

GENETIC ANALYSIS OF A TIME-SERIES IN ENDANGERED TIDEWATER GOBY:
EXTINCTION-COLONIZATION DYNAMICS OR DRIFT IN ISOLATION

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PROJECT SUMMARY

The classic metapopulation model of population structure implies that local population extinction of isolated habitat patches occurs regularly, dispersal among patches is routine, and that natural colonization of suitable unoccupied or restored habitats may occur within relatively short periods of time. Validation of metapopulation dynamics is important for conservation because it implies application of different management strategies than when fragmented populations remain isolated in the absence of dispersal. Our study focused upon tidewater goby, an endangered species that is managed based upon the supposition that it follows the classic metapopulation model of population structure throughout its geographic range even though ascertainment of this pattern was based upon study of a subset of the species range in southern California. We used genetic analysis of a time series to search for signals of extinction-colonization within isolated populations as indicated by genetic change associated with founder events. Our analysis focused upon northern California populations and consisted of repeat collections from 14 locations representing temporal durations spanning 1 to 23 years (mean 11.4 years) depending upon location. Comparison of repeat collections indicated within-site stability through time within sites across several metrics (allelic richness, expected heterozygosity, allele frequencies, and individual assignments), a result that is inconsistent with the classic metapopulation model. Instead strong between population genetic differentiation and within-site stability supports a model of drift in the absence of dispersal as the best-fit model for northern California populations of tidewater goby. Our findings show that tidewater goby exhibit different population structures across its geographic range (metapopulation vs drift in isolation) and that management strategies for northern California populations of endangered tidewater

goby should not rely on natural colonization of apparently suitable or restored habitats for species recovery.

Introduction

According to the classic definition a metapopulation consists of a set of isolated habitat patches that exist in a balance between local extinction and their recolonization through dispersal from occupied patches (Levins 1969). The primary method for assessing metapopulation dynamics in natural settings has been to conduct repeated field surveys within sampling units through time and record species presence/absence (e.g., Lafferty 1999a; Hanski 1999; Haag *et al.* 2005; Lamy *et al.* 2012). Site occupancy histories are then used to infer rates of extinction and colonization for the study populations. A concern with such approaches is that it is often impossible to distinguish between a true local population extinction and absence due to imperfect detection (MacKenzie *et al.* 2002; MacKenzie *et al.* 2003). Failing to detect a species is a common problem in field surveys, especially when abundance is low, field collections are difficult, or collection effort is limited (Gu & Swihart 2004).

Genetic analyses of repeat collections on a time series provide another tool for assessing metapopulation dynamics (Lamy *et al.* 2012). This approach relies upon site-specific changes in genetic structure across repeat collections as a signal of metapopulation dynamics. Shifts in genetic structure across repeat samples are expected under a metapopulation model due to extinction and subsequent recolonization by a divergent migrant pool and/or founder effects. An advantage of temporal genetic sampling in comparison to repeat field surveys is that genetic approaches allow for identification of extinction and colonization events that may have occurred

in the interval between repeat field surveys (the “rescue effect”; Hanksi 1994) or that may have been overlooked due to incomplete site occupancy histories. Temporal genetic analysis can also be used to differentiate between a true extinction-colonization event and those falsely implied due to non-detection. A problem with the approach is that temporal genetic change may occur as a result of a population bottleneck rather than an extinction-colonization cycle (Lamy *et al.* 2012). However, founder events associated extinction-colonization are expected to result in larger temporal shifts in genetic structure than bottlenecks. When combined temporal genetic analysis and repeat field survey approaches can provide a robust approach for evaluating metapopulation dynamics.

The tidewater goby (*Eucyclogobius newberryi*) is a small fish (maximum total length 60 mm) that is listed as endangered under the US Endangered Species Act. Tidewater goby are restricted to discrete brackish water lagoons and estuarine habitats along the California coast that are separated by long stretches (generally 1-20 km) of inhospitable ocean habitat (Swift *et al.* 1989). The coastal lagoons inhabited by tidewater goby are walled from the Pacific Ocean by sand bars nearly year around. The sand bars periodically breach causing rapid draining of the lagoons (Krauss *et al.* 2002) and subjecting tidewater goby within lagoons to sudden environmental shifts, such as loss of habitat and rapid changes in salinity and temperature. While tidewater goby have been shown to be highly tolerant of environmental variability, breaching events and human induced habitat degradation may occasionally lead to extinction of local populations (Swift *et al.* 1989; Lafferty *et al.* 1999b). Dispersal between lagoons is rare, as it requires simultaneous lagoon breaching and dispersal through ocean corridors. As larval forms are intolerant of sudden salinity increases, dispersal most likely occurs during the adult stage

(Hellmair & Kinziger Unpubl. Data). As an annual species the tidewater goby completes its entire life cycle in less than one year (Hellmair 2011). Abundance of tidewater goby can fluctuate dramatically on an inter-annual basis, and thus population dynamics of the species is more similar to insects than large vertebrates (Lafferty *et al.* 1999a; Kinziger Unpubl. Data). Each population appears to fluctuate independently (Lafferty *et al.* 1999a; Hellmair 2011) and has demography dependent on local factors effecting population growth rather than immigrants (Brown & Ehrlich 1980). For this reason the fundamental unit of conservation for tidewater goby is considered to be at population level rather than the individual.

Site occupancy histories generated from repeated field surveys of southern California populations indicate metapopulation dynamics with high site-specific annual extinction (0.37) and colonization rates (0.48) (Lafferty *et al.* 1999a). Based upon these findings tidewater goby is generally considered to follow a metapopulation dynamic range-wide (U.S. Fish and Wildlife Service 2005). However, metapopulation dynamics may not apply throughout the range of the species because the southern populations for which metapopulation dynamics were ascertained belong to a genetically and morphologically distinct management unit (Dawson *et al.* 2001; Ahnelt *et al.* 2004; Earl *et al.* 2010). Further, populations in southern California experience warmer environmental conditions than populations in the northern extent of the species range.

Investigations of spatial genetic structure indicate that tidewater goby is one of the most genetically subdivided vertebrates along the Pacific Coast of California (Crabtree 1985; Mendonca *et al.* 2001; Dawson *et al.* 2001; McCraney *et al.* 2010; Earl *et al.* 2010). While these patterns may be produced under metapopulation dynamics, a broad range of genetic structures is possible under extinction-colonization dynamics (Whitlock & McCauley 1990; Gilpin 1991;

Hastings & Harrison 1994; Wade & McCauley 1998). Thus, it is uncertain whether the strong genetic structuring of tidewater goby is reflective of metapopulation dynamics or a history of drift in the absence of the homogenizing effects of dispersal (McCrane *et al.* 2010).

The objective of this study was to use temporal genetic approaches to evaluate patterns of local population extinction and colonization for tidewater goby at the northern extent of the species range in California. Within-site temporal durations represented by our collections spanned 1 to 23 years (mean 11.4 years), depending on availability of archived material. We used microsatellite loci as the high allelic richness of this molecular marker makes it a powerful tool for detecting founder events (Luikart *et al.* 1998, 1999; Spencer *et al.* 2000). Genetic revolutions within sights were sought using several metrics, with high weight assigned to shifts in allelic richness and allele frequency because these metrics have been shown to be highly sensitive indicators of founder events (Nei *et al.* 1975; Nakajima *et al.* 1991; Spencer *et al.* 2000). We also included a comprehensive spatial genetic analysis of tidewater goby populations in our study region, as uncovering metapopulation dynamics with repeat genetic analysis requires that study populations be genetically delimited such that founder events associated with extinction-colonization cycles are likely to be detected (Lamy *et al.* 2012). Lastly, patterns resolved from temporal genetic analysis were interpreted within the context of extinction and colonization cycles inferred from occupancy histories derived from repeat field surveys.

Methods

Genetic collections and molecular methods

A total 1660 individuals from 36 spatially and temporally independent collections (mean sample size 46.1, range 17 to 67) were genotyped at 9 microsatellite loci (Table 1, Fig. 1). The data included 621 individuals from McCraney *et al.* (2010) combined with 1039 new individuals. Temporal data consisted of time series from 10 locations repeatedly sampled from two to four times. Collections dated from 1988, 1990, 1999, 2006, 2010, 2011, and 2013, spanning 1 to 23 years depending upon location. Spatial coverage included 19 geographically isolated sites, each judged to be demographically independent based upon their geographic isolation, including all extant tidewater goby populations in the North Coast Management Unit (Earl *et al.* 2010), except Freshwater Slough in Humboldt Bay. Inclusion of an additional 11 small sample size collections (mean sample size 3.8, range 1 to 14) increased the number of locations with time series data to 14 and spatial coverage to 21 sites. The small sample size collections were only used for the individual assignment based tests.

