.0)	7 (33.3)	21

genetic structure. The private-alleles method corrected for population size estimated an average of 7.78 immigrants per generation between populations. However, the assignment test correctly assigned 60.19% individuals to their presumptive subspecies (five groups compared; Table 5). Most notably, 83.9% of *pusillula* individuals were assigned correctly.

GENETIC DIVERGENCE

Pairwise comparisons of divergence based on Dce resulted in higher genetic distances among *pusillula* and the other putative subspecies. The greater divergence of *pusillula* was emphasized by the topology of the population UPGMA and neighbor-joining trees based on the pairwise Dce matrix. That matrix showed two major groups, a *pusillula* group and one containing all other populations sampled (Table 6, Fig. 2). The separation of *pusillula* from other populations was supported by 100% of bootstrap replicates. The distinctness of *pusillula* was also emphasized by its differentiation from the closest upland population (LG), which grouped with the remaining upland subspecies.

DISCUSSION

Our study corroborates earlier ones using mtDNA that failed to detect marked genetic differentiation among morphologically distinct subspecies of the Song Sparrow (Hare and Shields 1992, Zink and Dittmann 1993, Fry and Zink 1998). In particular, given previous evidence of recent population expansion of Song Sparrows across North America (Fry and Zink 1998), it is not surprising that our analysis of variation at hypervariable loci also indicated that generally low levels of differentiation exist among populations of song sparrows in the San

Francisco Bay region. However, low fixation indices are expected at polymorphic loci with many alleles and high heterozygosities (Jin and Chakraborty 1995). High polymorphism may bias estimates of F_{st} downward by as much as an order of magnitude (Charlesworth 1998) as compared to estimates of divergence in the same system assessed with less variable markers (Hedrick 1999). Despite those potential biases, however, the private alleles method of estimate Nm , which is not biased by high polymorphism, also indicated low levels of differentiation and relatively high rates of gene flow, amounting to about eight migrants per generation. Slatkin (1987) estimated that only values of $Nm < 1$ will result in fixation via genetic drift.

Despite the low level of genetic differentiation that we found, we believe our results provide important information concerning delineation of conservation units. First, we asked if upland populations of Song Sparrows were differentiated from tidal marsh populations. We found that microsatellite allele frequencies differed significantly among the upland populations of *gouldii* and adjacent populations in tidal marsh. Second, we tested for differentiation among tidal marsh populations and found significant differences among *pusillula* and populations resident in the northern bay tidal marshes (*maxillaris* and *samuelis*). Third, we estimated the amount of genetic structure attributable to subspecies and found some evidence of genetic structure among putative subspecies. The amount of variation detected was small (1–2%) among subspecies, but was significantly greater than zero. Additionally, variation among putative subspecies facilitated the successful assignment of 60% of all individuals to subspecies, and 83% of putative *pusillula* individuals to the correct subspecies. Finally, our UPGMA dendrogram constructed from

TABLE 6. Cavalli-Sforza and Edwards chord distance between Song Sparrow populations. Distances calculated with all nine loci except for BB (*M. m. morphna*), which only used eight (i.e. PSAP 335 was not included).

Subspecies	Population	M. m. gouldii		M. m. pusillula		M. m. samuelis		M. m. maxillaris		M. m. heermanni		M. m. morphna	
		MM	LG	PB	DM	PM	SC	GS	RR	CO	BB		
M. m. gouldii	MM	*****											
	LG	0.4380	*****										
M. m. pusillula	PB	0.4560	0.3990	*****									
	DM	0.4560	0.4230	0.2840	*****								
M. m. samuelis	PM	0.4130	0.4480	0.4110	0.4200	*****							
	SC	0.3540	0.3870	0.3580	0.3840	0.2860	*****						
M. m. maxillaris	GS	0.4000	0.3630	0.3810	0.4050	0.3700	0.3130	*****					
	RR	0.3450	0.3460	0.3780	0.3860	0.3440	0.2720	0.2930	*****				
M. m. heermanni	CO	0.4120	0.3850	0.4060	0.4400	0.3490	0.3120	0.3640	0.3210	*****			
M. m. morphna	BB	0.5110	0.4850	0.4630	0.4930	0.4800	0.4630	0.4670	0.4250	0.4790	*****		

