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Genetic variation among interconnected populations of *Catostomus occidentalis*: implications for distinguishing impacts of contaminants from biogeographical structuring

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Abstract

Exposure to contaminants can affect survivorship, recruitment, reproductive success, mutation rates and migration, and may play a significant role in the partitioning of genetic variation among exposed and nonexposed populations. However, the application of molecular population genetic data to evaluate such influences has been uncommon and often flawed. We tested whether patterns of genetic variation among native fish populations (Sacramento sucker, *Catostomus occidentalis*) in the Central Valley of California were consistent with long-term pesticide exposure history, or primarily with expectations based on biogeography. Field sampling was designed to rigorously test for both geographical and contamination influences. Fine-scale structure of these interconnected populations was detected with both amplified fragment length polymorphisms (AFLP) and microsatellite markers, and patterns of variation elucidated by the two marker systems were highly concordant. Analyses indicated that biogeographical hypotheses described the data set better than hypotheses relating to common historical pesticide exposure. Downstream populations had higher genetic diversity than upstream populations, regardless of exposure history, and genetic distances showed that populations from the same river system tended to cluster together. Relatedness among populations reflected primarily directions of gene flow, rather than convergence among contaminant-exposed populations. Watershed geography accounted for significant partitioning of genetic variation among populations, whereas contaminant exposure history did not. Genetic patterns indicating contaminant-induced selection, increased mutation rates or recent bottlenecks were weak or absent. We stress the importance of testing contaminant-induced genetic change hypotheses within a biogeographical context. Strategic application of molecular markers for analysis of fine-scale structure, and for evaluating contaminant impacts on gene pools, is discussed.

Keywords: AFLP, biogeography, ecotoxicology, microsatellites, pesticides

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Introduction

Molecular genetic tools and population genetic algorithms have been applied to assess the importance of historic and demographic factors that may influence partitioning of genetic variation among groups of organisms (Avise 1994).

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In addition to these typical forces, toxicity of anthropogenic stressors and associated habitat degradation may influence survivorship, recruitment, reproductive success, mutation rates and migration, thereby influencing partitioning of genetic variation among exposed and nonexposed populations (Anderson *et al*. 1994; Bickham & Smolen 1994; Fox 1995).

Application of molecular genetic approaches for examining population-level impacts of anthropogenic stressors is atypical, even though well-known examples such as

industrial melanism (Kettlewell 1973), acquisition of insecticide tolerance (McKenzie & Batterham 1994) and plant adaptation to heavy metals (Macnair 1987) are powerful reminders of the potential importance of anthropogenic stress as a force for evolutionary change. Some authors have recognized the potential relevance of ecotoxicology to evolution (Palumbi 2001) and conservation biology (Hansen & Johnson 1999), but widespread consideration of potential interactions between these fields remains peripheral. In addition to well-known documentation of industrial melanism and pest tolerance, there is a substantial body of literature that documents induced genetic change in contaminant-exposed populations. Effects range from increases in mutation rate (Dubrova *et al*. 1996; Yauk & Quinn 1996; Ellegren *et al*. 1997; Yauk *et al*. 2000) to erosion of genetic diversity (Murdoch & Hebert 1994; Street & Montagna 1996; Street *et al*. 1998) and acquisition of tolerance (see Klerks & Weis 1987; Belfiore & Anderson 2001 for reviews).

Ecotoxicological impacts on populations occur against a background of biogeography and history. Geographic and demographic forces may, in many cases, be of sufficient influence to override effects of multigenerational toxic stress on partitioning of genetic variation. Importantly, of those who have tested for contaminant impacts at the population genetic level, few have considered biogeographical explanations (Belfiore & Anderson 1998; Staton *et al*. 2001). High gene flow may prevent detectable induced genetic distinctions among groups (Colson 2002), whereas isolated populations may be at much higher risk of stressor-induced population genetic change. Although contaminant-induced genetic change in isolated populations has been detected using molecular markers (Theodorakis & Shugart 1997), it remains to be tested clearly whether genetic change associated with contaminant exposure is distinguishable from biogeographical structuring of genetic variation among interconnected populations at a large geographical scale.

Agricultural pesticide contamination of interconnected watersheds in the Central Valley of California presents a compelling long-term, landscape-scale, contamination scenario. The Sacramento/San Joaquin (Central Valley) watershed drains a large landscape area, and the valley bottom is a heavily agriculturalized landscape which has been under intensive cultivation since the 1930s. Pesticide application is intense and widespread throughout the valley. In 2000, 126 million pounds of pesticide active ingredient were applied to agricultural commodities covering nearly 9 million acres (California Department of Pesticide Regulation 2001). Agricultural pesticide application in the Central Valley has been well-documented for decades, and full-use reporting has been in place since 1991. Introduction of agricultural pesticides to watersheds through rainstorm runoff and irrigation return water, and resulting toxicity to test organisms, has been demonstrated widely (Kuivila & Foe 1995; Pereira *et al*. 1996; Domagalski *et al*. 1997; Werner *et al*. 2000; Werner *et al*. 2002). In addition, recent field caging and laboratory exposure studies in our laboratory have indicated that native fish (Sacramento sucker *Catostomus occidentalis*) were indeed exposed to DNA-damaging agents during storm runoff events following applications of pesticides in the Central Valley (Whitehead *et al*. submitted).

C. occidentalis, a catostomid species endemic to California, is well distributed throughout the Sacramento/San Joaquin Valley (Moyle 2002). Contrary to many Central Valley species, *C. occidentalis* is abundant across a wide range of habitats, including higher-elevation relatively undeveloped areas and low-elevation highly developed areas. They are therefore amenable to watershed-scale analysis of genetic structure.

Our overarching goal was to test whether impacts of pesticide exposure were discernable at the population level in *C. occidentalis*. Our two general working hypotheses were that long-term exposure to pesticide contamination had induced population genetic change in *C. occidentalis*, or that biogeographical factors were primarily responsible for genetic patterning among populations. Field sampling was designed to test for the influence of both historical habitat contamination by pesticides, and geographical structure of watersheds, on partitioning of genetic variation among populations. The first challenge was to test for structure among groups collected from different sites in order to evaluate the possibility that local factors could have provoked independent genetic trajectories. If structure exists, the second challenge was to test how the multipopulation data set supported various hypotheses of mechanisms which might influence genetic patterns among populations. Expectations of patterning of genetic variation across populations differed, considering the influence of biogeography vs. contamination stress. As such, we made predictions of how genetic variation may be partitioned among exposed and reference populations, and contrasted them to predictions based on biogeography.

Of the DNA-based methods available for analysis of population genetic structure, we selected amplified fragment length polymorphism (AFLP) and microsatellite markers. AFLP and microsatellite screening are complementary approaches for examining genetic variation among populations. AFLPs are inexpensive, easy, fast, generate a large number of polymorphic markers randomly distributed throughout the genome (Vos *et al*. 1995), and are highly reproducible and reliable (Jones *et al*. 1997; Bagley *et al*. 2001). As such, these markers have been useful for examining genetic variation below the species level (see Mueller & Wolfenbarger 1999; for review). However, AFLPs suffer from the inability to discern allelic states, and the resulting weakness for estimating some demographic parameters (Wong *et al*. 2001). In contrast, multiple microsatellite

alleles can be scored per locus, are highly variable and have proven powerful for examining genetic variation at fine taxonomic levels (Jarne & Lagoda 1996). Furthermore, novel approaches for evaluating demographic parameters, such as detecting evidence of recent bottlenecks (Garza & Williamson 2001), have been developed for microsatellites that take advantage of their unique physical properties. However, prior sequence information is required for microsatellite development, thereby restricting the number of loci available for screening to a number far fewer than possible with AFLPs. Combining few hypervariable codominant microsatellite markers with many dominant AFLP markers should prove to be a powerful approach for examining relationships among *C. occidentalis* populations distributed throughout the Central Valley.

Briefly, the goals of our study were twofold. First, we tested microsatellites and AFLPs for their relative consistency in detecting fine-scale genetic partitioning among geographically proximate, interconnected, *C. occidentalis* populations. Second, we tested whether patterns of population genetic variation were consistent with pesticide exposure history or, conversely, with expectations based on biogeography.

Materials and methods

Sampling strategy

The Sacramento sucker (*Catostomus occidentalis*) was selected for study because it is a widely distributed fish native to the Sacramento-San Joaquin basin. Fish were caught by seine net, backpack electrofisher and by boat-based electrofisher. Small fin clips (approximately 5 mm^2) were taken and air-dried. Sixty fin clips were collected from each of 12 populations spanning six rivers (Fig. 1, Table 1).

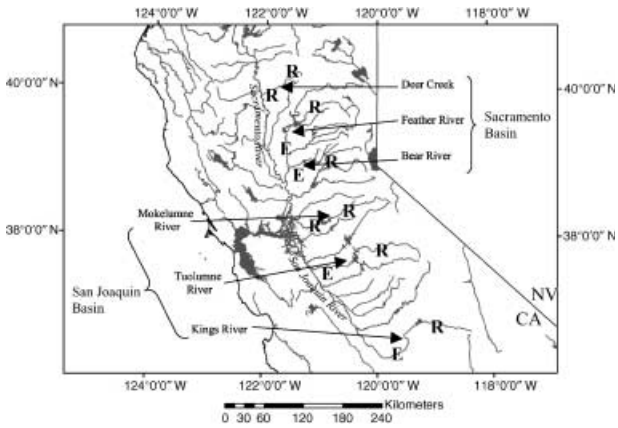

Fig. 1 Map of the California Central Valley, showing locations of sampling sites. R = reference site; E = pesticide exposed site.

