

Phylogeography of *Camassia quamash* in western North America: postglacial colonization and transport by indigenous peoples

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Abstract

Recent human activities have spread numerous plant species across the globe, yet it is unclear to what degree historical human activities influenced plant dispersal. In western North America, *Camassia quamash* was one of the most important food plants for indigenous peoples, who transported its propagules either intentionally or accidentally. We investigated how human and natural dispersal might have contributed to the current pattern of spatial genetic structure in *C. quamash* by performing phylogeographical surveys at two geographical scales. We sequenced two noncoding regions of chloroplast deoxyribonucleic acid (DNA) in 226 individuals from 53 populations of *C. quamash* as well as 126 individuals from 21 populations of the non-food plant *Zigadenus venenosus*. Contrary to the expectation of anthropogenic transport, *C. quamash* populations did not exhibit weaker genetic structure than *Z. venenosus* populations. We also failed to find convincing evidence for signatures of transport. Instead, our data showed strong effects of past glaciation and geographical barriers of the mountains in the Cascade Range, Olympic Peninsula and Vancouver Island. West of the Cascades, the species appears to have largely migrated northward from a southern refugium after deglaciation, whereas few populations having a highly divergent haplotype might have survived in southwestern Washington. Our data suggest that despite substantial ethnobotanical evidence for anthropogenic transport, the current pattern of genetic structure of *C. quamash* does not show any detectable signatures of transport by indigenous peoples and is better understood as the result of natural dispersal processes.

Keywords: anthropogenic dispersal, chloroplast DNA, ethnobotany, genetic structure, glacial refugia, *Zigadenus venenosus*

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Introduction

Knowledge of dispersal processes is critically important for understanding many key aspects of plant biology, including population dynamics, spatial genetic structure and range expansion (Cain *et al.* 2000). For many species, humans represent an important vector of dispersal. However, whereas human-mediated dispersal is clearly implicated in the many episodes of exotic plant

invasions during the last few centuries (Mack *et al.* 2000), and the early spread of domesticated crops (Zohary & Hopf 1993), the degree to which historical human activities influenced plant dispersal more broadly is unclear. Human transport in preindustrial times was not as extensive as that in recent times, but the potential for long-distance dispersal was considerable for some species (MacDougall 2003). However, the quantity of propagules dispersed by historical human activities, and the distances over which they were dispersed, may have been insufficient to create patterns in geographical ranges or genetic structure than can be readily discriminated from those created by natural dispersal.

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In North America, many plant species were utilized by indigenous peoples for various purposes (Moerman 1998; MacDougall 2003), and the effect of historical human populations on plant dispersal has been widely studied. One line of evidence involves disjunct occurrences of plant populations and their proximity to Indian villages or travel routes (Moseley 1931; Rousseau 1966; Keener & Kuhns 1997), although non-human causes are also possible in many of these cases (Murphy 2001; MacDougall 2003). Other studies report verbal accounts of human-mediated dispersal from indigenous descendants (Gilmore 1930) or written accounts in historical documents by early European explorers and settlers (MacDougall 2003), but the paucity of such data and potential observer bias have made it difficult to assess whether plant movement by indigenous peoples was sufficient to have detectable consequences. Genetic analysis of populations can provide critical insights in this respect because intensive plant movement should leave genetic signatures that are likely to persist for long periods of time (Le Corre *et al.* 1997; Fineschi *et al.* 2000; Mohanty *et al.* 2001; Harter *et al.* 2004). This is especially so for the chloroplast genome, which is maternally inherited in most angiosperms (Corriveau & Coleman 1988; Reboud & Zeyl 1994), moving in seeds or vegetative tissue but not pollen, and is thus an effective marker for a retrospective analysis of dispersal of propagules.

In this study, we report a phylogeographical study of common camas, *Camassia quamash* (Pursh) Greene (Agavaceae), which was one of the most important plant resources utilized by indigenous peoples throughout western North America (Beckwith 2004; Sultany *et al.* 2007). Ethnographical, ethnobotanical and archaeological evidence indicates that large quantities of camas bulbs were collected and consumed for at least 3000 years (Turner & Bell 1971; Gritzner 1994; Weiser 2006). Camas is abundant in grassland ecosystems of the Pacific Northwest (Fig. 1), which in many cases were intensively managed prior to European settlement with prescribed fire to increase the productivity of culturally important plants (especially *Camassia* species; MacDougall *et al.* 2004; Storm & Shebitz 2006). Previous literature frequently mentions anthropogenic transport of camas propagules, particularly in southwestern British Columbia, Canada and western Washington, United States. Bulbs were widely traded not only locally but also over long distances (Gunther 1973), and in addition to deliberate transport, propagules that were dropped or discarded by indigenous peoples may have assisted dispersal and spread into new areas as well (White 1999; Turner & Peacock 2005). These cultural practices have largely ceased over the past two centuries following European settlement.

