CUANDO LA DISPERSIÓN PROMUEVE LA ADAPTACIÓN: DIFERENCIACIÓN GENÉTICA Y MORFOLÓGICA EN EL ODONATO COSMOPOLITA *PANTALA FLAVESCENS*

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Se informa a la Escuela de Postgrado de la Facultad de Ciencias que la Tesis de Doctorado presentada por la candidata

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"No creas lo que tus ojos te dicen. Todo lo que muestran son limitaciones. Mira con tu comprensión, encuentra lo que ya sabes y verás el camino para volar" (Richard Bach, 1970, En: Jonathan Livingston Seagull)

BIOGRAFÍA

Ingrid Alvial nació en Talcahuano en abril de 1981. Realiza sus estudios primarios y secundarios en la enseñanza pública de esa ciudad y prosiguió sus estudios universitarios en la Universidad de Concepción, donde se titula de Biólogo en marzo del año 2005. El año 2009 finalizó sus estudios de Magíster en Ecología en la Universidad de Chile. Entre el año 2010 y 2012 trabajó como profesional en el Centro de investigación CEAZA, y desde el año 2013 a la actualidad, se dedicó de forma íntegra al Doctorado en Ciencias mención Ecología y Biología Evolutiva de la Universidad de Chile.

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RESUMEN

La estructuración de las poblaciones en el espacio-tiempo tiene vital importancia en procesos demográficos y evolutivos. Un patrón de baja estructura genética es comúnmente reportado en especies ampliamente distribuidas. Sin embargo, factores ecológicos locales o barreras geográficas podrían limitar la distribución de las especies y promover una estructura poblacional. *Pantala flavescens* Fabricius 1798, es uno de los insectos más ampliamente distribuidos en el mundo, con presencia en zonas tropicales y subtropicales de todos los continentes, con excepción de la Antártica, e incluso ha alcanzado islas remotas en el Pacífico Sur como Isla de Pascua. Esto lo convierte en un modelo ideal para estudiar los patrones de conectividad genética a gran escala geográfica. Conforme a lo anterior, en esta investigación se estudió la estructura genético-poblacional de *P. flavescens* mediante marcadores mitocondriales y nucleares en localidades continentales e insulares, con el objetivo de evaluar si existe un patrón de diferenciación genética acorde al aumento de la distancia geográfica, y en especial en islas remotas del Océano Pacífico. Como resultado de esta investigación: a) se reportó una alta conectividad y alto flujo génico a lo largo de sitios continentales de Centro y Sudamérica, a pesar de una separación geográfica mayor a 5000 kilómetros, b)

se observó estructura poblacional a larga escala geográfica, con bajos niveles de flujo génico entre sitios continentales de América y sitios insulares del Pacífico Sur y Océano Indico, c) se evidenció una escasa conectividad genética entre sitios continentales e islas del Pacífico Sur con Isla de Pascua, una de las islas más remotas del Océano Pacífico, y d) se reportó menor tamaño y diferente configuración alar en individuos de Isla de Pascua. Estos resultados demuestran que a pesar de la alta capacidad de dispersión y conocida conducta migratoria de *P. flavescens*, una barrera oceánica estaría limitando su flujo génico en islas remotas del Pacífico, como Isla de Pascua. Este aislamiento geográfico estaría afectando la población presente en Isla de Pascua, sugiriendo un efecto fundador y adaptación local debido a la pérdida en la capacidad de vuelo de la especie. Finalmente, este trabajo de investigación nos señala que especies con altas capacidades de dispersión también pueden ser afectadas por procesos de diferenciación y divergencia adaptativa, y de esta forma, nuestros resultados estarían aportando nuevos avances al entendimiento de cómo operan los procesos microevolutivos en condiciones de aislamiento geográfico.

ABSTRACT

Population structure in space and time has an important influence on evolutionary and demographic processes. Genetic structure pattern is commonly reported in highly widespread species; however, geographical or ecological factors can limit species distribution, promoting population structure. *Pantala flavescens* (Fabricius, 1798), is one of the most numerous and widespread odonate on Earth, absent only from Antarctica and most of Europe even reported in remote islands such in the South Pacific Ocean as Easter Island. In this sense, *P. flavescens* is an ideal model for studies about genetic connectivity at a long geograhical scale. This research studied the genetic and population structure in the Cosmopolitan dragonfly, *P. flavescens* through mitochondrial and nuclear markers in continental and insular localities, in order to determine if a genetic differentiation patterns is reflected according increase in geographic distance, and specially in highly remote Pacific islands. Indeed, the results of this research showed: a) high connectivity and gene flow across American continent, in despite of 5000 kilometers between the sampling sites; b) a signal of global genetic differentiation between American continental locations and those in the Indian and South Pacific Oceans was detected, and c) a lack of gene flow at isolated

islands such as Easter Island. In addition, linear and morphometric analysis reported significant differences in wing size and wing shape in individuals of Easter Island. These results show that despite the dispersal ability and migratory behavior of *P. flavescens*, the existence of physical barriers or terrestrial discontinuity may determine genetic structure along their global distribution and limit gene flow towards remote islands of Pacific. The high geographic isolation in Easter Island would be determining founder effect and local adaptation related to a decrease in migratory behavior. Finally, this research provides new insight about how the microevolutionary processes is functioning on cosmopolitan species, and about the effect of geographic isolation to gene flow.

INTRODUCCION GENERAL

Dispersión y conectividad genética

Los cambios en las densidades poblacionales son el resultado, por un lado, de las tasas de natalidad y mortalidad y por otro, de la movilidad, una tendencia innata para el desplazamiento activo o exposición al transporte pasivo (Schneider 1984). Además de sus implicancias en las dinámicas poblaciones, el movimiento determina el nivel de flujo génico entre poblaciones y afecta a procesos como adaptación local, especiación y evolución de rasgos de historia de vida (Dieckmann et al. 1999). Si bien, las causas del movimiento permanecen como una temática de estudio en ecología, éstas incluyen factores distales como la evasión de la competencia entre parientes, disminución de la endogamia y estocasticidad demográfica y ambiental; y factores proximales como la variación entre individuos, condiciones ambientales locales o antecedentes genéticos (Tesson & Edelaar 2013). En la mayoría de las especies, los organismos se mueven hasta su máxima capacidad fisiológica, estando este proceso bajo una fuerte presión selectiva (debido por ejemplo a la evasión de depredadores, la búsqueda y localización de alimento, y al encuentro de una pareja potencialmente reproductiva) que se traduce en estrategias específicas del individuo que le permiten permanecer y adaptarse a nuevos hábitats

(Bilton et al. 2001). A pesar de que dispersión y migración son los conceptos clásicos del movimiento de individuos, ambos términos evocan a condiciones y mecanismos diferentes. Lincoln et al. (1998) definen la dispersión simplemente como "el movimiento hacia el exterior de organismos o propágulos desde su punto de origen o liberación". De acuerdo a Bilton et al. (2001), quienes estudiaron el proceso de dispersión en invertebrados acuáticos, la dispersión podría o no implicar migración, colonización o flujo genético. En este sentido, si la dispersión conduce a una colonización exitosa y ésta es seguida por el entrecruce de generaciones subsecuentes, se dará lugar al flujo genético y a la transferencia de genes de una población a otra. Por otro lado, según Dingle & Drake (2007) la migración involucraría procesos de escape y/o colonización y la definen como una "adaptación específica a escenarios en los cuales los cambios en la calidad del hábitat en diferentes regiones ocurren de forma asincrónica, de tal que manera que el movimiento es obligado y permitiría un acceso temporal/frecuente a los recursos disponibles". En esta vía, los procesos de dispersión y flujo genético se vuelven fundamentales en las dinámicas evolutivas de las especies, ya que, por un lado, permiten la colonización de nuevos hábitats y la amplitud del rango de distribución de las especies, y por otro, disminuyen las probabilidades de extinción y aseguran la persistencia de las poblaciones (Monaghan et al. 2001). Si bien

la dispersión puede reducir fuertemente la cantidad de diferenciación genética entre poblaciones debido al alto movimiento de individuos, también puede conducir al aislamiento reproductivo e independencia evolutiva, cuando la dispersión es superada por barreras geográficas que impiden el movimiento y/o intercambio de individuos (Hughes et al. 2009). Por esta razón comprender los mecanismos de dispersión vs. conectividad entre poblaciones no solo es importante en términos del entendimiento de los procesos ecológicos y demográficos de las especies, sino que también es crítico para predecir la viabilidad y capacidad de recuperación de las especies frente a disturbios antropogénicos y naturales (Tesson & Edelaar 2013).

Dispersión y estructura genética

La estructura genética se define como la distribución azaroza de genotipos en el espacio y tiempo; y refleja las diferencias genéticas que se desarrollan entre los diferentes componentes de una o mas poblaciones (Eguiarte et al. 2007). El aislamiento reproductivo se considera común en las poblaciones naturales y según Fox et al. (2001) se describe como la distancia física que separa a los individuos que impide el apareamiento al azar completo (poblaciones panmicticas) y promueve el desarrollo de una estructura genética espacial. Sin embargo, estructura genética temporal puede también encontrarse cuando subgrupos de una

población se separan en el tiempo. Uno de los ejemplos mas extremos es el observado en el salmón rosado del Pacífico (*Oncorhynchus gorbuscha* Walbaum 1792), donde cohortes de años pares e impares muestran marcadas diferencias genéticas dentro del mismo río (Beacham & McIntosh 2012). La estructura poblacional puede cambiar profundamente el patrón evolutivo de una población, ya que afecta procesos tales como adaptación local, coadaptación, y a largo plazo, especiación (Roderick 1996). Por un lado, entender la adaptación local puede ser crucial para comprender la ecología de una población espacialmente estructurada, y por otro, determinar el grado de aislamiento de una población y/o su estructura genética nos puede dar indicios de que una población esta en proceso de adaptación local. Esto porque la selección de genotipos con una alta adecuación biológica es mas efectiva cuando un grupo esta genéticamente aislado (Fox et al. 2001).

Conforme a lo anterior, el aislamiento del hábitat surge como una de las fuerzas selectivas mas importantes en moldear las estrategias de dispersión de los organismos (Roff 1990, Denno et al. 2001) y, por consiguiente, en definir la estructura genética de las poblaciones. Especies ampliamente distribuidas o cosmopolitas, por lo general han mostrado una escasa estructura poblacional y alta similitud genética a lo largo de su distribución geográfica. Esto porque su capacidad de dispersión permite la alta conectividad y reduce las posibilidades de aislamiento geográfico

y/o reproductivo (Freeland et al. 2003, Nims et al. 2008, Lyons et al. 2012). Sin embargo, hay casos en que barreras geológicas y ecológicas (componentes biológicos) han logrado limitar el flujo genético y/o dispersión, y han dado paso a estructura poblacional (Estoup 1996, Artiss 2004, Voda et al. 2016, Mims et al. 2016). Por ejemplo, Estoup (1996) estimó tasas de diferenciación genética en poblaciones continentales e insulares del abejorro común (*Bombus terrestris* Linnaeus 1758), y encontró alta homogeneidad genética a escala continental, pero fuerte diferenciación genética en poblaciones insulares mediterráneas. De igual manera, Lyons et al. (2012), en base a frecuencias alélicas, describieron una población panmictica para Norte América en la mariposa monarca *Danaus plexippus* Fabricius (1796). La mariposa monarca ha sido conocida por su extrardinaria ruta migratoria entre México y Estados Unidos. No obstante, y similar a los resultados reportados por Estoup (1996), estos autores encontraron diferenciación genética en poblaciones insulares de la mariposa monarca ubicadas en Hawaii y Nueva Zelanda. En este sentido, nuevamente el océano fue una fuerte barrera al flujo genético y ha promovido la diferenciación genética en sistemas insulares.

Insularidad y flujo genético

Las islas oceánicas han sido importantes sistemas de estudio para muchas teorías ecológicas en biología evolutiva y biología de la

conservación; siendo reconocidas como verdaderos laboratorios naturales para estudiar procesos evolutivos (Emerson 2002). Ha sido comúnmente señalado que debido al aislamiento geográfico y, a menudo, su pequeño tamaño, las poblaciones insulares tienen menor diversidad genética que las poblaciones continentales (Hinten et al. 2003; White & Searle 2007; Boff et al. 2014). Sin embargo, los alcances del aislamiento geográfico sobre la diversidad genética y los procesos microevolutivos en sí, siguen siendo un tópico novedoso y extraordinario por descubrir en estudios ecológicos.

La biota en hábitats insulares tiene dos principales vías de llegada: por antiguas conexiones terrestres o extensiones de los actuales márgenes continentales y/o por el movimiento transoceánico que puede ser activo o pasivo dependiendo de las capacidades de dispersión de los organismos (Whittaker & Fernández 2007). A escalas microevolutivas, las islas oceánicas adquieren su biota estrictamente a través de la dispersión, siendo la frecuencia de la dispersión un principal determinante del establecimiento y diferenciación poblacional (Paulay & Meyer 2002). En efecto, la presencia o ausencia de especies en sistema insulares depende de la capacidad de dispersión de las especies, y del establecimiento de un tamaño poblacional lo suficientemente grande como para evitar la extinción y asegurar la persistencia de la población en el tiempo (Stefan 1984). De igual modo, la

diversidad genética dependerá de la razón entre pérdidas al tamaño poblacional al momento y después de la fundación, y de las ganacias al tamaño poblacional asociado a nuevas inmigraciones y mutaciones (deriva genética) (Frankhman 1997).

El aislamiento geográfico y el tamaño poblacional también pueden ser relacionados con la deriva genética, a través del efecto fundador. El término "efecto fundador" fue primero introducido por Mayr (1954) para describir los accidentes genéticos inherentes a un pequeño tamaño poblaiconal, importantes durante la colonización. Para explicar la divergencia de poblaciones periféricas, Mayr (1954), propuso que cambios genéticos extremadamente rápidos podrían suceder en el genoma de poblaciones pequeñas y localizadas que fueron fundadas por unos pocos individuos (deriva genética), y cortarían el intercambio genético con la población original o fuente. Mas aún, presiones selectivas que actúan sobre la nueva población son probablemente diferentes debido a que el ambiente podría presentar diferentes características. En este sentido, el efecto fundador tiene dos importantes consecuencias. Primero, la población fundadora tendrá menor variación genética que la población original desde la cual derivó; y segundo, las frecuencias alélicas en la población fundadora pueden diferir marcadamente desde aquellas de la población original (Brooker 2015). Por esta razón, se espera que las poblaciones localizadas en islas oceánicas remotas muestren

muy baja diversidad genética y marcada diferenciación genética en comparación a sus contrapartes continentales, como resultado de un tamaño poblacional reducido, lo que favorecería además procesos de divergencia adaptativa (Gillespie 2004).

Isla de Pascua o Rapa Nui es una de las islas mas remotas del Oceáno Pacífico, localizada a 3510 kilómetros aproximadamente del continente americano (Mieth & Bork 2005). Campos & Peña (1973), Segers & Dumont (1993) & Dumont et al. (1998), han dado cuenta de una flora y fauna muy empobrecida en la isla y con bajos niveles de endemismo, no solo en comparación al continente, sino que también se muestra depauperada en comparación a otras islas del Pacífico como Hawai (Jordan et al. 2005), Islas Galápagos (Schmitz et al. 2007) o islas de la Polinesia Francesa (Meyer & Claridge 2014). Las razones para ello serían el elevado aislamiento geográfico, su pequeño tamaño (166 km^2), su historia geológica reciente (0.24-0.11 Mya) (Stevenson et al. 2014) y las prácticas agrícolas que generaron fuerte erosión en los suelos y mermaron la vegetación nativa (Hunt 2007). En adición a lo anterior, Rapa Nui posee un clima subtropical con precipitaciones que oscilan entre los 630 mm/año a lo largo de la costa y los 2100 mm/año en su parte más alta; y con una temperatura promedio anual de 21°C, siendo el mes más cálido enero (24°C) y el mes más frío agosto (18°C) (Mann et al. 2008). Todas estas características, sumadas al constante viento que azota la isla, la convierten en un

laboratorio natural ideal para el estudio de procesos ecológicos y microevolutivos.

Planteamiento del problema

Como se señaló anteriormente, Isla de Pascua muestra bajos niveles de endemismos en insectos y casi la totalidad de las especies descritas son cosmopolitas. En efecto, el Odonato de la especie *Pantala flavescens* Fabricius 1758 es el único representante para su grupo que se encuentra presente en la isla. *P. flavescens* a la luz del conocimiento, es el odonato mas ampliamente distribuido, presente en todos los continentes y solo ausente en la Antartica (Dijkstra & Clausnitzer 2014). Varios autores han dado cuenta de las extraordinarias y particulares adaptaciones de *P. flavescens* para recorrer largas distancias de vuelo. Es así como esta especie posee un mecanismo especializado para la oxidación de grasas que utilizan como combustible durante el vuelo (Kallapur & George 1973), y sus alas poseen asombrosas propiedades antifatiga, aerodinámicas y de compensación del viento durante el vuelo (Li et al. 2014, Ren et al. 2013, Srygley & Dudley 2015). Estas capacidades les han permitido ser un reconocido migrante que sigue la abundancia temporal de hábitats dejados por las lluvias alrededor de la zona de convergencia intertropical (Buden 2010, May 2012, Hobson et al. 2012), y ha sido registrado en enjambres de cientos de individuos cruzando océanos (Feng et al. 2006) e

incluso altas altitudes geográficas (Borisov 2009). Además, *P. flavescens* es común en muchas islas del Pacífico como islas de Micronesia (Buden 2010), Fiyi (Marinov & Waqa-Sakiti, 2013), Samoa (Marinov et al. 2013), y otras islas en la Polinesia Francesa (Lieftinck 1966).

