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Population structure and dispersal in a patchy landscape: nuclear and mitochondrial markers reveal area effects in the spider *Theridion californicum* (Araneae: Theridiidae)

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Changes in land use have a major effect on patterns of biodiversity. However, few studies have examined the demographic and genetic shifts associated with a return to semi-natural habitat following extended periods of human disturbance. Here we examine patterns of population structure in a spider restricted to the Pacific coastal strip of North America that exhibits an exuberant colour polymorphism. We use mitochondrial DNA and AFLP markers to examine genetic structure and estimate gene flow. The results show contrasting, gender-specific patterns between these markers that suggest limited dispersal, combined with area effects most likely caused by expansion from refugial habitat patches following land-management changes in a region of the San Francisco East Bay. Colour-morph frequencies are not correlated with this complex genetic structure. Thus, unlike the classical area effects that were based on colour morphs, we demonstrate in *T. californicum* signals of historical contingency at neutral loci but not at the *Colour* locus, where traces of past events have been obliterated by balancing selection. *Biological Journal of the Linnean Society*, 2011, **104**, 600–620.

ADDITIONAL KEYWORDS: balancing selection – colour polymorphism – dispersal – disturbance – gene-flow – land-use change – range expansion.

INTRODUCTION

Landscape dynamics have played a key role in shaping biodiversity in the historical and recent past (Chiucchi & Gibbs, 2010). The current rapid rate and extent of habitat modification in particular has led to diverse effects on species, ranging from fragmentation and extinction (e.g. Mayer, Schiegg & Pasinelli, 2009), to hybridization (e.g. Dodd & Kashani, 2003), population expansion (Bloor, Kemp & Brown, 2008) and invasion of new habitats (Ewers & Didham, 2006). As a means to understand the processes underlying these patterns, numerous studies have applied phylogeographic methods based on molecular data, providing key insights into how populations have responded, and will continue to respond, to habitat modification. Most of these studies have focused on situations in which the habitat has been modified from a 'natural' state (e.g. Vandergast *et al*., 2007). Very few have examined phylogeographic shifts associated with the return of habitat to a natural state following extended periods of disturbance (notable exceptions include Chatzimanolis & Caterino, 2009). Here, we explore a situation where the landscape has undergone historical fragmentation and recent recovery and examine how a species with potentially limited dispersal has responded to such modifications.

The coastal region of western North America, in particular around the East Bay of San Francisco, is known for its high levels of endemism at the subspecies level (Davis *et al*., 2008). For example, the salamander *Batrachoseps attenuatus* (Plethodontidae) shows high levels of genetic diversity and deep divergences within the region (Martínez-Solano, Jockusch

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& Wake, 2007). Likewise, white-crowned sparrows, *Zonotrichia leucophrys nuttalli*, in the area show marked differences in song between closely contiguous areas (Baptista, 1976). At the same time, the East Bay region has had a long history of human impact, beginning with Native American occupation during the early Holocene (Jones, 1992). Before people arrived, the landscape appears to have been dominated by a mosaic of shrub land and woodland (Keeley, 2005), suggesting that neither natural fires nor extensive grazing played a key role in determining pre-human vegetation patterns. Early colonization by humans in the Holocene was associated with widespread burning and, later, extensive grazing, both processes leading to grassland-dominated systems. Beginning in the early 20th century, an effort was made to protect some portions of the San Francisco East Bay landscape from human impacts, with the establishment of parks for conservation, fire protection and recreation (Russell & McBride, 2003). As a result of these efforts, the latter half of the 20th century has seen a marked decrease in alien-dominated grasslands in many of the protected conservation areas of the East Bay as a consequence of colonization by shrubs from local refugial pockets of native vegetation, and succession to woodland (Edwards, 2002; Russell & McBride, 2003). Thus, the landscape existing prior to human colonization has, to some extent, been recreated. In order to understand how native species have been affected by these landscape changes, the current study focuses on a species of spider that is associated specifically with shrubs and woodland understory and is widespread in the East Bay region.

Theridion californicum (Theridiidae) has a maritime distribution along the west coast of North America from British Columbia to southern California (Levi, 1957). Within California, the species is not found east of the coastal mountain ranges, and is confined to a narrow coastal strip that is subject to cool coastal fog and moderate precipitation. The spider occupies a somewhat patchy habitat and lives near the ground. It has recently been shown to display a remarkably rich colour polymorphism (Oxford, 2009). *Theridion californicum* is an ideal candidate for studying the effects of recent expansion of the natural shrub-land habitat on the genetic population structure of a native species, in that it occurs within an environment that is not likely to be conducive to aerial dispersal (forest) and has a severely climate-limited east–west range.

The effects of past population expansions arising from landscape-scale changes have been examined in other organisms that show colour polymorphisms. For example, Cain & Currey (1963a) studied shell banding and colour morphs in the snail *Cepaea nemoralis* on the Marlborough Downs in southern England. Here, tracts of grassland far larger than the panmictic area of the snail are characterized by specific colour or banding morphs that abut other such areas dominated by different morphs. Because the steep clines in morph frequencies at the boundaries between areas do not generally coincide with obvious environmental discontinuities, and are highly stable over time, they have been termed 'area effects' (Cain & Currey, 1963a; Cowie & Jones, 1998; Davison & Clarke, 2010). Although Cain & Currey (1963a, b) originally argued that the mechanism maintaining area effects was selection by subtle environmental factors, the general consensus now is that they reflect the expansion, into newly available and unoccupied habitat, of genetically differentiated refugia populations (Goodhart, 1963; Cameron, Williamson & Morgan-Huws, 1977; Cameron, Carter & Palles-Clark, 1980; Ochman, Jones & Selander, 1983; Goodacre, 2001; Davison & Clarke, 2010). Area effects on a variety of scales have subsequently been described for colour morphs and molecular markers in a number of different species, but mostly snails (e.g. Ochman *et al*., 1983; Gould & Woodruff, 1990; Goodacre, 2001).

The current study set out to examine population structure and potential area effects in *T. californicum*. In a previous analysis (Croucher *et al*., 2011) of the same *T. californicum* populations, we addressed the role of selection in maintaining the colour polymorphism by determining whether colour was an outlier when judged against the background of neutral-locus frequency distributions. We found it was and concluded colour was subject to balancing selection. Here, we use the set of nuclear AFLPs analysed previously in terms of natural selection, together with mitochondrial sequence data, to examine the spatial distributions of both neutral markers and the colour phenotype. In particular, we wished to determine the extent to which the population structure reflects the recent increase in habitat availability in the San Francisco East Bay region. First, given that the spiders are likely to be relatively sedentary, our expectation is that their populations should be structured. Our specific hypothesis is that the newly available habitat was colonized from a single source (within or outside the East Bay area), resulting in a series of newly established populations showing effects of isolation by distance. Alternatively, populations may have arisen from multiple refuges within the area, resulting in a series of discrete populations with no signal of isolation by distance. Second, because of the local distribution and habitat specificity of the species, gene flow is expected to be sufficiently low as to prevent genetic homogenization of populations across the landscape. In particular, we

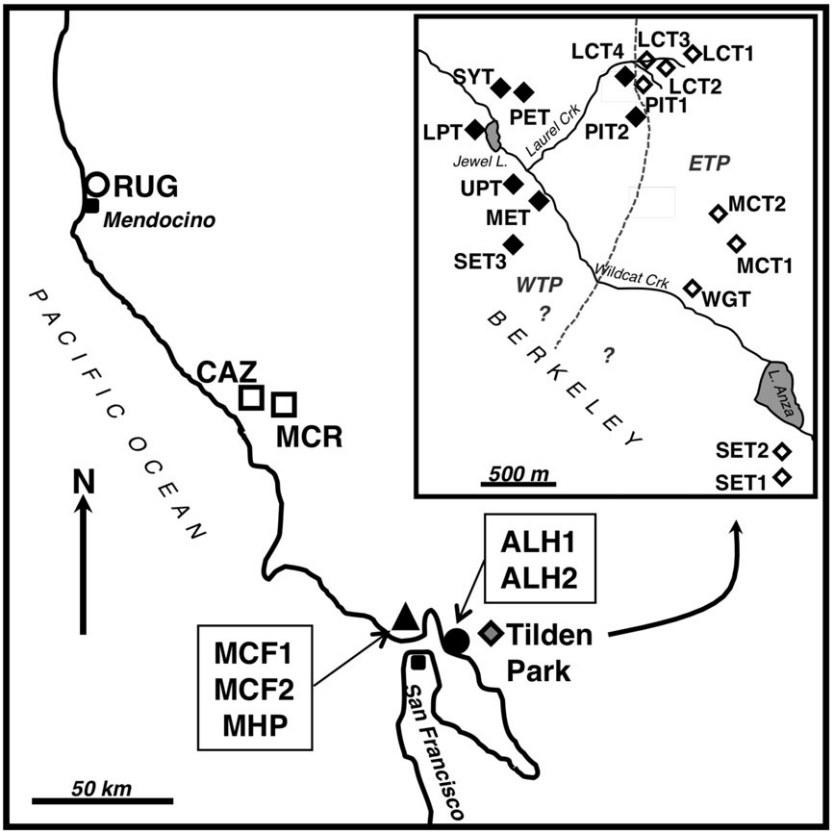

Figure 1. Map of the central northern coast of California, indicating the locations of the *T. californicum* sample sites (see Table 1). The approximate location of the division between East Tidlen Park (ETP) and West Tilden Park (WTP) is indicated by the dashed line; question marks indicate uncertainty because of lack of samples.

expect little evidence of long-distance movement (by means of ballooning on a silk thread) or even of short distance dispersal (by 'rappelling' on a bridging thread) (Bonte *et al*., 2003; Bell *et al*., 2005). Third, because the colour polymorphism has been shown to be subject to balancing selection (Croucher *et al*., 2011), we would expect different spatial patterns to emerge when considering colour and neutral molecular markers, with the latter better reflecting historical contingency.