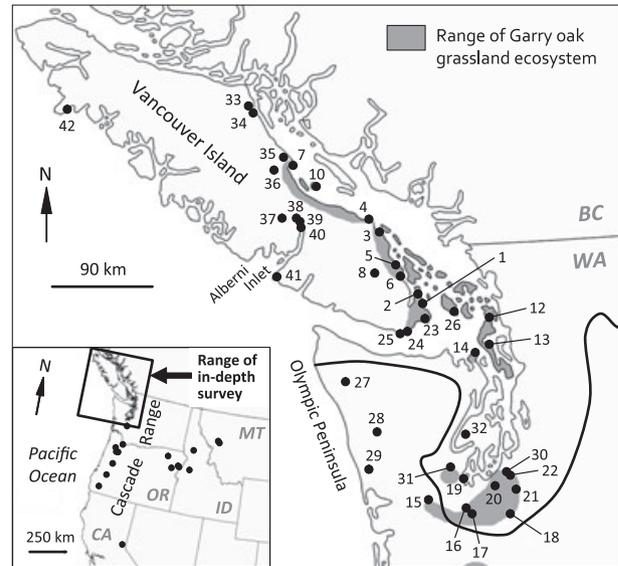


Fig. 1 Locations of the populations of *Camassia quamash* and/or *Zigadenus venenosus* sampled in this study. Populations used in the in-depth survey are numbered. See Fig. 2 and Table S1 for specific locations for each species. The grey areas indicate the main distribution of the Garry oak grassland ecosystem, and the thick line indicates the estimated maximum extent of the Cordilleran ice sheet (Booth 1987).

On Vancouver Island, camas bulbs were traded from the southeast, where *C. quamash* is very abundant, to the west coast and north end of the island (Turner & Bell 1971). They were also traded from the southwestern Olympic Peninsula northward, both within the Peninsula (Gritzner 1994) and subsequently to the west coast of Vancouver Island (Jewitt 1824). In addition, botanical composition of particular localities is quite suggestive of intentional transplanting. For example, the Somass River Estuary on central Vancouver Island (pop. 39; Fig. 1) is characterized by a suite of 'root vegetables' (e.g. *Potentilla anserina* and *Trifolium wormskioldii*) that were also important for indigenous peoples, but are atypical to the salt marsh environment. Because this habitat was managed by a local indigenous tribe and *C. quamash* is not widespread in the sites' peripheral area, the extensive population of camas has been hypothesized to be introduced from the southeastern Vancouver Island (Deur 2000). At a broader scale, Idaho was known as another area from where a large quantity of bulbs was traded over long distances (Harbinger 1964; Gritzner 1994), and the existence of extensive trading networks (Suttles 1998; Turner & Loewen 1998) suggests the possibility that the bulbs were transported even over the Cascade Range. Despite such considerable interest, no study to date has examined genetic evidence of human dispersal of camas propagules.

We performed a genetic analysis of *C. quamash* populations based on intensive sampling within the Pacific Northwest, as well as less-intensive sampling throughout the species range, using a phylogeographical approach and noncoding cpDNA sequences. Specifically, we addressed the influence of indigenous practices and natural postglacial migration processes on the current pattern of spatial genetic structure. In the Pacific Northwest, we tested three specific predictions of the hypothesis that indigenous peoples altered the spatial genetic structure of *C. quamash* via increased movement of propagules. (1) Genetic differentiation among populations (G_{ST} , N_{ST} , isolation by distance) should be lower and geographically disjunct haplotypes should be more common in *C. quamash* than in an ecologically similar species, *Zigadenus venenosus* S. Watson (meadow death camas; Melanthiaceae), which was not consumed by people because it contained a highly poisonous alkaloid (Turner & Bell 1971). (2) The above-mentioned camas population from Somass Estuary should be genetically most similar to populations in the hypothesized source region of southeastern Vancouver Island, rather than to populations from nearby areas where camas is less abundant. (3) Pattern of genetic similarity among populations should correspond to known trade routes of camas bulbs or cultural barriers of indigenous peoples. In the broader survey, we assessed evidence for camas bulbs having been transported over the Cascades. Specifically, a previous phylogenetic analysis of *Camassia* identified two distinct lineages largely from east or west of the Cascades (Fishbein *et al.* in press), allowing us to examine whether unusual haplotypes within the Pacific Northwest originated east of the Cascades.

