

Blue *hawaiiense* and Beyond: Conservation Genetics and Comparative Phylogeography of Four Hawaiian *Megalagrion* Damselfly Species (Odonata: Coenagrionidae)

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Abstract

Hawaii's endemic *Megalagrion* damselflies are rivaled in their beauty and diversity only by the degree of threat posed to them by anthropogenic disturbance. In this preliminary study of phylogeography and conservation genetics, we have sequenced about 660 base pairs (bp) of the mitochondrial COII gene from 191 damselflies from four species, including 31 populations that span a gradient of endangerment. We applied phylogeographic analyses in order to understand their biogeographic history. Unlike *Megalagrion xanthomelas* and *M. pacificum*, *M. calliphya* and *M. hawaiiense* rarely share haplotypes between populations and between islands, even within the larger Maui Nui super-island, suggesting that these latter two species do not disperse as well across land or water. Their phylogenies also better reflect the geological history of the islands. We applied conservation genetic analyses in order to understand their genetic health. Under a conservation genetic paradigm, populations with low genetic diversity are generally considered to be at greater risk of decline and extinction than populations with high genetic diversity. Applying this principle to *Megalagrion* populations gave both expected and surprising results. Expected results included measurements of high diversity in most populations of *M. calliphya*, *M. hawaiiense*, and Hawai'i Island populations of *M. xanthomelas*. Low diversity was observed in populations known to be bottlenecked or relictual, including O'ahu and Maui *M. xanthomelas*, and *M. pacificum*. Surprising results included low genetic diversity in O'ahu Ko'olau and Hawai'i Onomea *M. hawaiiense*, Moloka'i *M. pacificum*, and West Maui *M. calliphya*. We propose that these latter three populations be monitored and managed to maximize their long-term genetic health.

Introduction

Damselflies of the endemic Hawaiian genus *Megalagrion* are arguably Hawaii's most visible native stream insects. Found on all the main high islands, this genus has long fascinated biologists with its beauty and diversity (McLachlan, 1883; Perkins, 1899; Kennedy, 1928; Williams, 1936; Zimmerman, 1948; Polhemus, 1997). In fact, the approximately 23 species of this genus exploit the full known range of damselfly habitats, from ponds, anchialine pools, and low gradient streams, to fast mountain streams, acidic bogs, plant leaf axils, and even terrestrial habitats. *Megalagrion* appears to have arrived in Hawai'i about 11 million years ago, colonizing high islands that are now eroded away (Jordan *et al.*, 2003). It is an outstanding example of adaptive radiation, with bursts of speciation correlated with morphological innovations allowing the exploitation of broadly diverse and novel habitats (Jordan *et al.*, 2003). Fourteen of its species are endemic to single islands. Nine are found on more than one island, and two, *M. xanthomelas* and *M. pacificum* are known historically from the entire chain.

Extensive surveys carried out by the Hawai'i Biological Survey (HBS) in the 1990s led to a broad modern understanding of the range and abundance of *Megalagrion* species, and how these attributes have changed since the early days of Hawaiian entomology (e.g., Polhemus, 1993; Englund, 1999a). Most species, including some long believed extinct, were located. However, not all species and populations were found to be healthy, and some were merely remnants of once-thriving communities described by the early Hawaiian entomologists (see, for example, Englund, 2001). Because of this work, six species or subspecies of *Megalagrion* are currently considered Candidate Species under the U. S. Endangered Species Act (USFWS, 2004).

Polhemus (1993) highlighted the threats posed to *Megalagrion* by invasive species (fish, frogs, and other invertebrates) and habitat destruction correlated with human activity. Polhemus (1997) went further and posited two general classes of threats to Hawaiian damselflies: 1) phylogenetically related ecological vulnerability and 2) "biogeographic misfortune". *Megalagrion xanthomelas* and *M. pacificum* illustrate the first case. They are sister species that breed in coastal habitats and lower to middle stream reaches. This puts them in direct conflict with most human activity on the islands. Three other species (*M. leptodemas*, *M. oceanicum*, and *M. nigrohamatum nigrolineatum*) that are not closely related to each other illustrate the second case. They have the misfortune of being native to O'ahu, which has experienced more anthropogenic ecological disturbance than any other Hawaiian island. This has taken a heavy toll on Oahu's native insects.

Although few formal quantitative surveys of *Megalagrion* have been published (Englund, 1999a; Englund, 2001), workers from the HBS (R. Englund, N. Evenhuis, D. Preston, D. Polhemus, pers. comm.) and the U.S. Geological Survey (D. Foote, pers. comm.) have spent thousands of hours monitoring *Megalagrion* populations across the Hawaiian Islands, including many remote sites, within the context of their specific research goals. From this work, we have an excellent qualitative feel for the health of various *Megalagrion* populations based on ranges, abundances, ease of capture and sighting frequencies. In many cases, these observations span more than 15 years, and changes in population health over time have been observed. While a quantitative synthesis of this work is needed, our general knowledge of the health of some *Megalagrion* populations is excellent (e.g., see Polhemus, 2007). And while we have seen the exciting rediscovery of some *Megalagrion* populations (Evenhuis & Cowie, 1994; Englund, 1999b; Polhemus *et al.*, 1999), information on the health of others is disquieting.

The two main goals of conservation genetics are 1) the preservation of genetic diversity and the processes that foster it so that populations can meet the demands of a changing environment, and 2) the avoidance of inbreeding depression (Reed & Frankham, 2003). Inbreeding is a decrease in genetic diversity resulting from matings between related individuals. While many factors can decrease genetic diversity [e.g., certain life history and ecological strategies, natural selection, molecular structure of enzyme products, and many others—see Avise (2004)], inbreeding seems to trump all of these. That is, no matter what else is occurring, if inbreeding occurs, genetic diversity (heterozygosity and related measures) will decrease. This decrease in genetic diversity is often accompanied by inbreeding depression, a decrease in growth, survival or fertility (Avise, 2004).