Recientemente, Low et al. (2017) reportaron un alto flujo génico y ausencia de estructura genética para *P. flavescens* en Malasia, y anteriormente Christudhas & Mathai (2014) habían descrito un patrón similar a través de la India. Más aún, Troast et al. (2016), basados en secuencias mitocondriales de 49 individuos de *P. flavescens* colectados en Norte América, Sud América y Asia (India y Japón), estimaron altas tasas de flujo génico entre todos los sitios de estudio y sugirieron que esta especie debería ser considerada como una población panmictica global. No obstante, los autores también señalaron que el extremo aislamiento por e.g. en Isla de Pascua, podría ser un factor determinante de divergencia en esta especie. En efecto, Dumont & Verschuren (1993) quienes describieron la forma de vuelo de individuos adultos de *P. flavescens* en Isla de Pascua, anunciaron que los individuos de la isla habrían pérdido su capacidad para volar largas distancias como resultado de una selección hacia una conducta no migratoria. Esto porque se mostraron como pobres voladores (vuelos cortos y a baja altura) y tendían a agregarse y forrajear en áreas alejadas del viento. Mas tarde y en base a mediciones morfológicas, Samways

& Osborn (1998) también sugirieron una diferenciación de los individuos de Isla de Pascua con respecto a poblaciones continentales. Estos autores encontraron diferencias significativas en caracteres morfológicos relacionados al vuelo, con alas asimétricas, mas pequeñas y delgadas en individuos insulares. Ciertamente, el tamaño alar es un carácter determinante para la aptitud del vuelo en insectos (Roff 1990) y una mayor envergadura alar es primordial para facilitar el vuelo a larga distancia (Wakeling & Ellington 1997, Dudley 2000). Varios autores coinciden en que el aislamiento geográfico ha incidido en una perdida paulatina de la capacidad de vuelo en varios taxa de insectos (Roff 1990, Wagner & Liebherr 1992 y Denno et al. 2001), y en aves (Wrigth et al. 2016). Una pérdida en la capacidad de vuelo, típicamente debido a una reducción en la longitud del ala, se explicaría por el alto costo energético que requeriría mantener el aparato de vuelo a expensas de otros rasgos de historia de vida (Zera & Denno 1997, Langelloto et al. 2000). Aun más, Whittaker & Fernández (2007) agregan que, si la acumulación de estructuras morfológicas para mejorar la capacidad de dispersión exige una gran cantidad de energía, un dispersor superior podría terminar como un competidor inferior en condiciones de aislamiento geográfico. En este sentido, aunque buenos dispersores o exitosos migrantes tuvieran una mayor chance para alcanzar lugares remotos como islas oceánicas, estarían bajo riesgo constante de ser

eliminados fuera de ella, lo que puede conducir a dos escenarios: un aumento en la frecuencia de emigración/mortalidad de dicha especie y/o una modificación de sus capacidades dispersivas. Así, la estrategia de dispersión adoptada por una especie debería reflejar un balance entre la ventaja de tener alas para acceder adecuadamente a los recursos y un aumento en el éxito reproductivo de individuos menos voladores (Denno et al. 2001). En este sentido, los cambios morfológicos sugeridos para los individuos de Isla de Pascua, podrían ser el resultado de un aislamiento gradual que resultó en un cambio en las capacidades de dispersión.

Entonces siguiendo estas premisas, podemos esperar que *P. flavescens* muestre alta conectividad genética a lo largo de extensas distancias geográficas dentro de los continentes y en islas a las cuales su capacidad de dispersión les permita acceder (e.g. a lo largo de su ruta migratoria descrita en Islas Maldivas); y que individuos presentes en localidades aisladas como Isla de Pascua, distante a 3000 km del continente, en las cuales las posibilidades de dispersión activa son muy bajas, esta especie mostraría reducida variación genética y frecuencias alélicas muy diferentes al de poblaciones presentes en localidades continentales y otras localidades insulares, según lo que se espera por efecto fundador. Siguiendo lo anterior, el aislamiento geográfico en Isla de Pascua podría conducir a divergencia adaptativa en *P. flavescens*.

Conforme a lo anteriormente planteado, el objetivo general de esta tesis fue evaluar los efectos del aislamiento geográfico sobre la estructura genética (frecuencias alélicas y haplotipicas) y capacidades de dispersión (caracteres morfológicos asociados al vuelo) en una especie ampliamente distribuida, el odonato *Pantala flavescens*. Este trabajo de tesis fue estructurado en tres capítulos u objetivos específicos. El primer capítulo tuvo como objetivo determinar la estructura genético-poblacional de *P. flavescens* a nivel continental, a lo largo de un continuo geográfico entre localidades de Centro América y Sudamérica. El segundo capítulo tuvo como objetivo determinar y comparar la estructura genético-poblacional y diversidad genética entre el continente americano y localidades insulares dentro del Océano Indico (Islas Maldivas) y Pacífico Sur (Isla de Pascua e Isla Tonga). Finalmente, el tercer capítulo de esta tesis tuvo como objetivo evaluar y comparar los rasgos morfológicos y caracteres relacionados al vuelo (tamaño y forma alar) entre localidades continentales (Continente americano) e insulares del Océano Indico (Islas Maldivas) e Islas del Pacífico Sur (Isla de Pascua, Isla Tonga, Isla Fiji e Isla Cook).

Hipótesis de trabajo

A pesar de la alta capacidad de dispersión del Odonato *Pantala flavescens*, barreras naturales como el desierto de Atacama y extensas áreas oceánicas como el Océano Pacífico, pueden limitar su conectividad genética y determinar diferencias morfológicas y genéticas.

Hipotesis específicas:

H1: A escala continental, se espera que las condiciones de extrema aridez en el norte de Chile y sur de Perú, determinen diferencias significativas en la estructura genético-poblacional de *P. flavescens* comparada con poblaciones de Centro América y Sudamérica (Capítulo II).

H2: A mayor escala, se espera que en *P. flavescens* las barreras oceánicas generen mayores limitaciones al flujo genético que las barreras territoriales, encontrándose una menor conectividad entre poblaciones de islas insulares versus continentales, comparado con la conectividad entre poblaciones continentales (Capítulo III)

H3: El elevado aislamiento geográfico y las barreras oceánicas existente entre I. de Pascua con el continente determinan una mayor diferenciación morfológica entre caracteres relacionados al vuelo de *P. flavescens*, presentandose una menor capacidad de vuelo en poblaciones insulares versus aquellas continentales (Capítulo IV).

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CAPITULO I: ESTRUCTURA GENÉTICA A ESCALA CONTINENTAL EN *PANTALA FLAVESCENS*

LACK OF GENETIC STRUCTURE IN *PANTALA FLAVESCENS* AMONG CENTRAL AND SOUTH AMERICAN LOCATIONS (ODONATA: LIBELLULIDAE)

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ABSTRACT

Pantala flavescens is the most widespread odonate on Earth, absent only in Antarctica and a part of Europe. A recent study performed with sequences of mtDNA suggested the presence of one panmictic population of the species at a global scale. However, is necessary complement this information with nuclear and diploid markers that offers more information about the genetic variability of populations and that allow to test panmictic hyphotesis. Here, we

sequenced a fragment of the COI gene and genotyped eight microsatellite loci to analyze the population genetic structure and diversity in individuals collected in Central America (two locations in Costa Rica, separated by 147 km) and two locations in South America (one site in Chile and one site in Peru, separated by 52 km) to evaluate if desert conditions south of equator promove genetic differentiation in *P. flavescens*. The global F_{ST} and AMOVA estimated from COI and microsatellite data showed no evidence of genetic structure despite the >5,000 km of distance between both geographic regions. These results suggest a single panmictic population and extraordinary movement of *P. flavescens* along American continent, corroborating the previous studied performed in this species.

INTRODUCCION

Dispersal is a life-history trait that has important demographic and genetic effects on populations, influencing the dynamics, persistence, distribution, abundance and community structure of populations. Dispersal determines also the level of gene flow between populations, affecting processes such as local adaptation, speciation and the evolution of other life-history traits (Dieckmann et al. 1999). Additionally, dispersal plays a central role in the response of populations and species to ever-increasing global change, including climate change, habitat loss and

fragmentation, and biological invasions (Tesson & Edelaar 2013). Particularly, migration or active long dispersal movements provide a means of avoiding adverse environmental conditions, and can also be a way of exploiting alternative habitats or habitat patches, even when conditions in the resident habitat patch are favorable and likely to remain so (Dingle & Drake 2007).

In insects, migration is an interesting and varied phenomenon. The ability to fly has made insects successful colonists, enabling them to exploit habitats that would not otherwise be accessible and increasing their environmental impact, as well as the flexibility of their response to environmental contingencies, greatly increasing an insect's life history options (Rankin & Burchsted 1992). Among insects, several species of dragonflies, locusts, moths and butterflies are well-known migrants (Chapman et al. 2015). Studies conducted on long dispersal or migratory insects, have revealed little genetic differentiation across distribution sites (e.g., Wennenberg 2001; Daemen et al. 2001; Freeland et al. 2003; Lyons et al. 2012). However, environmental barriers and geographic isolation may limit the genetic structure of widely distributed populations (Artiss 2004). A common, known migrant is the monarch butterfly, *Danaus plexippus* (Linnaeus, 1758). Lyons et al. (2012) studied the genetic structure of North American migratory monarch populations and non-migratory populations in Hawaii and New Zealand. They reported lack of

genetic structure and one admixed population along North America, but they observed genetic divergence among the monarch populations of Hawaii and New Zealand. In this context, despite being widely distributed in North America, greater geographic distance seems to reduce levels of gene flow in this long dispersal insect as expected by the effect of isolation by distance. *Libellula quadrimaculata* (Linnaeus, 1758) is a dragonfly commonly distributed throughout Europe, Asia, and North America. Artiss (2004) studied the phylogeography of this species among these three continents. The study found three monophyletic lineages equivalent to populations in Asia (Japan), Europe and North America. Similarly, to the *D. plexippus*, for *L. quadrimaculata* oceans and larger geographic distances are barriers to gene flow, separating populations between continents.

Another well-known migrant, found throughout the tropics and extending well into the northern temperate zones of America and Asia, is *Pantala flavescens* (Fabricius, 1798) (May 2012). It is one of the most numerous and widespread odonate on Earth, absent only from Antarctica and most of Europe. It migrates widely, following the temporary abundance of habitat provided by rains (Dijkstra & Clausnitzer 2014). In the tropics, *P. flavescens* is adapted to follow prevailing winds of the Intertropical Convergence Zone (ITCZ), where rainy conditions and consequent formation of temporary ponds are likely (Corbet 1999). A multi-

generational migratory circuit for *P. flavescens*, covering about 14,000-18,000 km between East Africa and the Indian subcontinent, as outlined by Anderson (2009), and verified by Hobson et al. (2012). The first study conducted to explore genetic similarity of this species at a large scale (Troast et al. 2016) included sequences from North America (Canada and United States), South America (Guyana), Japan and India. Their results suggested high rates of gene flow among all included geographic regions; providing the first significant evidence that *P. flavescens* should be considered a global panmictic population. However, in view of the climatic and ecological gradient that exists between Central and South America, and especially desert habitats south the Equator; a study including samples of *P. flavescens* from tropical and subtropical latitudes would be important to determine whether there are genetic differences between populations and, consequently, gene flow limitation. In this context, the objective of the present study was to evaluate the population genetic structure of *P. flavescens* in two Central American locations (Costa Rica) and two locations in South America (Chile and Peru), both regions separated by 5,000 kilometers. We sequenced a fragment of the mitochondrial gene cytochrome oxidase I and genotyped individuals for eight microsatellite loci in order to evaluate if desert conditions south of equator are determining differences in genetic diversity and haplotype/allele frequencies between regions

and among locations within regions. The use of both types of markers together allows more robust interpretations about recent and past history of genotypes in the natural populations, because the rapid evolution of microsatellites offers a valuable pool of genetic variation that may be particularly useful when other methods show insufficient variability (Roderick 1996). In this sense, our study provided new insights about the extent of dispersal and population connectivity in locations separated by more than five thousand of kilometers in *P. flavescens*.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 87 adults of *Pantala flavescens* were collected from four localities across its range in Central (two locations in Costa Rica) and South America (one location in Chile and one location in Peru) (Table I, Figure 1). All specimens were collected using an entomological aerial net and preserved in 95% ethanol until DNA extraction and were dissected and 1 mm^3 of thoracic muscle was obtained from each for the DNA extraction using the salt method described by Aljanabi & Martinez (1997). A fragment of the mitochondrial gene Cytochrome Oxidase I (COI) was selected for sequencing, and additionally, eight microsatellites described for the species by Cao et al. (2015) were amplified.

Table I. General information on sampling sites of *Pantala flavescens* individuals used for this study.

Sampling site	Geographic coordinates	Altitude [ma.s.l.]	Sample size
Santa Rosa National Park, Guanacaste, Costa Rica	10°50'N, 85°37'W	290	17
Campus of University of Costa Rica, Heredia, Costa Rica	10°00'N, 84°06'W	1174	22
Municipal Park of Tacna, Tacna, Peru	18°06'S, 70°20'W	260	19
Azapa Valley, Arica, Chile	18°31'S, 70°10'W	269	29

Figure 1. Sampling locations of *Pantala flavescens*, located in Central and South America.

Mitochondrial DNA sequencing and data analysis.

A fragment of the COI gene was amplified for all individuals using the primers C1-N-2191 (5'-CCGGTAAAATTAAAATATAAACTTC-3') and C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3') described for insects by Simon et al. (1994). Our PCR protocol contained 2 µL of DNA (50 ng/µL), 2.5 µL of 10XPCR buffer (Invitrogen), 1.6 µL of $MgCl_2$ (50mM) (Invitrogen), 2 µL of dNTPs (2.5 mM) (Invitrogen), 14.6 µL of water, 1 µL of forward and reverse primers (50 ng/µL) and 0.3 µL of TaqDN A polymerase (Invitrogen). The procedure started at 95°C for 2 min, followed by 35 cycles of 95°C for 30 s, 50°C for 30s, 72°C for 30 s, and a final step of 72°C for 5 min. Amplifications were verified on a 1% agarose gel and sequencing in both directions was performed at Macrogen Inc. Sequences were checked using PROSEQ software (Filatov 2002) and aligned using MULTALIN software (Corpet 1998). Genetic variability within and among localities was assessed by describing the number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (Hd), average number of differences (k) and nucleotide diversity (Pi) using DNASP 4.9 (Rozas et al. 2003). To visualize mutational steps and differences in haplotype composition, a haplotype network was constructed using the median joining algorithm method in POPART software (Leigh & Bryant 2015). To detect possible differences among sites, the global F_{ST} and pairwise F_{ST} were estimated with ARLEQUIN software (Excoffier &

Lischer 2010). In order to detect a possible phylogeographic pattern, the average gene diversity within populations (H_S), total gene diversity across all populations (H_T), the number of substitution types (N_{ST}), and genetic differentiation over populations (G_{ST}), were calculated using PERMUT & CPSRR 2.0 (Ponds & Petit 1996). Further, to detect differences among regions (Central vs. South America) and differences between sites within regions, an analysis of molecular variance (AMOVA) was performed in ARLEQUIN Version 3.5 (Excoffier & Lischer 2010); the significance tests were conducted using 1,000 permutations and computing conventional F-Statistics from haplotype frequencies.

Microsatellite genotyping and data analysis

We genotyped all the individuals for the eight microsatellite loci described for *P. flavescens* by Cao et al. (2015). The PCR conditions used were as follows: 1.5 µL of DNA (50 ng/µL), 1.5 µL of $MgCl_2$ (50 mM) (Invitrogen), 0.5 µL of BSA 1%, 2 µL of 10 x PCR buffer (Invitrogen), 2 µL of dNTPs (2.5 mM) (Invitrogen), 4.15 µL of water, 0.5 µL of each primer, 0.5 of M13 and 0.15 µL of Taq polymerase (Invitrogen). For each PCR, we used the touchdown method described by Beheregaray & Sunnucks (2000) starting 10°C above to the annealing temperature of each microsatellite. PCR products with fluorescent primers (M13) were run in an ABI-PRISM 3010 sequencer (Perkin Elmer) using 500 ROX Size Standard (Applied

Biosystems) at the Pontificia Universidad Católica de Chile. The allelic data matrix was built using GENEMARKER software (Softgenetics Inc). MICROCHECKER software (Van Oosterhout et al. 2004) was used to identify possible genotyping mistakes and the presence of null alleles in the allelic data. The number of alleles per locus, and expected (H_E) and observed (H_O) heterozygosity were estimated using GENETIX software (Belkhir et al. 2000). This same software was used to test for linkage disequilibrium and for departures from Hardy Weinberg Equilibrium (HWE). 5,000 permutations on mono locus genotypes and 5,000 allele permutations were used, respectively. The allelic richness (AR) and the inbreeding coefficient (F_{IS}) were estimated with FSTAT software (Goudet 1995). To ensure that the analysis was performed with unrelated individuals, the r_{xy} index (Queller & Goodnight 1989) was estimated with IDENTIX software (Belkhir et al. 2001). 1,000 permutations were generated to build the simulated distribution. To detect possible differences among sites, global F_{ST} was estimated with GENETIX software. As with the COI gene, an AMOVA was conducted in ARLEQUIN Version 3.5 (Excoffier & Lischer 2010) to detect differences among regions (Central vs. South America) and differences between sites within regions. As complement to the F_{ST} index, we used a Bayesian approximation with STRUCTURE software (Pritchard et al. 2000) to infer the most probable number of populations. For this analysis, we used the admixture model and

the correlated allele frequencies model. The procedure was run 5 times for each K estimation (from K = 1 to K = 5) with a burn-in and an after burn-in of 200,000 MCMC each.

RESULTS

Mitochondrial DNA

The final COI alignment included 87 sequences of 444 bp. There were 35 polymorphic sites in the alignment, revealing a total of 37mutations, with no insertions or deletions. These polymorphic sites defined a total of 27 haplotypes. Haplotype sequences were deposited in the NCBI GenBank with accession numbers KY200583 to KY200609. Total haplotypic diversity (Hd) was 0.898 (variance = 0.0004). The number of polymorphic sites (S) ranged from 14 (Arica) to 18 (Tacna and Guanacaste); the average number of differences (k) ranged from 2.995 (Arica) to 4.220 (Guanacaste); and the number of haplotypes (h) ranged from 8 (Arica) to 13 (Guanacaste) (Table II). Two haplotypes were present in all sampling sites (Figure 2). The most common haplotype was found in 21 individuals (24 % of the total individuals) and the second most common haplotype was found in 16 individuals (18 %). Of the remaining haplotypes, 10 were found in a minimum of two individuals and 15 were found in a single individual and therefore at a single location. In this way, the haplotype network did not show an apparent geographical structure.