Tidewater goby were generally collected by a seine net in near shore waters. Individual fish or fin clips were preserved in 95% ethanol and whole genomic DNA was extracted using the Qiagen DNeasy® Blood and Tissue Kit following the manufacturers recommendations. Tidewater goby were genotyped at nine microsatellite loci (Table S1, Supporting information). Microsatellite primers, thermal cycling conditions, and reaction volumes are described by McCraney *et al.* 2010. Fluorescently labeled polymerase chain reaction products were visualized and genotypes scored using the Beckman–Coulter CEQ 8000 Genetic Analysis System. Allele scores were determined twice and discrepancies were either resolved or no score was assigned. Methods used here were identical to those used by McCraney *et al.* (2010) allowing combining of genotypic data across studies.

Analysis of Genetic Structure

Tests for conformance to Hardy–Weinberg proportions were generated using GENODIVE 2.0b25 (Meirmans & Van Tienderen 2004). Estimates of observed and expected heterozygosity were calculated in GenoDive and standardized private allelic richness and standardized allelic richness, equalized to a sample size of 30 genes using rarefaction were calculated with HP-RARE 1.1 (Kalinowski 2005). Tests of significant differences in genetic diversity were conducted using ANOVA in the software R (R Development Core Team).

Pairwise population differentiation (F_{ST}) and tests of their significance were estimated using GENODIVE. Correction for multiple tests was performed using the modified false discovery rate procedure (Benjamini & Yekutieli 2001), an approach that has greater power to detect significant differences than sequential Bonferroni correction (Rice 1989; Narum 2006). Patterns of spatial and temporal differentiation were visualized by constructing an unrooted neighbor-joining tree using PHYLIP (Phylogeny Inference Package, version 3.68; Felsenstein 1993). Trees were constructed using Cavalli-Sforza genetic distances and branch support was evaluated using 1000 bootstrap replicates.

To determine if our tidewater goby populations, which are distributed linearly along the California coast, conform to isolation by distance (IBD) model of gene flow we examined the relationship between genetic (F_{ST}) and geographic (km) distances. Geographic distances between pairs of populations were calculated from the linear distances between latitude and longitude positions using the software GEOGRAPHIC DISTANCE MATRIX GENERATOR (Ersts Unpubl.). A Mantel test with 1000 random permutations were performed to test the significance of the relationship between genetic and geographic distance using the software

IBDWS (Jensen *et al.* 2005). Only the 19 most contemporary collections from geographically isolated locations ($N > 20$ individuals/site) were used for the IBD analysis.

Bayesian cluster analysis was used to estimate the number of genetically distinct groups in our data using the software STRUCTURE 2.3.4 (Pritchard *et al.* 2000; Falush *et al.* 2003). Analyses were conducted using the full data set assuming the number of distinct populations ranged from $K = 1, \dots, 15$ distinct groups. Analyses were also run for each site alone assuming $K = 1, \dots, N$, where N was equal to the number of repeat samples within a site. The latter analysis was run to seek changes in population assignment affiliation across temporal collections, as would be expected with extinction-colonization. All simulations were run for 20,000 steps (with 10,000 discarded as burn-in) and 20 independent runs were conducted at each value of K . All analyses were run without prior population information using the admixture model, and the correlated allele frequencies model. The log probability of the data ($\ln \Pr(X|K)$) and the ad hoc statistic ΔK were used as indicators of the number of genetically groups in the data. Summaries of STRUCTURE output were generated using STRUCTURE HARVESTER (Earl & vonHoldt 2012) and graphical depictions were rendered with DISTRUCT (Rosenberg 2004). The 11 small sample size collections were included in the STRUCTURE analyses.

Field Surveys

Site occupancy (presence/absence) histories for the 14 sites analyzed using temporal genetic methods were taken from an unpublished occupancy database including 99 locations in northern California (Kinziger unpubl. data). Site occupancy was recorded on an annual time-step and the subset of data selected included the years between out temporal genetic collections. The data was assembled from field surveys, published papers, museum vouchers, and

unpublished reports. Records included those where search effort was judged sufficient to detect tidewater goby, but this did not eliminate the possibility that non-detection issues could have lead to errors regarding assertions of local population extinctions. The field-based site occupancy histories were used to generate naïve estimates of extinction-colonization (ie, without accounting for detection probabilities) and used for comparison to inferences derived from temporal genetic analysis.

Effective Population Size

Single point-in-time estimates of inbreeding effective size (N_{ei}) for all collections were generated using the linkage disequilibrium method with bias correction (Waples 2006; Waples & Do 2010) implemented in the software NEESTIMATOR 2.01 (Do et al. 2014). We excluded allele frequencies lower than 0.02 and used a jackknife procedure to construct 95% confidence intervals. Temporal estimates of variance effective size (N_{ev}) were generated using two approaches. First, we used the Bayesian coalescent model developed by Berthier *et al.* (2002) and implemented in CONE (Anderson 2005). We explored population sizes ranging from 2 to 10000, and report $N_{ev} > 10000$ as infinite. The number of importance-sampling repetitions was set to 100000. Second, N_{ev} was estimated following Jorde and Ryman (2007) using the program NEESTIMATOR. Allele frequencies lower than 0.02 were excluded and 95% confidence intervals were constructed using a jackknife procedure. For temporal estimates, generation time was set to one year, consistent with the annual life cycle of tidewater goby (Hellmair 2011), and estimates were generated for all possible pairwise combinations of years within sites.

Results

All loci were polymorphic ranging from 3 to 33 alleles with an average of 10.6 alleles per locus. Of the 15318 genotypes (1702 individuals at nine loci) only 184 genotypes were missing (Table S1, Supporting information). Of the 324 tests for conformance to Hardy–Weinberg proportions (36 populations at nine loci), none were significant following Bonferroni correction for multiple tests (critical value = 0.000154).

Genetic diversity measures were highly variable among collections for expected heterozygosity (mean 0.33, standard deviation 0.15, range 0.08 to 0.57), standardized allelic richness (mean 2.85, standard deviation 1.09, range 1.37 to 4.92), and standardized private allelic richness (mean 0.04, standard deviation 0.05, range 0.00 to 0.22) (Table 1). Distilling the dataset to include the 10 sites with multiple years of observation (an average of 2.6 years of data per site = 26 total observations) and comparison with a one-way ANOVA (sites) showed that variation among sites explained 98.5% of the total variation in both expected heterozygosity and standardized allelic richness and variation among years within sites only explained 1.5%.

Pairwise genetic differentiation (F_{ST}) among spatially isolated sites was high (mean 0.411, standard deviation 0.176, range -0.002 to 0.779,) and significant in all cases except for comparisons between two geographically proximate population pairs (less than 9 km apart) (BIG and STN; SAL and WHS; $P < 0.00711$; Table S2, Supporting Information). In contrast, temporal genetic differentiation within sites was non-significant at seven sites, significant but low at two sites (MCD and VRG; range 0.01091 to 0.07386), and significant and moderate at one site (ELK, $F_{ST} = 0.15022$)($P < 0.00711$).

The relationship between genetic and geographic distance was significant ($P < 0.0010$), but geographic distance only explained about 22 percent of the variation in genetic

differentiation between populations and there was a large amount of variance in genetic differentiation at all geographic distances (Fig. 2). The intercept of the relationship was well above zero (0.2186), which is inconsistent with the classic definition of an IBD model of gene flow (Hutchison & Templeton 1999).

Relationships depicted by the neighbor-joining tree were consistent with three geographically defined groups (north to south): (1) Northern group, including populations north of Humboldt Bay, (2) populations within Humboldt Bay and the Eel River estuary, and (3) Southern group, including populations south of the Lost Coast area in Mendocino County (Fig. 3). Despite geographic intermediacy, the Humboldt Bay group was separated by a long branch from the other two primary groups. Well-supported structure (bootstrap values > 80) was evident among populations within the northern and southern groups but branch lengths among the Humboldt Bay/Eel River estuary populations were relatively short and lacked bootstrap support. Temporal collections from a given sites were more closely related to one another than they were to any other site in all cases except for the comparison between BIG and STN. The latter result was the result of non-significant spatial differentiation between these sites.

