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The evolution of geographic parthenogenesis in *Timema* walking-sticks

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Abstract

Phylogenetic studies of asexual lineages and their sexual progenitors are useful for inferring the causes of geographical parthenogenesis and testing hypotheses regarding the evolution of sex. With five known parthenogens and well-studied ecology, *Timema* walking-sticks are a useful system for studying these questions. *Timema* are mainly endemic to California and they exhibit the common pattern of geographical parthenogenesis, with asexuals exhibiting more-northerly distributions. Neighbour-joining and maximum-parsimony analyses of 416 bp of mitochondrial cytochrome oxidase I (COI) from 168 individuals were used to infer general phylogenetic relationships, resulting in three major phylogeographical subdivisions: a Northern clade; a Santa Barbara clade; and a Southern clade. A nested cladistic analysis, comparing intra- and interspecific haplotypic variation on a geographical scale, revealed that the overall pattern of geographical parthenogenesis in *Timema* could be attributed to historical range expansion. These results suggest that geographical parthenogenesis is the result of more-extensive northerly dispersal of asexuals than sexuals.

Keywords: nested clade analysis, parthenogenesis, phylogeography, walking-sticks

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Introduction

Geographic parthenogenesis, first noted by Vandel in 1928, refers to the common pattern of asexuals being either more northerly, more widespread, residing in harsher habitats, or at higher elevations than sexual relatives (Bell 1982; Lynch 1984; Stearns 1987; Gaggiotti 1994; Peck *et al*. 1998). Why is geographical parthenogenesis a common pattern? This question has been difficult to address since the best explanation is one that combines historical geographical, ecological, and glaciation events with the assumption that asexuals have better dispersal ability than sexuals and are better able to persist at the edges of a geographical range (Bell 1982; Stearns 1987). Generally speaking, events or processes leading to speciation and the production of asexuals, coupled with competitive and demographic differences between sexuals and asexuals, have led to the geographical patterns we observe today. Understanding the geographical distribution of asexuality relative to sex should be important in determining why sex is maintained in most species (Bell 1982).

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Tests to distinguish between alternate hypotheses for the advantages of sex have been difficult to formulate (Barton & Charlesworth 1998). Consequently, many cases of geographical parthenogenesis have been explained qualitatively by comparing geographical patterns of asexuals relative to sexuals (Glesener & Tilman 1978). However, simply noting relative distributions has limited value for distinguishing between possible explanations for the evolution of sex. Hypotheses for the pattern of geographical parthenogenesis can be grouped into two categories: (i) ecological competition hypotheses, and (ii) demographic hypotheses. Competition between asexuals and sexuals is thought to play an important role in the establishment of asexuality (Cuellar 1977; Glesener & Tilman 1978; Barton & Charlesworth 1998). Under an ecological scenario, asexuals are better competitors or have an advantage over sexuals under certain ecological conditions. Asexuality is maintained in close proximity to sex due to competition (Cuellar 1977). In this case, geographical parthenogenesis is due to differences in competitive ability as they relate to ecology.

By contrast, demographic effects are linked to the twofold advantage of asexuality. If a population is expanding and an individual has a reproductive advantage, then the 'new' area will have more of that individual's offspring. As

well, parthenogenesis may be an advantage in a colonizing species since a single individual can establish a new population (Gerritsen 1980; Bell 1982; Stearns 1987; Peck *et al*. 1998). Asexuals may also be better able to persist at the edges of a geographical range or in marginal habitats (Bell 1982; Stearns 1987; Peck *et al*. 1998). When low population densities result in mate limitation, for example at the edge of a geographical range, there should be a higher proportion of individuals with parthenogenetic ability because asexuals are not limited by the necessity of finding a mate (Gerritsen 1980; Bell 1982; Peck *et al*. 1998). Those individuals found where population density is high (for example at the centre of a range) would have no such mate limitation and consequently sex would predominate (Bell 1982; Peck *et al*. 1998). With demographic effects, the pattern of geographical parthenogenesis is due to the ability of asexuals to spread to new areas faster than sexuals, because they colonize more readily in unstable metapopulations, or because they spread more rapidly over longer historical timespans.

There are five described parthenogenetic species of *Timema* walking-sticks, each with a morphologically similar sexual relative (Table 1) (Vickery 1993; Sandoval & Vickery 1996; Sandoval *et al*. 1998; Vickery & Sandoval 1999). Each parthenogen feeds on the same or similar host plants as its sexual counterpart, with asexual populations being predominately female. Of the parthenogens, four (*T. douglasi*, *T. shepardii*, *T. genevieve* and *T. tahoe*) fit the criteria of being all or more northerly in distribution than sexual relatives, with the fifth, *T. monikensis*, being only slightly southeast of its closest sexual relative (Fig. 1).

Previous phylogenetic work suggested that the genus *Timema* originated in the southern part of its current distribution at least 20 Ma and expanded northward (Sandoval *et al*. 1998). The onset of the most recent series of glaciations in North America 2–3 Ma may have served as a climatic trigger for extensive speciation in *Timema* (Sandoval *et al*. 1998). Though the glaciers did not reach California, glaciation events would have caused extensive cooling, resulting in host plants moving to lower altitudes (Sandoval *et al*. 1998). Consequently, *Timema* would have been able to disperse between mountain ranges during this time, with subsequent warm periods isolating *Timema* populations on different mountains, and promoting speciation. Sexual and asexual species should both exhibit range expansion under this scenario. If asexuals have higher dispersal ability and are better able to persist at the edges of a geographical range, as theory suggests, then *Timema* parthenogens should be at the leading edge of the overall genus expansion northward. The pattern of geographical parthenogenesis may therefore be a result of range expansion.

The purpose of this paper is twofold. Firstly, we greatly extended phylogenetic studies of Sandoval *et al*. (1998) to include recently collected and newly discovered species, and by the inclusion of extensive intraspecific samples, and we used this phylogeny to further evaluate the evolutionary history of this genus and describe general biogeographical patterns. Secondly, to test the hypothesis that geographical parthenogenesis in *Timema* is the result of range expansion, we used a nested cladistic analysis. This type of analysis has the ability to discriminate between the alternative hypotheses of range expansion, allopatric fragmentation, and restricted dispersal (restricted gene flow in sexual species) (Templeton *et al*. 1995; Templeton 1998),

Sexual Species	Sexual Species Host Plant	Morphological Asexual Counterpart	Asexual Species Host Plant
T. poppensis	A, B	*T. douglasi*	A
T. californicum	C, D, G, H	*T. shepardii*	C
T. cristinae	D, E, H	*T. monikensis*	D, I
T. podura	E, D, G, I	*T. genevieve*	E
T. bartmani	F	*T. tahoe*	F
T. knulli	B, D	—	—
T. landelsensis	C	—	—
T. petita	D	—	—
T. boharti	C, D, E, G	—	—
T. chumash	D, G, I	—	—
T. ritensis	K	—	—
T. nevadense	K	—	—
T. dorotheae	D	—	—
T. nakipa	C, D, E, G	—	—
*T. coffmani***	K	—	—
*T. morongensis***	J	—	—

Species marked by ** are not included in the phylogenetic analyses.

Table 1 Sexual: asexual *Timema* pairs and their host plant usage. Sexual *Timema* species that have no known asexual counterpart and their host plant usage are also shown. *T. coffmani* (Sandoval & Vickery 1999) was not sequenced and *T. morongensis* was discovered after this work was performed. Host plants: A = *Pseudotsuga menziesii* (Douglas fir); B = *Sequoia sempervirens* (Californian redwood); C = *Arctostaphylos* spp. (manzanita); D = *Ceanothus* spp.; E = *Adenostoma fasiculatum* (chamize); F = *Abies concolor* (white fir); G = *Quercus* spp. (oak); H = *Heteromeles arbutifolia* (toyon); I = *Cercocarpus* spp.; J = *Eriogonum* sp.; K = *Juniperus* spp. (juniper)

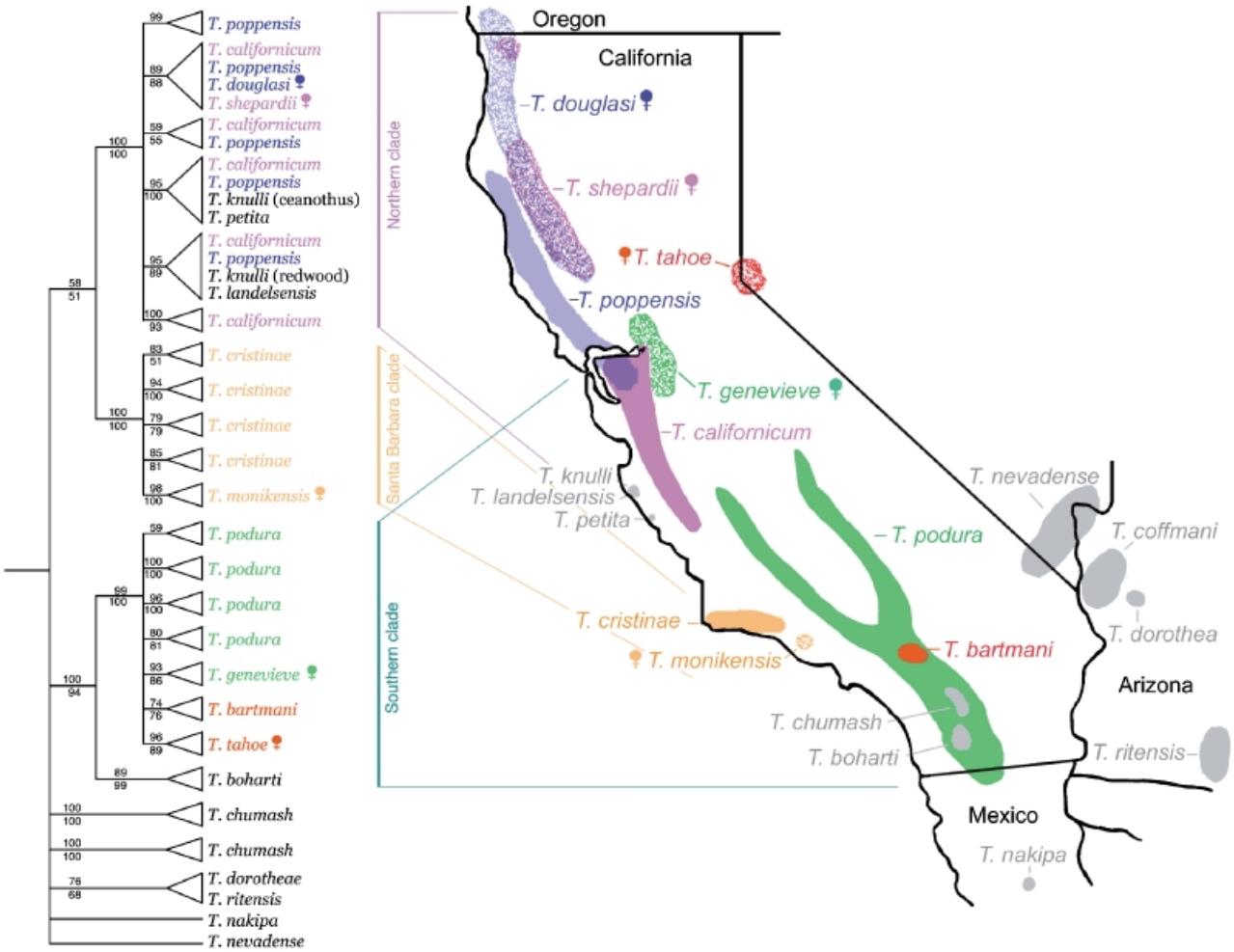


Fig. 1 General phylogenetic relationships within *Timema* obtained through neighbour joining (NJ) and maximum parsimony (MP) analyses of mitochondrial COI. Species positions are given within triangle tips. Asexual lineages are designated by female symbols. Numbers above branches are NJ bootstrap values and numbers below show MP bootstrap values. Fig. 2 shows detailed views of the Northern, Santa Barbara, and Southern clades. Also shown are the geographical distributions of all *Timema* species (adapted from Sandoval *et al*. 1998). The newly described species *T. morongensis* has been collected west of *T. chumash* but its full distribution is unknown.

and thus can be used to evaluate the evolutionary causes of geographical parthenogenesis.