The sampling strategy permitted comparisons between watersheds, rivers, upstream and downstream sites, as well as comparison of reference and exposed condition. Three of the rivers (Bear River, Deer Creek, Feather River) are in the Sacramento River basin and three (Mokelumne River, Tuolumne River, and Kings River) are in the San Joaquin River basin. Two populations were sampled per river – a 'downstream' population and an 'upstream' counterpart. All upstream populations (Deer-up, Feather-up, Bear-up, Mokelumne-up, Tuolumne-up and Kings-up) and two of the six downstream populations (Deer-dn and Mokelumne-dn) were from reference sites, whereas the four remaining downstream populations (Feather-dn, Bear-dn, Tuolumne-dn and Kings-dn) were from exposed sites.

Table 1 Populations sampled with location, elevation (in meters), characterization as pesticide exposed (Exp) or reference (Ref) site, and number of individuals used for AFLP and microsatellite analyses per population

Population	Location	Elevation (m)	Exp ref	AFLP (*n*)	Microsat (*n*)
Sacramento Basin					
Bear-dn	Bear River, downstream	26	**Exp**	40	59
Bear-up	Bear River, upstream	577	Ref	40	61
Deer-dn	Deer Creek, downstream	85	Ref	40	60
Deer-up	Deer Creek, upstream	523	Ref	40	55
Feather-dn	Feather River, downstream	24	**Exp**	40	55
Feather-up	Feather River, upstream	460	Ref	40	60
San Joaquin Basin					
Kings-dn	Kings River, downstream	94	**Exp**	40	66
Kings-up	Kings River, upstream	1057	Ref	40	63
Mokelumne-dn	Mokelumne River, downstream	18	Ref	40	58
Mokelumne-up	Mokelumne River, upstream	211	Ref	40	58
Tuolumne-dn	Tuolumne River, downstream	40	**Exp**	40	60
Tuolumne-up	Tuolumne River, upstream	420	Ref	40	61

Sampling site selection

Geographic information system (GIS) mapping of historical pesticide application patterns was used in the first level of screening of field sites for inclusion as exposed or reference sites. Data on pesticide applications were compiled from the California Department of Pesticide Regulation (CDPR) Pesticide Use Database (http://www.cdpr.ca.gov/dprdatabase.htm). Mapping indicated areas of intensive pesticide application, and sites where such application patterns overlapped rivers were selected for further characterization. These candidate sites were then investigated with reference to reported pesticide contamination (Kuivila & Foe 1995; MacCoy *et al*. 1995; Pereira *et al*. 1996; Domagalski 1997; Domagalski *et al*. 1997; Kratzer 1997; Domagalski *et al*. 1998). Candidate sites with no, or minimal, reported contamination were eliminated. Remaining candidate sites were further pruned according to the six criteria outlined below.

Exposed and reference sites were selected according to the following criteria: (1) some downstream/upstream counterparts must differ according to pesticide exposure history (downstream exposed sites, with upstream reference counterparts), whereas other counterparts must each have limited (or no) pesticide exposure history (downstream reference sites, with upstream reference counterparts); (2) dams should separate downstream/upstream counterparts in order to limit upstream gene flow; (3) pesticide history of 'exposed' sites should relate to historic presence of orchards rather than row crops; (4) sampling must cover a large landscape area because pesticide contamination is widespread throughout the Central Valley; (5) sampling sites must represent adequately the geographical hierarchy of the watershed; and (6) populations of a native fish species must be present at each of the proposed sampling sites.

Hypotheses and predictions

Our hypothesis is that physical geographical factors are primarily responsible for patterning of genetic variation among populations. Such geographical factors include the structure of watersheds (direction of river flow), barriers to migration (salinity) and geographical distance among populations. If this hypothesis is true, we would predict that (a) for each upstream population, the most closely related group would be its downstream counterpart, (b) all downstream populations within each watershed would be closely related (because river gradient between downstream populations is not as pronounced as between upstream/downstream counterparts), (c) a significant portion of genetic variation would be partitioned between populations grouped according to watershed and river, (d) all downstream populations would be more variable genetically than upstream counterparts (due to direction of gene flow), and (e) genetic distance would be correlated with geographical distance.

Our secondary hypothesis is that long-term pesticide exposure influenced patterning of genetic variation among populations. We tested for the influence of three general mechanisms whereby pesticide contamination may have induced genetic change. First, long-term exposure to pesticide contamination may have caused changes in frequencies of alleles linked closely to loci under selection. If this hypothesis is true, we would predict that alleles of candidate loci would have divergent frequencies consistently between exposed populations and their upstream (reference) counterparts, but would not be divergent between downstream reference populations and their upstream counterparts. Loci that demonstrated such a pattern would be candidates for further evaluation to test the hypothesis more rigorously. Also, we would predict that genetic similarity would be higher among exposed populations than between all other population pairings. Second, long-term exposure to pesticide contamination may have induced an increase in the frequency of heritable mutations in exposed populations. Indeed, recent studies in our laboratory have demonstrated pesticide runoff in the Central Valley to be genotoxic and mutagenic (Whitehead *et al*. submitted). If this hypothesis is true, we would predict that exposed populations would have a higher frequency of rare alleles, indicating increased mutation rates, than reference populations. Third, long-term exposure to pesticide contamination may have reduced sizes of exposed populations significantly compared to reference populations, resulting in a genetic bottleneck. If the bottleneck hypothesis is true, we would predict that exposed populations would exhibit reduced genetic variability at loci distributed throughout the genome, and that microsatellite markers would reveal evidence of recent bottlenecks, compared to reference populations. Hypotheses are not independent, but contaminant-associated predictions are contrasted to those relating to biogeographical expectations.

DNA extraction and quantification

Fin clips were extracted using Qiagen DNeasy tissue kit (Qiagen Inc., Valencia, CA, USA) or the Sigma Genelute mammalian genomic DNA kit (Sigma Scientific Technology, Inc., Houston, TX, USA). DNA concentrations were determined by fluorimetry with picogreen, and diluted to a final concentration of 24 ng/mL for AFLP analysis, or 5 ng/mL for microsatellite analysis.

AFLP procedure

The AFLP procedure (Vos *et al*. 1995) was based on the methods of Agresti *et al*. (2000), with modifications.

Table 2 AFLP selective-amplification primer combinations and microsatellite primers. See Tranah 2001) and Tranah *et al.* (2001) for microsatellite primer sequences. Listed are number of bands/alleles detected per locus and levels of polymorphism

AFLP selective primer extensions		No. bands	No. polymorphic bands
Eco	Mse		
AAC	CGTG	25	20
AAC	CTTG	37	26
ACC	CAAG	35	28
ACC	CGTG	26	21
ACT	CCTG	41	25
AGG	CAAG	30	24
AGG	CCTG	30	21
AGG	CGC	43	34
AGG	CGT	32	25
AGG	CTGG	48	36
Sum		347	260
Microsatellite primers		**No. alleles**	**No. polymorphic alleles**
Dlu 26a*		13	13
Dlu 243		11	11
Dlu 476		18	18
Dlu 488		25	25
Dlu 4158		13	13
Dlu 4183		21	21
Dlu 4184		17	17
Dlu 4296		25	25
Sum		143	143

*Locus excluded from further estimation of population genetic parameters.

Pre-amplification polymerase chain reaction (PCR) primers each had a one-base extension (Eco-A, Mse-C). Reactions were run on a PTC-100 thermalcycler (MJ Research Inc., Watertown, MA, USA) under the following conditions: 19 cycles of denaturing (94 °C for 20 s), annealing (56 °C for 30 s), and extension (72 °C for 2 min), a 30-min hold at 60 °C and an indefinite hold at 4 °C. Selective amplification primers (Table 2) each had three or four-base extensions [Eco-ANN, Mse-CNN(N)], and the thermalcycler profile started with 2 min at 94 °C, followed by nine cycles of 94 °C for 20 s, 66 °C for 30 s and 72 °C for 2 min, decreasing annealing temperature by 1 °C per cycle. This was followed by 24 more cycles of 94 °C for 20 s, 56 °C for 30 s and 72 °C for 2 min, a 30-min hold at 60 °C, then storage at 4 °C. *Eco*RI selective primers were end-labelled with a HEX fluorophore (Integrated DNA Technologies Inc., Coralville, IA, USA). PCR product was loaded onto 8% denaturing polyacrylamide gels and electrophoresed at 50 W for 60–120 min. GeneScan-500 ladder (Applied Biosystems, Warrington, UK) was used to size bands, and one individual sample was electrophoresed on all gels as an internal allelic standard. Bands were visualized by scanning gels on the FMBIO fluorescent imaging system (Hitachi Software Engineering America Ltd). Presence or absence of bands was scored manually as 1 or 0, respectively. Only clearly resolvable bands were scored. Monomorphic markers were scored, but globally monomorphic markers were omitted from further population genetic analyses. All samples from one population (Mokelumne-dn) were run through the AFLP procedure twice, and scores for individuals were compared among replicates to assess reproducibility of the AFLP process. Assumptions associated with interpretation of AFLP bands were as outlined in Gaudeul *et al*. (2000).

