

**A phylogenetic study of the Great Hawaiian Dragonfly or
Anax strenuus.**

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Abstract

The Great Hawaiian Dragonfly, *Anax strenuus*, is an endemic Hawaiian odonata found throughout the Hawaiian island chain. However, no research has been formally conducted on this species. This study creates a baseline genetic analysis of *A. strenuus* by examining the populations genetics of the species using phylogenetic methods. Thirty-two specimens representing *A. strenuus* and *Anax junius*, hypothesized to be an introduced species, were collected from thirteen populations and sequenced for Tuba, 16S, 12S, H3 and COII. DNA analysis of these genes resulted in data ranging from 98.6%-99.99% similarity with no distinction between populations on the same island or between islands. There was also no distinction between the lowland species (*A. junius*) and *A. strenuus*. This unexpected result implies that gene flow has been significant enough to maintain population cohesion among all *A. strenuus* and warrants more investigation into the genetic relationship and taxonomy of *A. strenuus* and *A. junius* using more divergent data. This research is currently underway.

Introduction

Nishida (1992) estimated that the Hawaiian Islands have 5,462 endemic insect species, which is around 94% of all insect species found on the islands. Of these native insects, twenty-seven are endemic odonta species, twenty-three which are damselflies and two which are dragonflies (Jordan et al. 2007). Increased worldwide interest in odonata and a noticeable decline in dragonfly populations have resulted in an influx of research on regional dragonfly species (Hassal and Thompson 2008, Bernard and Schmitt 2010, Bried and Mazzacano 2010).

Dragonflies are on the decline across countries where monitoring Odonata species is routinely performed which includes Japan, North America and mainland Europe (Hornung and Rice 2003, Kadoya et al. 2009, Suhonen et al. 2010). Korkeamaki and Suhonen (2002) compared population studies of twenty species of dragonflies found in central Finland from 1930 to 1975 and data collected from 1995 to 1996 only to discover that of the original two hundred and nineteen populations, ninety-eight had vanished. This pattern of waning biodiversity among dragonflies may be occurring across the globe (Settele and Kuhn 2009). Due to this trend, baseline studies and conservation efforts have been focused specifically odonata (Korkeamaki and Suhonen 2002, Hassal and Thompson 2008, van Dyke et al. 2008, Settele and Kuhn 2009).

The original 1992 Hawaiian Biological Survey outlined the locality of various arthropods (Nishida 1992). There were two notable results of this survey: a rekindled interest in the origin and future of Hawaiian insects and data that supported the hypothesis that endemic Hawaiian insects were on the decline (Roderick and Gillespie 1998, Jordan et al. 2007).

The Great Hawaiian Dragonfly or *Anax strenuus* was recognized as globally vulnerable from data collected in the Hawaiian Biological Survey. This species is arguably the largest dragonfly in the world, reaching a wingspan of seven and a half inches (Berger 2004). *Anax strenuus* was originally documented and recorded from the end of the nineteenth century to the mid-twentieth but since then the species has not been investigated (Hagen 1861, McLachlan 1874, Zimmerman 1948). Beyond cross-breeding attempts conducted by McLachlan (1874) to discover how closely related *A. strenuus* is to an introduced dragonfly, *Anax junius*, there is no published research on this species.

The purpose of this study was to generate a phylogeny of *A. strenuus* thus creating a baseline genetic analysis. There were two main goals: to quantify the gene flow of *A. strenuus* between the islands and to compare the genetic relationship between the two species of the genus *Anax* found in Hawaii.

Materials and Methods

Materials Examined

Thirty specimens of *A. strenuus* and two specimens *A. junius* were collected from thirteen different populations across the islands of Maui, Oahu, Molokai, Hawaii and Kauai (Appendix I, Table 1). All specimens were preserved in 100% ethanol.

DNA extraction, amplification and sequencing

Genomic DNA was extracted using Qiagen DNeasy protocol for animal tissue (QIAGEN., Valenci, CA, USA). Muscle tissues from the thorax and leg region were used. Vouchers of the specimens were

deposited into the Insect Genomics Collection (IGC) of the M.L Bean Museum, Brigham Young University, Provo, UT.

The molecular data set comprised five genes: Tuba, 16S, 12S, H3 and COII (Appendix I, Table 2). The primers and nested primers for all of the genes can be found in Table 3 within Appendix 1. All primers were obtained from Integrated DNA Technologies, Inc. These genes were amplified using three step polymerase chain reactions (PCR) at forty cycles with annealing temperatures of 50°C. All products were visualized using agarose gel electrophoresis. Purification was done using a montage PrepEase Purification 96-well plate and cycle-sequenced using BigDye Terminator chemistry (version 3, ABI). An ABI 3100 capillary sequencer at Brigham Young University's DNA Sequencing Center, was used to generate sequences. Complementary strands were sequenced with fragment overlap to reduce errors in sequencing.