Table II. Sample sizes and genetic characterization of nuclear and mitochondrial markers in the four sampling sites of *Pantala flavescens* individuals used for this study. N - number of individuals; h - number of haplotypes; S - number of segregating sites; Hd - haplotype diversity; k - average number of differences between pairs of haplotypes; Pi - nucleotide diversity; H_E- expected heterozygosity; H_O - observed heterozygosity; Ar - allelic richness; F_{IS} - inbreeding coefficient. (CR= Costa Rica)

Sampling site	N COI/ms	COI					Microsatellites			
		h	S	Hd	k	Pi	H_E	H_O	Ar	F_{IS} /p value
Guanacaste, CR	17/17	13	18	0.97	4.22	0.009	0.50	0.48	2.76	0.070 */0.154*
Heredia, CR	22/21	12	17	0.91	3.45	0.007	0.48	0.51	2.67	-0.038 */0.279*
Tacna, Peru	19/17	10	18	0.87	3.86	0.009	0.47	0.51	2.51	-0.031 */0.371*
Arica, Chile	29/29	8	14	0.79	2.99	0.007	0.49	0.58	2.61	-0.153 */0.004*

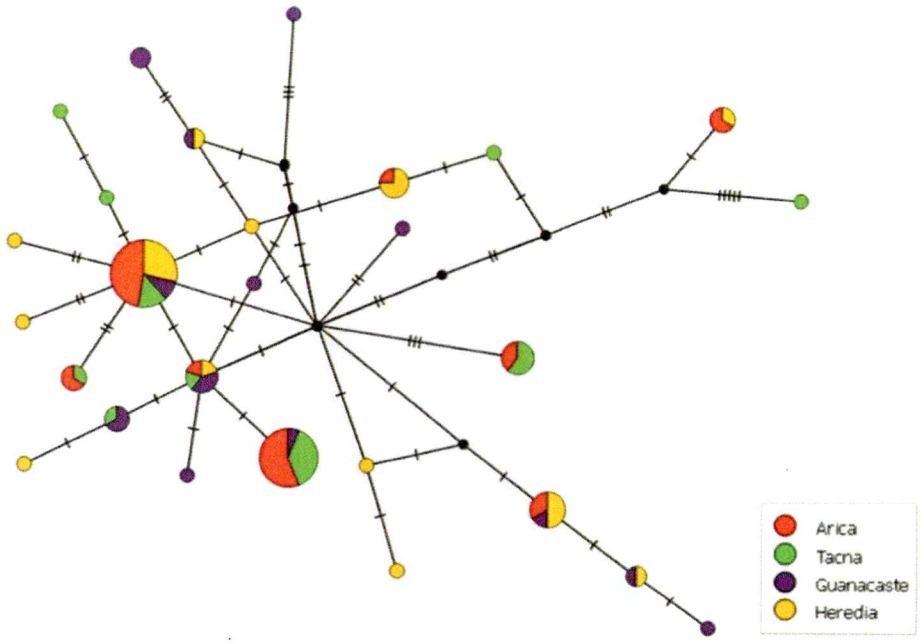

Figure 2. Median Joining (COI) haplotype network of Central (Guanacaste and Heredia) and South (Arica and Tacna) America. The area of each circle is proportional to the number of individuals. Each perpendicular line between haplotypes indicates a single mutational step.

Genetic diversity analysis revealed that total genetic diversity (H_T= 0.916, s.e. = 0.0355) was similar to the average gene diversity for all locations (H_S= 0.886, s.e. = 0.0373), which suggests no population differentiation across the distributional range. The analysis showed that total N_{ST} (0.038) was not significantly higher than G_{ST} (0.033), which indicates a lack phylogeographic structure (p = 0.655). Pairwise F_{ST} based on haplotype frequencies are showed in Table III. These comparisons showed significant differences (*p* value <0.05) among Arica (northern Chile and Guanacaste (northern Costa Rica), and Tacna (southern Peru) and Heredia (southern Costa Rica). Finally, although, the AMOVA revealed that 4.92% of the variance could be explained by the region factor (Table IV), the permutation analysis did not show evidence of genetic differences among regions (F_{CT}= 0.045, p = 0.330). Nor did the same analysis find genetic differences between sites within regions (F_{SC}= 0.004, p = 0.326).

Table III. Matrix of pairwise F_{ST} values (*p* values) between populations based on mitochondrial markers. Significant values to *p* <0.05.

	Arica	Tacna	Guanacaste
Arica	0		
Tacna	-0.002 (0.41)	0	
Guanacaste	0.060 (0.016)	0.028 (0.09)	0
Heredia	0.043 (0.05)	0.064 (0.007)	0.0103 (0.257)

Table IV. Results of the Analysis of Molecular Variance (AMOVA) of the COI gene for *Pantala flavescens* populations and geographical groups from Central and South America in this study.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	1.372	0.02070	4.52
Among populations within groups	2	0.952	0.00185	0.40
Within populations	83	36.274	0.43703	95.08
Total	86	38.598	0.45965	

Microsatellite analysis

General features of the eight microsatellites analyzed are shown in Appendix I. The mean number of alleles per locus per sample (observed allelic diversity) ranged from 2.51 (Tacna) to 2.76 (Guanacaste), the heterozygosity ranged from 0.47 (Tacna) to 0.50 (Guanacaste), the observed heterozygosity ranged from 0.48 (Guanacaste) to 0.58 (Arica), and the inbreeding coefficient ranged from -0.031 (Tacna) to 0.071 (Guanacaste), with a significant value in Arica (Chile) related to excess of heterozygosity observed in two of the loci SSR10 and SSR15 (Table II). The analysis by locus showed that all loci were polymorphic. The mean allele richness varied from 1.936 (SSR2) to 3.385 (SSR14) with a mean of 3.38 alleles per locus. Departures from HWE were detected in one (SSR2) out of the eight loci ($p < 0.01$) in only one sampling site (Arica). The mean r_{xy} values estimated for the groups of individuals collected in the four sites showed no statistical evidence (Permutation, $p > 0.05$) for highly related

individuals; suggesting that samples are composed of unrelated individuals. The AMOVA revealed that only 0.04% of the variance was explained by the region factor (Table V) and the permutation analysis showed no evidence of genetic differences among regions (F_{CT}= -0.004, p = 0.657). The same analysis found no genetic differences between sites within regions (F_{SC}= -0.008, p = 0.577).

Table V. Results of the Analysis of Molecular Variance (AMOVA) for *Pantala flavescens* populations and geographical groups from Central and South America in this study using microsatellite loci.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	0.267	-0.00447	0.04
Among populations within groups	2	1.326	-0.00793	0.06
Within populations	164	161.074	0.98216	99.9
Total	167	162.667	0.96975	

The Global F_{ST} showed no significant differences (F_{ST}= 0.0014, p = 0.3682) and Structure software showed that K = 1 had the highest likelihood, L (K) mean value and the lowest standard deviation (data not shown).

DISCUSSION

In this study, we used both the COI gene and microsatellite loci to infer the genetic structure of the most widespread odonate on Earth, *Pantala flavescens*. Microsatellites and mtDNA gave congruent results with no signals of genetic structure in Central

and South America, separated by >5000 km. These results corroborate the results of Troast et al. (2016), who suggested that *P. flavescens* may have a predominantly global panmictic population rather than a series of geographically isolated populations. Few studies have analyzed the haplotype/allelic diversity in other species of anisoptera covering broad geographical regions (Artiss et al. 2001; Freeland et al. 2003; Christudhas & Mathai 2014; Troast et al. 2016). Freeland et al. (2003) reported relatively high haplotype diversity in the absence of phylogeographic structuring in the migratory dragonfly, *Anax junius* (Drury, 1773). However, the proportion of individuals with unique haplotypes in the South (southern U.S.A. and Mexico) was significantly greater than that in the North (Canada and northern U.S.A.), which can be explained because *A. junius* is centered in the tropics (Corbet 1999) and therefore may have expanded into the northern part of its range only recently. Similar to *A. junius*, the lack of geographic structuring in American populations of *P. flavescens* may be explained by the mobility of this species, where migration is known to be a common event (Wojtusiak 1974; Anderson 2009; Hobson et al. 2012; May 2012). Overall, highly mobile insects must be adapted to a wide range of environmental conditions, but *P. flavescens* seems to have this capability at an unprecedented range.

In concordance with the results of Freeland et al. (2003), we also observed presence of more unique haplotypes in the sites located

in Central America (Costa Rica) than from sites in South America (Arica and Tacna). Similarly, we found that sites located in Costa Rica showed higher values of haplotypic diversity and allelic richness than in Arica and Tacna. Effectively, Arica showed the lowest values of number of haplotypes and genetic diversity, despite the greater sample size. This scenario of differences in freshwater disponibility could be the reflection of different times of larval development among tropical and subtropical latitudes (Hawking & Ingram 1994). The stable and predictable conditions in the North (Central America) are favoring the larval development time of resident and migratory populations, and this may help to maintain genetic variation at this site. Additionally, the high rainfall and consequently, the diversity of aquatic habitats, in tropical regions promote a high diversity of tropical odonates and greater probability of speciation events (Kalkman et al. 2008). On contrary and similar to other sites in desert areas, in Arica and Tacna, the extreme hydrothermal conditions and high solar radiation restrict the activity of adult dragonflies, while high summer temperatures and insufficient aeration in many reservoirs hamper the development of their larvae (Borisov 2006). In arid regions, the oviposition along the migration route at long distances might be detrimental because of the higher likelihood that water bodies might dry up before larval development is complete (May 2012). In this type of habitat, the adults could

contain this risk by mating and ovipositing in pools once a region of active rain is reached. However, in low desert climates sites, such as Arica and Tacna, we did not find larvae or exuviae during our five visits to these sampling sites. This may suggest an obligate migratory behavior of adults, making use of convergent winds to transport them to places where rain is falling (Corbet 1999).

Vast geographic distances and oceans could be potential barriers to genetic connectivity in widely distributed species (Artiss 2004; Lyons et al. 2012). However, distances >5000 km and desert climate in South American locations, are not barriers to dispersal of *P. flavescens*. Finally, although low levels of genetic differentiation accompanying absence of geographic structure were reported among Central and South American locations, new studies covering more sites along the global distribution of *P. flavescens*, especially including populations from very isolated sites, e.g., oceanic islands, are necessary to solidify and prove the hypothesis of a panmictic population.

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Appendix I. Summary of genetic values by microsatellite in four populations of *Pantala flavescens* from Central and South America. N - Sample size; Na - number of alleles; Ar- allelic richness (Ar), H_E- expected heterozygosity (H_E), H_O- observed heterozygosity (H_O), F_{IS} inbreeding coefficient (* $p<0.05$, ** $p<0.01$); r_{xy}- relatedness index, estimated per sampling site (in bottom line).

Sampling locations				
Loci	Guanacaste, Costa Rica	Heredia, Costa Rica	Arica, Chile	Tacna, Peru
SSR6				
N	16	21	29	14
Na	4	3	3	2
Ar	2.673	2.254	2.330	1.979
H_E	0.363	0.373	0.380	0.336
H_O	0.312	0.285	0.413	0.285
F_{IS}	0.171	0.256	-0.070	0.187
SSR28				
N	16	21	29	16
Na	4	4	4	4
Ar	3.340	2.930	3.124	2.750
H_E	0.638	0.566	0.629	0.556
H_O	0.562	0.619	0.724	0.562
F_{IS}	0.150	-0.067	-0.132	0.021
SSR9				
N	16	20	26	16
Na	4	4	4	3
Ar	2.749	2.595	2.823	2.859
H_E	0.527	0.486	0.568	0.552
H_O	0.437	0.600	0.615	0.437
F_{IS}	0.201	-0.209	-0.063	0.239
SSR14				
N	15	16	24	6
Na	5	5	5	3
Ar	3.448	3.906	3.189	3.000
H_E	0.615	0.642	0.566	0.652
H_O	0.600	0.437	0.333	0.500
F_{IS}	0.059	0.347	0.429**	0.318
SSR10				
N	17	21	28	16
Na	3	2	3	3
Ar	2.353	2.000	2.214	2.617
H_E	0.527	0.495	0.515	0.554
H_O	0.705	0.904	0.964	0.875
F_{IS}	-0.310	-0.818**	-0.864**	-0.555**
SSR24				
N	17	21	29	16
Na	4	5	5	4
Ar	3.091	3.168	3.332	2.949

H_E	0.558	0.480	0.577	0.443
H_O	0.647	0.523	0.620	0.500
F_{IS}	-0.128	-0.065	-0.057	-0.095
SSR15				
N	13	19	27	13
Na	3	3	2	2
Ar	2.456	2.531	1.999	2.000
H_E	0.446	0.480	0.475	0.488
H_O	0.538	0.526	0.778	0.692
F_{IS}	-0.166	-0.068	-0.625**	-0.384
SSR2				
N	16	20	21	11
Na	2	2	2	2
Ar	1.977	1.960	1.887	1.922
H_E	0.341	0.320	0.244	0.235
H_O	0.062	0.200	0.190	0.272
F_{IS}	0.827**	0.396	0.245	-0.111
r_{xy}	-0.054	-0.045	-0.032	-0.082

CAPÍTULO II: ESTRUCTURA GENÉTICA A GRAN ESCALA GEOGRÁFICA EN *PANTALA FLAVESCENS*

CONTINENTAL VERSUS OCEANIC CONNECTIVITY DETERMINE POPULATION STRUCTURE IN A HIGHLY DISTRIBUTED DRAGONFLY SPECIES, *PANTALA FLAVESCENS*

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ABSTRACT

How populations are structured in space-time have important implications for demographic and evolutionary processes of species. Whereas, low levels of genetic structure are expected in highly widespread species, geographical and/or ecological factors can limit species distributions, promoting population structure. The wandering glider, *Pantala flavescens* Fabricius, is one of the most widespread insects on Earth, absent only in Antarctica and a part of Europe and even reported in remote islands such as Pitcairn Island or Easter Island in the South Pacific Ocean. The presence

of *P. flavescens* in remote islands may occur with a reduction in the connectivity in these populations. In order to determine the population structure of *P. flavescens*, 220 individuals were collected from seven locations, four in continental America and three on oceanic Islands. COI sequences and microsatellites loci were used to characterize genetic diversity, genetic structure and reciprocal migration in the areas studied. Both COI and microsatellites showed a clear genetic structure with low levels of migration of *P. flavescens* between the American and Island locations. American locations did not show genetic structure even in locations separated by 5000 km. Conversely, insular locations showed genetic structure, low migration rates, and low genetic diversity. Interestingly, Easter Island, located at the middle of the southern Pacific Ocean, showed lowest values of genetic diversity and highest values of genetic differentiation, as a result of the distance from the other locations. These results show that despite the dispersal ability and migratory behavior of *P. flavescens*, the existence of physical barriers or terrestrial discontinuity can determine genetic structure along their global distribution. This study provides new insight about the effect of isolation on ecology and microevolutionary processes in cosmopolitan species.

Keywords: Genetic structure, COI, Microsatellites, migration, panmictic populations

INTRODUCTION

Migratory processes have consequences on population dynamics, determining gene flow between populations and affecting local adaptation, speciation and the evolution of life history traits (Dieckmann et al. 1999). In widespread species, genetic studies have indicated a higher genetic similarity or a lack of genetic structure along their geographical distribution, with absence of local adaptation (Freeland et al. 2003, Nims et al. 2008, Lyons et al. 2012). However, geological, climatological and ecological factors, may limit the genetic structure in widespread distribution populations (Estoup 1996, Artiss 2004, Voda et al. 2016, Mims et al. 2016). In this sense, habitat isolation arises as one of the most selective forces in shaping the natural dispersal of organisms (Roff 1990, Denno et al. 2001). For example, Estoup (1996) evaluated genetic differentiation among continental and insular populations of the bumble bee (*Bombus terrestris* L.), demonstrating genetic homogeneity and large gene flow at the scale of the European continent, but strong and significant genetic differentiation in insular Mediterranean populations. This divergence among populations was associated with founder effects and genetic drift; where the sea constitutes a geographical barrier for significant migration events and hence genetic flow from the continent to the islands. In the same way, Lyons et al. (2012) described an admixed or panmictic population of monarch

butterflies using microsatellites markers (*Danaus plexippus* L.) across eastern and western North America. However, these authors also identified genetic divergence among monarch butterflies from Hawaiian Islands, New Zealand and the North American populations, with a pattern of decreasing in genetic diversity and allelic richness with increasing distance from North America. Therefore, despite the large and nearly cosmopolitan distribution of this species, likely attributable to its dispersal tendencies, gene flow is limited across large geographic distances, and especially in oceanic islands, where barriers have isolated populations of the species.

In this context, oceanic islands are ideal models to evaluate the effects of geographic isolation on ecological and evolutionary process in cosmopolitan species. In fact, oceanic islands have been important study systems for ecologists, evolutionary and conservation biologists and are widely recognized as natural laboratories, for studying evolution due to their discrete geographical nature and diversity of species and habitats (Emerson 2002). Island populations are often small and isolated, and therefore experience increased levels of inbreeding and a greater impact of genetic drift (Furlan et al. 2012). Insular populations tend to have lower levels of genetic diversity and fitness than counterparts found in mainland populations (e.g. Hinten et al. 2003, White & Searle 2007, Boff et al. 2014).

Pantala flavescens Fabricius or the "wandering glider" is the most widespread odonate on Earth, absent only in Antarctica and a part of Europe (Dijkstra & Clausnitzer 2014). It migrates widely, following the temporary abundance of habitat provided by rains and it is among the odonates most often found in oceanic islands and in swarms well out to sea (Buden 2010, May et al. 2012). Recently, Troast et al. (2016) based on mitochondrial sequences of 49 individuals collected in North America, South America and Asia, reported high rates of gene flow among these sites and suggested that *P. flavescens* should be considered a global panmictic population. Low et al. (2017) reported high gene flow and minimal population structure in populations of *P. flavescens* in Peninsular Malaysia, while Christudhas & Mathai (2014) described similar pattern across India. Following these premises, we expect admixture or panmictic populations of *P. flavescens* along vast geographic distances within continents. Nevertheless, the presence of *P. flavescens* in remote pacific islands may presume low dispersal and effects of isolation in the genetic differentiation of these populations, as seen in other cosmopolitan species, *B. terrestris* or *D. plexippus*. Indeed, *P. flavescens* inhabit Easter Island or Rapa Nui (Samways & Osborn 1998, Dumont & Verschuren 1991), the most remote inhabited island in the world (Mieth & Bork 2005), located 3510 km from the American continent. Rapa Nui has relatively depauperate freshwater biota reflecting its young

geological age, small size, great isolation, and importantly its biotic losses in recent ecological history (Segers & Dumont 1993). Based on this information, we propose that the individuals of *P. flavescens* present in Easter Island would have low genetic diversity and few exchanges of individuals with the rest of populations around the world. Based on these premises, the main goal of this research was to determine the population genetic structure of *P. flavescens* present in continental (Central and South America) and insular locations (Easter Island, Tonga island and Maldives island) through nuclear and mitochondrial genetic markers and to evaluate population connectivity in these locations. To our knowledge, this is the first study employing genetic markers of different origins (mitochondrial and nuclear markers) and different evolutionary properties to elucidate the population genetic structure at a large scale in this widespread dragonfly. The results will give us new insights about the dispersal capability and barriers to gene flow for this widely-distributed species.

MATERIALS AND METHODS

Sample collection and DNA extraction

A total of 220 adult specimens of *Pantala flavescens* were obtained from seven locations across Central and South America, the Maldives Islands and two other islands in the South Pacific (Table I, Figure

1) from March 2015 to September 2016. All specimens were collected using an entomological net and preserved in 95% ethanol until DNA extraction. Individuals were dissected and 1 mm^3 of thoracic muscle was obtained from each for the DNA extraction using the salt extraction method described by Aljanabi & Martinez (1997). In order to detect geographic genetic structure, a partial region of the Cytochrome Oxidase I (COI) was amplified, as it is recognized as one of the most variable regions of mitochondrial DNA in dragonflies (Freeland et al. 2003). Additionally, eight microsatellites described by Cao et al. (2015) for *P. flavescens* were amplified.

Table I. Sampling locations, date of collection and number of specimens analyzed.