Materials and Methods

Taxonomy and geographic distributions

Timema are herbivorous stick insects, most of which inhabit chaparral vegetation (Vickery 1993; Sandoval *et al*. 1998; Law 2001; Nosil *et al*. 2002). This genus belongs to the order Phasmatoptera and it apparently represents the sister group to the rest of the phasmatids (Vickery 1993; Vickery & Sandoval 2001). The identification of parthenogenetic *Timema* species has relied on a combination of host plant use, colour, body morphology, laboratory rearing, and a lack of males (Vickery 1993; Sandoval & Vickery 1996; Vickery & Sandoval 2001).

Each parthenogenetic *Timema* species has a close morphological sexual counterpart (Table 1) which feeds on the same or overlapping hosts plants but does not overlap with it in range. The parthenogen *T. douglasi* was previously paired with *T. californicum* (Sandoval *et al*. 1998), but an apparently closer sexual, *T. poppensis*, has recently been sampled (Vickery & Sandoval 1999). *T. monikensis* has been described as the closest asexual relative of *T. cristinae* (Vickery & Sandoval 1998). Although *T. monikensis* has been shown to reproduce parthenogentically (Vickery & Sandoval 1998; Vickery & Sandoval 2001); males (of unknown viability) have also been collected in this species.

The host plant use and geographical distributions of other sexual species, including the recently discovered sexuals, *T. knulli*, *T. petita*, and *T. landelsensis*, are also given in Table 1 and Fig. 1. *T. knulli* is a previously described

species known only from pinned specimens, leading Sandoval & Vickery (1996) to suggest it was a synonym of *T. californicum*. However, in the spring of 1999, *T. knulli* was collected at Big Creek Reserve and it has since been re-described as a valid species (Vickery & Sandoval 2001).

Collection

Timema walking sticks were collected throughout California from as many geographically widespread sites as possible (Appendix). The focus of collecting was on extensively sampling the sexual: asexual pairs from as many geographically separate populations as possible. Since the nested cladistic analysis depends critically upon sampling, as well as finding the closest sexual relative for each asexual, the goal was to accurately represent extant genetic variation. For each site, *Timema* were shaken from their respective host plants using a sweep net. Long-term male mate-guarding behaviour is prevalent in this genus and adult females of sexual species are rarely found without a male riding on their back (Bartman & Brock 1995; Sandoval & Vickery 1996; Vickery & Sandoval 2001).

Walking-sticks were kept alive in jars until they could be processed for DNA sampling. For each collected insect 1–3 legs were removed with forceps and dried in silica gel, to be used for DNA extraction. If the legs were small, the head was also removed and stored in silica gel. The body was kept in 75% ethanol as a voucher specimen, and representative voucher specimens have been deposited in the Lyman Entomological Museum (McGill University, Montreal).

DNA samples

DNA was extracted from silica-gel stored legs. Usually one leg was large enough for this procedure, but in the case of smaller species or juvenile individuals, multiple legs from the same individual or the head were used. The tissue was crushed with a sterile glass pipette and suspended in 0.9 mL Lifton buffer (0.2 M sucrose, 0.05 M EDTA, 0.1 M Tris, 0.5% SDS). The DNA was extracted using a phenol chloroform protocol and 70% ethanol precipitation. Polymerase chain reaction (PCR) was performed using combinations of the mitochondrial COI primers S2183 (CAA CAT TTA TTT TGA TTT TTT GG) and S2195 (TTG ATT TTT TGG TCA TCC WGA AGT) with A2566 (CCT ATA GAI ART ACA TAA TG) and A3014 (TCC AAT GCA CTA ATC TGC CAT ATT). PCR product was processed using exonuclease I and shrimp alkaline phosphatase. Big Dye Cycle Sequencing was used to sequence a fragment about 450 bp long.

Sequence analysis and phylogeny reconstruction

Sequences were aligned by eye using the program SE-AL version 2.0 (Rambaut 2001). PAUP 4.0b8 (Swofford 2000) was used to analyse the data by neighbour joining (NJ) (under a Kimura two-parameter model of evolution) and maximum parsimony (MP) searching (heuristic search, simple addition of sequences, TBR branch swapping). Maximum likelihood was too computationally intensive for the total dataset. Trees were assessed for robustness using bootstrapping (1000 replicates for NJ and 200 for MP). As in Sandoval *et al*. (1998) three outgroups were used — the phasmatids *Baculum extradentatum* and *Anisomorpha buprestoidea*, and the cockroach *Blatella germanica*.

Nested cladistic analysis

We used nested cladistic analysis (NCA) to relate genetic and geographical distance and infer the population-genetic and demographic causes of the geographical distribution of haplotypes. The first step in this analysis is to determine the 95% statistical parsimony limit and estimate the haplotype network, as given by the algorithms in Templeton *et al*. (1992). The program TCS (Clement & Posada 2000) was used to estimate the mtDNA haplotype network for each sexual: asexual pair and to calculate the 95% statistical parsimony plausible limit. This limit is a measure of how many steps can separate two haplotypes with confidence that parsimony is supported.

Once the haplotype networks are constructed, the next step is to construct a nested cladogram, the full rules of which are given in Templeton *et al*. (1987), and Templeton & Sing (1993). Following the nesting procedure, each clade is tested for geographical structure, against the null hypothesis of no geographical associations (see Templeton *et al*. 1995; for full methodology). The two main test statistics are the clade distance, D_c (X), and the nested clade distance, D_n (X). D_c (X) is a measure of how geographically widespread haplotypes are within the nested *n*-step clade X, and D_n (X) is a measure of how geographically distant haplotypes are within the *n*-step clade X from all haplotypes in the clade within which it is nested (i.e. the clade at the next higher level) (Templeton *et al*. 1995). The average distance of interior to tip (I-T) clades is also measured for D_c and D_n (Templeton *et al*. 1995). The statistical significance of each of these test statistics is determined at the 5% level with 1000 permutations. The interpretation of these results is conducted using an inference key (Templeton *et al*. 1995; Templeton 1998; February 2001 updated version available at: http://bioag.byu.edu/zoology/crandall_lab/geodis.htm). These statistics were calculated using GEODIS 2.0 (Posada *et al*. 1999). A useful description and justification of nested clade analysis is provided in Templeton (2002).

There are three possible scenarios that could explain the observed patterns of geographical parthenogenesis: range expansion; allopatric fragmentation; and restricted dispersal. The methodology described above is unique in its ability to discriminate between these alternative hypotheses.

Firstly, if *Timema* parthenogens have experienced a range expansion north, then northerly populations should be younger (tip haplotypes) than southerly ones (interior haplotypes). There should also be fewer widespread haplotypes in northern areas and more haplotype variation in southerly pre-expansion regions (Templeton *et al*. 1995; Templeton 1998). If geographical sampling is adequate, it is possible to discriminate between continuous range expansion vs. colonization (abrupt establishment of populations in a new area).

A second possible model that could explain geographical distributions is allopatric fragmentation (Templeton *et al*. 1995; Templeton 1998). Immediately after the split, each new sexual population will reflect the prefragmented population and thus the populations will be indistinguishable from one another. If asexuality arose in one of the sexual populations and drove this sexual progenitor extinct, then as time increases, mutations occur independently in the isolated populations, and sexuals and asexuals become genetically differentiated.

Lastly, under a scenario of restricted dispersal (restricted gene flow in sexual species), the geographical extent of a haplotype tends to be associated with age; an older haplotype will be more widespread (Templeton *et al*. 1995; Templeton 1998). This situation would be indicated by the finding that young asexual haplotypes were less widespread than older sexual ones. When a new asexual haplotype starts to spread, it will often remain within the geographical range of its ancestors, especially under an isolation-by-distance model. In addition, because the ancestral sexual haplotype is expected to be most frequent near its geographical origin, most asexual derivatives of these haplotypes will also occur near this area.

The ability to differentiate between these alternative hypotheses is dependent upon adequate sampling. None of the events described above are mutually exclusive, and the nested cladistic analysis can assess these different events at various hierarchical levels within the cladogram. This methodology has been applied extensively to inferring intraspecific relationships and to a lesser degree, evolutionarily close interspecific relationships (Templeton 2002). Since a sexual: asexual pair should share a recent common ancestor, this connection can be treated as intraspecific. Therefore, the nested cladistic analysis was applied to each *Timema* sexual: asexual pair separately, and, in the case of evolutionary-close species, to those connections at the 95% statistical parsimony level.

Results

mtDNA variation and general phylogenetic relationships

The analysis of 416 bp of mitochondrial COI from 168 *Timema* individuals resulted in 122 haplotypes. New sequences (154) have been deposited at GenBank under the accession numbers AF409998–AF410151. This segment of COI is AT rich and GC deficient, as estimated from the dataset (A; 27.3%, C; 16.1%; G: 19.2%; T: 37.4%). About half (207) of the sequenced sites were constant. Of the 209 variable sites, 174 were parsimony informative. Third-position changes accounted for most of the informative sites (132 sites, 75.9%), followed by first and second position changes (32 sites, 18.4%; 10 sites, 5.7%, respectively). Most changes were synonymous (89.5%).