DNA sequence analysis

Sequence data was initially aligned in Geneious 6.1 to ensure the gene ends were homologues (Geneious 6.1). Genes were then assembled into contigs. Final alignments were created in MAFFT (Kato and Toh 2008).

Results

Assembly of the genes resulted in close similarities. Bp numbers varied from 387 to 690 but for all samples the genetic variability was around 99% (Appendix I, Table 4). There were no distinctions between populations or species.

Due the nature of the outcome, phylogenetic analysis of the species proved to be implausible and as such was not executed as originally planned. Further, DNA sequencing using a locus from the mitochondrial genome (cytB) is currently underway and preliminary results are encouraging.

Discussion

The results were not able to specifically quantify the pattern of gene flow between the populations due to the similarities among the specimens. Gene flow between populations, even as isolated as they seem, has been seen in other odonata species (Keller et al. 2010, Raebel et al. 2010). While dragonflies have the ability to fly the distances needed to continue gene flow between islands, it must also be noted that a low number of population cross over is needed to keep geographically separated populations genetically similar (Wang and Russel 2007, Chen and He 2009).

One of the main objectives of this research was to relate *A. strenuus* and *A. junius*. The almost nonexistent genetic variation may be in part due to the inferred past of *A. strenuus* (MacLachlan 1874). *Anax strenuus* has been assumed to be the endemic cousin of *A. junius* based on their similar coloring and morphology. The global abundance of *A. junius* may account for the ancestors of *A. strenuus* coming to the Hawaiian Islands. It can also be noted that while the data was fragmented and not well detailed, MacLachlan (1874) was able to breed the two species together with some success. This research is usually discounted due to its hobbyist nature.

More so than any other trait, *A. strenuus* and *A. junius* were identified by *A. strenuus*' greater body size. The minor genetic differences between the two species may account for this trait. Drastic genetic variation is not required for large ranges of morphologic differences (Chandra et al. 2011). In such cases, Southern Chinch bug (*Blissus insularis*) populations varied at most 3% when examining amplified fragment length polymorphisms (AFLP). The converse of Bergmann's rule may also account for the noticeable size variations between the highland and lowland specimens, but there could also be other factors such as differing concentrations in oxygen at different elevations during naiad development (Lehnert et al 2012).

To gain a better understanding of the relationship that *A. strenuus* has between differing populations and *A. junius*, genetic tests focusing on faster genes, SNPs or haplotypes should be done. Sets of data that result from these tests would allow a more accurate analysis to be executed, using the Hardy-Weinberg equation or AMOVA, a statistical analysis of molecular variance within species using a specific molecular marker.