In the Bayesian Cluster analysis of the complete data set the ad hoc statistic ΔK indicated the strongest level of structure at $K=2$ clusters, the log probability of the data arrived at a plateau at about $K=9$ clusters, but inspection of the distribution of individual assignments indicated biologically meaningful clustering at $K=11$ genetically distinct groups (Fig. S1, Supporting information). To improve power for identifying turn-over within sites, the Bayesian cluster analysis was conducted for each site alone. For 13 of the 14 sites with repeat collections the highest log probability of the data was at $K=1$ and visual inspection of $K>1$ revealed assignments

were generally symmetric to all populations indicative of the absence of population turn-over through time. The exception was ELK, where the ad hoc statistic ΔK and the log probability of the data indicated two clusters, and inspection of the distribution of individual assignments indicated samples collected in 2006 assigned to a different cluster than samples collected in 2011.

Repeat Field Surveys

Naïve inspection of occupancy histories for the 14 sites included in the temporal genetic analysis indicated four total extinction-colonization cycles, including one turn-over cycle each at TS and TEN, and two cycles of turn-over at MCD (Table 2). No extinction-colonization cycles were evident at the remaining 11 sites, but annual survey data were missing in many years opening the possibility that extinction-colonization events may have occurred but were undetected.

Effective Population Size

Although estimates of N_{ei} and N_{ev} were highly variable across collections, two consistent patterns emerged that were independent of the estimator used (Tables 1 and 3). First, both N_{ei} and N_{ev} fluctuated dramatically through time within sites consistent with the annual life history of tidewater goby and the large inter-annual variation in abundance observed in field surveys (Kinziger unpubl.). Second, estimates for N_{ei} and N_{ev} for populations from Humboldt Bay/Eel River estuary were often much less 50, whereas populations from outside of the zone generally had much higher effective sizes, suggesting that the former populations are vulnerable to extinction under the 50/500 rule (Franklin 1980; but see Jamieson & Allendorf 2012; Frankham *et al.* 2013).

Discussion

We used a temporal genetic sampling scheme aimed at detecting within population genetic revolutions associated with extinction and colonization events. Contrary to expectations under a metapopulation model we resolved a general pattern of genetic stability across repeat collections for northern California populations of endangered tidewater goby, even in metrics that are highly sensitive to founder events such as allele frequencies and allelic richness (Spencer *et al.* 2000). None of the temporal collections exhibited significant reductions in genetic diversity or changes in allele frequency as would be expected if one of these sites experienced extinction followed by colonization by a small number of founders. Individual assignment tests, which allowed analysis of small sample size collections, also revealed within site stability in genetic structure. The temporal duration represented by our sampling (5-20 years) was sufficiently long that that we would have had a high probability of detecting extinction-colonization events if they were occurring routinely, and especially if turn-over was occurring at the rates previously reported for southern California tidewater goby populations (Lafferty *et al.* 1999a; Appendix S1, Supporting Information).

Founder events from metapopulation dynamics are expected to produce larger temporal genetic change than population bottlenecks. The extent of temporal genetic change expected due extinction-colonization dynamics is a property of the extent to which focal populations exhibit fine-scale structuring in the native range (Lamy *et al.* 2012), the effective size of the founder group (Nei *et al.* 1975) and whether founders originate from a single or multiple sources (Slatkin 1977; Wade & McCauley 1988; Whitlock & McCauley 1990). The high degree of spatial

variation in allele frequencies, allelic richness, and heterozygosity among isolated populations of tidewater goby in our study region, combined with the potential for founder effects, suggests that within-site genetic change resulting from extinction-colonization cycles should have been detectable were they occurring. The small yet significant temporal change in allele frequency differentiation observed at two sites (MCD and VRG; range 0.01091 to 0.07386), was much less than overall pairwise differentiation between spatially isolated sites (mean 0.411). For this reason and because these sites exhibited temporal stability in allelic richness and assigned to a single group in Bayesian cluster analysis, we interpret small change in allele frequency as resulting from drift in isolation rather than extinction-colonization dynamics.

An exception was the temporal genetic pattern resolved in the ELK, which exhibited moderate genetic differentiation across temporal collections ($F_{ST} = 0.15022$) and assigned to two distinct groups in Bayesian Cluster Analysis (2006 vs 2011) but no evidence of change in allelic richness and clustering of temporal collections in tree-based analysis. The field-based detection history for this location indicated continuous site occupancy during the study period, a pattern that is inconsistent with extinction-colonization dynamics. Available evidence suggests that these patterns have likely resulted from introduction of an outside tidewater goby population. The event likely occurred concurrently with the introduction of Sacramento pikeminnow (*Ptychocheilus grandis*) to the same location in 2008 (Kinziger *et al.* 2013). The time of the introduction is intermediate between our temporal genetic collections (2006 and 2011), and our ELK collection clustered with a lower Eel River population (SLT) in the tree-based analysis, which was same source location identified for introduced Sacramento pikeminnow (Kinziger *et al.* 2013).

Comparison to Field Surveys

Naïve inspection of site occupancy histories derived from field surveys suggested four extinction-colonization cycles (two cycles at MCD, and one cycle each at TS and TEN; Table 2). Our repeat genetic sampling covered four of these putative cycles and in each case temporal genetic stability was resolved across multiple metrics at each site. Thus temporal genetic analysis did not support the hypothesized extinction-colonization implied by repeat field survey data in all cases that we could test. Instead, these results imply that field survey approaches failed to detect tidewater goby when it was actually present. Non-detection is likely a result of the large interannual fluctuations in abundance and the large habitat areas sometimes occupied by tidewater goby (see also Tanadini & Schmidt 2011; McCarthy *et al.* 2013). These findings emphasize the importance of accounting for non-detection issues when using repeat field surveys to make inference regarding metapopulation dynamics for tidewater goby.

Site occupancy histories for our 14 sites were generated post-hoc and consequently annual survey data were missing in many years opening the possibility that extinction-colonization events may have been overlooked. However, our temporal genetic analysis did not identify any extinction-colonization events that were undetected by repeat field surveys due the rescue effect or due incomplete records, despite coverage of a 20 year temporal duration at several locations in our repeat genetic analysis. These findings illustrate the complementary nature of genetic and field-based methods for discerning metapopulation dynamics, or signaling the absence thereof, especially when occupancy histories from revisitation field surveys are incomplete but historical genetic collections covering longer time-scales are available.

Drift in Isolation

Our comprehensive spatial collections of northern California tidewater goby indicate high among population variability in genetic diversity and strong differentiation between populations (Table 1; Fig. 1). The extent of differentiation was independent geographic distance separating populations including a high level of differentiation between populations separated by just a few kilometers (Fig. 2). The temporal genetic stability resolved within sites indicates that this pattern is not due to extinction-colonization dynamics, but instead is a result of drift within isolated populations combined with the absence of dispersal between these sites, at timescales of at least 20 years. Thus, despite being distributed in linear fashion along the California coast, our analysis does not support routine migration between adjacent populations as would be expected in a one-dimensional stepping-stone model of gene flow (Kimura & Weiss 1964). The exception to the overall pattern was two instances of non-significant genetic differentiation between geographically proximate populations (BIG vs. STN and SAL vs. WHS), which may be the result of gene flow between geographic proximate population or large effective population sizes and the absence of drift.

While contemporary patterns suggest the absence of dispersal, the tree-based analysis recovered three geographically defined groups (northern, Humboldt Bay/Eel River estuary, and southern) reflective of historical connectivity. The groups identified in the tree are separated by the presence of large tracts of coastline where tidewater goby appear to be naturally absent, including the Lost Coast, a 160 km roadless stretch of coastline characterized by steep topography separating the southern group and the Humboldt Bay/Eel River estuary. The northern group and the Humboldt Bay/Eel River estuary are separated by an 50 km of coastline that is uninhabited by tidewater goby. The close relationships among populations in each of these

regions are suggestive of connectivity within but reduced gene flow between these regions. Thus, the presence of intermediate step-stone populations that may restore connectivity is an conservation consideration for tidewater goby.