Microsatellite procedure

Primers for microsatellite analysis (Table 2) were developed by G. Tranah, and sequences are reported in Tranah (2001) and Tranah *et al*. (2001). Forward primers were end-labelled with one of three fluorophores: HEX, TET or FAM (Integrated DNA Technologies Inc., Coralville, IA, USA). PCR amplification was performed in 10-μL volumes containing 5 ng genomic DNA. Reaction mixtures were amplified using the following protocol: 95 °C for 1:30, then 30 (35 for HEX-labelled primer pairs) cycles of 95 °C for 1 min, 52 °C for 30 s, 72 °C for 1 min, followed by a final extension of 60 °C for 45 min. Alternately labelled PCR products from the eight loci were separated into three sets, containing two or three of differentially labelled locus products. Then, 1 μL (3 μL for Dlu 26a) of diluted PCR product for each of the loci of a given set were pooled together and combined with 2 μL H_2O, 1.45 μL 100% de-ionized formamide (Sigma), 0.5 μL blue dextran loading dye (ABI) and 0.05 μL ABI GeneScan-500 ROX internal size standard. One μl of a FAM-labelled 400 base pairs (bp) lane indicator (MJ Research, Inc.) was added into every sixth lane to aid in the

tracking of lanes during analysis. Samples were denatured at 95 °C for 6 min and chilled on ice. One μl aliquots of PCR product / size standard mixtures were loaded automatically into the 96 lanes of a 75 micron 5.5% denaturing polyacrylamide gel. Electrophoresis was performed on the BaseStation DNA Fragment Analyser (MJ Research, Inc.) in 1 × TBE running buffer with a 2-min prerun at 1900 V, a 30-s constant injection at 4000 V and a 6000 scan collection run at 2600 V (5000 scans per hour). PCR products were analysed and genotypic data (based on DNA fragment size) were generated using Cartographer (version 1.2.3) DNA fragment analysis software (MJ Research, Inc.).

Data analysis

General statistics. Fisher exact tests for independence of loci and tests for Hardy–Weinberg proportions for microsatellites were performed in GENEPOP version 3.3 (Raymond & Rousset 1995) using the Markov chain method with 1000 batches and 1000 iterations per batch. If linkage patterns indicated the possibility of family structure (> 10% of locus pairs indicating disequilibrium within a population), a maximum likelihood test for full siblings was performed in the program KINSHIP version 1.2 (developed by K.F. Goodnight; available at http://www.gsoftnet.us/GSoft.html) to rule out the presence of family structure. To investigate the presence of null alleles in our data set, frequency of null alleles for each microsatellite locus was estimated based on equation 3 from Brookfield (1996).

Population structure. For AFLPs, two methods were used to test the null hypothesis of panmixis. First, Weir & Cockerham's (1984) theta (θ) estimator of Wright's (1951) F_{ST} was calculated among all populations, and between all population pairs using TFPGA version 1.3 (Miller 1997). A bootstrapping procedure (1000 permutations) was included to build 95% confidence intervals for calculated θ-values. There is some concern whether allele frequency-based techniques are appropriate for calculating population parameters for dominant markers (Wong *et al*. 2001). Analysis of molecular variance (AMOVA) is based on phenetic distance measures which are independent of allele frequencies (Excoffier *et al*. 1992), and was used as a second approach to test for population differentiation. AMOVA F_{ST} correlates (Φ_{ST}) were calculated for each population pair, using 16 000 random permutations of the data, in ARLEQUIN version 2.000 (Schneider *et al*. 2000).

For microsatellites, θ was calculated for all populations and all population pairs using GENETIX version 4.02 (Belkhir 2000). Data sets were bootstrapped 1000 times to estimate 95% confidence intervals. Second, genetic structure among all population pairs was tested using Fisher's exact test, as described by Raymond & Rousset (1995) for genotype and allele frequency differences, for each locus, in GENEPOP. *P*-values and associated standard errors were calculated using a Markov chain with 100 batches and 1000 iterations per batch. The joint null hypothesis of no significant difference in allele or genotype frequencies across loci was tested following Bonferroni correction and by Fisher's method of summation of χ^2 statistics across loci (Ryman & Jorde 2001).

Genetic structure was tested further among population groups using the AMOVA procedure as described above for both marker systems. We tested for significant partitioning of genetic variation between population groups according to geographical hierarchy (watershed and river) and according to pesticide exposure history. Presence of alleles private to these groupings was used to provide supporting evidence of structure.

Band/allele frequencies. To test for the influence of contaminant-induced selection, populations were screened for loci with bands / alleles having downstream / upstream frequency differences consistent among downstream exposed/upstream reference counterparts, but inconsistent between downstream reference/upstream reference counterparts. To test for evidence of increased mutation rates we screened populations for private alleles (occur only in one population) and for rare private alleles (occur in only one individual). A higher frequency in exposed compared to reference populations would lend support to the mutation hypothesis.

Genetic diversity. For AFLP markers, genetic diversity was described as percent polymorphic loci. This was calculated as the proportion of all loci that are polymorphic ($0 <$ frequency < 1.0) in that population. Second, average heterozygosity across loci (H) was estimated based on Lynch & Milligan's (1994) Taylor expansion method for dominant markers (loci with ≤ 3 null alleles were pruned from the data set).

For microsatellite markers, unbiased estimates for expected heterozygosity were calculated for each locus according to Nei (1987) and averaged across loci to derive average heterozygosity for each population. Diversity was also measured by calculating the average proportion of alleles present across loci.

Genetic distance. For AFLP data, Nei's (1978) genetic distance ($D_{NEI\,78}$) was calculated for all population pairs using TFPGA. For microsatellite data, Cavalli-Sforza & Edwards's (1967) chord distance (D_{CSE}) and Goldstein *et al*.'s (1995) distance $(\delta\mu)^2$ were calculated for all pairs using GENETIX and RSTCALC (Goodman 1997), respectively. An unrooted dendrogram was constructed from the distance matrix using the neighbour-joining method (Saitou & Nei 1987) in the program MEGA version 2.1 (Kumar *et al*. 2001). One hundred bootstrap replicates were generated in PHYLIP

version 3.5 (Felsenstein 1995) to evaluate the support of tree nodes. Principle coordinate analysis was also performed on the distance matrix using the DCENTER and EIGEN subroutines in NTSYS-pc version 2.1 (Rohlf 2000). Relationships between genetic and geographical distances were evaluated using the Mantel procedure in ARLEQUIN, with 10 000 random permutations of rows and columns.

Genetic bottlenecks. Microsatellite data were used to test for the occurrence of recent bottlenecks using two empirical approaches. Allele frequency distributions were plotted for all loci in each population, and a low frequency of rare alleles leading to a mode-shift distortion of the typical J-shaped allele frequency distribution was considered evidence of a recent bottleneck (Luikart *et al*. 1998). Garza & Williamson's (2001) *M* is a ratio of the number of alleles to the range in allele size and was also used to test for evidence of recent bottlenecks in the program AGARST (Harley 2001). According to Garza & Williamson's simulations, and their review of data sets in natural populations, a population has undergone a recent bottleneck if $M < 0.68$ (Garza & Williamson 2001).

Correlation between markers. Spearman's coefficient of rank correlation (Sokal & Rohlf 1995) was calculated to test the relative consistency of AFLP and microsatellite markers in estimating population genetic parameters. Consistency of population rankings by the two AFLP genetic diversity estimators was compared to the two microsatellite diversity estimators (four comparisons in total). Ranking of population pairs by θ and genetic distance was also compared between markers. Correlations (and associated *P*-values) were calculated using SigmaStat statistical software (SPSS, Chicago, IL, USA).

Results

General statistics

Ten AFLP primer combinations produced 347 resolvable bands, of which 260 were polymorphic (Table 2). Of the 13 880 individual scores for the Mokelumne-dn population (347 loci, 40 individuals), only eight (0.13%) were scored differently between the two parallel AFLP runs. All eight microsatellite loci were highly polymorphic. Alleles per locus ranged from 11 to 25 (Table 2).

Deviations from Hardy–Weinberg equilibrium (HWE) and linkage equilibrium were detected for some microsatellite loci. Eleven of 96 locus–population combinations deviated from HWE ($P < 0.01$). Across loci, six of 12 populations were deviant from HWE. Maximum likelihood tests for full siblings for each population ruled out the influence of family structure in the data set. Expected number of null homozygotes was low (0–7) for most loci. However, locus Dlu 26a deviated from HWE in five populations, and expected number of null homozygotes ($r = 12$) was highest for this locus. As such, Dlu 26a was excluded from further population genetic analysis.

Genetic structure

For AFLP markers, calculations of θ and results from AMOVA indicated significant structuring of genetic diversity among sampled populations. Global θ and Φ_{ST} estimates over all populations were 0.11 (95% CI = 0.13–0.09) and 0.11 ($P < 0.0001$), respectively. For all pairwise population comparisons, 95% confidence intervals of θ estimates did not overlap with 0, indicating significant differences in allele frequencies among all populations. Correspondingly, all pairwise Φ_{ST} estimates among populations were highly significant ($P < 0.0001$).

For microsatellite markers, θ and Fisher's exact test also indicated significant genetic structure among populations. Global θ for all populations was 0.05 (95% CI = 0.04–0.06). Theta among all pairs of populations was significant ($P < 0.0001$) except for Kings counterpart populations ($P = 0.101$). Fisher's exact test indicated significant difference in allele and genotype frequencies between all population pairs. The joint null hypothesis of no difference in allele frequencies across loci was rejected following Bonferroni's correction for multiple comparisons ($P < 0.007$), and following Fisher's method of summation of χ^2 statistics across loci ($P < 0.001$). Both AFLP and microsatellite data indicated least genetic differentiation between Kings-dn and Kings-up, and greatest differentiation between Feather-up and Bear-up populations.

AMOVA analysis revealed significant partitioning of genetic variation at the two levels of geographical hierarchy (Table 3). Although the majority of variation was partitioned among individuals within groups, analysis of the AFLP data set indicated that grouping of populations according to watershed (populations of Sacramento and San Joaquin watersheds) did account for a small (1.87%) but significant ($P = 0.007$) portion of genetic variation, and grouping of populations according to river accounted for a greater portion of genetic variation (5.37%, $P < 0.0001$). Similarly, the microsatellite data set indicated a small (1.2%) but significant ($P = 0.001$) partitioning of variation among rivers. However, in contrast to AFLP data, AMOVA structure between populations of the two watersheds was not significant with microsatellite markers ($P = 0.320$).