Locality, country	Abb.	Geographic coordinates	Date of collection	Number of samples
Maldives Islands	MAL	04°10'N, 73°30'E	11/2015	11
Nukualofa, Tonga	TON	21°07'S, 175°13'W	09/2016	46
Easter Island, Chile	EIS	27°07'S, 109°22'W	08/2015	82
Guanacaste, Costa Rica	GUA	10°50'N, 85°37'W	04/2016	17
Heredia, Costa Rica	HER	10°00'N, 84°06'W	04/2016	22
Tacna, Peru	TAC	18°06'S, 70°20'W	03/2015	19
Arica, Chile	ARI	18°31'S, 70°10'W	03/2015	29

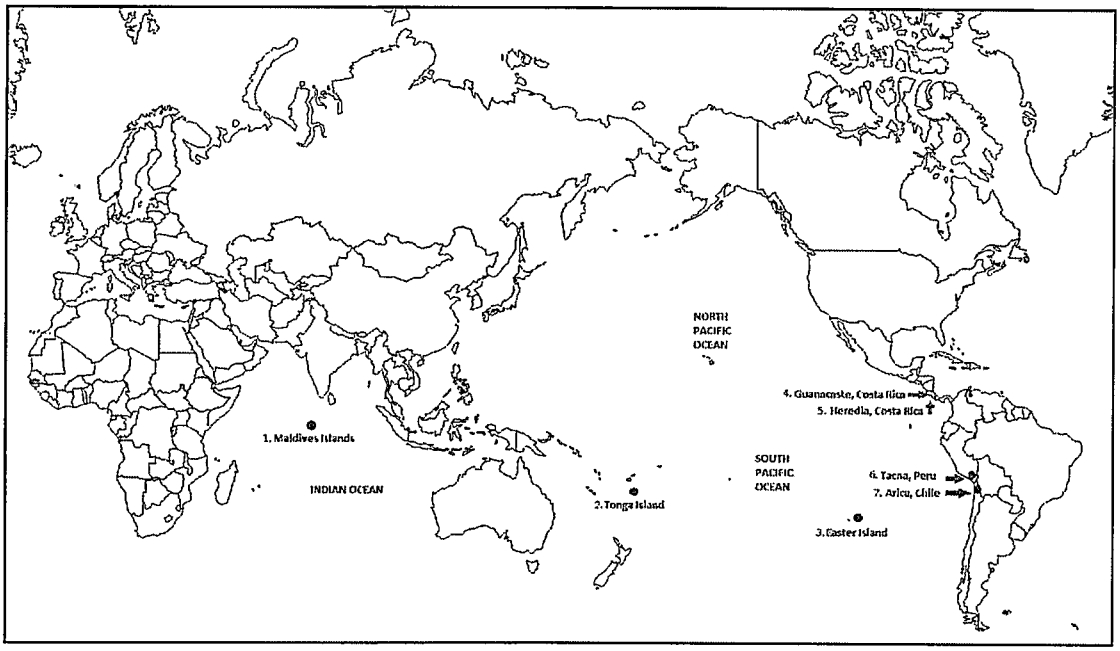

Figure 1. *Pantala flavescens* Sampling sites located in Indian Ocean, South Pacific Ocean and the coast of the American continent.

COI analysis

A portion of the Mitochondrial COI gene was amplified for the specimens sampled (Table I) using the universal primers N2191 (5′-CCGGTAAAATTAAAATATAAACTTC-3′) and J1718 (5′-GGAGGATTTGGAAATTGATTAGTTCC-3′) described by Simon et al. (1994). PCR reactions were performed following a modified protocol of Simon et al. (1994) that contains 2 µL of DNA (50 ng/µL), 2.5 µL of 10XPCR buffer (Invitrogen), 1.6 µL of $MgCl_2$ (50mM) (Invitrogen), 2 µL of dNTPs (2.5 mM) (Invitrogen), 14.6 µL of water, 1 µL of forward and reverse primers (50 ng/µL) and 0.3 µL of Taq polymerase (Invitrogen). The PCR procedure started at 95°C for 2 min, followed

by 35 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 30 s, and a final step of 72°C for 5 min. Amplifications were verified on a 1% agarose gel and sequencing in both directions was performed in Macrogen Inc. Sequences were aligned using MULTALIN online software (Corpet 1998) and were checked using PROSEQ software (Filatov 2002). Molecular diversity indices, such as number of polymorphic sites (S), haplotype diversity (h), and nucleotide diversity (π), were calculated using DNASP 4.9 software (Rozas et al. 2003). Genealogical relationships among haplotypes were further assessed using a median joining algorithm network constructed in POPART software (Leigh & Bryant 2015).

To test for the presence of phylogeographic structure within species, the average gene diversity within populations (H_S), total gene diversity across all populations (H_T), the number of substitution types (N_{ST}), and genetic differentiation over populations (G_{ST}) were calculated using PERMUT (Ponds & Petit 1996). PERMUT computes measures of diversity and differentiation from haploid population genetic data, testing whether the differentiation and diversity measures differ from the equivalent measures that do not consider the distances between haplotypes. If N_{ST} is higher than G_{ST}, this indicates the presence of a phylogeographic structure (Ponds & Petit 1996), i.e. closely related haplotypes are more often found in the same geographical area than would be expected by chance. Further, population pairwise

F_{ST} values were estimated using ARLEQUIN 3.5 software (Excoffier & Lischer 2010), and the significance was tested using 1,000 permutations and computing conventional F-Statistics from haplotype frequencies.

Microsatellites analysis

Samples from the seven locations were genotyped at eight microsatellite loci originally described by Cao et al. (2015) using a modified PCR protocol described by the same authors. The PCR conditions used were as follows: 1.5 µL of DNA (50 ng/µL), 1.5 µL of $MgCl_2$ (50 mM) (Invitrogen), 0.5 µL of BSA 1%, 2 µL of 10 x PCR buffer (Invitrogen), 2 µL of dNTPs (2.5 mM) (Invitrogen), 4.15 µL of water, 0.5 µL of each primer, 0.5 of M13 and 0.15 µL of Taq polymerase (Invitrogen). For the PCR procedure, a touchdown starting 10°C above the annealing temperature of each microsatellite was used. PCR products were run in an ABI-PRISM 3010 sequencer (Perkin Elmer) using 500 ROX Size Standard (Applied Biosystems) at the Pontificia Universidad Católica de Chile. The allelic data matrix was obtained using GENEMARKER software (Softgenetics Inc). MICROCHECKER software (Van Oosterhout et al. 2004) was used to identify possible genotyping mistakes and the presence of null alleles in the allelic data. The number of alleles per locus, expected (H_E) and observed (H_O) heterozygosity were estimated using GENETIX software (Belkhir et al. 2000). This same

software was used to test for linkage disequilibrium and for departures from Hardy Weinberg Equilibrium (HWE). 5,000 permutations on mono locus genotypes were used. Allelic richness (A_R) and the inbreeding coefficient (F_{IS}) were estimated with FSTAT software (Goudet 1995). To ensure that the analysis was performed with a significant number of unrelated individuals, the r_{xy} index (Queller & Goodnight 1989) was estimated with IDENTIX software (Belkhir et al. 2001). IDENTIX used multilocus data to estimate the relatedness between pairs of individuals within each locality, compared with a distribution of simulated genotypes constructed after an allele permutation among individuals. 1,000 permutations were generated to build the simulated distribution. Population structure was evaluated using three different methods: i) F_{ST} with GENETIX software (Belkhir et al. 2000), ii) a Bayesian approximation with STRUCTURE software (Pritchard et al. 2000) and iii) an iterative reassignment of individuals with FLOCK software (Duchesne & Turgeon 2012). GENETIX software computed pairwise F_{ST} from allelic frequencies and the statistical significance using 1,000 permutations. STRUCTURE software implements a model-based clustering method for inferring population structure using genotype data consisting of unlinked markers. STRUCTURE both identifies clusters from the data and assigns individuals to the cluster representing the best fit for the variation patterns found. The analysis was performed using the admixture model and the

correlated allele frequencies model. The procedure was run 8 times for each K estimation (from K = 1 to K = 7) with a burn-in of 200,000 MCMC iterations. LnP(D) values obtained for each K value were compared using the Structure Harvester software (Eare et al. 2012). The graphical displays of the STRUCTURE results (K =2) were generated using DISTRUCT software (Rosenberg 2004). We used FLOCK 2.0 (Duchesne & Turgeon 2012) to assign genetically similar individuals to k partitions using a positive feedback mechanism where the number of individuals assigned to each of k groups grows with each re-allocation. Allele frequencies were estimated for each of the k groups, and each genotype was reallocated to the group with the highest likelihood score following the multilocus maximum likelihood method of Paetkau et al. (1995). We carried out a total of 30 re-allocations and performed 50 runs for k = 2 to 6 with a log-likelihood value ranging from 0 to 0.6. To understand long-term migration rates of *P. flavescens*, we used MIGRATE-N software (Beerli 2002). MIGRATE-N uses a coalescent approach to estimate mutation-scaled migration rates (M), the mutation-scaled effective population size (θ) for each population, and the long-term effective population size (Ne) of each population (that is, 4Nem, where m is the mutation rate per site). Start values for the migration parameter were generated from the F_{ST} calculations. We used the default settings for MIGRATE-N, except for the following run options: (i) the Bayesian inference module using the Brownian

motion mutation model; (ii) one single long run using heating with temperatures of 1.0, 1.5, 3.0 and 10^3, (iii) sampling 10 replicates of each 100,000 in intervals of 100; (iv) after discarding the first 100,000 visits. The uniform prior distributions ranging from 0 to 100 with delta of 10 was used for both mutation scaled population size Θ and M. Values of 4Nm were divided by four to compare levels of gene flow between populations (i.e., Nm). Values of Nm were then summed to provide estimates of the overall immigration rate and emigration rate of each population following Anderson & Miekle (2010). MIGRATE is relatively robust to minor violations of assumptions (e.g., all potential source populations have been sampled) and can be precise with even a few loci (Beerli 2004).

RESULTS

COI analysis

A total of 41 haplotypes were obtained from the 220 individuals of *Pantala flavescens* sequenced for a 439 bp segment of COI (GeneBank Accession N°s: KY200583 to KY200609, and KY934249 to KY934261). The number of substitutions varied from 1 (EIS) to 19 (TAC) and the number of polymorphic sites (S) ranged from 1 (EIS) to 18 (TAC and GUA). As shown in Table II, genetic diversity (h) varied from 0.02 (EIS) to 0.97 (GUA) and nucleotide diversity (π) was the lowest in EIS (0.00006) and peaked in MAL (0.009).

Table II. Summary of genetic diversity indices in the COI gene of *Pantala flavescens*. Number of sequences (N), number of haplotypes (nh), private haplotypes (np), haplotype diversity (h) and nucleotide diversity (π) based on the COI analysis are shown.

Location	N	nh	np	h (±SD)	π (±SD)
MAL	10	8	5	0.933±0.077	0.009±0.005
TON	43	13	8	0.815±0.045	0.006±0.003
EIS	80	2	0	0.025±0.024	0.0001±0.0002
GUA	17	13	6	0.971±0.027	0.009±0.005
HER	22	13	5	0.909±0.045	0.007±0.004
TAC	19	10	4	0.877±0.056	0.008±0.005
ARI	29	8	0	0.791±0.051	0.006±0.004

Two common haplotypes were present in most locations, except for the Maldives. Interestingly, these two haplotypes represent the whole extent of diversity in Easter Island (Figure 2). The most common haplotype was found in 116 individuals (53 % of the total individuals), and the second was found in 16 individuals (7.3% of the total individuals). Of the remaining haplotypes, 14 were found in a minimum of two individuals and 25 were found in single individuals. Qualitatively, the haplotype network did not show any apparent geographical structure.

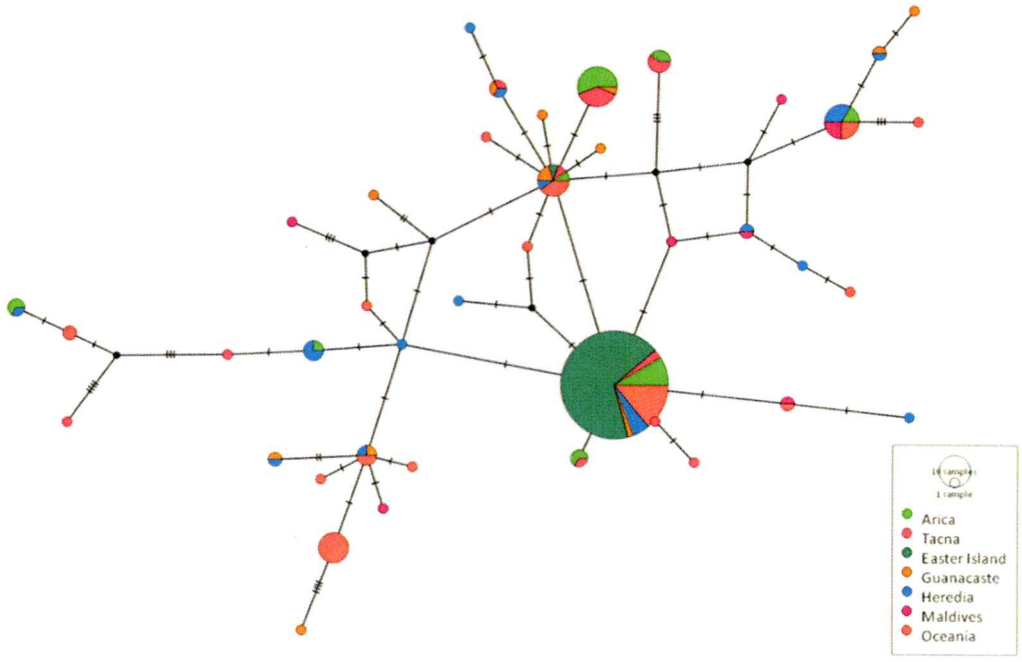

Figure 2. Median joining network showing relationship among COI haplotypes in *Pantala flavescens*. Circles represent haplotypes with sizes proportional to their respective frequencies. The population origin of haplotypes is indicated by colors. Tick marks represent deduced numbers of nucleotide substitutions along each branch.

The F_{ST} comparisons between pairs of locations are shown in Table III. In this analysis, most of the largest F_{ST} values were observed for paired comparisons between island locations (MAL, TON and EIS) and locations in the Americas (GUA, HER, TAC and ARI) ranging from 0.10 to 0.52. Most showed statistical differences (p <0.01), except for the comparisons of MAL and TON with GUA and HER. Pairwise comparison between locations in the Americas presented low F_{ST} values (<0.06), showing no statistical differences among these locations (p >0.01). On the other hand, pairwise comparisons

between Island locations (MAL, TON and EIS) showed F_{ST} ranging from 0.15 to 0.72, all showing statistical differences (p <0.01). Finally, Easter Island had the highest F_{ST} values (> 0.25) with all the sites, demonstrating statistically significant differences between this remote island and each of the other locations (p <0.001).

Table IV. Mean estimates of haplotype diversity among populations of *Pantala flavescens* along American and Polynesian subsets. G_{ST} and N_{ST} are based on mtDNA data and were estimated with PERMUT software (Ponds & Petit 1996).

Set and subset of data	N_{ST}	G_{ST}	P value
Global	0.248	0.149	0.014*
American	0.038	0.033	0.655
Polynesian	0.335	0.296	0.289

Table III. Matrix of pairwise F_{ST} values between populations based on mitochondrial (above diagonal) and microsatellite (below diagonal). Significant values after a Benjamini-Yekutieli correction (Benjamini & Yekutieli, 2001) based on the false discovery rate approach (*= significant differences p<0.01).

	MAL	TON	EIS	GUA	HER	TAC	ARI
MAL	-	*0.158**	*0.721**	0.046	0.024	*0.104**	*0.116**
TON	*0.109**	-	*0.254**	0.068	0.040	*0.131**	*0.115**
EIS	*0.119**	*0.199**	-	*0.520**	*0.355**	*0.450**	*0.377**
GUA	*0.344**	*0.248**	*0.410**	-	0.036	0.053	0.052
HER	*0.407**	*0.273**	*0.444**	0.003	-	0.065	0.051
TAC	*0.359**	*0.244**	*0.405**	0.002	0.003	-	0.022
ARI	*0.360**	*0.252**	*0.408**	0.022	0.041	0.011	-

Genetic diversity analysis revealed that total genetic diversity (H_T= 0.894) was higher than the average gene diversity for all sites (H_S= 0.760). PERMUT results showed a significantly larger N_{ST} than G_{ST} value across all populations for the mtDNA, which indicated that the genetic variability of *P. flavescens* was geographically structured across its distributional global. Nevertheless, the analysis performed for American sites and Island locations separately did not detect phylogeographic structure (Table IV).

Microsatellites analysis

Summary statistics for the microsatellite loci per location is shown in Table V. Detailed information is provided in Appendix I. A total of 56 alleles were detected across loci, with 5 alleles at locus SSR10 and 9 alleles at locus SSR24. Average A_R value was highest in TON (2.536) and lowest in EIS (1.899). The expected and observed heterozygosity per sites varied from 0.452 (EIS) to 0.671 (TON), and from 0.345 (GUA) to 0.586 (TON), respectively. Five out of the 28 comparisons showed evidence of linkage disequilibrium ($p < 0.01$); however, this disequilibrium was not observed in all locations. Departures from HWE were detected in seven of eight microsatellite loci ($p < 0.01$), but deviations were not associated with any specific locus or location (Appendix I). Analysis using MICROCHECKER suggested presence of null alleles by general excess of homozygote for most allele size classes in three of eight loci:

SSR24 (in Heredia, Easter Island and Tonga), SSR2 (in Tacna, Guanacaste, Heredia and Maldives), and SSR14 (in Arica, Easter Island and Tonga Island).

Table V. Summary information of the eight microsatellite loci averaged over the seven locations for *Pantala flavescens*. The number of individuals genotyped (N), allelic richness (A_R), and observed and expected heterozygosities (H_O and H_E) are shown for each sampling location.

	N	A_R	H_E	H_O
MAL	11	2.015	0.586	0.403
TON	46	2.536	0.671	0.471
EIS	82	1.899	0.452	0.407
GUA	17	2.086	0.508	0.345
HER	21	1.973	0.472	0.379
TAC	14	1.953	0.475	0.346
ARI	29	1.994	0.498	0.505

The mean r_{xy} values estimated for the groups of individuals collected in the seven sites showed statistical evidence (Permutation, $p > 0.01$) of highly related individuals only in EIS (Appendix 1). Pairwise F_{ST} values based on allelic frequencies ranged from 0.002 to 0.444 (Table III). Similarly, to the results of pairwise comparison based in mtDNA, western locations (MAL, TON and EIS) compared to the eastern locations (GUA, HER, TAC, ARI) showed higher and statistical significant F_{ST} values ($F_{ST} > 0.24$, $p < 0.01$). Further, locations in American continent (GUA, HER, TAC, ARI) showed lower and had no significant F_{ST} values ($F_{ST} < 0.04$, $p > 0.05$), while Island locations (MAL, TON, EIS) showed high and

statistically significant F_{ST} values (F_{ST} >0.10, p <0.01). These results were consistent with those obtained with STRUCTURE and STRUCTURE HARVESTER (Appendix II and III) and FLOCK software that suggested K = 2 as the most probable number of genetic groups (Figure 3). These analyses clearly separated western (Central and South American) and eastern locations (Polynesian and Maldives islands).