Conservation Implications

The current management paradigm for tidewater goby is based upon the supposition that it follows the classic metapopulation model of population structure throughout its entire geographic range (U.S. Fish and Wildlife Service 2005), even though ascertainment of this pattern was based upon study of a subset of the species range in southern California (which are also genetically and morphologically distinct from northern California populations; Lafferty *et al.* 1999a; Dawson *et al.* 2001; Ahnelt *et al.* 2004; Earl *et al.* 2010). Our findings show that tidewater goby likely exhibit different population structures across its geographic range, with southern areas exhibiting metapopulation dynamics (Lafferty *et al.* 1999a) and populations from the northern extent of the species range exhibiting drift in the absence of migration. For management these findings indicate that local population extinctions can be viewed as routine component of the species' life history in southern areas whereas in the northern extent of the species range local population extinctions should be considered more permanent due to absence of dispersal at short time-scales.

The combined signal of our spatial-temporal genetic analysis revealed strong between population genetic differentiation and high within-site temporal stability consistent with a best-fit model involving drift combined with the near absence of dispersal between populations. Thus, our findings suggest that natural dispersal may be too infrequent to restore genetic variation within populations or repopulate suitable habitats in northern California, especially at timescales

that are relevant for conservation. Tidewater goby recovery, which is measured by the number of occupied sites, should not rely upon natural dispersal for recovery and should consider artificial translocation as important recovery strategy in more northern areas. The source populations should be carefully reviewed, as large effective sizes combined with the absence of gene flow, suggests potential for local adaptation within isolated populations of tidewater goby in this region.

An area of special concern for tidewater goby conservation includes populations originating from Humboldt Bay/Eel River estuary. Populations in this area exhibited a consistent and significant reduction in genetic diversity (in both allelic richness and heterozygosity) and lower effective sizes in comparison to populations from outside this area (Tables 1 and 3; McCraney *et al.* 2010). Populations within the Humboldt Bay/Eel River estuary generally share a common set of alleles, albeit at different frequencies, as indicated by the close relationship among the populations from this region in the neighbor-joining tree and significant differentiation (F_{ST}) in pairwise comparisons (Fig. 3; Table S2, Supporting information). This pattern is likely a consequence of a historic demographic bottleneck followed by habitat fragmentation caused wetland reclamation projects that were used for agricultural development in this region (McCraney *et al.* 2010). Tidewater goby from Humboldt Bay/Eel River estuary also show reduced life history variation, including spawning during a single time period of short duration unlike populations from outside this area that spawn year around (Goldberg 1977; Swift *et al.* 1989; Swenson 1999; Hellmair 2011). The loss of genetic and life history diversity and the apparent extinction recovered in the occupancy histories for several populations in Humboldt Bay/Eel River estuary (Kinziger Unpubl.) signal that tidewater goby populations in this region

are high risk (Hellmair 2011; see also Newman & Pilson 1997; Keller 1998; Saccheri *et al.* 1998).

Use of human-mediated genetic rescue should be considered as management tool for restoring genetic and life history diversity within tidewater goby populations in Humboldt Bay/Eel River estuary owing to the low frequency of natural dispersal and presence of artificial barriers (e.g. tidegates) that restrict natural dispersal (see also McCraney *et al.* 2010). Genetic rescue has been used to successfully introduce genetic variation and increase fitness in small, low genetic diversity populations that are at risk of extinction (Tallmon *et al.* 2004; Hogg *et al.* 2006; Hedrick & Fredrickson 2010). Given its high level of genetic diversity, life history variation (Hellmair 2011), and geographic proximity, Big Lagoon appears to be an appropriate source location for such efforts.

Conclusion

Our analysis highlights the utility of using historical genetic collections archived in natural history museums to make inferences into metapopulation dynamics (see also Wandeler *et al.* 2007; Nielsen & Hansen 2008; Nachman 2013). To maximize power for detecting extinction-colonization dynamics genetic time series should include repeat collections spanning long temporal durations from as many independent sites as possible. Temporal genetic approaches are invaluable when site occupancy histories from field studies are lacking or incomplete, which is likely the case for many species. Temporal genetic analysis is an important tool for determining just how common classical metapopulation dynamics are in natural systems, as accumulating evidence indicate metapopulation structure is rare in invertebrates (Driscoll 2008; Driscoll *et al.* 2010; Lamy *et al.* 2012; but see also Fountain *et al.* 2014) and there are

relatively few well-established cases from other taxa (Hanski 1999; Lafferty 1999a; Haag *et al.* 2005).

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Figures

Figure 1. Distribution of sampling sites for tidewater goby in northern California, USA. Sites with repeat collections for temporal genetic analysis indicated by circles and additional collections used for spatial genetic analysis indicated by triangles.

Figure 2. Relationship between pairwise genetic differentiation (F_{ST}) and linear geographic distances (km) for 19 spatially isolated populations of northern California tidewater goby.

Figure 3. Unrooted neighbor-joining tree generated using PHYLIP. Branch lengths are equivalent to Cavalli-Sforza genetic distance. Bootstrap values are along branches. Site abbreviations as in Table 1.

- 1 Table 1. Site, collection year, sample ID, and within population microsatellite DNA genetic diversity [sample size (N), observed
- 2 heterozygosity (H_o), Hardy-Weinberg expected heterozygosity (H_e), allelic richness (A), rarified allelic richness (A_r), rarified number
- 3 of private alleles (A_p), inbreeding effective size (N_{ei}) and 95% confidence interval] for northern California tidewater goby.
- 4 Populations are listed from north to south.

Site	Year	Sample ID	N	H_o	H_e	A	A_r	A_p	N_{ei}	95% Confidence Interval	Latitude	Longitude
Tillas Slough	99	TS99	48	0.25	0.27	2.78	2.28	0	∞	∞ - ∞	41.93358889	124.1908611
Tillas Slough	11	TS11	59	0.28	0.26	3.22	2.39	0	∞	38- ∞	41.93358889	124.1908611
Lake Earl	99	ERL99	47	0.25	0.24	4.22	2.88	0.11	∞	120- ∞	41.8418	124.207
Lake Earl	06	ERL06	49	0.27	0.28	5.00	3.22	0.03	55	20- ∞	41.8418	124.207
Lake Earl	10	ERL10	1								41.8418	124.207
Lake Earl	11	ERL11	54	0.25	0.28	3.89	2.9	0.03	83	14- ∞	41.8418	124.207
Stone Lagoon	90	STN90	19	0.57	0.55	4.89	4.61	0.05	70	26- ∞	41.23327778	124.0837306

Stone Lagoon	06	STN06	46	0.52	0.52	6.33	4.87	0.22	∞	127- ∞	41.23327778	124.0837306
Stone Lagoon	10	STN10	1								41.23327778	124.0837306
Stone Lagoon	11	STN11	2								41.23327778	124.0837306
Big Lagoon	06	BIG06	47	0.60	0.56	6.67	4.92	0.16	∞	523- ∞	41.16393056	124.1304278
Big Lagoon	10	BIG10	49	0.53	0.54	6.11	4.63	0.05	1277	86- ∞	41.16393056	124.1304278
Big Lagoon	11	BIG11	45	0.54	0.55	5.78	4.66	0.15	160	42- ∞	41.16393056	124.1304278
McDaniel Slough	99	MCD99	40	0.14	0.16	2.00	1.77	0	3	1-15	40.85766667	124.1233944
McDaniel Slough	06	MCD06	31	0.18	0.18	1.78	1.7	0	3	1-36	40.85766667	124.1233944
McDaniel Slough	10	MCD10	12	0.20	0.19	1.78					40.85766667	124.1233944
McDaniel Slough	11	MCD11	59	0.14	0.14	1.78	1.48	0	1260	1- ∞	40.85766667	124.1233944
Arcata Wastewater												
pond 3	09	AWP09	45	0.08	0.08	1.44	1.37	0	1	0.1-8	40.85290833	124.0913972
Arcata Wastewater												
Raceway 1	10	AWR10	2								40.85297222	124.0916667
Gannon Slough	06	GAN06	48	0.22	0.22	2.11	1.89	0	∞	39- ∞	40.85198056	124.0800778