Here we seek to quantify the genetic health of populations from four *Megalagrion* species by analyzing mtDNA sequences with phylogenetic and conservation genetic techniques. In particular, we discuss the relationship between genetic diversity at a mitochondrial locus and the abundance, range, and known demographic history of populations from four species of *Megalagrion*. Genetic data can serve at least three purposes toward the goal of *Megalagrion* conservation. First, phylogenetic analysis of genetic data can help us to identify species and their genetically unique subunits that merit conservation attention (Moritz, 1994; Crandall *et al.*, 2000). Second, genetic diversity is an indicator of the health of the population. Although Lande (1988) maintained that demographic factors have a greater influence on population endangerment and extinction than genetic factors, recent meta-analyses of hundreds of individual taxa have shown that may not be true in many cases (DeSalle, 2005). These studies indicate that population health is often influenced by low genetic diversity (Spielman *et al.*, 2004) and the loss of heterozygosity can have a deleterious effect on population fitness (Reed & Frankham, 2003). Finally, genetic diversity can give us an idea of whether

and how recent perturbations have affected populations. For example, populations that have seen reductions in population size due to anthropogenic causes are expected to have lower genetic diversity than those that have not, due to inbreeding.

The four species analyzed here include populations of known and unknown health. These species were selected for the following reasons. First, all of them are widespread, being found on at least four separate islands. Second, *M. xanthomelas* and *M. pacificum*, which have recently been the subject of a more detailed phylogeographic analysis (Jordan *et al.*, 2005), are candidates for listing under the U.S. Endangered Species Act. Extensive work on these species by the HBS has given us an excellent qualitative sense of the health of their sampled populations. In particular, the *M. xanthomelas* population on O'ahu is known to have experienced recent severe bottlenecks (Englund, 2001). Because of this, we expect genetic diversity to be low in it, allowing us to use it as a reference point for comparison with results from other populations. *Megalagrion pacificum* is one of the most endangered of the Hawaiian damselflies, having been extirpated on Kaua'i and O'ahu. Third, we chose *M. hawaiiense* because it is morphologically variable across its range, and has been proposed for splitting into at least two separate species (Daigle, 2000). In particular, *M. hawaiiense* males display color variability. Most males of the species are red, but blue males occur in the Ko'olau Mountains of O'ahu, and males with red abdomens and aqua thoraces are found near waterfalls along the Hana coast of Maui. There has been disagreement in the literature about the taxonomic status of these color variants (Polhemus & Asquith, 1996; Daigle, 2000). Although *M. hawaiiense* has never been considered to be of conservation concern, its morphological variability suggests that certain populations may be unique, meriting specific conservation action. This species has not been monitored as much as the other three, and so we have less information about its health. Genetic data can give us a starting point for its conservation. Fourth, we chose *M. calliphya* to serve as a sort of positive control. It has never been considered threatened in any way, and its populations seem quite healthy across its range. In fact it has successfully colonized many human-created freshwater sources at Volcano Village on Hawaii Island, including rainwater cisterns and ditches (e.g., the moat around a USGS greenhouse described in the stream conference paper presented by Idelle A. Cooper & David Foote, "Response of *Megalagrion calliphya* to simulated drought events"). We therefore expect it to display higher levels of genetic diversity than populations that have been negatively impacted by human activities.

We have initiated this preliminary study because knowledge of population genetic diversity can contribute to an understanding of the conservation needs of *Megalagrion* damselflies. Here, we use DNA sequence data to identify conservation units within each species and discuss conservation, taxonomy, and biogeography. We also quantify genetic diversity across a gradient of population health levels, including populations known to be at risk and populations known to be healthy. Correlations of population health and genetic variability are then used to assess the health of poorly known populations. Inspection of results from this comparison suggest that several populations may need conservation attention. Our results from this preliminary study will guide us as we expand the scope of the project in the future by including more loci, individuals, populations, and analyses.

Materials & Methods

A total of 191 damselflies representing four species were sampled from 31 populations (Table 1, Fig. 1) over a 14-year period of time. In many cases, population sample sizes were limited by the difficulties of collecting rare organisms over diverse and rugged terrain. These sample sizes are smaller than would be optimal for a conservation genetics study, and haplotype frequencies estimated from them are thus less reliable than those from large populations. However, because of the exploratory nature of this study and the importance of the questions being addressed, we have chosen to retain these samples in order to maximize the information available to decision makers. DNA was extracted from thoracic or leg muscle using a Qiagen DNEasy kit.

Approximately 660 base pairs of the mitochondrial cytochrome oxidase II gene were amplified

Table 1. Populations, sample sizes, and haplotype distributions for four damselfly species included in the current study. X = *M. xanthomelas*, P = *M. pacificum*, H = *M. hawaiiense*, C = *M. calliphya*. Vertically adjacent numbers in boldface represent samples from neighboring locales that were aggregated for the population genetic analyses. Haplotype codes correspond to Figs. 2, 3, 4. Numbers following colons represent the number of individuals bearing a particular haplotype in a particular population.

