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Analysis of Genetic Variation in the Olympic Mudminnow (*Novumbra hubbsi*)

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Summary

The Olympic mudminnow (*Novumbra hubbsi*) is endemic to western Washington and inhabits wetland areas and low-gradient streams in the Chehalis River Basin, Puget Sound, and along the Olympic Coast. Available habitat has declined substantially and Olympic mudminnow are considered a state sensitive species and an increasing priority for conservation. Although information exists regarding the species life history and distribution, information regarding the status and long-term viability of populations, the boundaries that define populations and conservation units, and the level of gene flow among populations is much more limited. Genetic data has become increasingly useful for conservation planning and could provide important information for Olympic mudminnow; however, limited genetic data currently exists. In this study we developed a suite of microsatellite DNA markers specifically for Olympic mudminnow and we used these markers to genotype over 1,000 individuals collected from 23 locations distributed throughout the species range. We found that the level of genetic variation within collections varied considerably, but overall, most locations did not appear to be at risk of any detrimental effects associated with low genetic variation. We observed the greatest amount of genetic variation within collections from the Chehalis River Basin, which can be attributed to the fact that the Chehalis River was a glacial refugium for Olympic mudminnow. Overall there was a relatively high degree of genetic variation among collections (global $F_{ST} = 0.273$) and nearly all collection locations represented a genetically distinct population. Exceptions were multiple collections made within the Green Cove and Hopkins Ditch drainages, each of which represented a single population. Genetic relationships among populations appear to have been largely influenced by the glacial history of western Washington and the species limited dispersal ability and history of isolation in coastal areas. Prior to this study, the origins of Olympic mudminnow in eastern Puget Sound (native populations vs. undocumented introductions) were unknown. Our data indicate that these populations represent undocumented introductions of mudminnow from an unknown site or sites on the southern Olympic Coast. Information presented in this study will be useful for informing the conservation planning process for Olympic mudminnow and form the basis for future genetic monitoring efforts.

Introduction

Genetic data has become increasingly more useful and important for helping to manage species of conservation concern (Allendorf and Luikart 2007). One of the many advantages of incorporating genetic information into conservation and management plans is that genetic data often allows biologists to make inferences regarding species and populations when traditional types of data (e.g. abundance estimates, movement data) are unavailable. For example, genetic data can be used to estimate population size and effective population size (Luikart et al. 2010; Waples and Do 2010), determine if a population is stable or declining and perhaps the relative time frame the decline occurred (Williamson-Natesan 2005), and describe patterns of individual movement (DeHaan et al. 2011; Vollestad et al. 2012) and gene flow among populations (Smith et al. 2011; Homola et al. 2012). Genetic data can be especially important when information regarding the biology and demographic status of a species is limited, yet biologists and managers depend on biological and demographic data to move forward with conservation planning.

The Olympic mudminnow (*Novumbra hubbsi*) provides a good example of a case where information related to the species biology and the status of many populations is limited, yet this information is necessary for biologist and managers to move forward with conservation planning. The Olympic mudminnow is one of five species worldwide in the family Umbridae and is the only member of the genus *Novumbra*. Olympic mudminnow are endemic to western Washington and are generally found in marshy, wetland-type habitat with a muddy bottom, low water velocities, and abundant aquatic vegetation (Meldrim 1968; Harris 1974; Mongillo and Hallock 1999). They are tolerant of a wide range of environmental conditions but they generally avoid saline waters and swift moving currents (Meldrim 1968) and they do not often occur when large predatory fishes such as introduced largemouth bass (*Micropterus salmoides*) are present (Mongillo and Hallock 1999). The species' present distribution consists of four broad geographical areas in Washington State: 1) the Chehalis River Basin, 2) coastal drainages on the Olympic Peninsula (e.g., Lake Ozette, Quinault River, Copalis River), 3) south Puget Sound, and 4) east Puget Sound (Lake Washington and Snoqualmie River drainages). A substantial proportion of the wetland habitat in these areas has been lost or degraded in the past century (Mongillo and Hallock 1999) and as a result, the Olympic mudminnow is currently recognized as a state sensitive species and an increasing priority for conservation.

The distribution of Olympic mudminnow has been largely influenced by the glacial history of western Washington. At the maximum extent of the Pleistocene glaciation (approximately 15,000 years ago), Puget Sound and a large portion of western Washington was covered by the Puget Lobe of the Cordilleran Ice Sheet and Olympic mudminnow were restricted to a refugium in the Chehalis River Basin and coastal river systems on Washington's Olympic Peninsula that remained unglaciated (McPhail 1967; McPhail and Lindsey 1986). As the Puget Ice Lobe began to recede, a large freshwater lake formed at the southern end of present day Puget Sound and this lake drained to the south into the Chehalis River. Olympic mudminnow were able to access newly available freshwater habitat via the glacial connections between southern Puget Sound and the Chehalis River (McPhail 1967). Mudminnow generally avoid swift currents (Meldrim 1968) and presumably because of this they did not disperse beyond drainages in southern Puget Sound.

One notable exception to the pattern of Olympic mudminnow distribution is the species occurrence in drainages of eastern Puget Sound (Lake Washington and Snoqualmie River drainages). Mongillo and Hallock (1999) considered these populations introduced based on several lines of evidence including: mudminnow avoid swift currents and would likely have avoided the swift flowing waters that connected south Puget Sound to east Puget Sound during glacial recession; mudminnow have never been found in any other areas north of the Nisqually River system; no mudminnow were discovered following a rotenone treatment of Margaret Lake downstream of the Cherry Creek population; and mudminnow have only been observed at an elevation greater than 110 m in one location, however the sites in east Puget Sound are at elevations of 240 m (Cherry Creek) and 135 m (East Fork Issaquah Creek). Alternatively, Trotter et al. (2000) proposed that mudminnow in east Puget Sound may be native and that they reached these areas via waterways that connected east Puget Sound to south Puget Sound during glacial recession. The authors also suggested that mudminnow may have persisted at the glacial margins and then recolonized habitat in eastern Puget Sound following glacial recession (Trotter et al. 2000). Trotter et al. (2000) concluded that genetic data would be useful for resolving the question of the origins of mudminnow in eastern Puget Sound.

Although there is a basic understanding of the species biology, life history, and distribution, other information important for conservation including population abundance and trends (i.e. increasing, decreasing), the degree of migration and gene flow among populations,

and the long-term viability of mudminnow populations is limited. Genetic information could be useful for helping to infer the answers to these questions; unfortunately, genetic information for Olympic mudminnow is also limited. Lopez et al. (2000) conducted a phylogenetic analysis of Esocoid fishes using mitochondrial DNA (mtDNA) and found that Olympic mudminnow were more closely related to members of the genus *Esox* than they were to other mudminnow species. Pickens (2003) conducted a genetic analysis of Olympic mudminnow collected from seven locations using mtDNA and found almost no genetic variation. Pickens (2003) concluded that more variable nuclear markers (i.e. microsatellites) would be required to sufficiently characterize levels of genetic variation within mudminnow and to infer the genetic relationships among watersheds and sampling locations.

Based on the need for additional information regarding the status and genetics of Olympic mudminnow, our study had the following objectives:

1. Develop new, highly variable microsatellite markers from an Olympic mudminnow genomic library
2. Describe the levels of genetic variation within mudminnow collections from throughout the species range
3. Determine the spatial scale that constitutes a “population” of Olympic mudminnow
4. Determine the major evolutionary units/genetic groups of Olympic mudminnow to aid with possible designation of management or conservation units
5. Determine the origins of Olympic mudminnow populations located on the eastern side of Puget Sound

Materials and Methods

Sample collection

Olympic mudminnow were collected at 23 different locations distributed throughout the species range (Figure 1; Table 1). Collection sites were distributed among five broad geographic areas; south Puget Sound (n = 5), the Chehalis River Basin (n = 7), east Puget Sound (n = 3), Grays Harbor (n = 1), and the Olympic Coast (n = 7). Collections from Hopkins Ditch, a low-gradient stream in the Chehalis River Basin, and Green Cove, a wetland and low-gradient stream complex in the south Puget Sound area, included three replicate sample sites spaced approximately 1-2 km apart in Green Cove, and approximately 2.5 km apart in Hopkins Ditch.

Mudminnow were collected from 2010 to 2012 by dip netting, baited minnow traps, and electrofishing. Collections targeted 50 individuals per sampling site. A small piece of tissue was taken from the upper caudal fin of each individual captured and preserved in 100% non-denatured ethanol.

Laboratory Methods

Microsatellite marker development was conducted by Genetic Identification Services (GIS; Chatsworth, CA). Microsatellite loci were isolated from whole genomic DNA extracted by GIS from 10 Olympic mudminnow collected from Hopkins Ditch (n = 4), Green Cove (n = 3), and the ditch along Highway 109 (n = 3) using methods described by Jones et al. (2002). Six library enrichments were attempted simultaneously using biotin capture molecules designed for unique tetranucleotide motifs (AAAC₈, CATC₈, TACA₈, TAGA₈, CAGA₈, AAAG₈, AAAT₈, TGAC₈). Microsatellite-enriched libraries were produced by ligating Olympic mudminnow fragments into the *HindIII* cut site of pUC19 plasmid. Recombinant plasmids were electroporated into *Escherichia coli* DH5 α . Recombinant plasmids were examined using a blue-white screen to test for successful ligations into the vector. Successful clones were chosen randomly for sequencing analysis using ABI PRISM *Taq* dye terminator methodology and run on an Applied Biosystems 3730 (Life Technologies, Foster City, CA) genetic analyzer. Primers were designed for the flanking regions of 21 potential microsatellites discovered during sequencing analysis using DesignPCR version 1.03 (Research Genetics, Inc.).

The 21 potential microsatellites identified by GIS were screened for variation at Abernathy Fish Technology Center (AFTC) using Olympic mudminnow samples from throughout their range (n = 8). We extracted DNA from fin clips using Qiagen DNeasy 96 blood and tissue extraction kits (Qiagen Inc, Valencia, CA) following the manufacturer's instructions. Polymerase chain reactions (PCR) were carried out in 10 μ l volumes containing 2 μ l of template DNA, 5 μ l of 2x QIAGEN Multiplex PCR Master Mix (final concentration of 3mM MgCl₂), 2.8 μ l of water and 0.2 μ M of forward and reverse primer. PCR thermal conditions consisted of an initial activation step of 15 minutes at 95 °C, 30 cycles of 95 °C for 30 seconds, 90 seconds at primer specific annealing temperature, and 72 °C for 90 seconds, followed by a final extension of 72 °C for 10 minutes. Forward primers were 5'-end labeled with fluorescent dyes. Annealing temperatures for each multiplex PCR are provided in Appendix 1. The 21 amplified loci were

screened for variation using an Applied Biosystems 3130xl automated genotyping platform (Life Technologies) using an internal size standard (Genescan-500 LIZ, Life Technologies) and analyzed using GENEMAPPER software version 4.0 (Life Technologies).

DNA was then extracted from a total of 1,081 Olympic mudminnow from the 23 collection sites listed in Table 1 using Qiagen DNeasy 96 blood and tissue extraction kits. All individuals were genotyped at a subset of 13 loci (Appendix 1) following the procedures outlined above.

Statistical Analysis

Mudminnow were initially grouped according to collection sites for statistical analysis (Table 1). All collections were tested for departures from Hardy-Weinberg equilibrium (HWE) expectations using exact tests implemented in the program GENEPOP v4.0.7 (Raymond and Rousset 1995). We also used GENEPOP to test each collection for evidence of linkage disequilibrium (LD; i.e. non-random association among genotypes). HWE and LD tests were performed to ensure that collections from each location represented a random collection of individuals at that site and did not contain large numbers of closely related individuals or individuals from multiple spawning populations. We adjusted significance values for HWE and LD tests for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989).

We used the program CONVERT v1.31 (Glaubitz 2004) to estimate allele frequencies for each sampling location. We then determined the total number of alleles observed over all loci for each sampling location and the number of private alleles observed at each sampling location. We used the program GDA (Lewis and Zaykin 2001) to estimate measures of genetic diversity for each sampling location including the mean number of alleles per locus, expected heterozygosity, observed heterozygosity, and F_{IS} (a measure of departure from HWE). Additionally, we used the program HP-Rare v1.0 (Kalinowski 2005) to estimate allelic richness for each sampling location based on a minimum sample size of 58 genes. This program provides estimates of allelic richness that have been corrected for differences in sample size among different locations. We then grouped populations according to their geographic areas (east Puget Sound, south Puget Sound, Chehalis River Basin, Olympic Coast) to determine if there were significant differences in measures of genetic diversity among the different geographic areas. For this analysis, the single collection from Grays Harbor (Gillis Slough Pond) was included with the Olympic Coast

collection sites. We used permutation tests (1,000 permutations) implemented in the program FSTAT v2.9.3.2 (Goudet 2001) to determine if there were significant differences in observed heterozygosity, expected heterozygosity, allelic richness, and F_{IS} among the different groups.

We tested each sampling location for evidence of a recent genetic bottleneck (within the past 0.2-4.0 effective generations) using the program BOTTLENECK (Piry et al. 1999). This program tests for an excess of heterozygotes relative to the frequency of alleles in a population (Cornuet and Luikart 1996). We assumed a two-phased model of mutation with 90% stepwise mutations and 10% variance in non-stepwise mutations. The significance of genetic bottleneck tests was evaluated using a one-sided Wilcoxon test.