Although geographical structure did account for some variation among populations, grouping of populations according to exposure history (exposed vs. reference) did not (AFLP $P = 0.89$; microsatellite $P = 0.83$) (Table 3). More importantly, exposed population groupings vs. downstream reference population groupings did not account for significant partitioning of genetic variation across watersheds (AFLP $P = 0.34$; microsatellite $P = 0.07$), nor within watersheds

Table 3 Analysis of molecular variance (AMOVA) testing for partitioning of genetic variation among populations, grouped according to geography and exposure history, using AFLP and microsatellite markers. Populations were grouped according to basin (Sacramento vs. San Joaquin), river, exposure history of downstream populations and within-basin exposure history of downstream populations. Statistics included sum of squared deviations (sum of squares), estimation of variance components (variance), percentage of total variation contributed by each component (% total), Φ-statistics and *P*-value

Source of variation	d.f.	Sum of squares	Variance	% total	Φ-statistics	*P*-value
AFLP						
Geographical partition, basins						
Between groups	1	180.99	0.37	1.87	$\Phi_{CT} = 0.019$	0.007
Among populations/groups	10	939.61	1.94	9.91	$\Phi_{SC} = 0.118$	< 0.0001
Within populations	462	7982.40	17.28	88.21	$\Phi_{ST} = 0.101$	< 0.0001
Total	473	9103.00	19.59			
Geographical partition, rivers						
Between groups	5	735.12	1.05	5.37	$\Phi_{CT} = 0.054$	< 0.0001
Among populations/groups	6	385.48	1.19	6.10	$\Phi_{SC} = 0.115$	< 0.0001
Within populations	462	7982.40	17.28	88.54	$\Phi_{ST} = 0.064$	< 0.0001
Total	473	9103.00	19.51			
Exposure partition, exposed vs. downstream reference						
Between groups	1	72.20	0.07	0.38	$\Phi_{CT} = 0.004$	0.337
Among populations/groups	4	255.83	1.16	5.93	$\Phi_{SC} = 0.060$	< 0.0001
Within populations	231	4226.85	18.30	93.69	$\Phi_{ST} = 0.063$	< 0.0001
Total	236	4554.88	19.53			
Exposure partition, Sacramento exposed vs. downstream reference						
Between groups	1	92.30	0.65	3.21	$\Phi_{CT} = 0.032$	0.331
Among populations/groups	1	57.15	0.99	4.86	$\Phi_{SC} = 0.050$	< 0.0001
Within populations	115	2148.30	18.68	91.94	$\Phi_{ST} = 0.081$	< 0.0001
Total	117	2297.75	20.32			
Exposure partition, San Joaquin exposed vs. downstream reference						
Between groups	1	47.53	–0.01	–0.05	$\Phi_{CT} = 0.000$	0.665
Among populations/groups	1	47.75	0.76	4.05	$\Phi_{SC} = 0.040$	< 0.0001
Within populations	116	2078.56	17.92	96.00	$\Phi_{ST} = 0.040$	< 0.0001
Total	118	2173.84	18.66			
Microsatellite						
Geographical partition, basins						
Between groups	1	20.12	0.00	0.09	$\Phi_{CT} = 0.001$	0.322
Among populations/groups	10	183.03	0.13	4.51	$\Phi_{SC} = 0.045$	< 0.00001
Within populations	1420	3915.97	2.76	95.41	$\Phi_{ST} = 0.046$	< 0.00001
Total	1431	4119.11	2.89			
Geographical partition, rivers						
Between groups	5	114.96	0.03	1.20	$\Phi_{CT} = 0.012$	0.002
Among populations/groups	6	88.19	0.10	3.46	$\Phi_{SC} = 0.035$	< 0.00001
Within populations	1420	3915.97	2.76	95.34	$\Phi_{ST} = 0.047$	< 0.00001
Total	1431	4119.11	2.89			
Exposure partition, exposed vs. downstream reference						
Between groups	1	17.06	0.03	0.90	$\Phi_{CT} = 0.009$	0.064
Among populations/groups	4	36.41	0.05	1.89	$\Phi_{SC} = 0.019$	< 0.00001
Within populations	710	1944.67	2.74	97.21	$\Phi_{ST} = 0.028$	< 0.00001
Total	715	1998.13	2.82			
Exposure partition, Sacramento exposed vs. downstream reference						
Between groups	1	7.21	0.02	0.57	$\Phi_{CT} = 0.006$	0.334
Among populations/groups	1	4.59	0.02	0.55	$\Phi_{SC} = 0.006$	< 0.00001
Within populations	345	968.05	2.81	98.87	$\Phi_{ST} = 0.011$	< 0.00001
Total	347	979.85	2.84			
Exposure partition, San Joaquin exposed vs. downstream reference						
Between groups	1	21.99	0.08	3.00	$\Phi_{CT} = 0.030$	0.335
Among populations/groups	1	8.93	0.05	1.77	$\Phi_{SC} = 0.018$	< 0.00001
Within populations	365	976.62	2.68	95.23	$\Phi_{ST} = 0.048$	< 0.00001
Total	367	1007.53	2.81			

Table 4 Frequencies of AFLP bands and microsatellite alleles private among populations within a basin (A) or private to a population (B)

(A)	Sacramento River Watershed						San Joaquin River Watershed					
AFLP												
AAC–CTTG 213	9 / 40	11 / 40	7 / 39	1 / 39	1 / 38	5 / 39	—	—	—	—	—	—
ACT–CCTG 347	5 / 40	8 / 40	2 / 40	—	1 / 37	—	—	—	—	—	—	—
AGG–CGT 152	2 / 40	—	4 / 39	—	1 / 36	3 / 40	—	—	—	—	—	—
Microsatellite*												
243 187	—	—	1 / 120	—	—	3 / 120	—	—	—	—	—	—
488 267	—	—	—	—	—	—	3 / 132	3 / 126	—	1 / 116	2 / 120	—
488 283	1 / 118	—	—	—	—	1 / 120	—	—	—	—	—	—
4183 146	—	—	1 / 120	—	4 / 110	—	—	—	—	—	—	—
4183 222	1 / 118	—	—	—	1 / 110	—	—	—	—	—	—	—
4184 231	—	—	—	1/110	2 / 110	—	—	—	—	—	—	—
4296 226	1 / 118	—	—	—	—	1/120	—	—	—	—	—	—

(B)	Bear-dn	Bear-up	Deer-dn	Deer-up	Fea-dn	Fea-up	Kings-dn	Kings-up	Mok-dn	Mok-up	Tuol-dn	Tuol-up
AFLP												
AAC–CGTG 07	—	—	—	1 / 40	—	—	—	—	—	—	—	—
AAC–CGTG 141	—	—	1 / 40	—	—	—	—	—	—	—	—	—
AAC–CTTG 98	—	—	—	—	—	—	—	1 / 36	—	—	—	—
AAC–CTTG 217	—	—	—	—	—	—	—	—	—	—	1 / 39	—
ACC–CAAG 96	—	—	1 / 40	—	—	—	—	—	—	—	—	—
ACC–CAAG 248	—	—	—	—	—	—	—	—	—	—	—	2 / 40
ACT–CCTG 89	—	—	—	—	—		—	—	—	—	—	2 / 40
ACT–CCTG 175	—	—	—	—	—	—	1 / 40	—	—	—	—	—
AGG–CCTG 155	—	—	—	—	—	—	—	—	—	—	1 / 39	—
AGG–CGC 159	—	—	—	—	—	—	—	—	—	—	5 / 39	—
AGG–CGC 310	—	—	—	—	—	4/39	—	—	—	—	—	—
AGG–CGT 160	1/40	—	—	—	—	—	—	—	—	—	—	—
AGG–CTGG 157	—	—	—	—	—	—	—	—	—	—	1/39	—
AGG–CTGG 211	—	—	—	—	1/37	—	—	—	—	—	—	—
AGG–CTGG 280	—	—	—	—	—	4/39	—	—	—	—	—	—
AGG–CTGG 341	—	—	—	—	—	—	—	—	—	—	1/39	—
Microsatellite[a]												
243 179	—	—	1/120	—	—	—	—	—	—	—	—	—
488 191	—	—	3/120	—	—	—	—	—	—	—	—	—
4158 206	—	—	—	—	—	—	—	—	—	—	—	4/122
4183 218	1/118	—	—	—	—	—	—	—	—	—	—	—
4296 230	—	—	—	—	—	3/120	—	—	—	—	—	—
4296 234	—	—	1/120	—	—	—	—	—	—	—	—	—

*Microsatellite allele frequencies are per haploid genome. Abbreviations for Feather, Mokelumne, and Tuolumne Rivers are 'Fea', 'Mok', and 'Tuol', respectively

(within Sacramento AFLP $P = 0.33$; microsatellite $P = 0.33$; within San Joaquin AFLP $P = 0.66$; microsatellite $P = 0.33$).

The opccurrence of private bands provided supporting evidence for partitioning of population genetic variation according to geography. Three AFLP bands and six microsatellite alleles were private among Sacramento watershed populations (Table 4A), sometimes at high frequency.

Band/allele frequencies

We detected no consistently outstanding differences in band/allele frequencies of specific loci (or groups of loci) between counterpart populations of exposed rivers compared to counterparts of reference rivers. Of the 260 polymorphic AFLP loci screened, only five showed such patterns. Most of these five bands occurred at low frequency in exposed populations (average frequency = 0.058, equivalent to one to four individuals of approximately 40 sampled), and frequency differences between exposed and upstream counterparts were small (average frequency difference = 0.050). Because the frequency of AFLP bands that could reflect the pattern of selection occurred at low frequency, the occurrence of these patterns of variation were probably random. For microsatellite markers, of the 130 alleles resolved from all seven loci none showed the predicted pattern.