MATERIAL AND METHODS

STUDY REGION

The focal area was approximately 2.5 km by 3 km within the boundaries of the Charles Lee Tilden Regional Park, located on the Coastal Range and Hayward Fault area immediately east of the cities of Berkeley and Kensington in the hills of the San Francisco East Bay. The hills here peak at 581 m elevation (Vollmer Peak) and are bounded to the west by the Hayward Fault. The Wildcat Fault runs along the eastern side of the hills and bisects Tilden Park, manifesting itself as Wildcat Creek and Gorge (Fig. 1). The park consists of a patchwork of bay laurel (*Umbellularia californica*)/oak (*Lithocarpus densiflorus*, *Quercus* spp.)/redwood (*Sequoia sempervirens*) forest and more open areas, the latter being exposed to the westerly wind that blows marine fogs in from the Pacific Ocean. Until recently (early 1900s), the entire area was dominated by grasses (McBride & Heady, 1968; Russell & McBride, 2003). Following the establishment of the park in 1936, large sections are now covered by coastal scrub, predominantly coyote brush (*Baccharis pilularis*), with additional species such as California coffee berry (*Rhamnus californica*), California blackberry (*Rubus ursinus*), thimbleberry *Rubus parviflorus*, and poison oak (*Toxicodendron diversilobum*) (Edwards, 2002; Russell & McBride, 2003).

FOCAL TAXON

Theridion californicum (Banks, 1904) was first described from Mill Valley (Marin Co., CA, USA). The species is confined to shaded understory plants such

as blackberry, thimbleberry and poison oak, as well as stinging nettle *Urtica dioica*, woodland strawberry *Fragaria vesca* and redwood sorrel *Oxalis oregana*, and is most numerous along shady trails within the mixed oak, bay laurel or redwood forest. In total, 243 specimens of *T. californicum* were collected from beneath leaves or within rolled leaves of understory plants from 25 sampling localities in Northern California (Table 1 and Fig. 1). The majority of these localities (17) were within Tilden Park, with eight at more moderate distances. Sampling locations were carefully chosen so that they were separated by a broad range of geographic distances ranging from more than 200 km (Russian Gulch State Park – Tilden Park) to tens of metres within Tilden Park. Sampling locations within Tilden Park were selected using a number of criteria. First, sampling was limited by the availability of suitable habitat patches of 10–20 m in length (for example, much of the area to the north-east of Wildcat Creek in the centre of the park remains as unsuitable open grassland). Second, sample sites were located along the network of forest paths, such that adjacent sites were not obviously contiguous (> 20 m apart). Third, to account for the highly varied topography of the park, as many distinct paths at as many different altitudes as possible were sampled. As many paths are associated with local creek watersheds, this ensured a broad sampling. Fourth, sites were chosen so that they were separated by a variety of geographic distances. This material was previously analysed in Croucher *et al*. (2011). *Theridion californicum* exhibits a colour polymorphism comprising a common Yellow morph and up to ten more rare abdominal colour morphs with patterns of yellow, red, white and black pigmentation [see Oxford, (2009) for a detailed description of the colour morphs and Croucher *et al*. (2011) for morph frequencies in the present samples]. The morphs appear to be inherited in a simple Mendelian fashion through alleles at a single locus, with the Yellow morph acting as the bottom recessive (Oxford, 2009; Croucher *et al*., 2011). Each spider had previously been scored for the colour morph it exhibited, with the *Colour* locus treated as a dominant locus denoted as Yellow ('0') or Patterned ('1') (see Croucher *et al*., 2011).

DNA extraction, genotyping and sequencing

DNA extraction, AFLP profile generation using eight selective primer pairs, scoring and error checking procedures for these samples have been described in detail (Croucher *et al*., 2011). Analyses of these AFLP data identified 521 AFLP loci, of which 26 were apparently under balancing selection and two under positive selection (Croucher *et al*., 2011). Consequently, the analyses that follow are based upon the remaining set of 493 'neutral' AFLP loci.

In addition to the previously generated AFLP data (Croucher *et al*., 2011), for the current study approximately 700 bp of the mitochondrial cytochrome *c* oxidase subunit 1 (CO1) gene was PCR amplified using the standard universal barcoding primers LCO1 1498: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO1 2198: 5'-TAAACTTCAGGGTGACCA AAAAATCA-3' (Folmer *et al*., 1994). After trimming away the primer sequences, this resulted in a 658-bp fragment of the CO1 gene. The PCR cycle consisted of an initial denaturation step of 2 min at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 45 s annealing at 42 °C, 1.5-min extension at 72 °C, with a final extension of 10 min at 72 °C. PCR reactions consisted of 2 μL DNA (10–30 ng), 0.5 U Ampli*Taq* DNA polymerase (Applied Biosystems, Foster City, CA, USA), 1.8 mM magnesium chloride ($MgCl_2$), 0.2 mM of each dNTP, 0.4 mM of each primer in a 20-μL final volume. PCR products were cleaned of excess primer and nucleotides by treatment with Exonuclease 1 and Shrimp Alkaline Phosphatase and bi-directionally sequenced using Big Dye ver. 3.0 chemistry (Applied Biosystems). The affinity of the final sequences at both the nucleotide and amino acid level was verified by blasting against GenBank. All sequences are available from GenBank under accession numbers JF979383–JF979401.

Summary diversity and demographic statistics

DNA sequence traces were edited using SEQUENCHER ver. 4.6 (GeneCodes, Ann Arbor, MI, USA) and aligned and manipulated using MESQUITE ver. 2.72 (Maddison & Maddison, 2009) and CLUSTAL W ver. 2.0 (Larkin *et al*., 2007). Summary statistics for the AFLP data [gene diversity λ (Nei, 1987)] and Shannon's information index *I* (Shannon & Weaver, 1949) were computed using POPGENE (Yeh & Boyle, 1997). Gene (haplotype) diversity λ (Nei, 1987) among the mtDNA haplotypes was computed using ARLEQUIN ver. 3.5 (Excoffier & Lischer, 2010). ARLEQUIN was also employed to assess the possibility of recent population expansion in *T. californicum*. Tajima's *D* (Tajima, 1989) was estimated for the mtDNA haplotypes and its statistical significance assessed by comparing the observed values of Tajima's *D* to their distribution from 10 000 coalescent simulations. Significantly negative values of Tajima's *D* can be indicative of population growth (or selective sweeps) and significantly positive values indicate population contraction, genetic subdivision or diversifying selection (Eytan & Hellberg, 2010). The mismatch distribution among the mtDNA haplotypes was also computed and tested for its fit against models

Table 1. Sample localities, groups, genetic diversity and demographic expansion statistics

Group and locality	Code	Longitude	Latitude	*N*	*%Y*	h_{AFLP}	I_{AFLP}	h_{mtDNA}	Recent demographic expansion		Sudden spatial expansion		Tajima's *D*
									R	SSD	*R*	SSD	
West Tilden Park (WTP)				**96**	**54.2**	**0.372**	**0.536**	**0.595**	**0.41***	**0.16***	**0.41 NS**	**0.09 NS**	**–0.10 NS**
Selby Trail 3	SET3	–122.2674	37.9058	5	80.0	0.318	0.449	0.000	NA	NA	NA	NA	NA
Pine Tree Trail 2	PIT2	–122.2588	37.9133	1	100.0	–	–	–	NA	NA	NA	NA	NA
Lower Packrat Trail	LPT	–122.2697	37.9124	20	65.0	0.372	0.536	0.100	0.83 NS	0.01 NS	0.83 NS	0.01 NS	–1.87†
Sylvan Trail	SYT	–122.2677	37.9149	2	100.0	0.041	0.060	1.000	NA	NA	NA	NA	NA
Memory Trail 1	MET	–122.2659	37.9084	16	37.5	0.257	0.374	0.571	0.29 NS	0.12 NS	0.29 NS	0.04 NS	0.34 NS
Upper Packrat Trail 1	UPT	–122.2671	37.9093	22	59.1	0.437	0.625	0.649	0.32*	0.13 NS	0.32 NS	0.09 NS	0.37 NS
Peak Trail	PET	–122.2670	37.9148	23	39.1	0.441	0.630	0.763	0.44†	0.14*	0.44 NS	0.11 NS	–0.08 NS
Laurel Canyon Trail 4	LCT4	–122.2597	37.9148	7	42.9	0.378	0.535	0.762	0.46 NS	0.12 NS	0.46 NS	0.10 NS	0.85 NS
East Tilden Park (ETP)				**68**	**73.5**	**0.347**	**0.519**	**0.609**	**0.33 NS**	**0.09 NS**	**0.33 NS**	**0.06 NS**	**–0.98 NS**
Wildcat Gorge Trail 1	WGT	–122.2550	37.9032	6	83.3	0.153	0.217	0.600	0.06 NS	0.01 NS	0.06 NS	0.01 NS	–1.23 NS
Selby Trail 2	SET2	–122.2490	37.8937	10	80.0	0.194	0.279	0.644	0.44 NS	0.11 NS	0.44 NS	0.08 NS	–0.43 NS
Meadow Canyon Trail 2	MCT2	–122.2532	37.9075	2	50.0	0.041	0.059	1.000	NA	NA	NA	NA	NA
Laurel Canyon Trail 3	LCT3	–122.2580	37.9080	15	60.0	0.205	0.300	0.781	0.46*	0.12 NS	0.46 NS	0.11 NS	0.06 NS
Selby Trail 1	SET1	–122.2490	37.8921	18	77.8	0.254	0.373	0.571	0.24 NS	0.06 NS	0.24 NS	0.03 NS	0.04 NS
Pine Tree Trail 1	PIT1	–122.2591	37.9153	6	50.0	0.113	0.164	0.533	0.79 NS	0.20 NS	0.79 NS	0.16 NS	1.03 NS
Meadow Canyon Trail 1	MCT1	–122.2500	37.9053	1	100	–	–	–	NA	NA	NA	NA	NA
Laurel Canyon Trail 2	LCT2	–122.2570	37.9161	4	100	0.111	0.158	0.500	0.75 NS	0.19 NS	0.75 NS	0.14 NS	–0.71 NS
Laurel Canyon Trail 1	LCT1	–122.2549	37.9162	6	83.3	0.208	0.296	0.333	0.67 NS	0.26 NS	0.67 NS	0.06 NS	–1.13 NS
Albany Hill (ALH)				**30**	**33.3**	**0.437**	**0.626**	**0.674**	**0.43†**	**0.13 NS**	**0.43 NS**	**0.08 NS**	**2.70 NS**
Albany Hill 1	ALH1	–122.3040	37.8976	15	40.0	0.218	0.316	0.705	0.43*	0.11 NS	0.43 NS	0.09 NS	1.63 NS
Albany Hill 2	ALH2	–122.3049	37.8977	15	26.7	0.453	0.642	0.629	0.49 *	0.18 NS	0.49 NS	0.10 NS	2.35 NS
Mill Valley (MIV)				**23**	**69.6**	**0.450**	**0.639**	**0.708**	**0.47***	**0.12 NS**	**0.47 NS**	**0.10 NS**	**1.16 NS**
Cascade Falls, Mill Valley	MCF1	–122.5638	37.9127	10	50.0	0.378	0.538	0.733	0.72*	0.19*	0.72*	0.17*	1.23 NS
Cascade Trail, Mill Valley	MCF2	–122.5698	37.9129	9	77.8	0.442	0.630	0.750	0.32 NS	0.09 NS	0.32 NS	0.07 NS	0.62 NS
Hillside Park, Mill Valley	MHP	–122.5480	37.9098	4	100.0	0.290	0.415	0.833	0.75 NS	0.19 NS	0.75 NS	0.18 NS	0.65 NS
Guerneville (GUV)				**11**	**90.9**	**0.192**	**0.277**	**0.346**	**0.53 NS**	**0.10 NS**	**0.53 NS**	**0.04 NS**	**–1.77***
Mays Canyon Road	MCR	–122.8692	38.4857	10	90.0	0.192	0.277	0.378	0.51 NS	0.11 NS	0.51 NS	0.05 NS	–1.69*
Cazadero	CAZ	–123.0699	38.5069	1	100.0	–	–	–	NA	NA	NA	NA	NA
Russian Gulch (RUG)				**15**	**33.3**	**0.200**	**0.293**	**0.743**	**0.18 NS**	**0.08 NS**	**0.18 NS**	**0.04 NS**	**0.15 NS**
Russian Gulch	RUG	–123.8033	39.3299	15	33.3	0.200	0.2929	0.743	0.18 NS	0.08 NS	0.18 NS	0.04 NS	0.15 NS