To determine what constitutes a population of Olympic mudminnow, we first estimated the overall level of genetic variation among sample sites (F_{ST}) and the associated 95% confidence interval based on 1,000 bootstrap replicates using the program FSTAT. We also calculated estimates of F_{ST} for each of the four geographic areas described above (Gillis Slough was grouped with the Olympic Coast for this analysis as well) and conducted permutation tests with FSTAT to determine if there was a significant difference in the level of genetic variation among the different geographic areas. We then used FSTAT to estimate the level of genetic variation among each pair of sampling locations (pairwise F_{ST}). We used GENEPOP to perform tests of allele frequency heterogeneity to determine if there were significant differences in allele frequencies among all sampling locations. Significance values for allele frequency heterogeneity tests were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989).

We used two methods to determine the spatial genetic relationship among mudminnow collection sites. First we conducted a multi-dimensional factorial correspondence analysis (FCA) using the program GENETIX (Belkhir et al. 2004). This method provides a graphical approach similar to principal component analysis (PCA) for viewing the data where individuals or populations which are more genetically similar to one another cluster together on the graph. We plotted the first two variance components for each population. We also constructed a consensus neighbor-joining tree (NJ-tree) using the program PHYLIP v3.6 (Felsenstein 1993). We first generated 1,000 replicate datasets using a bootstrap procedure. We then estimated Cavalli-Sforza and Edwards' (1967) chord distances among sampling locations and we constructed a consensus NJ tree based on these values.

We conducted an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to determine how genetic variation was partitioned among sample locations and among the different geographic areas. We performed 4 separate AMOVA analyses with populations organized into two, three, five, and six different groups. Populations were grouped for AMOVA analysis according to geographic locations and based on genetic similarities observed from the FCA and NJ-tree analyses. The AMOVA with two groups included a coastal group (sample sites 16-23) and a Puget Sound and Chehalis River group (sample sites 1-15). The AMOVA with three groups included three groups identified in the FCA and NJ-tree analyses; a south Puget Sound and Chehalis River group (sites 1-12), a south Olympic Coast and east Puget Sound group (sites 13-19) and a north Olympic Coast group (sites 20-23). For the AMOVA with five groups, we organized collection sites into broad geographic areas including a south Puget Sound group (sites 1-5), an east Puget Sound group (sites 13-15), a Chehalis River group (sites 6-12), a Grays Harbor group (site 16), and an Olympic Coast group (sites 17-23). The AMOVA analysis with six groups included a south Puget Sound and Chehalis River group identified from the FCA and NJ-tree analysis (sites 1-12), a south Olympic Coast and east Puget Sound group identified in the FCA and NJ-tree analysis (sites 13-19), and the four collections from the North Olympic Coast (James Pond, N.F. Whale Creek, Steamboat Creek Bog, and Lake Ozette Pond) were each split out as separate groups based on the high level of differentiation they exhibited in the FCA and NJ-tree analyses. AMOVA analyses were conducted using the program ARLEQUIN v3.5.1.2 (Excoffier et al. 2005). Significance tests for the different variance components were based on 1,023 permutations.

Olympic mudminnow generally avoid swift currents and salt water (Meldrim 1968), and presumably they have limited dispersal ability. In order to determine if there was any migration and potential for gene flow among the different sampling locations, we used the program GeneClass2 (Piry et al. 2004) to identify any first generation migrants among sampling locations in our dataset. We used the Bayesian assignment method of Rannala and Mountain (1997) to determine the likelihood of observing each individual's genotype in the location (designated as populations for this analysis) it was collected from (L_home). We chose to use L_home to identify first generation migrants because we did not sample all potential source populations (see Piry et al. 2004 for details). Individuals were identified as first generation migrants if L_home was less than the threshold probability value (based on 1,000 simulations) of 0.01. For this

analysis the replicate sample sites from Green Cove and Hopkins Ditch were combined into a single population from each site based on the fact that no significant differences in allele frequencies were observed among the replicate sample sites (see below).

We also compared the level of variation among collection sites based on allele identity (F_{ST}) to the level of variation based on allele size (R_{ST}) to help infer the relative divergence time among the different sample sites. If populations have diverged relatively recently, differences among populations would be due primarily to genetic drift and estimates of F_{ST} would be similar to estimates of R_{ST} . Alternatively, if populations were historically diverged from one another, differentiation would also be the result of stepwise mutations and estimates of R_{ST} would be greater than estimates of F_{ST} (Hardy et al. 2003). We were particularly interested in the comparisons between the eastern Puget Sound populations and the other sample sites in our study given the competing theories regarding the origins of the eastern Puget Sound populations. If mudminnow recolonized habitat in eastern Puget Sound following glacial recession or if eastern Puget Sound populations were relict populations, we would expect estimates of R_{ST} to be significantly greater than estimates of F_{ST} . Alternatively, if mudminnow in eastern Puget Sound were the result of recent translocations, we would expect estimates of F_{ST} to be similar to estimates of R_{ST} for comparisons between eastern Puget Sound populations and potential source populations. We calculated pairwise estimates of F_{ST} and R_{ST} among all sample sites using the program SPAGeDi v1.2 (Hardy and Vekemans 2002) and we used a permutation test (1,000 permutations) to determine if there were significant differences among pairwise estimates of F_{ST} and R_{ST} .

Results

Nine of the 21 loci developed by GIS were discarded due to non-resolvable electropherograms. We were able to score the remaining 13 loci consistently and we developed three multiplex sets for these 13 loci (Appendix 1). The number of alleles per locus detected during initial screening efforts ranged from 4 (*Nhub13*) to 48 (*Nhub07*; Appendix 1).

Fifteen of the 23 collection sites we surveyed had at least one locus that was fixed for a single allele (showed no variation) and there were also several instances where a collection site had a locus with an allele at a frequency of 0.9 or greater (Appendix 2). Prior to Bonferroni correction, we observed 11 deviations from HWE out of 265 total tests. Following Bonferroni

corrections we observed no loci that deviated from HWE expectations at any sampling location. We observed 11 locus pairs out of 1,452 comparisons that showed evidence of linkage. There was no clear pattern of linkage among populations or pairs of loci. These results suggest that the samples collected from each site did not contain high numbers of closely related fish or fish from multiple distinct spawning populations.

The different measures of genetic diversity we estimated varied considerably among collection sites. The total number of alleles observed at a site ranged from 41 at Adna Wetland to 170 at Satsop Slough (Table 1). We observed private alleles (alleles found in only a single sampling location) in nine of the 23 collection sites. The number of private alleles observed ranged from one private allele observed in S. Hanaford Creek to 15 private alleles observed in Satsop Slough (Table 1). Six of the nine locations where we observed private alleles were sites on the Olympic Coast and the other three sites were within the Chehalis River Basin. The mean number of alleles per locus and allelic richness were both lowest in Adna Wetland (3.154 and 2.859, respectively) and greatest in Satsop Slough (13.077 and 11.322, respectively; Table 1). Expected heterozygosity and observed heterozygosity were both lowest in James Pond (0.373 and 0.380, respectively) and greatest in the collection from the ditch along Highway 109 (0.751 and 0.755, respectively; Table 1). When we calculated mean estimates of the total number of alleles, allelic richness, expected heterozygosity, and observed heterozygosity for the four different geographic areas, all estimates of genetic diversity were greatest in the Chehalis River Basin (Table 2) and permutation tests showed that observed and expected heterozygosity were significantly greater in the Chehalis River Basin compared to the coastal populations ($P < 0.05$). All other comparisons were not significant. Two of our sample sites showed evidence of a recent genetic bottleneck; S. Hanaford Creek (Wilcoxon test $P = 0.024$) and James Pond (Wilcoxon test $P = 0.012$).

The overall level of genetic variation among collection sites (i.e. global F_{ST}) was 0.273 and was significantly different from 0.0 (95% C.I. = 0.200-0.361). Estimates of F_{ST} for the four geographic areas were as follows: Chehalis River Basin $F_{ST} = 0.101$; south Puget Sound $F_{ST} = 0.167$; east Puget Sound $F_{ST} = 0.089$; and Olympic Coast (including Gillis Slough) $F_{ST} = 0.350$. Permutation tests showed that the F_{ST} estimate for the Olympic Coast was significantly greater than the other geographic areas and all other comparisons were not significant. Pairwise estimates of F_{ST} ranged from 0.002 for comparisons within the Green Cove and Hopkins Ditch

sites to 0.539 for the pairwise comparison between James Pond and Upper Cook Creek (Table 3). In general, the greatest pairwise F_{ST} estimates were for comparisons among collection sites from the Olympic Coast. Tests of allele frequency heterogeneity showed that there were significant differences in allele frequencies among all sampling locations with the exception of the comparisons among the three Hopkins Ditch sites and the comparisons among the three Green Cove sites.

The first axis on the FCA plot accounted for 17.93% of the total variation observed and separated the four collection sites from the northern Olympic Coast (Lake Ozette Pond, James Pond, Steamboat Creek Bog, and N.F. Whale Creek) from all other collection sites (Figure 2). The second axis on the FCA plot accounted for 13.19% of the total variation and separated collection sites in the Chehalis River Basin and south Puget Sound from collection sites from the southern Olympic Coast (Upper Cook Creek, Conner Creek, ditch along Hwy 109) and east Puget Sound (Figure 2). Gillis Slough, which drains into Grays Harbor, clustered with the south Olympic Coast and east Puget Sound collection sites. Similar to the FCA plot, the consensus NJ-tree grouped collection sites into three main groups: one group consisting of collection sites from south Puget Sound and the Chehalis River Basin; one group consisting of collection sites from the south Olympic Coast, Gillis Slough, and east Puget Sound; and one group consisting of collection sites from the north Olympic coast (Figure 3). The majority of the branches on the tree had greater than 50% bootstrap support. The Woodard Creek and Adna Wetland collections grouped intermediate to the collections from the north Olympic Coast and the south Olympic Coast/east Puget Sound; however bootstrap support for these nodes was less than 50%.

We conducted four separate AMOVA analyses to examine how variation was partitioned among mudminnow collection sites. We observed the greatest amount of genetic variation among groups (18.78%) when sampling sites were organized into six separate groups and the least amount of genetic variation among groups (6.64%) when sampling sites were organized into two separate groups (Table 4). Significance tests showed the level of variation among groups and among collections sites within groups was significantly different from 0.0 ($P < 0.0001$) for all four AMOVA analyses but the level of variation among individuals within collection sites was not significantly different from 0.0 for any of the AMOVA analyses.

In order to determine the potential for gene flow among populations, we conducted an analysis to identify first generation migrants. Out of the 1,081 individuals we collected for

analysis, we identified 26 potential first generation migrants (Table 5). Five of these individuals were migrants among collection sites within the Chehalis River Basin, three individuals were migrants among collection sites within east Puget Sound, and the remaining 18 were migrants among collection sites on the Olympic Coast. Most collection sites where we identified potential first generation migrants contained only a single migratory individual; however, Conner Creek and the ditch along Hwy 109 each had four fish identified as potential migrants and James Pond and N.F. Whale Creek each had five fish identified as potential migrants (Table 5).

We compared pairwise estimates of F_{ST} and R_{ST} to infer the divergence history among collection sites. Permutation tests identified 30 comparisons out of 253 total where pairwise R_{ST} was not significantly greater than pairwise F_{ST} suggesting the two collection sites being compared had diverged relatively recently (Table 6). Eleven of these 30 comparisons involved collection sites in east Puget Sound. We observed non-significant comparisons among the three east Puget Sound collection sites as well as among the three east Puget Sound sites and collection sites on the south Olympic Coast. The remaining pairwise comparisons where R_{ST} was not significantly greater than F_{ST} involved collection sites within the Chehalis River Basin ($n = 14$; including the comparisons among the Hopkins Ditch sites), comparisons among the Green Cove sites in south Puget Sound ($n = 3$), and comparisons among coastal sites ($n = 2$).

Discussion

Microsatellite marker development

A previous study of genetic variation in Olympic mudminnow utilized mtDNA and found very little variation among individuals (Pickens 2003). That study concluded that more variable nuclear microsatellite loci would likely be necessary to accurately characterize levels of genetic variation in Olympic mudminnow (Pickens 2003). To meet the objectives of our study, we developed new microsatellite loci specifically for Olympic mudminnow and used a suite of 13 of these loci to genotype over 1,000 individuals. These new loci showed moderate to high levels of variation in Olympic mudminnow (4-48 alleles per locus) and allowed us to conduct the fine-scale analyses of genetic variation described above. The number of alleles observed at each locus and the levels of genetic diversity observed (e.g. allelic richness, heterozygosity) were equivalent to what we have observed in other fish species (DeHaan and Ardren 2005; Ardren et al. 2007). In addition to the analyses described in this study, these loci could be useful for a number of future

genetic studies of Olympic mudminnow designed to monitor changes in genetic variation and population status over time, examine patterns of individual movement and gene flow in greater detail, and perhaps estimate census and effective population sizes. A paper describing the primer development process has recently been published in the journal *Conservation Genetics Resources* (Adams et al. 2013).

Genetic variation within sampling locations

An understanding of the level of genetic variation within populations is important for population monitoring and developing effective conservation plans. Populations with greater levels of genetic variation may have increased fitness and be less susceptible to extirpation (Quattro and Vrijenhoek 1989; Saccheri et al. 1998; Reed and Frankham 2003). Furthermore, populations with increased genetic variation may be better suited to adapt to future changes in habitat and other environmental characteristics (Allendorf and Luikart 2007). In the case of Olympic mudminnow, information on population abundance trends (i.e. stable, increasing, decreasing) is limited and information on genetic variation within sample sites and populations could be used as another means to infer population viability. Although there are not specific levels of genetic variation that are necessary for long term population viability, comparisons among populations can be useful for identifying populations with reduced genetic variation which may face a greater risk of decline or extirpation. Our estimates of genetic variation ranged widely among the 23 locations we sampled for this study. Comparisons among collection sites showed that some sites had relatively low genetic variation and may be at risk as a result. For example, within the Chehalis River Basin, estimates of variation for the Adna Wetland collection were among the lowest we observed whereas estimates of variation for the Chehalis Oxbow and Satsop Slough collections were two to four times greater. Populations such as Adna Wetland with low levels of variation might represent relatively small or declining populations and may warrant increased population monitoring or additional conservation measures.