Locality sampled	Sample size				Haplotypes: Frequency
	X	P	H	C	
O'ahu					
Tripler Army Medical Center (TAMC)	20		7		Xa:20 HWa:6, HWc:1 HWa:2, HWb:4
Mount Ka'ala			6		
Ko'olau Mtns. Waikane Stream					
Moloka'i		6	8	4	Pa:6 HWc:7, HWm:1 – Ca:2, Cb:2 Xa:5, Xb:6, Xc:2, Xe:1 Pa:6, Pe:1, Pf:1 HWb:1 – Cb:3, Ci:1, Cj:2, Ck:1, Cm:1, Cr:1
Kalaupapa Peninsula					
Waikolu Valley					
Pelekunu Valley & taro patch near Keawenui Stream	14				
Wa'ilau Valley		9			
Kamakou TNC Preserve			1	9	
Lāna'i					
Pipeline seepage, Maunalei Gulch	6				Xa:4, Xc:2 Xa:2, Xb:2
Kō'eke Lodge Ponds	4				
Maui					
East Maui, Cape Hanamānoa Region	4	4	2		Xb:1, Xd:3 Pb:3, Pd:1 – HWf:2 HWf:2, HWj:1 – Cf:2, Cg:1, Ch:2, Cp:1 HWk:1 – Ch:2, Cs:1, Ct:1 HWg:1, HWl:1 HWg:1 – Ce:3, Cu:1
East Maui, Haipua'ena Stream			3	6	
East Maui, East Wa'ilua Iki Stream			1	4	
East Maui, Upper Hanawī			2		
West Maui, Olowalu Stream			1	4	
West Maui, Mt. 'Eke					
Hawai'i					
Kohala Mtns., upper Waikoloa Stream, N. of Waimea				1	Cd:1
Kohala Mtns., SE of Pu'u Pili				1	Cr:1 HWd:2, HWi:1 HWd:3 Xf:3, Xg:3, Xh:1, Xj:3, Xnr:1, Xr:1 HWe:4
Kohala Mtns., upper Alakahi Stream at head of Waipi'o Valley			3		
Kohala Mtns., Honokāne Nui Stream			3		
Waikāunalo Co. Park	12		4		
Onomea Str. above garden					
Hawai'i Volcanoes National Park, Ola'a Pu'u Unit				11	Cc:6, Cd:2, Cg:1, Co:1, Cq:1 Xf:7, Xh:6, Xp:1, Xq:1 Xa:1, Xb:1, Xf:6, Xh:2, Xi:4, Xk:1, Xn:1
Kāwā Springs	15				
Kaloko Springs	16				
Total	91	19	41	40	

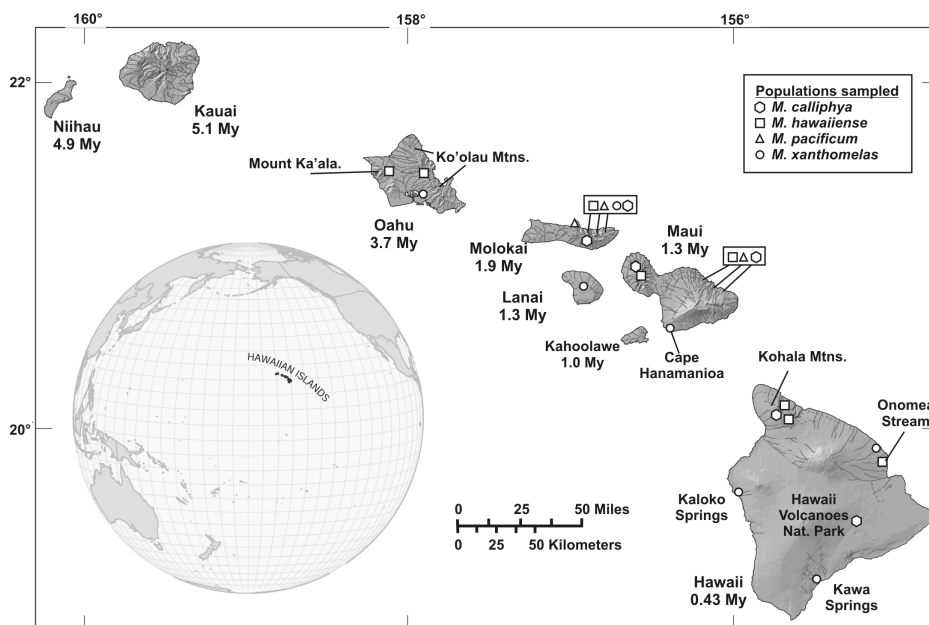


Figure 1. Map of the high Hawaiian Islands, showing sample sites for *Megalagrion calliphya*, *M. hawaiiense*, *M. pacificum*, and *M. xanthomelas*. Island ages based on K-Ar dating are shown (Clague & Dalrymple, 1987).

for each individual damselfly using the polymerase chain reaction (PCR) and primers C2-J-3102 (aaatggcaacatgagcacaayt) and TK-N-3773 (gagaccagtactgtcttcagtcate) from Jordan *et al.* (2003). Standard PCR was carried out with the AmpliTaq gold enzyme under the following conditions: 10 minutes at 95 °C followed by 35 cycles of 30 sec. at 95 °C, 30 sec. at 50 °C, and 60 sec. at 72 °C. PCR products were purified by using a Qiagen Qiaquick purification kit. Cycle sequencing using each of the PCR primers was carried out following the protocols from the manufacturer of the dye terminators (ABI or Beckman Coulter). Sequencing products were purified by using Sephadex spin columns or ethanol precipitation. DNA sequences were visualized on either an ABI 377, 3100, or Beckman Coulter CEQ 8000 instrument.

Individual primer sequences were compared and corrected for base-calling errors by using Sequencher 3.0 (Genecodes Corp., Ann Arbor, Michigan) or CodonCode Aligner (CodonCode Corp., Dedham, Massachusetts). Final alignments were generated using ClustalW (Thompson *et al.* 1994), and were adjusted by eye using MEGA (Kumar *et al.*, 2001).