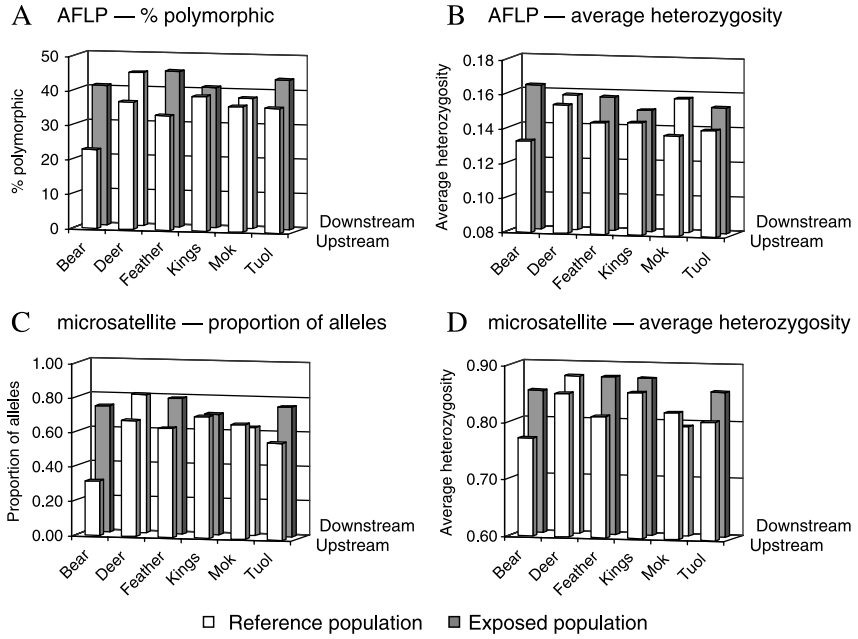

Fig. 2 Population genetic diversity calculated by AFLP-derived diversity estimates. (A) Percentage of all loci that are polymorphic; (B) average heterozygosity based on Lynch and Milligan's Taylor expansion method, including pruning of loci with fewer than three null individuals, and by microsatellite-derived diversity estimates; (C) average proportion of alleles present across loci; (D) Nei's unbiased expected heterozygosity. Dark and light bars represent exposed and reference populations, respectively. Front bars represent upstream populations, and back bars represent downstream counterparts.

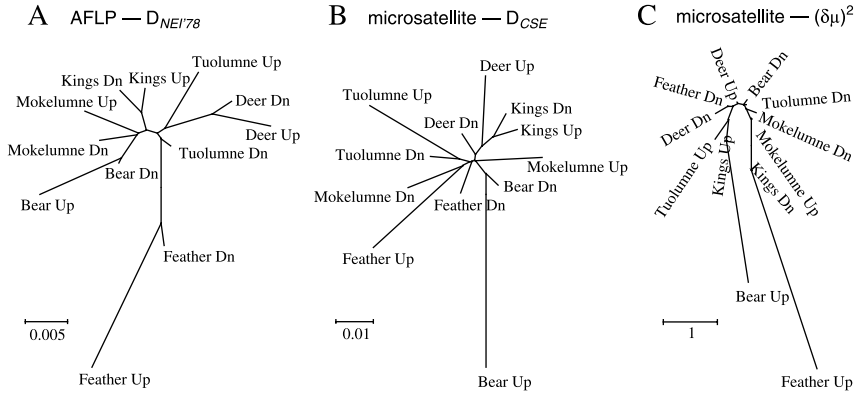

Fig. 3 Dendrograms of genetic relationships among populations. The neighbour-joining method was applied to (A) $D_{NEI'78}$ matrix derived from AFLP data; (B) D_{CSE} matrix derived from microsatellite data; (C) $(\delta\mu)^2$ derived from microsatellite data.

Private bands / alleles occurred at a low frequency, and these were distributed evenly among populations regardless of exposure history or position in the watershed (Table 4B). No bands/alleles were private among exposed, nor among reference, populations.

Genetic diversity

For AFLP markers, diversity in downstream populations (H = 0.157, SD = 0.005; % polymorphic = 44.86, SD = 3.004) was higher than in upstream populations (H = 0.143, SD = 0.007; % polymorphic = 36.45, SD = 6.202) (P = 0.003 for H, and P = 0.014 for percentage polymorphic), but diversity was not different between exposed and downstream reference groups (P = 0.702 for H, and P = 0.654 for percentage polymorphic; Fig. 2A,B).

Microsatellite markers indicated similar patterns of genetic diversity among populations (Fig. 2C,D). Diversity in downstream populations (proportion of alleles present = 0.712, SD = 0.072; H = 0.825, SD = 0.041) was higher than in upstream populations (proportion of alleles present = 0.569, SD = 0.123; H = 0.776, SD = 0.032) (P = 0.035 for proportion of alleles present, and P = 0.045 for H), but diversity was not different between exposed and downstream reference groups (P = 0.825 for proportion of alleles present and P = 0.633 for H).

Genetic distance

Genetic groupings reflected expected geographical relationships. Pairwise genetic distances derived from AFLP markers ($D_{NEI'78}$) indicated that the most closely related population to each upstream population was the downstream counterpart (Fig. 3A). Counterpart populations tended to cluster together, and Feather-up and Bear-up populations were most distinct from the rest. The smallest

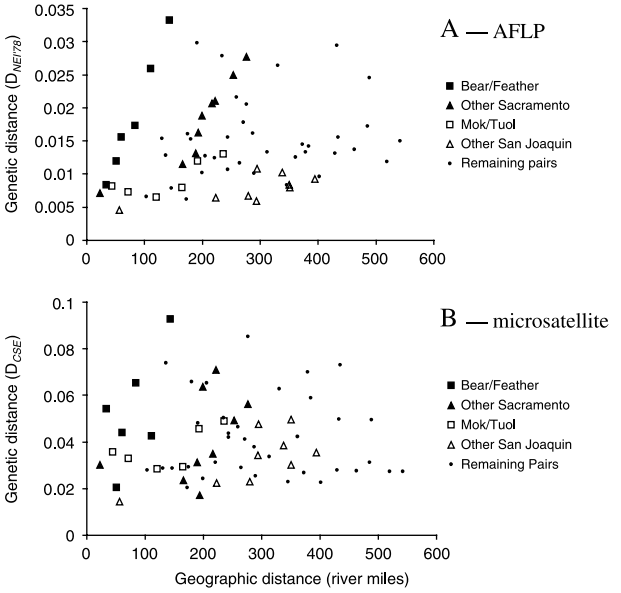

Fig. 4 Relationship between genetic and geographical distances between all population pairs. (A) Nei's genetic distance derived from AFLP data; (B) Cavalli-Sforza–Edwards' chord distance derived from microsatellite data. Closed symbols are Sacramento basin pairs. Closed squares are Bear/Feather pairs. Open symbols are San Joaquin pairs. Open squares are Mokelumne/Tuolumne pairs. Dots represent all remaining pairs between watersheds.

distance measured was between Kings-dn and Kings-up populations, and the largest was between Feather-up and Bear-up. These results mirror the extremes of genetic similarity detected with θ and Φ_{ST}.

Microsatellite data based on D_{CSE} reflected closely patterns of relatedness indicated by AFLP markers, despite low bootstrap support (< 50%) for nodes from all trees. Downstream populations were typically the most closely related population to each upstream counterpart (Fig. 3B), and Feather-up and Bear-up were outliers. The smallest and largest distances were between Kings-dn and Kings-up, and Feather-up and Bear-up, respectively. In contrast, clustering of populations by $(\delta\mu)^2$ had little correspondence to geographical proximity (Fig. 3C), nor to patterns of relatedness indicated by AFLP and microsatellite D_{CSE} distances. Most populations grouped together in a single dense cluster. Only Bear-up and Feather-up were resolved as outliers. Finally, principal coordinate analysis indicated that exposed populations did not cluster separately from reference populations for either AFLP or microsatellite markers (A. Whitehead, unpubl. data).

There was no significant correlation between genetic and geographical distances among all population pairs for distances derived from either AFLP ($r = 0.1537$, $P = 0.211$) or microsatellite ($r = -0.0111$, $P = 0.482$) markers (Fig. 4). However, correlations were evident at local scales between geographical and AFLP-derived distances. At the watershed scale correlation was weak, but significant, among all Sacramento populations ($r = 0.58$, $P = 0.02$), but not significant among all San Joaquin populations ($r = 0.32$, $P = 0.10$). Within watersheds, strength of correlation increased. Distances were correlated between Feather and Bear populations ($r = 0.993$, $P = 0.044$), and between Mokelumne and Tuolumne populations ($r = 0.7752$, $P = 0.039$). Microsatellite-derived distances showed a relationship to geographical distance only between Mokelumne and Tuolumne populations ($r = 0.5528$, $P = 0.039$).

Table 5 Spearman's rank order correlation coefficients (and associated *P*-values) between estimates of population genetic parameters, including measures of genetic diversity, F_{ST} and genetic distance, derived from AFLP and microsatellite markers. Diversity measures for AFLP markers were percent loci that were polymorphic (% polymorphic) and Lynch and Milligan's estimator of average heterozygosity ($H_{L\&M}$), and for microsatellite markers were Nei's (1987) expected heterozygosity (H_E) and proportion of alleles present in the population (prop alleles). Genetic distances were Nei's (1978) distance ($D_{NEI'78}$) and Cavalli-Sforza–Edwards' chord distance (D_{CSE}) for AFLP and microsatellite markers, respectively

Marker		Correlation	
AFLP	Microsatellite	coefficient	*P*-value
Genetic diversity			
% polymorphic	H_E	0.86	< 0.001
% polymorphic	Prop alleles	0.92	< 0.001
$H_{L\&M}$	H_E	0.48	0.105
$H_{L\&M}$	Prop alleles	0.66	0.017
F_{ST}			
Theta	Theta	0.54	< 0.001
Genetic distance			
$D_{NEI'78}$	D_{CSE}	0.53	< 0.001

Genetic bottlenecks

Bear-up is the only population for which test results indicated evidence of recent bottlenecks. For Bear-up $M = 0.625$, whereas M was greater than 0.680 for all other populations. Allele frequency distributions in most populations were J-shaped, except for in Bear-up (A. Whitehead, unpubl. data). There were 72–83% fewer low-frequency alleles in Bear-up compared to other population distributions, again indicating a recent bottleneck in that population.