Tests for recent genetic bottlenecks can also be useful for identifying populations that may have recently declined. Two of the sampling locations we surveyed, James Pond and South Hanaford Creek, showed evidence of a recent genetic bottleneck based on a heterozygote excess. James Pond has almost completely dried up multiple times in recent years and mudminnow collected from James Pond for our study were likely the offspring of a small number of

individuals that survived in small pockets of habitat when the size of the pond was constricted (P. Crain, Olympic National Park, *personal communication*). Although abundance data does not exist for the South Hanford Creek collection site, fewer mudminnow were observed at this site during sampling efforts than other nearby sites within the Chehalis River Basin (USFWS, *unpublished data*). It is important to keep in mind that the bottleneck test we performed is able to detect a bottleneck that occurred within approximately the last four generations (Cornuet and Luikart 1996) and a genetic bottleneck may have occurred outside this time period at some of the other collection sites in our study.

Both the glacial history of western Washington and the species life history appear to have an important influence on the level of genetic variation within Olympic mudminnow collections. Species typically show increased genetic variation within populations located in or near areas of former glacial refugia compared to populations from formerly glaciated areas that were presumably founded by small numbers of individuals following glacial recession (Bernatchez and Wilson 1998; Costello et al. 2003; Stamford and Taylor 2004). During the Pleistocene glaciation, the Chehalis Basin was a refugium for Olympic mudminnow and habitat in Puget Sound was colonized by migrants from the Chehalis Basin following glacial recession (McPhail 1967; McPhail and Lindsey 1986). Comparisons among the four geographic areas (Chehalis River Basin, south Puget Sound, east Puget Sound, Olympic Coast) showed that genetic variation was significantly greater in collections from the Chehalis River Basin compared to the other three geographic regions. Much of the freshwater habitat that mudminnow occupy on the Olympic Coast also remained ice free during the Pleistocene glaciation; however, genetic variation within collections from the Olympic Coast was substantially lower than estimates from the Chehalis River Basin. This likely reflects the long history of isolation among many of these areas resulting from the species' limited capacity for dispersal in coastal areas. The only hydrologic connection among many of the sites we sampled from the Olympic Coast would have been via the Pacific Ocean. Given that mudminnow are a freshwater species that avoid salinity (Meldrim 1968), migration among these sites would not have been possible. Historically isolated populations often contain private alleles not found in other populations (Slatkin 1985; Allendorf and Luikart 2007). Nearly all of the collection sites on the Olympic Coast contained multiple private alleles, providing further evidence of the long isolation history among these collection sites.

Genetic variation among sampling locations

Genetic data have been widely used to help delineate populations in order to guide conservation planning (Pearse and Crandall 2004; Waples and Gaggiotti 2006; Ardren et al. 2011). Prior to establishing conservation guidelines for Olympic mudminnow, it is important that biologists have a clear understanding of what constitutes a population for the species. We employed a hierarchical sampling strategy involving collections from different geographic areas, multiple collection sites within each geographic area, and multiple collections within two sites, in order to determine the spatial scale that constitutes a population. The overall level of genetic variation we observed ($F_{ST} = 0.273$) was relatively high compared to other species with similar life histories (e.g. Oregon chub, *Oregonichthys crameri*; DeHaan et al. 2012) and suggests that a strong degree of population structure exists. The level of genetic variation among collection sites varied among the four geographic areas we surveyed. Although we observed a difference in the overall F_{ST} estimates for the Chehalis River Basin, south Puget Sound, and east Puget Sound (range, 0.089 to 0.167), permutation tests showed that there was no significant difference in the overall F_{ST} estimates among these three geographic areas. The overall F_{ST} estimate for the Olympic Coast was nearly two to four times greater than the other geographic areas ($F_{ST} = 0.350$) and permutation tests showed there was a significantly greater level of variation among coastal collection sites. Historically isolated populations typically show increased levels of genetic divergence due to limited genetic exchange and increased genetic drift (Costello et al. 2003; Currrens et al. 2009). As noted above, mudminnow in coastal habitats have been historically isolated from one another since the only hydrologic connection among populations is via the Pacific Ocean. Historically limited gene flow among mudminnow from the Olympic Coast explains the higher level of variation among coastal sampling sites relative to the other geographic areas.

The pairwise F_{ST} estimates among the 23 sampling locations were relatively high and we observed significant allele frequency differences among nearly all sampling locations. The exception to this pattern was the multiple collections from Green Cove and Hopkins Ditch where we observed pairwise F_{ST} estimates near 0.0 and no differences in allele frequencies among collections. These data suggest that each sampling location represents a genetically distinct population of mudminnow and that the two large stream and wetland complexes (Green Cove

and Hopkins Ditch) each contain a single population. Relatively high pairwise F_{ST} estimates indicate that little genetic exchange occurs among most of the populations we sampled. The analysis of first generation migrants also suggested little migration and gene flow occurs; only 26 first generation migrants were detected out of 1,081 individuals. Most of the individuals identified as potential migrants were migrants from nearby populations within the same geographic area (e.g. South Hanford Creek and Hopkins Ditch in the Chehalis River Basin; People's Creek and Cherry Creek in E. Puget Sound). Although mudminnow generally avoid currents, individuals may be able to migrate among these locations via off-channel habitats or during flood events. It is important to note that we were only able to sample a fraction of the known mudminnow populations and putative migrants may represent fish from unsampled populations assigned to the most likely population. Five fish collected from James Pond were identified as migrants from North Fork Whale Creek and vice versa. The only connection between these two areas would be via the Pacific Ocean therefore migration among these sites is not possible. James Pond has dried up multiple times in recent years and the population showed evidence of a recent bottleneck. Examination of the allele frequencies for James Pond shows that this population was fixed for several alleles found at high frequencies in North Fork Whale Creek. Identification of migrants among these two populations is likely the result of genetic similarity due to increased genetic drift when the population in James Pond was reduced to small numbers in recent years.

Analyses from several other species have demonstrated the important influence that glacial history has on the genetic relationships among populations (Bernatchez and Wilson 1998; Costello et al. 2003; Stamford and Taylor 2004). The FCA and NJ-tree analyses both highlight the effects of the Pleistocene glaciation on the genetic relationship among Olympic mudminnow populations. Following deglaciation, mudminnow colonized habitat in south Puget Sound from the Chehalis River refugium (McPhail 1967; McPhail and Lindsey 1986). This shared evolutionary history is reflected in both the FCA plot and the NJ-tree analysis where populations from these two distinct major drainages clustered together. Coastal mudminnow populations were highly diverged from the Chehalis Basin and south Puget Sound in these analyses. Many areas along the Olympic Coast remained ice free during the Pleistocene glaciation (McPhail 1967) and our data suggest that mudminnow persisted in these habitats independently of fish from the Chehalis River Basin during the Pleistocene glaciation. Our results were similar to

those of Rosenfeld (1983) who observed significant differences in morphometric and meristic characteristics between Olympic mudminnow from coastal populations and populations in the Chehalis River Basin. Rosenfeld (1983) attributed these differences to a historic barrier to gene flow in the area of the Montesano Hills created during the Pleistocene glaciation. Additional genetic analysis of populations near the Montesano Hills could help to determine if the genetic division between coastal mudminnow and fish from the Chehalis Basin occurs in the same populations observed by Rosenfeld (1983).

Within the coastal populations there appears to be further genetic divergence; populations from the southern Olympic Coast (sites 16-19 from the Quinault River Basin south to Grays Harbor), grouped separately from populations from the north Olympic Coast (Sites 20-23, north of the Quinault River). Furthermore, there was more similarity among populations from the south Olympic Coast compared to the populations from the north Olympic Coast, which were much more divergent from one another. Mudminnow have a broader distribution on the southern Olympic Coast (Mongillo and Hallock 1999) and populations may be periodically connected via off-channel habitats and during flood events. Alternatively, our collections from the north Olympic Coast represent nearly all of the sites mudminnow have been documented north of the Quinault River (Mongillo and Hallock 1999), and the only hydrologic connection among sample sites is via the Pacific Ocean. The difference in clustering patterns between the north and south Olympic Coast likely reflects the difference in the degree of isolation among populations.

Often times biologists and fisheries managers organize populations into conservation or management units in order to address the specific conservation needs of different populations and to set recovery goals. Previously biologists and fisheries managers have utilized several types of information to designate recovery units including behavioral, ecological, and genetic information (Nielsen 1995; Waples 1995; USFWS 1996). In instances where genetic information does not exist, fish populations are often grouped into conservation units according to their watershed or river basin of origin (e.g. USFWS 1998). We conducted four separate AMOVA analyses to determine how much genetic variation there was among different population groupings that could represent putative conservation units. Simply grouping populations according to their geographic region accounted for only 9.82% of the total genetic variation we observed, and there was nearly twice (19.0%) as much genetic variation among populations within groups when populations were grouped in this manner. AMOVA analyses based on the

relationships observed from the FCA and NJ-tree analyses accounted for a much higher degree of genetic variation among groups. We observed the greatest amount of genetic variation among groups (18.78%) when we designated six separate groups. This analysis separated each population from the north Olympic Coast into a separate group, which may not be reasonable from a management perspective. The best alternative based on the genetic information may be to have a Chehalis and south Puget Sound group, a south Olympic Coast Group, and a north Olympic Coast group, since these three groups were apparent from both the FCA and NJ-tree analyses and the AMOVA analysis with these groups showed the second highest level of variation among groups (15.83%). The question of where to place the east Puget Sound populations is complicated given the unique origins of these populations (see below). In the end, it is important that additional types of data including behavioral, ecological, morphological, etc. are also considered if biologists decide to designate multiple conservation units for the species.

Origins of populations in eastern Puget Sound

When the origins of a population are in question, genetic data often provide a useful means for determining the population's true history (Small et al. 2006; Small et al. 2011). Population history can have important implications for conservation and management; historically isolated populations may contain unique genetic material not found in other populations (Small et al. 2006; Ardren et al. 2011; Small et al. 2011) and these populations are often recognized as high priorities for conservation given their evolutionary potential (Lesica and Allendorf 1995). However, it is important to be able to distinguish historically isolated populations from undocumented introductions that may be a lower priority for conservation. Competing theories exist regarding the origins of Olympic mudminnow in eastern Puget Sound. Mongillo and Hallock (1999) concluded that mudminnow in eastern Puget Sound represented undocumented introductions whereas Trotter et al. (2000) suggested that these fish may have colonized eastern Puget Sound from the Chehalis River refugium or may represent historically isolated populations that persisted at the glacial margins during the Pleistocene glaciation.

Data from this study provide important insight into the origins of these populations. Populations that have been historically isolated often contain private alleles not observed in other populations (Slatkin 1985; Allendorf and Luikart 2007). Coastal mudminnow populations have been historically isolated and we observed several private alleles in these populations; however

no private alleles were observed in the eastern Puget Sound populations as we might expect if these were relict populations that persisted near the glacial margins in eastern Puget Sound. If mudminnow from the Chehalis refugium colonized areas in eastern Puget Sound, we would expect some degree of genetic similarity between these populations and the Chehalis River Basin and south Puget Sound populations. The FCA plot and the NJ-tree both indicated that populations in eastern Puget Sound were most similar to populations from the southern Olympic Coast and were quite differentiated from populations in the Chehalis River Basin and south Puget Sound. Pairwise estimates of F_{ST} provided further evidence that Olympic mudminnow in eastern Puget Sound were most similar to populations from the southern Olympic Coast. Comparisons between F_{ST} and R_{ST} can be useful for inferring the history of divergence between populations (Hardy et al. 2003; Peterson and Ardren 2009). Comparisons between F_{ST} and R_{ST} and permutation tests suggest that populations in eastern Puget Sound were historically diverged from most of the other populations we surveyed but were more recently diverged from populations from the southern Olympic Coast (i.e. no significant difference between F_{ST} and R_{ST}). Collectively these data suggest that the eastern Puget Sound populations were not historically isolated and that they are likely recent introductions from the southern Olympic Coast.

Introduced populations often show reduced levels of genetic variation and/or the signature of a genetic bottleneck when compared to natural populations, particularly when small numbers of founding individuals are used (Mock et al. 2004; Stephen et al. 2005). Interestingly, levels of genetic variation within the eastern Puget Sound populations were similar to those that we observed in other populations, and no genetic bottleneck was detected in these populations. The bottleneck test that we used is sensitive to a bottleneck that occurred within approximately the past four generations (Cornuet and Luikart 1996) and its possible that sufficient time has elapsed to allow the populations to recover from any genetic bottleneck associated with a founding event. The fact that genetic variation in these populations was equivalent to other natural populations suggests that either large numbers of individuals were introduced, or that multiple introductions occurred. Recent anecdotal evidence suggests that the latter situation was indeed the case. Accounts exist of multiple translocations of mudminnow from areas on the southern Olympic Coast to private ponds in the Cherry Creek drainage and additional translocations may have occurred into the Issaquah Creek drainage (USFWS, *unpublished data*).