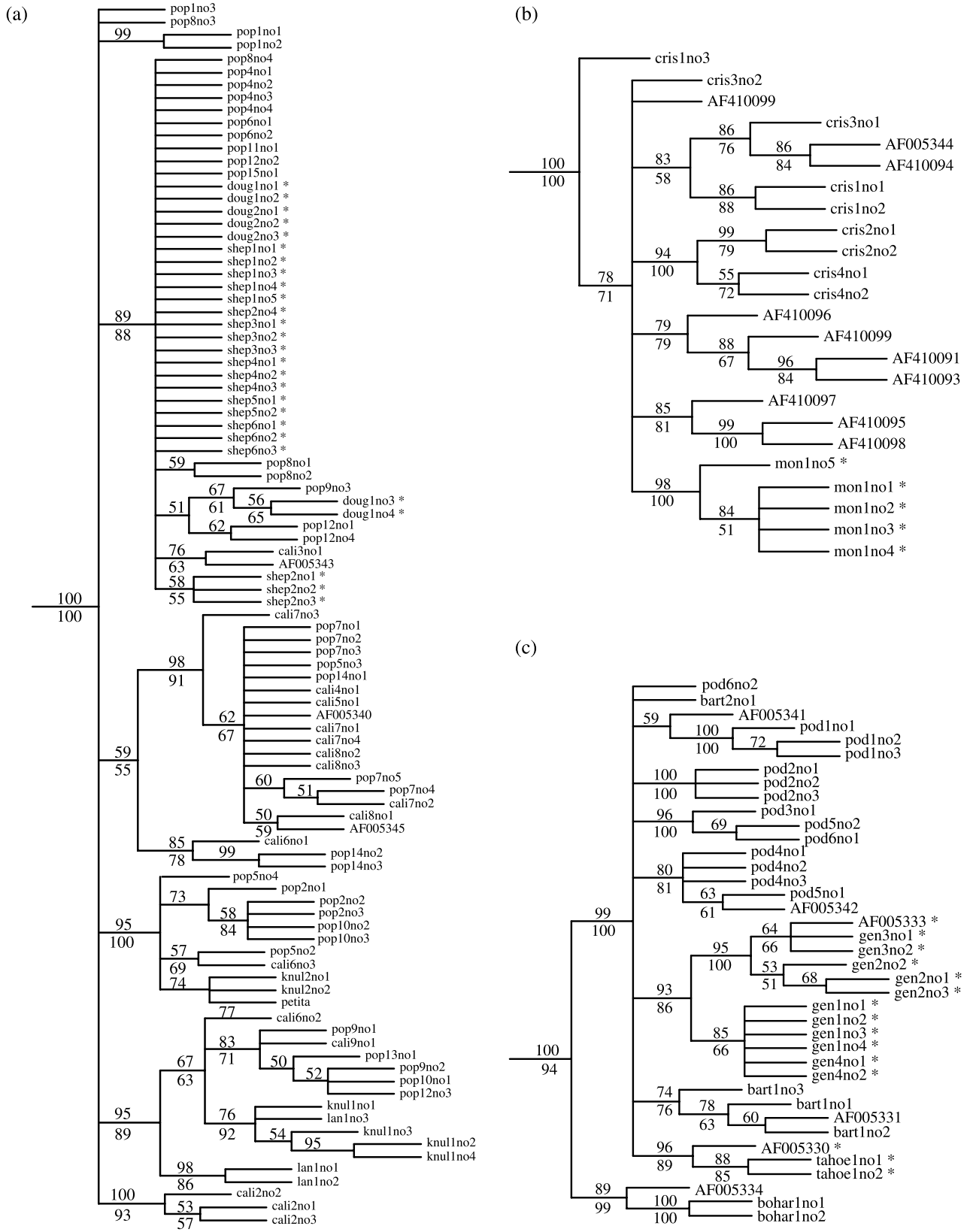
The topologies of the neighbour joining and maximum parsimony tree were almost identical, resulting in three major phylogeographical subdivisions: a Northern coastal clade (hence called Northern), a Santa Barbara clade, and a Southern interior clade (hence called Southern) (Fig. 1). The southernmost sexual species *T. chumash* and *T. nakipa*, the Arizona species *T. ritensis* and *T. dorotheae*, and the Nevada species *T. nevadense*, were not contained within these subdivisions and were basal to these groupings (Fig. 1). The bootstrap values for these divisions were high. Divergence between the Northern and Santa Barbara clades ranged from 12–15%, with the Southern clade being 16–19% from either the Northern or Santa Barbara clades.

The two sexual: asexual pairs *T. poppensis*: *T. douglasi* and *T. californicum: T. shepardii*, in the Northern clade were sampled in the Coastal Mountain Range from just below San Francisco to the Oregon border (Fig. 1). Unexpectedly, the parthenogenetic taxa *T. douglasi* and *T. shepardii* were very closely related, having either almost identical or identical haplotypes (Fig. 2A). These asexual species both grouped with some individuals from the most northern *T. poppensis* populations. *T. californicum* and *T. poppensis*, the two sexual species, were polyphyletic. Furthermore, *T. poppensis* and *T. californicum* found in the same geographical area, in particular just below San Francisco Bay, were very closely related (under 1% divergence) and in some cases had the same haplotype.

In addition to these sexual: asexual pairs, the newly sampled species *T. knulli*, *T. petita* and *T. landelsensis* demonstrated interesting phylogenetic relationships. Populations of *T. knulli* on different host plants (redwood and *Ceanothus*) were genetically distinct for COI, with average divergence of 4.13 ± 0.34%. *T. knulli* on *Ceanothus* was more closely related to *T. petita* than it was to *T. knulli* on redwood. Conversely, *T. knulli* on redwood was genetically closer to *T. landelsensis* found on *Archytostaphylos* (manzanita) than to other *T. knulli*.

The Santa Barbara clade contained the sexual: asexual pair *T. cristinae*: *T. monikensis*. The asexual formed a monophyletic group that was nested within its maternal sexual progenitor (Fig. 2B).

The Southern clade contained the sexual species *T. boharti*, as well as the sexual: asexual pairs *T. podura: T.*


(a)
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pop8no3
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pop4no4
pop6no1
pop6no2
pop11no1
pop12no2
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genevieve and *T. bartmani: T. tahoe* (Fig. 2C). There was a bifurcation in this group, with *T. boharti* being basal to the rest of the taxa in this clade. *T. genevieve* was clearly monophyletic and comprised two genetically distinct populations that were separated by about 200 km. Both of these populations were northeast of San Francisco and were far from the closest sexual relative *T. podura*. *T. podura* populations from different locations generally had haplotypes that were most similar to other individuals from the same locality. Monophyly of the sexual: asexual pair *T. podura: T. genevieve* was not strongly supported by bootstrapping, nor was there any suggestion of a lack of monophyly. The asexual *T. tahoe*, sampled only in the White Fir (*Abies concolor*) near Lake Tahoe, was also monophyletic. Monophyly of the sexual: asexual pair *T. bartmani: T. tahoe* was also not strongly supported by bootstrapping, but there was no evidence for a lack of monophyly.

Nested cladistic analysis

Three distinct and separate networks were constructed, which were concordant with the phylogenetic subdivisions described above. The 95% statistical parsimony limit for the dataset was 8 steps. The number of mutational steps between each one of these subdivisions was a minimum of 30 steps. Since this method was designed to infer intraspecific processes, each sexual: asexual pair was first allocated to a single network and the analyses were conducted as such. However, the Northern clade contained many closely related haplotypes for the sexual: asexual pairs *T. poppensis*: *T. douglasi*, and, *T. californicum*: *T. shepardii*, as well as haplotypes for *T. knulli*, *T. petita*, and *T. landelsensis*. Because these taxa were not separable based on the mtDNA dataset, the analysis was performed using all of these species to form the Northern cladogram (Fig. 3). The Santa Barbara cladogram (Fig. 4) contains *T. cristinae* and *T. monikensis*, as in the phylogenetic tree. Even though *T. podura*: *T. genevieve* and *T. bartmani*: *T. tahoe* were joined within the same network (in the Southern clade) at the 8 step level (Fig. 5), it made no difference to the results if they were divided into separate analyses or analysed together.

The Northern clade

As in the NJ and MP phylogeny, *T. douglasi* and *T. shepardii* were both closely related to *T. poppensis* (clades 3–6 and 3–7) while separated from *T. californicum* (clade 3–5) (Fig. 4). *T. knulli* was separated into two disjoint networks, with clade 3–2 containing *T. knulli* on redwood and clade 3–4 having *T. knulli* on *Ceanothus*. *T. poppensis* and *T. californicum* from various localities were nested within all three higher level clades (5–1, 5–2, and 5–3).

There were two types of ambiguities within this structure: circularities and separation of clades by more steps than given under 95% statistical parsimony. Circularities where there were unobserved haplotypes (zeros) between represented haplotypes were broken and haplotypes were grouped according to the rules in Templeton & Sing (1993) and Clement & Posada (2000). Circularities in clades 1–12, 1–18, and 1–32 could not be resolved and haplotypes were therefore grouped at that level. These circularities corresponded to the result that these haplotypes were either almost identical or identical to one another. The second ambiguity was the separation of clades 4–1, 3–4, 4–2, 2–3, 4–3, and 3–10 by more steps than given for 95% statistical parsimony. However, these *b*-step networks matched the inferred relationships in the phylogeny above, with each of those clades supported by a high bootstrap value. The agreement between the phylogeny and this network provided confidence that these subdivisions were valid under maximum parsimony.

The nested contingency analysis for the Northern cladogram network showed statistical significance ($P < 0.05$ indicating significant associations between haplotype and geography, depicted in Fig. 3 with a '*') mainly for higher level clades. No tests were significant at the 1-step clade level, two were significant at the 2-step level (2–8 and 2–14), four were significant at the 3-step level (3–4, 3–6, 3–7, 3–10), and all were significant at higher levels (4–2,

Fig. 2 (*Opposite*) Neighbour-joining and maximum parsimony trees for the Northern, Santa Barbara, and Southern clade subdivisions of *Timema* walking-sticks. In all cases, numbers above branches indicate NJ bootstrap values and numbers below are MP bootstraps. Parthenogenetic species are designated by asterisks. (A) Phylogenetic relationships among *Timema* species in the Northern clade. Species codes are as follows: pop = *T. poppensis*; doug = *T. douglasi*; cali = *T. californicum*; shep = *T. shepardii*; knul1 = *T. knulli* (redwood); knul2 = *T. knulli* (ceanothus); lan = *T. landelsensis*; petita = *T. petita*. The first number after each species code designates a population location while the second number refers to a different individual. For example, pop1no1 is *T. poppensis* from locality one, individual number one. AF005343- AF005345 refer to *T. californicum* that were sequenced by Sandoval *et al*. (1998). (B) Phylogenetic relationships between *T. cristinae* and *T. monikensis* in the Santa Barbara clade. Species codes are as follows: cris = *T. cristinae*, and mon = *T. monikensis*. The first number refers to a locality while the second number designates individual. AF005340 refers to *T. cristinae* that was collected and sequenced by Sandoval *et al*. (1998) and AF410091-AF410099 were sequenced by Nosil *et al*. (2002). (C) Phylogenetic relationships among *Timema* species in the Southern clade. Species codes are as follows: pod = *T. podura*; gen = *T. genevieve*; bart = *T. bartmani*; tahoe = *T. tahoe*; bohar = *T. boharti*; with numbers referring to sampling location and individual, respectively. The designations AF005341 and AF005342 refer to *T. podura*; AF005333 refers to *T. genevieve*, and AF005334 refers to *T. boharti*; these individuals, as well as the *T. tahoe* AF005330 and the *T. bartmani* AF005331, were sequenced by Sandoval *et al*. (1998).