Mutation-scaled effective population size (θ) estimated by Migrate-N, showed lower values in oceanic islands (MAL = 0.233, EIS = 0.179 and TON = 0.281) in comparison with values obtained in the American locations (ARI = 0.387, TAC = 0.451, GUA = 0.722, HER = 0.399).

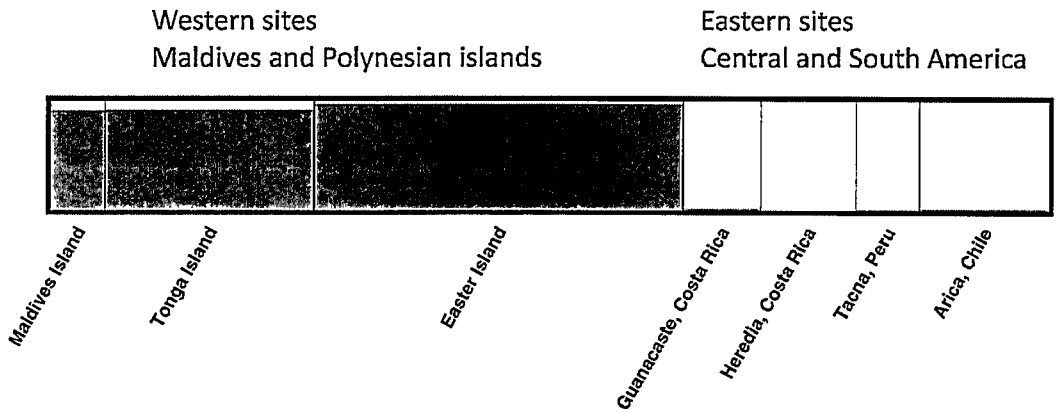

Figure 3. Graphical display of population structure showing the best cluster number (K=2) based on STRUCTURE results constructed using DISTRUCT software (Rosenberg 2004). FLOCK software suggested the same scenario.

Further, historical migration rates (M) among all locations (Table VI), revealed relatively high values within Central and South American locations (1.3 to 3.4), suggesting connectivity among the locations in America. Similarly, we observed similar migration values (0.3 to 3.7) between pairs of locations in the west (Indian and Pacific islands). The lowest values where observed among locations from the western area and America (0.57 to 1.1). Nonetheless, when we compared immigration versus emigration rates, our results showed larger values of immigration in American locations, converse to what was observed in the western locations (Figure 4).

Table VI. Mutation-scaled migration rates (M) among the seven populations of *Pantala flavescens*. Grey colors indicate highest and lowest values of M (light grey= 0.5-1.5, dark grey = 1.6-3.9).

From ⇨ To ⇩	Western sites (Maldives and Polynesian islands			Eastern sites (Central and South America)			
	MAL	TON	EIS	GUA	HER	TAC	ARI
MAL	-	3.740	3.681	1.139	1.027	0.622	0.749
TON	0.759	-	1.096	0.728	0.611	0.575	0.711
EIS	0.659	2.587	-	0.614	0.583	0.637	0.741
GUA	2.112	1.110	3.994	-	2.952	1.346	1.973
HER	1.082	0.974	1.686	3.003	-	2.760	3.420
TAC	2.072	2.498	1.959	2.260	3.284	-	1.824
ARI	1.121	2.400	3.028	2.697	1.638	1.171	-

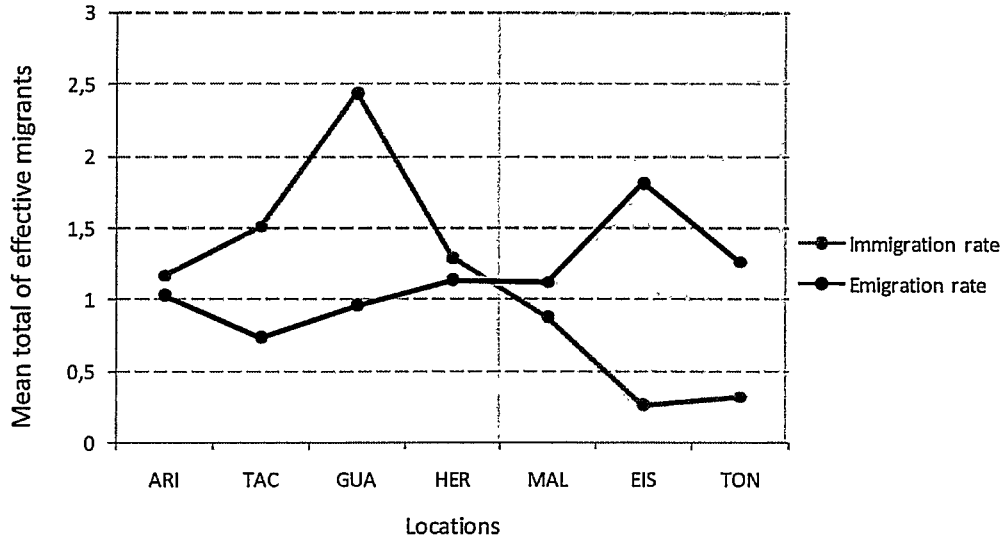

Figure 4. Overall immigration and emigration rates (ΣNe) obtained in the seven locations studied of *P. flavescens*.

DISCUSSION

Widespread species exhibit generally higher genetic similarity or a lack of genetic structure along their geographical distribution (Freeland et al. 2003, Lyons et al. 2012). In the cosmopolitan dragonfly, *Pantala flavescens* we have previously observed high genetic connectivity only along American continent (Alvial et al. 2017). In addition, Troast et al. (2016) suggested high rates of gene flow among samples from North America, South America and Asia, using PCR-amplified cytochrome oxidase one (CO1) mitochondrial DNA data. In contrast, in this study using mitochondrial and microsatellites markers, we detected a signal of global genetic differentiation between American continental

locations and those in the Indian and South Pacific Oceans and a lack of gene flow to isolated islands such as Easter Island. In this sense, oceanic barriers or terrestrial discontinuities may limit the genetic structure of high dispersal populations, suggesting a scenario of reduced genetic diversity as the geographic isolation increases toward remote islands.

Global patterns of genetic differentiation

The main differences observed in this study genetically separate American locations from western locations (insular systems). Analysis based on mitochondrial and microsatellites genetic markers showed similar results, pointing out a genetic differentiation among western (Maldives and Polynesian Islands) and eastern locations (Central and South America). It is important to point out that COI sequences do not show evidence of genetic differences between the individuals from MAL and TON when compared with those in GUA and HER in Central America, probably due to the low power offered by the 439 bp sequence of this gene. Nevertheless, the global pattern of genetic differentiation obtained with STRUCTURE and FLOCK software and the lack of connectivity or migration between both geographic regions strengthens the phylogeographic pattern detected. With exceptions of host-parasite associations (e.g. Brown et al. 1997, Stireman et al. 2005), patterns of genetic differentiation are scarce in

widespread species (Estoup 1996, Failloux et al. 2002, Artiss 2004, Lyons et al. 2012). In the four-spotted skimmer (*Libellula quadrimaculata* L.), a widespread and migratory dragonfly, Artiss (2004) described a pattern of genetic differentiation concordant with its distribution in North America, Asia and Europe. Therefore, despite the large and continuous holarctic distribution of this species, likely attributable to its dispersal capabilities, historical events (glaciations) have isolated populations and geographic barriers, such as Atlantic Ocean, have limited gene flow between continental regions. In the South Pacific, while the mass of islands in Polynesia, Micronesia and Melanesia have facilitated the colonization and diversification of many terrestrial invertebrates (Plaisance et al. 2008, Hembry & Balukjian 2016, Shaw & Gillespie 2016), the ocean also has been a major biogeographical barrier to dispersal (Palumbi 1994, Failloux et al. 2002, Gaither 2010). Similarly, Failloux et al. (2002) observed genetic differentiation in populations of the Dengue virus vector, *Aedes aegypti* L. identifying three major clusters as result of geographical barriers, which together with migration and colonization events have limited gene flow and generated the actual population structure in *Aegypti* forms. In the Indo-Pacific, Gaither et al. (2010) and Brigss (1999) have determined that the mass of islands of the Indonesian archipelago constitute a major biogeographical barrier to the dispersal of many marine

invertebrates. Gaither et al. (2010) used mitochondrial-sequence comparisons to evaluate the efficacy of biogeographical barriers on populations of snappers (*Lutjanus fulvus* F.) across their natural ranges. These authors found high levels of population structure in *L. fulvus* at all geographical scales, predicting that population divergence in the Indonesian archipelago may be a product of geographical isolation enhanced by oceanographic currents that limit gene flow to and from those islands, and adaptation to unusual ecological conditions.

Contrasting pattern within American continent and oceanic islands

The hypothesis of a terrestrial continuum versus oceanic barriers is clear when genetic diversity and genetic differentiation are evaluated in both geographic regions. Effectively, continental locations in America presented low values of F_{ST} (<0.06) showing no statistical differences inwardly (p >0.01). Moreover, we observed relatively higher genetic diversity (h) and nucleotide diversity (π) in the continental sites, and lower values in the insular populations. Despite these differences, all values of nucleotide diversity were <0.009. Insular populations are expected to have low effective population sizes (Ne) and reduced genetic diversity, because are likely to have experienced population bottlenecks at some point in their evolutionary history and only a fraction of the original genetic diversity of the population

would be maintained (Frankham 1997). Genetic diversity enhances the colonization ability of species on a short-term ecological time scale by increasing the probability that the population will survive, grow and reproduce under novel conditions (Crawford & Whitney 2010). Considering the level of admixture and the values of immigration rates observed, the American population of *P. flavescens* demonstrated a high interchange of individuals along this large geographic area suggesting colonization abilities and potential to adapt to changing environmental conditions. Thereby, under the existence of long continental extensions or suitable habitats for nymph development, e.g. intermediate islands, as has been described in the migratory route over Indian and South Africa (Hobson et al. 2012), high gene flow can be expected. However, without the presence of continental bridges such as in the case of remote islands, like Easter Island, *P. flavescens* shows population discontinuities. A similar pattern of a panmictic population along continental sites versus genetic differentiation in insular locations was described by Lyons et al. (2012) in populations of monarch butterflies. These authors demonstrated a pattern of decreasing in genetic diversity and allelic richness with increasing distance from North America toward populations in Hawaii and New Zealand. On the other hand, geographic isolation in oceanic islands has promoted the local adaptation and diversification in many groups of organisms. Examples of this

adaptation and diversification are shown in the work of Schmitz et al. (2007) on the butterflies of the Galapagos Islands and in Roderick & Gillespie (1998) on terrestrial Hawaiian arthropods. Schmitz et al. (2007) described the phylogenetic reconstruction of the endemic genus *Galagete*, based on a single colonization event by a common ancestor and posterior radiation and diversification coincident with the chronological emergence of the major islands. Roderick & Gillespie (1998) described patterns of speciation within Hawaiian arthropod lineages associated with repeated colonization of new islands, such that the most ancestral groups were on the oldest islands.

Genetic differentiation in Easter Island

The presence/absence of species in insular systems depends of a combination between dispersal capabilities and the settlement of a population large enough to avoid extinction and ensure persistence of the population (Stefan 1984). Consequently, genetic variation on islands is determined by the net effects of loss at foundation, subsequent loss in a finite population, and gains arising from secondary immigration and new mutations (Frankhman 1997). Oceanic islands acquire their biota strictly through dispersal, and dispersal frequency (gene flow) is a major determinant of whether populations can become established and whether they differentiate from each other (Paulay & Meyer 2002).

If dispersal is too rare to lead to successful colonization, then an organism cannot become established. The absence of large suites of taxa, i.e., a ''disharmonic'' biota, is one of the most striking feature of islands (Carlquist 1974). As was expected, Easter Island showed the highest genetic differences, the lowest genetic diversity, the lowest effective population size and the lowest number of migrants compared with all other locations. Despite the strong and constant rains (1070 mm average annual rainfall) that can provide suitable habitat to immature individuals, Easter Island has low availability of freshwater such as rivers or streams, and as such, scarce habitat available for nymph development. In concordance, Borisov (2006) mentioned that the distribution of dragonflies is primarily determined by the presence of reservoirs suitable for development of their preimaginal stages, which is strongly depend precipitations and local climate. Besides, agricultural activities have isolated and reducted the few freshwater ponds. Habitat loss leads directly to the loss of individuals resulting in loss of genetic diversity, and decreased population sizes in the remnant habitats and increased isolation can altering the genetic composition of a population through genetic drift and inbreeding (Zhang et al. 2012). At that time, low genetic diversity reported for *P. flavescens* in this remote island, could be determined by the small population size, the scarce of availability habitats and the

island´s geographic isolation, which prevents arrival of new migrants and may complicate the establishment of individuals that have been able to migrate to the island. Further, Easter Island also showed the lowest immigration rate and highest emigration rate in comparison to the other sites. Its small size (171 km^2) and long distance from continent (almost 4000 km from the Chilean coast) explain these results, and corroborate MacArthur & Wilson´s expected equilibrium model of island biogeography (Whittaker & Fernández 2007), where new forms will arise because of *in situ* radiation rather than immigration, as an island becomes ever more isolated. Over time, the equilibrium theory of island biogeography has been applied to other island diversity relationships, such as correlations between island area and genetic diversity, and species diversity and genetic diversity (Johnson et al. 2000, McGlaughlin et al. 2014). For example, Johnson et al. (2000) proposed a theoretical model to predict rates of migration and extinction of island organisms based on population genetic data, and found that island size and island isolation were identified as major determinants of genetic diversity, corroborating that was observed in Easter Island population.

The biota in insular habitats may arrive in one of two ways, via former terrestrial connections or previous extensions of actual continental margins; and by transoceanic movement, which can be active or passive, depending from the dispersal capabilities of

organism (Whittaker & Fernandez 2007). The actual freshwater fauna and flora of Easter Island is composed of mainly cosmopolitan species (Campos & Peña 1973); probably transported by humans. Dumont et al. (1998) suggested that most species were imported in the last few centuries since people started visiting the island regularly, and *P. flavescens* could be one of this species. If the arrival of *P. flavescens* occurred through passive transport mediated by man, we can suppose that this event would have been between the 15th and 16th centuries, coincident with the arrival of the first settlers to Rapa Nui or Easter Island (Englert 1948). Easter Island legends tell that the first settlers arrived after their native land, "Hiva", had been submerged. Hiva was the native land of "Hotu Matu`a", first king of Rapa Nui or Easter Island, who is said to have arrived on the island in the second half of the sixteenth century. Surprisingly, Macmillan (1924) concluded that an archipelago of considerable extent must have foundered close to Easter Island in the 15th century, coincident with the arrival of the first settlers. If the theory of ancient lands now submerged were true, the existence of intermediate islands close to Easter Island could have facilitated the active dispersal of *P. flavescens* to Easter Island from western sites, before the arrival of the first habitants. In our analysis, we reported the lowest values of genetic diversity in Easter Island. This, and the presence of only two haplotypes shared with most of the other

sites, more the high values of relatedness and the presence of inbreeding, are suggesting a recent colonization event from other Polynesian islands, because founder effects that are generally considered to accompany colonization of new islands may lead to rapid differentiation that we still don't observed in Easter Island (Roderick & Gillespie 1998). The classically founder principle is that a species immigrating to a remote island will establish by means of a tiny founding population. This new population contains only a subset of the genetic variability from the source population and it subsequently receives no further mixture. Genetic variation can be increased after establishment by mutation and re-sorting or genetic drift (the chance alteration of allele frequencies from one generation to the next) (Whittaker & Fernandez 2007). Additionally, the relatedness index values estimated for the groups of individuals collected in the seven sites showed statistical evidence for highly related individuals in Easter Island population. It is generally held as axiomatic that an increase of inbreeding in small populations reduces fitness in animals and it is intuitively reasonable that the loss of a broad genetic base is likely to increase the probability of extinction (Frankham 2005). The small population size inferred in Easter Island and the genetic isolation suggested by the actual results, give us reason to promote the conservation and preservation of the insular population of *P. flavescens*. However, further studies are

necessary to elucidate the mystery around the arrival of the wandering glider on Easter Island.

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Appendix I. Summary of genetic values by microsatellite in the seven populations of *P. flavescens*. Sample size (N), number of alleles (Na), allelic richness (Ar), expected heterozygosity (He), observed heterozygosity (Ho), inbreeding coefficient (F_{IS}) (* p-value <0.01) and the relatedness index (r_{xy}) in the last line, estimated per sampling site.