Conclusions and Future Research

Olympic mudminnow have become an increasing priority for conservation given the amount of habitat that has been lost or degraded and the species limited geographic distribution. Given that limited data exist regarding several important aspects of the species life history and current population status, prioritizing populations for conservation can be challenging. Data presented in this study including tests for population bottlenecks, estimates of genetic variation, and the level of gene flow among populations will be useful to biologists and managers responsible for developing conservation plans for the species. For example, populations such as Adna Wetland that showed low levels of genetic variation may represent good candidates for increased population monitoring and conservation measures such as habitat protection. Data from our study also provide important insights into the origins of populations in eastern Puget Sound. Given that these populations are undocumented introductions, they likely do not merit the same prioritization for conservation as some of the historically isolated and genetically divergent populations on the Olympic Coast. In addition to providing data useful for developing and implementing conservation planning, our study also forms the basis for future genetic monitoring efforts for Olympic mudminnow (Schwartz et al. 2007; Turner et al. 2007). Periodic re-sampling of populations included in this study will allow biologists to track genetic change in these populations over time and provide additional information regarding population viability.

There are numerous sites in western Washington that contain Olympic mudminnow that we did not sample for this study and future collections and analyses from these areas may provide additional information regarding the species' natural distribution and evolutionary history, and help identify additional introduced populations. For example, the distribution of Olympic mudminnow on the north Olympic coast is limited; however this area is relatively undeveloped and additional undiscovered populations may exist. Collections of additional mudminnow from the north Olympic coast will likely help to further refine potential conservation area boundaries and provide additional insight into the evolutionary history of the species. Hagen and Moodie (1979) collected mudminnow in Puyallup Creek, northeast of the Nisqually River, and collections and genetic analyses of mudminnow from this area would also help to further characterize the species native distribution. We observed deep divergence between coastal populations and populations from the Chehalis Basin based on our genetic data,

a pattern that was previously observed from morphometric and meristic data as well (Rosenfeld 1983). Additional collections and genetic (i.e. mitochondrial DNA sequencing) and morphometric analyses may be useful to further explore the level of historic divergence between coastal and Chehalis Basin populations and to determine if additional taxonomic classification is warranted.

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Table 1. Collection site numbers, collection site names, geographic area of each sample site, number of individuals collected at each site, and measures of genetic diversity for 23 Olympic mudminnow collection locations. Measures of genetic diversity include: total number of alleles observed at each collection site (A_T), number of private alleles observed at each collection site (A_P), mean number of alleles per locus (A_M), allelic richness (A_R), expected heterozygosity (H_{exp}), observed heterozygosity (H_{obs}), and F_{IS} (a measure of departure from HWE). Collection site numbers correspond to map locations in Figure 1.

Collection site number	Sampling location	Geographic area	Number of individuals	A_T	A_P	A_M	A_R	H_{exp}	H_{obs}	F_{IS}
1	Green Cove 1	S. Puget Sound	45	92	0	7.077	6.529	0.604	0.603	0.002
2	Green Cove 2	S. Puget Sound	50	102	0	7.846	7.187	0.590	0.602	-0.020
3	Green Cove 3	S. Puget Sound	50	96	0	7.385	6.736	0.591	0.597	-0.011
4	Woodard Creek	S. Puget Sound	50	70	0	5.385	4.920	0.562	0.550	0.022
5	Spurgeon Creek	S. Puget Sound	50	96	0	7.385	6.808	0.653	0.651	0.003
6	Hopkins Ditch 1	Chehalis River	47	127	0	9.769	8.906	0.711	0.715	-0.006
7	Hopkins Ditch 2	Chehalis River	50	126	0	9.692	8.708	0.725	0.729	-0.006
8	Hopkins Ditch 3	Chehalis River	50	121	0	9.308	8.570	0.706	0.675	0.044
9	S. Hanaford Creek	Chehalis River	34	111	1	8.538	8.413	0.732	0.740	-0.011
10	Adna Wetland	Chehalis River	46	41	0	3.154	2.859	0.423	0.428	-0.014
11	Chehalis Oxbow Lake	Chehalis River	43	160	12	12.308	11.125	0.708	0.688	0.028
12	Satsop Slough	Chehalis River	50	170	15	13.077	11.322	0.729	0.728	0.002
13	Peoples Creek	E. Puget Sound	50	81	0	6.231	5.658	0.605	0.603	0.004
14	Cherry Creek	E. Puget Sound	50	93	0	7.154	6.623	0.658	0.669	-0.017
15	E.F. Issaquah Creek	E. Puget Sound	50	57	0	4.385	4.159	0.530	0.518	0.022
16	Gillis Slough Pond	Grays Harbor	43	123	4	9.462	8.711	0.703	0.678	0.035
17	Conner Creek	Olympic Coast	50	147	3	11.308	9.976	0.703	0.717	-0.019
18	Ditch along Hwy 109	Olympic Coast	47	132	2	10.154	9.119	0.751	0.755	-0.004
19	Upper Cook Creek	Olympic Coast	49	52	0	4.000	3.699	0.414	0.388	0.063
20	N.F. Whale Creek	Olympic Coast	44	68	4	5.231	4.926	0.408	0.426	-0.046
21	Steamboat Creek Bog	Olympic Coast	33	45	5	3.462	3.418	0.384	0.394	-0.027
22	James Pond	Olympic Coast	50	51	0	3.923	3.774	0.373	0.380	-0.018
23	Lake Ozette Pond	Olympic Coast	50	75	9	5.769	5.339	0.557	0.526	0.057

Table 2. Mean estimates of genetic variation for the four geographic areas samples were collected from. Estimates for the Olympic Coast also include the Gillis Slough collection site which drains into Grays Harbor. Abbreviations are as follows: n = total number of collection sites within the geographic area; A_T = total number of alleles; A_R = allelic richness; H_{obs} = observed heterozygosity; H_{exp} = expected heterozygosity.

Geographic Area	n	A_T	A_R	H_{obs}	H_{exp}
Chehalis River Basin	7	122.286	8.558	0.671	0.676
Olympic Coast (including Gillis Slough)	8	86.625	6.120	0.537	0.541
South Puget Sound	5	91.200	6.436	0.600	0.600
East Puget Sound	3	77.000	5.480	0.597	0.598

Table 3. Pairwise estimates of genetic variation (F_{ST}) among 23 Olympic mudminnow collection sites. Values in bold represent comparisons that did not show significant differences in allele frequencies based on tests of allele frequency heterogeneity.

Collection site #	Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Green Cove 1														
2	Green Cove 2	0.006													
3	Green Cove 3	0.002	0.003												
4	Woodard Creek	0.251	0.261	0.268											
5	Spurgeon Creek	0.170	0.182	0.187	0.210										
6	Hopkins Ditch 1	0.095	0.102	0.103	0.207	0.088									
7	Hopkins Ditch 2	0.094	0.107	0.108	0.186	0.078	0.003								
8	Hopkins Ditch 3	0.100	0.107	0.108	0.202	0.092	0.004	0.002							
9	S. Hanaford Creek	0.110	0.123	0.124	0.167	0.067	0.037	0.023	0.033						
10	Adna Wetland	0.286	0.302	0.296	0.315	0.279	0.248	0.236	0.246	0.235					
11	Chehalis Oxbow Lake	0.136	0.146	0.146	0.228	0.131	0.068	0.073	0.078	0.070	0.212				
12	Satsop Slough	0.134	0.142	0.145	0.216	0.102	0.074	0.071	0.075	0.063	0.226	0.055			
13	Peoples Creek	0.301	0.303	0.300	0.290	0.302	0.268	0.257	0.264	0.242	0.347	0.230	0.243		
14	Cherry Creek	0.262	0.264	0.262	0.282	0.276	0.225	0.215	0.221	0.210	0.308	0.180	0.189	0.061	
15	E.F. Issaquah Creek	0.357	0.354	0.351	0.385	0.369	0.319	0.315	0.316	0.307	0.412	0.277	0.287	0.126	0.080
16	Gillis Slough	0.272	0.281	0.279	0.263	0.230	0.190	0.186	0.193	0.185	0.311	0.162	0.173	0.154	0.096
17	Conner Creek	0.222	0.229	0.230	0.222	0.208	0.167	0.166	0.170	0.159	0.292	0.145	0.145	0.200	0.149
18	Ditch along Hwy 109	0.222	0.229	0.228	0.242	0.220	0.168	0.167	0.173	0.169	0.295	0.150	0.160	0.179	0.139
19	Upper Cook Creek	0.401	0.397	0.398	0.402	0.378	0.339	0.337	0.332	0.347	0.472	0.317	0.322	0.301	0.252
20	N.F. Whale Creek	0.421	0.421	0.424	0.454	0.396	0.369	0.361	0.366	0.377	0.482	0.345	0.336	0.414	0.389
21	Steamboat Creek Bog	0.441	0.445	0.443	0.335	0.394	0.372	0.362	0.371	0.351	0.516	0.370	0.354	0.372	0.366
22	James Pond	0.440	0.441	0.443	0.472	0.414	0.390	0.378	0.386	0.394	0.487	0.365	0.353	0.433	0.409
23	Lake Ozette Pond	0.401	0.410	0.408	0.382	0.362	0.342	0.332	0.343	0.330	0.461	0.330	0.328	0.378	0.350

Table 3. Continued

Collection site #	Location	15	16	17	18	19	20	21	22
1	Green Cove 1								
2	Green Cove 2								
3	Green Cove 3								
4	Woodard Creek								
5	Spurgeon Creek								
6	Hopkins Ditch 1								
7	Hopkins Ditch 2								
8	Hopkins Ditch 3								
9	South Hanaford Creek								
10	Adna Wetland								
11	Chehalis Oxbow Lake								
12	Satsop Slough								
13	Peoples Creek								
14	Cherry Creek								
15	EF Issaquah Creek								
16	Gillis Slough	0.198							
17	Conner Creek	0.244	0.138						
18	Ditch along Hwy 109	0.226	0.135	0.040					
19	Upper Cook Creek	0.325	0.235	0.279	0.277				
20	North Fork Whale Creek	0.465	0.364	0.391	0.372	0.515			
21	Steamboat Creek Bog	0.445	0.360	0.358	0.343	0.498	0.500		
22	James Pond	0.485	0.384	0.415	0.398	0.539	0.030	0.529	
23	Lake Ozette Pond	0.420	0.320	0.341	0.314	0.472	0.349	0.393	0.373

Table 4. Analysis of molecular variance (AMOVA) results. The level of variation among groups and among collection sites within groups was significantly different from 0.0 for all analyses. The level of variation among individuals within collection sites was not significantly different from 0.0 for any of the AMOVA analyses.

Number of groups	Population groupings	Percent of variation		
		Among groups	Among collection sites within groups	Among individuals within collection sites
2	Sites 1-15; Sites 16-23	6.64%	23.21%	0.20%
3	Sites 1-12; Sites 13-19; Sites 20-23	15.83%	15.73%	0.20%
5	Sites 1-5; Sites 13-15; Sites 6-12; Site 16; Sites 17-23	9.82%	19.00%	0.21%
6	Sites 1-12; Sites 13-19; Site 20; Site 21; Site 22; Site 23	18.78%	13.28%	0.20%

Table 5. Numbers of individuals identified as first generation migrants. The first column in the table represents the location where a putative migrant individual was collected from and the following columns represent the putative source population of that individual.

Collection location	Source population										
	Hopkins Ditch	Chehalis Oxbow	Satsop Slough	S. Hanaford Creek	Peoples Creek	Cherry Creek	E.F. Issaquah Creek	Hwy 109 Ditch	Conner Creek	James Pond	N.F. Whale Creek
Hopkins Ditch				2							
Chehalis Oxbow			1								
Satsop Slough		1									
S. Hanaford Creek	1										
Peoples Creek											
Cherry Creek					1		1				
E.F. Issaquah Creek						1					
Hwy 109 Ditch									4		
Conner Creek								4			
James Pond											5
N.F. Whale Creek										5	

Table 6. Population pairs that showed no significant difference between pairwise estimates of F_{ST} (the level of variation among populations based on allele identity) and R_{ST} (the level of variation among populations based on allele size) based on 1,000 permutations.

