DINÁMICA EVOLUTIVA DE LA HONESTIDAD EN UN SISTEMA DE COMUNICACIÓN PLANTA-POLINIZADOR

Tesis
Entregada A La
Universidad De Chile
En Cumplimiento Parcial De Los Requisitos
Para Optar Al Grado De

Doctora en Ciencias Biológicas con mención en Ecología y Biología Evolutiva

Facultad De Ciencias

Por

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Septiembre, 2015

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FACULTAD DE CIENCIAS

UNIVERSIDAD DE CHILE

INFORME DE APROBACIÓN

TESIS DE DOCTORADO

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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Ha sido aprobada por la comisión de Evaluación de la tesis como requisito para optar al grado de Magíster en Ciencias Biológicas con mención en Ecología y Biología Evolutiva, en el examen de Defensa Privada de Tesis rendido el día 19 de Enero de 2015.

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“El secreto de la genialidad es el de conservar el espíritu del niño hasta la vejez, lo cual quiere decir nunca perder el entusiasmo”

(Aldous Huxley)

BIOGRAFÍA

Nací en Santiago de Chile, el 19 de Mayo de 1978. En el año 1996 me trasladé a Valparaíso, para cursar la carera de Agronomía, en la Pontificia Universidad Católica de Valparaíso. Durante mis estudios de pregrado me interesé por la investigación científica, especialmente por la mejora genética de los cultivos. Así, entre los años 2001 al 2005 colaboré en diferentes proyectos de investigación relacionados con el mejoramiento genético vegetal y conservación de recursos fitogenéticos, en el Laboratorio de Fitogenética molecular. En el año 2004 me incorporé al programa de Magíster en Producción Agroambiental dictado en la Facultad de Agronomía de la misma Universidad. Al terminar este programa postulé al Programa de Doctorado en Ciencias Biológicas, con mención en Ecología y Biología Evolutiva. Ingresé al programa en el año 2006, incorporándome al Laboratorio de Ecología Evolutiva, bajo la tutela del Dr. Rodrigo Medel.

AGRADECIEMIENTOS

Este trabajo no habría sido posible sin la ayuda de numerosas personas que me guiaron, apoyaron y soportaron en las diferentes etapas de este proyecto. En primer lugar le agradezco a Rodrigo Medel por la confianza al haberme integrado al laboratorio y por todo el soporte durante el desarrollo de este trabajo. A Carezza Botto y a mis compañeros del Laboratorio, Gastón, Mauricio, Carmen, Maureen, Claudia, Gabriel, Roxana, Marcela, y Carlos, por las conversaciones, la ayuda en terreno, con el invernadero y con los análisis de datos. A mi familia y amigos, soportar mis ausencias y darme ánimo en los momentos difíciles. Sobre todo por los buenos momentos y por apoyar siempre con entusiasmo mi camino.

Este trabajo fue financiado por CONICYT a través de las Becas 21060185, 24080048, 29090020 y 23100196. Además de ser apoyada por los proyectos Anillo – PBCT ACT-34/2006 y FONDECYT 1150112 (R. Medel).

ÍNDICE DE MATERIAS

LISTA DE TABLAS

Capítulo I

Capítulo II

Capítulo III

Capítulo IV

LISTA DE FIGURAS

Capítulo I

Capítulo II

Capítulo III

Capítulo IV

RESUMEN

En sistemas mutualistas planta- polinizador, las plantas emiten señales hacia los polinizadores para indicar la presencia de recompensas, obteniendo de esta forma el servicio de polinización. Las recompensas a menudo están escondidas, lo que obliga a los polinizadores a guiarse por las señales para obtener las recompensas, lo que crea un conflicto de interés que genera la oportunidad de que aparezcan individuos engañadores. La herbácea *Mimulus luteus* se estudió para abordar el problema de la honestidad en la señalización. La honestidad de las señales se examinó evaluando la significancia en las correlaciones entre señales y recompensas. Se testearon diferentes hipótesis para mecanismos de mantención de honestidad. Usando una aproximación genética cuantitativa se examinó el potencial evolutivo de la honestidad en una población. El principio de la desventaja de Zahavi fue puesto a prueba como un potencial mecanismo para explicar la evolución y mantención de señales honestas y una aproximación geográfica, basada en la Teoría del Mosaico Geográfico de la coevolución, fue usado para examinar el contexto ecológico de la honestidad en un sistema de comunicación planta-polinizador. Los resultados indican que la comunicación entre *Mimulus luteus* y sus polinizadores ocurre a través de señales honestas, que probablemente se mantienen honestas por el potencial costo de engañar, que implicaría la perdida del servicio de polinización. La guía de néctar es una señal importante para indicar la cantidad de néctar contenido en las flores. El análisis de la arquitectura genética de los atributos involucrados en la señalización y recompensas, demostró la ausencia de correlaciones genéticas y la presencia de heredabilidades significativas para sólo dos de los rasgos estudiados, por lo tanto, la evolución de esta población estaría limitada por la ausencia de variación genética aditiva en algunos atributos. Se concluye que *Mimulus luteus* se comunica con sus polinizadores a través de señales honestas, obteniendo de esta forma el servicio de polinización. Los patrones de selección impuestos por los polinizadores a escala geográfica deberán ser investigados con mayor detalle, para aumentar el entendimiento de los mecanismos que producen la amplia variación en los fenotipos observados.

Palabras clave: *Mimulus luteus,* comunicación planta- polinizador, honestidad de señales, señalización, recompensas, heredabilidad, correlaciones genéticas, correlaciones fenotípicas, principio de la desventaja de Zahavi, Teoría del Mosaico Geográfico de la coevolución

ABSTRACT

In plant-pollinator mutualistic systems, plants emit signals to pollinators to indicate the presence of rewards, thereby obtaining the pollination service. Rewards usually are concealed, forcing pollinators to rely on these signals to obtain the rewards, creating a conflict of interest that gives rise to the opportunity of deceitful individuals. The herbaceous *Mimulus luteus* was studied to approach the problem of honest signaling. Honesty of signals was examined by evaluating the significance of correlation between signals and rewards. Different hypothesis for mechanisms for the evolution and maintenance of honesty were tested. Using a quantitative genetic approach, the evolutionary potential for honesty was examined in one population. Zahavi's handicap principle was tested as hypothesis for maintenance of honesty in the system and a geographic perspective based on of the geographic mosaic theory of coevolution was finally used to examine the ecological context of honesty in a plant-pollinator communication system. The results indicate that the communication between *M. luteus* and its pollinators occurs through honest signals, which are probably maintained by the potential cost of loosing the pollination service. The nectar guide is an important signal to indicate the amount of nectar contained in the flowers. The quantitative genetic analysis of advertisement and rewards traits demonstrated the absence of significant genetic correlations and the presence of significant heritabilities for only two floral traits, therefore the evolution of the population is constrained by lack of additive genetic variation in some traits. It is concluded that *Mimulus luteus* communicates with its pollinators through honest signals, thereby obtaining the pollination service. Patterns of selection imposed by different pollinators at la geographic scale need to be further investigated in more detail, to increase our understanding of the mechanisms that produce the large variation in the observed phenotypes.

Key words: *Mimulus luteus,* plant-pollinator communication, signal honesty evolution, advertising, rewards, heritability, genetic correlations, phenotypic correlations, Zahavis's handicap principle, geographic mosaic theory of coevolution.

CAPITULO I

INTRODUCCIÓN GENERAL

1. TEORÍA DE SEÑALES

En el contexto de la economía, la teoría de señales aborda el problema de la reducción de la asimetría de la información entre dos partes en una interacción (revisado en Connelly et al., 2011). Las asimetrías de la información surgen cuando algunos individuos poseen una información que otros individuos no conocen, pero que les permitiría tomar mejores decisiones si la tuvieran (Connelly et al., 2011). De esta forma, existe un emisor que posee una información que no está disponible para un receptor y que en general, es útil para éste. El emisor comunica esta información a través de señales que el receptor interpreta, modificando su comportamiento según su interpretación (Connelly et al., 2011). La teoría de señales se enfoca en entender como las partes resuelven asimetrías de información sobre una cualidad latente y no observable (Connelly et al., 2011).

2. SEÑALIZACIÓN EN LA COMUNICACIÓN ANIMAL

En el contexto de la comunicación animal, la pregunta central es si las señales utilizadas en la comunicación son honestas o no, y cuales son los mecanismos que determinan el resultado de la interacción (Searcy & Nowicki, 2005).

El rol de la confiabilidad de las señales en la comunicación animal ha sido ampliamente estudiado y debatido (ver revisiones en Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005), generando controversias tanto en la definición de comunicación y los términos relacionados (Scott-Philips, 2008; Rendall et al., 2009; Carazo & Font, 2010; Ruxton & Schaefer, 2011), como en los mecanismos que promueven la evolución y mantención de señales honestas (Zahavi, 1975; Grafen, 1990, Számadó, 2011; Fraser, 2012; Grose, 2011; Higham, 2014).

En la revisión de Ruxton & Shaefer (2011) se proporcionan las siguientes definiciones, que serán las que se utilizarán en este trabajo:

1. Comunicación: aplicado a rasgos que fueron seleccionados para la función de comunicar, es decir, señales, es lo que ocurre cuando el valor del atributo de un organismo (el emisor), estimula los sistemas sensoriales de otro organismo (el receptor), causando un cambio en su comportamiento.
2. Señales: Atributos de comunicación de los emisores, que evolucionaron para provocar cambios en el comportamiento de los receptores, donde tanto los emisores como los receptores, en promedio, se benefician de esos efectos.
3. Señales honestas: Señales que contienen información útil para el receptor porque generan un cambio en su comportamiento, que produce, en promedio, beneficios, es decir, ganancias en fitness.

4. Comunicación engañosa: ocurre cuando, en promedio, la respuesta del receptor a las señales, beneficia al emisor pero no al receptor. Esto generalmente implica situaciones en las que el receptor no es capaz de identificar el contexto con precisión, por lo que responde a la señal con respuestas que en otro contexto habrían sido beneficiosas.

Dado que la selección natural actúa a nivel individual, se esperaría que los señalizadores tiendan a beneficiarse mediante el engaño en la comunicación, sin embargo, como la selección natural también actúa sobre los receptores, estos sólo deberían responder a señales que en promedio les reporten beneficios. Pero si los receptores no responden a las señales, entonces es difícil entender la existencia de un sistema de señalización deshonesto (Searcy & Nowicki, 2005).

Desde esta argumentación se desprenden las siguientes preguntas. (1) ¿Son las señales realmente confiables?; (2) ¿Qué mecanismos mantienen la confiabilidad a pesar de los evidentes beneficios de la deshonestidad para los señalizadores?; (3) Si las señales son deshonestas, ¿responderán los receptores de igual forma a estas señales?, y si es así, ¿porqué? (Searcy & Nowicki, 2005).

Zahavi en 1975, propuso un marco teórico para explicar la evolución y mantención de señales honestas, mediante la presencia de señales "*Handicap*" (desventajosas). En este modelo, la honestidad se mantiene por el costo estratégico pagado en el equilibrio, que es adicional al costo de eficacia (necesario para la transmisión de la información), y que solos los señalizadores honestos serán capaces de pagar (Zahavi, 1975, Grafen, 1990, Maynard Smith & Harper, 2003).