Two types of phylogenetic analysis were performed on unique haplotypes from each data set: 1) maximum likelihood (ML) with bootstrapping and 2) Bayesian. Initial data exploration (not shown) demonstrated that, without fail, outgroup taxa were connected to the ingroup by relatively long branches, and that this could affect hypothesized ingroup relationships. Therefore, all analyses were performed without outgroups. Three separate phylogenetic analyses were performed. *Megalagrion xanthomelas* and *M. pacificum* are closely related and were analyzed together, and the other two species were analyzed separately. In order to correct for multiple substitutions at nucleotide sites and for variation in substitution rates among sites, we selected the simplest ML model that did not differ significantly from the most complex (best fitting) model (GTR+I+G) for each data set. This was done using the likelihood ratio test procedure of Frati *et al.* (1997), the Akaike information criterion method of Buckley *et al.* (2002) and PAUP* 4.10b (Swofford, 1998). This simplest adequate model reduces computing time and the variance of estimated parameters. We used this appropriate model to perform heuristic ML searches in PAUP* with 10 random addition sequence replicates and TBR branch swapping. Parameter estimates for the appropriate model were fixed after being estimated on an initial

Table 2. Population and sampling parameters for four species of Hawaiian damselflies. Haplotype and nucleotide diversity and their sampling variances were calculated for entire species, without subdivision. The genetic distance shown is the maximum value calculated between haplotypes within each species using the model indicated.

	N	Number Haplotypes	Aligned Sequence Length (bp)	Number Variable Sites/Pars. inform.	ML Model	Maximim Corrected Genetic Distance	Haplotype Diversity +/-V	Nucleotide Diversity +/-V
<i>M. calliphya</i>	40	20	653	27/19	HKY+I	0.028	0.95 +/- 0.017	0.0085 +/- 0.0046
<i>M. hawaiiense</i>	41	13	643	26/12	GTR+I	0.025	0.90 +/- 0.023	0.0105 +/- 0.0056
<i>M. pacificum</i>	19	6	660	32/20	HKY+G	0.0031	0.60 +/- 0.12	0.0011 +/- 0.0009
<i>M. xanthomelas</i>	91	16	660	32/20	HKY+G	0.032	0.82 +/- 0.03	0.0116 +/- 0.0060

neighbor joining tree. The parameter values were re-estimated on the resulting ML tree, and the heuristic search was performed again. If the results of the two heuristic searches agreed, we searched no further. ML bootstrap analysis was performed under the appropriate model with parameter values fixed based on estimates from the ML tree. We performed 200 pseudoreplicates in PAUP* by using a heuristic search with one random addition sequence replicate and retaining one tree.

Bayesian analysis was performed by using MrBayes 2.01 (Huelsenbeck & Ronquist, 2001). Unique haplotypes for each data set were analyzed using the Metropolis coupled Markov chain Monte Carlo method. Four chains, three heated and one cold, were run for 1 million generations each. Only sample points occurring after stationarity of the negative log likelihood score (-lnL) was achieved were used in calculating posterior probabilities. Stationarity was assessed by graphing the -lnL for every 100th generation, and looking for the point where these values leveled off near their optimal value. The burnin value (generations discarded) was different for each data set (2000–12000 generations).

Population genetic parameters were estimated using the Arelequin software package (Schneider *et al.*, 2000). We estimated both gene (haplotype) and nucleotide diversity for 22 populations. In some cases, neighboring locales were aggregated to increase sample sizes (Table 1). Gene diversity is roughly equivalent to the expected heterozygosity of diploid data. It represents the probability that two randomly selected haplotypes from a population will be different. Nucleotide diversity is the probability that two randomly selected homologous nucleotides from the population will be different. Estimates of these parameters become less reliable as sample sizes decrease.

Results

Aligned sequence lengths, the number of variable sites, and appropriate ML nucleotide substitution models are shown by species in Table 2. All sequences generated are available from GenBank. Please contact the first author for accession numbers and alignments. Alignments ranged from 643–660 bp in length, and were unambiguous. There were no insertions or deletions. Maximum corrected genetic distances within species (Table 2) ranged from 0.0031 for *M. pacificum* to 0.032 for *M. xanthomelas*, a ten-fold difference. Total numbers of haplotypes found per species varied from 6 for *M. pacificum* to 20 for *M. calliphya* (Table 2).

Phylogeographic patterns varied between species. We found 3 main clades for *M. xanthomelas* and *M. pacificum*, corresponding to *M. xanthomelas* predominantly from O'ahu and Maui Nui, *M. pacificum*, and *M. xanthomelas* from Hawai'i (Fig. 2). Many haplotypes were shared between populations and even between islands. On the other hand, *M. hawaiiense* populations rarely shared haplotypes, and only once was a haplotype found on 2 islands (HWc, Fig. 3). We found support for three clades, one comprised mainly of Maui and Hawai'i haplotypes, another of O'ahu haplotypes, and a third of mainly Moloka'i haplotypes. Moloka'i haplotypes were intermediate between the other two clades, in essence bridging the gap between O'ahu and Maui in a manner consistent with the geological history of the islands. Finally, the 4 individuals from Onomea Stream on Hawai'i all bore a haplotype that differed by a minimum of 11 and a maximum of 14 substitutions (0.019–0.025 substitutions/site) from the rest of the species. Phylogeography of *M. calliphya* also shows little sharing of haplo-