Materials and methods

Study system

Camassia quamash is widely distributed in western North America. Along the Pacific coast, the species mainly occurs in the Garry oak (*Quercus garryana*) grassland ecosystem (Fig. 1), with some populations known from other types of open habitats, including bogs, estuaries and forest clearings. *Camassia quamash* is polycarpic, requires several years for seedlings to reach maturity and reproduces mostly sexually by seeds and rarely vegetatively by offset bulbs (Thoms 1989; Stevens *et al.* 2000; Ranker & Hogan 2002). Individual flowering plants consist of an oval bulb (~2 cm long), which produces long, keeled, grass-like leaves and a stem with a racemose inflorescence. Except for flowers and fruits, *Z. venenosus* is quite similar to *C. quamash* in terms of life history and morphological traits (Turner & Kuhnlein

1983; Kruckeberg 1996). The two species often grow sympatrically in Garry oak grasslands, so the possibility of confusing the poisonous bulbs of *Z. venenosus* with those of *C. quamash* was a concern for indigenous peoples (Turner & Bell 1971). Confusion was avoided by gathering camas bulbs in early summer when the distinctive ellipsoidal capsules and blue-violet flowers of *C. quamash* remained on the stem (Gritzner 1994). *Zigadenus venenosus* plants were weeded in some regions (Beckwith 2004), but we know of no ethnobotanical evidence of trading or transplanting of this species over large distances. Seeds of both species develop in dry capsules and lack any obvious mechanism for dispersal.

Sampling

Sampling was conducted at two spatial scales: an in-depth survey of populations of both *C. quamash* and *Z. venenosus* within British Columbia and western Washington State, and a broader but less intensive survey of *C. quamash* populations from most of the species' range. In the in-depth sampling, we sampled 35 and 21 populations of *C. quamash* and *Z. venenosus* respectively (Fig. 1; Table S1, Supporting information). Although the geographical ranges of the two species mostly overlap within this region, *Z. venenosus* does not occur along the west coast of Vancouver Island and the Olympic Peninsula. In each population, leaf material was collected from six plants except for population 2, where only four plants of *C. quamash* were available (*C. quamash*: $n = 208$; *Z. venenosus*: $n = 126$). The broader survey was aimed at assessing whether unusual haplotypes within the in-depth survey might have been transported from elsewhere, and also at improving our ability to draw inferences about postglacial colonization. For this broader survey, we sampled a single plant in each of 18 populations of *C. quamash* from across the rest of the species' distribution range (Fig. 1; Table S1, Supporting information). Leaves were collected between April and July of 2007–2008, except for two populations (BPP and TNC) for which leaves were collected in 2001.

DNA extraction, PCR and sequencing

Total DNA was extracted from ~1 cm² of leaf tissue using DNeasy Plant Mini Kit (Qiagen). On the basis of a preliminary screening of cpDNA variation in noncoding regions with 11 primer pairs, we selected two regions for each species: rpl32-trnL and psbJ-petA for *C. quamash*, and rpl32-trnL and rpl16 intron for *Z. venenosus* (Small *et al.* 1998; Shaw *et al.* 2007). Polymerase Chain Reactions (PCRs) were performed in a total volume of 50 µL consisting of the following components: 3–5 µL of template DNA, 1X reaction buffer (Invitrogen), 50 mM

MgCl₂, 10 mM dNTPs, 0.2 μM each primer and 3.75 U Taq DNA Polymerase (Invitrogen). The cycling conditions were template denaturation at 80 °C for 5 min; followed by 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 50 °C for 1 min, followed by a ramp of 0.3 °C/s to 65 °C and primer extension at 65 °C for 4 min; followed by a final extension step of 5 min at 65 °C (Shaw *et al.* 2007). The PCR products were purified on spin columns (Epoch Biolabs) and sequenced directly with the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturers' protocols with reaction mixtures diluted five times. The resulting dye-labelled fragments were purified with Sephadex G-50 (Sigma-Aldrich) and electrophoresed on an ABI PRISM 3730 automated sequencer (Applied Biosystems). All the cpDNA regions included a series of As or Ts, beyond which sequencing reactions failed to amplify in some samples. Thus, we restricted the analysis to subregions between these homopolymer regions and either forward (rpL32-F and petA; Shaw *et al.* 2007) or reverse primers (rpL16R1516; Small *et al.* 1998). In many cases, sequencing was performed in one direction using the above-mentioned primers; however, in some cases, ambiguous base pairs needed to be verified by sequencing in the opposite direction using the other amplification primers or an internal primer (rpL16F71-ZV1, 5'-AAAAAATTGACCGACTCCCGG-3'). Samples of rare haplotypes and rare combinations of haplotypes within populations were sequenced twice to exclude PCR errors. In *C. quamash*, sequences of rare haplotypes were also checked against sequences of a related species *C. leichtlinii*, because these species occasionally hybridize where they grow sympatrically (Uyeda & Kephart 2006; Fishbein *et al.* in press).