Grafen (1990) desarrolló un modelo teórico para validar el marco teórico propuesto por Zahavi, y desde entonces, el "principio de la señal desventajosa" se convirtió una de las teorías más ampliamente conocidas en comportamiento animal y ecología del comportamiento, siendo además adoptada en otros campos, como la psicología evolutiva y la evolución humana (revisado por Higham, 2014). Sin embargo, esta teoría ha recibido bastantes críticas (Lachmann et al., 2001; Grose, 2011; Számadó, 2011; Fraser, 2012, Higham, 2014), en las que se argumenta que la presencia de costos en la producción de la señal no es una característica necesaria ni suficiente para la señalización honesta (Számadó, 2011). Revisiones recientes (Vehrencamp, 2000, Hurd & Enquist, 2005, Searcy & Nowicki, 2005; revisado por Számadó, 2011), están de acuerdo en que la honestidad en la señalización se puede generar a partir de diferentes mecanismos, particularmente cuando: (1) hay intereses comunes, (2) hay restricciones, como en el caso de índices, en los cuales el nivel de señalización está ligado al nivel de lo que se informa, por lo que no es posible engañar, o (3) por el costo de las señales en las interacciones en las que existe un conflicto de interés. Este último punto es el que genera controversia, pues Számadó (2011) concluye que la honestidad en la comunicación es mantenida por el potencial costo de engañar y no por el costo pagado por los señalizadores honestos en el equilibrio, siendo este costo estratégico, contrario a lo propuesto por Zahavi- Grafen, innecesario e insuficiente para explicar la existencia de un sistema de señalización honesto, incluso cuando se habla de sistemas en los que existe un conflicto de interés (Számadó, 2011).

3. COMUNICACIÓN PLANTA POLINIZADOR.

Los sistemas de señalización planta-animal, ofrecen modelos excepcionalmente útiles para testear directamente algunos aspectos de la teoría de señales, sin embargo, no han sido tan ampliamente estudiados como los sistemas de señalización animal (Schaefer et al., 2004). La interacción entre plantas y polinizadores es un mutualismo que, por definición, es una interacción inter-específica en la que ambas partes se benefician, es decir, la inversión que cada organismo hace sobre el otro produce un retorno que supera su costo (Connor, 1995). En esta interacción, los polinizadores obtienen recompensas tales como néctar, polen, ceras y otros elementos necesarios para su supervivencia y/o reproducción. A cambio de estas recompensas, las plantas obtienen el servicio de polinización, incrementando su eficiencia reproductiva (Waser et al., 1996).

Sin embargo, esta interacción no está libre de conflicto. Dado que la producción de recompensas representa, por lo general, un costo energético para las plantas (Pleasants & Chaplin, 1983; Southwick, 1984; Pyke, 1991; Ordano &

Ornelas, 2005; pero ver Leiss et al., 2004), cualquier estrategia que reduzca este costo sin reducir la visita de los polinizadores debería ser favorecido por la selección natural (Benitez- Vieyra et al., 2010). Por otro lado, los polinizadores se beneficiarán al visitar plantas que les porporcionen la mayor cantidad y/o calidad de recompensas (Waddington & Holden, 1979; Rathcke, 1992), por lo que se esperaría que la selección actue sobre las capacidades cognitivas de los polinizadores, para hacer más eficiente la recolección de recompensas, optimizando su costo energético (Chittka et al., 2001; Raine & Chittka, 2008).

Para identificar las recompensas, los polinizadores utilizan ciertas características de las flores como colores, olores, simetría o guías de néctar (Giurfa & Lehrer, 2001), que podrían transmitir información útil a acerca de la calidad y/o cantidad de las recompensas (Weiss, 1991, Schaefer et al., 2004), actuando como elementos de señalización.

En este sentido, una señal honesta debería estar correlacionada con la cantidad o calidad de recompensa, siendo el caso más obvio, cuando las señales son emitidas por las mismas recompensas (e.g. Hansen et al., 2006; Wright & Schiestl, 2009). Sin embargo, en muchos casos las recompensas no pueden ser detectadas directamente por los polinizadores, por lo que estos deben seleccionar las flores que visitan utilizando otros atributos que se correlacionan significativamente con las recompensas (e.g. Cresswell & Galen, 1991) y que actúan como señales.

Las señales de las plantas, a diferencia de las señales emitidas por animales, son relativamente constantes en el espacio y tiempo, sin la posibilidad, por ejemplo, de modificarse en presencia de depredadores, además de ser a menudo menos complejas y dinámicas que las señales animales (Schaefer et al., 2004). Por otro lado, el interés de los receptores es bastante claro, en la mayoría de los casos los animales responden a esta señales en busca de recompensas producidas por las plantas, generalmente alimentarias, lo que permite una evaluación relativamente fácil, tanto de la honestidad de las señales, como de las consecuencias en fitness al reaccionar a ellas (Schaefer et al., 2004). Preguntas importantes que se pueden abordar usando un sistema de comunicación planta- animal se refieren a la honestidad en la señalización: ¿Emiten las plantas señales confiables acerca del contenido y calidad alimenticia de frutos y flores?, ¿Qué tan común es el engaño en estos sistemas? (Schaefer et al., 2004), ¿Qué mecanismos permiten la evolución y mantención de señalización honesta?

Una señal honesta en este caso, estará correlacionada significativamente con la calidad o la cantidad de recompensa. Sin embargo, si bien las correlaciones fenotípicas entre atributos morfológicos florales y recompensas son comunes (Plowright, 1981, 1987; Cresswell & Galen, 1991; Duffield et al., 1993; Mitchell & Shaw, 1993; Campbell, 1996; Davis, 1997; Klinkhamer & van der Veen-van Wijk, 1999; Armbruster et al., 2005; Fenster et al., 2006; Gomez et al., 2008; Benitez-Vieyra et al., 2010), el engaño en la emisión de señales, respecto de las recompensas ofrecidas, ha sido descrito en una gran cantidad de especies (Dafni, 1984; Schiestl, 2005; Renner, 2006). La evolución de flores sin recompensas se ha descrito en al menos 32 familias de angiospermas (Renner, 2006). Hoy se estima que alrededor de un tercio de las especies de orquídeas atraen a sus polinizadores mediante engaño alimentario y otras 400 especies mediante engaño sexual (Cozzolino & Widmer, 2005). Este escenario ofrece la oportunidad de investigar acerca de los mecanismos que promueven la evolución de los sistemas de comunicación entre plantas y polinizadores hacia sistemas de señalización honesta o deshonesta.

Jersákova et al. (2006) revisaron los posibles mecanismos mediante los cuales podrían evolucionar sistemas de polinización por engaño en orquídeas, siendo la hipótesis de que la polinización por engaño promueve la polinización cruzada, dado que es poco probable que los polinizadores visiten varias flores de la misma planta si estas no ofrecen recompensas, la que ha recibido el mayor sustento, tanto teórico como empírico (Thakar et al., 2003; Smithson & Gigord, 2001; Jersákova et al., 2006).

Benitez Vieyra et al. (2010) evaluaron la oportunidad de engaño en la emisión de señales de *Turnera ulmifolia* L. mediante la estimación de selección sobre la precisión de la señal. Hipotetizaron que un bajo nivel de precisión a nivel intra-individual en la correlación entre señal y recompensa (precisión de la señal) permitiría a las plantas una reducción en la producción de recompensas, lo que constituiría una forma de engaño hacia los polinizadores, sin embargo, detectaron selección fenotípica significativa sobre este atributo, lo que estaría restringiendo la evolución de señales deshonestas en este sistema.

4. EVOLUCIÓN DE LA HONESTIDAD

El proceso de adaptación ocurre cuando el fitness varía de manera predecible con una característica fenotípica determinada genéticamente, cambiando la distribución del rasgo entre generaciones consecutivas (Harder & Johnson, 2009). Por lo tanto, el análisis del proceso adaptativo debe evaluar dos aspectos fundamentales: (1) selección fenotípica, o la asociación entre la variación de un atributo y el fitness dentro de las generaciones y (2) en qué medida la herencia genética crea parecido entre los padres y su descendencia; (Harder & Johnson, 2009).

La selección fenotípica requiere que el fitness varíe sistemáticamente con la variación de los atributos entre los individuos (Lande & Arnold, 1983). Existen numerosos estudios en los que se ha evaluado la presencia de selección fenotípica sobre atributos florales (Kingsolver et al., 2001; Geber & Griffen, 2003; Ashman & Morgan, 2004).

Sin embargo, la capacidad de la selección fenotípica de alterar la distribución de un atributo entre generaciones dependerá de la presencia de variación genética, estimada como herdabilidad (h^2) (Falconer & Mackay, 1996; Lynch & Walsh, 1998), y de su asociación genética con otros rasgos, estimada mediante correlaciones genéticas (Conner, 2006).

Ashman & Majetic (2006), revisaron la presencia de valores significativos de h^2 y r_g reportados para atributos florales de distintas clases, (principalmente sexuales, de atracción y de sistemas de apareamiento) en 41 especies de plantas hermafroditas. En promedio, el 39% de la variación fenotípica se podía atribuir a variación genética, indicando una considerable capacidad de respuesta a las presiones selectivas (Harder & Johnson, 2009). Las correlaciones genéticas revisadas por Ashman & Majetic (2006) fueron en su mayoría positivas ($r_g \approx 0,4$) (Harder & Johnson, 2009).

Como se mencionó anteriormente, las correlaciones fenotípicas entre atributos morfológicos florales y recompensas son relativamente comunes (Plowright, 1981, 1987; Cresswell & Galen, 1991; Duffield et al., 1993; Mitchell & Shaw, 1993; Campbell, 1996; Davis, 1997; Klinkhamer & van der Veen-van Wijk, 1999; Armbruster et al., 2005; Fenster et al., 2006; Gomez et al., 2008; Benitez-Vieyra et al., 2010), sin embargo la naturaleza adaptativa de estas correlaciones, dependerá de la arquitectura genética subyacente de los rasgos.

Por una parte, la ausencia de variación genética aditiva limitará la respuesta a las presiones de selección (Falconer & Mackay, 1996; Lynch & Walsh, 1998) tanto de los polinizadores, como de cualquier otro factor de selección presente en la población. Por otra parte, la presencia de correlaciones genéticas positivas entre rasgos de señalización y recompensa limitaría la evolución de señales deshonestas, independiente de los costos que la producción de las recompensas tenga para las plantas, en este caso la honestidad de las señales se explicaría por la imposibilidad de evolucionar deshonestidad, lo que indicaría la presencia de un índice.

5. EL MOSAICO GEOGRÁFICO DE LA HONESTIDAD.

La teoría del mosaico geográfico de la co-evolución, propuesta por Thompson (1994; 2005) plantea un marco teórico para la co-evolución, que resalta la importancia de considerar el contexto geográfico en los estudios evolutivos de interacciones antagonistas y mutualistas. De acuerdo con esta teoría, tres procesos promueven la dinámica coevolutiva, (1) mosaicos de selección geográficos, (2) Puntos cálidos y fríos de co-evolución entremezclados, (3) remezcla de caracteres (Thompson, 2005). Esta teoría ha recibido sustento empírico en sistemas de interacción planta-animal, como por ejemplo, en sistemas de herbivoría (Thompson & Fernandez, 2006; Muola et al., 2010), sistemas de depredación de semillas (Benkman, 1999; Rey et al., 2005; Parchman & Benkman, 2008), sistemas de interacción parásito hospedero en plantas (Medel et al., 2010) y sistemas mutualistas planta- polinizador (Anderson & Johnson, 2008; Gomez et al., 2009a, 2009b).