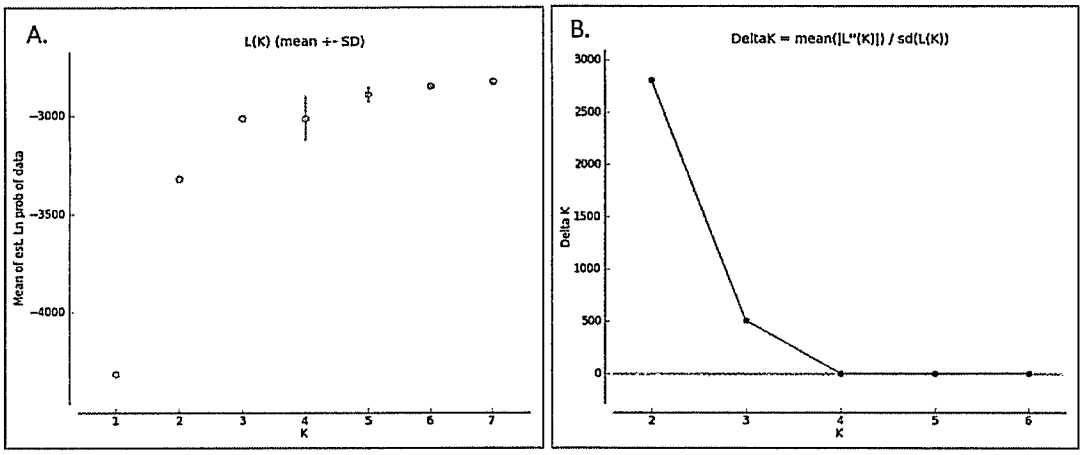
Loci	Arica	Tacna	Guanacaste	Heredia	Maldives Is.	Easter Is.	Tonga Is.
SSR6							
N	29	13	16	21	10	78	43
Na	3	2	4	3	4	3	5
Ar	1.683	1.599	1.761	1.728	2.550	2.305	2.665
He	0.3549	0.3107	0.3633	0.3730	0.6650	0.6220	0.7144
Ho	0.3793	0.2308	0.3125	0.2857	0.6000	10.000	0.7442
Fis	-0.051	0.294	0.171	0.257	0.150	-0.604*	-0.029
SSR9							
N	26	10	12	18	9	81	45
Na	3	2	4	5	2	3	4
Ar	2.097	1.913	2.564	2.197	1.908	1.853	2.285
He	0.5496	0.5000	0.6701	0.5617	0.4938	0.4552	0.6114
Ho	0.4231	0.2000	0.4167	0.4444	0.6667	0.4074	0.6222
Fis	0.249	0.633	0.415	0.236	-0.297	0.111	-0.007
SSR28							
N	29	13	16	21	11	80	41
Na	4	3	4	4	3	3	5
AR	2.352	2.029	2.427	2.169	1.928	1.712	2.221
He	0.6296	0.5296	0.6387	0.5669	0.4587	0.3680	0.5895
Ho	0.7241	0.5385	0.5625	0.6190	0.4545	0.0875	0.5610
Fis	-0.133	0.023	0.151	-0.068	0.057	0.765*	0.061
SSR14							
N	26	4	16	17	2	55	37
Na	5	2	5	5	1	4	7
Ar	2.225	1.929	2.389	2.400	1.000	1.490	2.791
He	0.5836	0.4688	0.6289	0.6142	0.0000	0.2433	0.7411
Ho	0.3077	0.2500	0.5625	0.4118	0.0000	0.0727	0.5676
Fis	0.488*	0.571	0.137	0.356	NA	0.706*	0.247*
SSR10							
N	29	12	17	21	9	68	44
Na	3	3	4	3	3	4	4

Ar	1.947	2.076	1.995	1.810	2.112	1.889	2.322
He	0.5155	0.5174	0.4931	0.4206	0.5494	0.4649	0.6222
Ho	0.8621	0.7500	0.3529	0.5714	0.5556	0.4265	0.6591
Fis	-0.663*	-0.414	0.312	-0.337	0.048	0.090	-0.048
SSR15							
N	28	10	15	19	7	79	44
Na	2	3	3	3	2	4	4
Ar	1.864	2.223	1.996	2.033	1.670	1.949	2.195
He	0.4841	0.5800	0.5178	0.5263	0.3367	0.4893	0.5473
Ho	0.4643	0.4000	0.2667	0.3684	0.1429	0.4051	0.3864
Fis	0.059	0.357	0.511	0.324	0.625	0.178	0.304*
SSR2							
N	23	14	16	21	10	80	42
Na	2	2	2	3	4	5	6
Ar	1.496	1.642	1.584	1.569	2.609	2.230	2.786
He	0.2580	0.3367	0.3047	0.2846	0.6850	0.5869	0.7421
Ho	0.1304	0.0000	0.0000	0.1429	0.3000	0.8125	0.7381
Fis	0.511	1.000*	1.000*	0.516	0.597*	-0.379*	0.017
SSR24							
N	20	10	14	16	10	79	34
Na	4	3	4	3	4	4	7
Ar	2.293	2.213	1.977	1.883	2.347	1.767	3.027
He	0.6087	0.5550	0.4515	0.4316	0.5800	0.3858	0.8019
Ho	0.7500	0.4000	0.2857	0.1875	0.5000	0.0506	0.4118
F_{IS}	-0.208	0.327	0.399	0.587*	0.189	0.870*	0.498*
r_{xy}	-0.029	-0.0706	-0.0654	-0.016	-0.2394	0.0234*	-0.0172

Appendix II. Table output of the Evanno method implemented in Structure Harvester showing the largest value in the Delta K column for K=2.

K	Mean LnP(K)	Stdev LnP(K)	Ln′(K)	|Ln″ (K)|	Delta K
1	-4312.525000	0.305894	-	-	-
2	-3321.337500	0.244584	991.187500	686.00000	2804.760133
3	-3016.150000	0.597614	305.187500	302.987500	506.995060
4	-3013.950000	109.997078	2.200000	116.487500	1.059005
5	-2895.262500	38.002817	118.687500	76.650000	2.016956
6	-2853.225000	4.388866	42.037500	19.162500	4.366162
7	-2830.350000	10.966182	22.875000	-	-

Appendix III. Plots of (A) mean LnP(D) (the natural log of the probability of the data from STRUCTURE output) with vertical lines showing standard deviation, and (B) Delta-K (the second-order rate of change of K) versus K, for the 7 populations from across the species range, showing a highest delta-K of 2. Plots are from the STRUCTURE HARVESTER output.



CAPÍTULO III: VARIACIÓN MORFOLÓGICA A GRAN ESCALA GEOGRAFICA EN *PANTALA FLAVESCENS*

Large-scale geographical variation in wing size and wing shape of the cosmopolitan dragonfly *Pantala flavescens* (Fabricius 1798) (Odonata: Libellulidae)

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ABSTRACT

Dispersal and local patterns of adaptation play a major role on the ecological and microevolutionary trajectory of natural populations. In the case of flying species, the morphology of wings is one of the key life-history traits that relates directly with dispersal. In order to detect changes in morphology, measurements of body size, forewing size, forewing shape, hindwing size and hindwing shape adults of *Pantala flavescens* were obtained and compared among continental and insular locations across American continent, South Pacific Islands and Maldives Islands to infer if the geographic isolation determine morphological differentiation. Effectively, our results showed significant

differences in morphological traits among localities and the sex of *P. flavescens*. The traits that most explained the morphological variation were the forewing size, abdomen: thorax ratio, extension of anal loop and width of anal field. Easter Island individuals would have shorter and curved forewings, and compressed anal loop in hindwings, corroborating to previous descriptions in this remote island, and showing that insular individuals would have reduced their wings as result of geographic isolation, resulting in loss of the capability to migrate long distances. A trade-off between dispersal capability and reproduction is suggested, mainly associated to shorter thorax in males. On the other hand, individuals from Tacna (southern Perú) and Heredia (Costa Rica) would have extended anal loop and anal field and would be specially adapted for migration or long-distance dispersal.

INTRODUCTION

Dispersal is one of the more important biological traits in determining ecological and evolutionary process in the natural populations, affecting local adaptation, population and community dynamics and genetic structure (Doligez & Part 2008). Dispersal relate the ability to colonize new habitats, enlargement of their distributional range (Lester et al. 2007), and to determine survival of a species in a patchy landscape (Sekar 2012) providing flexibility as a response to environmental contingencies (Tesson

& Edelaar 2013). In insects, the ability to fly has influence their success, enabling them to exploit new habitats, even when the resident habitat conditions are favorable and it has given a mean of avoiding adverse environmental conditions (Rankin & Burchsted 1992). In this context, body size and wing size are among the key traits in determining flight capability and dispersal behavior in insects (Corbet 1999, Etienne & Olff 2004). In effect, research performed on flight morphology in dragonflies suggests that wing size and relative wing breadth are important determinants of flight performance with larger, broader wings facilitating longer distance flight (Wakeling & Ellington 1997, Corbet 1999, Dudley 2000).

In general, loss of flight capability means a loss of ability to migrate distances greater than a few hundred meters (Roff 1994), thus allowing isolation and the opportunity for the local adaptation (Roff 1994, Denno et al. 2001). In this way, it is not surprising that organisms that inhabit isolated areas can have shorter or reduced wings. Indeed, Darwin (1859) hypothesized that flightlessness occurs on oceanic islands where dispersing individuals should experience higher mortality than non-dispersing individuals. When arrived on an island, weakly-flying insects will survive if they avoid being carried away by the wind, favoring the development of winglessness forms (Darwin 1859). As it results, islands have imposed severe restriction to the power of dispersal

which is reduced by natural selection because wing shape largely determines the high energetic costs of flight (Roff 1994). Examples given by Williamsom (1981) of flightless forms include twenty endemic species of beetle on Tristan de Cuhna, all but two of which have reduced wings. Although there is no long-term evidence of decrease in wing size due to loss of dispersion in dragonflies, significant differences in morphological characters have been reported among species that differ in their dispersal behavior (e.g. migrant vs. non-migrant dragonflies) (Johansson et al. 2009, McCauley 2013, Suarez & Sarmiento 2016). In this context, comparisons at large-scale geographical in traits related to flight performance, could provide evidences about microevolutionary process such as local adaptation or natural selection in response to a variety of habitats conditions.

Pantala flavescens (Fabricius 1798) or the "wandering glider" is the most widespread odonate on Earth, absent only in Antarctica and a part of Europe (Dijkstra & Clausnitzer 2014). It migrates widely, following the temporary abundance of habitat provided by rains and it is among the odonates most often found in oceanic islands and in swarms well out to sea (Buden 2010, May 2013). *P. flavescens* is the only dragonfly present in Easter Island, the most remote inhabited island in the world, located at 3510 km from the American continent (Dumont & Verschuren 1993, Samways & Osborn 1998). In this remote island, Dumont & Verschuren (1993) pointed

out that adults of *P. flavescens* seem to have abandoned the long-distance dispersal behavior because they are poor flyers and tend to aggregate and forage in wind sheltered areas, and show a perching reflex at sudden wind rises. The authors argued that this normally migrant species is prevented to migrate from Easter Island by natural selection, which strongly favors non-migrant behavior, while poor larval nutrition physically prevents those which retain migratory behavior. Under the same assumption, Samways & Osborn (1998) compared some morphological traits and behavioral features in adult of *P. flavescens* from Easter Island and South Africa. Similarly, the individuals of Easter Island showed reduced and more asymmetrical hind wings, and had lower flight height than the continental population. To evaluate if the geographic isolation is determined by morphological changes in individuals of Easter island, more robust analyses are necessary. In this sense, geometric morphometric methods are a powerful tool to prove changes in wing shape of insects (Daly 1985), allowing us to summarize morphological data numerically and graphically, to express and test hypothetical relationships. In this way, the use of both linear and geometric morphometry will allow us a more exhaustive evaluation about morphological differentiation of *P. flavescens* in Easter Island. Accordingly, with the antecedents, the objective of this research was to compare body size, wing size ad wing shape

in continental and insular locations of *P. flavescens*, to evaluate morphological differentiation in relation to geographic isolation.

MATERIALS AND METHODS

Collection of samples

A total of 238 adult individuals of *Pantala flavescens* were used for the analysis. 157 individuals were collected from six locations using an entomological aerial net and preserved in 95% ethanol, 12 individuals were donated from Maldives Islands and 69 were revisited and measured from museum collections (Table I).

Table I. Locations, geographic coordinates, number of individuals, source and individuals used for the analysis.

Region	Location	Coordinates	N	Source
Central America	Guanacaste, Costa Rica	10°50′N, 85°37′W	17	Personal collection
	Heredia, Costa Rica	10°00′N, 84°06′W	29	Personal collection (22) and University of Costa Rica Museum (7)
South America	Arica, Chile	18°31′S, 70°10′W	34	Personal collection
	Tacna, Peru	18°06′S, 70°20′W	20	Personal collection
South Pacific Islands	Easter Island	27°07′S, 109°22′W	47	Personal collection
	Nukualofa, Tonga Island	21°07′S, 175°13′W	32	Personal collection (29) and Zealand Arthropod Collection (4)
	Rarotonga, Cook Islands	21°13′S, 159°46′W	29	New Zealand Arthropod Collection (18) and Auckland Museum (11)
	Viti Levu, Fiji Islands	17°49′S, 178°0′E	18	New Zealand Arthropod Collection (15) and Auckland Museum (3)
Maldives Islands	Kandufushi Island	2°32′N, 72°59′E	4	Donated of private collection by Ch. Anderson
	South Malé Atoll	3°57′N, 73°28′E	8	Donated of private collection by Ch. Anderson

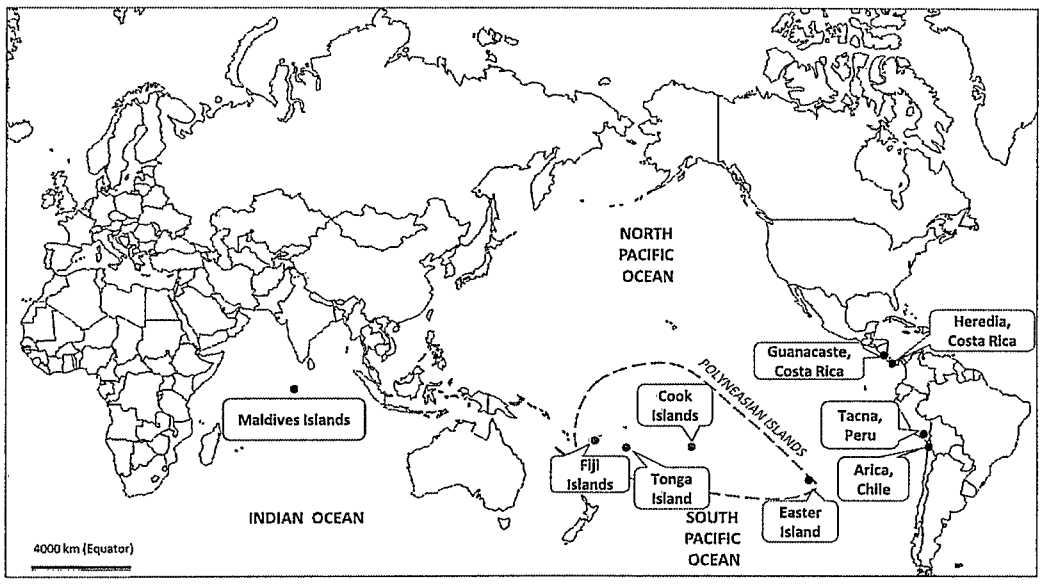

Figure 1. Distribution of study locations of *Pantala flavescens*.

Traditional morphometry

Morphological measures were obtained from 226 individuals (77 females and 149 males) shown in Table I. All individuals were measured using a digital caliper (CD-6 digital caliper, Mitutoyo Corporation, Tokyo, Japan) under a Stereoscope microscope at 10X. Eight morphological measures were obtained, namely body length (BD, distance between the anterior end of the postclypeus and the tip of the anal appendages), abdomen length (AL, distance between the anterior end of the first abdominal segment and the tip of the anal appendages), thorax length (TL, distance among the anterior margin of the prothorax to the posterior margin of the metathorax), head width (HW), forewing length (FWL, distance from the costal

vein base to the end of vein R2), forewing height (FWH, wing width from the nodus to the end of vein R4), hindwing length (HWL, distance from the base of the costal vein to the end of R2) and hindwing height (HWH, distance from first antenodal cross vein to the end of anal loop) (Figure 2). These eight morphological measures allowed to obtain seven ratios, which were included into statistical analysis: abdomen: thorax ratio (A.T), thorax to body length (T.BL), head width to body length (H.BL), forewing length to body length (FWL.BL), forewing height to body length (FWH.BL), hindwing length to body length (HWL.BL), and hindwing height to body length (HWH.BL). Ratios were used for scaling morphometric variables to remove variation in general body size as proposed by Daly (1985) and Marinov & Mchugh (2010).

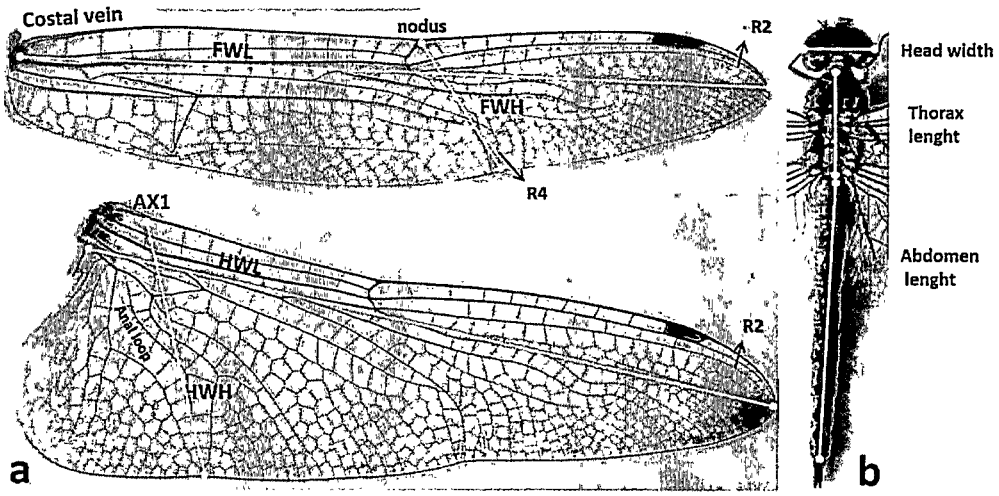

Figure 2. Morphological linear measures selected for wing size (a) and corporal size (b) analysis.

In order to determine a global statistical difference among sexes and populations based in the seven ratios established, a non-parametric permutation-based multivariate analysis of variance (PERMANOVA, 999 permutations) was performed. A Linear Discriminant Analysis was used to identify the combination of variables that better explains differences among populations. This analysis is a multivariate technique that describes the relationship between two variable sets by calculating the linear combinations that are maximally correlated (Tabachnick & Fidel 2001). In the present study, the seven-morphometric ratio were stepwise introduced as predictor variables into the discriminant analysis. The next analysis considered the variable explaining differences among populations obtained with the Linear Discriminant Analysis. For these variable, a Generalized Linear Model (GLM) was used to find differences between localities and sex (the independent variables in the model). The link function used was Gaussian and a *posteriori* test paired Tukey's HSD was used. All statistical analysis was performed in the R software (R Core Team 2016); the Permanova used the "vegan" library (Oksanen et al. 2016) and the discriminant analysis used "MASS" library (Venables & Ripley 2002).

Geometric morphometry

To detect differences in the shape of the wings, a landmark-based geometric morphometric analysis was performed. Morphometric

measures were done in 280 wings corresponding to 140 individuals that were collected from seven locations along America, South America, Maldives Island, Tonga Island and Easter Island (Table I). Each wing (140 right forewings and 140 right hindwing) were photographed at a fixed position using a digital camera (14 mega pixels camera Olympus SP-810UZ). To capture the shape of the wings, 30 landmarks (12 in forewing and 18 in hindwing, Figure 3) were placed on the digital images using the software TpsDig (Rohlf 2003a). Landmarks selection was following Johansson et al. (2009) and Suarez & Sarmiento (2016), who found that using vein nodes as landmarks they capture adequately wing shape and facilitates the comparative analyses. The software TpsRelw (Rohlf 2003b) was used to obtain superimposed landmark coordinates. This software rotates, scales, and translates landmark coordinates into alignment using a generalized least square superimposition method based on the generalized Procrustes analysis (Rohlf & Slice 1990). This procedure removes size differences among individuals and leaves purely allometric shape variation for subsequent analysis. Then, a Principal Component Analysis was performed for the relative warp to visualize shape variations; deformation grids were produced by regression of the shape variables on the canonical variables, also using the TpsRelw software. To assess differences in wing shape configuration, a Procrustes ANOVA (Goodall 1991) was performed with the Procrustes distances among individuals of *P.*

flavescens. All statistical analyses were performed with the "Geomorph" library (Adams & Otarola 2013) implemented in the R 3.1.1 software (R Core Team 2016).