Analyses

Sequences were aligned manually using BIOEDIT 7.0.9 (Hall 1999). We retained indels by coding them as single characters (i.e. single mutation events), whereas mononucleotide repeat regions with length variations were not used for analysis. As a result, we analysed 898 bp for *C. quamash* (EMBL/GenBank/DDBJ accession nos. AB512687–AB512722) and 1070 bp for *Z. venenosus* (AB512723–AB512738). Polymorphic sites are listed in Table S2 (Supporting information).

The following analyses were employed to compare population genetic structure between species using data from the in-depth survey. First, we estimated overall measures of genetic differentiation among populations, G_{ST} and N_{ST} , following the procedure described by Pons & Petit (1995, 1996) using PERMUT 1.0 (<http://www.pierroton.inra.fr/genetics/labo/Software/>).

Because N_{ST} takes similarities between haplotypes into account, N_{ST} is significantly larger than G_{ST} when closely related haplotypes are found in the same populations. The difference between these statistics was tested with 1000 permutations. Second, we calculated correlations between geographical and genetic distance between all possible pairs of populations. We employed two different measures of geographical distance (linear distance and log-transformed linear distance) and two measures of genetic distance [F_{ST} and Slatkin's (1995) linearized F_{ST}]. ARLEQUIN 3.11 (Excoffier *et al.* 2005) was used to calculate Pearson's correlation coefficients for all four possible combinations of these measures and to assess their significance with 1000 permutations. For these analyses, we conducted two separate analyses for *C. quamash* either using all populations or excluding five populations on the Pacific coast (pops. 27, 28, 29, 41 and 42), where *Z. venenosus* was not found. Finally, we compared haplotype distributions between the species to examine possible geographically disjunctive haplotypes. Statistical parsimony networks of haplotypes were obtained with TCS (Templeton-Crandall-Sing) 1.21 (Clement *et al.* 2000) using the algorithms of Templeton *et al.* (1992).

The geographical structure of genetic variation in *C. quamash* in the in-depth survey was further analysed using spatial analysis of molecular variance (SAMOVA), which defines groups of geographically proximate populations that are maximally differentiated from each other (Dupanloup *et al.* 2002). The SAMOVA program (<http://web.unife.it/progetti/genetica/Isabelle/samova.html>) iteratively seeks the configuration of a user-defined number (K) of groups of populations that maximizes F_{CT} , the proportion of total genetic variance resulting from differences between groups. Groups defined early in the iterative process can subsequently be spatially interrupted by others, so there is some possibility of group disjunctions. We ran the program for $K = 2, 3, \dots, 15$ using data from the in-depth survey. All configurations of $K = 2$ up to $K = 5$ included $K-1$ groups each comprised only of a single population, plus one group comprised of all others; these configurations were not considered informative. All configurations with $K \geq 6$ showed very similar results, both in terms of F_{CT} and the geographical pattern of population grouping; therefore, we report the configuration ($K = 6$) with the smallest number of single-population groups. We then examined qualitatively whether the resulting group structure corresponded to natural geographical barriers or to known camas trade routes, with the latter determined by surveying ethnographical literature (see Introduction). Also, we tested whether the structure corresponded to cultural barriers, which were evaluated by a conservative consensus classification of native

languages (Goddard 1996). Sampled populations were classified according to three linguistic groups in our region, Wakashan, Chimakuan and Salishan, and the correspondence between SAMOVA and linguistic groups was assessed with a randomization test on a 6×3 contingency table (Roff & Bentzen 1989). To assess the possibility of unusual haplotypes being transported over the Cascades as well as to make broader inferences about species' phylogeography, we analysed genealogical relationships of all haplotypes, including those detected in the broad survey, using the TCS network.

Results

Comparison of population genetic structure between *Camassia quamash* and *Zigadenus venenosus*