Correlation between markers

A high degree of correlation was detected between population genetic parameters estimated by AFLP and microsatellite markers (Table 5). Spearman's rank order correlations

were significant for three of the four comparisons of genetic diversity measures between markers. Correlations between AFLP and microsatellite-derived pairwise θ and genetic distances were also highly significant ($P < 0.001$).

Discussion

Genetic structure was detected among all population pairs, and at the river and watershed scales of geographical hierarchy. Biogeographical structuring of genetic variation effectively described both AFLP and microsatellite data sets. However, no signal of contaminant-induced genetic change, which would result in genetic patterns contrary to biogeographical expectations, was detected. Data derived from AFLP and microsatellite markers were highly correlated for estimates of population genetic parameters, as well as for relationships among populations.

Biogeographical patterns

Genetic patterns among populations, from both AFLP and microsatellite data sets, supported predictions derived from biogeographical hypotheses. Both markers detected fine-scale structure among interconnected populations of *C. occidentalis* across rivers of the Central Valley watershed. Although differences between populations were statistically significant, one must be careful to not assume automatically that statistical significance of population structure always indicates biological significance (Waples 1998; Hedrick 1999). In the current study, however, the null hypothesis of panmixis was rejected, and biogeographical factors appeared to be of sufficient influence to explain patterns of variation among groups, despite low F_{ST} values. Downstream populations were more closely related and had higher genetic diversity than upstream populations, and the downstream counterpart was most similar to each upstream population. These results imply that directional gene flow from upper to lower watershed was important in structuring variation among populations. Data for both markers also indicated structure at the scale of rivers and watersheds, as revealed by both AMOVA and private bands/alleles. Although a relationship between genetic and geographical distance was not detected across population pairs throughout the Central Valley, relationships among local populations fit an isolation-by-distance model. Correspondingly, Slatkin (1993) examined several models and found that, for groups with high gene flow, only nearby groups will exhibit isolation-by-distance. Other than for migratory salmonids, the genetic structure of native species from large western watersheds has not been well characterized. To our knowledge, this is the first study to demonstrate conspecific biogeographical structuring of genetic variation in resident native fish from a large interconnected western watershed.

Contaminant-induced patterns

Multigenerational contaminant stress appeared to be of insufficient force to influence genetic divergence against the background of biogeography and low F_{ST} values. Patterns of genetic variation among populations did not support predictions derived from contaminant-induced genetic change hypotheses. Band/allele frequency differences among populations provided no evidence of pesticide-induced selection or increased mutation rates. There was no significant partitioning of genetic variation between exposed and reference populations. In addition, exposed populations were not genetically distinct from reference groups, and there was no evidence of recent bottlenecks in exposed populations.

There may be two broad reasons why our data set failed to support contaminant-related predictions. First, contaminant stress may not have induced genetic change in exposed populations. Insufficient time may have passed since the onset of contaminant stress, or perhaps the intensity of contaminant stress was insufficient to induce a population genetic response detectable above the background biogeographical signal. Whereas *C. occidentalis* is relatively long-lived (spawning usually occurs in the fourth year of life; Moyle 2002), organisms with shorter generation times may have responded within the given time frame.

Although relatively little is known about *C. occidentalis* life history, fishes native to the Sacramento and San Joaquin river basins are generally considered to have good dispersal ability (Moyle 2002). Gene flow among exposed and reference populations may have been high enough to dilute habitat-induced changes in allele frequencies, mutation rates or changes in effective population size (see Slatkin 1987 for review). If populations are connected sufficiently, local stress would have to be intense enough to overcome the diluting influence of migration between exposed and reference populations. If populations are subdivided sufficiently, local stress would have to be intense enough to offset the effects of random genetic drift. Recent studies hypothesizing habitat-induced selection (Colson 2002) and increased mutation rates (Dahl *et al*. 2001; Theodorakis *et al*. 2001) reported that gene flow was probably sufficient to offset the influence of local stressors. Finally, disturbed habitats, such as agriculturally developed areas, may act as sinks for migrants from more stable environments, thereby contributing to erosion of a contaminant-induced population genetic signal. Theodorakis *et al*. (2001) found that the predominant direction of kangaroo rat migration was into radiation-contaminated sites from clean sites, supporting the hypothesis that immigration masked contaminant effects on long-term residents.

The second broad reason for no contaminant-induced signal is that contaminant stress may have induced genetic change in exposed populations, but our approach failed to detect it. For example, several molecular markers showed

little differentiation of Norwegian Scots pine populations (Karhu *et al*. 1996). However, local selective forces were of sufficient intensity to overcome the homogenizing effect of high gene flow. This drove strong local differentiation in presumably few adaptively relevant loci controlling date of bud set. In the current study, AFLP markers were used because they have the power to sample a large portion of the genome reliably, relative to other markers. This increases their potential for detecting signatures of selection at adaptive (or linked) loci. However, genetic change associated with adaptation to contaminants may often involve one or a few major gene changes (Macnair 1991), and finite sampling of the genome may fail to detect such changes. In addition, changes in environmental conditions may not always drive genetic adaptation, as populations may adapt through phenotypic plasticity (Thompson 1991). Microsatellite markers were selected because they have proven variable enough to detect fine population structure, and due to high mutability, may be more sensitive to environmental mutagens than other markers. Chemical exposures have been shown to induce microsatellite mutation *in vitro* (Jackson *et al*. 1998; Zienolddiny *et al*. 2000; Lopez *et al*. 2002). In field studies, microsatellites have been used to detect increased mutation rates in barn swallow family groups exposed to environmental mutagens (Ellegren *et al*. 1997). However, the application of these markers to anonymous individuals in a population may have low power for detecting increased mutation rates.

Study design considerations

It is important to analyse contaminant-induced population genetic change within a phylogenetic or biogeographical context (Staton *et al*. 2001) in order to avoid spurious correlations. Regrettably, few of the studies reviewed by Belfiore & Anderson (2001) that reported contaminant-induced population genetic change explored alternate phylogenetic or biogeographical explanations for genetic patterns. Such limited analyses risk supporting contaminant-induced expectations with spurious correlations. Notable are a few study designs that followed several lines of evidence to support contaminant-related hypotheses. For example, Theodorakis & Shugart (1997) examined radionuclide contamination-induced effects on gene pools of isolated mosquitofish populations. Fish transplanted from a clean site to an isolated radionuclide contaminated site (approximately 18 years prior to the study) were more similar genetically to fish from a second isolated contaminated site than to fish from the parental (clean) population, suggesting selective convergence. Genetic diversity also differed between populations from exposed sites and reference sites. Furthermore, several random amplified polymorphic DNA marker (RAPD) bands correlated with higher fecundity (Theodorakis & Shugart 1997), higher survivorship (Theodorakis *et al*. 1999), lower genotoxic responses in laboratory exposures (Theodorakis & Shugart 1998) and occurred at higher frequency in populations from contaminated sites compared to populations from reference sites (Theodorakis & Shugart 1997). A correlation between frequency of these markers and selection coefficient in contaminated sites was also observed. In another study, increased minisatellite mutation was detected in herring gulls from heavily industrialized nesting sites in the Great Lakes (Yauk & Quinn 1996), and mutation rates were correlated negatively with distance of nesting site to steel mills and urbanization (Yauk *et al*. 2000). The authors found that population substructure could not account for differences in mutation rates among sampled nesting colonies (Yauk & Quinn 1999). Robust field sampling designs, coupled with testing of alternate hypotheses, are necessary study components in order to reduce false correlations and inappropriate conclusions (Belfiore & Anderson 1998). In the current study, a thorough and replicated field sampling design, appropriate for testing alternate hypotheses, was critical for establishing the primacy of biogeography over current and historical contaminant stress.

Molecular markers

Examining genetic differences among closely related and geographically proximate groups of organisms with high levels of genetic exchange presents methodological and statistical challenges (Waples 1998). In the current study, two state-of-the-art molecular markers were used which differ in their strengths and limitations. We compared the relative consistency of applying many bi-allelic dominant markers (347 AFLP loci) to few multiallelic codominant markers (seven microsatellite loci) for detecting fine-scale structure among many interconnected populations. Our results indicated a high degree of concordance between marker systems for detecting structure and estimating population parameters in Central Valley *C. occidentalis*. In congruence with our results, Mariette *et al*. (2001) reached similar conclusions regarding genetic differentiation between *Pinus pinaster* populations derived with AFLP and microsatellite analyses. Furthermore, a high degree of concordance was detected between AFLP and microsatellite-derived dendrograms and principle coordinate plots of relationships among coconut populations collected from across their geographical range (Teulat *et al*. 2000). In the current study, genetic vs. geographical distance relationships, and genetic structure between watersheds, were not demonstrated as clearly with microsatellites as with AFLPs. Our 260 polymorphic bi-allelic AFLP loci may have had greater statistical power than the seven multiallelic microsatellite loci for detecting such relationships.

Using computer simulations of different evolutionary scenarios, Mariette *et al*. (2002) examined correlation

between AFLP or microsatellite estimates of gene diversity and the true whole genome diversity. In some scenarios sampling of more loci is necessary, for which AFLPs may be more efficient. Given five microsatellite loci compared to 200 AFLP loci (conditions most similar to the current study), correlations for the AFLP marker system were as strong, or stronger, than microsatellite-derived estimates. The authors illustrated that to achieve similar predictive accuracy, at least four times more dominant markers are to be used than codominant markers, and in cases of high gene flow at least 10 times. Our use of nearly 40 times more polymorphic AFLP markers than microsatellite markers falls well within this range of comparable predictive capacity.