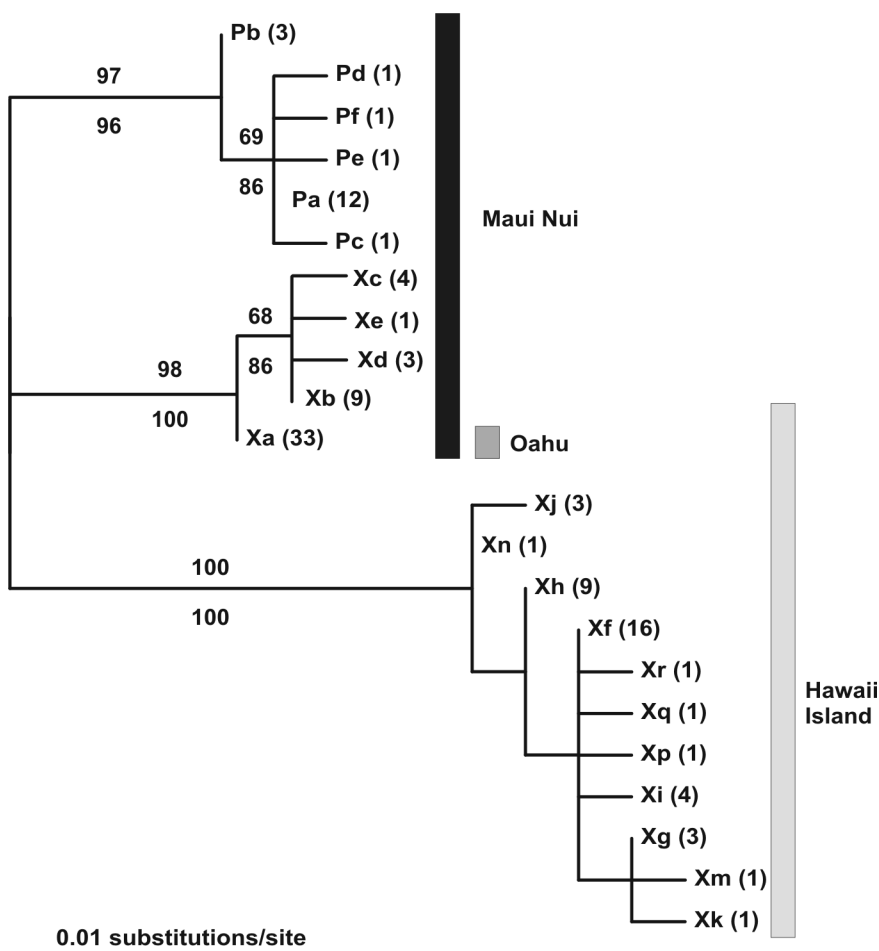


Figure 2. Maximum likelihood (ML) phylogram of 16 haplotypes from 91 individuals of *Megalagrion xanthomelas* and 6 haplotypes from 19 individuals of *M. pacificum*, generated using the HKY+G model in both maximum likelihood and Bayesian analyses. Numbers above the line are ML bootstrap values (200 pseudoreplicates) and numbers below the line are Bayesian posterior probabilities (one million generations). Due to the widespread nature of these haplotypes, identification of specific populations on the tree is not feasible. See Table 1 for specific haplotype distributions.

types between islands, but the distribution of two haplotypes serves to unite the islands in a manner consistent with geological history: haplotype Ca, from Moloka'i, shows little difference from Maui haplotypes, and haplotype Cg was found on East Maui and Hawai'i (Fig. 4).

Genetic diversity calculations for the entire species were telling (Table 2). In terms of both haplotype and nucleotide diversity, *M. pacificum* scores the lowest, with *M. xanthomelas* next in haplotype diversity. The other 2 species generally displayed the most genetic diversity in the study. Genetic diversity calculations for individual populations were much more variable (Fig. 5). In general, populations of *M. calliphya* displayed the most haplotype and nucleotide diversity, while *M. xanthomelas* displayed high haplotype diversity with lower nucleotide diversity. Once again, *M. pacificum* showed the least genetic diversity by either measure. *Megalagrion hawaiiense* was surprising, in that while its nucleotide diversity was comparable to *M. xanthomelas*, its haplotype diversity was generally lower.

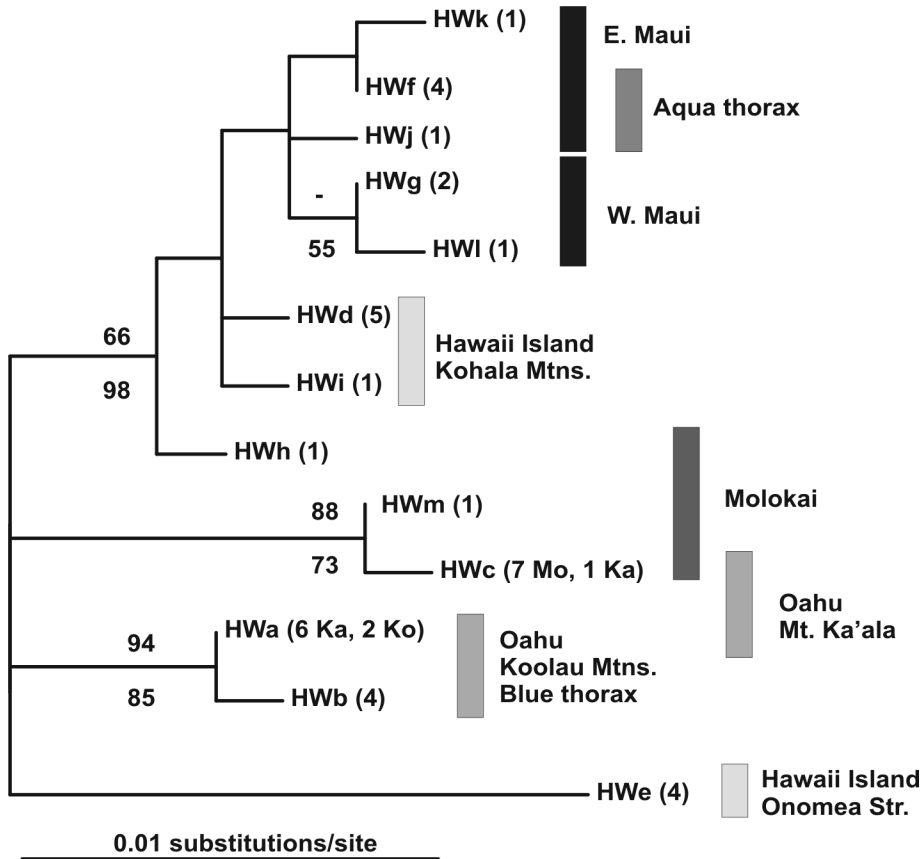


Figure 3. ML phylogram of 13 haplotypes from 41 individuals of *M. hawaiiense* generated as in Fig. 2, but using a GTR+I model. The occurrence of the blue and aqua color polymorphisms are noted.