In the in-depth survey, 11 and 8 haplotypes were detected in *C. quamash* and *Z. venenosus* respectively, and the number of haplotypes was comparable between the species (Table 1). Contrary to the expectation of anthropogenic transport, G_{ST} and N_{ST} were not lower in *C. quamash* than in *Z. venenosus* populations, both before and after excluding five populations on the Pacific coast where *Z. venenosus* was not found (Table 1). In both species, there was no significant difference between G_{ST} and N_{ST} . Correlations between geographical and genetic distance varied depending on the distance measures used; although significant correlations were found in *C. quamash* (strongest correlation, $r = 0.187$, $P < 0.001$ for all populations; $r = 0.215$, $P < 0.001$ with five populations excluded) but not in *Z. venenosus* ($r = 0.134$, $P = 0.07$). The distribution of haplotypes was similar for the two species in that several haplotypes exhibited disjunct distributions. In *C. quamash*, haplotype G occurred disjunctively in northern Vancouver Island and southwestern Washington (Fig. 2a). Similarly, in *Z. venenosus*, haplotype E occurred disjunctively within Vancouver Island, although to a lesser extent (Fig. 2b). Haplotypes showed clear spatial clustering, with occasional

outlying occurrences (Fig. 2). For example, haplotype A in both species was not only found mostly around southeastern Vancouver Island but also in southwestern Washington at low frequencies. Both species showed divergent haplotypes (K of *C. quamash* and H of *Z. venenosus*) in southwestern Washington.

Spatial genetic structure in *C. quamash*

The group structure revealed by SAMOVA ($K = 6$, $F_{CT} = 0.663$) showed genetic peculiarities of two populations in southwestern Washington (groups V and VI; Fig. 3), both of which were associated with the divergent haplotype K (Fig. 2a). Among multiple-population groups, group I was composed of populations containing predominantly haplotype B. Many of these populations were from the west coast of the Olympic Peninsula and Vancouver Island (Fig. 3), suggesting that the mountains in these regions acted as effective barriers to east–west dispersal, although the grouping of west-coast populations is also congruent with a major coastal route of bulb trading. The association of three populations on the east coast of Vancouver Island (pops. 4, 7 and 35; Fig. 3) with the west-coast populations suggests a possible colonization corridor through the Alberni Inlet towards the east coast. The disjunct occurrence of group II reflects that of haplotype G. Groups I and II are adjacent both on Vancouver Island and in southwestern Washington, but the disjunct populations of group II are likely to be of the same origin because haplotypes B and G were distinguished by one long (17-bp) indel (Table S2, Supporting information). The association of the Somass River Estuary (pop. 39) with west-coast populations did not support the ethnobotanical hypothesis that the species was introduced to this site from southeastern Vancouver Island. There was no significant association between SAMOVA and linguistic groupings ($\chi^2 = 12.52$, $P = 0.247$).

In the broad survey, we detected seven additional haplotypes (L–R; Fig. 4). The TCS network of all 18 haplotypes clearly showed genetic divergence between

Table 1 Sample size, number of haplotypes and indices of genetic differentiation for populations of *Camassia quamash* and *Zigadenus venenosus* in the in-depth survey

Data set	No. of populations	No. of plants	No. of haplotypes	$G_{ST} \pm SE$	$N_{ST} \pm SE\dagger$
<i>Camassia quamash</i>	35	208	11	0.661 ± 0.062	0.653 ± 0.091
<i>Camassia quamash</i> —West Coast†	30	178	10	0.646 ± 0.068	0.663 ± 0.096
<i>Zigadenus venenosus</i>	21	126	8	0.643 ± 0.094	0.642 ± 0.105

†Data set of *C. quamash* without five populations along the west coast of Vancouver Island and the Olympic Peninsula (pops. 27, 28, 29, 41 and 42; Fig. 1).

‡No significant differences between G_{ST} and N_{ST} were observed.

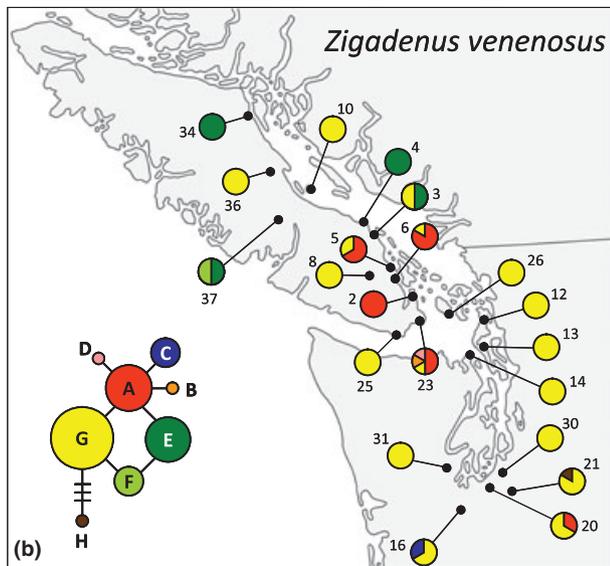
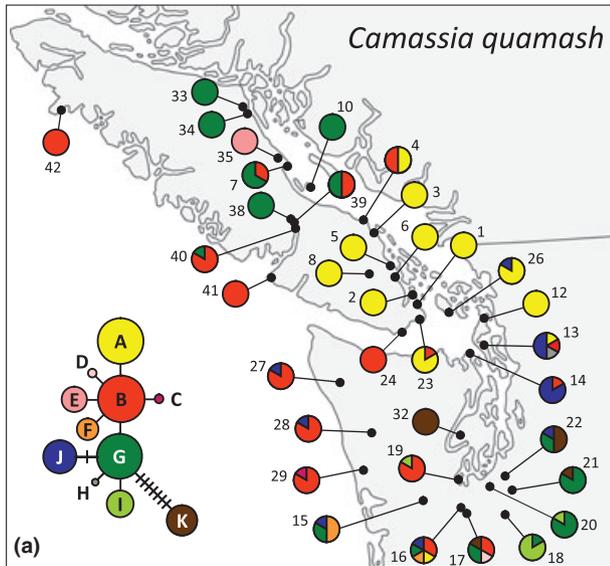