*$P < 0.5$; †$P < 0.01$.

N, sample size; *%Y*, percentage of Yellow vs. Patterned morphs in population; h_{AFLP}, AFLP gene diversity; I_{AFLP}, Shannon's information index; h_{mtDNA}, mtDNA gene diversity; *R*, Harpending's Raggedness Index (mismatch distribution); SSD, sum of squared deviations from demographic model (mismatch distribution); NS, not significant; NA, not available.

of recent demographic expansion (Slatkin & Hudson, 1991; Rogers & Harpending, 1992; Schneider & Excoffier, 1999) and a sudden spatial expansion (Ray, Currat & Excoffier, 2003; Excoffier, 2004), with the significance of Harpending's Raggedness statistic R and the sum of square deviations (SSD) being determined by 10 000 bootstrap replicates in ARLEQUIN.

Spatial autocorrelation analysis

The spatial genetic structure of *T. californicum* was examined for evidence of isolation-by-distance through spatial autocorrelation analyses as implemented by SPAGEDI (Hardy & Vekemans, 2002). For the mitochondrial data, Moran's I index (Sokal & Oden, 1978) was employed as a measure of correlation between observations of the same type (haplotypes), made at locations of a given geographic distance. For the dominant neutral AFLP data, the kinship coefficient $F_{(d)}$ (Hardy, 2003) was employed. Although the kinship coefficient requires that the inbreeding coefficient f be known, the estimator is quite robust to moderate errors in its specification (Hardy, 2003). The analysis was therefore run using several values for the inbreeding coefficient, with little qualitative difference in the outcome. The results presented are based on a value of $f = 0.0185$. This value is the average F_{IS}, weighted by sample size, as determined by Approximate Bayesian Computation using the software ABC4F (Foll, Beaumont & Gaggiotti, 2008) using the AFLP data and the final population meta-sample groupings (see below). For each marker set, autocorrelation was evaluated over ten distance classes of similar frequency, including one class for individuals found in the same location (zero distance). Moran's I and $F_{(d)}$ were assessed for statistical significance using a randomization test that permuted individuals over locations in 10 000 replications. Spatial autocorrelation was examined independently for the *Colour* locus. Additional tests for significant correlation between genetic and geographic distance matrices used Mantel tests (Mantel & Valand, 1970), as implemented by ZT (Bonnet & Van de Peer, 2002).

Analysis of molecular variance and spatial analysis of molecular variance

The genetic relationships among the sample populations were evaluated by estimating Φ_{ST}, an analogue of Wright's F_{ST} that takes the evolutionary distance between haplotypes into account (Excoffier, Smouse & Quattro, 1992; Excoffier & Smouse, 1994). Estimates of Φ_{ST} were generated through analysis of molecular variance (AMOVA) using the ARLEQUIN software (Excoffier & Lischer, 2010) and tested for statistical significance by randomization (10 000 replicates per comparison). For the mitochondrial data, the evolutionary distance between haplotypes was simply taken as the square of the number of pairwise differences between sequences (the use of more sophisticated distance measures, such as Kimura's 2-parameter model, weighting transitions twice that of transversions, made little difference to the estimates). For the AFLP data, the number of pairwise differences was also employed, this time measured between AFLP profiles (phenotypes) for each pair of individuals. One of the strengths of AMOVA is its robustness to a wide variety of distance data. However, as AFLP data are dominant, masking heterozygous genotypes, they actually represent a 'phenotype' and therefore inferences about the underlying diploid genetics should be treated cautiously.

For both the mtDNA and AFLP data sets, many population comparisons yielded very low Φ_{ST} values, indicating that the respective populations were genetically indistinguishable. The data were therefore grouped into statistically and genetically meaningful meta-samples on the basis of several analyses. The first was a spatial analysis of molecular variance using the software SAMOVA (Dupanloup, Schneider & Excoffier, 2002). This approach aims to find the set of k maximally differentiated populations by generating a Voronoi tessellation of the projected geographical coordinates of the samples, and arbitrarily dividing the samples into k-groups. At each step, Φ_{CT} is estimated through AMOVA, and the edges dividing the k-groups are updated through a simulated annealing procedure such that Φ_{CT} is maximized. The SAMOVA analysis focused upon the mitochondrial data, partly because the Φ_{ST} estimates indicated that these data contained a stronger signal of population structure than the AFLP data and because the software was not stable under the large AFLP data set. Initial analyses on the entire mitochondrial data set, together with the pairwise Φ_{ST} estimates, indicated that the samples from Mill Valley (MIV), MCF1, MCF2 and MHP, could be pooled. Similarly, the Albany Hill (ALH) samples, ALH1 and ALH2, were pooled, as were the samples from the vicinity of Guerneville (GUV), CAZ and MCR. Particular interest was paid to the genetic structure of the Tilden Park samples. When SAMOVA was run for values of k from 1 to 6, the maximal Φ_{CT} value ($\Phi_{CT} = 0.4430$) was obtained for $k = 3$; partitioning Tilden park into a western set of samples {PET, UPT, LPT, MET, SET3} and an eastern set {SET1, SET2, LCT1, LCT2, LCT3, LCT4, WGT, PIT1, MCT2}. SYT ($N = 2$) remained as singleton. The two samples (MCT1 and PIT2), each comprising only a single individual, were excluded from this analysis.

Multidimensional scaling analysis

To better visualize the genetic landscape of *T. californicum*, the pairwise Φ_{ST} estimates from both the mitochondrial and AFLP data sets were transformed to principle coordinates through a multidimensional scaling analysis (MDS) using the ISOMDS function in the R-package MASS (R Development Core Team, 2008). For one to six dimensions, individual solutions were iterated until the improvement in Kruskal's stress, *S*, was less than 0.0001. The optimum dimensionality was determined by a 'scree' test (Table 2). For the mitochondrial data, a clear 'elbow' was detected for the two-dimensional solution with higher-dimensional solutions not yielding a substantial reduction in stress. For the AFLP data, the two-dimensional solution was also selected, although such a clear 'elbow' was not obvious. The three-dimensional solution did not provide a substantially better separation of the data. The results of the MDS analyses were used to revise the grouping of the Tilden Park populations into two distinct meta-samples (see Results).

Median-joining network

The evolutionary relationships among the mitochondrial DNA haplotypes, and their relative distributions among the meta-samples for the entire data set, were visualized by constructing a median-joining (MJ) network (Bandelt, Forster & Röhl, 1999) using the software Network 4.510 (http://www.fluxus-engineering.com).

Gene flow among *T. californicum* populations

The program MIGRATE-N 3.0 (Beerli & Felsenstein, 1999, 2001; Beerli, 2006) was used to assess dispersal patterns and effective population sizes among *T. californicum* populations within a Bayesian coalescent framework. Analyses were run at two levels. First, to examine dispersal patterns over moderate to long distances. MIGRATE-N analyses were carried out using the meta-samples. Second, to examine short-range dispersal, MIGRATE-N analyses were run only on the original ungrouped Tilden Park samples. The program was used to estimate migration rates between pairs of populations (N_em) and θ ($= N_e\mu$), where N_e is the effective population size, m is the migration rate between two populations and μ is the mutation rate per generation at the locus considered. In each case, a full migration model was examined, allowing unrestricted migration among all groups. The runs consisted of a burn-in discarding 25 000 genealogies, 400 000 sampled genealogies and an adaptive heating scheme spread across four chains. For these analyses, the AFLP profiles were treated as long haplotypic sequences at a single locus. It is possible that this approach could lead to a systematic and slight underestimation of N_em as heterozygosity is obfuscated by the dominant nature of AFLP data; however, attempts to convert each AFLP locus into a single nucleotide polymorphism (SNP) (after converting them to 'codominant' data using the program ABC4F (Foll *et al.*, 2008), with each marker being treated as a single locus, simply highlighted how uninformative are single AFLP loci. Only migration estimates, for which the 95% Bayesian comparison intervals did not include zero, were considered valid ('significant'). The MIGRATE-N analyses supplement traditional estimates of $M = N_em$, calculated under the island model of population structure (Wright, 1931) by ARLEQUIN.