Discussion

Comparative phylogeography

Megalagrion xanthomelas and *M. pacificum* share many more haplotypes between populations and islands than either of the other two species. Jordan *et al.* (2005) used phylogeographic analysis to propose that *M. xanthomelas* and *M. pacificum* do not often cross ocean channels, and that haplotype sharing seems to have occurred within the boundaries of contiguous or once contiguous islands (Hawai'i and Maui Nui). Populations of the two additional species analyzed here, *M. calliphya* and *M. hawaiiense* have many fewer shared haplotypes than *M. xanthomelas* and *M. pacificum*, suggesting that they have not often crossed ocean channels, but also that they do not experience as much gene flow within islands as *M. xanthomelas* and *M. pacificum*.

Megalagrion xanthomelas and *M. pacificum* show the greatest genetic differentiation between the Hawai'i Island clade and those from O'ahu and Maui Nui (Fig. 2). Two north island haplotypes found on Hawai'i are in low frequency and seem to be the result of limited immigration. This general pattern is consistent with the geological history of Hawai'i Island, which has never been connected to Maui Nui. *Megalagrion calliphya* and *M. hawaiiense*, however, both show much less differentiation between Hawai'i Island haplotypes and those found on Maui Nui (Figs. 3, 4). Many elements of their phylogeographic patterns roughly recapitulate the chronological history of the island

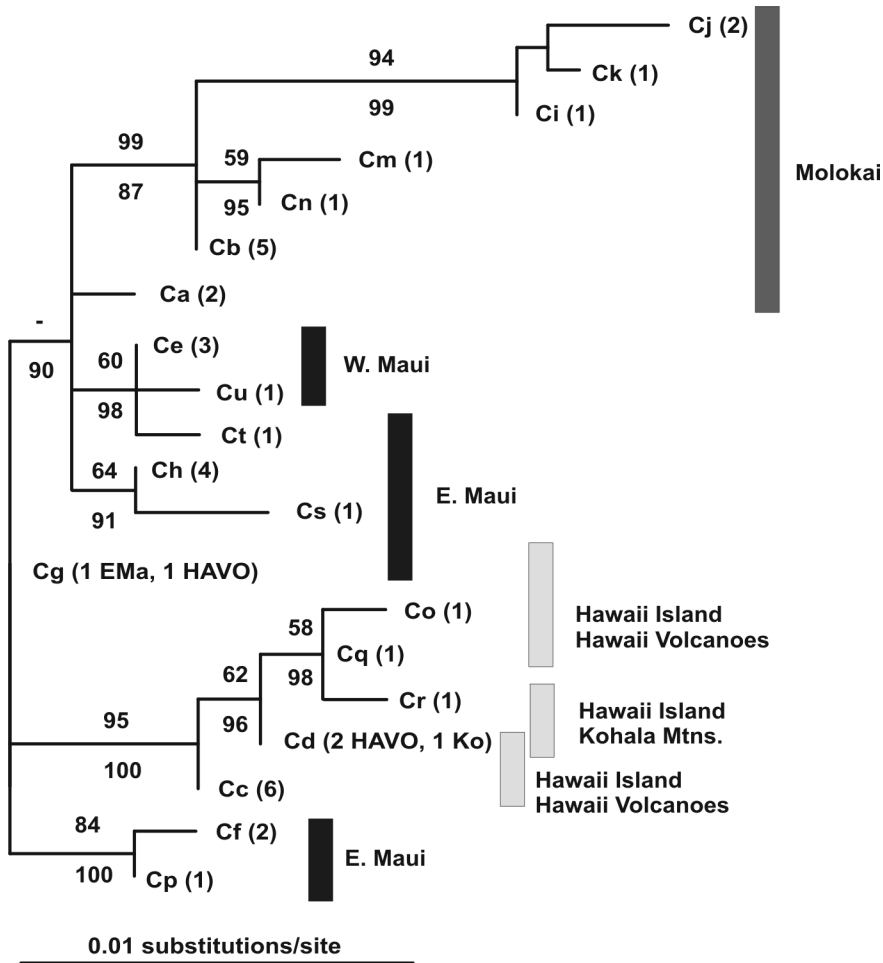


Figure 4. ML phylogram of 20 haplotypes from 40 individuals of *M. calliphya* generated as in Fig. 2, but using the HKY+I model.

creation (e.g., clustering of Moloka'i and O'ahu haplotypes, and Maui and Hawai'i Island haplotypes). These geological signatures differ from those of *M. xanthomelas* and *M. pacificum*, where more recent and local processes (e.g., hybridization and gene flow) are thought to be responsible for genetic patterns within Maui Nui and Hawai'i (Jordan *et al.*, 2005).

High levels of mtDNA divergence in *M. xanthomelas* led Jordan *et al.* (2005) to explore the possibility of incipient speciation of Hawai'i Island populations. This idea was ultimately rejected in light of both nuclear DNA sequences and morphology that support the monophyly of *M. xanthomelas*. Jordan *et al.* (2005) also noted that genetic distances between Hawai'i Island and O'ahu/Maui Nui populations of *M. xanthomelas* appear higher than they should based on the geological age of Hawai'i Island. They hypothesized that this might be due to an ancient event of introgressive hybridization between Maui Nui *M. pacificum* and *M. xanthomelas*. This hypothesis is supported by our observation of low genetic distances between Hawai'i Island and Maui Nui populations of *M. hawaiiense* and *M. calliphya* (Figs. 3, 4), which may better reflect the baseline inter-island genetic distance for *Megalagrion* in the absence of introgression.