Fig. 2 Statistical parsimony networks and spatial distribution of cpDNA haplotypes detected in the in-depth survey for (a) *Camassia quamash* and (b) *Zigadenus venenosus*. Each circle in the parsimony networks represents a single haplotype, sized proportional to the logarithm of its relative abundance, and each cross-hatch on the lines connecting haplotypes represents one mutational difference, with indels coded as single characters.

the east and west sides of the Cascades, indicating that none of the haplotypes found in the in-depth survey (including the divergent haplotype K) originated from east of the Cascades. Two clades (haplotype K and the others) are supported west of the Cascades. Of the 11 haplotypes detected in the in-depth survey, four common haplotypes (A, B, G and I) were also found further south in the range of the broad survey (i.e.

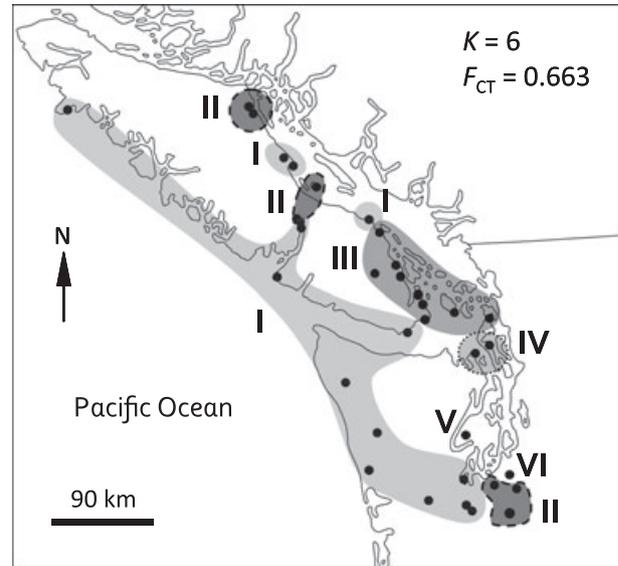


Fig. 3 Configuration of population groupings of *Camassia quamash* defined by spatial analysis of molecular variance (SAMOVA). The analysis detected six groups of populations ($F_{CT} = 0.663$). Two populations are not grouped with any other populations (groups V and VI).

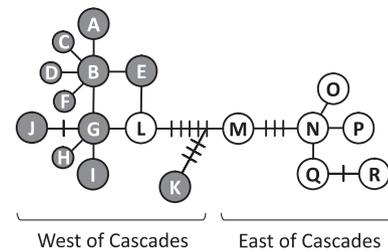


Fig. 4 Statistical parsimony networks of haplotypes of *Camassia quamash* detected both in the in-depth survey and broad survey. The grey haplotypes (A–K) indicate those found in the in-depth survey, whereas the open haplotypes (L–R) indicate those found exclusively in the broad survey. Each cross-hatch on the lines connecting haplotypes represents one mutational difference, with indels coded as single characters. The sizes of circles do not reflect relative abundance of haplotypes (some circles are smaller for ease of presentation).

Oregon or southern Washington; See Table S3 and Fig. S1, Supporting information, for the distribution of haplotypes).

Discussion

Overall, we found little support for the hypothesis that movement of propagules via indigenous activities altered the spatial genetic structure of *Camassia quamash*. There were no differences in the predicted directions between *C. quamash* and the nonconsumed *Zigadenus venenosus* for measures of genetic differentiation and

isolation by distance. Some haplotypes were disjunctively distributed, but such disjunctions were found for both species and thus do not provide convincing evidence of anthropogenic transport in *C. quamash*. We also found no support for the hypothesis of long-distance transplantation to the Somass Estuary population (pop. 39), or the possibility of transporting propagules over the Cascades. Although the genetic similarity among populations along the west sides of the Olympic Peninsula and Vancouver Island was consistent with an historical trade route along this corridor (Jewitt 1824; Gritzner 1994), this pattern was also consistent with natural northward expansion since the last glacial maximum and with high-elevation barriers to east–west gene flow, as discussed below. Thus, although we cannot rule out some influence of indigenous movement of propagules on the spatial genetic structure of camas populations, any such effect appears minimal relative to natural processes, such as migration history, and ongoing dispersal and drift.