En el caso de la interacción entre plantas y polinizadores, los fenotipos florales responden a las presiones selectivas impuestas por los polinizadores (Fenster et al., 2004; Harder & Johnson, 2009), sin embargo, esta respuesta depende de varios factores, como por ejemplo, la consistencia espacial y temporal del ensamble, la calidad y cantidad del polen depositado, tasa de visitas, la composición de la flora acompañante, entre otros (Schemske & Horvitz, 1989; Johnston, 1991; Maad & Alexandersson, 2004; Nattero et al., 2010).

Dada la diversidad y complejidad de escenarios de selección que predice la teoría de mosaico geográfico, se esperan distintos resultados respecto de la confiabilidad de señales en diferentes poblaciones de una misma especie. Esta situación podría estar afectada no solo por presiones de selección diferenciales, sino que también por el ambiente abiótico en que se encuentran las poblaciones, y por condiciones intrínsecas de los individuos que conforman la población, siendo todos estos factores en su conjunto los que moldean el fenotipo floral y por lo tanto, la confiabilidad de las señales. Debido a esto, la aproximación al estudio de la dinámica evolutiva de la honestidad de señales debe incluir una aproximación geográfica, que permitirá un entendimiento mayor de los procesos que definen el resultado evolutivo.

6. OBJETIVOS

Basado en lo anterior, este trabajo examinará factores involucrados en la evolución y mantención de sistemas de señalización honesta, utilizando como modelo de estudio a la especie *Mimulus luteus,* para explorar y responder las siguientes preguntas: (1) ¿Es honesta la señalización en *M. luteus*?, (2) ¿Existen restricciones a la evolución de la honestidad? (3) ¿Qué impide o promueve la propagación de estrategias deshonestas?.

Para esto se estudiaron diez poblaciones de *Mimulus luteus*, que fueron cultivadas en jardín común para evaluar rasgos fenotípicos asociados con la señalización, como tamaño de señales, niveles de recompensas y en el caso de seis de estas poblaciones, la producción de óvulos como una aproximación de costo. Además, en una de estas poblaciones se estudió en detalle la base genética de los rasgos asociados a la señalización, mediante el uso de un diseño genético cuantitativo de hermanos completos anidados en medios hermanos.

En el capítulo II, se determinó la base genética de los rasgos asociados al sistema de señalización en una de las poblaciones, ubicada en el centro de la distribución, para examinar el potencial evolutivo de los atributos involucrados en el sistema de señalización.

En el capítulo III se describen los niveles de honestidad y costo de la emisión de señales en seis de las diez poblaciones de *M. luteus* incluidas en el estudio, examinando las correlaciones fenotípicas de los rasgos para determinar si la comunicación es honesta y explorar los mecanismos que podrían explicar el resultado.

Por último, en el capitulo IV, se describe el mosaico geográfico de la honestidad, para examinar el efecto que el contexto geográfico puede tener sobre los resultados evolutivos de esta interacción, estudiando el patrón de honestidad en las diez poblaciones de *M. luteus* incluidas en este estudio.

7. MODELO DE ESTUDIO: *MIMULUS LUTEUS* L. (PHRYMACEAE)

El género *Mimulus* se ha establecido como una planta modelo para estudios evolutivos, debido a su impresionante variabilidad fenotípica, distribución global y además, la presencia de una serie de atributos que lo hacen especialmente adecuado para estudios de ecología evolutiva (Wu et al., 2007). La mayoría de las 160–200 especies de *Mimulus* pertenecen a dos grandes radiaciones, que se centran en Norte América Oeste y Australia. (Vickery, 1978; Beardsley & Olmstead, 2002; Beardsley et al., 2004). Si bien la mayoría de los estudios evolutivos se han llevado a cabo en los *Mimulus* norteamericanos, principalmente en los complejos de especies de *M. guttatus*, y *M. cardinalis* (revisado por Wu et. al., 2007), en los últimos años, se ha incrementado en gran medida el número de estudios que involucran a las especies sudamericanas, y principalmente, pero no exclusivamente, a *M. luteus* (i.e. Medel et al., 2003, 2007; Botto- Mahan et al., 2004; Carvallo & Medel, 2005, 2010; Pohl et al., 2006;

Cooley et al., 2008, 2011; Cooley & Willis, 2009; Medel, 2011; Esterio et al., 2013). En Sud América el género está representado desde Colombia hasta Chile y se extiende hacia las provincias centrales de Argentina en la vertiente Oriental de la Cordillera de los Andes (von Bohlen, 1995).

Mimulus luteus se distribuye en Chile y Argentina, asociado a cursos de agua, entre los 29° y los 45° de Latitud Sur, desde el nivel del mar hasta los 3650 m.s.n.m., ocupando principalmente territorio chileno, mostrando una gran variación en los fenotipos florales, que ha sido asociada a la diversidad de ambientes ecológicos (Medel et al., 2007). *M. luteus* es una especie herbácea, hidrofílica, perenne, con flores hermafroditas, auto-compatibles, con anteras insertas y un estigma bilobulado que se cierra al contacto mecánico (Carvallo & Medel, 2010). La corola amarilla penta-lobulada presenta una conspicua guía de néctar de color rojo en el lóbulo inferior de tamaño, intensidad y forma variable a nivel intra- e inter-poblacional y las flores producen una pequeña cantidad de néctar que tiende a acumularse durante el día (Medel et al., 2003).

El periodo de floración y fructificación se extiende desde mediados de diciembre hasta fines de febrero, con un máximo de floración entre mediados de enero y mediados de febrero. (von Bohlen, 1995; Medel et al., 2003; Botto-Mahan et al., 2004; Medel et al., 2007). Esta especie presenta una amplia variación en la composición de los ensambles de polinizadores entre diferentes poblaciones, desde poblaciones en las que no se observan polinizadores hasta poblaciones con ensambles de polinizadores taxonómicamente diversos (Medel et al., 2007;

Cooley et al., 2008). Las flores de *M. luteus* permanecen abiertas por 3,6 días cuando han sido polinizadas y 5,9 días cuando no lo han sido (Medel et. al., 2003). Si bien esta especie ha sido descrita como auto-compatible, se ha observado que la autopolinización automática produce menor número de frutos por planta y una disminución en la producción de semillas en comparación con plantas que han recibido polen externo (Carvallo & Medel, 2010). Por lo tanto, esta especie requiere de vectores para asegurar una polinización efectiva (Medel et. al., 2003; Pohl et. al., 2006; Carvallo & Medel, 2010). Botto-Mahan et al. (2004) y Pohl et al. (2006) demostraron que la guía de néctar emite radiación en longitudes de onda que son percibidas por los polinizadores. Medel et al. (2003) detectaron selección fenotípica disruptiva sobre la guía de néctar en una población, atribuible a la preferencia divergente de picaflores y abejas. Medel et al. (2007) estudiaron siete poblaciones de *M. luteus* y observaron gran variación en los fenotipos florales en cuanto a tamaño de corola y guía de néctar, asociándose la variación a distintos ensambles de polinizadores. Sus resultados sugieren que el fenotipo floral de *M. luteus* es un rasgo relativamente lábil que es moldeado por los distintos ensambles de polinizadores en las poblaciones locales.

Para este estudio se seleccionaron diez poblaciones de *M. luteus,* distribuidas entre los 30° y los 37° de latitud Sur y entre los 600 y los 3300 metros sobre el nivel de mar, a lo largo de la Cordillera de Los Andes (Tabla 1, Fig 1).

Tabla 1: Distribución geográfica de los sitios de colecta de semilla y tamaños muestrales obtenidos para las diez poblaciones estudiadas.

Población	**Abreviación**	**N**	**Latitud**	**Longitud**	**Altitud (m.a.s.l.)**	**Región**
La Laguna	**LL**	64	30° 16' S	69° 57' O	3.341	IV
El Toro	**ET**	41	30° 57' S	70° 31' O	1.740	IV
Juncal	**PJ**	89	32° 51' S	70° 80' O	2.284	V
Farellones	**FAR**	85	33° 21' S	70° 15' O	2.657	RM
El Yeso	**EY**	92	33° 37' S	70° 01' O	2.580	RM
Laguna Teno	**LTE**	94	35° 05' S	70° 29' O	1.453	VII
Laguna Maule	**LMA**	80	35° 59' S	70° 27' O	2.262	VII
Termas de Chillán	**TCH**	99	36° 54' S	71° 24' O	1.805	VIII
Antuco	**ANT**	74	37° 23' S	71° 26' O	932	VIII
Ralco	**RAL**	83	37° 55' S	71° 33' O	603	VIII

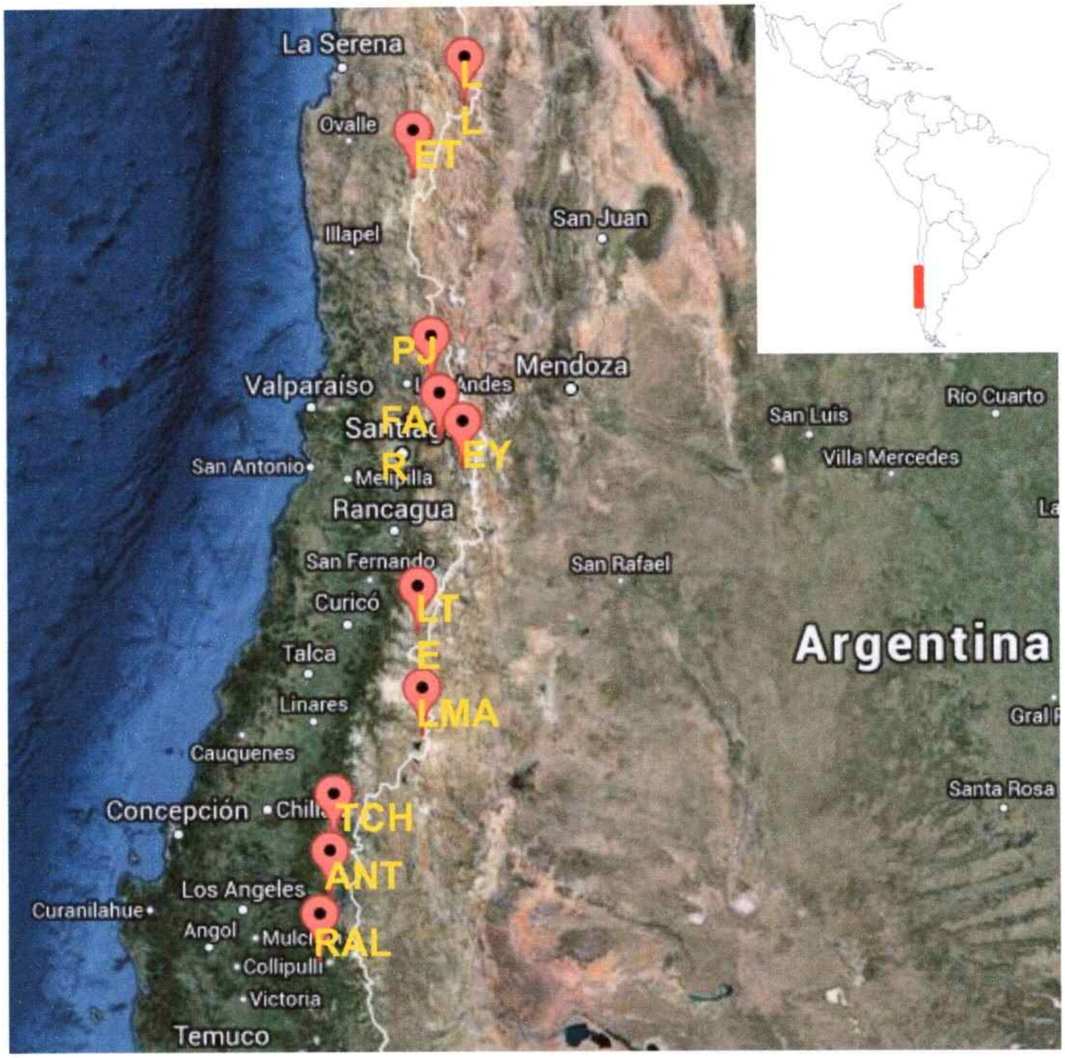

Figura: 1: Ubicación geográfica de los sitios de colecta de semilla, indicados por la marca roja. Los nombres de las poblaciones se indican según las siguientes siglas: La Laguna(LL), El Toro (ET), Juncal (PJ), Farellones (FAR), El Yeso (EY), Laguna Teno (LTE), Laguna del Maule (LMA), Termas de Chillán (TCH), Antuco (ANT), y Ralco (RAL).