Gannon Pond	06	PND06	17	0.23	0.20	1.56	1.56	0	∞	3- ∞	40.84561111	124.0811778
Gannon Pond	10	PND10	1								40.84561111	124.0811778
Jacoby Creek	06	JAC06	48	0.16	0.15	1.78	1.63	0	60	6- ∞	40.84342222	124.0815167
Jacoby Creek	10	JAC10	2								40.84342222	124.0815167
Rocky Gulch	10	ROK10	14								40.82795	124.0778889
Wood Creek	06	WDC06	48	0.10	0.12	1.44	1.37	0	3	1-12	40.78543611	124.1004417
Elk River	06	ELK06	42	0.27	0.27	2.00	1.84	0	54	4- ∞	40.75745833	124.1713278
Elk River	11	ELK11a	2								40.75745833	124.1713278
Elk River	11	ELK11b	59	0.21	0.21	2.00	1.78	0	34	10-1401	40.75745833	124.1713278
White Slough	06	WHS06	50	0.23	0.25	2.67	2.23	0	82	17- ∞	40.701925	124.2164583
Salmon Creek	11	SAL11a	59	0.27	0.25	2.44	2.11	0	27.3	3- ∞	40.67939444	124.2146833
Salmon Creek	11	SAL11b	60	0.24	0.24	2.56	2.2	0.03	401	4- ∞	40.67939444	124.2146833
Eel River	06	EEL06	45	0.27	0.30	2.78	2.55	0.02	72	19- ∞	40.65419722	124.2931306
Eel River	10	EEL10	47	0.25	0.25	2.67	2.27	0	∞	88- ∞	40.65419722	124.2931306
Salt River	10	SLT10	52	0.27	0.25	3.00	2.48	0.06	∞	38- ∞	40.60851667	124.3226972

Salt River	11	SLT11	3								40.60851667	124.3226972
Ten Mile River	99	TEN99	29	0.42	0.43	3.67	3.25	0	∞	31- ∞	39.54591667	123.7589889
Ten Mile River	13	TEN13	2								39.54591667	123.7589889
Virgin Creek	88	VRG88	58	0.51	0.51	4.44	3.66	0.06	540	67- ∞	39.47161389	123.8036139
Virgin Creek	99	VRG99	67	0.57	0.56	4.56	3.8	0.03	191	63- ∞	39.47161389	123.8036139
Virgin Creek	06	VRG06	50	0.59	0.57	4.33	3.7	0.04	∞	112- ∞	39.47161389	123.8036139
Virgin Creek	10	VRG10	47	0.54	0.56	4.56	3.71	0.08	∞	97- ∞	39.47161389	123.8036139
Pudding Creek	90	PUD90	20	0.44	0.43	3.11	2.96	0	86	6- ∞	39.45394722	123.8067056
Pudding Creek	06	PUD06	51	0.45	0.44	3.22	2.94	0	1394	57- ∞	39.45394722	123.8067056
Pudding Creek	10	PUD10	49	0.42	0.42	3.33	3.01	0	∞	42- ∞	39.45394722	123.8067056
Davis Lake	90	DL90	20	0.41	0.43	4.00	3.73	0.02	182	22- ∞	38.99013056	123.7011889
Davis Lake	13	DL13	56	0.42	0.44	5.55	4.31	0.12	64	25-5456	38.99013056	123.7011889

5

6

7 Table 2. Field survey presence (1) and absence (0) site occupancy histories for the 14 sites included in the temporal genetic analysis.

8 Points of genetic collections indicated by an asterisk. Empty cells indicate no field survey was conducted that year. Field survey data

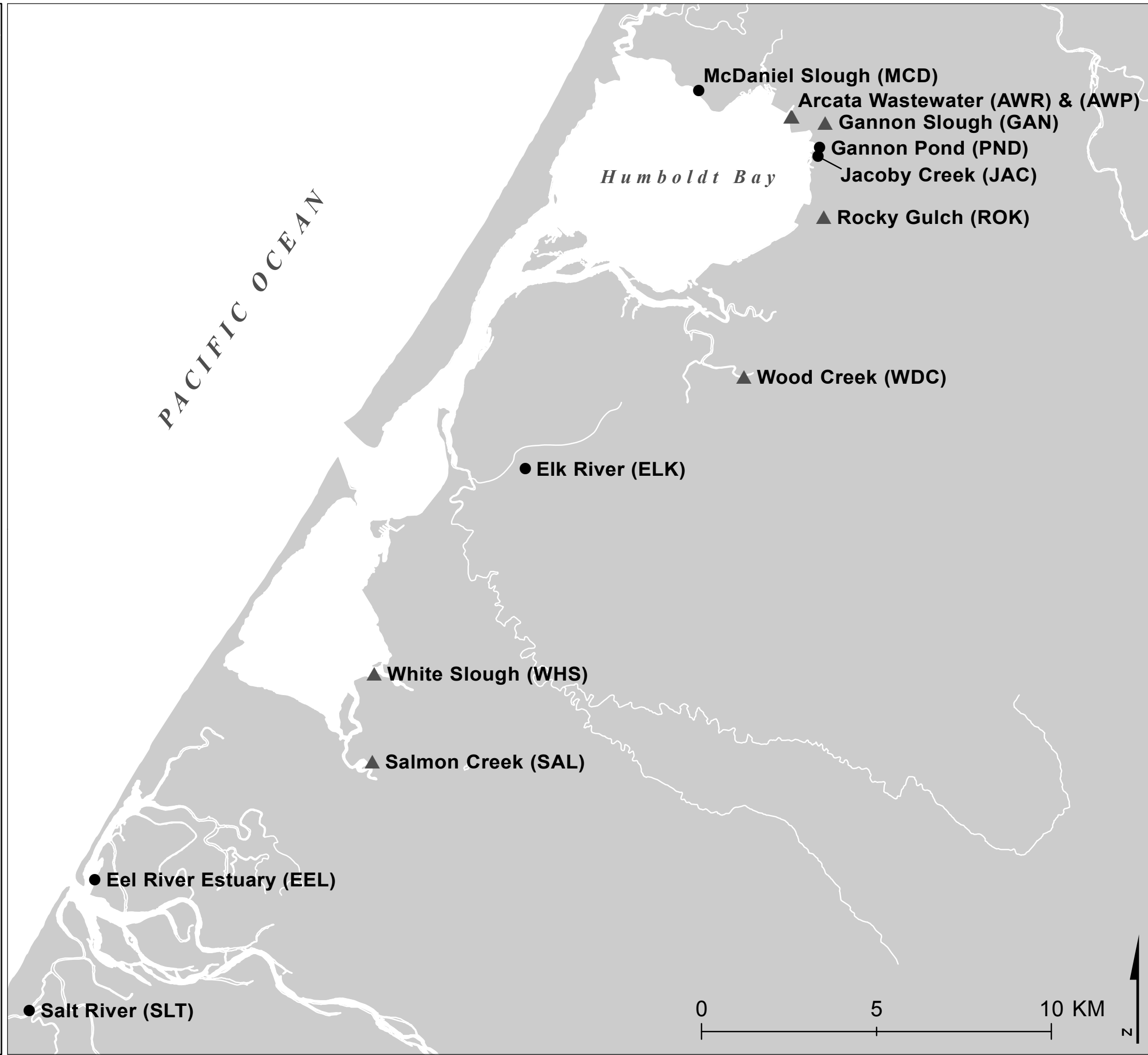
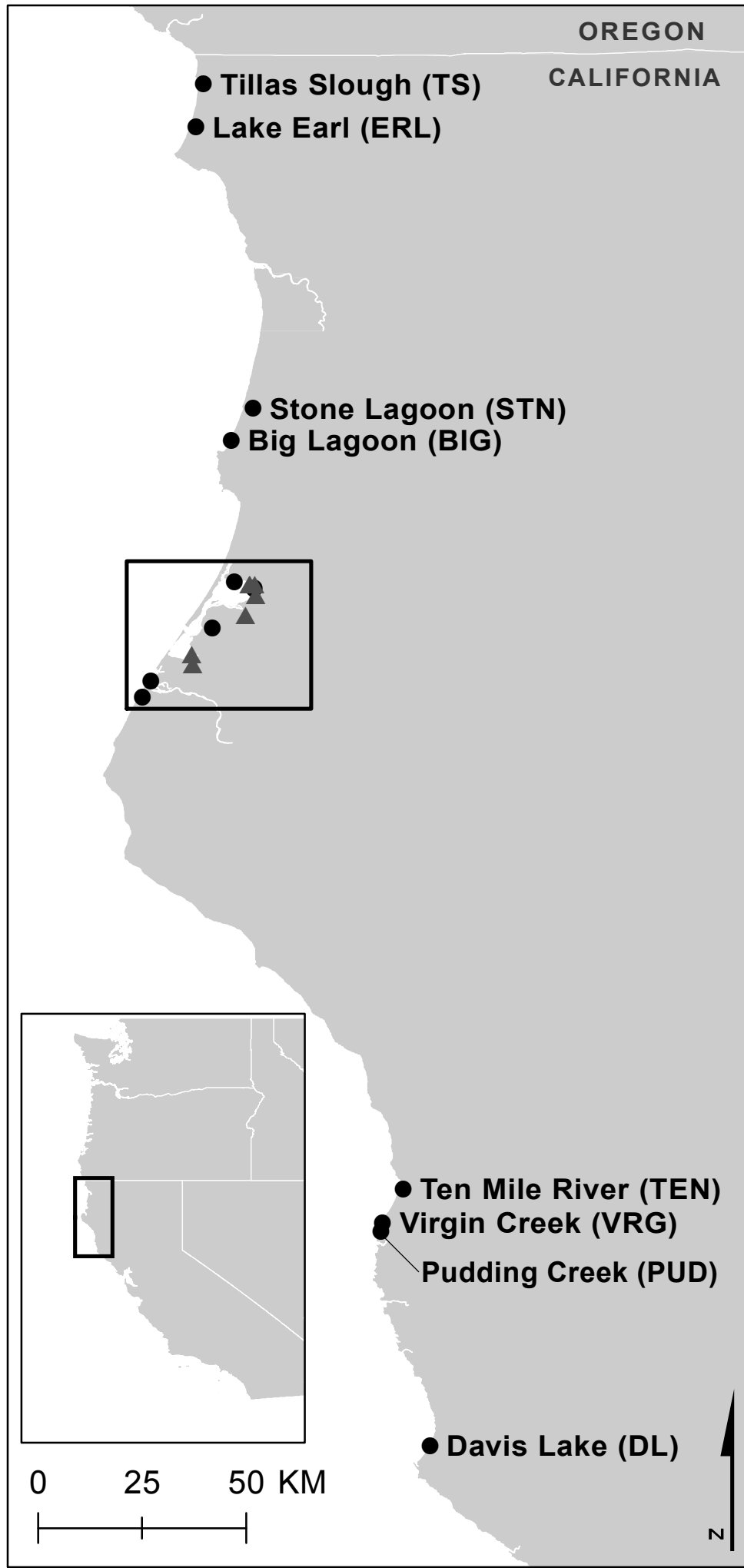
9 only includes years for which there was at least one survey.