Sample site 1	Sample site 2	F_{ST}	R_{ST}	P -value
Peoples Creek	Cherry Creek	0.061	0.159	0.106
Peoples Creek	Conner Creek	0.200	0.243	0.050
Peoples Creek	Cook Creek	0.301	0.245	0.145
Peoples Creek	E.F. Issaquah Creek	0.126	0.112	0.571
Peoples Creek	Gillis Slough	0.153	0.155	0.122
Cherry Creek	Cook Creek	0.252	0.238	0.134
Cherry Creek	E.F. Issaquah Creek	0.080	0.028	0.848
Cherry Creek	Gillis Slough	0.096	0.084	0.253
E.F. Issaquah Creek	Cook Creek	0.325	0.229	0.436
E.F. Issaquah Creek	Gillis Slough	0.198	0.113	0.619
E.F. Issaquah Creek	Hwy 109 Ditch	0.226	0.334	0.068
Green Cove 1	Green Cove 2	0.006	0.006	0.480
Green Cove 1	Green Cove 3	0.002	-0.007	0.915
Green Cove 2	Green Cove 3	0.003	0.008	0.236
Chehalis Oxbow	Satsop Slough	0.055	0.060	0.122
Chehalis Oxbow	Adna Wetland	0.212	0.302	0.096
Hopkins Ditch 1	Hopkins Ditch 2	0.003	0.004	0.291
Hopkins Ditch 1	Hopkins Ditch 3	0.004	0.014	0.148
Hopkins Ditch 1	Spurgeon Creek	0.088	0.086	0.215
Hopkins Ditch 1	Adna Wetland	0.248	0.291	0.114
Hopkins Ditch 2	Hopkins Ditch 4	0.002	-0.003	0.803
Hopkins Ditch 2	Spurgeon Creek	0.078	0.089	0.168
Hopkins Ditch 2	Adna Wetland	0.236	0.284	0.137
Hopkins Ditch 3	Spurgeon Creek	0.092	0.108	0.175
Hopkins Ditch 3	Adna Wetland	0.246	0.320	0.097
Adna Wetland	Green Cove 2	0.302	0.353	0.056
Adna Wetland	Spurgeon Creek	0.279	0.272	0.308
Adna Wetland	Woodard Creek	0.315	0.175	0.699
Gillis Slough	Cook Creek	0.235	0.142	0.405
James Pond	N.F. Whale Creek	0.030	0.013	0.588

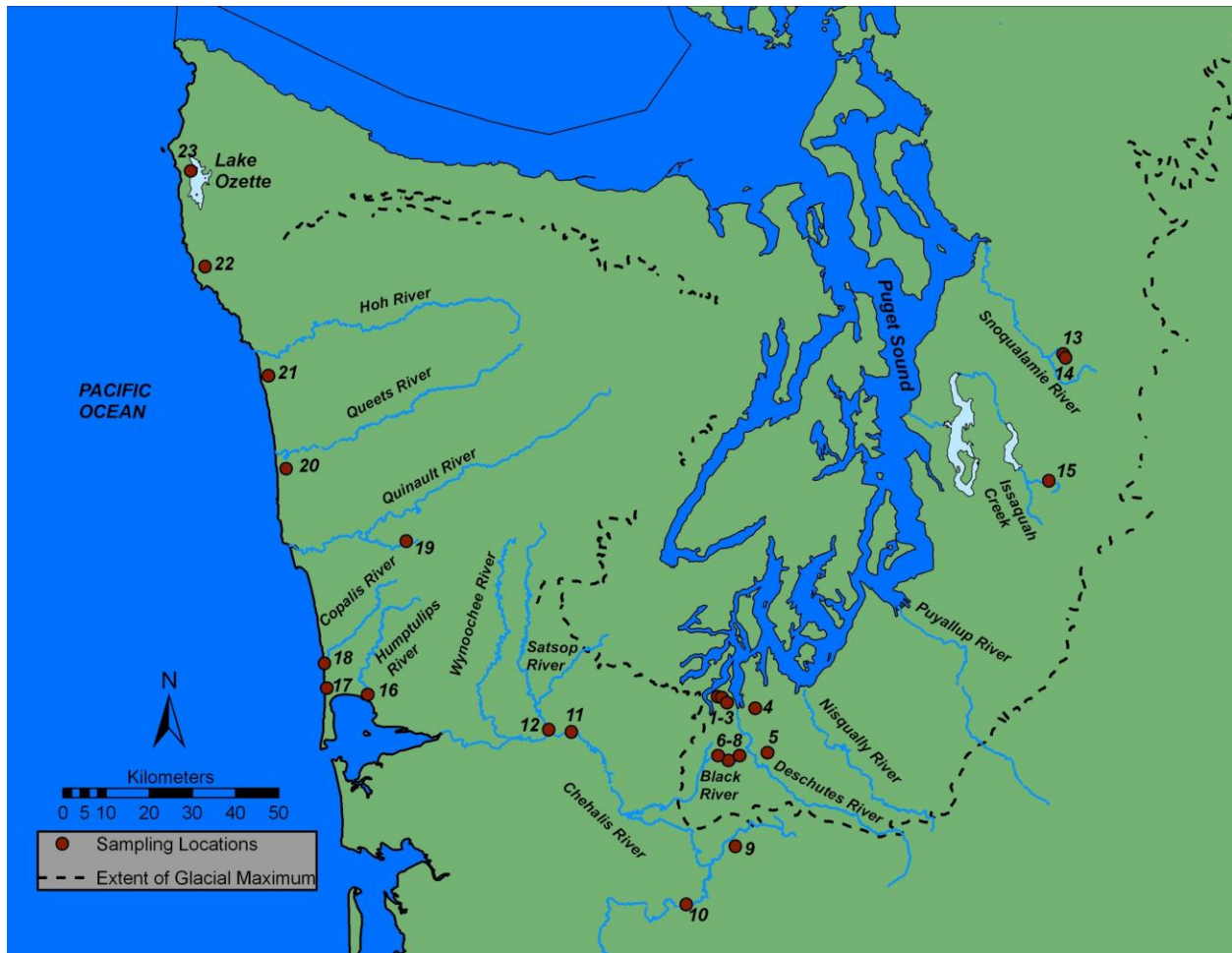


Figure 1. Locations where Olympic mudminnow were collected for this study. The dashed line represents the maximum extent of the Puget Ice Lobe during the last glacial period. Sampling location numbers correspond to numbers in Table 1.

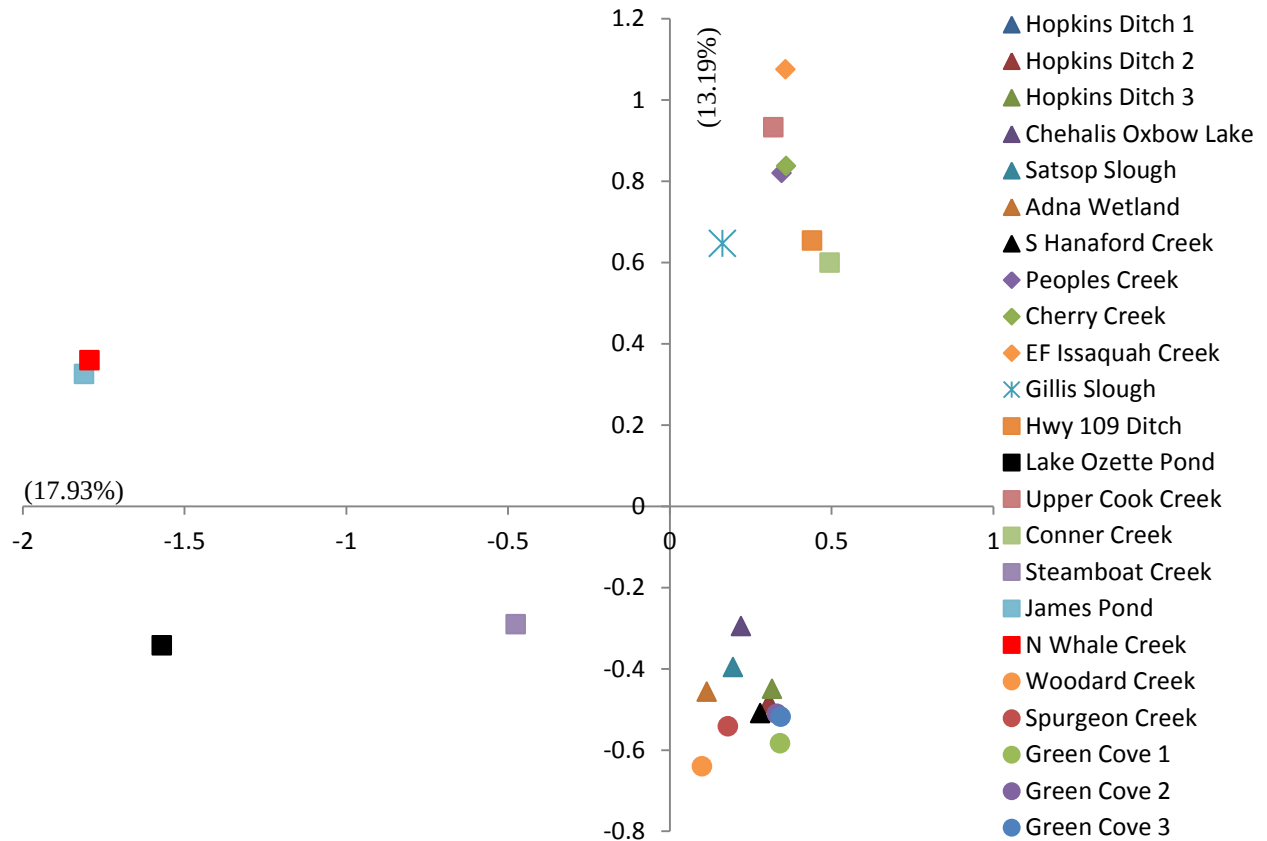


Figure 2. Correspondence analysis (FCA) of 23 Olympic mudminnow collections. Each point on the graph represents one of the collection sites in our analysis. Circles correspond to collection sites in south Puget Sound, triangles correspond to collection sites in the Chehalis River Basin, diamonds correspond to collection sites in east Puget Sound, squares correspond to collection sites on the Olympic Coast, and the asterisk corresponds to the collection site in Grays Harbor. Numbers in parentheses represent the percent of variation accounted for by each axis.

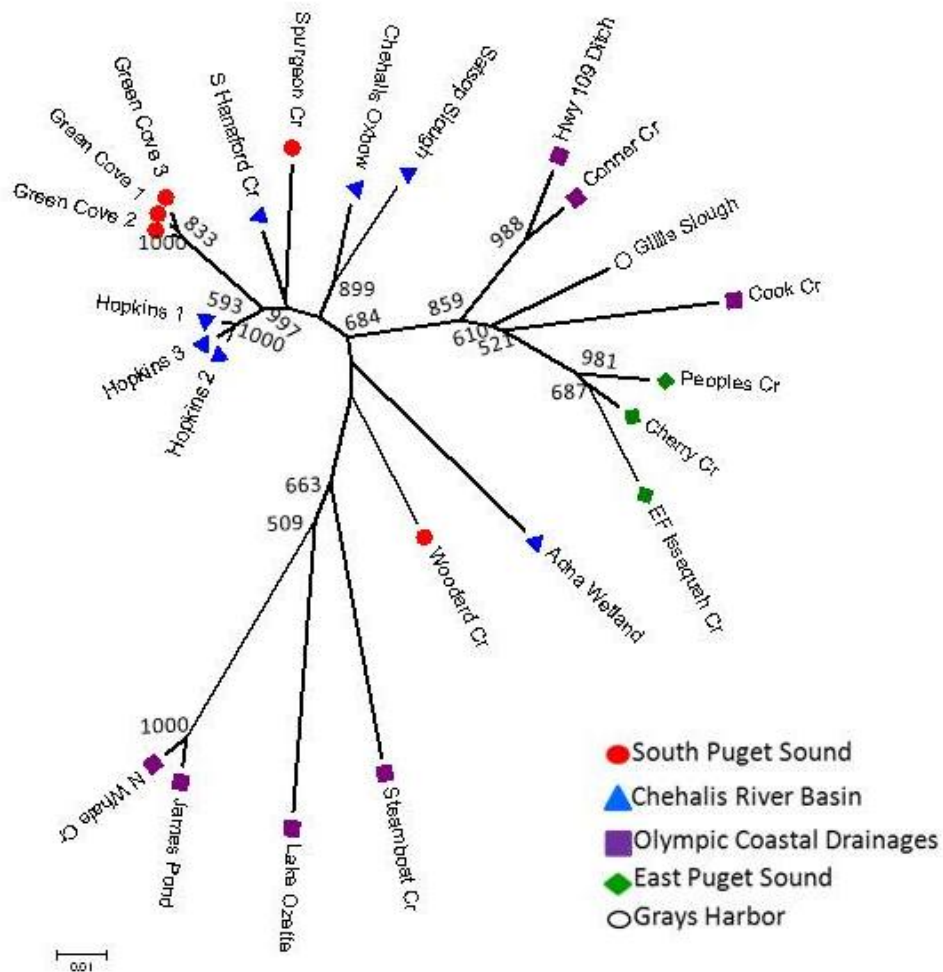


Figure 3. Consensus neighbor-joining tree based on Cavalli-Sforza and Edwards' (1967) chord distances for 23 Olympic mudminnow collection sites. Numbers at the nodes represent the number of bootstrap replicates out of 1000 that showed the displayed topology and only bootstrap values greater than 50% are displayed. Shapes after each collection site name correspond to the broad geographic areas samples were collected from.

Appendix 1. Information for the microsatellite loci developed for this project. T_A refers to the annealing temperature of each locus and N_T represents the total number of alleles we observed for that locus during our initial primer screen efforts. NA under the multiplex column denotes loci that could not be reliably scored and were omitted from data analysis.