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CAPÍTULO II

EVOLUTIONARY POTENTIAL FOR SIGNAL HONESTY IN *MIMULUS LUTEUS* (PHRYMACEAE)

ABSTRACT

In plant pollinator mutualisms signals are used by plants to advertise the presence of rewards to pollinators. Plants benefit from pollinator visits by obtaining the pollination service, whereas the pollinators rely on these signals to guide their foraging behavior. This scenario originates a conflict of interest, due to energetic costs invested by each party, and therefore opposite selection pressures for signal honesty are expected. The estimation of the quantitative genetic basis of the traits under selection is essential to understand the evolutionary output for signal honesty. In this study, we investigated the genetic basis of floral traits in one population of *Mimulus luteus.* Significant heritabilities were detected for only two out of seven traits examined. Floral tube length (0.13) and nectar guide area (0.18). We conclude that honest communication evolution is not restricted in this plant-pollinator communication system by underlying genetic correlations, however, since additive genetic variability for important advertising and rewarding traits, corolla area, nectar traits and pollen, was not detected, evolution of the floral phenotype is not completely free of constraints.

Key words: Heritability, genetic correlation, signal honesty evolution, *Mimulus luteus*, plan-pollinator communication.

INTRODUCTION

The honesty of signals and its role in communication has mainly been investigated in animals (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005), however, this topic is also of high relevance in plant-animal interactions (Schaefer et al, 2004). Plant- animal interactions, and in particular, plant-pollinator interactions are interesting systems to test hypotheses regarding reliable signaling, because pollinators, in many cases, must rely on flower signals to access hidden rewards (Schaefer et al., 2004).

According to Ruxton & Schaefer (2011), a signal is a communicative trait of a signaler organism, that evolved to trigger changes in the behavior of perceivers that benefit (on average) both signalers and perceivers. Honest signals are defined as containing useful information, i.e. the behavioral change in the perceiver led by the signal, produces (on average) fitness gains for the perceiver, because the information is useful to it. Conversely, deceptive communication occurs in situations where (on average) the response of the perceiver benefits the informer but not the perceiver (Ruxton & Schaefer, 2011).

Following this definition, in a plant-pollinator interaction, an honest signal is a flower attribute used by pollinators to guide their foraging behavior, because it provides reliable information on reward status in the flowers, because it is significantly correlated with the quality and/or quantity of reward contained in the flower. Such a signal will benefit the pollinator, which benefits from visiting flowers with high reward quality and quantity (Waddington & Holden, 1979) and the plant, by obtaining the pollination service.

Although, in general, advertising floral traits are positively correlated to rewards, and therefore provide useful information to pollinators (Plowright, 1981, 1987; Cresswell & Galen, 1991; Duffield et al., 1993; Mitchell & Shaw, 1993; Campbell, 1996; Davis, 1997; Klinkhamer & van der Veen-van Wijk, 1999; Armbruster et al., 2005; Fenster et al., 2006; Gomez et al., 2008; Benitez- Vieyra et al., 2010), deceptive signals are also widely spread among angiosperms, and the evolution of unrewarding flowers with conspicuous displays has been described in at least 32 Angiosperms families (Renner, 2006). This poses the question on the mechanisms by which honest signaling evolves and is maintained in plant pollinator interactions.

According to signaling theory, honest signals can be generated by three main mechanisms: (1) shared interest, (2) constraints, i.e. indices, where signaling level is linked to level of what is informed, making impossible to cheat, and (3) due to the costs involved in the signaling interaction, in situations of conflicted interests between parties (Zahavi, 1975, Grafen, 1990, Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005; Számadó, 2011).

Because reward production commonly represents an energetic cost for plants (Pleasants & Chaplin, 1983; Southwick, 1984; Pyke, 1991; Ordano & Ornelas, 2005; but see Leiss et al. 2004), any strategy decreasing this cost without affecting its reproductive success should be favored by natural selection (Benitez- Vieyra et al., 2010). But, pollinators will benefit from optimizing its foraging behavior, visiting plants that provide the highest amount and quality of rewards (Waddington & Holden, 1979; Rathcke, 1992), and therefore, natural selection should be acting on its cognitive capacities to allow the detection of the most rewarding flowers, therefore, optimizing the energetic expenditure (Chittka et al., 2001; Raine & Chittka, 2008). This situation generates a conflict of interest between the parties and therefore, conflicting selection on the reliability of the signals should be expected.

The adaptive process requires the occurrence of two important factors, phenotypic selection on floral traits and genetic variation for those selected traits (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Absence of additive genetic variation will constrain the adaptive response to selection (Falconer & Mackay, 1996; Lynch & Walsh, 1998) either from pollinators or from any other selection factor in the population. Genetic correlations between signals and rewards can constrain the evolution of honest or deceptive signals, even if additive genetic variability is available and strong phenotypic selection is acting upon phenotypes (Conner, 2002; Conner, 2006). If signals and rewards are positively genetically correlated, a dishonest strategy will not be possible, and the signaling trait would become an index, linked to the advertised reward.

Although phenotypic selection has been widely documented for floral traits in many plant species (Ashman & Morgan, 2004; Harder & Barret, 2006), genetic variation and heritability for these traits, and particularly for floral reward traits has been much less frequently studied (Mitchell, 2004) In Ashman & Majetic (2006) review, average heritability reported for reward traits (i.e. aspects of nectar production such as rates, volume, concentration, and sugar content, and nectary size), was 0.29, whereas for corolla morphology traits averaged 0.46. The number of studies reporting heritability for rewards was significantly lower than studies reporting genetic basis for other floral traits.

According to Mitchell (2004), only six studies have estimated genetic basis for nectar traits (Mitchell & Shaw, 1993, Campbell, 1996; Boose, 1997; Klinkhamer & van der Veen-van Wijk, 1999, Vogler et al., 1999, Worley & Barret, 2000). Kaczorowski et al. (2008) investigated the genetic basis for nectar traits and floral morphology, detecting significant heritabilities for nectar volume and energy content, being these traits positively genetically correlated between them. However, they did not detect significant genetic correlations between signals and rewards. Significant genetic correlations between nectar traits and some aspect of floral morphology were detected only by Mitchell land Shaw (1993) and Klinkhamer & van der Veen-van Wijk (1999).

Understanding the quantitative genetics of the traits involved in the signaling interaction will provide insights on mechanisms for signal honesty, and the estimation of the potential outputs for the signals, in response to phenotypic selection.

The main objectives of this study were to determine whether the signals emitted by *M. luteus* are honest and to estimate the quantitative genetic basis of floral trait variation, to explore the evolutionary potential for honesty in this system. There fore, the data was used to answer the following questions: (1) Does *M. luteus* emit honest signals to its pollinators? (2) Is there significant genetic variation for and genetic correlations among floral traits in *M. luteus*? (3) Is there potential for this genetic variances and correlations to constrain the evolution of honest signaling?

MATERIALS AND METHODS

1. STUDY MODEL: *MIMULUS LUTEUS* L.

Mimulus luteus L. (Phrymaceae) is hydrophilic herbaceous, perennial species that inhabits streams and wetlands in the South American Andes from 0 – 3650 m.a.s.l. between 29° -45°S (von Bohlen, 1995). In Chile, flowering and fruiting occur form December to February. This species produces zygomorphic flowers with yellow corollas composed of five petals. In most cases, the lower lobe presents a conspicuous red spot, which acts as a nectar guide. The guide size is more variable between than within plants (Medel et al., 2003). The flowers emit radiation in wavelengths that can be detected by pollinators (Botto-Mahan et al., 2004; Pohl et al., 2006). The flowers produce small amounts of nectar, which accumulates during the day (von Bohlen, 1995, Medel et al., 2003, Botto-Mahan et al., 2004, Medel et al., 2007). Even though this species is described as self-compatible, previous studies performed in different populations indicated that automatic self-pollination produces a significantly less seed by, compared to cross-pollinated plants (Medel et al., 2003; Pohl et al, 2006; Carvallo & Medel, 2010). Therefore, the species is dependant on vectors for effective pollination (Medel et al., 2003; Pohl et al, 2006; Carvallo & Medel, 2010).

2. EXPERIMENTAL DESIGN

To create a quantitative genetic design of full-sibs nested within half seeds, to investigate the genetic structure of signaling related traits in *M. luteus*, .one fruit per individual was collected haphazardly from 200 individuals in one population of *Mimulus luteus* located in Juncal (32°51'S, 70°08'W, 2284 m.a.s.l., V Region, Chile), 80 km northeast of Santiago, during February 2006. This is a high Andean population that is completely covered by snow at least three months per year. The vegetation type corresponds to associations of the subalpine belt (Hoffmann et al., 1998).

Ten seeds per fruit were germinated in the greenhouse by mid August 2006. Only one randomly selected individual per fruit was transplanted to and individual pot and grown in the Greenhouse of the Evolutionary Ecology

Laboratory, at Facultad de Ciencias at Universidad de Chile in Santiago, Metropolitan Region, Chile.

The parentals were grown from August 2006 to January 2007 and the genetic families from January 2007 to June 2007, in homogeneous environmental conditions in terms of substrate, irrigation, pest and diseases management, temperature and luminosity.

For the parental plants, at flowering stage, crossings were made to obtain maternal full sibs families by crossing each male with three females; the target was to have 90 maternal full sib families within 30 half sibs paternal families. Plants were randomly designated as female or male without replacement. The seed from the crosses was harvested and prepared immediately for the second crop cycle, to grow the half sibs- full sibs' genetic design.

Six individuals from each harvested cross were randomly selected to be sown and grown in individual pots in the greenhouse, resulting in 68 maternal full-sib families nested within 30 paternal half-sib families, with 1 to 6 individuals within each family, totalling 351 individuals organized in a complete randomized design. Each individual was characterized for floral attributes, advertising traits and rewards, at flowering stage. At least three flowers per individual were measured and averaged to obtain the individual values.

3. PHENOTYPE CHARACTERIZATION

Traits evaluated included floral tube length (FTL) in mm, nectar guide area (NGA), and corolla area (CA) in mm^2, nectar volume (NV), sugar content in the nectar, measured as soluble solids (SS) in °brix, number of pollen grains per flower (POL), and number of ovules per flower (OV). To measure FTL, NGA, and CA, lateral and frontal photographs were taken in three to five flowers per individual on the first day of anthesis (Figure 1).