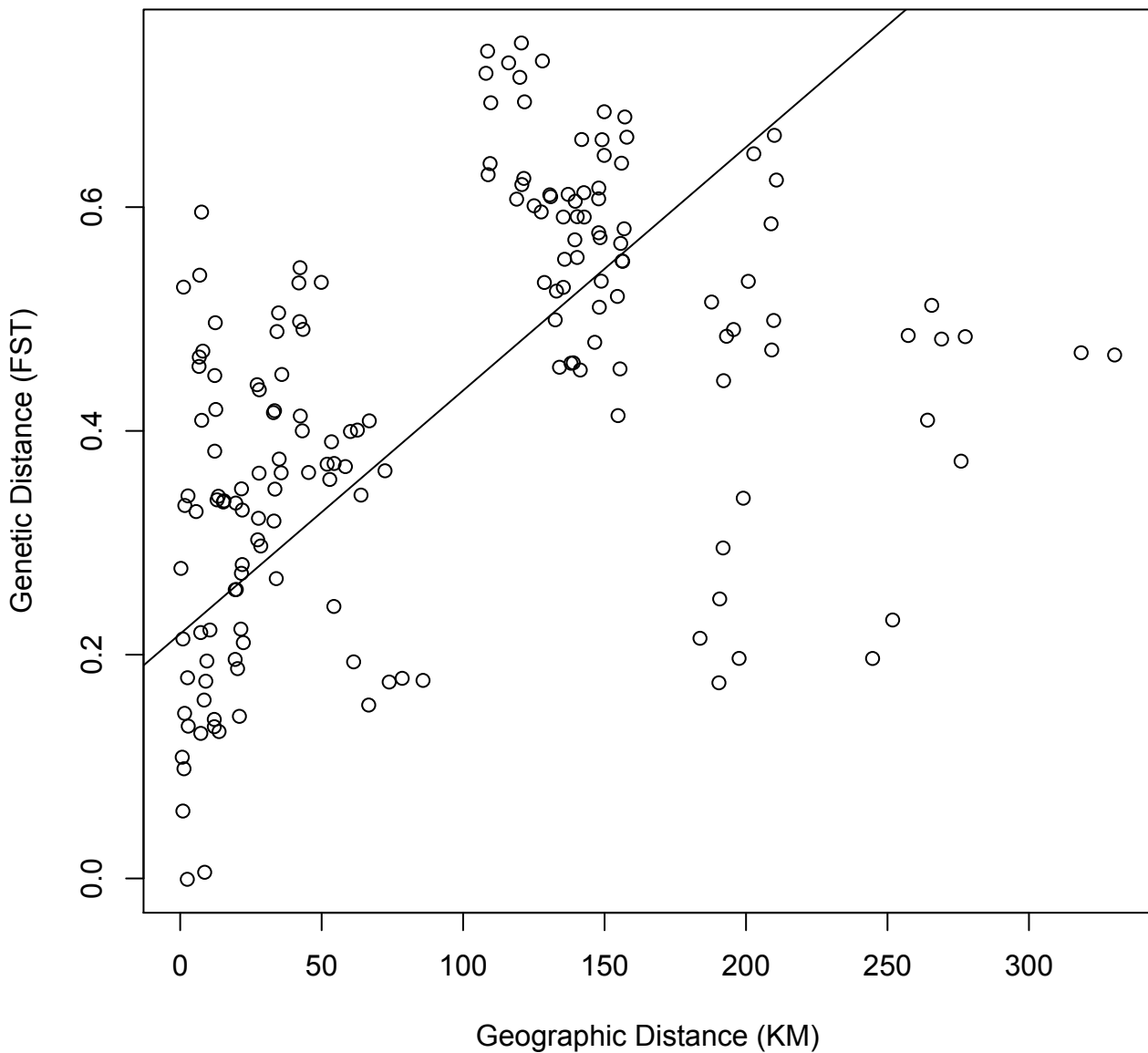
Site (Abbreviation)	1988	1990	1993	1996	1997	1999	2003	2004	2006	2007	2008	2009	2010	2011	2013
Tillas Slough (TS)						1*	0	1			1		1	1*	
Lake Earl (ERL)						1*	1	1	1*	1	1		1*	1*	
Stone Lagoon (STN)	1*	1	1	1		1	1	1	1*	1	1	1	1*	1*	
Big Lagoon (BIG)									1*	1	1	1	1*	1*	
McDaniel Slough (MCD)						1*	0	0	1*	0	0		1*	1*	
Gannon Pond (PND)									1*				1*		
Jacoby Creek (JAC)									1*	1	1	1	1*		
Elk River (ELK)									1*		1	1	1	1*	
Eel River (EEL)									1*				1*		
Salt River (SLT)													1*	1*	
Ten Mile River (TEN)						1*	1		0					1	1*