Table 2. Multidimensional scaling and 'Scree' test of pairwise Φ_{ST} values

	Kruskal's stress (*S*)	
No. of dimensions (*k*)	mtDNA (Φ_{ST})	AFLP (Φ_{ST})
1	21.869	33.047
2	**13.485**	**24.561**
3	11.239	18.888
4	9.334	14.848
5	7.731	12.151
6	6.515	10.476

Optimum dimensionality ($k = 2$) indicated in bold.

RESULTS

Marker summary information

In the course of a previous study (Croucher *et al.*, 2011), we generated a total of 521 reproducible and polymorphic AFLP markers (i.e. present in at least one but not all individuals) from 243 individuals of *T. californicum* from California. That study aimed to detect signatures of selection both among the AFLP loci and at the *Colour* locus, and showed that 26 of the AFLP markers (and *Colour*) were putatively under balancing selection and two under positive selection.

Analysis of the 658-bp mitochondrial CO1 gene fragment revealed 19 *T. californicum* haplotypes in 238 individuals. There were 22 variable positions. The majority of haplotypes were rare, with 12 being singletons and three haplotypes (H1: $N = 69$; H2: $N = 61$, H7: $N = 76$) accounting for 86.5% of the individuals. This yielded an overall Nei's (1987) gene diversity of 0.74.

Estimates of gene diversity (AFLP and mtDNA) and Shannon's information index (AFLP) varied considerably among the population samples, no doubt attributable in part to sample size. However,

at the meta-sample level (defined below), these estimates were remarkably homogeneous (see Table 1; mean values among population groups: h_{AFLP} = 0.352 ± 0.043, I_{AFLP} = 0.506 ± 0.060; h_{mtDNA} = 0.556 ± 0.089). Although variable, estimates of h_{AFLP} and I_{AFLP} among all population samples were, as expected, highly correlated (R^2 = 0.99, P < 0.001). However, gene diversity estimates based on AFLP data (h_{AFLP}) were not correlated with those based on mtDNA data (h_{mtDNA}) (R^2 = –0.19, NS).

Spatial population structure of *T. californicum*

Spatial autocorrelation analysis of both the mitochondrial and the AFLP data yielded qualitatively similar results and neither was strongly consistent with an 'isolation-by-distance' model of genetic divergence (Fig. 2A, B, respectively). Although, both mitochondrial haplotypes and AFLP phenotypes were on average highly similar for individuals sampled in the same location (Moran's I = 0.2774 and $F_{(d)}$ = 0.1226, respectively), by less than 100 m, mitochondrial haplotypes were not more similar than expected by chance (Moran's I = 0.0118) and AFLP phenotypes were less similar than expected, although not significantly so ($F_{(d)}$ = –0.0571). With increasing distance classes, the kinship coefficient for the AFLP data tended towards zero, indicating no correlation between genetic similarity and geographic distance. At mean distances of 168 m (I = 0.2478) and 383 m (I = 0.2107), mitochondrial haplotypes again exhibited significantly greater similarity than expected and then became significantly less similar than expected by approximately 1.5 km, with Moran's I tending to zero at greater distances. When the *Colour* locus was analysed (Fig. 2C), this too showed higher than expected similarity among individuals within the same location; however, the kinship coefficient was small ($F_{(d)}$ = 0.0489) and tended towards zero after 100 m.

Preliminary SAMOVA analyses using the mitochondrial data had indicated the presence of an approximately east–west divide among the densely sampled Tilden Park region (see Material and methods). Given the lack of an apparent isolation-by-distance signal in the AFLP data, and only a very local signal in the mitochondrial data, the relationships among the sample populations within genetic space were visualized by MDS analysis. The sample points in Figure 3A and B are marked according to their final groupings into meta-samples (see below). The first thing to note is that the AFLP data resulted in axes with a much greater unit of scale than the mitochondrial data, reflecting the overall much greater level of diversity present in the AFLP data. Second, if one focuses only on the Tilden Park samples (marked as WTP: West Tilden Park; and ETP: East Tilden Park), one can see that these samples are projected in a similar pattern along the first principle coordinate axis for both data sets, with the majority of samples that are left of the zero line in Figure 3A being left of the zero line in Figure 3B, and vice versa. Furthermore, this pattern agrees quite well with the results of the initial SAMOVA analysis and in both plots the geographic relationships among the sample populations are to some extent preserved; however, the clustering is clearly tighter for the mitochondrial data, again indicating a stronger geographical signal in the mitochondrial data than the AFLP data, as suggested by the spatial autocorrelation. The non-Tilden Park samples also group according to geographical location, and this signal too is stronger for the mitochondrial data (Fig. 3A). For example, MCF1, MCF2 and MHP (all from Mill Valley: MIV) cluster together, as do MCR and CAZ (Guerneville: GUV), and ALH1 and ALH2 (Albany Hill: ALH). Although these samples tend to be projected to the periphery of the Tilden Park samples, they are not clearly separated from them – again indicating that genetic distinctness does not increase simply with geographic distance. As both mitochondrial and AFLP-based MDS analyses exhibited broadly concurrent patterns within Tilden Park (despite the fact that correlation between the mitochondrial and AFLP Φ_{ST} distance matrices did not quite reach significance: Mantel test, r = 0.13, P = 0.08564, 10 000 randomizations), the pairwise Φ_{ST} distance matrices for both types of marker were merged using the SDM algorithm (Criscuolo *et al*., 2006) and subject to an unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, yielding two distinct sets of sample populations which genetically and geographically define the final meta-samples for Tilden Park (Fig. 4A): a western set: WTP {SET3, PIT2, LPT, SYT, MET, UPT, PET, LCT4} and an eastern set: ETP {WGT, SET2, MCT2, LCT3, SET1, PIT1, MCT1, LCT2, LCT1}. The same process was repeated for the pairwise Φ_{ST} distance matrices among the six final meta-samples (Fig. 4B), yielding three distinct clusters: {MIV}, {GUV, ETP} and {RUG, WTP, ALH}. Unlike the geographically proximate Tilden Park samples, the meta-samples do not cluster clearly according to geography, supporting a lack of geographic structuring over moderate distances. For the meta-samples, the lack of correlation between geographic distance and genetic distance was confirmed using Mantel tests between the matrix of mean pairwise great circle distances and the pairwise Φ_{ST} distance matrices for both the mtDNA (r = 0.17, P_{exact} = 0.2056) and the AFLP data (r = 0.10, P_{exact} = 0.3361). The mitochondrial and AFLP Φ_{ST}

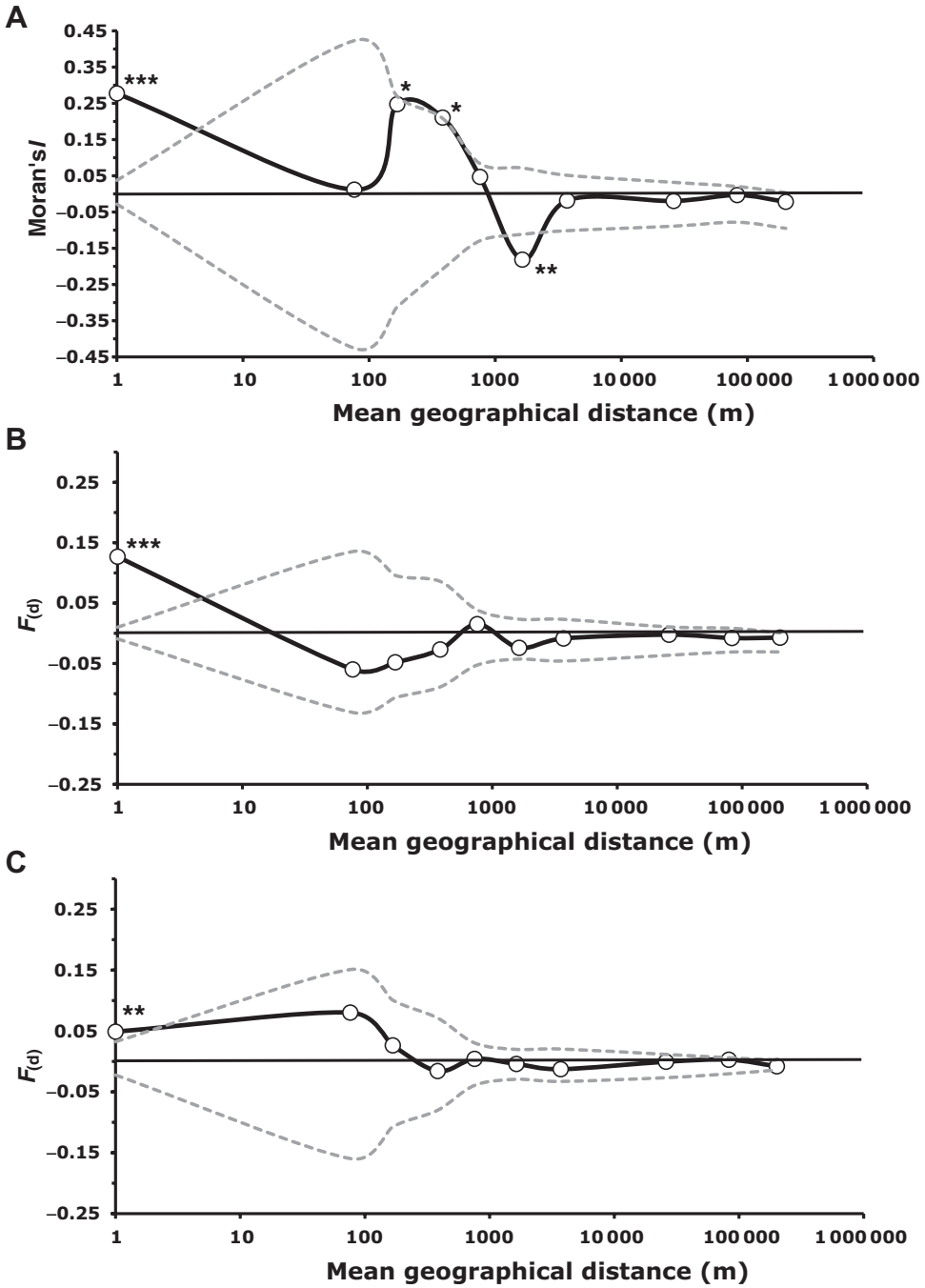

Figure 2. Spatial autocorrelelograms among *T. californicum* samples. A, mitochondrial DNA. B, neutral AFLP. C, *Colour*.

distance matrices were also not correlated ($r = -0.15$, $P_{exact} = 0.3486$). Mantel tests among the samples within ETP and WTP were not meaningful as many Φ_{ST} values were zero.