In addition to the importance of selecting appropriate markers, statistical estimators of population genetic parameters vary in their suitability for comparing closely or distantly related taxa. For example, simulations by Chakraborty & Leimar (1987) indicated that, when gene flow is high, Weir & Cockerham's θ performs better than other F_{ST} estimators. Goldstein & Pollock (1997) reviewed the performance of microsatellite genetic distance estimators for phylogenetic reconstruction. They concluded that measures not based on any mutation model, and which detect the degree of overlap between allele frequency distributions (such as D_{CSE}), perform better for closely related taxa than measures based on the stepwise mutation model [such as $(\delta\mu)^2$]. Indeed, in the current study, population relationships derived from $(\delta\mu)^2$ had little correspondence to biogeographical relationships that were reflected by AFLP data and microsatellite data based on D_{CSE}. One reason for this discrepancy may be that the microsatellite loci applied did not evolve according to the stepwise mutation model. Large gaps between allelic size classes in some loci (A. Whitehead, unpubl. data) tend to support this explanation. Others have found that D_{CSE} performed better than $(\delta\mu)^2$ for reflecting relationships among geographically proximate populations, for example in brook charr (*Salvelinus fontinalis*) (Angers & Bernatchez 1998) and European catfish (*Silurus glanis*) (Triantafyllidis *et al*. 2002). However, Angers & Bernatchez (1998) illustrated the potential power of combining the two types of genetic distances, using measures such as D_{CSE} to construct tree topologies and measures such as $(\delta\mu)^2$ to adjust branch lengths.

Depending on the nature of the question, different classes of markers vary in their inherent value for evaluating the impact of anthropogenic stressors on populations. For example, signals of selection may be detected most effectively by examining a large number of markers distributed throughout the genome. Vigouroux *et al*. (2002) detected selective sweeps in maize using microsatellite markers. However, 501 loci were screened, of which 10 showed evidence for selection. This is a far greater number than is feasible for most studies. In contrast, multilocus dominant markers may hold more promise for detecting the signatures of selection because they can be used to screen efficiently a large portion of the genome. For example, AFLP band frequencies have correlated with salt tolerance in barley (Pakniyat *et al*. 1997) and cold tolerance in ryegrass (Skot *et al*. 2002). Alternatively, Staton *et al*. (2001) outlined the merits of including focused single-locus approaches, with loci of known functional links to adaptation, for detecting selection. Enhanced mutation may be detected most sensitively by highly mutable loci such as microsatellites and minisatellites. Enhanced mutation rates have been detected in organisms from habitats contaminated with radiation (Dubrova *et al*. 1996; Ellegren *et al*. 1997) and industrial chemicals (Yauk & Quinn 1996; Yauk *et al*. 2000). Microsatellites are probably the most powerful markers for detecting evidence of recent bottlenecks according to their unique physical characteristics among markers (Cornuet & Luikart 1996; Luikart *et al*. 1998; Garza & Williamson 2001; Whitehouse & Harley 2001). Co-dominant markers may prove more reliable than dominant markers for estimating demographic parameters based on allele frequencies (Wong *et al*. 2001), and may be included to test alternate contaminant-independent mechanisms of genetic partitioning, such as inbreeding. Combined, large numbers of dominant markers and few highly variable codominant markers may be the most sophisticated approach for examining the influence of various mechanisms driving genetic partitioning among populations and species.

Conclusions

The emergence of population-level effects from long-term contaminant exposure may depend on factors not typically considered by ecotoxicologists. Interconnected populations may be at less risk from negative effects than more isolated populations, and gene flow to or from impacted habitats may dilute local effects. Evolutionary or biogeographical history could influence how contemporary populations respond to stressors. In the current study, two marker systems indicated concordant structure among geographically proximate fish populations. Biogeographical history imprinted a signal clearly detectable by a robust field sampling design, and was primarily responsible for patterns of variation among Central Valley *C. occidentalis*. Examination of population genetic pattern differences with reference only to contaminant-related hypotheses can lead to flawed inferences and erroneous results.

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References

Agresti JJ, Seki S, Cnaani A *et al.* (2000) Breeding new strains of tilapia: development of an artificial center of origin and linkage map based on AFLP and microsatellite loci. *Aquaculture*, **185**, 43–56.

Anderson S, Sadinski W, Shugart L *et al.* (1994) Genetic and molecular ecotoxicology: a research framework. Napa Conference on Genetic and Molecular Ecotoxicology, October 12–15, 1993; Yountville, CA, USA. *Environmental Health Perspectives*, **102**, 3–8.

Angers B, Bernatchez L (1998) Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution*, **15**, 143–159.

Avise JC (1994) *Molecular Markers, Natural History and Evolution*. Chapman & Hall, New York.

Bagley MJ, Anderson SL, May B (2001) Choice of methodology for assessing genetic impacts of environmental stressors: polymorphism and reproducibility of RAPD and AFLP fingerprints. *Ecotoxicology*, **10**, 239–244.

Belfiore NM, Anderson SL (1998) Genetic patterns as a tool for monitoring and assessment of environmental impacts: the example of genetic ecotoxicology. *Environmental Monitoring and Assessment*, **51**, 465–479.

Belfiore NM, Anderson SL (2001) Effects of contaminants on genetic patterns in aquatic organisms: a review. *Mutation Research*, **489**, 97–122.

Belkhir K (2000) GENETIX 4.02, Windows™ program for population genetics, 4.02. http://www.University-montp2.fr/~genetix/genetix.htm.

Bickham JW, Smolen MJ (1994) Somatic and heritable effects of environmental genotoxins and the emergence of evolutionary toxicology. *Environmental Health Perspectives*, **102**, 25–28.

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology*, **5**, 453–455.

California Department of Pesticide Regulation (2001) Summary of pesticide use report data 2000. California Department of Pesticide Regulation, Sacramento, CA.

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution*, **32**, 550–570.

Chakraborty R, Leimar O (1987) Genetic variation within a subdivided population. In: *Population Genetics and Fishery Management* (eds Ryman N, Utter F), pp. 89–120. University of Washington Press, Seattle.

Colson I (2002) Selection and gene flow between microenvironments: the case of *Drosophila* at Lower Nahal Oren, Mount Carmel, Israel. *Molecular Ecology*, **11**, 1311–1316.

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Dahl CR, Bickham JW, Wickliffe JK, Custer TW (2001) Cytochrome b sequences in black-crowned night-herons (*Nycticorax nycticorax*) from heronries exposed to genotoxic contaminants. *Ecotoxicology*, **10**, 291–297.

Domagalski JL (1997) Pesticides in surface and ground water of the San Joaquin–Tulare basins, California: analysis of available data, 1966 through 1992. United States Geological Survey water-supply paper 2468. United States Geological Survey, Sacramento, CA.

Domagalski JL, Dubrovsky NM, Kratzer CR (1997) Pesticides in the San Joaquin River, California: inputs from the dormant sprayed orchards. *Journal of Environmental Quality*, **26**, 454–465.

Domagalski JL, Kratzer CR, Brown LR, Gronberg JM, Burow KR (1998) Water quality in the San Joaquin-Tulare Basins, California, 1992–95. United States Geological Survey, Circular 1159. United States Geological Survey, Sacramento, CA.

Dubrova YE, Nesterov VN, Krouchinsky NG *et al.* (1996) Human minisatellite mutation rate after the Chernobyl accident. *Nature (London)*, **380**, 683–686.

Ellegren H, Lindgren G, Primmer CR, Moller AP (1997) Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature (London)*, **389**, 593–596.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Felsenstein J (1995) PHYLIP *(Phylogeny Inference Package), 3.57c*. Department of Genetics, SK-50. University of Washington, Seattle, WA.

Fox GA (1995) Tinkering with the tinkerer: pollution versus evolution. *Environmental Health Perspectives*, **103**, 93–100.

Garza JC, Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology*, **10**, 305–318.

Gaudeul M, Taberlet P, Till-Bottraud I (2000) Genetic diversity in an endangered alpine plant, *Eryngium alpinum* L. (Apiaceae), inferred from amplified fragment length polymorphism markers. *Molecular Ecology*, **9**, 1625–1637.

Goldstein DB, Pollock DD (1997) Launching microsatellites: a review of mutation processes and methods of phylogenetic inference. *Journal of Heredity*, **88**, 335–342.

Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, Feldman MW (1995) Genetic absolute dating based on microsatellites and the origin of modern humans. *Proceedings of the National Academy of Sciences USA*, **92**, 6723–6727.

Goodman SJ (1997) R-ST Calc: a collection of computer programs for calculating estimates of genetic differentiation from microsatellite data and determining their significance. *Molecular Ecology*, **6**, 881–885.

Hansen LJ, Johnson MB (1999) Conservation and toxicology: integrating the disciplines. *Conservation Biology*, **13**, 1225–1227.

Harley EH (2001) AGARST. *A Program for Calculating Allele Frequencies, GST and RST from Microsatellite Data. 2.0*. University of Cape Town, Cape Town.

Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Jackson AL, Chen R, Loeb LA (1998) Induction of microsatellite instability by oxidative DNA damage. *Proceedings of the National Academy of USA*, **95**, 12468–12473.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424–429.

Jones CJ, Edwards KJ, Castaglione S *et al.* (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding*, **3**, 381–390.

Karhu A, Hurme P, Karjalainen M *et al.* (1996) Do molecular markers reflect patterns of differentiation in adaptive traits of conifers? *Theoretical and Applied Genetics*, **93**, 215–221.

Kettlewell HBD (1973) *The Evolution of Melanism*. Clarendon Press, Oxford.

Klerks PL, Weis JS (1987) Genetic adaptation to heavy metals in aquatic organisms: a review. *Environmental Pollution*, **45**, 173–206.