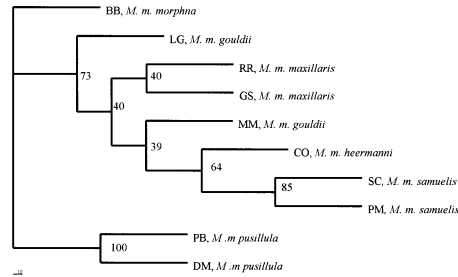
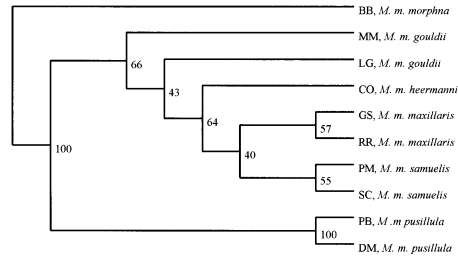


FIG. 2. Cavalli-Sforza and Edwards chord distance UPGMA and neighbor-joining (B) phenograms calculated from eight microsatellite loci. Numbers to the right of branch indicate bootstrap support over 100 replicates.

Dce separated *pusillula* from the remaining populations.

The subspecies designation was developed as a taxonomic device for classifying geographically variable species, rather than as an evolutionary unit (Mayr 1969). One set of criteria for the designation of a subspecies includes that, "Members of a subspecies share a unique geographic range or habitat, a group of phylogenetically concordant phenotypic characters, and a unique natural history relative to other subdivisions of the species" (O'Brien and Mayr 1991:1188). Subspecific designations also attempt to reflect long-term historical gene-pool separation as indicated by concordance at multiple independent loci (Avise and Ball 1990, Ball and Avise 1992). The Song Sparrows in the San Francisco Bay region have been studied extensively by researchers interested in plumage and morphological variation, erythrocyte antigen frequencies, and variation in song type (Marshall 1948, Mulligan 1963, Ferrell 1966, Aldrich 1984). However, those studies have yielded equivocal results regarding concordance in

the pattern of phenotypic traits, and have brought into question current subspecific designations. Whereas our results describe a statistically significant pattern of differentiation in multiple independent microsatellite loci in that region, the differences we found were less striking than expected based on patterns of morphological variation already described. Taken together, those results suggest that Song Sparrow populations in San Francisco Bay lack long-term historical isolation, and they challenge the current subspecies designation (similar examples for other species of birds are described by Zink et al. 2000).

Despite obvious uncertainty about the evolutionary history and status of Song Sparrow populations in San Francisco Bay, we believe their further conservation remains defensible via the designation of management units (Moritz et al. 1995). Moritz et al. (1995) deemed a management unit a functionally separate population or groups of populations. The designation of a management unit aims at identifying the geographic scale for monitoring and managing populations, with its only criterion being the statistically significant divergence of allele frequencies, irrespective of the phylogeny of alleles (Moritz et al. 1995). Given the statistically significant differences in allele frequencies between *pusillula* and *samuelis*, and between *gouldii* and the remaining tidal marsh subspecies, our analysis of Song Sparrow populations in San Francisco Bay indicate three relevant groupings, *pusillula*, *gouldii*, and *samuelis-maxillaris-heermanni*.

One difficulty when identifying management units based on statistically significant differences in allele frequencies is distinguishing between statistically significant and biologically significant differences (Hedrick 1999, Waples 1998). For instance, with larger sample sizes and the higher sensitivity of hypervariable loci, very small differences in allele frequency are expected to be judged as statistically significant (Moritz et al. 1995). To avoid attributing biological significance to statistical significance in weakly diverged groups, Moritz et al. (1995) recommended integrating genetic and ecological evidence.

Ecological evidence strengthens the argument for establishing conservation units based on the groupings listed above. In particular, the tidal marsh Song Sparrows in the San Francisco

Bay region display a pattern of morphological convergence that has been described in other tidal marsh organisms as possible adaptations to the tidal wetland environment (reviewed in Greenberg and Droege 1990). That pattern of morphological convergence includes larger bills, reduced rust coloration in plumage, and increased melanism or dark coloration (Greenberg and Droege 1990). In the San Francisco Bay region, *maxillaris* has a bill that is 40% greater in depth than *gouldii* (Marshall 1948, Greenberg and Droege 1990), and the salt marsh populations generally display patterns of pigmentation consistent with the notion of adaptation for background matching of darker wetland soils (Marshall 1948, Greenberg and Droege 1990). Another possible adaptation to the tidal marsh environment concerns salt tolerance; *pusillula* has been shown to maintain bodyweight while drinking saline solutions, whereas *gouldii* was unable to do so (Basham and Mewaldt 1987).