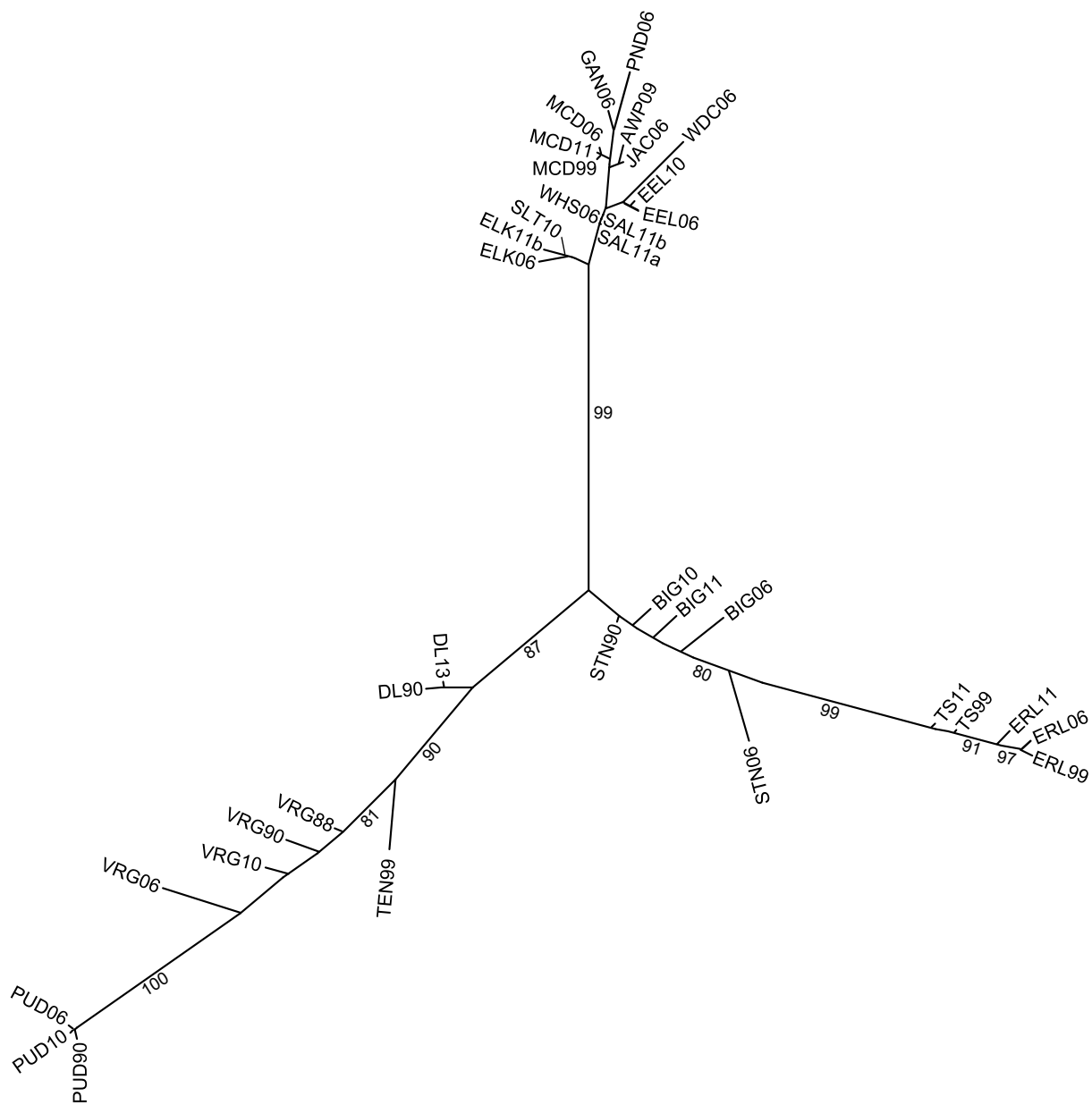
Table 3. Site, years of temporal collections, number of generations between temporal collections (gens), and estimates of variance effective size (N_{ev}) and 95% confidence intervals using the methods of Anderson (2005) and Waples 2005) for northern California tidewater goby. Populations are listed from north to south.

Site	Collection			N_{ev} (Berthier	95%	N_{ev} (Jorde	95%
	Years	Gens	2002)	Confidence	Interval	and Ryman	Confidence
						2007)	Interval
TS	99	11	12	∞	3334- ∞	∞	∞ - ∞
ERL	99	11	12	1708	360- ∞	2897	1446-4843
ERL	99	06	7	∞	2101- ∞	∞	∞ - ∞
ERL	06	11	5	∞	∞ - ∞	∞	∞ - ∞
STN	90	06	16	∞	1611- ∞	∞	∞ - ∞
BIG	06	10	4	637	154- ∞	193	∞ - ∞
BIG	06	11	5	∞	∞ - ∞	∞	∞ - ∞
BIG	10	11	1	380	49- ∞	∞	∞ - ∞
MCD	06	11	5	27	12-63	19	15-28
MCD	99	06	7	36	15-94	24	14-80
MCD	99	11	12	254	78-4011	315	∞ - ∞
ELK	06	11	5	16	8-30	8	5-23
EEL	6	10	4	116	39- ∞	125	∞ - ∞
VRG	88	99	11	417	205-1311	251	136-1588
VRG	88	06	18	339	188-686	133	91-252

VRG	88	10	22	507	275-1112	145	77-1192
VRG	99	06	7	223	106-710	224	126-1009
VRG	99	10	11	508	214-3765	252	141-395
VRG	06	10	4	∞	474- ∞	∞	∞ - ∞
PUD	90	06	16	1460	245- ∞	563	∞ - ∞
PUD	06	10	4	1829	113- ∞	∞	∞ - ∞
PUD	90	10	20	∞	1095- ∞	∞	∞ - ∞
DL	90	13	23	8139	1010- ∞	867	∞ - ∞







0.2

Supplementary Information

Appendix S1. Probability of at least one site going extinct by year n across m sites

To determine if our temporal genetic collections were sufficiently spaced such that an extinction event would have likely occurred between survey events we calculated the probability of at least one extinction at a site by n years (PE_n) as:

$$PE_n = 1 - (1 - P_e)^n \quad (1)$$

Where P_e is the annual site-specific extinction probability. As we obtained temporal collections from multiple sites, we also calculated probability of at least one site going extinct by year n across m sites as:

$$PE_n^* = 1 - \prod_{i=1}^m (1 - P_{\{e,i\}})^{n_i} \quad (2)$$

Assuming a 10 year time interval at 5 sites and an annual site-specific extinction rate of 0.37 (Lafferty et al. 1999) the probability of at least one site extinction among our field sites was 1. These results indicate that we would have a high probability of detecting metapopulation cycles if they were occurring at the rates previously reported for southern California tidewater goby populations.

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Lafferty KD, Swift CC, Ambrose RF (1999) Postflood persistence and recolonization of endangered tidewater goby populations. *North American Journal of Fisheries Management*, **19**,618-622.

Supplementary Tables and Figures.

Supplementary Table 1. Microsatellite loci details including allelic richness across all populations, size range (including amplified flanking regions and microsatellite repeats) in base pairs (bp), Hardy-Weinberg expected heterozygosity (H_e), and references.

Locus	Allelic Richness	Size Range (bp)	H_e	Reference
ENE5	4	291-301	0.27	Earl et al 2010
ENE6	8	218-232	0.3	Earl et al 2010
ENE8	14	127-181	0.4	Earl et al 2010
ENE9	33	116-218	0.62	Earl et al 2010
ENE12	10	193-220	0.31	Earl et al 2010
ENE13	3	244-250	0.04	Earl et al 2010
ENE16	9	150-186	0.4	Earl et al 2010
ENE2	6	143-161	0.36	Mendonca et al. 2001
ENE18	8	110-126	0.29	Earl et al 2010

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- Mendonca, H., Smith, J, Brinegar C (2001) Isolation and characterization of four microsatellite loci in the tidewater goby (*Eucyclogobius newberryi*) *Marine Biotechnology*, **3**, 91-95.

Supplementary Table 2. Genetic differentiation (F_{ST}) between population pairs (below diagonal) and p-values from permutation tests for significance (above diagonal). Comparisons between temporal collections highlighted in blue and significant tests for genetic differentiation in red.

TS11	ERL99	ERL06	ERL11	STN90	STN06	BIG06	BIG10	BIG11	MCD99
0.559144	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.17116	--	0.758324	0.374163	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.15176	-0.00323	--	0.9993	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.14215	0.00052	-0.00810	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.21548	0.22401	0.18960	0.18175	--	0.50335	0.213079	0.337666	0.613539	0.0001
0.17887	0.19241	0.16200	0.15498	-0.00087	--	0.027097	0.026297	0.076692	0.0001
0.18600	0.21783	0.19041	0.18325	0.00384	0.00722	--	0.071593	0.770123	0.0001
0.15904	0.19149	0.16309	0.15502	0.00169	0.00813	0.00532	--	0.518748	0.0001
0.17701	0.21172	0.18366	0.17556	-0.00242	0.00553	-0.00255	-0.00065	--	0.0001
0.69624	0.73301	0.70932	0.69711	0.50276	0.49489	0.44459	0.43921	0.44957	--
0.68107	0.71822	0.69223	0.68057	0.46796	0.46485	0.41831	0.41504	0.42403	0.07386
0.71600	0.75407	0.73207	0.71962	0.55159	0.53225	0.48529	0.47593	0.48879	0.00805
0.74672	0.77891	0.75323	0.73941	0.58319	0.54588	0.49386	0.49700	0.50549	0.33887
0.62017	0.66629	0.64042	0.62898	0.40682	0.41331	0.36929	0.36580	0.37483	0.20481
0.62568	0.68102	0.65046	0.63894	0.39213	0.40005	0.35585	0.35973	0.36252	0.34564
0.69410	0.72999	0.70544	0.69326	0.50381	0.49071	0.44294	0.44134	0.45045	0.16039
0.73065	0.76514	0.74002	0.72894	0.56246	0.53278	0.49117	0.48547	0.49769	0.45012
0.56952	0.59812	0.57277	0.55771	0.34220	0.35061	0.31359	0.29513	0.31647	0.36329
0.60936	0.64629	0.62169	0.60713	0.39906	0.39027	0.35695	0.35043	0.36282	0.43073
0.61141	0.63727	0.61157	0.60122	0.39057	0.39948	0.36273	0.35390	0.37026	0.23049
0.60523	0.63001	0.60597	0.59571	0.38992	0.40073	0.36494	0.35620	0.37088	0.24446
0.62580	0.65261	0.62881	0.61857	0.41481	0.42289	0.38414	0.37524	0.39134	0.20383
0.58373	0.60879	0.58492	0.57577	0.35659	0.37726	0.34263	0.33751	0.34005	0.26999
0.61292	0.64533	0.62127	0.61098	0.40001	0.40900	0.37227	0.36721	0.36811	0.27140
0.57708	0.59214	0.56661	0.55339	0.36148	0.36436	0.33252	0.31631	0.34263	0.39537
0.48207	0.51695	0.49229	0.48527	0.23315	0.24981	0.20851	0.25918	0.21466	0.60128
0.39228	0.45583	0.43342	0.43018	0.19353	0.20649	0.16829	0.20804	0.17554	0.51352
0.35680	0.41291	0.39267	0.38933	0.17100	0.18474	0.15271	0.18648	0.15978	0.47989