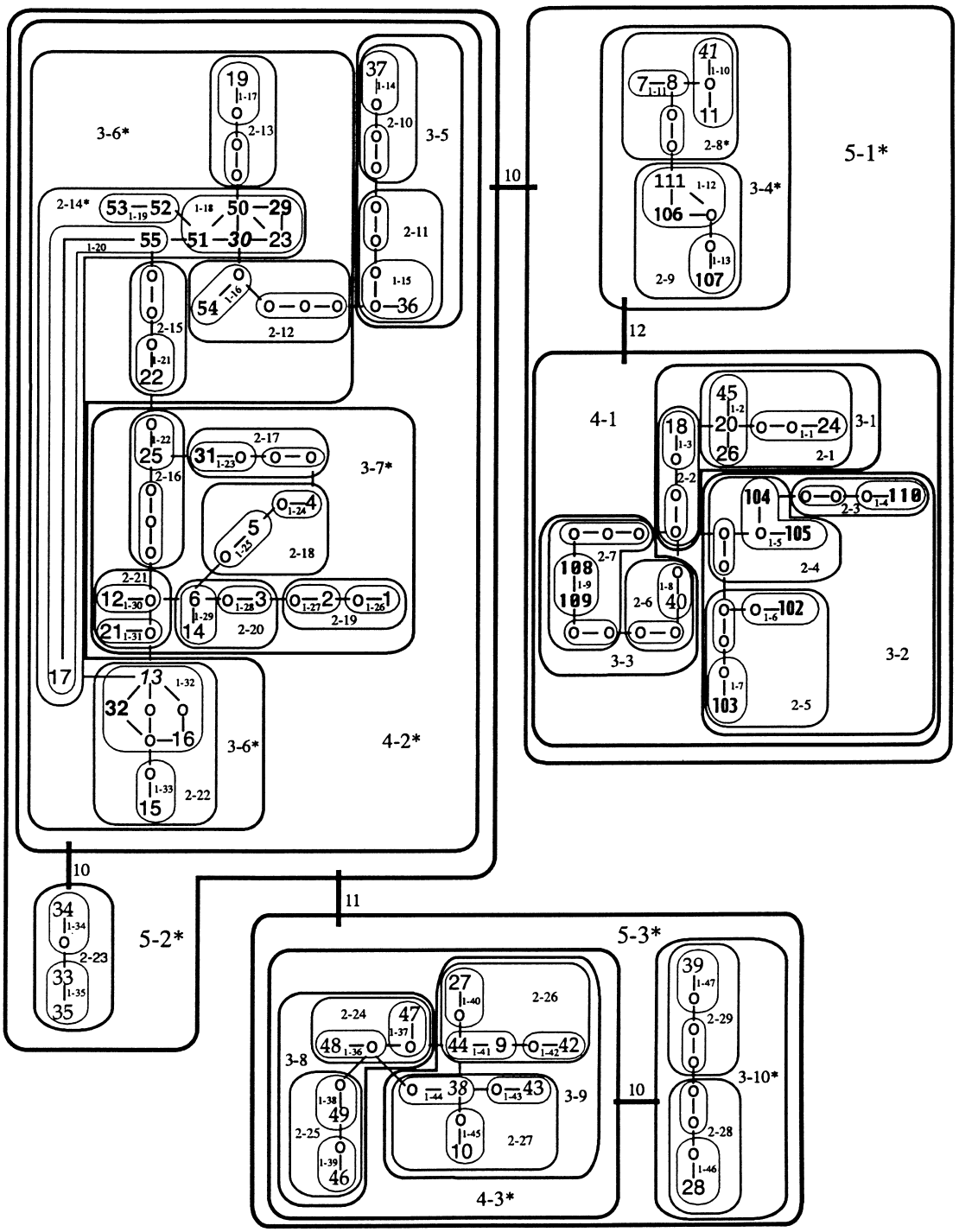

Fig. 3 The Northern cladogram. Numbers and fonts represent different haplotypes and species as follows: 1–28 = *T. poppensis*; 29–32 = *T. douglasi*; 33–49 = *T. californicum*; 50–55 = *T. shepardii*; 102–105 = *T. knulli* (redwood); 106–107 = *T. knulli* (ceanothus); 108–110 = *T. landelsensis*; 111 = *T. petita* (see Appendix for details). Haplotypes that are italicised contain more than one species, as follows: *13, 38, 41* = *T. poppensis*, and *T. californicum*; 30 = *T. douglasi* and *T. shepardii*. Lines between haplotypes represent a single mutational step supported at the 95% statistical parsimony level. Zeros are inferred intermediates. Numbers beside heavy solid lines denote that many mutational steps between clades. The nested clade level is given in a hierarchical manner; 1-*n* for 1-step clades, 2-*n* for 2 step clades, … , 5-*n* for 5 step clades. The whole cladogram is a 6–1 step clade. Clades marked by *, as well as the whole cladogram, were significant at the 5% level by a chi-square test of geographical structure.

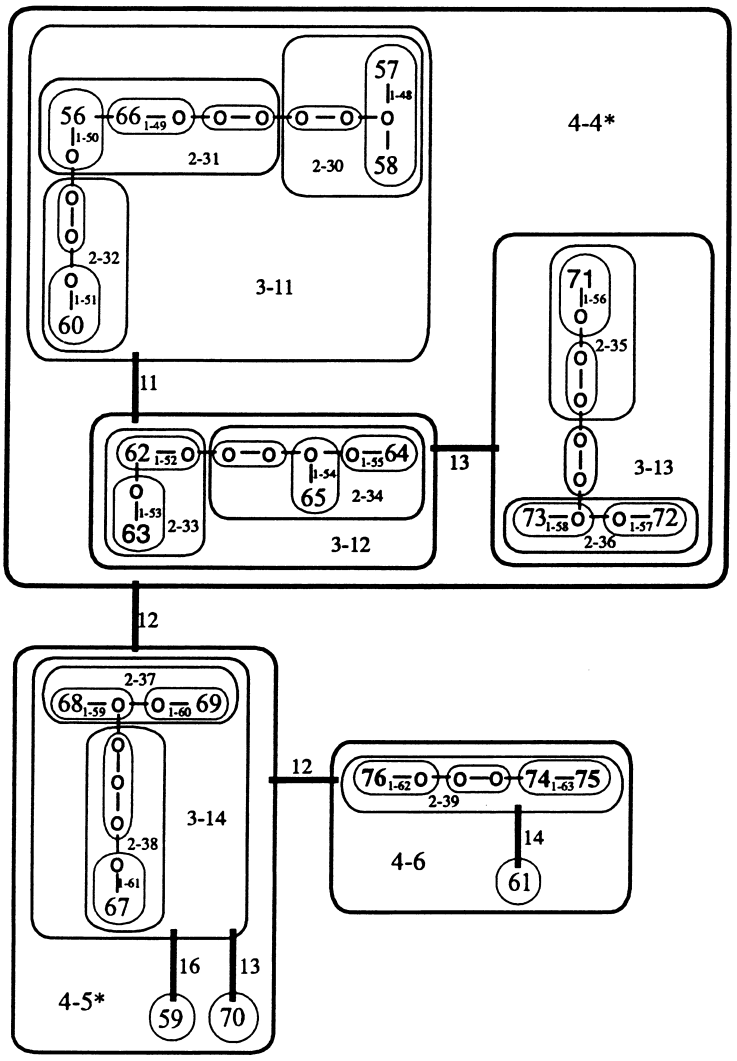

Fig. 4 The Santa Barbara cladogram. Numbers and fonts represent different haplotypes and species as follows: 56–73 = *T. cristinae*; 74–76 = *T. monikensis* (see Appendix for details). Lines between haplotypes are inferred mutational steps at the 95% parsimonious level, with zeros representing hypothetical intermediates. Heavy lines with numbers denotes that number of mutational steps between clades. Clade levels are designated as for the Northern cladogram (Fig. 3). The whole cladogram is a 5–4 step clade. Geographical associations, as determined by a chi-square test, were significant at the 5% level for those clades marked by *, as well as for the whole cladogram.

4–3, 5–1, 5–2, 5–3, 6–1). The results of the nested geographical analysis (D_n and D_x) are not shown, but are available upon request. The chain of inference results for significant clades are shown in Table 2. The clades containing both sexual and asexual species gave results of restricted dispersal by distance (2–14), contiguous range expansion (3–6), contiguous range expansion or long distance colonization (4–2), and long distance colonization (5–2). Fragmentation or isolation by distance was inferred for clade 4–1 (containing *T. knulli* on redwood, *T. landelsensis*, *T. poppensis*, and *T. californicum*). Overall, the sampling design was inadequate to differentiate between contiguous range expansion and long distance colonization for all taxa within the Northern cladogram.

The Santa Barbara Clade

The chi-square test for geographical structure was significant for most higher clade levels in this cladogram (clades 4–4, 4–5, and 5–4; significant Fig. 4 clades marked by a '*'). As in the other cladograms, there were more steps than statistically significant between *b*-step networks (Fig. 4). The nested analysis results (D_n and D_x) are available upon request. Each grouping reflected the structure seen in the NJ and MP phylogenetic tree. The inferred pattern for all significant clades was range expansion (Table 3). However, *T. monikensis*, the parthenogen, grouped together with only one *T. cristinae* haplotype (clade 4–6). Since the contingency analysis was not significant, there was insufficient evidence to infer geographical structure within this clade. At the total cladogram level, the overall inference was one of contiguous range expansion.