Marked differences in phenotype, despite low estimates of genetic divergence at microsatellite loci, beg the question, How can morphological differences be maintained in the face of high gene flow? Possible explanations for a pattern of weak resolution at microsatellite loci and marked variation in phenotype include (1) recency of divergence and insufficient lineage sorting, due possibly to large effective population sizes and short geological time scale; (2) high current gene flow at neutral loci, but strong selection at loci controlling morphological or plumage characteristics; (3) high current gene flow but low historical gene flow with low present-day differentiation being a result of introgression between subspecies or; (4) high gene flow with morphological and plumage variation resulting from phenotypic plasticity. If the last explanation were responsible for the observed pattern of genetic and phenotypic divergence in bay Song Sparrows, it becomes difficult to attribute biological significance to the statistically significant differences in allele frequencies that we found. However, if there is a heritable component to morphology, and observed variation in morphological traits is the result of selection, then the divergence of populations in morphological and plumage characteristics strengthens the biological significance of our genetic results because adap-

tive traits may not be measured at neutral loci (Karhu et al. 1996).

The case favoring the conservation of Song Sparrow populations in San Francisco Bay as management units rests on significant differences in microsatellite allele frequencies and possible adaptive differences in morphology. Moritz et al. (1995) proposed to include management units as distinct population segments protected under the U.S. Endangered Species Act. Among the putative subspecies studied here, *pusillula* is the most threatened by habitat loss and fragmentation (Marshall and Dedrick 1994). In addition, the diversion of freshwater into south San Francisco Bay has resulted in a reduction in salinity that has been accompanied by changes in plant composition of the salt marshes, which may result in changes in the selective pressures (Basham and Mewaldt 1987). Those factors, combined with the evidence of divergence in microsatellite loci reported here, suggest the prioritization of *pusillula* for conservation. The next task is to determine how to manage the grouping of *samuelis*-*maxillaris*-*heermanni*. We recommend that if the differences in morphology and plumage Marshall (1948) described can be demonstrated to be heritable and the result of selection, it then becomes logical to define the management units by habitat. In the absence of specific research on the heritability of morphology and plumage, and the potential for directional selection to act on those traits differently in relation to habitat, arguments either for or against designation of a separate management unit for the salt-marsh-inhabiting *samuelis* and brackish-marsh-inhabiting *maxillaris* remain weak.

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APPENDIX. Allele frequencies for nine microsatellite loci of populations sampled. Sample sizes are provided in the first row of every locus. Population abbreviations and locality are in Table 1.