Kratzer CR (1997) Transport of dizainon in the San Joaquin River Basin, California. United States Geological Survey, Open-File Report 97–411. United States Geological Survey, Sacramento, CA.

Kuivila KM, Foe CG (1995) Concentration, transport and biological effects of dormant spray pesticides in the San Francisco Estuary, California. *Environmental Toxicology and Chemistry*, **14**, 1141–1150.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA: *Molecular Evolutionary Genetics Analysis. 2.1*. Pennsylvania State Univeristy, University Park, PA.

Lopez A, Xamena N, Marcos R, Velazquez A (2002) Germ cells microsatellite instability. The effect of different mutagens in a mismatch repair mutant of *Drosophila* (spel1). *Mutation Research*, **514**, 87–94.

Luikart GL, Allendrof FW, Cornuet JM, Sherwin WB (1998) Distortion of allele frequency distributions provides a test for recent population bottlenecks. *Journal of Heredity*, **89**, 238–247.

Lynch M, Milligan BG (1994) Analysis of population genetic structure with RAPD markers. *Molecular Ecology*, **3**, 91–99.

MacCoy D, Crepeau KL, Kuivila KM (1995) Dissolved pesticide data for the San Joaquin River at Vernalis and the Sacramento River at Sacramento, California, 1991–94. United States Geological Survey, Open-File Report 95–110. United States Geological Survey, Sacramento, CA.

Macnair MR (1987) Heavy metal tolerance in plants: a model evolutionary system. *Trends in Ecology and Evolution*, **2**, 354–359.

Macnair MR (1991) Why the evolution of resistance to anthropogenic toxins normally involves major gene changes: the limits to natural selection. *Genetica (Dordrecht)*, **84**, 213–219.

Mariette S, Chagne D, Lezier C *et al.* (2001) Genetic diversity within and among *Pinus pinaster* populations: comparison between AFLP and microsatellite markers. *Heredity*, **86**, 469–479.

Mariette S, Le Corre V, Austerlitz F, Kremer A (2002) Sampling within the genome for measuring within-population diversity: trade-offs between markers. *Molecular Ecology*, **11**, 1145–1156.

McKenzie JA, Batterham P (1994) The genetic, molecular and phenotypic consequences of selection for insecticide resistance. *Trends in Ecology and Evolution*, **9**, 166–169.

Miller MP (1997) *Tools for Population Genetic Analysis (TFPGA), 1.3*. Department of Biological Sciences, Northern Arizona University, Flagstaff.

Moyle PB (2002) *Inland Fishes of California*. University of California Press, Berkeley.

Mueller UG, Wolfenbarger LL (1999) AFLP genotyping and fingerprinting. *Trends in Ecology and Evolution*, **14**, 389–394.

Murdoch MH, Hebert PDN (1994) Mitochondrial DNA diversity of brown bullhead from contaminated and relatively pristine sites in the Great Lakes. *Environmental Toxicology and Chemistry*, **13**, 1281–1289.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Pakniyat H, Powell W, Baird E *et al.* (1997) AFLP variation in wild barley (*Hordeum spontaneum* C. Koch) with reference to salt tolerance and associated ecogeography. *Genome*, **40**, 332–341.

Palumbi SR (2001) Humans as the world's greatest evolutionary force. *Science (Washington DC)*, **293**, 1786–1790.

Pereira WE, Domagalski JL, Hostettler FD, Brown LR, Rapp JB (1996) Occurrence and accumulation of pesticides and organic contaminants in river sediment, water and clam tissues from the San Joaquin River and tributaries, California. *Environmental Toxicology and Chemistry*, **15**, 172–180.

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

Rohlf FJ (2000) *ntsys-pc — Numerical Taxonomy and Multivariate Analysis System, 2.1*. Exeter Software, Setauket, NY.

Ryman N, Jorde PE (2001) Statistical power when testing for genetic differentiation. *Molecular Ecology*, **10**, 2361–2373.

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN: *a software for population genetics data analysis. 2.000*. University of Geneva, Geneva.

Skot L, Sackville Hamilton NR, Mizen S, Chorlton KH, Thomas ID (2002) Molecular genecology of temperature response in *Lolium perenne*: 2. Association of AFLP markers with ecogeography. *Molecular Ecology*, **11**, 1865–1876.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science (Washington DC)*, **236**, 787–792.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Sokal RR, Rohlf FJ (1995) *Biometry: the Principles and Practice of Statistics in Biological Research*. W.H. Freeman, New York.

Staton JL, Schizas NV, Chandler GT, Coull BC, Quattro JM (2001) Ecotoxicology and population genetics: the emergence of 'Phylogeographic and evolutionary ecotoxicology'. *Ecotoxicology*, **10**, 217–222.

Street GT, Lotufo GR, Montagna PA, Fleeger JW (1998) Reduced genetic diversity in a meiobenthic copepod exposed to a xenobiotic. *Journal of Experimental Marine Biology and Ecology*, **222**, 93–111.

Street GT, Montagna PA (1996) Loss of genetic diversity in harpacticoida near offshore platforms. *Marine Biology (Berlin)*, **126**, 271–282.

Teulat B, Aldam C, Trehin R *et al.* (2000) An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tagged microsatellites (SSRs) and AFLPs. *Theoretical and Applied Genetics*, **100**, 764–771.

Theodorakis CW, Bickham JW, Lamb T, Medica PA, Lyne TB (2001) Integration of genotoxicity and population genetic

analyses in kangaroo rats (*Dipodomys merriami*) exposed to radionuclide contamination at the Nevada Test Site, USA. *Environmental Toxicology and Chemistry*, **20**, 317–326.

Theodorakis CW, Elbl T, Shugart LR (1999) Genetic ecotoxicology IV: survival and DNA strand breakage is dependent on genotype in radionuclide-exposed mosquitofish. *Aquatic Toxicology (Amsterdam)*, **45**, 279–291.

Theodorakis CW, Shugart LR (1997) Genetic ecotoxicology II: population genetic structure in mosquitofish exposed *in situ* to radionuclides. *Ecotoxicology*, **6**, 335–354.

Theodorakis CW, Shugart LR (1998) Genetic ecotoxicology III: the relationship between DNA strand breaks and genotype in mosquito fish exposed to radiation. *Ecotoxicology*, **7**, 227–236.

Thompson JD (1991) Phenotypic plasticity as a component of evolutionary change. *Trends in Ecology and Evolution*, **6**, 246–249.

Tranah GJ (2001) Molecular genetic analysis of hybridization and population structure in endangered sturgeon and sucker species. PhD Thesis. University of California, Davis, CA.

Tranah GJ, Agresti JJ, May B (2001) New microsatellite loci for suckers (Catostomidae): primer homology in Catostomus, Chasmistes, and Deltistes. *Molecular Ecology Notes*, **1**, 55–60.

Triantafyllidis A, Krieg F, Cottin C *et al.* (2002) Genetic structure and phylogeography of European catfish (*Silurus glanis*) populations. *Molecular Ecology*, **11**, 1039–1055.

Vigouroux Y, McMullen M, Hittinger CT *et al.* (2002) Identifying genes of agronomic importance in maize by screening microsatellites for evidence of selection during domestication. *Proceedings of the National Academy of Sciences USA*, **99**, 9650–9655.

Vos P, Hogers R, Bleeker M *et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Werner I, Deanovic LA, Connor V *et al.* (2000) Insecticide-caused toxicity to *Ceriodaphnia dubia* (Cladocera) in the Sacramento-San Joaquin River Delta, California, USA. *Environmental Toxicology and Chemistry*, **19**, 215–227.

Werner I, Deanovic LA, Hinton DE *et al.* (2002) Toxicity of stormwater runoff after dormant spray application of diazinon and esfenvalerate (Asana (®)) in a French Prune Orchard, Glenn County, California, USA. *Bulletin of Environmental Contamination and Toxicology*, **68**, 29–36.

Whitehouse AM, Harley EH (2001) Post-bottleneck genetic diversity of elephant populations in South Africa, revealed using microsatellite analysis. *Molecular Ecology*, **10**, 2139–2149.

Wong A, Forbes MR, Smith ML (2001) Characterization of AFLP markers in damselflies: prevalence of codominant markers and implications for population genetic applications. *Genome*, **44**, 677–684.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

Yauk CL, Fox GA, McCarry BE, Quinn JS (2000) Induced minisatellite germline mutations in herring gulls (*Larus argentatus*) living near steel mills. *Mutation Research*, **452**, 211–218.

Yauk CL, Quinn JS (1996) Multilocus DNA fingerprinting reveals high rate of heritable genetic mutation in herring gulls nesting in an industrialized urban site. *Proceedings of the National Academy of Sciences USA*, **93**, 12137–12141.

Yauk CL, Quinn JS (1999) Genetic structure among breeding herring gulls (*Larus argentatus*) from the Great Lakes and Eastern Canada. *Journal of Great Lakes Research*, **25**, 856–864.

Zienolddiny S, Svendsrud DH, Ryberg D, Mikalsen AB, Haugen A (2000) Nickel (II) induces microsatellite mutations in human lung cancer cell lines. *Mutation Research*, **452**, 91–100.

This study forms part of Andrew Whitehead's PhD thesis on the effects of pesticide contamination on native fish populations. His interests include the integration of laboratory and field studies for examining acclimation and adaptation responses of native organisms to stressful environments. Susan Anderson is an ecotoxicologist and her research program focuses on evaluating genotoxic and reproductive effects of contaminant exposure in aquatic organisms. Kathy Kuivila's research studies the fate and effects of pesticides in aquatic ecosystems. Jen Roach is a population geneticist with interests relating to the characterization and conservation of terrestrial and aquatic species. Bernie May's research program applies a variety of molecular techniques (AFLPs, allozymes, microsatellites, introns, etc.) to study genomic variation in natural and aquacultural populations

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