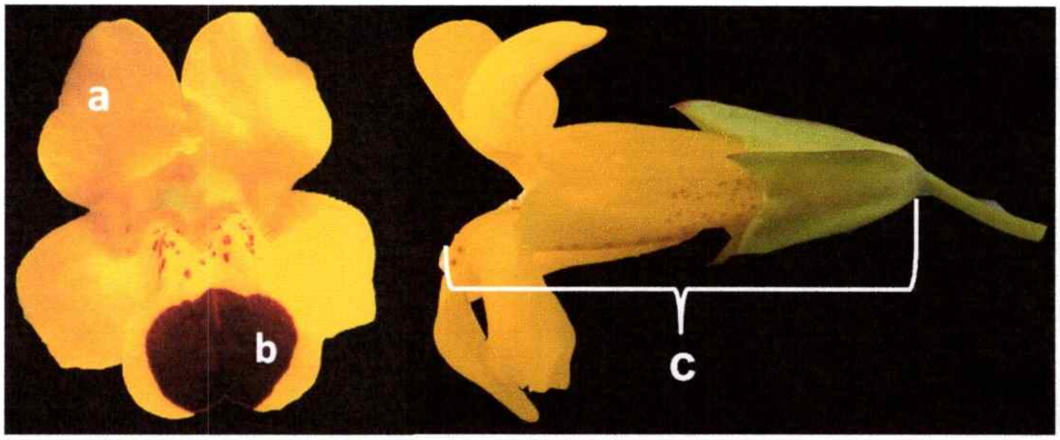

Figure 1: Floral traits measured as putative signals emitted to pollinators. (a) Corolla area (CA), which includes the nectar guide area, (b) Nectar guide area (NGA) (to calculate the total NGA area all the red spots within the flower "throat" were added to the main red area), and (c) Floral Tube Length.

Each photograph included a graded scale, allowing the measurement of tube length in mm and the conversion of a linear measurement in pixels, to obtain the areas of corollas and nectar guides in mm^2, using the software Image J (available at http://rsb.info.nih.gov/ij/download.html (Figure 1).

Nectar volume was measured using calibrated 5μl glass capillary tubes (Drummond Scientific, Broomall, PA USA). In every sample extracted, nectar volume was registered by measuring the height of the nectar column in the micro-cap, using a digital caliper (Mitutoyo America Corporation, Aurora, IL, USA), this number was transformed afterwards to micro-liters (μl). Soluble solids concentration was measured using a temperature-calibrated handheld Brix refractometer (PAL-1, ATAGO CO., LTD., Tokyo, Japan). To estimate the number of pollen grains and ovules, three flower buds were collected on the day previous to anthesis, to avoid pollen losses at flower opening. The flower buds were fixed in Formalin-Acetic-Alcohol (FAA) solution for 24 hours, and then storage in ethanol 70% until processed in the laboratory.

Pollen grains and ovules were counted using a particle counter (Beckman Coulter Z1, GMI, Inc.). For the counting, the complete amount of pollen (4 anthers) or ovules (full content in the ovary) were extracted and suspended in 1 ml 1% NaCl solution. An aliquot of 0.5 ml was diluted in 10 ml of solution, to be counted in the particle counter. Dilution factors were accounted when calculating the total number of pollen grains or ovules per sample. Numbers reported correspond to the average per flower per plant.

4. STATISTICAL ANALYSIS

4.1. Signal Honesty

Signal honesty was examined through Pearson's correlations between variables, using average values per individual.

Reward traits, nectar volume (NV), soluble solids content in the nectar (SS) and number of pollen grains (POL) were correlated with signaling traits, corolla area (CA), and nectar guide area (NGA). A significant correlation coefficient was considered an honest signal, whereas a non significant correlation was considered as dishonest. To examine a potential trade-off in resources allocation for signal or reward production, the number of ovules produced per flower (on average per individual) was correlated with signaling and reward traits. All the analyses were performed using JMP (JMP 10, SAS Institute Inc. North Carolina U.S.A.). *P*-values for the pairwise correlations were set to 0.05 and sequential Bonferroni correction for multiple tests (Rice, 1989) was used to test for statistical significance.

4.2. Quantitative genetics

Phenotypic variation in *Mimulus luteus* floral, rewards and gametes traits was partitioned into genetic and environmental components of variation using a generalized mixed model approach. Restricted Maximum Likelihood (REML) analyses were used to estimate the additive genetic variance-covariance matrix, along with heritabilities (h^2) and genetic correlations (r_g) for the seven floral traits. REML is preferred to the traditional ANOVA approach when the data is unbalanced (Shaw, 1987).

The software ASReml 3 (VSN International Ltd.) was used to perform the analyses. ASReml-generated standard errors for h^2 and r_g were used to determine if these genetic parameters were significantly different from zero (as in Elle, 1998). Because heritabilities vary between 0 and 1, whereas genetic correlations range between -1 and 1, one- and two- tailed one sample *t*-tests were respectively used to determine its significance The *t*-statistic was calculated by dividing h^2 or r_g by the standard error generated by ASReml (Zar, 1999). *P*-values for the t-test were set to 0.05 and sequential Bonferroni correction for multiple tests (Rice, 1989) was used to test for statistical significance.

RESULTS

1. FLORAL TRAIT VARIATION AND SIGNAL HONESTY

Between March and May 2007, 351 individuals composing a quantitative genetic design of full sibs nested within half sibs design were characterized for floral traits.

Advertising traits (tube length (FTL), nectar guide area (NGA) and corolla area (CA)), reward traits (nectar volume (NV), sugar content in the nectar (SS), and number of pollen grains per flower (POL)), and the number of ovules per flower (OV) were measured in three to five flowers per individual. Trait mean, standard deviation (SD), standard error, and coefficient of variation for all traits are shown in Table 1.

The least variable trait was the flower tube length (CV= 9), followed by corolla area, whereas the most variable was the number of ovules per flower (CV= 78). Rewarding traits and nectar guide area presented a similar intermediate variation (CV= 20 – 40) (Table 1).

Table 1: Means, sample sizes (N) standard deviations (SD), standard errors (SE) and coefficients of variation (CV) for all seven floral traits in one population, Juncal (PJ) of *Mimulus luteus*. Floral traits include: Floral Tube Length (FTL), Nectar Guide Area (NGA), Corolla Area (CA), Nectar Volume (NV), Soluble Solids in the nectar (SS), Number of pollen grains per flower (POL) and Number of ovules per flower (OV).

Floral Trait	N	Mean	SD	SE	CV
Floral tube lenght (FTL)(mm)	327	33.37	2.96	0.16	8.86
Nectar Guide Area (NGA)(mm^2)	350	2.48	0.75	0.04	30.31
Corolla area (CA) (mm2)	350	507.63	101.60	5.43	20.02
Nectar Volume (NV) (µL)	325	1.41	0.38	0.02	27.12
Soluble solids (SS) (°brix)	294	6.59	2.62	0.15	39.73
# Pollen grains (POL)	284	74466.50	29523.42	1751.89	39.65
# Ovules (OV)	269	617.12	480.32	29.29	77.83

Pearson's correlations were examined to determine significant associations between traits (Table 2). Significant phenotypic correlations were detected between most measured traits.

Table 2: Pearson's correlations coefficients (r) for seven floral traits in one population of *Mimulus luteus*. Floral traits include: Floral Tube Length (FTL), Nectar Guide Area (NGA), Corolla Area (CA), Nectar Volume (NV), Soluble Solids in the nectar (SS), Number of pollen grains per flower (POL) and Number of ovules per flower (OV). Bold numbers indicate significant correlations at $p<0.05$ after sequential Bonferroni correction (Rice, 1989).

	FTL	NGA	CA	NV	SS	POL	OV
FTL	-						
NGA	**0.19**	-					
CA	**0.52**	**0.28**	-				
NV	**0.43**	**0.26**	**0.28**	-			
SS	0.05	**0.19**	0.07	0.14	-		

POL	**0.27**	**0.17**	0.16	**0.19**	**0.24**	-	
OV	0.11	0.04	0.14	0.13	**0.26**	**0.17**	-

Advertising traits, FTL, CA and NGA were significantly correlated with rewards, NV, SS and POL, indicating that these flower attributes are reliable indicators for the amount and quality of rewards present in the flowers (Table 2). All flower morphology attributes, FTL, CA and NGA were significantly correlated, between each other, and all correlations detected were positive (Table 2). The number of ovules per flower was significantly and positively correlated with the number of pollen grains and the amount of sugar in the nectar, no negative correlations with ovule production were detected (Table 2).

2. HERITABILITY AND GENETIC CORRELATIONS

Variance-covariance genetic matrix was estimated for seven traits in *Mimulus luteus*. Significant heritabilities were detected for only two traits, floral tube length (h^2= 0.13) and nectar guide area (h^2= 0.18) (Table 3). Significant genetic correlations were not detected between any of the traits studied, despite the significant correlations observed at the phenotypic level.

Table 3: Narrow sense heritabilities (on the diagonal) and genetic correlations (off-diagonal) for seven floral traits in one population (PJ) of *Mimulus luteus*. Floral traits include: Floral Tube Length (FTL), Corolla Area (CA), Nectar Guide Area (NGA), Nectar Volume (NV), Soluble Solids in the nectar (SS), Number of pollen grains per flower (POL) and Number of ovules per flower (OV). Bold numbers indicate significant correlations at *p<0.05* after sequential Bonferroni correction (Rice, 1989).

	FTL	**NGA**	**CA**	**NV**	**SS**	**POL**	**OV**
FTL	**0.13**						
NGA	0.28	**0.18**					
CA	0.70	0.01	0.10				
NV	0.13	0.52	0.65	0.13			
SS	0.08	0.13	-0.02	0.16	0.19		
POL	0.36	0.26	0.59	0.10	0.32	0.18	
OV	0.06	0.16	0.45	0.02	0.15	0.10	0.18

DISCUSSION

In this study, the association between visual signals and rewards in one population of *Mimulus luteus* was examined to assess the honesty of signals in this communication system. Moreover, the quantitative genetics of traits involved in the advertising and reward system was estimated, to allow the exploration of the mechanisms and the potential for honesty evolution in this plant pollinator signaling system.

1. SIGNAL HONESTY

Nectar guide area was significantly positively correlated with all rewards measured (nectar volume and concentration and pollen amount). The size of the flowers, i.e. flower tube length and corolla area, was significantly positively correlated with nectar volume, whereas floral tube length was correlated also to the amount of pollen contained in the flower (Table 2). Therefore, these floral attributes can be reliably used by pollinators to guide their foraging behavior, and if pollinators are responding to them, as reported in previous work (Medel et al., 2003), these attributes constitute honest signals to pollinators.

According to signaling theory, a potential explanation for the occurrence of honest signaling when interest between parties are in conflict, as in plant pollinator interaction, is that the signals are indexes, i.e. the signaling attribute is physically linked to the trait informed and therefore its impossible to cheat (Searcy & Nowicki, 2005). Since in *Mimulus luteus* nectar is hidden within the flowers and therefore the pollinators can not directly assess the rewards contained in flowers, other floral attributes are used to guide reward recollection (Medel et al., 2003). If these floral attributes (i.e. corolla and nectar guide size and shape) were indexes, significant genetic correlations between signaling and reward traits, generated by pleiotropy or by strong linkage disequilibrium may be constraining the decoupling of the signal and the reward in response to selection towards dishonesty (Conner, 2002; Conner, 2006). These genetic correlations could be generated from a strong and stable correlational selection upon the association of signal and reward across generations (Sinervo & Svensson, 2002), imposed by pollinators' choice.