The Southern Clade

As for the other species pairs, higher clade levels were significant for the nested contingency analysis (clade 3–18, 4–9, 4–10, 5–5; Fig. 5 significant clades marked by a '*'). There

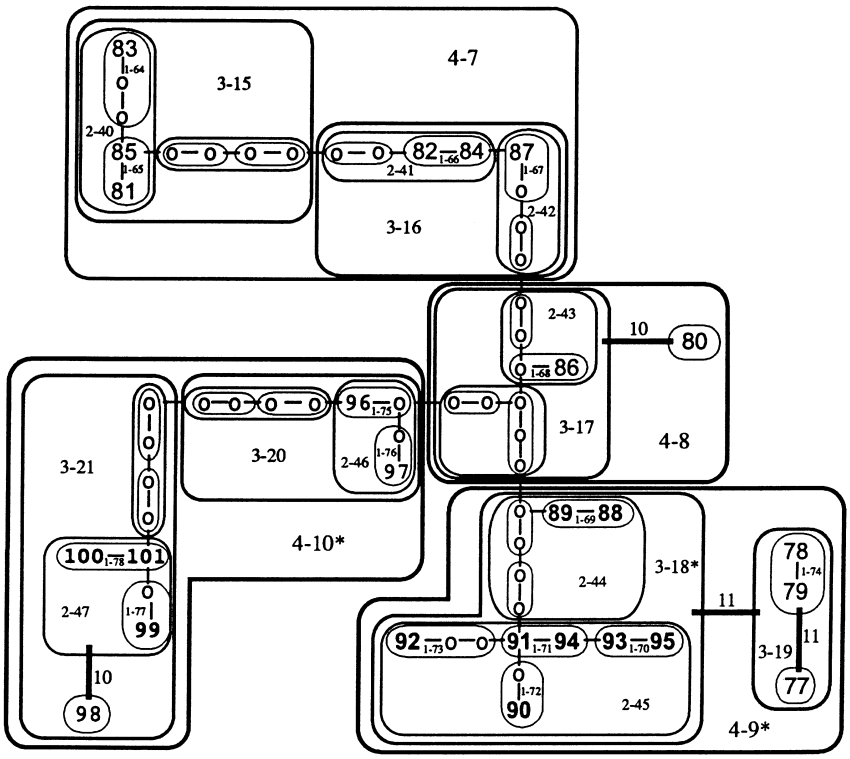

Fig. 5 The Southern cladogram. Numbers and fonts represent different haplotypes and species as follows: 77–87 = *T. podura*; 88–95 = *T. genevieve*; 96–98 = *T. bartmani*; 99–101 = *T. tahoe* (see Appendix for details). Solid lines represent one mutational step, with zeros being intermediate haplotypes not sampled. Statistical parsimony is supported at the 95% level. Heavy lines have a greater number of mutational steps between clades, as given by the number beside. The whole cladogram is a 5–5 step clade. Chi-square tests were significant for those clades marked by *, as well as the whole cladogram, at the 5% level, indicating geographical structure.

were few ambiguities in this nested clade analysis; some connections were a greater number of steps than allowed under 95% statistical parsimony. However, the topology of the cladogram (Fig. 5) shown matched that of the phylogenetic tree (Fig. 2C). The nested results (D_n and D_x) are available upon request. The chain of inference is shown in Table 4. Clade 3–18 contained *T. genevieve* from two geographically distant populations (clades 2–44 and 2–45). The chain of inference for this analysis is consistent with contiguous range expansion within the asexual lineage (Table 4).

At the next higher clade level, 4–9, *T. genevieve* nested with *T. podura* from the Sierra Madre mountains and Sequoia National Park, and the inference was consistent with past fragmentation. The geographical structure of the *T. bartmani*: *T. tahoe* clades was significant only at the 4–10 level (Fig. 5, marked by a '*'). As in the Northern cladogram, the parthenogenetic *T. tahoe* haplotypes were nested interior to their sexual counterparts. The nested clade analysis for this clade indicates range expansion with long distance colonization. At the total cladogram level, the inference was contiguous range expansion (Table 4).

Discussion

Phylogeography of Timema

Timema appears to have originated in the south and expanded north, consistent with previous inferences (Sandoval *et al*. 1998). There are three distinct phylogenetic subdivisions, the Northern, Santa Barbara, and Southern clades, which correspond well with the geography of California, and the sexual species from southernmost California, Arizona, and Mexico are basal to these subdivisions. One notable difference to previous findings is the placement of *T. chumash*. Previous work suggested that this species was a close sister group to *T. podura* sampled from the same area. The phylogeny presented in this paper demonstrates that *T. chumash* is actually a basal species that does not cluster with *T. podura*.

The Northern clade contains numerous polytomies with low or no divergence, suggesting that these taxa are of very recent origin (see also Law & Crespi 2002). Unexpectedly, *T. shepardii* appears more closely related to *T. poppensis* (and *T. douglasi*) than to *T. californicum*, its presumed closest sexual relative. There are five possible hypotheses that could account for this finding: (i) *T. poppensis* is actually the closest sexual relative; (ii) there is a species/gene tree ambiguity for mitochondrial COI; (iii) the closest *T. californicum* was not sampled or has become extinct; (iv) *T. shepardii* is a hybrid of *T. poppensis* and *T. californicum*, exhibiting the morphology and host plant use similar to *T. californicum* but having mtDNA close to *T. poppensis*; and (v) *T. poppensis* and *T. californicum* (and thereby *T. douglasi* and *T. shepardii*) are not different species. It is not possible to distinguish between these hypotheses without data from nuclear loci.

Table 2 Inference chain for nested geographical analysis of the Northern cladogram using the inference key given in Templeton (1998). The localities for sexual species are given by the following letter designations: N = north of San Francisco, SF = San Francisco, B = below San Francisco. The asexuals *T. douglasi* and *T. shepardii* are north of San Francisco

Clade	Species	Chain of Inference	Inferred Pattern
2–8	*T. poppensis* (SF, N) *T. californicum* (SF)	1-2-3-4-no	Restricted gene flow with isolation by distance
2–14	*T. poppensis* (N) *T. douglasi* *T. shepardii*	1-2-3-4-no	Restricted isolation by distance in nonsexual species; restricted gene flow in sexual species
3–6	*T. poppensis* (N) *T. douglasi* *T. shepardii*	1-2-11-12-no	Contiguous range expansion
3–7	*T. poppensis* (N) *T. douglasi*	1-2-11-17-no	Inconclusive
4–1	*T. poppensis* (N) *T. californicum* (SF) *T. knulli* redwood (B) *T. landelsensis* (B)	1-2-3-4-9-10-no	Geographic sampling scheme inadequate to discriminate between fragmentation or isolation by distance
4–2	*T. poppensis* (N) *T. douglasi* *T. shepardii* *T. californicum* (B)	1-2-3-5-6-13-14-no	Sampling design inadequate to discriminate between contiguous range expansion and long distance colonization
5–2	*T. poppensis* (N) *T. douglasi* *T. shepardii* *T. californicum* (B)	1-2-11-12-13-yes	Long distance colonization
6–1	*T. poppensis* (SF, N) *T. douglasi* *T. californicum* (SF, B) *T. shepardii* *T. knulli* redwood (B) *T. knulli* ceanothus (B) *T. landelsensis* (B) *T. petita* (B)	1-2-3-5-6-13-14-no	Sampling design inadequate to discriminate between contiguous range expansion and long distance colonization

Table 3 Inference chain for nested geographical analysis of the Santa Barbara cladogram using the inference key given in Templeton (1998)

Clade	Species	Chain of Inference	Inferred Pattern
4–4	*T. cristinae*	1-2-11-12-no	Contiguous range expansion
4–5	*T. cristinae*	1-2-11-12-no	Contiguous range expansion
5–4	*T. cristinae* *T. monikensis*	1-2-11-12-no	Contiguous range expansion

Table 4 Inference chain for nested geographical analysis of the Southern cladogram using the inference key given in Templeton (1998)

Clade	Species	Chain of Inference	Inferred Pattern
3–18	*T. genevieve*	1-2-11-12-no	Contiguous range expansion
4–9	*T. podura* *T. genevieve*	1-2-3-5-15-no	Past fragmentation
4–10	*T. bartmani* *T. tahoe*	1-2-11-12-13-14-yes	Long distance colonization
5–5	*T. podura* *T. genevieve* *T. bartmani* *T. tahoe*	1-2-11-12-no	Contiguous range expansion

Phylogenetic and host plant evidence from the redescribed species *T. knulli* suggests that *T. knulli* on *Ceanothus* and *T. knulli* on redwood are quite genetically distinct. Although *T. petita* has been described as a new species, it is closely related to *T. knulli* on *Ceanothus*. Since these species feed on the same host plant species, and their male genitalia only differs in size, these taxa may not be different species. No intermediate variants have been collected but the geographical area between *T. petita* and *T. knulli* has not been extensively sampled. *T. landelsensis* does appear to be the sister species of *T. knulli* (redwood).

The Santa Barbara clade is geographically limited to the coastal region around Santa Barbara. The asexual in this clade, *T. monikensis*, is unusual in several respects: (i) it is the only asexual not showing geographical parthenogenesis; (ii) it is the only asexual in which females produce males (of uncertain viability) to any notable degree, though reproduction is predominantly by parthenogenesis; and (iii) although the mtDNA phylogeny indicates definitively that *T. cristinae* is the maternal ancestor of *T. monikensis*, the collected males have genitalia and behaviour more similar to *T. chumash* (C. Sandoval, personal communication), suggesting that *T. monikensis* may be a hybrid of *T. chumash* and *T. cristinae*. Presumably, either a hybrid origin of the asexual, or the presence of facultative parthenogenesis, may be related to the lack of geographical parthenogenesis in this clade.

In the Southern clade, each asexual, *T. genevieve* and *T. tahoe*, is monophyletic. Since the base of this clade is unresolved, it is difficult to infer relationships between the sexual: asexual pairs *T. podura*: *T. genevieve* and *T. bartmani*: *T. tahoe*. Host plant use and morphology agree that these species pairs are each other's closest relatives, as does the nested cladogram. *T. boharti* is basal to the Southern clade and is geographically located at the southernmost limits of the genus, consistent with the idea that *Timema* originated in the south and has since expanded north. Except for one sampled population of *T. podura*, the sexuals *T. podura* and *T. bartmani* are found in the 100 km surrounding *T. boharti*, indicating that these species may not have expanded their range very much. By contrast *T. genevieve* is separated from the geographically closest *T. podura* by 250 km and *T. tahoe* is > 500 km north of *T. bartmani*, suggestive that asexuals have better dispersal ability than their sexual counterparts (see Law & Crespi 2002).

The phylogenetic split of *Timema* into the Northern, Santa Barbara, and Southern clades matches the geography of California remarkably well, with the distributions of *Timema* corresponding to the mountain ranges (i.e. the Coast Ranges bordering the Pacific Ocean, the Sierra Nevada and its foothills in eastern California, and several desert mountain ranges in Arizona and Nevada). Few phylogeographical studies have been conducted on California taxa, but there are many species known to exhibit geographical patterns defined by the mountain ranges (e.g. Tan & Wake 1995; Gervais & Shapiro 1999; Rodríguez-Robles *et al*. 1999). As in *Timema*, phylogeographical analyses of the Californian Newt, *Taricha torosa*, showed that northern populations had low sequence divergence while southern and central populations were quite differentiated, suggesting that northern newts are relatively young (Tan & Wake 1995). Furthermore, the Tan & Wake (1995) study interpreted phylogeographical patterns to reflect dispersal of subspecies from south to north, and from north to south, corresponding to geological changes in the California coastal region. The observation that *T. poppensis* and *T. californicum* are polyphyletic may also reflect vicariance and dispersal due to sea level changes and land formation around San Francisco. The phylogeny of the Californian mountain kingsnake (*Lampropeltis zonata*) also shows geographical structuring between northern and southern clades that roughly corresponds to that seen in *Timema* (Rodríguez-Robles *et al*. 1999). In addition, the southern subspecies of kingsnakes are basal with respect to more northerly populations. These two studies, combined with our analysis of *Timema*, suggest that species distributions in California have been highly influenced by dispersal, and that this region shows concordant phylogeographical splits between north and south in diverse taxa.