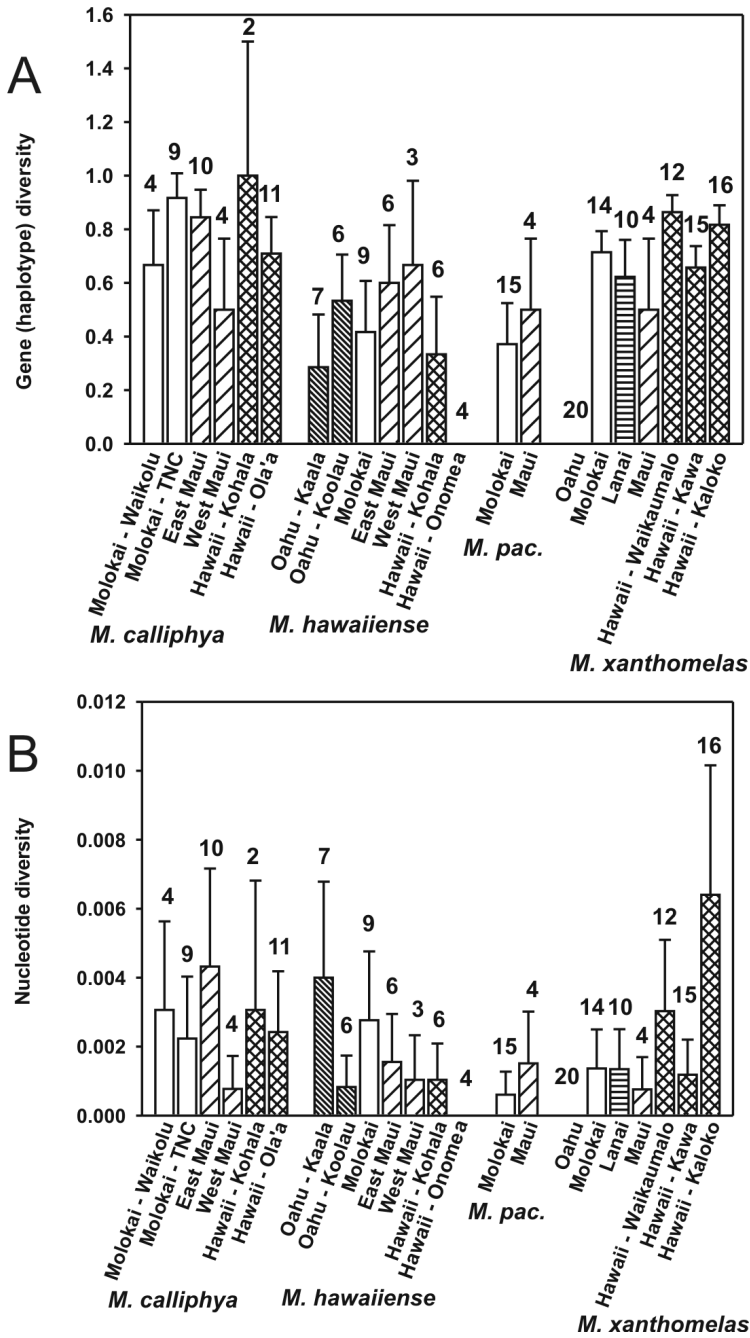


Figure 5. Comparison of population genetic parameters calculated for all populations of all species sampled using the Arlequin software package (Schneider *et al.*, 2000). Numbers above bars represent sample sizes. Whiskers are the sampling variance in each case. **A:** Gene or haplotype diversity, which is roughly equivalent to heterozygosity for diploid data; **B:** Nucleotide diversity, which is the probability that two homologous nucleotides sampled at random from the data set will be different.

Although we do not support species-level recognition for the Hawai'i Island populations of *M. xanthomelas*, we do feel that these genetically unique populations should be managed as a separate entity within this species. Of the 43 *M. xanthomelas* sampled from Hawai'i Island, 2 individuals from Kaloko bore haplotypes common on O'ahu and/or Maui Nui. The rest bore haplotypes that can be diagnosed from Maui Nui and O'ahu haplotypes at 11 nucleotide sites. Although the presence of the 2 northern haplotypes at Kaloko technically means that Hawai'i Island is not diagnosable from the other islands, the 11 diagnosable sites in the other 41 individuals represent a great deal of genetic uniqueness and we recommend that this be taken into account as Hawai'i Island populations are managed. Diagnosable island subunits were not observed within *M. pacificum*, *M. calliphya*, or *M. hawaiiense*. Significantly, this includes the finding of no genetically distinguishing characteristics for the O'ahu blue and Maui aqua populations of *M. hawaiiense* (Fig. 3), countering the argument that these color variants represent unique species (Daigle, 2000).

Conservation genetics

Long-term monitoring of several populations included in this study has shown them to be relatively healthy. Damselflies in these populations are abundant, the geographical area occupied is not unnaturally restricted (as *M. xanthomelas* is on O'ahu), and no significant declines in population size have been observed. Chief among these are populations of *M. calliphya* from Hawai'i Volcanoes National Park (HAVO) and East Maui. Other populations are known to be but small remnants of larger populations, or populations that were perhaps never very large. Included in these are *M. xanthomelas* populations from O'ahu and East Maui, which were both described as abundant by early Hawaiian entomologists (e.g., Perkins, 1913). *Megalagrion pacificum* is generally rare throughout its range, though it is locally abundant in populations on North Moloka'i (R. Englund, pers. comm.).

Results of the genetic diversity analyses confirm some of our previous knowledge (e.g., Englund, 2001) and offer surprises in some cases and important warnings in others. First the confirmations: Hawai'i Island *M. xanthomelas* show a great deal of diversity, although in spite of a large sample size, the Kawa population has less nucleotide diversity than Waikaumalo or Kaloko. The Kaloko nucleotide diversity is especially high because of the presence of 2 individuals there from the northern *M. xanthomelas* clade. Likewise, genetic diversity in *M. calliphya* is relatively high. In particular, the Moloka'i, East Maui and HAVO populations of *M. calliphya* display high nucleotide diversity, confirming the relative health of this species. This is not surprising to those who have spent time at Volcano, and have observed *M. calliphya* successfully using human and pig-created habitats.