Locus/ alleles	<i>gouldii</i>		<i>pusillula</i>		<i>samuelis</i>		<i>maxillaris</i>		<i>heermanni</i>	<i>morphna</i>
	MM	LG	PB	DM	PM	SC	GS	RR	CO	BB
MME 1	20	18	27	29	20	28	21	31	21	25
140	—	0.028	—	—	—	—	—	—	—	—
144	—	—	—	—	0.025	—	—	—	0.071	0.100
146	0.125	0.194	0.056	0.138	0.250	0.179	0.238	0.290	0.167	0.360
148	0.225	0.111	0.093	0.069	0.200	0.143	0.167	0.065	0.071	0.080
150	—	0.028	0.019	0.017	—	—	—	—	0.048	0.020
151	0.075	—	0.019	—	—	—	—	—	—	—
152	0.375	0.389	0.630	0.431	0.275	0.393	0.381	0.435	0.405	0.360
154	—	—	0.019	0.052	—	0.018	—	0.016	0.048	—
156	—	—	0.019	0.052	0.050	0.054	0.024	—	—	—
158	0.125	0.222	0.148	0.207	0.200	0.196	0.167	0.161	0.190	0.080
160	0.075	—	—	0.017	—	—	—	—	—	—
162	—	0.028	—	—	—	—	—	—	—	—
164	—	—	—	0.017	—	0.018	0.024	0.032	—	—
MME 3	19.0	17.5	23.0	24.5	15.5	23.5	17.0	28.5	18.0	25.0
162	—	—	—	—	—	—	—	0.053	0.028	0.140
164	—	—	—	—	—	0.021	—	—	—	—
168	—	—	—	—	0.032	—	—	—	—	0.060
170	0.184	0.229	0.087	0.082	0.032	0.191	0.147	0.123	0.028	0.020
172	0.526	0.429	0.239	0.204	0.419	0.383	0.441	0.544	0.500	0.600
174	—	0.029	—	—	—	—	0.059	0.035	0.111	—
176	—	0.029	—	—	—	—	0.118	—	0.056	—
178	—	0.143	0.109	0.082	0.065	—	0.176	0.053	0.028	0.100
180	0.263	0.086	0.239	0.449	0.355	0.340	0.059	0.140	0.194	0.060
182	0.026	0.057	0.326	0.184	0.097	0.064	—	0.053	0.056	0.020
MME 7	19.0	17.5	23.0	23.5	15.5	23.5	17.0	27.5	17.0	24.0
110	0.105	0.143	—	0.021	0.065	0.021	0.088	0.164	0.029	0.167
114	0.026	0.029	0.022	0.021	0.097	0.170	0.029	0.036	0.029	0.083
116	—	0.057	0.022	—	0.065	0.043	0.029	0.145	0.059	0.042
118	0.132	—	—	—	—	0.064	0.029	0.073	0.029	—
120	—	0.057	—	—	0.032	0.064	0.088	0.091	0.059	0.063
122	0.211	0.114	0.174	0.064	—	—	—	0.036	—	0.083
124	0.026	—	0.022	—	0.097	0.043	0.029	0.036	0.029	—
126	0.105	0.114	0.174	0.106	0.129	0.277	0.176	0.127	0.176	0.042
128	0.237	0.143	0.065	0.149	0.097	0.085	0.147	0.182	0.059	0.104
130	—	0.114	0.174	0.191	0.129	0.064	0.088	0.018	0.206	0.146
132	0.132	0.057	0.152	0.298	0.226	0.106	0.088	0.073	0.235	0.146
133	—	0.029	—	—	—	—	0.029	—	—	—
134	—	0.143	0.130	0.128	—	0.021	0.088	0.018	0.059	—
136	—	—	0.065	0.021	0.065	0.021	0.088	—	0.029	0.042
138	0.026	—	—	—	—	—	—	—	—	0.021
140	—	—	—	—	—	0.021	—	—	—	0.021
MME 8	21	18	27	27	20	28	21	30	21	25
208	0.167	0.167	—	0.019	0.075	0.071	0.048	0.083	0.143	—
210	—	—	—	—	—	—	—	—	0.024	—
212	—	—	—	—	—	0.018	—	—	—	—
213	0.199	0.333	0.093	0.056	—	0.089	0.143	0.050	0.095	—
214	—	—	—	—	—	—	—	—	0.024	—
215	—	0.083	0.111	0.056	0.075	0.107	0.095	0.033	0.071	0.100
217	0.143	0.028	0.148	0.148	0.025	0.071	0.024	0.117	0.024	0.140
218	0.095	—	0.204	0.148	0.225	0.143	0.190	0.167	0.095	0.100
219	0.238	0.083	0.093	0.148	0.150	0.143	0.048	0.167	0.095	0.140
220	0.024	0.028	0.037	—	0.200	0.179	0.143	0.133	0.238	0.020
221	—	—	—	—	—	—	—	—	0.024	—
222	—	—	—	—	—	—	—	0.017	0.024	—
223	—	0.111	0.148	0.185	0.025	0.071	0.071	0.050	0.071	0.220
225	0.024	—	—	—	0.100	—	0.048	0.017	—	0.040
227	—	—	—	—	—	—	—	0.017	—	—
228	—	0.028	—	—	—	—	0.024	—	—	0.20

APPENDIX. Continued.