Geographic Parthenogenesis

The Northern Clade. Overall, the analysis for higher clade levels in the Northern cladogram shows range expansion, consistent with previous phylogeographical work (Sandoval *et al*. 1998) that the genus has expanded. However, the major implication of this previous work was that *Timema* originated in the south and the direction of expansion was only towards the north. Contrary to this suggestion, there are two notable indications that some ancestral haplotypes occur in the north. First, the inferred pattern for clade 2–8 is restricted gene flow with isolation by distance. Tip haplotypes in this clade belonging to both *T. poppensis* and *T. californicum* are located near San Francisco. The interior haplotypes are those of *T. poppensis* from San Francisco and from 200 km north. Consistent with a model of restricted gene flow, the tip (young) haplotypes have a geographical range nested within the range immediately interior to it (Templeton *et al*. 1995). This result suggests that some northern *T. poppensis* haplotypes are ancestral. Second, the overall inference for the Northern cladogram is range expansion. Under a model of range expansion, old haplotypes should be sampled from the pre-expansion areas and should be interior to the cladogram (Templeton *et al*. 1995). Again, some northern *T. poppensis* haplotypes are interior, suggestive that they may be ancestral. One hypothesis that would be consistent with ancestral *T. poppensis*

haplotypes is that *T. poppensis* has expanded from northerly, coastal, glacial refugia populations.

A peculiarity in the NCA is the observation that the asexuals *T. douglasi* and *T. shepardii* are found interior to the sexual species, *T. poppensis*. Usually, haplotypes of recent origin and low frequency should occur at the tips of a cladogram (Excoffier & Langaney 1989). Assuming that haplotypes of asexuals would be of more recent origin since they arise from their sexual progenitors, asexuals should predominate at the tips of the cladogram. Since the asexual haplotypes fall into circularities with, and are closely related to, *T. poppensis*, these asexuals may have arisen from interior sexual haplotypes and be so recent in origin that their haplotypes have not had time to diverge, and consequently are 'acting' like old haplotypes within the nested design. Consistent with this hypothesis is the result of restricted dispersal by distance for clade 2–14. When a new haplotype arises its will often remain within the geographical range of it progenitor (Templeton *et al*. 1995). If these haplotypes are of very recent origin, there will have been insufficient time for divergence and consequently these haplotypes will nest immediately adjacent to ancestral haplotypes.

Although range expansion has occurred, there is inadequate sampling to discriminate between contiguous range expansion and long distance colonization for most clades. In terms of evaluating the hypothesis that geographical parthenogenesis is the result of range expansion, the evidence indicates that both restricted dispersal (clade 2–14) and contiguous range expansion (clade 3–6) play a role in the geographical structure. Further support that parthenogen distribution is the result of range expansion is the observation that despite the fact that *T. douglasi* and *T. shepardii* have few haplotypes, both of these taxa are remarkably widespread in distribution.

The Santa Barbara Clade. Within the *T. cristinae*: *T. monikensis* pair there is an overall inference of contiguous range expansion. *T. monikensis*, the parthenogen, groups together with only one *T. cristinae* haplotype, suggesting that there may be other un-sampled haplotypes. Furthermore, there are as many as 16 steps between *T. cristinae* haplotypes sampled from the same geographical location. There are two plausible explanations for this result: (1) sampling effort was not adequate to characterize all *T. cristinae* haplotypes, or (2) *T. cristinae* has remarkably high within-species divergence over small spatial scales (Nosil *et al*. 2002), presumably due to large population sizes maintained over long time periods. Even though this pair does not fit the pattern of geographical parthenogenesis, inferred contiguous range expansion does explain the geographical structure. The primary inferred direction of this range expansion is southeast, from clade 4–5 with *T. cristinae* to 4–6 with *T. monikensis* haplotypes.

The Southern Clade. Within the parthenogen *T. genevieve* (clade 3–18), contiguous range expansion is inferred. Haplotypes within the nested clades 2–44 and 2–45 are separated from one another by 150 km, suggesting that *T. genevieve* is a good disperser or that there are missing intermediates. With respect to its sexual counterpart *T. podura*, however, the inference is one of fragmentation (clade 4–9). This result suggests that the geographical distribution of *T. genevieve* is due to a past fragmentation event and that the pattern of geographical parthenogenesis is not due to range expansion. At the next higher level (total cladogram), the inference is one of contiguous range expansion, regardless of whether or not the *T. bartmani*: *T. tahoe* pair is included. There are two plausible scenarios that could account for different inferences at different levels. In the first scenario, *T. podura* has expanded northward. At some point during this expansion, the parthenogen *T. genevieve* originated and both species continued to expand north. Since *T. genevieve* is expected to be a better disperser, this asexual would be at the leading edge of this expansion. By definition, the origin of asexuality can be considered a fragmentation event since the two lineages are no longer able to exchange genes.

T. genevieve continued to expand north while *T. podura* lagged. The second scenario is similar except that the fragmentation event could be inferred within *T. podura* before the origination of *T. genevieve*. In this case, when *T. genevieve* arose, it drove its progenitor population extinct and continued to expand (and diverge) northwards. Both hypotheses could account for the pattern of geographical parthenogenesis since the actual position of *T. genevieve* is the result of range expansion and not fragmentation. Under either supposition, the observation that *T. genevieve* has some internal haplotypes in the nested design can be examined. In the first scenario, *T. genevieve* would be expected to be old since the lineage originated before the fragmentation event; indeed, Law & Crespi (2002) have inferred that *T. genevieve* is an ancient asexual (over 800 000 years old), while other, more northerly asexuals are of considerably more recent origin. Under the second hypothesis, *T. genevieve* haplotypes would be ancestral if the progenitor extinction event occurred a long time ago. The observation that there are two highly diverged and geographically separated populations of *T. genevieve* supports this notion.

Long distance colonization is inferred for the *T. bartmani*: *T. tahoe* clade (4–10). As in other species pairs, *T. tahoe* is internal to its sexual relative *T. bartmani*. This internalization falls between *T. bartmani* haplotypes from two different, but geographically close, populations, suggesting that the *T. tahoe* haplotypes are ancestral. Examination of the

clade distance values for the 4–10 clade indicates significance with *T. bartmani* from only one population (haplotypes 96 and 97), and that *T. tahoe* is distantly located from this population. These *T. bartmani* haplotypes are more internal, and presumably older, than *T. tahoe*. It is difficult to accurately determine relationships within this part of the cladogram since there are not many sampled populations or haplotypes. Since the NCA indicates that the asexual distribution is the result of long distance colonization, the pattern of geographical parthenogenesis can be attributed to range expansion.

Conclusions

Observations that asexuals tend to be geographically more northerly in distribution have usually been explained by assuming a higher dispersal rate of asexuals at the leading edge of species range expansion (Bell 1982; Stearns 1987). This study is the first of its kind that has tried to address this pattern of geographical parthenogenesis in a rigorous fashion. In each case of parthenogenesis, the major inference is that geographical structure is the result of range expansion. Regardless of whether or not *Timema douglasi* and *T. shepardii* are different species, the observation that they exhibit few widespread haplotypes supports the idea of a high asexual dispersal rate, as well as inferences of range expansion. Although *T. monikensis* does not fit the pattern of geographical parthenogenesis, this species may be a hybrid, with its distribution determined by both of its parental types, or a facultative parthenogen. The *T. genevieve* lineage has apparently been fragmented from its sexual progenitor, but the overall more northerly pattern is consistent with a hypothesis of range expansion. *T. tahoe* is geographically north and far from its sexual counterpart, the apparent result of long distance colonization. The cases of *T. genevieve* and *T. tahoe*, since each is geographically far from respective sexuals, are also consistent with theory suggesting that asexuals have better dispersal ability than sexuals.

Studies of the geographical distributions of asexuality relative to sex have long been thought to be able to provide insight into the advantages of sex. Geographic parthenogenesis in *Timema* is most likely the result of differential dispersal rates of sexuals and asexuals, such that species with both reproductive modes have moved north, but asexuals have moved faster and farther. Previous studies on geographical distributions of asexuals, not just geographical parthenogenesis, have also supported the idea that the ability of asexuals to rapidly colonize vacant habitats is important in determining distributions (Chaplin & Ayre 1997; Schön *et al*. 2000). The ability to disperse limits competition between asexuals and sexual progenitors, although this outcome may only confer an evolutionary short-term advantage to asexuality in most cases.

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This work forms part of the thesis of Jennifer Law, a recent member of the Behavioural Ecology Research Group at Simon Fraser University. J. L.'s research focuses on the phylogeographical analysis of taxa that provide insight into outstanding questions in evolutionary biology, such as the evolution of sex and the causes of adaptive radiation. Bernard Crespi is a faculty member at Simon Fraser University, whose research program involves the combined phylogenetic, ecological, and behavioural analysis of social behaviour, sex, tritrophic relationships, and speciation.