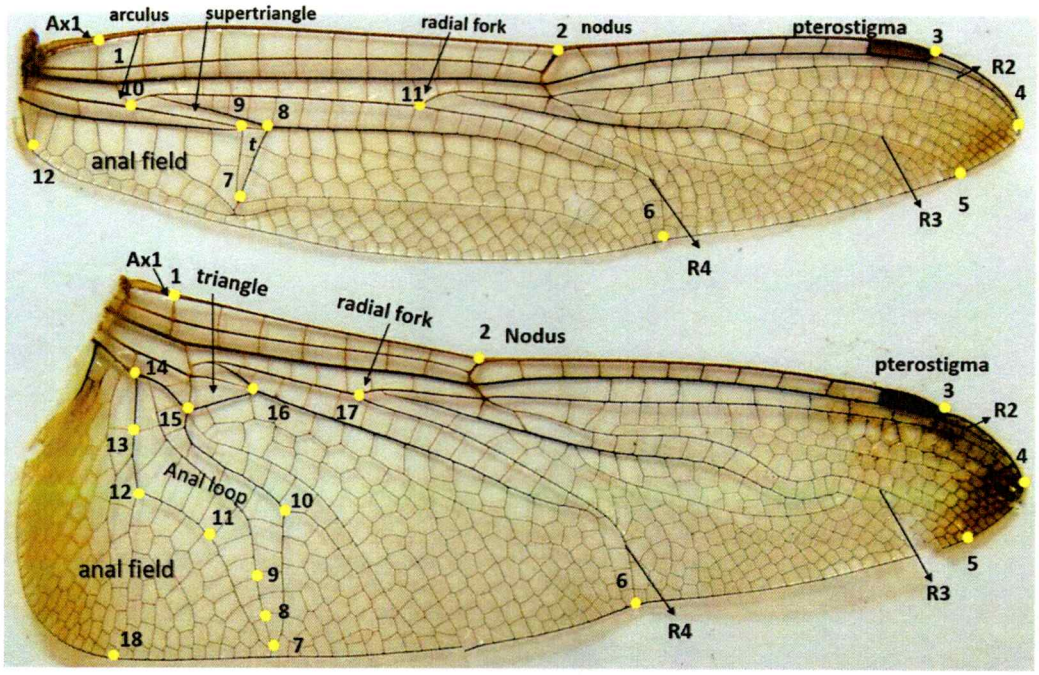

Figure 3. Forewing (above) and hindwing (under) landmarks recorded for each individual of *Pantala flavescens*. To both wing landmarks are: 1, costal vein base (Ax1); 2, nodus; 3, end of pterostigma; 4, end of vein R2; 5, end of vein R3; and 6, end of R4. Additional landmarks in forewing are: 7-9, within triangle (t) area; 10, in arculus; 11, in radial fork; and 12, margin of anal field. Additional landmarks in hindwing are: 7-15, within anal loop area; 16, extreme of triangle area, 17, radial fork; and 18, lower margin of anal field.

RESULTS

Traditional morphometry

The PERMANOVA (999 permutations) showed a significant difference in morphological measures in the interaction of populations and

sex (Λ = 0.01042, F= 2.9956, *P*= 0.006). Linear Discriminant Analysis showed that variables that most explain the differentiation between localities were: forewing length (FWL.BL), forewing height (FWH.BL) and abdomen: thorax ratio (A.T) (Figure 4 and 5). In this way, this analysis indicated that individuals from Easter Island (PAS) present shorter and thinner forewings than individuals from all other localities analyzed. Besides, this analysis reflected that females of all localities would have the largest abdomen: thorax ratio, with exception of Easter Island females, which showed a shorter abdomen: thorax ratio. Generalized Linear Models corroborated the result obtained with the Linear Discriminant Analysis showing significant differences in the interaction localities and sex for forewing length (FWL.BL), forewing height (FWH.BL) and abdomen: thorax ratio (A.T) (Table II). Tukey's HSD *a posteriori* analysis showed significant differences ($P < 0.001$) in forewing length and forewing height mainly due to the smaller values observed in female and males in Easter Island (Fig. 5a and Fig 5b). In the case of the abdomen: thorax ratio, the analysis showed clear higher values for females than males in all sites but an inverse pattern in Easter Island (Figure 5c).

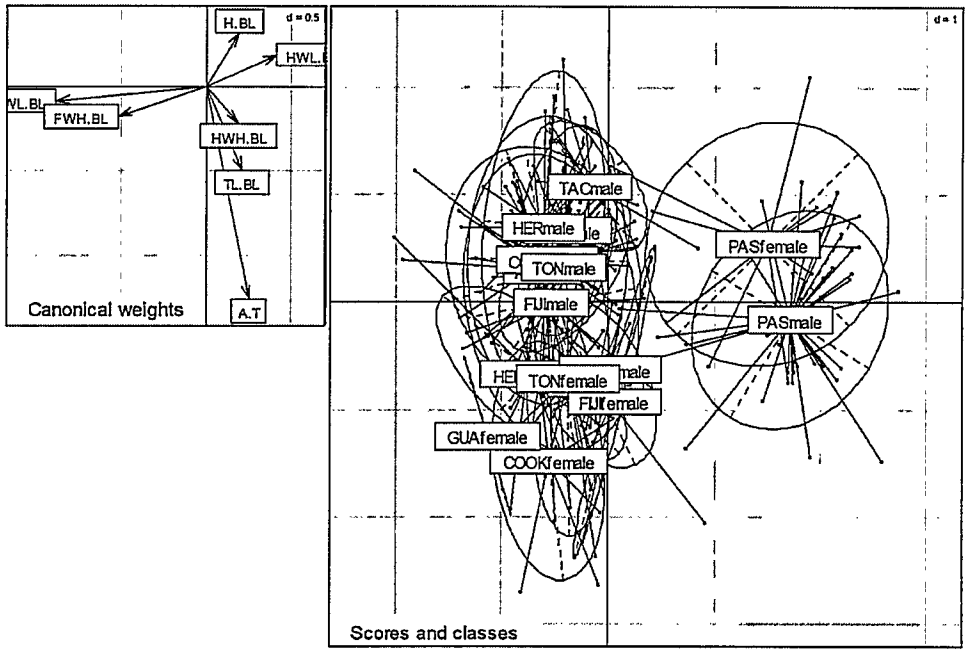

Figure 4. Linear Discriminant Analysis showing combination of variables that best separate sex and populations of *Pantala flavescens*.

Table II. Results of GLM analysis performed on results of PCA conducted on females and males of *Pantala flavescens* from seven localities.

	Df	Deviance	Resid.	Df	Resid.	Pr(>F)
FWL.BL NULL	225	0.590				
Site	7	0.273	218	0.316	30.026	<0.001
Sex	1	0.021	217	0.294	16.519	<0.001
Site: sex	7	0.021	210	0.273	2.344	0.02
FWH.BL NULL	225	0.035				
Site	7	0.016	218	0.019	29.887	<0.001
Sex	1	0.000	217	0.018	0.293	0.03
Site: sex	7	0.002	210	0.016	5.075	<0.001
A.T. NULL	225	18.109				
Site	7	2.5309	218	15.578	7.350	<0.001
Sex	1	2.4888	217	13.09	50.596	<0.001
Site: sex	7	2.7599	210	10.33	8.015	<0.001

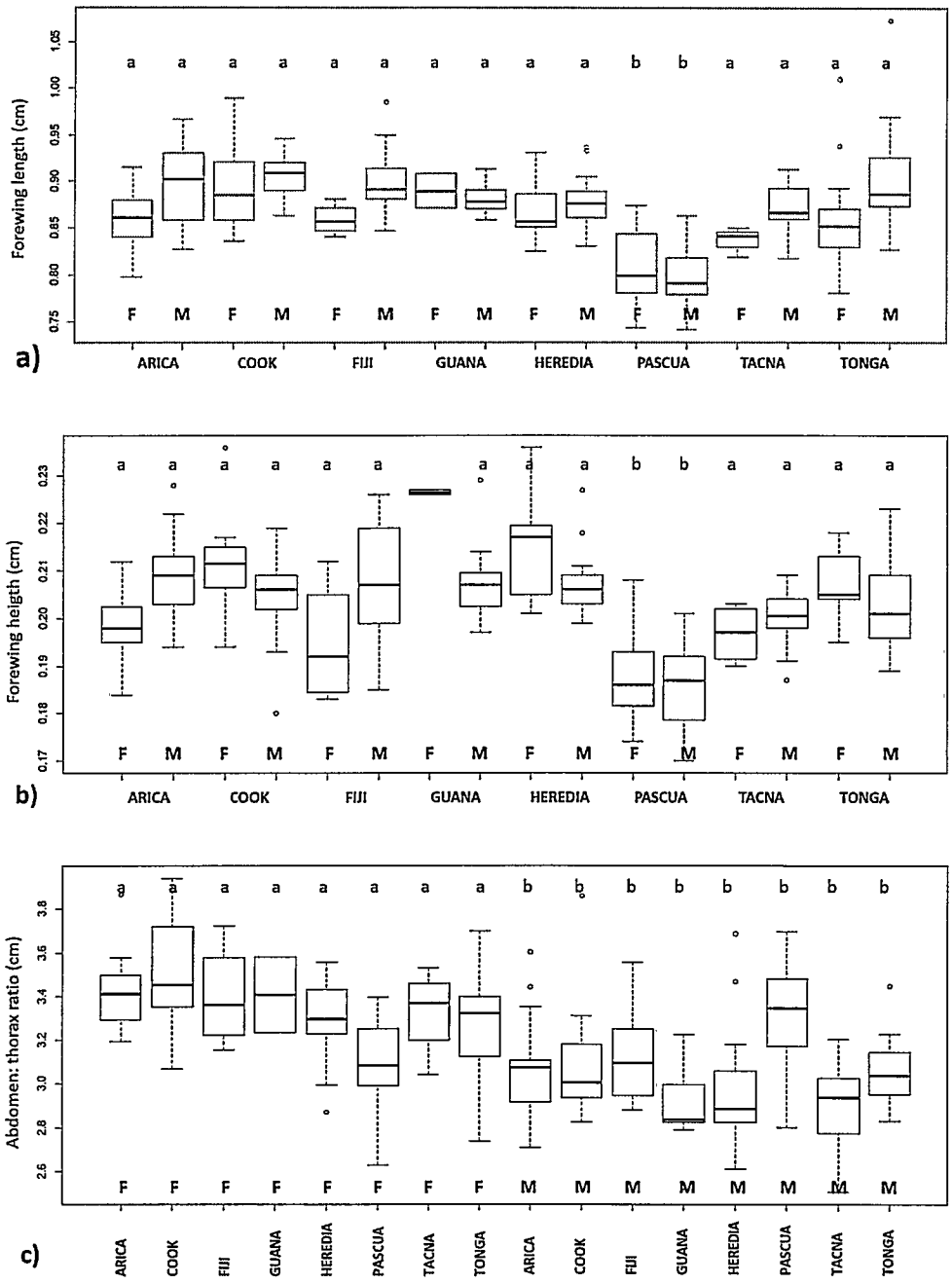

Figure 5. Box plot of forewing length (a), forewing height (b) and abdomen: thorax ratio (c) measured in females and males of *Pantala flavescens*. Different letters in the box represent statistical differences (a> 0.05, b<0.05).

Geometric morphometry

Figure 6 shows the landmark configuration of the Procrustes superimposed coordinates for the wings. Wing shape showed statistical differences among the localities ($P < 0.001$); landmarks 8 and 9 of the forewing and 11, 12 and 13 of the hindwing were more different than the other landmarks. The first two relative warps of the forewing and hindwing explained 38.3% and 48.24% of the wing shape variation, respectively. The first relative warp of the forewing explained 21.9% of the wing configuration (Figure 6) and was related with compression in the middle of the forewing, indicating forewings less compressed to the left side of the diagram in PAS and ARI, and forewings more compressed to the right of the diagram for GUA and TON. The second relative warp of the forewing explained 16.30 % of the shape variation (Figure 6), and it was related to width of anal field (Figure 7); this located wider anal field to the upper part of the diagram in TAC and compressed anal field to the lower of diagram in the other populations.

The first and second relative warp of hindwing explained 34.67% and 13.51 % of the wing shape respectively (Figure 6); and were mainly related with configuration of anal loop and width of anal field (Figure 7). Individuals located to the right of the first relative warp would have more extended (large) anal loop, such as

TAC, while individuals located to the left of the diagram would have more compressed o shorter anal loop, for example PAS. Meanwhile, individuals located to the upper part of the diagram present wider anal field, such as HER, and individuals located to the upper part of the diagram would have narrow anal field, for example ARI.

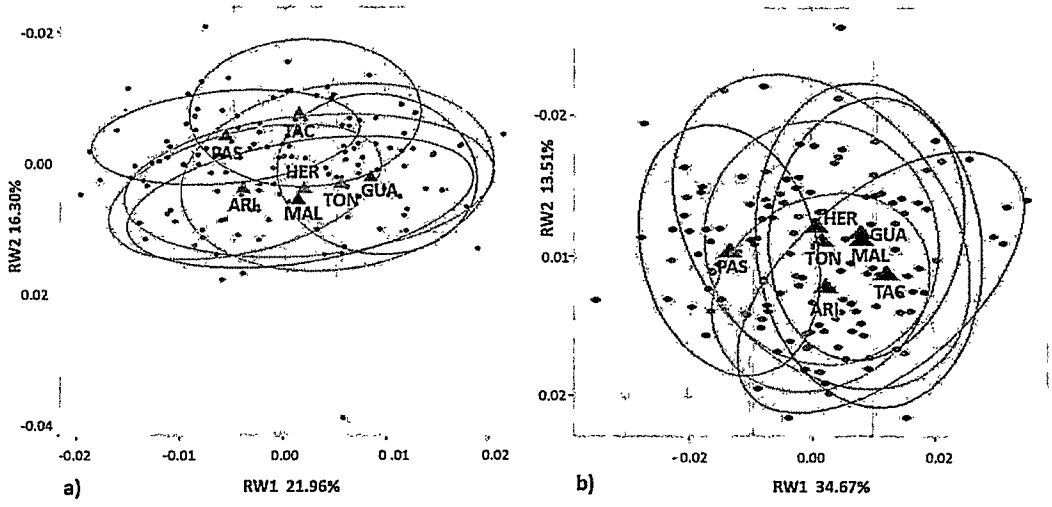

Figure 6. Scatter plots of Procrustes shape coordinates of forewings (left) and hindwings (right) of *Pantala flavescens*.

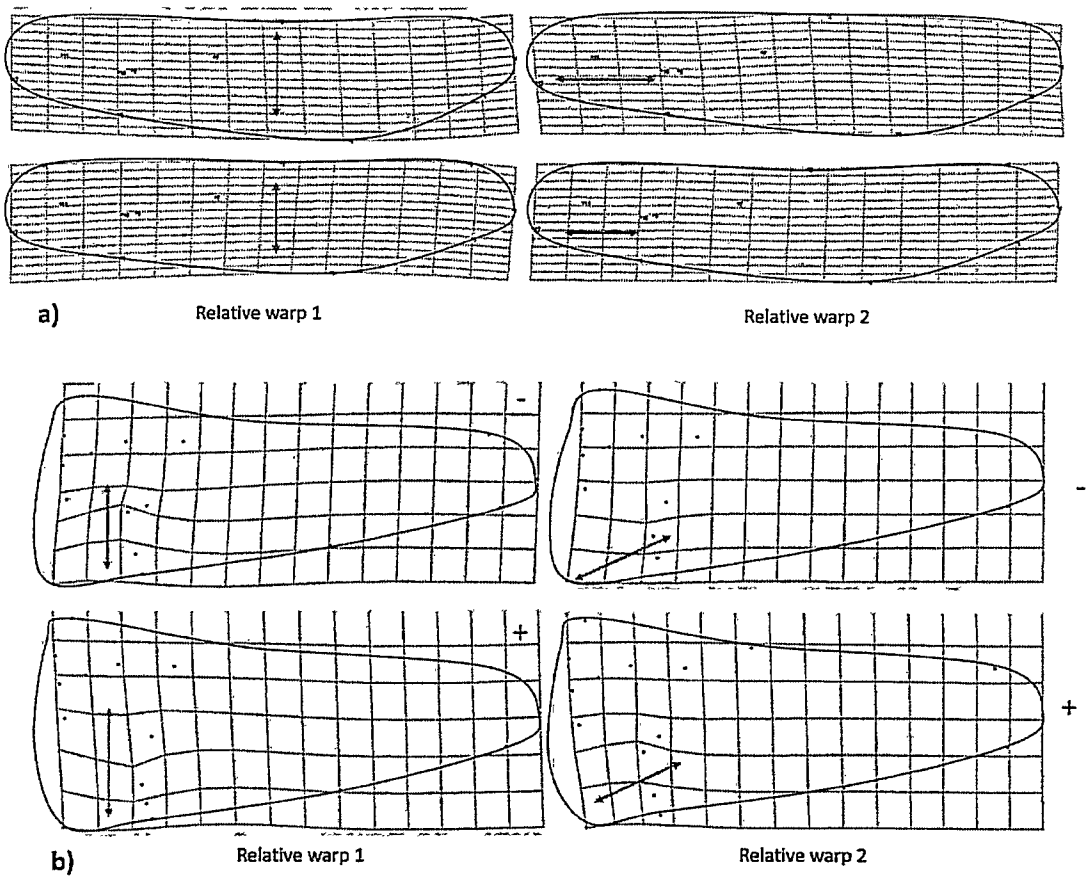

Figure 7. Thin-plate spline transformations showing consensus shapes of forewings (a) and hindwing (b) generated with the TpsRelw software (Rohlf, 2003c). Deformation grids were produced by regression of the shape variables on the canonical variables.

DISCUSSION

Understanding the relationship between morphology and dispersal provide a valuable framework for predicting which populations or species are most likely to be affected by dispersal limitation due to fragmentation of habitats or environmental pressures (McCauley

2013) and to understanding how microevolutionary process (natural selection and adaptation) has shaped dispersal behavior (Johansson et al. 2009). Species or populations that have more limited dispersal due to morphological constraints may experience a greater loss of population connectivity or genetic flow when the distances between habitats increase (Alvial et al. 2017) or if the quality of the inter-habitat matrix decreases (McCauley 2013). In this research, the analysis clearly showed differences in wing size and wing shape among localities with a marked difference in Easter Island. In this way, individuals from Easter Island showed smaller and thinner forewings than the individuals collected in all other sites.