Analyses of molecular variance within Tilden Park strongly supported this grouping into WTP and ETP meta-samples (Table 3), with 41.04% of mitochondrial variation occurring between WTP and ETP and only 7.73% of the variation occurring among populations within these meta-samples; yielding a Φ_{ST} of 0.4877 ($P < 0.00001$). The AFLP data yielded a similar level of variation (7.19%) among populations within the meta-samples, but had less power to differentiate the meta-samples, with only 11.52% of the variation partitioned between WTP and ETP; yielding a Φ_{ST} of 0.1871 ($P < 0.00001$). The *Colour* locus revealed little

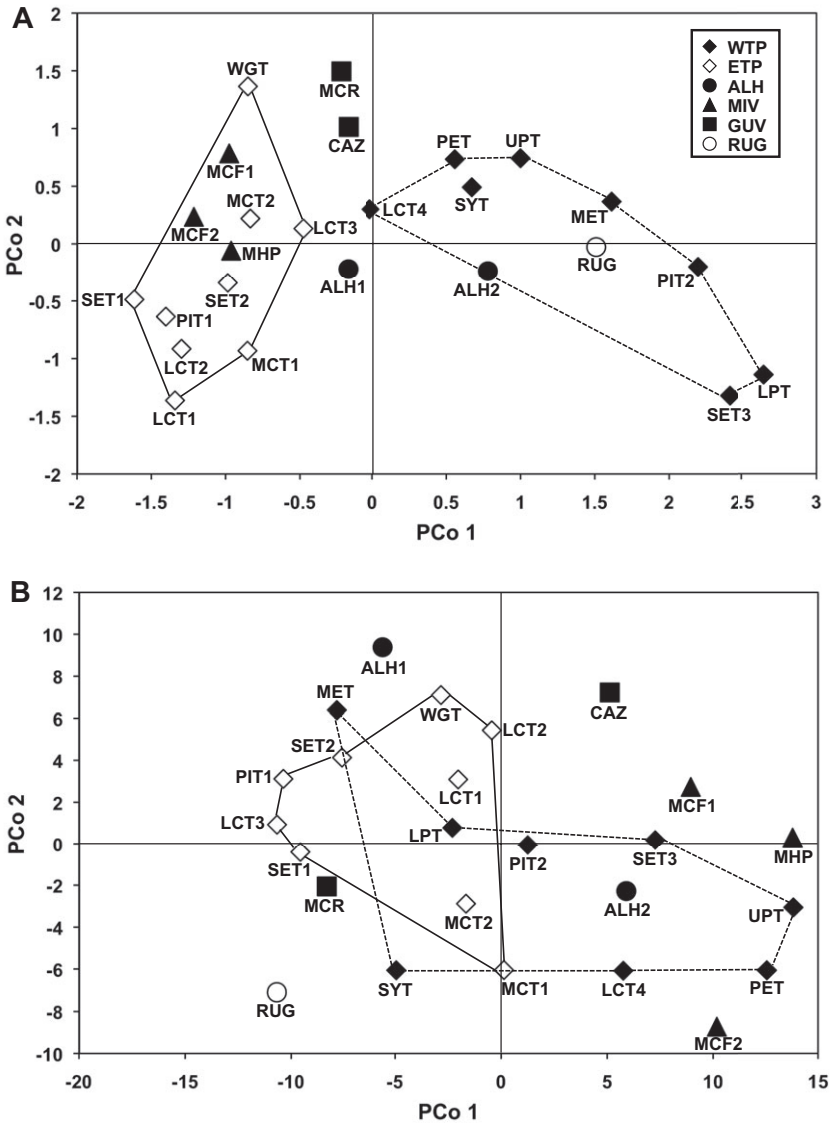

Figure 3. Multidimensional scaling plots (two dimensions) of pairwise Φ_{ST} matrices among the *T. californicum* sample populations. A, mitochondrial DNA. B, neutral AFLP. Markers follow the final meta-sample groupings (see Table 1). Maximum convex polygons connecting populations within East Tilden Park (ETP; dotted lines) and within West Tilden Park (WTP; solid lines) are shown.

variation among populations within meta-samples (0.38%) and only moderate variation between ETP and WTP (7.19%); yielding a non-significant Φ_{ST} of 0.0757. When all samples were included in the AMOVA, the percentage of variation among populations within meta-samples remained similar (mitochondrial: 6.09%; neutral AFLP: 7.62%; *Colour*: 0.70%), but the percentage of variation among the meta-samples was generally much lower [mitochondrial: 26.96% ($\Phi_{ST} = 0.3305$; $P < 0.00001$); neutral AFLP: 6.61% ($\Phi_{ST} = 0.1423$; $P < 0.00001$); *Colour*: 10.87% ($\Phi_{ST} = 0.1157$; $P = 0.0028$)]. Overall, these results indicate a lack of genetic structure over moderate to long geographic distances and confirm the expectation that the *Colour* locus does not effectively discriminate between populations.

Evolutionary relationships among mitochondrial haplotypes

The evolutionary relationships among the mitochondrial DNA haplotypes, and their relative distributions among the meta-samples for the entire data set, were visualized by constructing an MJ network (Fig. 5). The pie diagrams at the nodes (haplotypes) are approximately scaled according to the relative

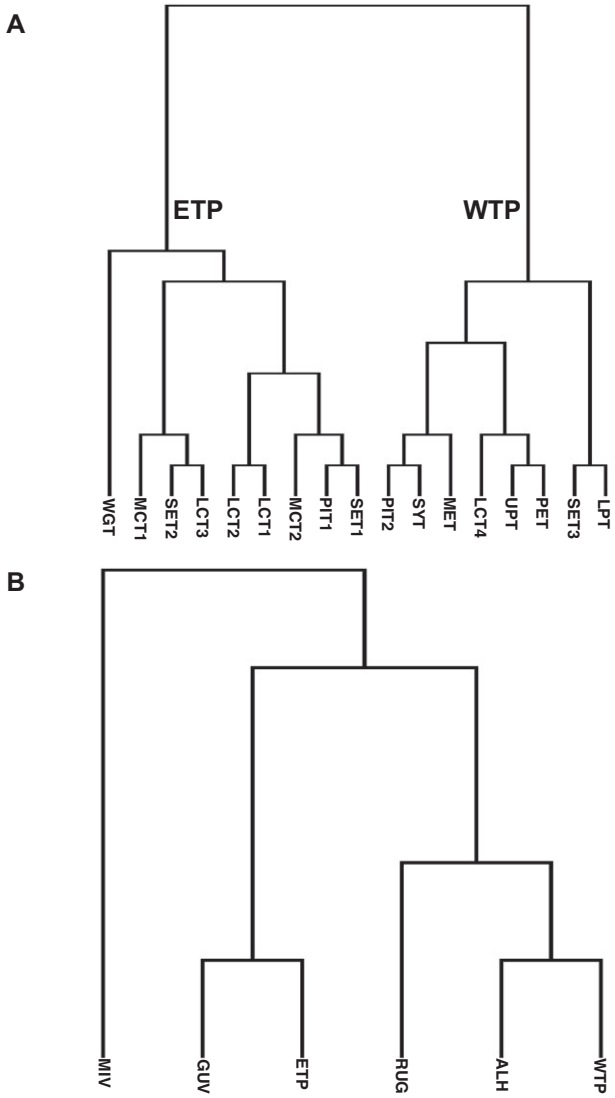

Figure 4. Unweighted pair group method with arithmetic mean (UPGMA) cluster analysis of combined mitochondrial DNA and neutral AFLP pairwise Φ_{ST} distance matrices. A, Tilden Park samples, indicating the resolution into West Tilden Park (WTP) and East Tilden Park (ETP) meta-samples. B, all six meta-samples, indicating the lack of geographical structuring.

frequency of each haplotype within each meta-sample, after adjusting for sample size. The edges indicate the mutational steps connecting each haplotype with the changes traced from the most common haplotype (H1) and the base pair position of the mutation from the 3' end of primer LCO1 1498 (the coding strand) indicated. As may be expected from the weak geographical structuring among the meta-samples, there is little evidence for local patterns of evolution among the haplotypes (Fig. 5) – haplotypes are scattered among the meta-samples and are not obviously found together with their mutational neighbours. However, considering the most common haplotypes (H1, H2 and H7, Fig. 5): H7 is strongly associated with WTP (57.44% of WTP haplotypes), ALH (40.00%) and RUG (46.67%), and was found only three times in ETP (4.69% of ETP haplotypes) and not at all in MIV or GUV. Conversely, H2 was frequent in ETP (56.25% of ETP haplotypes) and MIV (39.13%), absent from RUG and rare in WTP, occurring only five times (5.31% of WTP haplotypes). This difference probably drives the division of the six meta-samples into the three groups {MIV}, {ETP, GUV} and {WTP, ALH, RUG} (Fig. 4B). The most common haplotype, H1, was found in all meta-samples and was approximately equally frequent in WTP (27.66% of WTP haplotypes) and ETP (28.13% of ETP haplotypes).