Locus/ alleles	<i>gouldii</i>		<i>pusillula</i>		<i>samuelis</i>		<i>maxillaris</i>		<i>heermanni</i>	<i>morphna</i>
	MM	LG	PB	DM	PM	SC	GS	RR	CO	BB
229	0.167	0.111	0.148	0.241	0.125	0.089	0.143	0.150	0.071	—
230	—	—	—	—	—	0.018	0.024	—	—	—
231	0.024	—	—	—	—	—	—	—	—	—
234	—	0.028	0.019	—	—	—	—	—	—	0.140
MME 12	20.0	18.0	24.0	28.0	17.5	26.0	21.0	24.0	21.0	24.0
182	0.075	—	—	0.018	0.057	0.038	—	0.063	—	—
188	0.450	0.583	0.604	0.679	0.571	0.577	0.643	0.500	0.333	0.021
200	0.025	—	—	—	—	—	—	—	—	0.542
206	0.075	0.056	—	0.036	—	—	0.024	0.021	—	0.021
212	0.050	0.028	0.042	0.036	0.114	0.058	0.048	0.063	0.214	—
218	0.100	0.083	0.104	0.089	0.143	0.212	0.119	0.125	0.238	0.021
224	—	0.139	0.167	0.125	—	0.019	0.048	0.125	—	0.021
230	0.050	0.056	—	—	0.057	0.058	0.071	0.042	0.119	0.083
236	0.025	0.028	0.083	0.018	0.057	0.019	0.048	0.042	0.024	0.042
242	0.125	0.028	—	—	—	0.019	—	0.021	0.071	0.208
250	0.025	—	—	—	—	—	—	—	—	0.042
ESCU 1	21	18	27	26	19	28	21	28	21	21
132	0.214	0.333	0.241	0.558	0.289	0.304	0.262	0.232	0.214	0.190
134	—	0.028	—	—	—	—	—	—	—	—
138	0.071	0.028	0.204	0.077	0.079	0.036	0.167	0.125	—	0.095
140	0.024	—	0.019	—	—	0.018	—	—	0.048	—
142	0.048	0.139	0.130	—	0.132	0.071	0.048	0.071	0.071	—
144	0.119	—	—	0.019	0.026	0.054	0.048	0.071	0.024	0.048
146	0.024	0.111	0.037	0.038	0.053	0.107	0.095	0.107	0.095	0.143
148	0.095	0.139	0.037	0.058	0.184	0.107	0.095	0.214	0.238	0.357
149	—	0.028	—	—	—	—	—	—	—	—
150	0.357	0.083	—	—	0.158	0.107	0.024	0.054	0.143	—
151	0.024	0.028	0.222	0.154	0.026	0.071	0.143	0.089	0.071	—
152	0.024	0.028	0.037	0.077	0.053	0.089	0.048	0.036	0.048	0.167
154	—	0.028	—	—	—	0.018	0.024	—	0.024	—
156	—	—	0.019	0.019	—	0.018	0.024	—	—	—
160	—	—	—	—	—	—	0.024	—	—	—
162	—	0.028	0.056	—	—	—	—	—	—	—
170	—	—	—	—	—	—	—	—	0.024	—
GF 235	21	18	27	26	19	27	21	30	20	25
184	0.048	—	0.019	0.212	0.132	0.037	0.048	0.050	0.025	—
186	—	—	—	—	—	0.019	—	—	—	—
188	—	0.028	0.111	0.019	—	0.019	—	—	—	0.020
190	—	—	—	—	—	—	—	—	0.025	—
192	0.024	0.167	0.019	0.019	—	0.093	0.048	0.100	0.025	0.060
194	0.190	—	0.130	0.154	0.132	0.056	0.143	0.100	0.050	0.100
196	—	0.028	0.074	0.096	0.053	0.167	0.024	0.050	0.050	0.020
198	0.143	0.028	0.074	—	0.053	0.130	0.095	0.050	0.100	0.180
200	0.214	0.333	0.056	0.115	0.237	0.130	0.262	0.250	0.275	0.020
202	0.048	—	0.056	—	0.026	0.056	—	0.017	0.025	0.300
204	—	—	—	—	—	0.019	0.024	0.083	0.025	—
206	—	0.028	0.019	—	—	—	0.024	0.050	0.025	0.140
208	—	0.028	0.037	0.077	—	—	—	—	0.025	—
210	0.143	0.056	0.056	0.058	0.026	—	0.119	0.050	0.050	0.020
212	0.071	0.056	0.056	0.077	0.053	0.037	0.024	0.033	0.050	0.020
214	—	0.028	—	—	0.026	—	—	—	0.025	0.020
216	—	0.056	0.019	0.038	0.053	0.019	0.048	0.033	—	—
218	0.024	0.028	0.019	0.019	—	0.019	0.024	—	—	0.020
220	0.024	—	—	—	—	0.056	—	0.017	—	—
222	—	—	0.019	0.019	—	—	—	—	0.025	—
226	0.048	0.056	—	—	0.026	0.019	0.024	—	0.025	0.020
228	—	—	0.074	0.058	0.105	0.037	—	—	0.050	0.040
230	—	0.028	0.037	—	0.053	0.074	0.048	0.067	0.125	—
232	—	0.028	0.093	0.038	—	—	0.048	0.033	—	0.020
236	—	0.028	0.037	—	—	—	—	0.017	—	—