Locus Name	Forward Primer Sequence (5'-3')	Reverse Primer Sequence (5'-3')	Multiplex	T_A	N_T
<i>Nhub01</i>	TTC-CAC-TGA-CCA-AAT-CTC-AG	ACC-CCA-AAA-AGA-GAA-AAC-AG	Novumbra 1	60	24
<i>Nhub02</i>	CCC-AAT-ACC-ACA-CAA-ACT-AGA	TTG-GCC-CTA-CAA-CAA-AAA	Novumbra 1	60	8
<i>Nhub04</i>	AAG-GAG-AGA-GAA-GGA-GAG-AAA-C	TGC-TTT-GAA-AGA-CAA-CCA-A	Novumbra 1	60	15
<i>Nhub05</i>	GCT-TCA-GAA-AAC-AAA-TGA-CTG	GGG-AAA-TAG-ATA-GAA-AGT-GGG-A	Novumbra 1	60	13
<i>Nhub12</i>	ATC-CGA-TTA-GAG-AGC-CAA-TG	GCG-TAA-ACG-GTT-CAT-CTT-G	Novumbra 1	60	14
<i>Nhub03</i>	AGC-CAG-AGA-ACA-GGA-GGA-A	CCA-ACA-ACG-AGA-GGT-GAT-G	Novumbra 2	60	24
<i>Nhub09</i>	ACC-CGA-AAG-AAG-TCC-CTG	GAT-GCC-CAA-CCT-CAG-AAA	Novumbra 2	60	33
<i>Nhub10</i>	TTT-TTC-TGT-CAC-TCC-TCT-GTT-C	AAG-CGT-TGG-CTA-TTT-CTC-A	Novumbra 2	60	15
<i>Nhub11</i>	CAC-TGG-AAA-AAC-TCC-TTC-AG	CTG-CCT-CAT-TTG-AAA-GAC-AT	Novumbra 2	60	21
<i>Nhub06</i>	TGC-AGA-ACA-CAA-ATT-GAG-TG	GGT-TCA-GTC-TCC-CAG-TCT-TT	Novumbra 3	52	28
<i>Nhub07</i>	TGC-TAG-GTG-TTG-GAA-GAG-TAA	CCT-GAA-TAG-TTG-CGA-TGT-G	Novumbra 3	52	48
<i>Nhub08</i>	TGA-GAG-AGC-CAC-AGT-AAG-TGT-C	TTG-TCC-AGC-GTG-ACT-CAA	Novumbra 3	52	19
<i>Nhub13</i>	CCC-TAT-CGG-ATT-TCC-TCA	CAG-GCA-TTT-TTT-CGT-TTT-C	Novumbra 3	52	4
<i>Nhub14</i>	TCC-TTG-TCA-CTG-TTA-GTA	GAT-CAT-AAA-TGC-TTA-CAG	NA		
<i>Nhub15</i>	CGG-TAA-TAT-CAC-GTA-GGG-TTC	TCG-GAT-GCA-AAT-TAG-GTT-C	NA		
<i>Nhub16</i>	CCT-CTT-TTC-CCT-GTC-TGA-A	AGC-TTC-CTT-CTT-TTC-TCC-AC	NA		
<i>Nhub17</i>	CCC-GGT-TAG-GGT-TAG-AGG	AAC-TCT-GGC-AAT-TTC-TGT-CTG	NA		
<i>Nhub18</i>	TTC-CCG-GTT-AGG-GTT-AGA-G	AGC-CTG-ACC-ATA-TCT-TGT-TTG	NA		
<i>Nhub19</i>	GGC-AAT-TTC-TGT-CTG-TTT-CTC	CGC-TCC-ATA-TTT-TCC-TTC-A	NA		
<i>Nhub20</i>	ATT-GGT-CAG-GAG-AAA-CTA-TGT-G	AAA-AGG-CGA-AAA-ACT-TGT-G	NA		
<i>Nhub21</i>	AAC-TCT-GGC-AAT-TTC-TGT-CTG	CCC-GGT-TAG-GGT-TAG-AGG	NA		

Appendix 2. Allele frequencies for the 13 loci used in this study. Collection site numbers correspond to locations listed in Table 1 and Figure

1. Shaded cells represent sites where a locus was fixed or nearly fixed for a single allele.

Locus	Allele	Collection Site Number																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Nhub01	274	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.955	0.000	1.000	0.000
Nhub01	278	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000
Nhub01	282	0.033	0.020	0.050	0.000	0.040	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.034	0.000	0.000	0.000
Nhub01	286	0.067	0.060	0.080	0.000	0.030	0.064	0.020	0.050	0.074	0.000	0.023	0.010	0.000	0.000	0.000	0.081	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	290	0.022	0.040	0.030	0.041	0.000	0.075	0.060	0.040	0.059	0.337	0.047	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	294	0.211	0.120	0.170	0.000	0.020	0.032	0.100	0.030	0.132	0.000	0.105	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000
Nhub01	298	0.267	0.370	0.250	0.041	0.460	0.053	0.070	0.060	0.191	0.000	0.093	0.290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	302	0.122	0.170	0.160	0.010	0.050	0.223	0.140	0.100	0.206	0.000	0.023	0.080	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.985	0.000	0.250
Nhub01	306	0.144	0.040	0.040	0.204	0.000	0.213	0.260	0.310	0.147	0.000	0.058	0.090	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.090
Nhub01	310	0.022	0.040	0.030	0.174	0.160	0.106	0.070	0.070	0.074	0.239	0.047	0.040	0.000	0.030	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.140
Nhub01	314	0.078	0.100	0.110	0.357	0.020	0.064	0.080	0.150	0.074	0.413	0.070	0.000	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.010
Nhub01	318	0.011	0.010	0.000	0.174	0.210	0.053	0.070	0.030	0.044	0.011	0.012	0.000	0.000	0.000	0.000	0.000	0.030	0.021	0.041	0.000	0.000	0.000	0.060
Nhub01	322	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.080	0.000	0.000	0.000	0.010	0.000	0.160	0.210	0.116	0.130	0.032	0.225	0.000	0.000	0.000	0.360
Nhub01	326	0.011	0.000	0.000	0.000	0.000	0.085	0.080	0.080	0.000	0.000	0.000	0.000	0.030	0.020	0.000	0.023	0.170	0.181	0.357	0.000	0.000	0.000	0.010
Nhub01	330	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.035	0.010	0.200	0.080	0.120	0.163	0.120	0.149	0.245	0.000	0.000	0.000	0.010
Nhub01	334	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.070	0.020	0.380	0.170	0.290	0.151	0.060	0.053	0.061	0.000	0.000	0.000	0.000
Nhub01	338	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.030	0.030	0.020	0.010	0.047	0.060	0.213	0.061	0.000	0.000	0.000	0.010
Nhub01	342	0.011	0.030	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.105	0.150	0.320	0.260	0.190	0.000	0.070	0.021	0.010	0.000	0.000	0.000	0.000
Nhub01	346	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.105	0.030	0.020	0.040	0.000	0.070	0.010	0.032	0.000	0.000	0.000	0.000	0.000
Nhub01	350	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.010	0.010	0.060	0.180	0.012	0.010	0.128	0.000	0.000	0.000	0.000	0.000
Nhub01	354	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.081	0.010	0.000	0.000	0.000	0.093	0.040	0.032	0.000	0.000	0.000	0.000	0.030
Nhub01	358	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.023	0.000	0.032	0.000	0.000	0.000	0.000	0.020
Nhub01	362	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.058	0.020	0.085	0.000	0.000	0.000	0.000	0.000
Nhub01	366	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.110	0.000	0.000	0.000	0.047	0.020	0.000	0.000	0.000	0.000	0.000	0.010
Nhub01	370	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.160	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	374	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	378	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.011	0.000	0.000	0.000	0.000	0.000
Nhub01	382	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	394	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub01	398	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub12	154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub12	158	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub12	162	1.000	1.000	1.000	0.130	1.000	0.979	0.960	1.000	0.853	1.000	0.930	0.960	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.977	0.000	1.000	0.000

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Nhub12	166	0.000	0.000	0.000	0.750	0.000	0.021	0.040	0.000	0.118	0.000	0.047	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	1.000	0.000	0.430
Nhub12	170	0.000	0.000	0.000	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.570
Nhub12	174	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub12	178	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.047	0.010	0.011	0.092	0.000	0.000	0.000	0.000
Nhub12	182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.080	0.000	0.000	0.186	0.030	0.000	0.837	0.000	0.000	0.000	0.000
Nhub12	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.420	0.460	0.370	0.337	0.130	0.223	0.071	0.000	0.000	0.000	0.000
Nhub12	190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	0.190	0.260	0.279	0.210	0.106	0.000	0.000	0.000	0.000	0.000
Nhub12	194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.370	0.270	0.360	0.058	0.250	0.202	0.000	0.000	0.000	0.000	0.000
Nhub12	198	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.010	0.035	0.170	0.309	0.000	0.000	0.000	0.000	0.000
Nhub12	202	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.060	0.106	0.000	0.000	0.000	0.000	0.000
Nhub12	206	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.021	0.000	0.000	0.000	0.000	0.000
Nhub12	210	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.011	0.000	0.000	0.000	0.000	0.000
Nhub12	214	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
Nhub04	231	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub04	235	0.000	0.000	0.000	0.000	0.000	0.053	0.070	0.050	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.020	0.128	0.796	0.000	0.000	0.000	0.000
Nhub04	239	0.089	0.110	0.100	0.070	0.200	0.192	0.160	0.250	0.221	0.000	0.047	0.090	0.040	0.000	0.000	0.209	0.290	0.436	0.204	0.000	0.000	0.000	0.000
Nhub04	243	0.789	0.820	0.750	0.920	0.230	0.319	0.370	0.350	0.441	0.772	0.291	0.150	0.650	0.380	0.240	0.023	0.470	0.319	0.000	0.159	0.000	0.200	0.000
Nhub04	247	0.111	0.070	0.150	0.010	0.070	0.287	0.340	0.220	0.162	0.011	0.174	0.180	0.010	0.220	0.110	0.430	0.220	0.106	0.000	0.011	0.000	0.090	0.000
Nhub04	251	0.011	0.000	0.000	0.000	0.270	0.128	0.050	0.110	0.074	0.217	0.128	0.050	0.060	0.080	0.000	0.174	0.000	0.011	0.000	0.193	0.000	0.080	0.000
Nhub04	255	0.000	0.000	0.000	0.000	0.190	0.011	0.000	0.020	0.029	0.000	0.081	0.230	0.100	0.120	0.320	0.070	0.000	0.000	0.000	0.159	0.000	0.210	0.000
Nhub04	259	0.000	0.000	0.000	0.000	0.030	0.011	0.000	0.000	0.074	0.000	0.140	0.150	0.000	0.000	0.080	0.012	0.000	0.000	0.000	0.102	0.000	0.330	0.000
Nhub04	263	0.000	0.000	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.081	0.010	0.130	0.200	0.250	0.023	0.000	0.000	0.000	0.182	0.000	0.090	0.000
Nhub04	267	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.050	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000
Nhub04	271	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000
Nhub04	275	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.000	0.000
Nhub04	279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000
Nhub04	392	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.620
Nhub04	400	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.300
Nhub04	404	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080
Nhub04	416	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.227	0.000	0.000
Nhub04	420	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.364	0.000	0.000
Nhub04	424	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.227	0.000	0.000
Nhub04	432	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000
Nhub04	436	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.000	0.000
Nhub05	347	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.174	0.060	0.128	0.000	0.000	0.000	0.000	0.000
Nhub05	351	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.120	0.128	0.000	0.000	0.000	0.000	0.000
Nhub05	363	0.000	0.000	0.000	0.000	0.000	0.021	0.010	0.020	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub05	367	0.000	0.000	0.000	0.520	0.000	0.011	0.000	0.000	0.044	1.000	0.628	0.430	1.000	0.990	1.000	0.826	0.800	0.745	1.000	1.000	0.985	1.000	0.450