Appendix I

Collection data. Species codes refer to Fig. 2, haplotype numbers refer to Figs 3–5, and for host plant codes see Table 1

Haplotype number	Code	GenBank number	Species	Host plant	County	Location	GPS Coordinates
1	pop1no1	AF409998	*T. poppensis*	A	Napa	Poppe Road., Howell Mtn	38 35 725 N 122 26 669 W
2	pop1no2	AF409999	*T. poppensis*	A	Napa	Poppe Road., Howell Mtn	38 35 725 N 122 26 669 W
	pop1no3	AF410000	*T. poppensis*	A	Napa	Poppe Road., Howell Mtn	38 35 725 N 122 26 669 W
3	pop8no3	AF410003	*T. poppensis*	A	Napa	Ink Grade Road., Howell Mtn	38 35 725 N 122 26 669 W
4	pop8no1	AF410001	*T. poppensis*	A	Napa	Ink Grade Road., Howell Mtn	38 35 725 N 122 26 669 W
5	pop8no2	AF410002	*T. poppensis*	A	Napa	Ink Grade Road., Howell Mtn	38 35 725 N 122 26 669 W
6	pop8no4	AF410004	*T. poppensis*	A	Napa	Ink Grade Road., Howell Mtn	38 35 725 N 122 26 669 W
7	pop2no1	AF410005	*T. poppensis*	A	Santa Clara	Loma Prieta Way	37 06 252 N 121 52 770 W
	pop2no2	AF410006	*T. poppensis*	A	Santa Clara	Loma Prieta Way	37 06 252 N 121 52 770 W
	pop2no3	AF410007	*T. poppensis*	A	Santa Clara	Loma Prieta Way	37 06 252 N 121 52 770 W
	pop10no2	AF410026	*T. poppensis*	A	Sonoma	Tin Barn Road.	38 37 093 N 123 17 535 W
8	pop10no3	AF410027	*T. poppensis*	A	Sonoma	Tin Barn Road.	38 37 093 N 123 17 535 W
	pop7no4	AF410011	*T. poppensis*	A	Santa Clara	Loma Prieta Way	37 06 252 N 121 52 770 W
9	pop7no5	AF410012	*T. poppensis*	A	Santa Clara	Loma Prieta Way	37 06 252 N 121 52 770 W
10	pop5no3	AF410014	*T. poppensis*	A	Santa Clara/Cruz	Summit Road.	37 13 340 N 122 05 270 W
11	pop5no4	AF410015	*T. poppensis*	A	Santa Clara/Cruz	Summit Road.	37 13 340 N 122 05 270 W
12	pop4no1	AF410016	*T. poppensis*	A	Humboldt	King Mtn	40 08 223 N 124 04 358 W
	pop4no2	AF410017	*T. poppensis*	A	Humbold	King Mtn	40 08 223 N 124 04 358 W
	pop15no1	AF410034	*T. poppensis*	D	Humboldt	Shively	40 26 759 N 123 58 581 W
	doug2no1	AF410042	*T. douglasi*	A	Humboldt	Bald Hills Road. mile 9	41 12 264 N 123 57 508 W
13	doug2no3	AF410043	*T. douglasi*	A	Humboldt	Bald Hills Road. mile 9	41 12 264 N 123 57 508 W
14	pop4no3	AF410018	*T. poppensis*	A	Humboldt	King Mtn	40 08 223 N 124 04 358 W
15	pop4no4	AF410019	*T. poppensis*	A	Humboldt	King Mtn	40 08 223 N 124 04 358 W
16	pop6no1	AF410020	*T. poppensis*	A	Humboldt	King Mtn 2	40 08 589 N 124 05 047 W
17	pop6no2	AF410021	*T. poppensis*	A	Humboldt	King Mtn 2	40 08 589 N 124 05 047 W
18	pop9no1	AF410022	*T. poppensis*	A	Sonoma	Fish Rock Road. 3	38 50 317 N 123 31 029 W
19	pop9no3	AF410024	*T. poppensis*	A	Sonoma	Fish Rock Road. 3	38 50 317 N 123 31 029 W
	pop10no1	AF410025	*T. poppensis*	A	Sonoma	Tin Barn Road.	38 37 093 N 123 17 535 W
20	pop9no2	AF410023	*T. poppensis*	A	Sonoma	Fish Rock Road. 3	38 50 317 N 123 31 029 W
21	pop11no1	AF410028	*T. poppensis*	A	Sonoma	Fish Rock Road. 2	38 49 389 N 123 34 885 W
22	pop12no1	AF410029	*T. poppensis*	A	Sonoma	Fish Rock Road. 4	38 53 117 N 123 22 505 W
23	pop12no2	AF410030	*T. poppensis*	A	Sonoma	Fish Rock Road. 4	38 53 117 N 123 22 505 W
24	pop12no3	AF410031	*T. poppensis*	A	Sonoma	Fish Rock Road. 4	38 53 117 N 123 22 505 W
25	pop12no4	AF410032	*T. poppensis*	A	Sonoma	Fish Rock Road. 4	38 53 117 N 123 22 505 W
26	pop13no1	AF410033	*T. poppensis*	A	Sonoma	Fish Rock Road. 1	38 49 389 N 123 34 885 W
27	pop14no1	AF410035	*T. poppensis*	B	Santa Clara/Cruz	Summit Road.	37 01 692 N 121 44 415 W
	pop14no2	AF410036	*T. poppensis*	B	Santa Clara/Cruz	Summit Road.	37 01 692 N 121 44 415 W
28	pop14no3	AF410037	*T. poppensis*	B	Santa Clara/Cruz	Summit Road.	37 01 692 N 121 44 415 W
29	doug1no1	AF410038	*T. douglasi*	A	Mendocino	Orr Springs Road.	39 12 047 N 123 17 636 W

Appendix I *Continued*

Haplotype number	Code	GenBank number	Species	Host plant	County	Location	GPS Coordinates
	doug1no2	AF410039	*T. douglasi*	A	Mendocino	Orr Springs Road.	39 12 047 N 123 17 636 W
	shep1no4	AF410065	*T. shepardii*	C	Mendocino	Orr Springs Road.	39 11 559 N 123 15 707 W
30	shep1no5	AF410066	*T. shepardii*	C	Mendocino	Orr Springs Road.	39 11 559 N 123 15 707 W
	doug1no3	AF410040	*T. douglasi*	A	Mendocino	Orr Springs Road.	39 12 047 N 123 17 636 W
31	doug1no4	AF410041	*T. douglasi*	A	Mendocino	Orr Springs Road.	39 12 047 N 123 17 636 W
32	doug2no2	AF410043	*T. douglasi*	A	Humboldt	Bald Hills Road. mile 9	41 12 264 N 123 57 508 W
33	cali2no1	AF410045	*T. californicum*	C, D, E	San Luis Obispo	Cuesta Ridge, Santa Lucia Mtns	35 21 430 N 120 39 351 W
34	cali2no2	AF410046	*T. californicum*	C, D, E	San Luis Obispo	Cuesta Ridge, Santa Lucia Mtns	35 21 430 N 120 39 351 W
35	cali2no3	AF410047	*T. californicum*	C, D, E	San Luis Obispo	Cuesta Ridge, Santa Lucia Mtns	35 21 430 N 120 39 351 W
36	cali3no1	AF410048	*T. californicum*		Montery	Arroyo Seco	36 14 640 N 121 29 134 W
37		AF005343	*T. californicum*		Montery	Arroyo Seco	36 14 640 N 121 29 134 W
	cali4no1	AF410049	T. californicum		Santa Clara/Cruz	Loma Prieta	37 06 252 N 121 52 770 W
	cali5no1	AF410050	*T. californicum*		Santa Clara	Corralitos Canyon	37 03 480 N 121 49 460 W
	pop7no1	AF410008	*T. poppensis*	A	Santa Clara/Cruz	Loma Prieta × Boche	37 06 252 N 121 52 770 W
	pop7no2	AF410009	*T. poppensis*	A	Santa Clara/Cruz	Loma Prieta × Boche	37 06 252 N 121 52 770 W
38	pop7no3	AF410010	*T. poppensis*	A	Santa Clara/Cruz	Loma Prieta × Boche	37 06 252 N 121 52 770 W
39	cali6no1	AF410051	*T. californicum*	C, E, G	Santa Clara/Cruz	Summit Road.	37 13 343 N 122 05 271 W
40	cali6no2	AF410052	*T. californicum*	C, E, G	Santa Clara/Cruz	Summit Road.	37 13 343 N 122 05 271 W
	cali6no3	AF410053	*T. californicum*	C, E, G	Santa Clara/Cruz	Summit Road.	37 13 343 N 122 05 271 W
41	pop5no2	AF410013	*T. poppensis*	B	Santa Clara/Cruz	Summit Road.	37 13 343 N 122 05 271 W
42	cali7no2	AF410055	*T. californicum*		Santa Clara/Cruz	Loma Prieta × Boche	37 06 400 N 121 52 500 W
43	cali7no3	AF410056	*T. californicum*		Santa Clara/Cruz	Loma Prieta × Boche	37 06 400 N 121 52 500 W
	cali7no4	AF410005	*T. californicum*		Santa Clara/Cruz	Loma Prieta × Boche	37 06 400 N 121 52 500 W
44	—	AF005344	*T. californicum*		Santa Clara/Cruz	Loma Prieta	37 06 252 N 121 52 770 W
45	cali9no1	AF410058	*T. californicum*	C, G	Santa Clara/Cruz	Summit Road.	37 02 720 N 121 45 190 W
46	cali8no1	AF410059	*T. californicum*	G	Santa Clara	Lick Obs, Mt Hamilton	37 20 590 N 121 38 188 W
47	cali8no2	AF410060	*T. californicum*	G	Santa Clara	Lick Obs, Mt Hamilton	37 20 590 N 121 38 188 W
48	cali8no3	AF410061	*T. californicum*	G	Santa Clara	Lick Obs, Mt Hamilton	37 20 590 N 121 38 188 W
49	—	AF005345	*T. californicum*	G	Santa Clara	Lick Obs, Mt Hamilton	37 20 590 N 121 38 188 W
50	shep1no1	AF410062	*T. shepardii*	C, G	Mendocino	Orr Springs Road.	39 11 559 N 123 15 707 W
51	shep1no2	AF410063	*T. shepardii*	C, G	Mendocino	Orr Springs Road.	39 11 559 N 123 15 707 W
	shep1no3	AF410064	*T. shepardii*	C, G	Mendocino	Orr Springs Road.	39 11 559 N 123 15 707 W
	shep5no1	AF410077	*T. shepardii*	C	Mendocino	Elk Mtn	39 16 729 N 122 55 546 W
	shep5no2	AF410078	*T. shepardii*	C	Mendocino	Elk Mtn	39 16 729 N 122 55 546 W
	shep2no4	AF410070	*T. shepardii*	C	Mendocino	Hopland Research Stn	38 57 320 N 123 07 479 W
	shep3no1	AF410071	*T. shepardii*	C	Sonoma	King Ridge Road.	38 35 734 N 123 09 589 W
	shep3no3	AF410073	*T. shepardii*	C	Sonoma	King Ridge Road	38 35 734 N 123 09 589 W
	shep4no2	AF410075	*T. shepardii*	C	Mendocino	near Laytonville	39 04 211 N 123 07 948 W
	shep4no3	AF410076	*T. shepardii*	C	Mendocino	near Laytonville	39 04 211 N 123 27 948 W
	shep6no2	AF410080	*T. shepardii*	C	Del Norte	Big Flat Road.	41 42 035 N 123 48 574 W
	shep6no3	AF410081	*T. shepardii*		Del Norte	Big Flat Road.	41 42 035 N 123 48 574 W
	shep2no1	AF410067	*T. shepardii*	C	Mendocino	Hopland Research Stn	38 57 320 N 123 07 479 W
52	shep2no2	AF410068	*T. shepardii*	C	Mendocino	Hopland Research Stn	38 57 320 N 123 07 479 W