No significant genetic correlations between traits were detected in our quantitative genetic analysis, which implies that no restriction for the occurrence of a dishonest advertisement strategy is imposed by the underlying genetic correlation structure between traits (Conner, 2002; Conner, 2006). Therefore, a mechanism based on the costs involved in the signaling system might be involved in the maintenance of signal honesty (Zahavi, 1975; Grafen, 1990; Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005; Számadó, 2011).

We evaluated the correlation between the signals and the amount of ovules produced, to explore potential trade-offs due to resources allocation in the production of the signal, as a potential explanation for signal honesty, following "Zahavi's handicap principle", which states that the cost in the production of the signal will prevent dishonesty (Zahavi, 1975; Grafen, 1990). However, no significant correlations between signals and the amount of ovules were detected. Moreover, significant positive correlations between the amount of sugar in the nectar and the amount of pollen with the number of ovules were detected; therefore, a reproductive trade-off can not be invoked as a mechanism for the maintenance of honesty, at least with the available data.

According to Számadó (2011), it is not the realized cost paid by honest signalers what maintains honesty, but the potential cost of cheating. As long as this potential cost is high enough, the realized cost for honest signalers can be zero or even negative (Hurd, 1995; Számadó, 1999; Lachmann et al., 2001).

A previous study reported pollinator mediated selection on the nectar guide phenotype (Medel et al., 2003), moreover, it was determined that hummingbirds and bees exhibited divergent preferences for corolla and nectar guide sizes in *M. luteus*, which resulted in disruptive selection (Medel et al., 2003). Although it was demonstrated that the insect pollinator assemblage preferred flowers with large corollas and large nectar guides, a significant correlation between corolla and nectar guide sizes with nectar traits was not detected (Medel et al., 2003), and one of the potential explanations proposed was the hypothesis by Little (1983), which argues a that a dishonest strategy can be successful when newly emerged insects are attracted to flowers by dishonest signals before they learn to recognize the signals. However, if the pollinator assemblage becomes more constant, pollinators will learn to recognize reliable signals and cheater individuals will loose the pollination service, which is demonstrated by phenotypic selection acting on reliable signals, which has been reported by some recent studies (e.g. Armbruster et al., 2005; Benítez-Vieyra et al., 2010), and therefore, the "punishment of the cheater" mechanism is a plausible explanation for the presence of honest signals in this population.

Gonzalez-Browne (2014) evaluated the correlation between signals and rewards in this same population, Juncal, in the field during the summer of 2012-13, detecting significant phenotypic correlations between corolla area, nectar volume and number of pollen grains and also, a strong correlation between corolla area and nectar guide area. In this same study, significant phenotypic selection gradient was detected for corolla area. Moreover, although in the other population studied, El Yeso, a significant correlation gradient for nectar volume and nectar guide size was detected, indicating that pollinators favor plants showing a positive advertisement-reward association (Gonzalez-Browne, 2014). Gonzalez- Browne (2014) characterized the pollinator assemblage in both populations studied, Juncal and El Yeso, determining that 6 different species were pollinators for the populations, being the assemblages similar between them. A pollen limitation experiment was performed by Gonzalez-Browne (2014), confirming the results reported by Medel et al., (2003) and Carvallo & Medel (2010) in other populations, which indicate that *Mimulus luteus* even though self-compatible, has probably some mechanism to prevent self- pollination, because the reproductive success of cross pollinated plants was much higher than those with automatic self-pollination, therefore supporting the idea that pollination service is important for *M. luteus*.

All this evidence demonstrates that the pollinator service is important for *M. luteus* and therefore, the potential cost of loosing it, could be maintaining the honesty of the signals.

2. EVOLUTIONARY POTENTIAL FOR SIGNAL HONESTY

Since Darwin it has been hypothesized that the evolution in floral traits is the result of natural selection mediated by pollinators (reviewed by Armbruster & Muchhala, 2009) and also, in more recent reviews, the result of selection pressures imposed by other antagonistic agents (Strauss, 1997; Strauss & Whittall, 2006).

According to Harder & Johnson (2009), despite selection on individual traits acting only sporadically within populations and inconsistently among populations; experimental studies of pollination function usually demonstrate the adaptive nature of floral and inflorescence traits, which, along with the patterns revealed by phylogenetic studies, is consistent with a key role for adaptation of pollination systems in floral diversification (Harder & Johnson, 2009).

For the adaptive process and evolution to take place, two important conditions must be met, phenotypic selection acting upon floral traits and genetic variation for those selected traits (Falconer & Mackay, 1996; Lynch & Walsh, 1998). Therefore, the absence of additive genetic variation will constrain the adaptive response to selection (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

The genetic basis of advertisement and rewards was examined in an attempt to understand the evolutionary potential of this communication system, in terms of the ability to respond to the selection pressures potentially imposed by biotic and abiotic factors on the traits involved in advertising and rewarding pollinators.

Significant heritabilities were detected only for two traits, floral tube length, and nectar guide area (Table 3), which is an indication that the system is limited to respond to selection pressures imposed on reward amounts or corolla area, due to lack of available additive genetic variation for this traits.

The determination of the genetic basis of floral traits has been investigated by several authors in different plant systems (reviewed by Ashman & Majetic, 2006). Although genetic variation and heritability for floral reward traits has been much less frequently studied (Mitchell, 2004), in our study the heritabilities detected were much lower than the average values reported in the literature. According to Ashman & Majetic (2006), average heritability reported for reward traits (i.e. aspects of nectar production such as rates, volume, concentration, and sugar content, and nectary size), was 0.29, whereas for corolla morphology traits averaged 0.46. The result of the low and non significant heritabilities detected in our study could be the result of historically strong selection on floral attributes. It has been argued that traits closely related to fitness, under strong directional selection, may have depleted additive genetic variance and lower heritability than morphometric or physiological traits (Fisher, 1930). However, Price & Schluter (1991) suggested that low heritability of fitness related traits can be due to high level of residual variance, rather than reduced additive genetic variance. Indeed, empirical evidence gathered on the wild generally supports the idea that low heritability of fitness linked traits arises because of large residual variance (Houle, 1992). It has been argued that low heritabilities for nectar traits may be the result of high environmental variances, caused by extreme sensitivity to environmental conditions, as well as the variability in pollinator, florivore, and herbivore activity in the field (Rathcke, 1992). In the latter case, high environmental variation may pose a serious constraint to the effectiveness of pollinators as selective agents on nectar traits.

Based on our results, we conclude that honesty evolution in this population is not restricted by genetic correlations, or trade-offs between rewards and reproductive structures (ovules). Which implies that, at least form this perspective, the population can evolve and maintain honest signals. However, since we were not able to detect significant additive genetic variation for corolla area and nectar volume, these two traits cannot evolve in response to selective pressures, which will restrict the evolution of the system if phenotypic selection upon these traits is confirmed.

ACKNOWLEDGMENTS

We thank Gastón Carvallo, Carlos Pino, and Carmen Gloria Ossa, for their invaluable contribution to field, greenhouse and laboratory work and Roxana Cares for her support in data analysis. This research was funded and supported by Anillo - PBCT ACT-34/2006 and CONICYT 21060185; 24080048, 29090020, and 23100196 scholarships to PS.

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CAPÍTULO III

SIGNAL HONESTY IN *MIMULUS LUTEUS* L. (PHRYMACEAE) AND "ZAHAVI'S HANDICAP PRINCIPLE"

ABSTRACT

Signaling theory is primarily concerned with honesty in communication when information asymmetry occurs between two parties. In the context of mutualistic plant-pollinator systems, the plants emit signals to pollinators to indicate the presence of rewards within the flowers, obtaining the pollination service in return. Rewards are, in most cases, concealed within the flowers, which obligate the pollinators to rely on these signals to obtain them. This scenario originates a conflict of interest, providing the opportunity for the development of dishonest strategies, which poses the question of which are the mechanisms that will promote honesty in such a system. Zahavi's handicap principle states that to stabilize an honest signaling system, the signals must be costly for the emitters, and therefore, only honest individuals will be able to afford signal production. To address this question, six populations of *Mimulus luteus* were studied, to examine the variation in the associations between signals and rewards, and estimate the potential cost of signal production, to test the applicability of Zahavi's principle to this system. High variation was detected for all traits among populations. Significant associations between signals, corolla area, and nectar guide area, and rewards, nectar volume and number and pollen grains, were detected. However the associations were variable between populations. It was not possible to detect direct costs of production in any of the honest signals indentified. Therefore we were not able to verify Zahavi's handicap principle as the mechanism to explain honest signals. An alternative mechanism proposed is the "punishment of the cheater" as the most plausible mechanism for stabilizing honesty in this system. However the realization of the cost by that mechanism remains to be tested. It was concluded that the communication between *M: luteus* and its pollinators is an honest communication system.

Key words: *Mimulus luetus,* plant-pollinator communication, signal honesty, deceptive pollination, signaling cost, Zahavi's handicap principle.

INTRODUCTION

1. SIGNALING THEORY

Signaling theory is useful to describe a communication interaction between two parties, an emitter and a receiver, which have differential access to a piece of information that is communicated through signals. (Connelly et al. 2011). And is mainly concerned on how those asymmetries on information about a latent and non observable attribute are resolved between parties (Connelly et al., 2011).

In the context of animal communication, the central question is whether the signals used to communicate are honest or not, and which are the mechanisms that will determine the result of the interaction (Searcy & Nowicki, 2005).

The role of signal reliability in animal communication has been widely studied and debated (see reviews by Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005), generating controversies in both the definition of communication and related terms (Scott-Philips, 2008; Rendall et al., 2009; Carazo & Font, 2010; Ruxton & Schaefer, 2011), and on the mechanisms that will promote and maintain honest signaling systems (Zahavi, 1975; Grafen, 1990, Számadó, 2011; Fraser, 2012; Grose, 2011; Higham, 2014).

Ruxton & Schaefer (2011) defined a signal as a communicative trait of a signaler organism, that evolved to trigger changes in the behavior of perceivers that benefit (on average) both signalers and perceivers. Honest signals are defined as containing useful information, i.e. the behavioral change in the perceiver led by the signal, produces (on average) fitness gains for the perceiver, because the information is useful to it. Conversely, deceptive communication occurs in situations where (on average) the response of the perceiver benefits the informer but not the perceiver (Ruxton & Schaefer, 2011).

Because natural selection acts at the individual level, it is expected that signalers will benefit by providing dishonest signals in situations of conflicting interests, however, since natural selection is also acting on the perceivers, these should only respond to signals that, on average, are beneficial for them (Searcy & Nowicki, 2005). But, if perceivers do not respond to signals, it's hard to understand the existence of a deceiving communication system, posing the following questions: (1) Do signals convey reliable information?; (2) Which are the mechanisms that maintain reliability in spite of the evident benefits of cheating?; (3) If the signals are deceiving, will the perceivers respond to them anyways?, and if so, why? (Searcy & Nowicki, 2005).

Zahavi (1975) proposed a theoretical background to explain the evolution and maintenance of honest signals, stating that honesty can be maintained by "handicaps". Handicaps are defined as signals that are costly to produce, containing an "efficacy production cost", which is associated with ensuring that the message is received; and a "strategic production cost", which will prevent dishonest individuals to produce the signal, and therefore maintain the honesty of the system (Higham, 2014). Grafen (1990) used a game theoretical approach to argue that strategic costs could create honesty, validating Zahavi's theoretical background, and since then, the "Handicap Principle" has become the theoretical framework more widely accepted and cited as the mechanism by which honest signals are maintained, when a conflict of interest occurs between interacting organisms (Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005; Higham, 2014). However, this theory has been criticized by several authors (Lachmann et al., 2001; Grose, 2011; Számadó, 2011; Fraser, 2012, Higham, 2014).