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Nhub05	371	0.811	0.810	0.850	0.350	0.270	0.202	0.220	0.170	0.265	0.000	0.012	0.070	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.015	0.000	0.000
Nhub05	375	0.022	0.010	0.020	0.130	0.190	0.330	0.410	0.390	0.485	0.000	0.244	0.160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub05	377	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
Nhub05	379	0.044	0.040	0.020	0.000	0.280	0.149	0.180	0.130	0.191	0.000	0.116	0.170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub05	381	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.500
Nhub05	383	0.111	0.140	0.110	0.000	0.010	0.277	0.170	0.260	0.015	0.000	0.000	0.090	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub05	387	0.011	0.000	0.000	0.000	0.000	0.011	0.010	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub05	395	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub05	399	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub02	221	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub02	229	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.260
Nhub02	233	0.000	0.000	0.000	0.010	0.040	0.000	0.000	0.000	0.000	0.000	0.035	0.110	0.150	0.090	0.000	0.361	0.010	0.000	0.000	1.000	0.000	1.000	0.630
Nhub02	237	0.022	0.010	0.000	0.860	0.730	0.192	0.250	0.250	0.441	0.000	0.128	0.250	0.390	0.060	0.080	0.128	0.330	0.138	0.276	0.000	0.985	0.000	0.110
Nhub02	241	0.544	0.610	0.700	0.000	0.020	0.255	0.170	0.260	0.338	0.598	0.465	0.460	0.440	0.690	0.880	0.279	0.420	0.192	0.510	0.000	0.015	0.000	0.000
Nhub02	245	0.300	0.300	0.210	0.070	0.200	0.340	0.320	0.300	0.118	0.000	0.244	0.120	0.000	0.040	0.040	0.186	0.170	0.181	0.041	0.000	0.000	0.000	0.000
Nhub02	249	0.133	0.080	0.090	0.000	0.010	0.075	0.130	0.110	0.103	0.000	0.093	0.050	0.000	0.040	0.000	0.012	0.060	0.340	0.174	0.000	0.000	0.000	0.000
Nhub02	253	0.000	0.000	0.000	0.000	0.000	0.138	0.110	0.060	0.000	0.402	0.035	0.000	0.000	0.000	0.000	0.012	0.010	0.128	0.000	0.000	0.000	0.000	0.000
Nhub02	257	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.020	0.000	0.000	0.000	0.010	0.020	0.080	0.000	0.012	0.000	0.021	0.000	0.000	0.000	0.000	0.000
Nhub13	189	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000
Nhub13	193	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.209	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub13	197	0.000	0.000	0.000	0.000	0.000	0.021	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.081	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub13	201	0.856	0.850	0.830	1.000	1.000	0.979	0.920	0.980	0.897	1.000	0.907	1.000	0.210	0.510	0.190	0.663	0.980	0.692	1.000	0.000	0.470	0.000	0.000
Nhub13	205	0.144	0.150	0.170	0.000	0.000	0.000	0.060	0.020	0.103	0.000	0.093	0.000	0.790	0.490	0.810	0.047	0.020	0.309	0.000	0.000	0.530	0.000	0.000
Nhub03	192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.030	0.021	0.000	0.000	0.000	0.000	0.000
Nhub03	196	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.510	0.255	0.000	0.091	0.000	0.000	0.000
Nhub03	200	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.100	0.128	0.000	0.125	0.000	0.110	0.000
Nhub03	204	0.000	0.000	0.000	0.000	0.090	0.043	0.010	0.020	0.059	0.000	0.000	0.000	0.000	0.000	0.000	0.488	0.030	0.011	0.010	0.205	0.000	0.170	0.040
Nhub03	208	0.000	0.000	0.000	0.050	0.230	0.021	0.010	0.010	0.132	0.011	0.226	0.000	0.380	0.310	0.310	0.105	0.000	0.053	0.663	0.171	0.000	0.150	0.410
Nhub03	212	0.011	0.020	0.000	0.210	0.200	0.181	0.230	0.240	0.324	0.804	0.107	0.040	0.000	0.000	0.000	0.174	0.000	0.011	0.327	0.261	0.000	0.430	0.210
Nhub03	216	0.000	0.010	0.000	0.580	0.310	0.277	0.250	0.190	0.191	0.000	0.024	0.100	0.140	0.090	0.120	0.105	0.030	0.000	0.000	0.148	0.212	0.140	0.090
Nhub03	220	0.278	0.140	0.260	0.120	0.000	0.149	0.130	0.160	0.191	0.011	0.131	0.130	0.100	0.050	0.040	0.012	0.070	0.032	0.000	0.000	0.742	0.000	0.050
Nhub03	224	0.233	0.240	0.200	0.000	0.010	0.064	0.080	0.090	0.029	0.000	0.262	0.040	0.030	0.080	0.020	0.058	0.100	0.085	0.000	0.000	0.000	0.000	0.160
Nhub03	228	0.222	0.320	0.310	0.040	0.010	0.085	0.130	0.130	0.000	0.000	0.012	0.000	0.040	0.080	0.240	0.000	0.060	0.255	0.000	0.000	0.046	0.000	0.040
Nhub03	232	0.244	0.260	0.230	0.000	0.120	0.075	0.080	0.090	0.059	0.000	0.000	0.150	0.120	0.230	0.260	0.000	0.040	0.096	0.000	0.000	0.000	0.000	0.000
Nhub03	236	0.011	0.010	0.000	0.000	0.030	0.043	0.020	0.020	0.000	0.000	0.024	0.020	0.160	0.130	0.010	0.000	0.030	0.053	0.000	0.000	0.000	0.000	0.000
Nhub03	240	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.174	0.000	0.030	0.030	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	244	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.010	0.000	0.000	0.048	0.190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Nhub03	248	0.000	0.000	0.000	0.000	0.000	0.032	0.050	0.030	0.000	0.000	0.024	0.120	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	252	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	256	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.010	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	260	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	264	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	268	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	272	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	284	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub03	296	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	179	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	1.000	1.000	1.000	1.000
Nhub09	183	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.750	0.760	0.970	0.907	0.150	0.170	1.000	0.000	0.000	0.000	0.000
Nhub09	187	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.080	0.000	0.000	0.000	0.096	0.000	0.000	0.000	0.000	0.000
Nhub09	191	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
Nhub09	195	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	199	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	203	0.000	0.000	0.000	0.160	0.130	0.032	0.020	0.030	0.015	0.000	0.000	0.000	0.010	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	207	0.000	0.000	0.000	0.010	0.240	0.011	0.030	0.050	0.132	0.000	0.012	0.000	0.040	0.040	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	211	0.000	0.020	0.020	0.010	0.010	0.032	0.020	0.030	0.000	0.000	0.000	0.010	0.090	0.020	0.000	0.000	0.050	0.053	0.000	0.000	0.000	0.000	0.000
Nhub09	215	0.089	0.110	0.060	0.030	0.070	0.181	0.210	0.190	0.074	0.000	0.048	0.010	0.080	0.100	0.030	0.023	0.030	0.011	0.000	0.000	0.000	0.000	0.000
Nhub09	219	0.067	0.050	0.050	0.100	0.200	0.117	0.120	0.090	0.103	0.000	0.012	0.010	0.000	0.000	0.000	0.012	0.100	0.043	0.000	0.000	0.000	0.000	0.000
Nhub09	223	0.000	0.010	0.010	0.020	0.000	0.202	0.170	0.180	0.177	0.000	0.107	0.040	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	227	0.044	0.060	0.040	0.400	0.010	0.096	0.140	0.050	0.147	0.000	0.095	0.060	0.000	0.000	0.000	0.000	0.180	0.053	0.000	0.000	0.000	0.000	0.000
Nhub09	231	0.311	0.220	0.220	0.260	0.110	0.064	0.100	0.060	0.147	0.522	0.143	0.060	0.000	0.000	0.000	0.000	0.060	0.021	0.000	0.000	0.000	0.000	0.000
Nhub09	235	0.222	0.140	0.210	0.010	0.230	0.021	0.070	0.080	0.059	0.467	0.095	0.040	0.000	0.000	0.000	0.000	0.070	0.053	0.000	0.000	0.000	0.000	0.000
Nhub09	239	0.089	0.170	0.230	0.000	0.000	0.096	0.010	0.090	0.088	0.011	0.024	0.020	0.000	0.000	0.000	0.000	0.120	0.202	0.000	0.000	0.000	0.000	0.000
Nhub09	243	0.089	0.040	0.050	0.000	0.000	0.032	0.050	0.040	0.044	0.000	0.012	0.160	0.000	0.000	0.000	0.000	0.080	0.064	0.000	0.000	0.000	0.000	0.000
Nhub09	247	0.067	0.150	0.100	0.000	0.000	0.096	0.010	0.040	0.015	0.000	0.012	0.090	0.000	0.000	0.000	0.000	0.080	0.053	0.000	0.000	0.000	0.000	0.000
Nhub09	251	0.000	0.020	0.000	0.000	0.000	0.021	0.020	0.030	0.000	0.000	0.024	0.070	0.000	0.000	0.000	0.000	0.050	0.117	0.000	0.000	0.000	0.000	0.000
Nhub09	255	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.010	0.021	0.000	0.000	0.000	0.000	0.000
Nhub09	259	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.010	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000
Nhub09	263	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
Nhub09	267	0.022	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	271	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	275	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	287	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub09	291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
<i>Nhub09</i>	295	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub09</i>	299	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub09</i>	307	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub09</i>	311	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	239	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.170
<i>Nhub10</i>	243	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
<i>Nhub10</i>	247	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.400	0.309	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	251	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.012	0.000	0.000	0.000	0.000	0.035	0.000	0.106	0.000	0.000	0.258	0.000	0.800
<i>Nhub10</i>	255	0.000	0.030	0.000	0.000	0.000	0.085	0.090	0.070	0.029	0.250	0.047	0.010	0.220	0.190	0.360	0.000	0.020	0.202	0.071	0.125	0.000	0.000	0.010
<i>Nhub10</i>	259	0.489	0.630	0.570	0.460	0.000	0.181	0.190	0.300	0.103	0.000	0.070	0.070	0.240	0.280	0.310	0.140	0.010	0.011	0.918	0.739	0.742	0.640	0.000
<i>Nhub10</i>	263	0.200	0.150	0.170	0.240	0.330	0.117	0.240	0.160	0.279	0.728	0.174	0.280	0.540	0.490	0.330	0.302	0.020	0.000	0.010	0.125	0.000	0.360	0.000
<i>Nhub10</i>	267	0.022	0.010	0.040	0.040	0.210	0.330	0.220	0.230	0.191	0.011	0.605	0.050	0.000	0.010	0.000	0.337	0.040	0.064	0.000	0.011	0.000	0.000	0.000
<i>Nhub10</i>	271	0.078	0.100	0.110	0.000	0.440	0.181	0.150	0.120	0.177	0.000	0.070	0.140	0.000	0.030	0.000	0.128	0.130	0.011	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	275	0.211	0.080	0.100	0.000	0.020	0.043	0.010	0.050	0.088	0.000	0.000	0.260	0.000	0.000	0.000	0.035	0.130	0.117	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	279	0.000	0.000	0.010	0.000	0.000	0.021	0.050	0.030	0.000	0.000	0.012	0.080	0.000	0.000	0.000	0.012	0.080	0.117	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	283	0.000	0.000	0.000	0.000	0.000	0.032	0.050	0.040	0.044	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.140	0.043	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	287	0.000	0.000	0.000	0.020	0.000	0.011	0.000	0.000	0.059	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.020	0.021	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	291	0.000	0.000	0.000	0.230	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub10</i>	295	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.015	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub11</i>	216	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.021	0.000	0.000	0.000	0.000	0.000
<i>Nhub11</i>	220	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.023	0.570	0.628	0.000	0.000	0.000	0.000	0.000
<i>Nhub11</i>	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000	0.070	0.210	0.266	0.000	0.000	0.000	0.000	0.000
<i>Nhub11</i>	228	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.116	0.070	0.011	0.020	0.000	0.000	0.000	0.000
<i>Nhub11</i>	232	0.000	0.030	0.060	0.000	0.110	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.220	0.060	0.110	0.047	0.030	0.011	0.674	0.000	0.076	0.000	0.000
<i>Nhub11</i>	236	0.046	0.040	0.050	0.120	0.000	0.149	0.080	0.190	0.118	0.000	0.000	0.000	0.260	0.330	0.610	0.244	0.020	0.000	0.286	0.000	0.288	0.000	0.000
<i>Nhub11</i>	240	0.011	0.010	0.010	0.040	0.050	0.053	0.150	0.100	0.177	0.000	0.000	0.000	0.110	0.250	0.000	0.221	0.000	0.000	0.020	0.000	0.121	0.000	0.020
<i>Nhub11</i>	244	0.011	0.040	0.000	0.380	0.090	0.149	0.200	0.200	0.221	0.424	0.012	0.030	0.360	0.190	0.080	0.198	0.000	0.000	0.000	0.000	0.515	0.000	0.000
<i>Nhub11</i>	248	0.273	0.090	0.230	0.310	0.170	0.160	0.150	0.090	0.103	0.326	0.035	0.040	0.010	0.000	0.000	0.047	0.000	0.011	0.000	0.000	0.000	0.000	0.350
<i>Nhub11</i>	252	0.386	0.420	0.310	0.150	0.030	0.149	0.100	0.070	0.162	0.022	0.395	0.240	0.000	0.020	0.000	0.035	0.000	0.032	0.000	0.046	0.000	0.030	0.110
<i>Nhub11</i>	256	0.080	0.100	0.160	0.000	0.110	0.149	0.100	0.190	0.118	0.228	0.128	0.100	0.040	0.020	0.200	0.000	0.020	0.011	0.000	0.034	0.000	0.060	0.230
<i>Nhub11</i>	260	0.102	0.160	0.120	0.000	0.170	0.117	0.120	0.090	0.059	0.000	0.058	0.160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.216	0.000	0.300	0.180
<i>Nhub11</i>	264	0.068	0.040	0.030	0.000	0.130	0.021	0.030	0.030	0.015	0.000	0.012	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.159	0.000	0.030	0.070
<i>Nhub11</i>	268	0.000	0.000	0.000	0.000	0.050	0.011	0.010	0.010	0.000	0.000	0.291	0.090	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.057	0.000	0.070	0.040
<i>Nhub11</i>	272	0.023	0.050	0.030	0.000	0.020	0.032	0.050	0.020	0.000	0.000	0.023	0.060	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.171	0.000	0.100	0.000
<i>Nhub11</i>	276	0.000	0.020	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.114	0.000	0.100	0.000
<i>Nhub11</i>	280	0.000	0.000	0.000	0.000	0.050	0.011	0.010	0.010	0.000	0.000	0.047	0.140	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.080	0.000	0.070	0.000
<i>Nhub11</i>	284	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.000	0.100	0.000
<i>Nhub11</i>	288	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.010	0.000