Previous research on flight morphology in dragonflies suggested that wing size and relative wing breadth are important determinants of flight performance; presence of larger, broader wings facilitate longer-distance flight (Johansson et al. 2009, McCauley 2013, Suarez & Sarmiento 2016). Broad wing is essential to gliding abilities of dragonflies because reduce the energetic costs of long-distance flight (Wakeling & Ellington 1997, Dudley 2000). "Gliders" can remain airborne at the expense of minimum activity of the wing muscles, and are for the most part migrants (Corbet 1962). Thus, dragonflies that migrate on continental scales generally have larger wings and a wider basal portion of the hindwings (Samways & Osborn 1998, Corbet 1999). In this sense, our

results suggested that Easter Island individuals do not present this capability to migrate long distances. This evidence agrees with the study performed by Dumont & Verschuren (1993) and Samways & Osborn (1998), who argued that migrant species is prevented from migrating from remote island by natural selection, which strongly favors non-migrant behavior, as it was early hypothesized by Darwin (1859).

Another important result of our study showed that males of all sites have less abdomen: thorax ratio than females. The thorax houses the musculature that powers flight and relative thorax size can strongly affect flight performance (Schilder & Marden 2004, Marinov & McHugh 2010). Abdomen: thorax ratio is an indirect measure of thorax size, thus < abdomen: thorax ratio may be indicating more development of thoracic muscles in males, and > abdomen: thorax ratio would be related to larger abdomen in females. However, Easter Island individuals showed an opposite pattern. Males from Easter Island would have shorter thorax and consequently, less development of thoracic muscles. We think that the lost in migratory behavior in islands may facilitate others life-history traits, e.g. reproduction, as was reported by Langerotto et al. (2000) and Denno et al. (2001). For example, Langerotto et al. (2000), observed a trade-off between flight capability and reproduction in males of the salt-marsh-inhabiting planthopper *Prokelisia dolus* (Wilson 1982), with flightless males

acquiring mating more successfully and leading more offspring than macropterous males. Similarly, Denno et al. (2001) observed that flightless females of the delphacid planthopper *Toya venilia* (Fennah 1959), showed higher fecundity, reproduced at an earlier age and produced larger progeny than their flight-capable counterparts. In this way, larger abdomen in males from Easter Island may facilitate the adoption of the tandem position and increase success and frequency of copulation. In effect, larger males of dragonflies have shown a higher mating probablility and higher lifetime mating success than smaller males (Tsubaki & Obo 1987, Michiels & Dhondt 1991).

In addition to results described by linear morphometry, our results of geometric morphometry analysis also give important insights about the wing shape of *P. flavescens* among continental and insular locations. This analysis showed that the more important traits in determining differences in wing shape were extension of anal loop in hindwing, width of anal field in hindwing and compression in the middle of the forewing (bend of the forewing). Indeed, individuals of Easter Island present shorter (compressed) anal loop and curved forewings (less compressed in the middle) than all other individual analyzed. On the contrary, individuals from Tacna (Peru) and Heredia (Costa Rica) showed more extended anal loop and wider anal field than the other locations. Anal loop in the hindwing is an important trait in flight capability. Previous

studies have shown that migratory species strongly differ from nonmigratory species in the shape of the anal lobe (Johansson et al. 2009) and many temporary pool-dwellers are gliders and typically possessing an enlarged anal field in the hindwing (Corbet 1999). Corroborating these studies, adults of *P. flavescens* from Easter Island showed shorter anal loop and narrow anal field, giving more evidences about a nonmigratory behavior in this remote island. Even more, individuals of Easter Island showed, through geometric morphometry analysis, wider forewings.

Wing shape can be a good predictor of adaptation to different selection pressures. In birds, several studies have demonstrated that wing shape is affected by migration distance (Kaboli et al. 2007), sexual selection (Stiles et al. 2005), and foraging strategies (Bullen & McKenzie 2007). In dragonflies, migration and mate guarding are the factors that more likely to affect wing shape (Johansson et al. 2009). More recently, Dellicour et al. (2017) mentioned that inter-specific shape variations can be observed when local conditions may select shapes, e.g., shapes associated with fragmented habitats, temperature, precipitation or elevation; and intra-specific shape variation may also be related to internal factors like genetic diversity, body size (i.e. allometry), and developmental factors such as temperature.

Corbet (1962) pointed out that *P. flavescens* is evidently an obligate migrant. This species can glide and then be carried by the wind at considerable heights, and over long distances. In addition, Li et al. (2014) showed that the wings of *P. flavescens* present excellent antifatigue properties through the biological coupling and cooperation effects of the morphology, configuration and structure. However, as it was hypothesized by Darwin (1859), islands are extreme examples of situations where powers of dispersal are reduced by natural selection and any attempt to disperse could be a severe disadvantage. Thus, Easter Island population of *P. flavescens* seem to have lost their migratory behavior. As was it predicted by Corbet (1962), the type of dispersal in dragonflies is related to its habitat. In isolated, permanent habitats, like Easter Island, dragonflies show specializations that prevent or reduce dispersal; in less isolated habitats, like Tonga, Cook or Fiji Islands, dispersal is neither assisted nor prevented, and occurs as result of fortuitous flights. In temporary habitats, like desert areas of southern Peru, dragonflies are specially adapted for dispersal.

Overall, isolated and remote Islands are model systems to study microevolutionary process because geographic isolation. In this context, individuals inhabiting Easter Island would be under selection and local adaptation and have shown that remote Pacific

Island provide many opportunities for regional and comparative studies of general ecological and evolutionary biology.

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DISCUSION GENERAL

Comprender la interacción entre capacidades de dispersión y conectividad genética entre poblaciones, no solo es importante en términos del entendimiento de los procesos ecológicos y demográficos de las especies, sino que también es crítico para predecir la viabilidad y capacidad de recuperación de las especies frente a disturbios antropogénicos y naturales (Tesson & Edelaar 2013). El objetivo de esta investigación fue determinar los alcances del aislamiento geográfico sobre la estructura genética en una especie con altas capacidades de dispersión. Para ello, patrones de frecuencias alélicas y haplotipicas se estudiaron en individuos del odonato *Pantala flavescens* colectados en localidades continentales (Centro y Sudamérica) e insulares (Islas Maldivas e Islas del Pacífico Sur), de manera de establecer el grado de conectividad genética a largas distancias geográficas y/o a través de barreras oceánicas. Además, mediante herramientas de morfometria tradicional y geométrica se evaluó la existencia de diferenciación morfológica, para evaluar si el aislamiento geográfico ha determinado una diferenciación en caracteres relacionados al vuelo.

Como primera aproximación (Capítulo I de esta tesis), se estimó la diferenciación genética utilizando el índice F_{ST} en el continente americano (Costa Rica, Perú y Chile), para evaluar si las condiciones de clima desértico al sur del Ecuador determinan diferencias genéticas en individuos de *P. flavescens* colectados en estas localidades. F_{ST} mide la variación de las frecuencias alélicas y haplotípicas entre poblaciones, y por tanto la diferenciación genética entre ellas (Eguiarte et al. 2007). En muchos aspectos F_{ST} es un parámetro ideal que nos da una idea de la historia de las poblaciones estudiadas, dando información acerca de la importancia evolutiva del flujo génico y la deriva génica. Como era de esperar para esta especie con alta distribución geográfica, nuestros análisis no reportaron estructura poblacional entre Centro y Sudamérica a pesar de los 5000 km que distancia ambas zonas geográficas, y de las evidentes diferencias climáticas entre ellas. Estos resultados fueron corroborados además por un análisis molecular de la varianza (MANOVA) el cual tampoco reportó diferencias significativas entre ambas regiones geográficas. No obstante, a pesar del alto flujo génico sugerido entre ambas regiones geográficas, nuestros resultados sugieren una mayor diversidad genética y mayor presencia de haplotipos únicos en las localidades ubicadas en Centro América. Una mayor diversidad genética en estas zonas tropicales podría estar relacionada con la hipótesis de rapidez evolutiva, la cual tiene su base en la

idea que las altas temperaturas en los trópicos conducen a mayores tasas de mutación (Miraldo et al. 2016); además la mayor frecuencia e intensidad de precipitaciones otorga una mayor disponibilidad de hábitats acuáticos para las ninfas de odonatos y, por ende, mayor diversidad de especies y mayor diversidad genética (Orr 2006).

Como segunda aproximación, se estimó la estructuración y grado de conectividad genética a gran escala geográfica incluyendo las localidades americanas e islas oceánicas del Pacífico Sur (Isla Tonga, Isla de Pascua) y del Indico (Islas Maldivas). Similar a la primera aproximación, se estimaron tasas de conectividad genética o flujo génico, y tasas de inmigración y emigración entre todas estas localidades. Los resultados de esta segunda investigación refuerzan, por un lado, la existencia de una alta conectividad genética a lo largo del continente americano (bajos valores de F_{ST}), y por otro, entregan evidencias de estructura poblacional entre los individuos colectados en el continente americano y los individuos de *P. flavescens* colectados en islas oceánicas (principalmente Isla Tonga e Isla de Pascua, en el Pacífico Sur). Este patrón de estructura genética observado entre localidades al este y al oeste de la región del Pacifico Sur, sería el resultado de procesos históricos más que contemporáneos, debido a la presencia de una señal filogeográfica que fue revelada por marcadores mitocondriales (Templeton et al. 1995).

De igual modo, el patrón de diferenciación genética observado en las distintas localidades sería de tipo aislamiento por distancia, ya que la mayor diferenciación genética y menor diversidad genética, fue reportada en la isla mas aislada geográficamente como es Isla de Pascua (Figura 1).

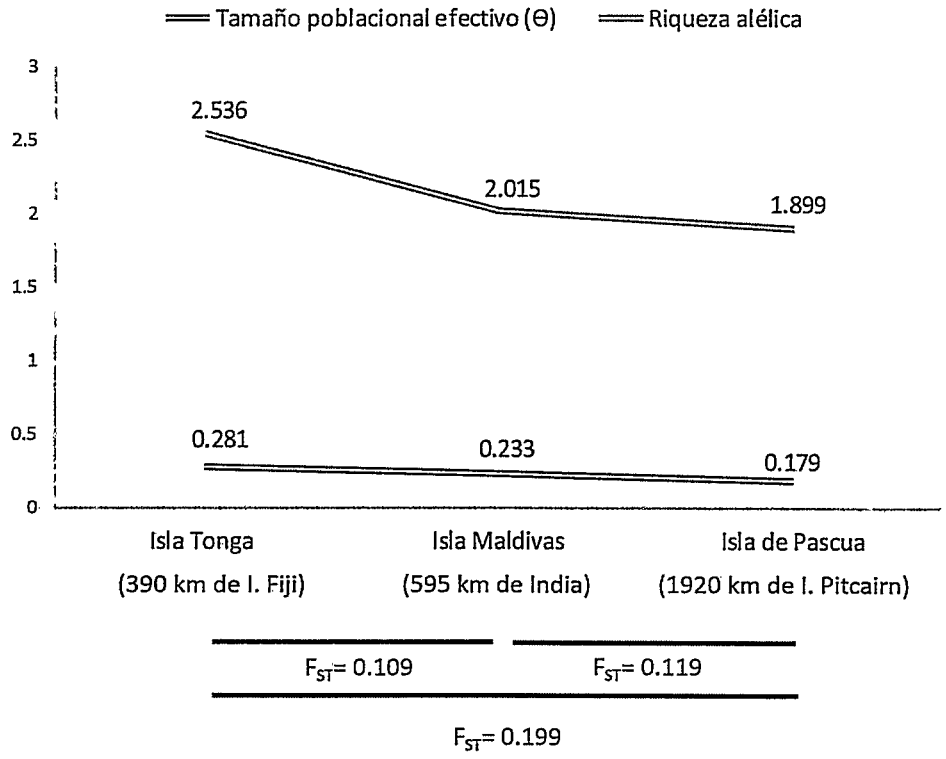

Figura 1. Relación entre tamaño poblacional efectivo (Θ), riqueza alélica y distancia desde la localidad más cercana en los tres sistemas insulares estudiados. Diferenciación genética fue medida por el índice FST en base a frecuencias alélicas.

Un patrón de alta conectividad genética a lo largo de una distribución continental con diferenciación genética en islas ha

sido reportado en otras especies cosmopolitas como la mariposa monarca (Lyons et al. 2012) y el abejorro común (Estoup 1996), en las cuales, al igual que para la especie en estudio, barreras oceánicas ha determinado diferenciación genética y posible especiación en poblaciones insulares. Entonces, la baja diversidad genética reportada en Isla de Pascua sería principalmente resultado de su pequeño tamaño (171 km^2); la escasez de hábitat acuáticos disponibles, los cuales ya se encuentran fragmentados y sometidos a fuertes presiones agrícolas; y su lejanía del continente (casi 4000 km desde la costa chilena), la que impide el arribo de nuevos migrantes y/o dificulta el establecimiento de individuos que han logrado alcanzar la isla. La baja diversidad genética registrada por marcadores microsatélites y mitocondriales en Isla de Pascua, además de los bajos tamaños poblaciones estimados, y los altos valores para coeficiente de endogamia, descritos en el tercer capítulo de esta tesis, estarían dando cuenta de un "efecto fundador" en la isla. Esto debido a que un efecto fundador tiene dos importantes consecuencias. Primero, la población fundadora tendrá menor variación genética que la población original desde la cual derivó; y segundo, las frecuencias alélicas en la población fundadora pueden diferir marcadamente desde aquellas de la población original (Brooker 2015). Si bien, con el tamaño muestral y los marcadores utilizados en esta investigación, no se reportan nuevos haplotipos para la isla; la

presencia de solo dos haplotipos, los cuales también son los haplotipos más comunes encontrados tanto en la población americana como polinésica, y la frecuencia de este haplotipo en la isla, donde solo un individuo de los 90 colectados mostró el segundo haplotipo; estaría reforzando un efecto fundador y a la vez, sugiriendo un evento de colonización reciente de *P. flavescens* en Isla de Pascua. Aún más, las tasas de mutación y la deriva genética tampoco parecen haber favorecido el surgimiento de nuevos haplotipos en la isla. Este último punto podría estaría asociado a que la isla es relativamente joven en términos geológicos (0.24-0.11 Mya) y tendría además una historia ecológica reciente.

De igual manera en este segundo capítulo de la tesis, fueron estimadas las tasas de inmigración y emigración para cada localidad, observando altas tasas de emigración y bajas tasas de inmigración en sistemas insulares, donde Isla de Pascua mostró el número mas bajo de migrantes efectivos. Este patrón de inmigración y emigración reportados en localidades insulares y en especial, en Isla de Pascua, estaría corroborando lo esperado bajo el modelo de biogeografía de Islas de MacArthur & Wilson (1967), el cual predice que el número de especies en una isla debería aumentar con el área de la isla y disminuir con la distancia de la isla al continente; y por lo tanto, poblaciones en islas de gran tamaño exhibirían menores tasas de extinción y poblaciones en islas

distantes del continente tendrían menores tasas de colonización o inmigración, y por tanto de diversidad genética (Figura 1).

Johnson et al. (2000) señalan que debido a que poblaciones insulares están a menudo aisladas de las poblaciones continentales, ellas deberían divergir en el tiempo (tanto genética como morfológicamente) desde las poblaciones fuente respectivas debido a la deriva genética, cambios en las presiones de selección, o ambos. Efectivamente, el aislamiento genético reportado en Isla de Pascua es sustentado por los análisis de variación morfológica, contemplados en la tercera aproximación de esta tesis (Capítulo III), los cuales mostraron diferencias significativas en rasgos morfológicos relacionados al vuelo, como tamaño y forma del ala, y que efectivamente están dando cuenta de una pérdida en la conducta migratoria de *Pantala flavescens* en Isla de Pascua. En efecto, los individuos de Isla de Pascua mostraron alas reducidas en tamaño y con distinta configuración alar la que estuvo principalmente relacionado a una menor extensión del área anal en el ala posterior. Estos caracteres son importantes en la aptitud de vuelo tanto en odonatos como en otros insectos y ha sido reconocido que alas mas grandes son características en especies migratorias de odonatos ya que permiten una mayor capacidad de vuelo (Johansson et al. 2009, Suarez & Sarmiento 2016). No obstante, Dellicour et al. (2007) señala que variaciones intraespecíficas en la forma del ala también pueden estar asociadas

a factores internos como diversidad genética, tamaño corporal y factores de desarrollo como temperatura, siendo muy importante realizar un estudio ecológico mas acabado para dimensionar el impacto de factores ambientales locales sobre rasgos del fitness y del desarrollo en individuos de *P. flavescens* en Isla de Pascua.

Otro resultado importante de destacar es la presencia de un mayor tamaño y/o largo de abdomen en machos de *P. flavescens* en I. de Pascua. Este resultado por un lado estaría dando cuenta de un menor desarrollo de músculos torácicos implicados en el vuelo (Schilder & Marden 2004), y por otro, podría estar involucrado en la mejora de otros rasgos asociados al fitness como la reproducción, ya que un mayor largo de abdomen podría facilitar la adopción de la posición "tándem" durante la copulación. En efecto, machos mas grandes de odonatos han mostrado una mayor probabilidad y éxito de apareamiento que machos de menor tamaño (Tsubaki & Ono 1987, Michiels & Dhondt 1991). En este sentido, dado que el largo del abdomen se relaciona de forma positiva al largo total del individuo (Marinov & McHugh 2010), podriamos esperar un mayor éxito de apareamiento en machos de *P. flavescens* presentes en Isla de Pascua. Entonces, como resultado de esta investigación vemos que *P. flavescens* muestra alta capacidad de dispersión y de conectividad genética a lo largo de extensas áreas geográficas, pero cuando hay continuos o puentes terrestre que faciliten su dispersión, como se observó en las localidades del

continente americano separadas por una distancia aproximada de 4000 km, o como se ha visto entre islas de la Polinesia Francesa; sin embargo, sin la presencia de puentes terrestres o ante una barrera oceánica de grandes extensiones (e.g. mayor a 1500 km que es la distancia entre Isla de Pascua e Islas Pitcairn), esta especie cosmopolita puede mostrar diferenciación genética y morfológica como resultado de los procesos microevolutivos y ecológicos que operan bajo aislamiento poblacional. En este sentido, la población de *P. flavescens* en Isla de Pascua, tiene altas probabilidades de estar sujeta a adaptación local debido al cumplimiento de los supuestos de efecto fundador que fueron inferidos para esta población insular. La capacidad de especiación local para compensar la deficiencia de especies fundadoras ha sido bien ilustrada en otros artrópodos como Lepidopteros de la familia Pyralidae en otras islas del Pacífico por Munroe (1996). Conforme a lo anterior, dado el actual proceso de diferenciación de la poblacion de *P. flavescens* en Isla de Pascua, y dado que una baja diversidad genética se asocia a una mayor capacidad de extinción (Amos & Harwood 1998), es que esta investigación desea promover el desarrollo de posteriores estudios ecológicos y evolutivos en este valioso laboratorio natural y, desea incentivar actividades de educación ambiental que contribuyan a la conservación y preservación de esta especie en la isla.

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