Recent reviews (Vehrencamp, 2000, Hurd & Enquist, 2005, Searcy & Nowicki, 2005; reviewed by Számadó, 2011) agree that signal honesty can be generated by three main mechanisms: (1) shared interest, (2) constraints, i.e. indices, where signaling level is linked to level of what is informed, making impossible to cheat, and (3) due to the costs involved in the signaling interaction, in situations of conflicted interests between parties (Zahavi, 1975, Grafen, 1990, Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005; Számadó, 2011).

The third point is the one generating the controversy, since Számadó (2011) concludes that honesty in communication is maintained by the potential cost of cheating and not by the realized cost paid by honest signalers at the equilibrium, being this strategic cost, contrary to Zahavi-Grafen proposal, neither a necessary nor a sufficient condition for honest signaling and thus, honest signals need not to be costly, even under conflict of interest (Számadó, 2011).

2. PLANT POLLINATOR COMMUNICATION

Plant- animal communication systems provide useful models to test some of the hypothesis posed by signaling theory, however, these systems have not been as widely studied as animal communication systems (Schaefer et al., 2004).

The interaction between plant and pollinators is a mutualism which is defined as an inter-specific interaction in which both parties obtain benefits, i.e. the investment that each party makes on the other produces a benefit that outweighs its cost (Connor, 1995). In this interaction, pollinators obtain rewards such as nectar, pollen, waxes, scents, and other elements required for its survival and/ or reproduction. In exchange, plants obtain the pollination service, increasing its reproductive success (Waser et al., 1996).

However, this interaction is not free of conflict. On the one side, because the production of rewards represents, in general, an energetic cost for the plants (Pleasants & Chaplin, 1983; Southwick, 1984; Pyke, 1991; Ordano & Ornelas, 2005; but see Leiss et al., 2004), any strategy reducing this cost without reducing pollinators visits will be favored by natural selection (Benitez- Vieyra et al., 2010). On the other side, pollinators will benefit form visiting the most rewarding plants (Waddington & Holden, 1979; Rathcke, 1992), and therefore, selection favoring pollinators cognitive capacity to optimize the energetic expenditure for reward recollection should be expected (Chittka et al., 2001; Raine & Chittka, 2008).

Following Ruxton & Schaefer (2011) definition, in a plant-pollinator interaction, an honest signal is a flower attribute used by pollinators to guide their foraging behavior, because it provides reliable information on reward status in the flowers, because it is significantly correlated with the quality and/or quantity of reward contained in the flower. Such a signal will benefit the pollinator, which benefits from visiting flowers with high reward quality and quantity (Waddington & Holden, 1979) and the plant, by obtaining the pollination service.

The most obvious case of honest signals is when the signals are emitted directly by the rewards (e.g. Hansen et al., 2006; Wright & Schiestl, 2009). However, in many cases, the rewards can not be detected directly by pollinators, and therefore, they must rely on other floral attributes, advertising floral traits significantly associated with the rewards, to select the flowers to visit (e.g. Cresswell & Galen, 1991).

Although, in general, advertising floral traits are positively correlated to rewards, and therefore provide useful information to pollinators (Plowright, 1981, 1987; Cresswell & Galen, 1991; Duffield et al., 1993; Mitchell & Shaw, 1993; Campbell, 1996; Davis, 1997; Klinkhamer & van der Veen-van Wijk; 1999; Armbruster et al., 2005; Fenster et al., 2006; Gomez et al., 2008; Benitez- Vieyra et al., 2010), deceptive signals are also widely spread among angiosperms, and the evolution of unrewarding flowers with conspicuous displays has been described in at least 32 Angiosperms families (Renner, 2006). This poses the question on the mechanisms by which honest signaling evolves and is maintained in plant pollinator interactions.

Mimulus luteus produces yellow penta-lobulated flowers with a conspicuous red nectar guide in the landing petal, highly variable within and among populations (Medel et al., 2007) which is used by pollinators to guide its foraging behavior (Medel et al., 2003), and therefore is an interesting system to investigate the mechanisms that will maintain honesty in communication.

The main objectives of this study were to determine whether the signals emitted by *Mimulus luteus* are honest, and to test Zahavi's handicap principle as an explanation for the output.

MATERIALS AND METHODS

1. STUDY MODEL: *MIMULUS LUTEUS* L.

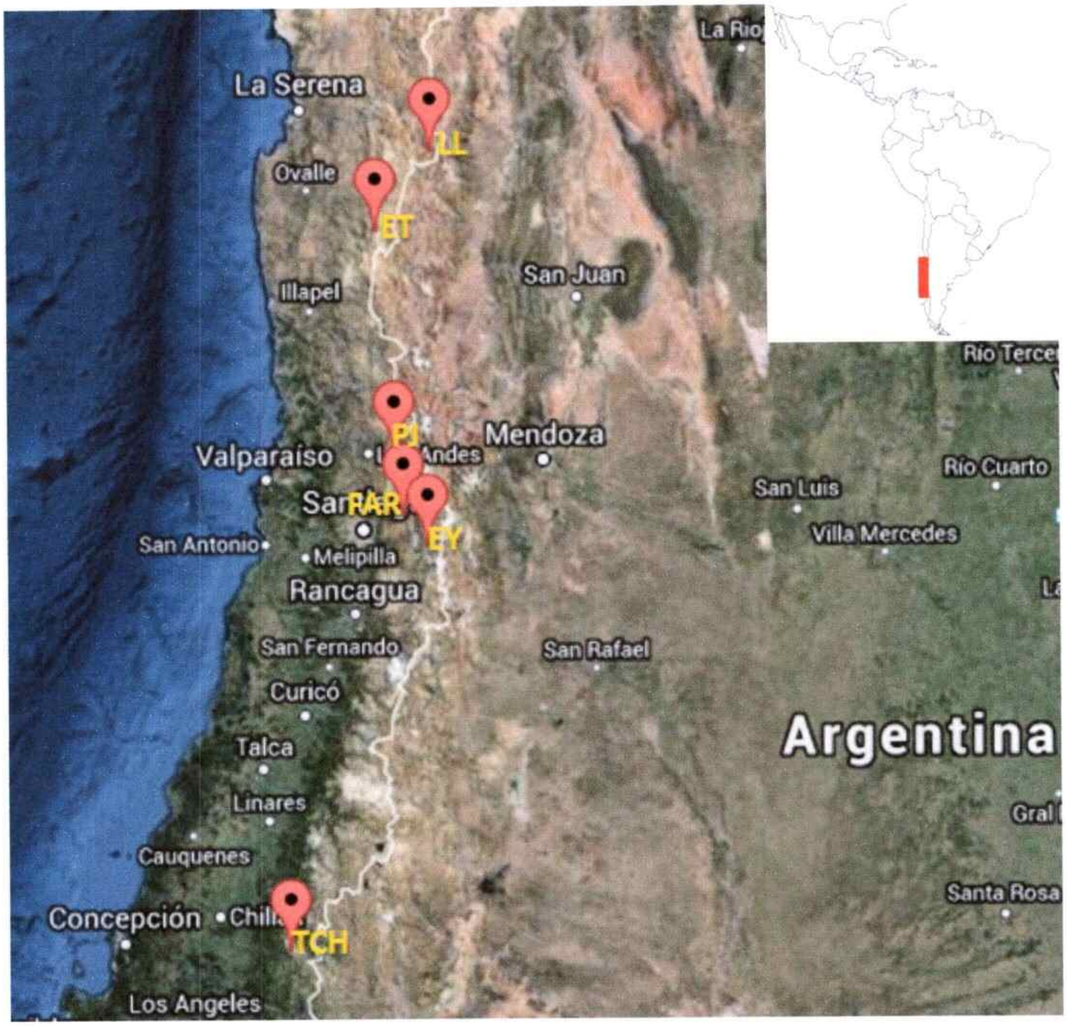
Mimulus luteus L. (Phrymaceae) is hydrophilic herbaceous, perennial species that inhabits streams and wetlands in the South American Andes from 0 – 3650 m.a.s.l. between 29° -45°S (von Bohlen, 1995). In Chile, flowering and fruiting occur form December to February. This species produces zygomorphic flowers with yellow corollas composed of five petals. In most cases, the lower lobe presents a conspicuous red spot, which acts as a nectar guide. The guide size is more variable between than within plants (Medel et al., 2003). The flowers emit radiation in wavelengths that can be detected by pollinators (Botto-Mahan et al., 2004; Pohl et al., 2006). The flowers produce small amounts of nectar that accumulates during the day (von Bohlen, 1995; Medel et al., 2003; Botto-Mahan et al., 2004; Medel et al., 2007). Even though this species is described as self-compatible, previous studies performed in different populations indicated that automatic self-pollination produces a significantly less seed by, compared to cross-pollinated plants (Medel et al., 2003; Carvallo & Medel, 2010). Therefore, the species is dependant on vectors for effective pollination (Medel et al., 2003; Carvallo & Medel, 2010).

Six populations of *Mimulus luteus* were selected for this study, located along the Andes between 30° and 37° SL, between Coquimbo and Bio-Bio Regions in Chile, covering a transect of c.a. 750 km between the extremes of the distribution (Table 1; Figure 1).

Table 1: Geographic location of seed collection sites, and sample sizes obtained for all six populations studied.

Population	**Abbreviation**	**N**	**Latitude**	**Longitude**	**Altitude (m.a.s.l.)**	**Region**
La Laguna	**LL**	64	30° 16' S	69° 57' O	3.341	IV
El Toro	**ET**	41	30° 57' S	70° 31' O	1.740	IV
Juncal	**PJ**	89	32° 51' S	70° 80' O	2.284	V
Farellones	**FAR**	85	33° 21' S	70° 15' O	2.657	RM
El Yeso	**EY**	92	33° 37' S	70° 01' O	2.580	RM
Termas de	**TCH**	99	36° 54' S	71° 24' O	1.805	VIII

Figure: 1: Geographic location of seed collection sites as pointed by the red mark. Populations were abbreviated as: La Laguna(LL), El Toro (ET), Juncal (PJ), Farellones (FAR), El Yeso (EY) and Termas de Chillán (TCH).



During February and March 2008 the populations were visited to collect seed. One fruit per individual was randomly collected on at least 100 individuals per population. The seed was germinated and only one randomly selected individual per capsule was grown in a common garden in the Greenhouse of the Evolutionary Ecology Laboratory, at Facultad de Ciencias at Universidad de Chile in Santiago, Metropolitan Region, Chile, obtaining between 41 and 99 individuals per population (Table 1) The plants were grown in individual pots, distributed in complete randomized design during August 2008 to June 2009, in homogeneous environmental conditions in terms of substrate, irrigation, pest and diseases management, temperature and luminosity.