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
<i>Nhub11</i>	292	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.034	0.000	0.090	0.000
<i>Nhub11</i>	296	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000
<i>Nhub11</i>	316	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	144	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.102	0.000	0.000	0.000
<i>Nhub06</i>	148	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.227	0.000	0.180	0.000
<i>Nhub06</i>	152	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.000	0.000	0.000	0.193	0.000	0.240	0.000
<i>Nhub06</i>	156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.058	0.000	0.000	0.000	0.227	0.000	0.390	0.000
<i>Nhub06</i>	160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.106	0.000	0.239	0.000	0.160	0.000
<i>Nhub06</i>	164	0.000	0.000	0.000	0.204	0.010	0.000	0.000	0.030	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.020	0.096	0.000	0.011	0.121	0.030	0.000
<i>Nhub06</i>	168	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.207	0.000	0.110	0.010	0.090	0.000	0.000	0.010	0.085	0.000	0.000	0.182	0.000	0.020
<i>Nhub06</i>	172	0.011	0.000	0.000	0.235	0.000	0.064	0.041	0.020	0.000	0.565	0.140	0.060	0.000	0.000	0.000	0.186	0.020	0.096	0.000	0.000	0.197	0.000	0.110
<i>Nhub06</i>	176	0.122	0.150	0.070	0.296	0.210	0.064	0.102	0.020	0.074	0.000	0.023	0.040	0.000	0.000	0.000	0.023	0.050	0.000	0.000	0.000	0.152	0.000	0.160
<i>Nhub06</i>	180	0.100	0.050	0.090	0.174	0.170	0.096	0.143	0.100	0.088	0.000	0.000	0.010	0.030	0.170	0.000	0.070	0.020	0.011	0.000	0.000	0.000	0.000	0.240
<i>Nhub06</i>	184	0.000	0.000	0.020	0.000	0.020	0.021	0.061	0.080	0.103	0.000	0.023	0.030	0.380	0.100	0.000	0.174	0.100	0.011	0.000	0.000	0.212	0.000	0.060
<i>Nhub06</i>	188	0.000	0.010	0.000	0.000	0.000	0.096	0.143	0.100	0.118	0.000	0.140	0.070	0.020	0.050	0.140	0.058	0.100	0.043	0.010	0.000	0.091	0.000	0.020
<i>Nhub06</i>	192	0.000	0.020	0.010	0.000	0.040	0.043	0.041	0.010	0.088	0.000	0.058	0.040	0.000	0.020	0.150	0.047	0.080	0.064	0.225	0.000	0.046	0.000	0.000
<i>Nhub06</i>	196	0.089	0.110	0.050	0.000	0.030	0.096	0.092	0.140	0.059	0.000	0.174	0.160	0.070	0.240	0.110	0.058	0.080	0.064	0.245	0.000	0.000	0.000	0.010
<i>Nhub06</i>	200	0.089	0.100	0.120	0.071	0.000	0.106	0.031	0.080	0.059	0.000	0.047	0.020	0.100	0.020	0.130	0.012	0.110	0.181	0.174	0.000	0.000	0.000	0.040
<i>Nhub06</i>	204	0.078	0.090	0.130	0.000	0.060	0.053	0.071	0.130	0.147	0.011	0.105	0.010	0.000	0.000	0.010	0.023	0.010	0.032	0.204	0.000	0.000	0.000	0.230
<i>Nhub06</i>	208	0.000	0.020	0.000	0.000	0.190	0.117	0.082	0.100	0.177	0.207	0.012	0.010	0.010	0.000	0.000	0.035	0.020	0.053	0.020	0.000	0.000	0.000	0.080
<i>Nhub06</i>	212	0.011	0.110	0.030	0.000	0.060	0.117	0.031	0.090	0.029	0.011	0.000	0.000	0.020	0.020	0.410	0.000	0.060	0.000	0.041	0.000	0.000	0.000	0.030
<i>Nhub06</i>	216	0.011	0.020	0.020	0.000	0.010	0.053	0.071	0.020	0.000	0.000	0.023	0.270	0.000	0.160	0.010	0.000	0.030	0.021	0.041	0.000	0.000	0.000	0.000
<i>Nhub06</i>	220	0.000	0.000	0.000	0.000	0.000	0.011	0.020	0.010	0.000	0.000	0.012	0.040	0.070	0.020	0.000	0.000	0.030	0.000	0.041	0.000	0.000	0.000	0.000
<i>Nhub06</i>	224	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.015	0.000	0.070	0.000	0.030	0.080	0.000	0.000	0.030	0.043	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	228	0.000	0.000	0.020	0.000	0.040	0.011	0.010	0.020	0.000	0.000	0.035	0.020	0.070	0.020	0.000	0.000	0.030	0.032	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	232	0.044	0.030	0.060	0.000	0.020	0.032	0.031	0.000	0.029	0.000	0.023	0.000	0.010	0.000	0.000	0.012	0.040	0.021	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	236	0.078	0.110	0.170	0.000	0.040	0.000	0.000	0.010	0.000	0.000	0.012	0.000	0.170	0.010	0.040	0.023	0.020	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	240	0.111	0.030	0.050	0.000	0.100	0.000	0.010	0.010	0.000	0.000	0.000	0.050	0.010	0.000	0.000	0.093	0.060	0.021	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	244	0.067	0.040	0.020	0.000	0.000	0.011	0.010	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.035	0.020	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	248	0.044	0.050	0.050	0.000	0.000	0.011	0.010	0.010	0.000	0.000	0.047	0.020	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	252	0.133	0.040	0.070	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	256	0.000	0.020	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.010	0.011	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	260	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	264	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub06</i>	280	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub07</i>	182	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
<i>Nhub07</i>	186	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.160	0.370	0.012	0.050	0.011	0.000	0.000	0.000	0.000	0.100
<i>Nhub07</i>	190	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.050	0.128	0.000	0.000	0.000	0.000	0.260

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Nhub07	194	0.000	0.000	0.010	0.000	0.000	0.021	0.020	0.050	0.000	0.000	0.000	0.010	0.000	0.020	0.080	0.023	0.050	0.043	0.000	0.000	0.000	0.000	0.130
Nhub07	198	0.000	0.030	0.010	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.058	0.050	0.120	0.160	0.030	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.070
Nhub07	202	0.011	0.050	0.040	0.000	0.000	0.106	0.160	0.300	0.000	0.000	0.000	0.070	0.090	0.100	0.050	0.116	0.000	0.043	0.010	0.000	0.000	0.000	0.030
Nhub07	206	0.044	0.050	0.010	0.000	0.000	0.021	0.070	0.030	0.000	0.000	0.023	0.000	0.000	0.140	0.000	0.093	0.100	0.096	0.010	0.000	0.030	0.000	0.060
Nhub07	210	0.022	0.000	0.030	0.000	0.030	0.085	0.120	0.090	0.000	0.000	0.000	0.000	0.010	0.130	0.000	0.093	0.080	0.064	0.071	0.012	0.000	0.000	0.080
Nhub07	214	0.033	0.020	0.060	0.000	0.120	0.096	0.120	0.090	0.000	0.000	0.035	0.060	0.080	0.000	0.000	0.116	0.010	0.075	0.265	0.023	0.030	0.000	0.130
Nhub07	218	0.078	0.070	0.050	0.020	0.210	0.138	0.090	0.110	0.000	0.000	0.047	0.010	0.100	0.020	0.000	0.012	0.000	0.032	0.306	0.233	0.167	0.130	0.050
Nhub07	222	0.244	0.130	0.230	0.082	0.220	0.043	0.050	0.040	0.000	0.364	0.000	0.000	0.010	0.010	0.000	0.000	0.000	0.085	0.092	0.081	0.167	0.150	0.070
Nhub07	226	0.233	0.280	0.250	0.184	0.070	0.117	0.100	0.050	0.000	0.303	0.058	0.000	0.100	0.030	0.000	0.105	0.040	0.085	0.112	0.116	0.000	0.150	0.020
Nhub07	230	0.111	0.130	0.110	0.010	0.040	0.085	0.070	0.050	0.017	0.000	0.012	0.000	0.000	0.000	0.000	0.035	0.010	0.000	0.061	0.058	0.000	0.140	0.000
Nhub07	234	0.011	0.030	0.060	0.000	0.020	0.085	0.070	0.070	0.000	0.000	0.035	0.080	0.000	0.050	0.000	0.047	0.030	0.043	0.010	0.186	0.121	0.070	0.000
Nhub07	238	0.000	0.000	0.010	0.163	0.030	0.043	0.030	0.030	0.000	0.000	0.047	0.010	0.030	0.000	0.000	0.081	0.050	0.106	0.000	0.047	0.242	0.030	0.000
Nhub07	242	0.000	0.000	0.010	0.316	0.090	0.011	0.010	0.010	0.000	0.000	0.000	0.010	0.010	0.000	0.000	0.081	0.120	0.032	0.000	0.047	0.030	0.080	0.000
Nhub07	246	0.000	0.010	0.000	0.071	0.130	0.011	0.000	0.000	0.052	0.000	0.023	0.000	0.000	0.010	0.000	0.000	0.060	0.032	0.000	0.047	0.106	0.000	0.000
Nhub07	250	0.044	0.030	0.010	0.000	0.030	0.021	0.030	0.000	0.069	0.000	0.023	0.010	0.000	0.040	0.000	0.000	0.020	0.043	0.000	0.023	0.015	0.010	0.000
Nhub07	254	0.100	0.040	0.050	0.010	0.000	0.021	0.010	0.020	0.069	0.000	0.058	0.000	0.000	0.000	0.030	0.012	0.040	0.021	0.000	0.023	0.061	0.160	0.000
Nhub07	258	0.033	0.030	0.000	0.000	0.010	0.011	0.020	0.010	0.017	0.258	0.070	0.020	0.000	0.070	0.400	0.047	0.020	0.000	0.000	0.035	0.000	0.050	0.000
Nhub07	262	0.022	0.010	0.010	0.020	0.000	0.000	0.010	0.010	0.086	0.076	0.000	0.000	0.320	0.020	0.020	0.000	0.040	0.011	0.000	0.012	0.000	0.010	0.000
Nhub07	266	0.000	0.000	0.000	0.092	0.000	0.032	0.010	0.030	0.017	0.000	0.035	0.000	0.020	0.000	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	270	0.000	0.000	0.000	0.010	0.000	0.011	0.000	0.010	0.086	0.000	0.035	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.012	0.030	0.000	0.000
Nhub07	274	0.000	0.010	0.010	0.000	0.000	0.000	0.010	0.000	0.086	0.000	0.023	0.010	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	278	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.035	0.010	0.000	0.010	0.000	0.012	0.020	0.000	0.000	0.012	0.000	0.000	0.000
Nhub07	282	0.011	0.040	0.040	0.000	0.000	0.011	0.000	0.000	0.017	0.000	0.012	0.100	0.000	0.000	0.000	0.000	0.010	0.011	0.051	0.000	0.000	0.000	0.000
Nhub07	286	0.000	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.010	0.011	0.010	0.035	0.000	0.020	0.000
Nhub07	290	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.023	0.010	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	294	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.086	0.000	0.023	0.030	0.000	0.000	0.000	0.000	0.050	0.021	0.000	0.000	0.000	0.000	0.000
Nhub07	298	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.000	0.050	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	302	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.040	0.000	0.000	0.000	0.000	0.030	0.011	0.000	0.000	0.000	0.000	0.000
Nhub07	306	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.035	0.000	0.000	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	310	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	314	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.035	0.000	0.012	0.030	0.100	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	318	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.012	0.000	0.010	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	322	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	326	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.081	0.110	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	330	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.047	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	334	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	338	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.070	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	346	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	350	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	370	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.035	0.030	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Locus	Allele	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Nhub07	374	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	386	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	394	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	398	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub07	418	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub08	275	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.020	0.000
Nhub08	279	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.693	0.000	0.770	0.000
Nhub08	283	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.171	0.000	0.000	0.000
Nhub08	287	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.210	0.000
Nhub08	291	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000	0.000
Nhub08	367	0.000	0.000	0.000	0.000	0.000	0.032	0.020	0.020	0.000	0.000	0.023	0.010	0.000	0.010	0.000	0.000	0.010	0.000	0.000	0.000	0.000	0.000	0.000
Nhub08	371	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.160	0.000	0.128	0.020	0.117	0.000	0.000	0.000	0.000	0.000
Nhub08	375	0.000	0.000	0.000	0.000	0.050	0.053	0.030	0.040	0.044	0.000	0.047	0.010	0.150	0.210	0.600	0.047	0.320	0.160	0.357	0.000	0.000	0.000	0.000
Nhub08	379	0.133	0.180	0.090	0.000	0.160	0.128	0.120	0.130	0.088	0.000	0.128	0.150	0.130	0.190	0.060	0.349	0.350	0.202	0.429	0.000	0.000	0.000	0.000
Nhub08	383	0.544	0.550	0.630	0.000	0.100	0.500	0.330	0.350	0.103	0.141	0.291	0.050	0.440	0.270	0.180	0.151	0.210	0.330	0.214	0.000	0.000	0.000	0.000
Nhub08	387	0.178	0.120	0.110	0.090	0.080	0.117	0.210	0.130	0.294	0.000	0.140	0.130	0.140	0.150	0.160	0.093	0.050	0.106	0.000	0.000	0.000	0.000	0.000
Nhub08	391	0.067	0.060	0.060	0.290	0.220	0.053	0.100	0.060	0.059	0.228	0.047	0.020	0.000	0.000	0.000	0.035	0.030	0.064	0.000	0.000	0.000	0.000	0.000
Nhub08	395	0.022	0.030	0.040	0.350	0.110	0.021	0.020	0.040	0.147	0.000	0.081	0.290	0.140	0.010	0.000	0.128	0.000	0.021	0.000	0.000	0.000	0.000	0.000
Nhub08	399	0.056	0.040	0.070	0.200	0.030	0.085	0.130	0.230	0.088	0.000	0.058	0.040	0.000	0.000	0.000	0.012	0.010	0.000	0.000	0.000	0.000	0.000	0.130
Nhub08	403	0.000	0.020	0.000	0.000	0.050	0.011	0.020	0.000	0.118	0.294	0.093	0.070	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.390
Nhub08	407	0.000	0.000	0.000	0.070	0.190	0.000	0.010	0.000	0.044	0.326	0.035	0.050	0.000	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.636	0.000	0.220
Nhub08	411	0.000	0.000	0.000	0.000	0.010	0.000	0.010	0.000	0.000	0.011	0.000	0.140	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
Nhub08	415	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.015	0.000	0.035	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
Nhub08	419	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040
Nhub08	423	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.333	0.000	0.110
Nhub08	431	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.030	0.000	0.010
Nhub08	435	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Nhub08	443	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
Nhub08	447	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020

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