GENE FLOW AMONG *T. CALIFORNICUM* POPULATIONS

There is clear differentiation between WTP and ETP (Figs 5, 6). The high frequency of mtDNA haplotype H2 in ETP and its relative absence in WTP is evident both among the individual Tilden Park samples and at the meta-sample level. The latter also shows how this haplotype is absent from the more northern samples GUV and RUG. Similarly, the high frequency of H7 in WTP and its relative absence from ETP is also clear. The mitochondrial differentiation between WTP and ETP is also strongly demonstrated by the pairwise Φ_{ST} values among the original samples and the corresponding estimates of migration M ($= N_e m$) given in the Appendix, Table A1. Pairwise estimates of Φ_{ST} among samples within ETP were low, with 25 out of 36 (69.44%) values being zero and yielding correspondingly infinite estimates of M [average $\Phi_{ST} = 0.0590$; median $\Phi_{ST} = 0.0000$; median M (excluding ∞) = 3.64]. No values of Φ_{ST} achieved statistical significance at the 5% level. Pairwise estimates of Φ_{ST} among samples within WTP were slightly higher than for ETP, with only 12 out of 28 (42.86%) values being zero [average $\Phi_{ST} = 0.1590$, median $\Phi_{ST} = 0.0318$; median M (excluding ∞) = 1.50]. Seven values of Φ_{ST} (25.00%) achieved statistical significance at the 5% level. Pairwise estimates of Φ_{ST} between ETP and WTP were much higher than the estimates within these meta-samples [average $\Phi_{ST} = 0.4730$, median $\Phi_{ST} = 0.4385$; median M (excluding ∞) = 0.57]. Only six out of 72 (8.33%) Φ_{ST} values were zero {and these comparisons all involved samples with either small sizes [MCT1 ($N = 1$), MCT2 ($N = 2$), SYT ($N = 2$)] or that were geographically close (LCT3 and LCT4)} and 36 values of Φ_{ST} (50.00%) achieved statistical significance at the 5% level. The overall Φ_{ST} (original samples pooled) between WTP and ETP was 0.4232 ($P < 0.00001$; $M = 0.73$).

Equivalent values of pairwise Φ_{ST} among the original samples and their corresponding estimates of

Table 3. AMOVA on the final meta-sample groupings

	Percentage of variation					
	Tilden Park only			All samples		
Source of variation	mtDNA	AFLP neutral	*Colour*	mtDNA	AFLP neutral	*Colour*
Among groups	41.04	11.52	7.19	26.96	6.61	10.87
Among populations within groups	7.73	7.19	0.38	6.09	7.62	0.70
Within populations	51.23	81.29	92.43	66.95	85.77	88.43
Φ_{ST}	0.4877‡	0.1871‡	0.0757 NS	0.3305‡	0.1423‡	0.1157†
Φ_{CT}	0.4104‡	0.1152*	0.0719*	0.2696‡	0.0661 NS	0.1087†

*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; NS, non-significant (10 000 permutations).

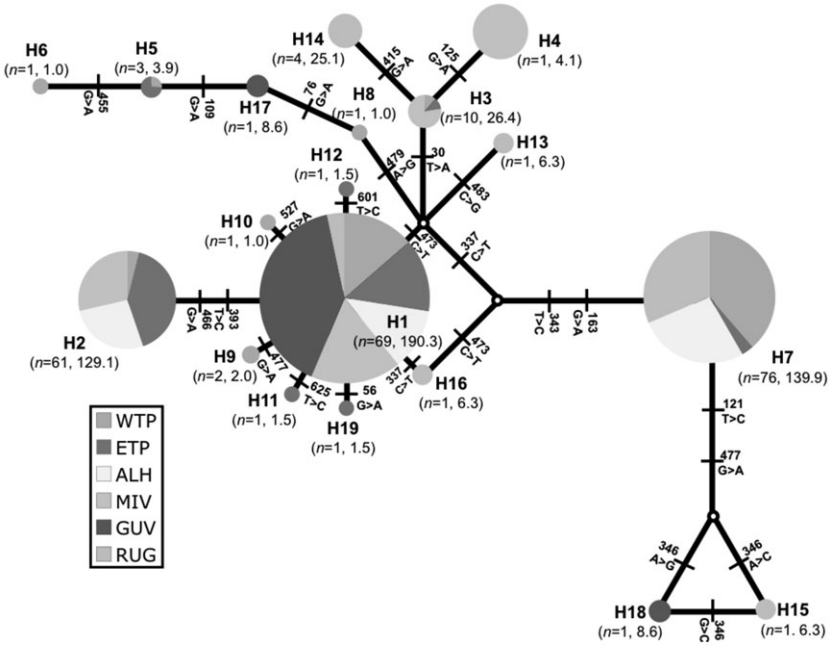

Figure 5. Median-joining network among *T. californicum* mitochondrial haplotypes. The node pies are approximately scaled by the haplotype relative frequency within each meta-sample. Mutational changes are indicated on the graph edges.

migration M ($= N_e m$) for the neutral AFLP data are given in the Appendix, Table A2. In all cases, the neutral AFLP data yielded much lower values of Φ_{ST} and greater estimates of M than the mitochondrial data. Nonetheless, the overall pattern of genetic differentiation was very similar, with samples within ETP showing less differentiation and more gene flow among each other than samples within WTP, and little gene flow between WTP and ETP. Pairwise estimates of Φ_{ST} among samples within ETP were low, with 21 out of 36 (58.33%) values being zero and yielding correspondingly infinite estimates of M [average $\Phi_{ST} = 0.0025$; median $\Phi_{ST} = 0.0000$; median M (excluding ∞) = 40.15]. As for the mitochondrial data, no values of Φ_{ST} achieved statistical significance at the 5% level. Pairwise estimates of Φ_{ST} among samples within WTP were again higher than for ETP, with only 12 out of 28 (42.86%) values being zero [average $\Phi_{ST} = 0.0637$, median $\Phi_{ST} = 0.0000$; median M (excluding ∞) = 5.18]. Eight values of Φ_{ST} (28.57%) achieved statistical significance at the 5% level. Pairwise estimates of Φ_{ST} between WTP and ETP samples were again higher than for the estimates within these meta-samples [average $\Phi_{ST} = 0.0969$, median $\Phi_{ST} = 0.0491$; median M (excluding ∞) = 3.92]. Twenty-five out of 72 (34.72%) Φ_{ST} values were zero and 31 values of Φ_{ST} (43.06%) achieved statistical significance at the 5% level. The overall Φ_{ST} (original

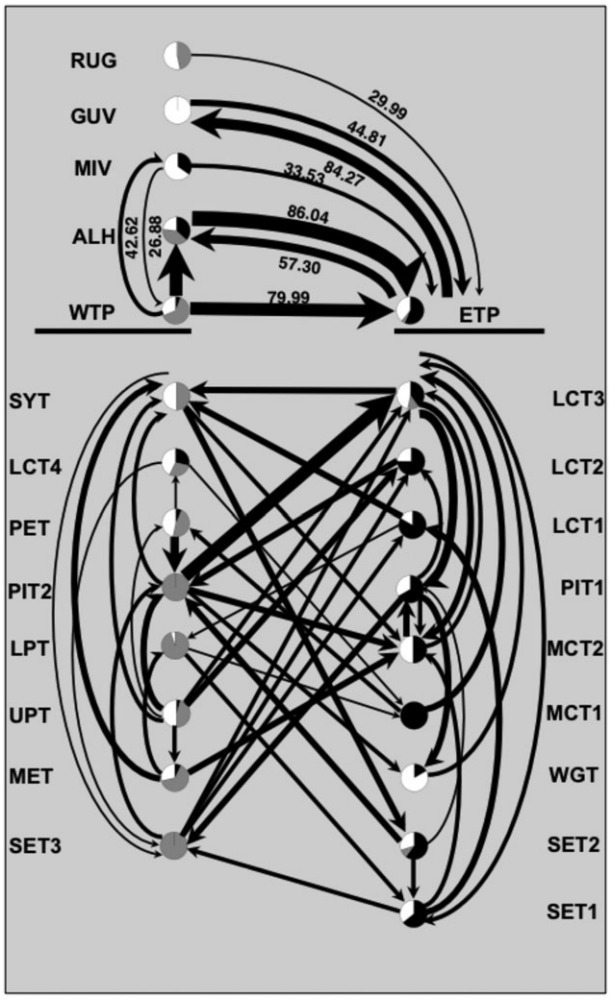

Figure 6. Schematic illustrating migration estimates M ($= N_e m$) from MIGRATE-N among the meta-samples (top half) and among the original Tilden Park samples (lower half), based on AFLP data. Sample population order from top to bottom corresponds approximately to their order from north to south. Arrows are scaled approximately proportional to the size of M. For actual M estimates within Tilden Park, see Appendix (Table A3). The pie diagrams indicate the frequency of key mitochondrial haplotypes within each sample: H2 (black); H7 (grey); all other haplotypes (white).

samples pooled) between WTP and ETP was 0.1225 ($P < 0.00001$; $M = 3.58$).

Bayesian coalescent estimates of migration rates using MIGRATE-N on the AFLP data revealed that the patterns of gene flow between populations of *T. californicum* were not only highly asymmetrical, but complex (Fig. 6, and Appendix Table A3). Examination of the lower half of Figure 6 (Tilden Park) reveals that notable patterns of migration within the WTP and ETP meta-samples are not typically between nearest neighbouring sites (Fig. 1), but rather over longer distances; for example: in WTP from SET3 to PIT2 but not from SET3 to MET; in ETP from LCT3 to SET1 but not from LCT3 to LCT2 (but see Discussion). It is also clear from the lower half of Figure 6 that, although there is complex interconnectivity between WTP and ETP, migration is biased from west to east. This west-to-east bias in migration was confirmed by the MIGRATE-N analyses at the meta-sample level (upper half of Fig. 6): no migration from ETP to WTP was inferred, compared with a value of $M = 79.99$ from WTP to ETP. Patterns of migration among the meta-samples (RUG, GUV, MIV, ALH, WTP and ETP) were also asymmetric. In general, migration appears biased towards the south and the east rather than towards the west and the north; however, the overall pattern is one of complex population interaction. MIGRATE-N analyses were also performed among the meta-samples using the mitochondrial data. No valid migration events were inferred (all estimates of M were low and included zero in the Bayesian 95% comparison interval). Analyses were not carried out among the original Tilden Park sample populations because the mitochondrial data – representing a single and not highly variable locus – were not informative.