Knowledge of transport by indigenous peoples

According to the ethnobotanical literature, indigenous peoples traditionally transported propagules of *C. quamash*, either intentionally or accidentally, within particular regions quite frequently, and also over long distances, at least occasionally. This led us to predict that genetic structure would be weaker in *C. quamash* than in *Z. venenosus* populations. Our data did not support this prediction, and although our in-depth survey covered only a part of the species' distribution ranges, G_{ST} values for both species (0.643–0.661) were comparable with the average of 124 angiosperm species in which the chloroplast genomes were maternally inherited ($G_{ST} = 0.637$; Petit *et al.* 2005) and much higher than that in two edible European trees, *Castanea sativa* ($G_{ST} = 0.43$; Fineschi *et al.* 2000) and *Prunus avium* ($G_{ST} = 0.29$; Mohanty *et al.* 2001), which have been extensively spread by humans.

These results are surprising, given the numerous descriptions of anthropogenic transport of this species. Three plausible factors could explain why indigenous practices did not leave any detectable signatures in the current spatial genetic structure. First, indigenous peoples may have traded predominantly inviable bulbs. Raw bulbs were also known to be traded and to be cheaper than dried or baked bulbs (Harbinger 1964), but harvested bulbs were usually baked in underground pits before consumption to convert nondigestible carbohydrate, inulin, to fructose (Turner & Kuhnlein 1983; Gritzner 1994). In addition, raw bulbs have a relatively high moisture content and therefore do not store well for extended periods of time (Turner

& Kuhnlein 1983; Beckwith 2004). Therefore, dried or baked bulbs would have been preferred, particularly in long-distance trading trips. Second, transplanting of propagules may have occurred only across relatively limited spatial scales. Some indigenous groups disseminated seeds or transplanted bulbs to enhance production (Suttles 1951; White 1999), but such management activities may have been largely restricted to within local indigenous territories. With respect to the extensive populations of *C. quamash* and other food plants in tidal marshes at the mouth of the Somass River, Deur (2000:217) noted that 'the presence of abundant camas ... might be logically attributed to transplanting ... from the large camas meadows at southeastern Vancouver Island'. However, the species also occurs nearby on some steep rocky hilltops and in coastal bluffs (H. Tomimatsu, personal observation). Thus, it seems most likely that propagules were transplanted to the Somass Estuary from nearby populations and propagated on site (assuming that this population was not natural). Finally, any populations that originated via indigenous transplantation might already have disappeared after indigenous management practices ceased following European settlement (Deur 2000). Transplanted bulbs might be less likely to persist in these new areas in competition with locally adapted species within the same community. Additionally, in sites with relatively deep soil where fire management by indigenous peoples appears to have historically maintained open conditions suitable for *C. quamash* (Turner 1999; MacDougall *et al.* 2004), land has largely been either converted to agricultural and urban development, or encroached by conifers (Gedalof *et al.* 2006; J. James, personal communication), with many remaining populations being found in sites with shallow soil where this habitat type is maintained naturally (Vellend *et al.* 2008).

Postglacial colonization

Many of the patterns we observed are interpretable with respect to the geological history of the region. One of the earliest regional comparative phylogeographical studies involved plants from the Pacific Northwest of North America (Soltis *et al.* 1997; Brunsfeld *et al.* 2001). Our broad survey, as well as a previous phylogenetic study (Fishbein *et al.* in press), showed a relatively deep genetic divergence between populations east and west of the Cascade Range (Fig. 4). This is consistent with an 'ancient vicariance' scenario (Brunsfeld *et al.* 2001) in which populations became isolated following the major uplift of the Cascades during the late Pliocene. A similar pattern was reported in allozyme data for Douglas fir (Li & Adams 1989).