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Appendix I

Table 1. The specimens used for molecular analysis including taxa codes and collection data					
Taxa Code	Species	Collection Date	Location	Latitude/Longitude	Notes
OD532	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail stream. Kawaikoi stream junction.	22° 13'7"N 159° 60'4"W	Adult
OD533	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail stream. Kawaikoi stream junction.	22° 13'7"N 159° 60'5"W	Adult
OD534	strenuus	8/1/2012	Hawaii: Kauai YMCA road trail. Kawaikoi stream	22° 13'1"N 159° 5'9"W	Adult
OD535	strenuus	8/1/2012	Hawaii: Kauai YMCA road trail. Kawaikinana		Adult
OD536	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail. Boardwalk. Swamp kill	22° 15'4"N 159° 5'9"W	Adult
OD537	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail stream. Kawaikoi stream junction.	22° 13'7"N 159° 6'5"W	Adult
OD539	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail. Swamp.	22° 15'6"N 159° 59'8"W	
OD540	strenuus	8/1/2012	Hawaii: Kauai YMCA road trail. Kawaikinana stream		
OD541	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail stream. Kawaikoi stream junction.	22° 13'7"N 159° 6'5"W	
OD542	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail stream. Kawaikoi stream junction.	22° 13'7"N 159° 6'5"W	
OD543	strenuus	8/3/2012	Hawaii: Kauai R. Beck Family Farm	22° 20'3"N 159° 1'7"W	
OD544	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail stream. Kawaikoi stream junction.	22° 13'7"N 159° 6'5"W	
OD545	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail. Swamp.	22° 15'6"N 159° 59'8"W	
OD546	strenuus	8/2/2012	Hawaii: Kauai Alakai swamp trail. Swamp.	22° 15'6"N 159° 59'8"W	
OD547	strenuus	8/6/2012	Hawaii: Molokai Main road before Pepe'opae boardwalk	21° 11'8"N 156° 90'8"W	Adult
OD548	strenuus	8/5/2012	Hawaii: Molokai Waikolu Nature conservancy road stream crossing	21° 13' 9"N 156° 9'2"W	
OD549	strenuus	8/6/2012	Hawaii: Molokai Bridge before tunnel on Kawela intake	21° 11' 6"N 156° 90'8"W	
OD550	strenuus	8/6/2012	Hawaii: Molokai Bridge before tunnel on Kawela intake	21° 11' 6"N 156° 90'8"W	
OD551	strenuus	8/5/2012	Hawaii: Molokai Waikolu Nature conservancy road stream crossing	21° 13'N 156° 92"W	
OD552	strenuus	8/6/2012	Hawaii: Molokai Bridge before tunnel on Kawela intake	21° 11'N 156° 90'8"W	
OD553	strenuus	8/6/2012	Hawaii: Molokai Bridge before tunnel on Kawela intake	21° 11'N 156° 90'8"W	
OD555	strenuus	8/15/2012	Hawaii: Maui Hana State Hwy 360	20° 49'1"N 156° 8'W	
OD556	strenuus	8/15/2012	Hawaii: Maui Hana State Hwy 360	20° 49'1"N 156° 8'W	
OD557	strenuus	8/15/2012	Hawaii: Maui Hana State Hwy 360	20° 49'1"N 156° 8'W	
OD558	strenuus	8/15/2012	Hawaii: Maui Hana State Hwy 360	20° 49'1"N 156° 8'W	

OD562	strenuus	3/30/2012	Hawaii: Oahu. Kava Vinui trail, Laie Summit		
OD567	junius	6/15/2012	Hawaii: Hawaii Island Aima Kapa	19° 67'6"N 156° 2'4	Sea Level- tidal marsh
OD578	strenuus	11/29/2012	Hawaii: Oahu, Kava Vinui trail, Laie Summit	21° 59'1"N 157° 59'6"	
OD579	strenuus	11/29/2012	Hawaii: Oahu, Kava Vinui trail, Laie Summit	21° 59'1"N 157.59'6"W	
OD584	strenuus	11/29/2012	Hawaii: Oahu, Kava Vinui trail, Laie Summit	21° 59'1"N 157.59'6"W	
OD587	strenuus	11/29/2012	Hawaii: Oahu, Kava Vinui trail, Laie Summit	21° 59'1"N 157.59'6"W	
OD588	junius	1/26/2013	Hawaii: Oahu, BYU-Hawaii campus		

Table 2. Genes sequenced in relationship to specimens

Taxa Code	DDVTUBAF/ DDVTUBAR	12S	16S	COII	H3	Taxa Code	DDVTUBAF/ DDVTUBAR	12S	16S	COII	H3
OD532						OD549					
OD533						OD550					
OD534						OD551					
OD535						OD552					
OD536						OD553					
OD537						OD555					
OD539						OD556					
OD540						OD557					
OD541						OD558					
OD542						OD562					
OD543						OD567					
OD544						OD578					
OD545						OD579					
OD546						OD584					
OD547						OD587					
OD548						OD588					

Genes	Primers
Tuba	DDVubaAF: 5'-GARCCCTACAAYTCYATTCT
	DDVTubAR was 5'- GAACCRGTKGGRCACCAGTC
Tuba nested primers	TH_TubaAF 5'-ACAYTC VGAYTGCTTCATGG
	TH_TubAR 5'-CGGTACARGAKRCAGCAVAT
16S	16Sa: 5'-CGCCTGTTTATC AAAAACAT
	16Sb: 5'-CTC CGGTTGAA CTC AGA TGA
12S	ai: 5'-AACTACGATTAGATACCCTATTAT
	bi: 5'-AAGAGCGACGGGCGATGTGT
H3	AF: ATGGCTCGTACCAAGCAGACVGC
	AR: ATATCCTTRGGCATRATRGTGAC
COII	R-lys: 5'-GAGACCAGTACTTGCTTTCAGTCATC
	9b: 5'-GTA CTTGCTTTCAGTCATCTWATG

Genes	Specimens	bp	Percent
Tuba	24	597	99.985
12S	7	450	99.99
16S	6	562	99.99
COII	3	690	98.6
H3	8	387	99.99

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