Recent demographic change statistics are given in Table 1. For simplicity, we focus on the values given for each of the meta-sample groupings (Table 1, in bold). Analysis of the mismatch distribution under a model of recent demographic expansion and under a model of sudden spatial expansion indicated that the latter was a better fit to the data, with neither Harpending's raggedness R nor the sum of squared deviations (SSD) being a significantly poor fit to the data for any meta-sample. It is also the case that SSD values were consistently lower for the sudden spatial expansion model than for the demographic expansion model, indicating a better fit. Tajima's D was not significant for any meta-sample.

DISCUSSION

Results from the mitochondrial (COI sequence data) and nuclear (AFLP) markers reveal very different patterns of population structure and provide insights into historic and ongoing processes structuring populations in the area.

Population structure

We set out to determine the extent to which populations of *T. californicum* were structured over the newly restored habitat in the San Francisco East Bay Hills, and in particular whether the structure reflects expansion from a single or multiple sources. Analyses

of spatial autocorrelation for both marker types indicated that, although individuals within sample populations were likely to be more genetically similar than expected by chance, beyond approximately 2 km neither marker showed any correlation with geographic distance. At distances less than 100 m, the neutral AFLP data suggested that individuals were less genetically similar than would be expected by chance – indicating that neighbouring populations are not genetic neighbours. The mitochondrial data indicated that at approximately 100 m distance neighbouring populations were on average no more related than expected by chance (Moran's *I* approaches zero), but between approximately 168 m and 383 m mitochondrial haplotypes were again more similar than expected by chance. This suggests that the genetic 'patch' structure (Sokal, 1983) for *T. californicum*, at least for the maternally inherited mitochondrial DNA, is less than *c*. 400 m. The lack of isolation-by-distance that we demonstrate here has also been found in other spiders, suggesting that long-distance dispersal by ballooning might be less effective than normally assumed (Ramirez & Haakonsen, 1999; Bonte *et al*., 2003; Bell *et al*., 2005; Oxford, 2005).

Despite evidence for only very local structuring and a lack of an isolation-by-distance signal, AMOVA, SAMOVA, MDS and cluster analysis all indicated that Tilden Park could be divided into two relatively distinct and differentiated genetic units – a western meta-sample (WTP) and an eastern meta-sample (ETP). Both the AFLP and mitochondrial data supported this conclusion, suggesting that populations on either side of Tilden Park have been established from a number of sources, with subsequent population expansion leading to the appearance of a 'barrier' to dispersal between these meta-samples. However, this barrier does not coincide with any existing landscape features.

The spatial patterns of molecular markers in *T. californicum* in Tilden Park, dividing populations into ETP and WTP groups, are reminiscent of area effects (Cain & Currey, 1963a) that reflect the expansion, into newly available and unoccupied habitat, of genetically differentiated refugia populations (Goodhart, 1963; Cameron *et al*., 1977, 1980; Ochman *et al*., 1983; Goodacre, 2001; Davison & Clarke, 2010). A similar explanation for differentiation in a two-dimensional array of populations has been made for the spider *Enoplognatha ovata* in Nidderdale (England, UK) (Oxford & Shaw, 1986; Oxford, 2005). We have argued that suitable habitat for *T. californicum* may have become widespread in the East Bay region only in the past 50 years or so: The natural wooded vegetation of the area was burned and replaced by extensive grasslands, starting in the mid Holocene with human arrival. Active management maintained these grasslands until the area was acquired as a state park, allowing expansion of the original shrub and woodland habitat (Russell & McBride, 2003). Therefore, most likely explanation for the genetic signatures characterizing ETP and WTP areas is that differentiation (continuous drift and founder/bottleneck events) occurred in remnant patches of suitable habitat once isolated in a matrix of unsuitable grassland. With the re-establishment of scrub and, eventually, woodland, these refugial populations may have expanded until they met at the boundaries identified in this paper. If this scenario is correct, it raises the question of why, in the north of Tilden Park at least, the boundary is so clearly defined. We have no information on the longevity of this boundary, but its clarity suggests that migration across it, at least for the mitochondrial markers, must be low. After discussing population differentiation for the two types of molecular marker, we will return to the question of gene flow.

In all analyses, the mitochondrial data exhibited a greater degree of genetic differentiation and structuring (e.g. higher Φ_{ST} values) than did the neutral AFLP data. This is to be expected given that the maternally inherited haploid mitochondrial genome experiences one fourth of the effective population size (N_e) of the diploid nuclear genome and therefore is subject to higher levels of drift. Although both the mitochondrial and neutral AFLP data reveal common overall patterns in the results presented here, for example the genetic division between WTP and ETP, and the lower estimates of average Φ_{ST} and higher rates of migration within ETP than within WTP, close inspection of Appendix Tables A1 and A2 quickly reveals that individual pairwise estimates of Φ_{ST} (and M) for the two types of marker are not correlated. This is also true for the pairwise Φ_{ST} values among the meta-samples (Mantel test: $r = -0.15$, $P_{exact} = 0.3486$). This suggests that the differences in the estimated values are not simply because of the lower effective population size of the mitochondrial genome, but reflect either differing population histories for the nuclear loci and the mitochondrial genome or the same population history (with at least two source populations), but with differences in gene flow for the two types of marker, as we suggest below.

Gene flow between populations

The actual values of M ($= N_e m$) presented for *T. californicum* must be treated with caution. The following discussion focuses entirely on estimates of M from the Bayesian coalescent analyses even although 'traditional' F_{ST} (Φ_{ST})-based estimates of M ($= N_e m$) were calculated (see Appendix, Tables A1 and A2).

Although F_{ST} (Φ_{ST})-based estimates of M provide useful general comparisons between the AFLP and mitochondrial data, this transformation is always fraught with difficulties (Whitlock & McCauley, 1999). The data presented here – distinctly asymmetric gene flow, between non-adjacent populations – clearly violate Wright's (1931) 'island model', which assumes, among other things, constant numbers of individuals within populations and equal contributions from each to the migrant pool. Furthermore, even the Bayesian estimates of M must be treated cautiously. MIGRATE-N assumes that populations have had stable sizes and migration rates for a long time (Kuhner, 2008), such that inferred migration rates among recently split or non-equilibrium populations are likely to be inaccurate. Consequently, a high-rate of inferred migration between recently established populations may reflect a lack of divergence from a common source population rather than true connectivity between the populations. Nonetheless, even if we disregard the exact magnitude of inferred values of M, the general patterns of asymmetry observed here are likely to be realistic.

Our primary prediction was that gene flow should be sufficiently low to prevent genetic homogenization of populations over the landscape. This is clearly supported, although the patterns revealed by the neutral AFLP and mitochondrial data are quite different. MIGRATE-N indicated no valid migration events among any of the six meta-samples with respect to the mitochondrial data, whereas the neutral AFLP data yielded a complex pattern of asymmetrical gene flow among the meta-samples, predominantly, but by no means exclusively, towards the south and east (Fig. 6, upper half), and most notably from WTP to ETP and not vice versa. Although MIGRATE-N analyses were not carried out for the mitochondrial data among the original samples from Tilden Park, the neutral AFLP-based analysis again showed that migration patterns were complex and highly asymmetric (Fig. 6, lower half; and Appendix, Table A3), with the overall majority of gene flow between samples from WTP to ETP. Of course, the inferred patterns of gene flow identified here act as a surrogate for the effects of all the meta-populations that have not been sampled and should not be taken to imply direct links among the studied populations.

At both the meta-sample and local level (among samples within Tilden Park) the pattern of gene flow deduced from AFLP data is in stark contrast to the mitochondrial haplotype frequencies displayed in the pie diagrams in Figure 6. Haplotype H7, for example, is essentially absent from ETP, despite apparently extensive nuclear gene flow into ETP sample populations from WTP populations, where H7 is common.

Consequently, the disparities between the mitochondrial (haploid, maternally inherited) and nuclear (AFLP, diploid) markers imply fundamental differences between male and female dispersal abilities in *T. californicum*. In females, local dispersal is apparently limited and results in very local structuring of mtDNA diversity. In males, however, there is a greater tendency to disperse locally, which is not unexpected as sub-adult and adult males must wander in search of females. Local dispersal may occur via short range 'rappelling' (Bonte *et al*., 2003) and, consequently, the west-to-east asymmetry in gene flow observed for the neutral AFLP markers may simply be a function of the predominantly west-to-east winds characteristic of the Pacific coast.

CONCLUSION

Records of land-use change in the San Francisco East Bay suggest that spatial expansions from isolated pockets of suitable habitat may have occurred very recently (within the last 50–100 years). Based on the analysis of mismatch distributions, similar spatial expansions resulting from a return to 'natural' habitat may have also occurred in the other sample locations (for example, Russian Gulch State Park (RUG) and Albany Hill (ALH) are also recently protected/recreational natural areas). We do not know how long this pattern has been established and so it is impossible to determine how stable the pattern may be, whether the observed boundary is moving and if the two areas (ETP and WTP) will merge with time. Future monitoring of the genetic structure of the Tilden Park area, together with wider sampling within the southern area of the Park and the surrounding area, to determine exactly where the boundary lies, would therefore be of great value.

The very distinct molecular area effects apparent in Tilden Park are unexpected; spiders are normally considered rather mobile organisms. However, local, historically generated population substructuring has previously been reported in another theridiid spider, *Enoplognatha ovata*. This species shows marked micro-area effects for colour and molecular markers on a scale of hundreds of metres in Nidderdale (England, UK) (Oxford & Shaw, 1986; Oxford, 2005). Here morph-frequency patterns show remarkable spatial and temporal stability, implying little migration between populations (Oxford, 2005). These patterns were probably established in the late 1940s during major habitat disturbance and so the 'ghost' of this event, in the form of differentiation of allele frequencies, has persisted for at least 60 years (= 60 generations) and shows little sign of decay. Tilden Regional Park was designated in 1936, but re-establishment of suitable habitat may have started

well before this time. The *Enoplognatha* example demonstrates just how long historical perturbations can exert an influence on populations if gene flow is relatively limited. In *T. californicum*, the signal of past population fragmentation and consequent differentiation, so striking for the molecular markers, has been lost at the *Colour* locus, possibly implying stronger selection for colour in this species than in *E. ovata*.