West of the Cascades, many species examined thus far, including *Tolmiea menziesii* (Soltis *et al.* 1989) and *Tellima grandiflora* (Soltis *et al.* 1991), have exhibited divergence between areas generally north and south of the Washington–Oregon boundary, suggesting that these species spread from separate northern and southern glacial refugia to form their present-day continuous distributions. Possible northern refugia include unglaciated areas of central Alaska and northwestern Vancouver Island (Soltis *et al.* 1997). However, in *C. quamash*, the pattern of postglacial range expansion appears to differ from that in these other species, although our data also support two strongly divergent clades (haplotypes A–L and K; Fig. 4). The widespread occurrence of four common haplotypes in one of these clades (haplotypes A, B, G and I) suggests that the current distribution of cpDNA variation to the west of the Cascades is largely the result of migration from a single refugium. If the species migrated northward from south of the last glacial maximum, reduction in haplotype richness with latitude is expected to result from bottlenecks along extended migration routes (Hewitt 1996). Within the range of the in-depth survey, fewer haplotypes were found in British Columbia (4) than in Washington (10; Fig. 2a), consistent with this expectation. Although we only sampled a single plant per population in the broad survey, we can still compare haplotype richness between the in-depth (north) and broad surveys (south, west of Cascades) by standardizing sampling effort using rarefaction (Leberg 2002). The number of haplotypes is lower in the in-depth survey (4.31 after rarefaction) than that in the broad surveys (5), again supporting migration from a southern refugium. The divergent haplotype K was found only in a small geographical area (Figs 2a and 3), and raises an intriguing possibility that some populations survived glaciation in southwestern Washington, a previously unrecognized area of putative refuge. Although the populations having this haplotype (pops. 21, 22 and 32) were located north of the southern extent of the Cordilleran ice sheet at its last maximum, recent phylogeographical studies suggest that ice-free habitats existed even in putatively glaciated areas within the limits of the ice sheet (Marr *et al.* 2008; Provan & Bennett 2008). Moreover, our data for *Z. venenosus* also detected a relatively divergent haplotype (H) in the same area (Fig. 2b). Further studies, using nuclear markers and examining phylogeographical concordance across species, are needed to substantiate the presence and location of the northern refugium. The difference between *C. quamash* and those species with clear north–south differentiation may be explained by the fact that previous studies mostly focused on species occurring in mesic coniferous forests (Soltis *et al.* 1997; Brunsfeld *et al.*

2001; Carstens *et al.* 2005), whereas *C. quamash* largely grows in open habitats such as Garry oak grasslands. Species with similar habitat requirements may be more likely to have shared the same refugia and similar colonization history.

On a smaller geographical scale, our in-depth survey revealed some patterns suggestive of mountainous barriers to gene flow. The SAMOVA grouping of west-coast populations along with a few populations on eastern Vancouver Island (group I; Fig. 3) suggests migration routes along the Pacific coast and the Alberni Inlet. Given that *C. quamash* does not occur at high elevations in these areas (e.g. there are no observational records >382 m in the Biogeoclimatic Ecosystem Classification Database of British Columbia; <http://www.for.gov.bc.ca/hre/becweb/>), the mountain ranges are likely to have worked as topographical barriers for dispersal. Other haplotypes such as A and G showed patchy and disjunct distributions (Fig. 2a), suggestive of long-distance dispersal in range expansion (Allen *et al.* 1996; Le Corre *et al.* 1997). Alternatively, the disjunct occurrences of some haplotypes might be relicts of once widespread distributions. For example, low-elevation areas of northern Vancouver Island were deglaciated relatively earlier (Hebda 1983), so haplotype G might have rapidly spread to areas close to its current northern range limit and subsequently been replaced by other haplotypes in some intervening areas.

Conclusions

Our data suggest that the current pattern of spatial genetic structure of *C. quamash* is largely the result of natural processes, including vicariance, the existence of multiple refugia, postglacial migration and topographical barriers for dispersal. The patterns we observed can be understood largely without invoking any effect of dispersal by indigenous peoples, although we cannot rule out some such influence. In North America, ecologists have only recently begun to appreciate the impacts of indigenous peoples on ecosystem processes, and their effects on plant dispersal have not been fully examined (MacDougall 2003; Delcourt & Delcourt 2004). Our study provides a strong indication that in addition to ethnobotanical and archaeological evidence, genetic approaches can yield important insights into the degree to which regional dynamics of species were historically modified by indigenous activities. Historical human-mediated dispersal has the potential to leave long-term imprints on spatial patterns of genetic variation (e.g. Petit *et al.* 2001). However, historical evidence that such dispersal occurred does not imply that it was of sufficient magnitude to create such a genetic imprint.

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Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Populations of *Camassia quamash* and *Zigadenus venenosus* sampled in this study

Table S2 Polymorphic sites in chloroplast DNA sequences used to distinguish haplotypes of *Camassia quamash* and *Zigadenus venenosus*

Table S3 Composition of haplotypes in *Camassia quamash* and *Zigadenus venenosus* populations

Fig. S1 Spatial distribution of cpDNA haplotypes detected in the broad survey for *Camassia quamash*.

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