APPENDIX. Continued.

Locus/ alleles	<i>gouldii</i>		<i>pusillula</i>		<i>samuelis</i>		<i>maxillaris</i>		<i>heermanni</i>	<i>morphna</i>
	MM	LG	PB	DM	PM	SC	GS	RR	CO	BB
238	0.024	—	—	—	—	—	—	—	—	—
240	—	—	—	—	0.026	0.019	—	—	—	—
PSAP335	21	18	26	29	20	27	21	30	21	0
97	—	0.167	—	0.017	0.025	0.019	0.024	0.033	0.024	—
99	0.405	0.139	0.308	0.224	0.075	0.222	0.262	0.200	0.262	—
101	0.048	0.111	0.115	0.172	0.275	0.130	0.214	0.150	0.071	—
103	0.048	0.167	0.154	0.138	0.100	0.056	0.143	0.050	0.143	—
105	0.310	0.278	0.096	0.276	0.400	0.370	0.143	0.383	0.214	—
107	0.190	—	0.115	0.069	0.050	0.130	0.071	0.133	0.143	—
109	—	—	—	—	—	—	—	—	0.024	—
111	—	0.028	—	—	—	—	—	—	0.024	—
113	—	0.028	0.019	0.017	0.075	0.019	—	0.033	0.048	—
115	—	0.056	0.173	0.069	—	0.056	0.119	—	0.048	—
117	—	0.028	0.019	0.017	—	—	—	0.017	—	—
119	—	—	—	—	—	—	0.024	—	—	—
MME 2	21	18	27	27	20	28	21	30	21	16
118	—	—	—	—	—	—	—	0.033	0.024	—
124	—	—	0.056	0.130	—	—	—	—	—	0.063
128	—	—	—	—	0.050	—	—	—	—	—
130	—	—	—	—	0.025	0.018	0.024	—	—	0.031
132	—	—	—	—	0.050	0.036	—	—	—	—
134	—	—	—	0.037	0.025	0.018	—	—	—	—
136	—	—	—	—	—	—	0.024	0.017	0.071	—
138	—	—	0.037	0.167	—	—	—	—	—	—
140	0.048	0.139	0.167	0.204	—	0.089	0.024	0.100	0.119	—
142	0.071	—	0.019	0.037	0.075	0.071	0.095	0.050	0.024	0.188
144	0.238	0.083	0.167	0.093	0.125	0.161	0.286	0.117	0.071	—
146	0.024	0.028	0.019	—	0.050	0.071	—	0.017	0.024	0.469
148	0.024	—	0.167	0.111	0.050	0.054	0.024	0.067	0.024	—
150	0.071	0.194	0.185	0.185	0.150	0.089	0.048	0.150	0.214	0.063
152	0.262	0.278	0.093	0.019	0.125	0.214	0.214	0.300	0.143	0.031
154	0.071	0.111	0.056	—	0.100	0.089	0.119	0.100	0.119	0.031
156	0.048	—	—	—	0.025	0.071	0.143	0.033	—	—
158	—	—	0.019	—	0.125	0.018	—	—	0.071	—
160	—	0.028	—	—	0.025	—	—	0.017	0.048	0.063
162	0.095	0.028	—	—	—	—	—	—	0.048	—
164	0.024	0.056	0.019	—	—	—	—	—	—	—
166	0.024	—	—	—	—	—	—	—	—	—
168	—	0.056	—	0.019	—	—	—	—	—	0.031