O'ahu and Maui *M. xanthomelas* populations are thought to be relictual and their low genetic diversity is thus not surprising. The *M. xanthomelas* population in Honolulu, however, is an extreme case of genetic inbreeding, consistent with documented demographic bottlenecks (Englund, 2001). Although our sample size here was high (20 individuals), they all bore the same mtDNA haplotype, suggesting a lack of genetic diversity for meeting the challenges of a changing environment (Reed & Frankham, 2003). The future health of *M. xanthomelas* on O'ahu may depend on translocations of individuals from Moloka'i populations that also harbor the O'ahu mitochondrial haplotype. In contrast, *M. hawaiiense* from Moloka'i also appear to be quite genetically diverse.

Results of conservation genetic analysis for some populations were surprising. Among the pleasant surprises was *M. pacificum*, which, although rare on East Maui, appears to harbor more genetic diversity there than on Moloka'i. This is all the more surprising since our calculations here did not include an extremely divergent mtDNA haplotype from the Kīpahulu Valley (Jordan *et al.*, 2005). O'ahu Mt. Ka'ala populations of *M. hawaiiense* are also quite diverse in spite of the fact that extensive habitat has been lost in this region. Results of concern include those from *M. hawaiiense* populations from the Ko'olau Mountains of O'ahu, the Kohala Mountains of Hawai'i, and the West Maui Mountains, which show very little genetic diversity. This species has not previously been on the radar of conservationists, but the genetic data suggest that perhaps it should be. Although not diagnosable, its population at Onomea Stream is genetically quite distinct from the rest of the species and merits further conservation attention.

Finally, *Megalagrion pacificum* and *M. xanthomelas*, which enjoy some of their highest num-

bers on Moloka'i, show relatively low genetic diversity there. These populations, which have been considered the most healthy in the species, occur in some of the most pristine streams in Hawai'i (Polhemus, 1993). Nevertheless, in spite of a sample size of 15, the Moloka'i *M. pacificum* show a stark lack of genetic diversity. This suggests that the long term outlook for those populations may not be good, although their low genetic diversity could also be the result of historical bottlenecks caused by sea level fluctuations, and therefore present no cause for alarm (Jordan *et al.*, 2005). In any case, these populations should be carefully monitored, stream flows should be assured and any detection of invasive fish in these streams should be followed by aggressive eradication efforts.

An alternative perspective on the data can be gained by considering the diversity results by island (Fig. 5). Patterns among species within islands are not constant. Rather than all species doing well on relatively pristine Moloka'i, or poorly on O'ahu, results vary. This in part may be due to local extirpations: O'ahu has entirely lost *M. pacificum*, so its genetic diversity cannot be considered. *Megalagrion calliphya* does not occur on O'ahu, but its sister species, *M. leptodemas* is in desperate straits there (Polhemus, 1993). And yet, *M. hawaiiense* on Mt. Ka'ala has high nucleotide diversity. Moloka'i populations of *M. calliphya* and *M. hawaiiense* are quite healthy, but *M. xanthomelas* and *M. pacificum* show disappointingly low genetic diversity levels there. Hawai'i Island populations generally show high diversity, but *M. hawaiiense* bucks this trend.

It is worth noting that the results of this paper may represent a best-case scenario, as the data included in it are from damselflies sampled up to 14 years ago. The sampled populations may have declined in the meantime. In May 2005, for example, we failed to find any *M. xanthomelas* at Waikamalo State park on Hawai'i Island. The stream was teeming with introduced fish and frogs. This example emphasizes the need for steps to be taken to immediately secure the genetic diversity that remains in this important endemic Hawaiian insect genus. Such efforts should begin with centralized record keeping and data sharing between all the agencies that have stewardship over, or interest in, *Megalagrion*. Populations with low genetic diversity should be managed in ways that increase gene flow and population sizes. Both of these factors are directly correlated with genetic diversity. These goals could be accomplished by ensuring adequate water flow, excluding and removing invasive species, and, in some cases, translocation.

In summary, we have assessed levels of genetic diversity in *Megalagrion* populations of known health. We have then estimated genetic diversity in a variety of *Megalagrion* populations and inferred their health. Based on these estimates, we propose that several populations may merit renewed conservation attention. We have shown that genetic diversity can be low in populations that have otherwise been considered healthy. Many examples in the literature suggest that low genetic diversity may be a sign of population endangerment and decreased fitness (Reed & Frankham, 2003; Spielman *et al.*, 2004). We have also shown that measuring genetic diversity may be a quick and reasonable method for assessing the health of populations that have not been extensively monitored. This approach shows promise in *Megalagrion*, in spite of small some sample sizes and analysis of a single locus. In the future, the expansion of this approach to remaining *Megalagrion* populations will be beneficial, and will improve on estimates of genetic diversity by sampling many more loci such as microsatellites and more individuals per population.

Acknowledgments

Specimens and field assistance were kindly provided by Ron Englund, Neal Evenhuis, Dan Polhemus, David Preston, Adam Asquith, Forrest Starr, Kim Martz, Bill Puleloa, Jen Morris, and David Foote. Thanks to Gordon Nishida and the Bishop Museum for maintaining and loaning valuable specimens. We are grateful for the extensive and insightful comments of an anonymous reviewer. This work was funded by the U.S. National Science Foundation Doctoral Dissertation Improvement program (Grant no. 0073314 to SJ), the U.S. Geological Survey Biological Resource Division, the UConn Department of Ecology and Evolutionary Biology, the Connecticut State Museum of Natural History's Penner and Slater Funds, the Canon National Parks Science Scholars Program, Sigma Xi, and the Bucknell University Department of Biology. We express our sincere

gratitude to the Hawai‘i Division of Aquatic Resources for organizing and funding the 2005 Stream Symposium at which these results were first presented and to J. Michael Fitzsimons and Neal Evenhuis for editing these conference proceedings. This paper is dedicated to the children of the *Kanu ‘o ka ‘Āina* Charter School, who have the privilege of sharing air, water, land, and time with these marvelous insects. Through them it will always be so.

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