Appendix I *Continued*

Haplotype number	Code	GenBank number	Species	Host plant	County	Location	GPS Coordinates
53	shep2no3	AF410069	*T. shepardii*	C	Mendocino	Hopland Research Stn	38 57 320 N 123 07 479 W
54	shep4no1	AF410074	*T. shepardii*	C	Mendocino	near Laytonville	39 04 211 N 123 27 948 W
55	shep6no1	AF410079	*T. shepardii*	C	Del Norte	Big Flat Road.	41 42 035 N 123 48 574 W
56	—	AF005340	*T. cristinae*		Santa Barbara	Santa Ynez Mtns Hwy 154	34 31 000 N 119 48 000 W
57	cris1no1	AF410082	*T. cristinae*	D	Santa Barbara	Ojala	34 29 488 N 119 18 367 W
58	cris1no2	AF410083	*T. cristinae*	D	Santa Barbara	Ojala	34 29 488 N 119 18 367 W
59	cris1no3	AF410084	*T. cristinae*	D	Santa Barbara	Ojala	34 29 488 N 119 18 367 W
60	cris3no1	AF410085	*T. cristinae*	D, H	Santa Barbara	Santa Ynez Mtns Gaviota	34 29 269 N 120 13 569 W
61	cris3no2	AF410086	*T. cristinae*	D, H	Santa Barbara	Santa Ynez Mtns Gaviota	34 29 269 N 120 13 569 W
62	cris2no1	AF410087	*T. cristinae*	D	Santa Barbara	Santa Ynez Mtns Refugio Road.	34 30 950 N 120 04 389 W
63	cris2no2	AF410088	*T. cristinae*	D	Santa Barbara	Santa Ynez Mtns Refugio Road.	34 30 950 N 120 04 389 W
64	cris4no1	AF410089	*T. cristinae*	E	Santa Barbara	Santa Ynez Mtns Refugio Road.	34 30 897 N 120 04 278 W
65	cris4no2	AF410090	*T. cristinae*	E	Santa Barbara	Santa Ynez Mtns Refugio Road.	34 30 897 N 120 04 278 W
66	—	AF410094	*T. cristinae*				34 31 000 N 119 48 000 W
67	—	AF410096	*T. cristinae*				34 31 000 N 119 48 000 W
68	—	AF410099	*T. cristinae*				34 31 000 N 119 48 000 W
69	—	AF410091	*T. cristinae*				34 28 000 N 119 46 111 W
	—	AF410093	*T. cristinae*				34 28 000 N 119 46 111 W
70	—	AF410092	*T. cristinae*				34 31 500 N 119 51 000 W
71	—	AF410097	*T. cristinae*				34 31 500 N 119 51 000 W
72	—	AF410095	*T. cristinae*				34 30 200 N 119 50 100 W
73	—	AF410098	*T. cristinae*				34 30 200 N 119 50 100 W
	mon1no1	AF410100	*T. monikensis*	I	Los Angeles	Santa Monica Mtn	34 07 172 N 118 50 441 W
	mon1no3	AF410101	*T. monikensis*	I	Los Angeles	Santa Monica Mtn	34 07 172 N 118 50 441 W
74	mon1no4	AF410102	*T. monikensis*	I	Los Angeles	Santa Monica Mtn	34 07 172 N 118 50 441 W
75	mon1no2	AF410103	*T. monikensis*	I	Los Angeles	Santa Monica Mtn	34 07 172 N 118 50 441 W
76	mon1no5	AF410104	*T. monikensis*	I	Los Angeles	Santa Monicka Mtn	34 07 172 N 118 50 441 W
77	—	AF005342	*T. podura*	E	Tulare	Sequoia NP	35 35 000 N 118 32 000 W
78	—	AF410105	*T. podura*	E	Santa Barbara	Hwy 166	35 05 687 N 120 07 477 W
	—	AF410107	*T. podura*	E	Santa Barbara	Hwy 166	35 05 687 N 120 07 477 W
79	—	AF410106	*T. podura*	E	Santa Barbara	Hwy 166	35 05 687 N 120 07 477 W
	—	AF410108	*T. podura*	E	San Diego	Tecate Divide	32 40 152 N 116 19 004 W
	pod2no2	AF410109	*T. podura*	E	San Diego	Tecate Divide	32 40 152 N 116 19 004 W
80	pod2no3	AF410110	*T. podura*	E	San Diego	Tecate Divide	32 40 152 N 116 19 004 W
81	pod3no1	AF410111	*T. podura*	D	Riverside	Hwy 243–1	33 48 907 N 116 47 462 W
82	pod5no1	AF410112	*T. podura*	D	Riverside	Hwy 243–3	33 47 842 N 116 46 591 W
83	pod5no2	AF410113	*T. podura*	D	Riverside	Hwy 243–3	33 47 842 N 116 46 591 W
84	—	AF005341	*T. podura*	E	Riverside	Hwy 243–3	33 47 842 N 116 46 591 W
85	pod6no1	AF410114	*T. podura*	D, E	Riverside	Hwy 243–2	33 51 096 N 116 49 568 W
86	pod6no2	AF410115	*T. podura*	D, E	Riverside	Hwy 243–2	33 51 096 N 116 49 568 W
	pod4no1	AF410116	*T. podura*	D	San Diego	Palomar Observatory	33 21 543 N 116 52 170 W
	pod4no2	AF410117	*T. podura*	D	San Diego	Palomar Observatory	33 21 543 N 116 52 170 W
87	pod4no3	AF410118	*T. podura*	D	San Diego	Palomar Observatory	33 21 543 N 116 52 170 W
88	gen1no1	AF410119	*T. genevievae*	E	Lake	Hwy 20 Road. mk 38.22	38 59 747 N 122 31 307 W

Appendix *Continued*

Haplotype number	Code	GenBank number	Species	Host plant	County	Location	GPS Coordinates
	gen1no2	AF410120	*T. genevievae*	E	Lake	Hwy 20 rd mk 38.22	38 59 747 N 122 31 307 W
	gen1no3	AF410121	*T. genevievae*	E	Lake	Hwy 20 rd mk 38.22	38 59 747 N 122 31 307 W
	gen1no4	AF410122	*T. genevievae*	E	Lake	Hwy 20 rd mk 38.22	38 59 747 N 122 31 307 W
	gen4no1	AF410128	*T. genevievae*	E	Colusa	Cook Springs Road.	39 15 768 N 122 30 029 W
89	gen4no2	AF410129	*T. genevievae*	E	Colusa	Cook Springs Road.	39 15 768 N 122 30 029 W
90	gen2no1	AF410123	*T. genevievae*	E	Santa Clara	Mine Road.	37 25 431 N 121 30 336 W
91	gen2no2	AF410124	*T. genevievae*	E	Santa Clara	Mine Road.	37 25 431 N 121 30 336 W
92	gen2no3	AF410125	*T. genevievae*	E	Santa Clara	Mine Road.	37 25 431 N 121 30 336 W
93	gen3no1	AF410126	*T. genevievae*	E	Santa Clara	Del Puerto Road.	37 23 433 N 121 28 403 W
94	gen3no2	AF410127	*T. genevievae*	E	Santa Clara	Del Puerto Road.	37 23 433 N 121 28 403 W
95	—	AF005333	*T. genevievae*	E	Santa Clara	San Ardo/Del Puerto Road.	37 23 433 N 121 28 403 W
	—	AF005331	*T. bartmani*	F	San Bernadino	Camp Meadow	34 09 813 N 116 54 377 W
	bart1no1	AF410130	*T. bartmani*	F	San Bernadino	Camp Meadow	34 09 813 N 116 54 377 W
96	bart1no2	AF410131	*T. bartmani*	F	San Bernadino	Camp Meadow	34 09 813 N 116 54 377 W
97	bart1no3	AF410132	*T. bartmani*	F	San Bernadino	Camp Meadow	34 09 813 N 116 54 377 W
98	bart2no1	AF410133	*T. bartmani*	F	San Bernadino	Running Springs	34 12 600 N 117 05 900 W
99	—	AF005330	*T. tahoe*	F	NEVADA	Hwy 50 × 28	39 00 000 N 120 00 000 W
100	tahoe1no1	AF410134	*T. tahoe*	F	NEVADA	Hwy 50 × 28	39 00 000 N 120 00 000 W
101	tahoe1no2	AF410135	*T. tahoe*	F	NEVADA	Hwy 50 × 28	39 00 000 N 120 00 000 W
102	knul1no1	AF410142	*T. knulli*	B	Montery	Big Creek Reserve	36 04 306 N 121 36 019 W
103	knul1no2	AF410143	*T. knull*	B	Montery	Big Creek Reserve	36 04 306 N 121 36 019 W
104	knul1no3	AF410144	*T. knulli*	B	Montery	Big Creek Reserve	36 04 306 N 121 36 019 W
105	knul1no4	AF410145	*T. knulli*	B	Montery	Big Creek Reserve	36 04 306 N 121 36 019 W
106	knul2no1	AF410146	*T. knulli*	D	Montery	Big Creek Reserve	36 04 261 N 121 35 734 W
107	knul2no2	AF410147	*T. knulli*	D	Montery	Big Creek Reserve	36 04 261 N 121 35 734 W
108	lan1no1	AF410148	*T. landelsensis*	C	Montery	Big Creek Reserve	36 11 365 N 121 33 150 W
109	lan1no2	AF410149	*T. landelsensis*	C	Montery	Big Creek Reserve	36 11 365 N 121 33 150 W
110	lan1no3	AF410150	*T. landelsensis*	C	Montery	Big Creek Reserve	36 11 365 N 121 33 150 W
111	petita	AF410151	*T. petita*	D	San Luis Obispo	Hwy 1, near Simeon	35 43 724 N 121 18 963 W
112	—	AF005335	*T. nakipa*	C, D	MEXICO – Baja	Sierra San Pedo Martir NP	
113	—	AF005334	*T. boharti*	C, D, E	San Diego	Hwy 79 × Julian	33 04 75 N 116 36 000 W
114	bohar1no1	AF410136	*T. boharti*	D, E	San Diego	Laguna Mtn	32 53 600 N 116 25 300 W
115	bohar1no2	AF410137	*T. boharti*	D, E	San Diego	Laguna Mtn	32 53 600 N 116 25 300 W
116	—	AF005336	*T. nevadense*	J	San Bernadino	Mid Hills	
117	—	AF005337	*T. dorotheae*		ARIZONA	Hualpai Mtns	
118	—	AF005338	*T. ritensis*	G, J	ARIZONA	Tucson	
119	chum1no1	AF410138	*T. chumash*	D, G	Los Angeles	Mt. Baldy	34 11 433 N 117 40 737 W
120	chum2no1	AF410139	*T. chumash*	D	Riverside	Hwy 243–2	33 51 096 N 116 49 568 W
121	chum2no2	AF410140	*T. chumash*	D	Riverside	Hwy 243–2	33 51 096 N 116 49 568 W
122	chum3no1	AF410141	*T. chumash*	G	San Bernadino	Hwy 38	34 06 420 N 116 58 387 W