0.37116	0.42884	0.40793	0.40438	0.18293	0.19699	0.16577	0.19511	0.17099	0.50023
0.37295	0.43511	0.41313	0.40960	0.18666	0.19668	0.16683	0.19471	0.17488	0.50693
0.51007	0.55973	0.53468	0.52859	0.31478	0.32751	0.28557	0.31690	0.28425	0.67309
0.46498	0.51855	0.49929	0.49589	0.31678	0.32557	0.28504	0.31304	0.28332	0.61759
0.48424	0.53497	0.51624	0.51220	0.33087	0.33986	0.29749	0.32888	0.29540	0.62550
0.46786	0.51202	0.47854	0.46985	0.21413	0.23101	0.19508	0.23238	0.19661	0.58085
0.41161	0.45933	0.43291	0.42699	0.19376	0.20594	0.17676	0.21463	0.17591	0.51624

MCD06	MCD11	AWP09	GAN06	PND06	JAC06	WDC06	ELK06	ELK11b	WHS06	SAL11a	SAL11b
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0002	0.144786	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.06953	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.29995	0.33334	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.12416	0.17939	0.21405	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.22727	0.34193	0.52850	0.10837	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.08992	0.13612	0.09804	0.06027	0.27712	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.35342	0.47132	0.59557	0.40945	0.53916	0.45762	--	0.0001	0.0001	0.0001	0.0001	0.0001
0.33527	0.37998	0.40885	0.27069	0.39710	0.34008	0.45734	--	0.0001	0.0001	0.0001	0.0001
0.39488	0.44948	0.41915	0.33838	0.49670	0.38188	0.46599	0.15022	--	0.0001	0.0001	0.0001
0.19787	0.25806	0.25823	0.18752	0.33552	0.19577	0.34161	0.24352	0.21977	--	0.469253	0.626837
0.21565	0.27275	0.28054	0.21075	0.34822	0.22288	0.33646	0.23668	0.19433	-0.00074	--	0.121988
0.17978	0.23027	0.23110	0.18858	0.34844	0.17727	0.33099	0.24478	0.21132	-0.00241	0.00510	--

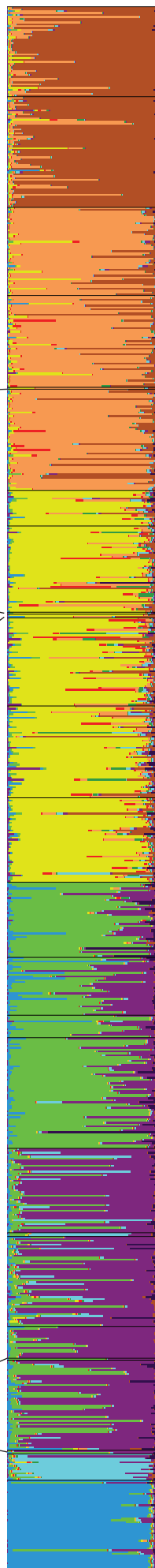
0.23360	0.31166	0.41709	0.29900	0.35215	0.32146	0.30744	0.30695	0.31108	0.13943	0.11100	0.14143
0.23354	0.30268	0.43676	0.29712	0.36221	0.32191	0.32925	0.34079	0.33750	0.15946	0.12966	0.15869
0.35050	0.41634	0.34797	0.26799	0.41804	0.31944	0.44131	0.10422	0.14491	0.13129	0.13563	0.14658
0.58152	0.64624	0.66026	0.53382	0.51054	0.60741	0.66042	0.50868	0.55501	0.52832	0.52498	0.54886
0.49329	0.55372	0.55496	0.45684	0.42692	0.51518	0.55289	0.45133	0.48182	0.46014	0.46561	0.48098
0.46473	0.51859	0.52417	0.42684	0.39542	0.48551	0.53288	0.41358	0.45177	0.43163	0.43733	0.45232
0.48520	0.54443	0.55717	0.44972	0.41124	0.51289	0.56169	0.42679	0.47347	0.44867	0.45400	0.47094
0.49025	0.55158	0.56756	0.45541	0.41368	0.52019	0.57271	0.43589	0.47919	0.45440	0.46086	0.47695
0.65811	0.71505	0.74690	0.61264	0.58237	0.68771	0.74099	0.57537	0.64517	0.61730	0.61556	0.63429
0.60918	0.65508	0.67389	0.57366	0.54346	0.63274	0.67475	0.55327	0.60790	0.58010	0.58055	0.59650
0.61710	0.66252	0.68058	0.58066	0.55187	0.63925	0.68525	0.56120	0.61706	0.59114	0.59147	0.60665
0.54788	0.62420	0.66415	0.49870	0.47233	0.58510	0.64768	0.49795	0.53368	0.49060	0.48457	0.51295
0.48960	0.54934	0.56053	0.44755	0.43149	0.51094	0.56055	0.45457	0.47429	0.45274	0.45061	0.47166

[illegible]

0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
--	0.09599	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.00736	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.29083	0.32792	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.46335	0.49925	0.53266	--	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.42509	0.45126	0.46812	0.12558	--	0.002	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
0.40500	0.43024	0.43402	0.11798	0.01091	--	0.011399	0.0038	0.0001	0.0001	0.0001	0.0001	0.0001
0.41899	0.44816	0.44986	0.14438	0.03425	0.00817	--	0.522348	0.0001	0.0001	0.0001	0.0001	0.0001
0.43175	0.46051	0.45690	0.17631	0.03781	0.01091	-0.00073	--	0.0001	0.0001	0.0001	0.0001	0.0001
0.56270	0.59965	0.60984	0.21686	0.15640	0.11688	0.10383	0.14252	--	0.116788	0.921308	0.0001	0.0001
0.53619	0.56356	0.58097	0.23598	0.14849	0.11634	0.10080	0.13300	0.00792	--	0.442856	0.0001	0.0001
0.54390	0.57080	0.59117	0.22206	0.15132	0.12010	0.10955	0.14758	-0.00839	-0.00005	--	0.0001	0.0001
0.41502	0.44488	0.51517	0.19358	0.20619	0.19465	0.22596	0.24309	0.35730	0.35468	0.35660	--	0.178082
0.39524	0.41290	0.47134	0.16724	0.17743	0.17618	0.20990	0.22737	0.33434	0.33400	0.33347	0.00547	--

Supplementary Figure 1. The proportion of each individual's multilocus genotype assigned to each of 11 groups inferred by Bayesian cluster analysis with STRUCTURE. Populations are ordered north to south (top to bottom).

TS99
TS11
ERL99
ERL06
ERL10
ERL11
STN90
STN06
STN10
STN11
BIG06
BIG10
BIG11
MCD99
MCD06
MCD10
MCD11
AWP09
AWR10
GAN06
PND06
PND10
JAC06
JAC10
ROK10
WDC06



ELK06
ELK11a
ELK11b
WHS06
SAL11a
SAL11b
EEL06
EEL10
SLT10
SLT11
TEN99
TEN13
VRG88
VRG99
VRG06
VRG10
PUD90
PUD06
PUD10
DL90
DL13