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APPENDIX

Table A1. Pairwise Φ_{ST} values (above diagonal) and corresponding estimates of M ($=N_em$) (below diagonal), generated by ARLEQUIN, for mitochondrial CO1 data among samples from Tilden Park

	WTP								ETP								
	SET3	PIT2	LPT	SYT	MET	UPT	PET	LCT4	WGT	SET2	MCT2	LCT3	SET1	PIT1	MCT1	LCT2	LCT1
SET3	. . .	0.0000	0.0000	0.4737	0.1303	**0.3158**	**0.3619**	**0.5270**	**0.8776**	**0.6988**	0.9160	**0.5311**	**0.8284**	**0.8897**	1.0000	**0.9215**	**0.9349**
PIT2	∞	. . .	0.0000	0.0000	0.0000	0.0000	0.0576	0.1667	0.7778	0.5370	0.6000	0.3109	0.7652	0.8000	1.0000	0.8182	0.8824
LPT	∞	∞	. . .	0.5287	**0.2079**	**0.3846**	**0.4400**	**0.6494**	**0.8738**	**0.7745**	**0.8923**	**0.6317**	**0.8535**	**0.8918**	0.9310	**0.9063**	**0.9157**
SYT	0.56	∞	0.45	. . .	0.0000	0.0000	0.0000	0.0000	0.2800	0.1946	0.0000	0.0000	**0.4909**	0.4684	0.0000	0.4563	0.6185
MET	3.34	∞	1.91	∞	. . .	0.0017	0.0844	0.1159	**0.4207**	**0.3842**	0.3615	**0.2571**	**0.5433**	**0.5108**	0.4652	**0.5105**	**0.5739**
UPT	1.08	∞	0.80	∞	301.41	. . .	0.0059	0.0000	**0.2332**	**0.2744**	0.1942	**0.1517**	**0.4188**	**0.3865**	0.3605	**0.3971**	**0.4682**
PET	0.88	8.18	0.64	∞	5.42	84.29	. . .	0.0000	**0.1463**	**0.2096**	0.0905	0.0746	**0.3510**	**0.3103**	0.2482	**0.3187**	**0.3959**
LCT4	0.45	2.50	0.27	∞	3.82	∞	∞	. . .	0.0341	0.0071	0.0000	0.0000	0.1846	0.1282	0.0000	0.1244	0.2553
WGT	0.07	0.14	0.07	1.29	0.69	1.64	2.92	14.15	. . .	0.1208	0.0000	0.0227	0.2255	0.2560	0.4546	0.3333	0.4828
SET2	0.22	0.43	0.15	2.07	0.80	1.32	1.89	70.00	3.64	. . .	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MCT2	0.05	0.33	0.06	∞	0.88	2.08	5.03	∞	∞	∞	. . .	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
LCT3	0.44	1.11	0.29	∞	1.44	2.80	6.20	∞	21.50	∞	∞	. . .	0.0807	0.0239	0.0000	0.0115	0.1108
SET1	0.10	0.15	0.09	0.52	0.42	0.69	0.92	2.21	1.72	∞	∞	5.69	. . .	0.0000	0.0000	0.0000	0.0000
PIT1	0.06	0.13	0.06	0.57	0.48	0.79	1.11	3.40	1.45	∞	∞	20.44	∞	. . .	0.0000	0.0000	0.0000
MCT1	0.00	0.00	0.04	∞	0.57	0.89	1.51	∞	0.60	∞	∞	∞	∞	∞	. . .	0.0000	0.0000
LCT2	0.04	0.11	0.05	0.60	0.48	0.76	1.07	3.52	1.00	∞	∞	43.00	∞	∞	∞	. . .	0.0000
LCT1	0.03	0.07	0.05	0.31	0.37	0.57	0.76	1.46	0.54	∞	∞	4.01	∞	∞	∞	∞	. . .

Φ_{ST} values in bold are statistically significant at $P < 0.05$.

Table A2. Pairwise Φ_{ST} values (above diagonal) and corresponding estimates of M ($=N_em$) (below diagonal), generated by ARLEQUIN, from AFLP data among samples from Tilden Park

	WTP								ETP								
	SET3	PIT2	LPT	SYT	MET	UPT	PET	LCT4	WGT1	SET2	MCT2	LCT3	SET1	PIT1	MCT1	LCT2	LCT1
SET3	. . .	0.0000	0.0259	0.0000	**0.1240**	0.0676	0.0683	0.0000	**0.0805**	**0.1101**	0.0000	**0.1677**	**0.1562**	**0.1335**	0.0000	0.0450	0.0258
PIT2	∞	. . .	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0769	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000
LPT	18.80	∞	. . .	0.0000	**0.0441**	**0.1720**	**0.1683**	**0.0615**	0.0203	**0.0393**	0.0000	**0.0649**	**0.0600**	**0.0495**	0.0000	0.0000	0.0000
SYT	∞	∞	∞	. . .	0.0000	0.1399	0.1249	0.0077	0.0720	0.0000	0.1132	0.0000	0.0000	0.0000	0.1154	0.0519	0.0000
MET	3.53	∞	10.84	∞	. . .	**0.2534**	**0.2428**	**0.1707**	0.0358	**0.0241**	0.0000	**0.0275**	**0.0179**	0.0000	0.0000	0.0109	0.0299
UPT	6.90	∞	2.41	3.07	1.47	. . .	0.0000	0.0550	**0.2035**	**0.2406**	0.1132	**0.2775**	**0.2661**	**0.2346**	0.0000	**0.1787**	**0.1659**
PET	6.82	∞	2.47	3.50	1.56	∞	. . .	0.0572	**0.1956**	**0.2346**	0.1008	**0.2697**	**0.2581**	**0.2255**	0.0000	**0.1666**	**0.1636**
LCT4	∞	∞	7.63	64.81	2.43	8.58	8.24	. . .	**0.0852**	**0.1329**	0.0000	**0.1859**	**0.1734**	**0.1463**	0.0000	0.0513	0.0260
WGT	5.71	∞	24.16	6.44	13.46	1.96	2.06	5.37	. . .	0.0025	0.0000	0.0053	0.0024	0.0138	0.0000	0.0000	0.0000
SET2	4.04	∞	12.21	∞	20.25	1.58	1.63	3.26	61.79	. . .	0.0000	0.0000	0.0129	0.0106	0.0000	0.0037	0.0024
MCT2	∞	6.00	∞	3.92	∞	3.92	4.46	∞	∞	∞	. . .	0.0000	0.0000	0.0000	0.0642	0.0000	0.0000
LCT3	2.48	∞	7.20	∞	17.70	1.30	1.35	2.19	40.67	∞	∞	. . .	0.0147	0.0000	0.0000	0.0104	0.0167
SET1	2.70	∞	7.84	∞	27.42	1.38	1.44	2.38	119.25	81.35	∞	34.98	. . .	0.0000	0.0000	0.0000	0.0234
PIT1	3.25	∞	9.60	∞	∞	1.63	1.72	2.92	23.31	126.77	∞	∞	∞	. . .	0.0000	0.0281	0.0028
MCT1	∞	0.00	∞	3.83	∞	∞	∞	∞	∞	∞	24.00	∞	∞	∞	. . .	0.0000	0.0000
LCT2	10.61	∞	∞	9.13	45.40	2.30	2.50	9.24	∞	∞	∞	39.62	∞	15.37	∞	. . .	0.0000
LCT1	18.91	∞	∞	∞	16.22	2.51	2.56	18.70	∞	179.53	∞	25.43	23.37	163.50	∞	∞	. . .

Φ_{ST} values in bold are statistically significant at $P < 0.05$.

Table A3. Estimates of the mean number of migrants exchanged per generation M ($=N_em$) and the mean population mutation rate θ ($N_e\mu$), generated by MIGRATE-N, from AFLP data among samples from Tilden Park

		M														
		Source														
		WTP							ETP							
Sink	θ	SET3	PIT2	LPT	SYT	MET	UPT	PET	LCT4	WGT	SET2	MCT2	LCT3	SET1	PIT1	MCT1	LCT2	LCT1
SET3	0.090	...	...	...	28.92	...	...	...	28.70	...	...	...	...	38.33	43.29	...	...	...
PIT2	0.086	36.42	...	...	...	...	50.26	57.22	...	...	45.09	...	...	...	...	...	42.85	...
LPT	0.094	...	...	...	...	37.82	...	...	...	...	...	...	...	...	...	...	...	26.65
SYT	0.087	...	31.79	...	...	43.40	41.45	...	...	...	...	...	47.76	...	...	...	...	45.35
MET	0.095	...	...	...	...	...	40.85	...	...	...	...	...	...	...	...	...	...	...
UPT	0.099	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
PET	0.097	...	...	...	...	...	28.83	...	...	...	...	...	...	...	...	39.20	...	...
LCT4	0.091	...	...	...	...	...	...	33.43	...	...	...	...	...	...	...	...	...	...
WGT	0.084	...	32.06	...	...	...	...	...	...	...	...	...	...	...	50.02	...	...	...
SET2	0.093	...	...	...	43.95	...	...	...	...	...	...	...	...	...	...	...	...	...
MCT2	0.084	...	45.79	...	34.54	48.74	...	...	...	...	...	...	44.79	32.25	34.14	...	...	...
LCT3	0.089	32.36	78.77	...	...	...	38.26	...	...	35.95	...	37.15	...	...	...	40.56	...	...
SET1	0.092	...	...	32.36	...	...	...	...	...	...	34.32	...	33.99	...	...	...	...	...
PIT1	0.088	...	...	...	...	...	...	...	...	...	30.01	40.78	53.74	...	...	...	...	...
MCT1	0.097	...	...	28.17	...	...	...	...	27.69	...	...	...	...	...	...	...	...	...
LCT2	0.081	31.33	...		...	...	42.16	...	...	...	...	...	...	...	34.89	...	...	...
LCT1	0.075	38.89	...		...	...	...	...	...	...	...	...	...	49.77	...	...	...	...