2. SIGNAL HONESTY AND SIGNALING COST

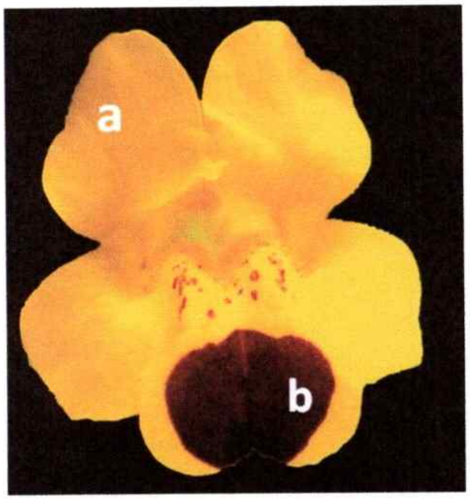
Signals (corollas and nectar guides), rewards (nectar and pollen) and the number of ovules produced per flower were evaluated. To measure corolla and nectar guide sizes, frontal photographs were taken in three to five flowers per individual on the first day of anthesis. Each photograph included a graded scale, allowing the conversion of a linear measurement in pixels, to obtain the areas in mm^2, of selected areas within the picture using the software Image J (available at http://rsb.info.nih.gov/ij/download.html (Figure 2).

Rewards provided by *M. luteus* flowers are nectar and pollen. Although pollen is also the male component of fitness, and therefore, we could expect a trade-off with reward production, it also plays an important role as a reward, since it is an essential nutritional source for pollinators and especially for Hymenoptera (Doul, 1975).

Nectar volume was measured using calibrated 5µl glass capillary tubes (Drummond Scientific, Broomall, PA USA). In every sample extracted, nectar volume was registered by measuring the height of the nectar column in the micro-cap, using a digital caliper (Mitutoyo America Corporation, Aurora, IL, USA); this number was transformed afterwards to micro-liters (µl). Soluble solids concentration was measured using an automatic temperature compensated (ATC) handheld digital brix refractometer (Atago PAL-1, ATAGO CO., LTD., Tokio, Japan).

To estimate the number of pollen grains and also the number of ovules in each flower, three flower buds per individual were collected on the day previous to anthesis, to avoid pollen losses at flower opening. The flower buds were fixed in Formalin-Acetic-Alcohol (FAA) solution for 24 hours, and then storage in ethanol 70% until processed in the laboratory. Pollen grains and ovules were counted using a particle counter (Beckman Coulter Z1, GMI, Inc. USA).

Figure 2: Traits measured as putative signals emitted to pollinators. (a) Corolla area (CA), which includes the nectar guide area, and (b) Nectar guide area (NGA), to calculate the total NGA area all the red spots within the flower "throat" were added to the main red area.



FLORAL TRAIT VARIATION

Analyses of variance (ANOVAs) were performed for each trait separately, using population as the fixed factor to analyze floral trait variation. Trait values were calculated as an average value per trait per individual. The number of individuals per population ranged between 41 and 99 (Table 1), 3 to 5 flowers were sampled per individual. All the analyses were performed using JMP (JMP 10, SAS Institute Inc. North Carolina U.S.A.).

SIGNAL HONESTY AND SIGNAL COST ESTIMATION

Signal honesty in each population was examined through Pearson's correlations.

Reward traits, nectar volume (NV), soluble solids content in the nectar (SS) and number of pollen grains (POL) were correlated with signaling traits, corolla area

(CA), and nectar guide area (NGA). A significant correlation coefficient was considered an honest signal, whereas a non significant correlation was considered as dishonest. To examine a potential trade-off in resources allocation for signal or reward production, the number of ovules produced per flower (on average per individual) was correlated with signaling and reward traits.

Multiple regression analyses were used to examine traits associations when the two signals assessed were correlated to the same reward. In these analyses, signaling traits were used as the predictor variables regressed on reward traits, the response variables.

All the analyses were performed using JMP (JMP 10, SAS Institute Inc. North Carolina U.S.A.). *P*-values for the pairwise correlations were set to 0.05 and sequential Bonferroni correction for multiple tests (Rice, 1989) was used to test for statistical significance.

RESULTS

1. FLORAL TRAITS VARIATION AMONG POPULATIONS

The first objective of this study was to describe the variation of the traits associated to the signaling system, i.e. signals, rewards, and the female component of fitness, ovules.

The signals considered were corolla area (CA) and nectar guide area (NGA). The rewards produced by the flowers are nectar, for which the amount was measured as nectar volume (NV) and energetic quality as the percentage of dissolved solids (i.e. sugar) in the nectar (SS), and the number of pollen grains produced by the flowers (POL).

The number of ovules per flower (OV) was measured to estimate the female fitness component. One way ANOVAs were performed to analyze the variation for each trait among populations and Tukey's test was used for population mean comparison. Highly significant differences between populations were detected for all traits evaluated (Table 2).

The corolla area was highly variable among populations. Extreme values for corolla area were observed in the populations in the extremes of the distribution range.

La Laguna (LL), the northernmost population, presented the smaller average CA (264 mm^2), whilst Termas de Chillán (TCH), the southernmost population, presented the biggest corollas, on average 833 mm^2, more than three times bigger than the average observed in LL.

Intermediate CA sizes were observed in the populations in the center of the distribution, ranging from 450 to 696 mm^2; notice that the increase in size does not really follow the northern- southern gradient for this trait.

For nectar guide areas, the lower values, not significantly different from each other, were observed in the extreme populations LL and TCH, with average values of 3.18 and 5.75 mm^2, respectively. The highest value for NGA was observed in Juncal (PJ), with an average of 25.5 mm^2, five to eight times bigger than the smaller averages. Nectar guide areas in rest of the populations ranged between 15 to 20 mm^2, not significantly different from each other.

Regarding the variation in reward traits, the smaller amount of nectar volume was detected in LL, followed by TCH, producing on average 0.57 and 1.44 µL of nectar per flower, respectively. The highest nectar volume production was observed in PJ, with an average of 2.89µL per flower, being five times higher than the smaller volumes observed.

Nectar quality was evaluated by measuring the sugar contained in the nectar, as soluble solids (SS). The highest values for SS were observed in LL and TCH with an average of 15.88 and 13.81%. The rest of the populations produced similar amounts of SS, ranging from 11.78% in ET to 10.08% in PJ, with values not significantly different between them.

Table 2: Means ± SE in floral traits for six populations of *Mimulus luteus*. Corolla Area (CA) and Nectar Guide Area (NGA) are in mm^2. Nectar Volume (NV) is in μL. Soluble Solids (SS) is in percentage. Number of pollen grains (POL) and number of ovules (OV) are the number estimated per flower. A significant effect of population (ANOVA) is denoted by asterisks: $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***). Significant groupings in each row are indicated by the superscript letters a, b, c, d, and e ($P < 0.05$, Tukey's studentized range test)

Population	**CA*** **	**NGA*** **	**NV*** **	**SS*** **	**POL *** **	**OV*** **
LL (n= 64)	264.30 ± 14.77^{e}	3.18 ± 0.54^{c}	0.57 ± 0.07^{d}	15.88 ± 0.85^{a}	$16{,}953 \pm 1{,}118^{d}$	$1{,}189 \pm 88^{bc}$
ET (n= 41)	449.48 ± 31.05^{d}	19.89 ± 3.51^{ab}	1.56 ± 0.16^{bc}	11.78 ± 0.87^{bc}	$21{,}246 \pm 2{,}166^{d}$	$1{,}641 \pm 230^{abc}$
PJ (n= 89)	695.91 ± 18.36^{b}	25.50 ± 2.27^{a}	2.89 ± 0.13^{a}	10.08 ± 0.57^{c}	$43{,}736 \pm 1{,}619^{bc}$	$1{,}509 \pm 123^{bc}$
FAR (n= 85)	670.18 ± 17.24^{b}	18.35 ± 1.76^{b}	2.02 ± 0.14^{b}	10.53 ± 0.59^{c}	$48{,}584 \pm 2{,}047^{ab}$	$2{,}296 \pm 214^{a}$
EY (n= 92)	573.73 ± 17.92^{c}	14.94 ± 1.37^{b}	1.88 ± 0.12^{b}	11.03 ± 0.56^{c}	$40{,}831 \pm 1{,}830^{c}$	$1{,}690 \pm 158^{ab}$
TCH (n= 99)	832.62 ± 20.29^{a}	5.75 ± 0.40^{c}	1.44 ± 0.08^{c}	13.81 ± 0.54^{ab}	$51{,}426 \pm 1{,}751^{a}$	973 ± 139^{c}
R^2 Adj	**0.540**	**0.227**	**0.298**	**0.155**	**0.367**	**0.196**

2. SIGNAL HONESTY AND COST

Pearson's correlations between attractive flower traits, corolla area (CA) and nectar guide area (NGA), and rewards, nectar volume (NV), nectar sugar content (SS) and number of pollen grains (POL) were examined to assess the reliability of signals in each population. The results of the analyses are shown in Table 3.

Significant and positive correlations between signals and rewards were detected in all populations, except in Juncal (PJ).

Corola area was positively and significantly correlated to the amount of pollen grains in four populations (ET, FAR, EY, and TCH), and it was also correlated to nectar volume in four populations (LL, ET, FAR, and EY), and to the amount of sugar in the nectar (SS) in LL. Whereas, nectar guide area was significantly and positively correlated to nectar volume in only one population, LL.

Pearson's correlations between signals, CA and NGA, and the amount of ovules (OV) produced per flower were also examined, in order to identify trade-offs which would imply the existence of costs in signal production. No significant correlations with the number of ovules were detected in any of the studied populations (Table 3).

Table 3: Pearson's correlation coefficients (r) for signal-reward, and signal- ovules associations in six populations of *Mimulus luteus*. Floral traits considered as signals are Corolla area (CA) and Nectar guide area (NGA). Reward traits are Nectar volume (NV), Sugar content (SS), and pollen grains per flower (POL). Ovules per flower (OV) are evaluated as cost component. Bold numbers indicate significant correlations at *p<0.05* after sequential Bonferroni correction (Rice, 1989).

			Rewards			
Population	**N**	**Signals**	**NV**	**SS**	**POL**	**OV**
LL	**64**	**CA**	**0.42**	**0.52**	0.32	0.23
		NGA	**0.41**	0.16	0.19	-0.27
ET	**41**	**CA**	**0.46**	0.11	**0.69**	-0.07
		NGA	0.27	0.19	0.37	0.10
PJ	**89**	**CA**	0.23	0.13	0.23	-0.02
		NGA	-0.12	0.16	0.13	-0.09
FAR	**85**	**CA**	**0.46**	0.15	**0.41**	-0.14
		NGA	0.24	0.08	0.09	-0.16
EY	**92**	**CA**	**0.59**	0.21	**0.32**	-0.11
		NGA	0.29	0.05	0.16	0.16
TCH	**99**	**CA**	0.15	**0.30**	**0.39**	-0.04
		NGA	0.18	0.11	-0.02	-0.43

DISCUSSION

The main objective of this study was to evaluate the applicability of Zahavi's handicap principle to explain the occurrence and maintenance of honest signals in a plant pollinator communication system, where plants advertise through attractive floral features the presence of rewards valuable for the pollinators.

We used the Chilean monkeyflower, *Mimulus luteus*, as the study model, since this species present interesting floral features that are perfectly suited for the study of signaling systems. *M. luteus* produces yellow flowers with a red nectar guide in the landing petal; both corollas and nectar guides can easily be identified as signals to pollinators.

Evidence from previous studies suggests that hummingbirds and bees exhibit divergent preferences for corolla and nectar guide sizes in *M. luteus*, which resulted in disruptive selection at a local scale (Medel et al., 2003). According to Medel et al. (2007), *M. luteus* is pollinated as well by Diptera and Lepidoptera; this diversity in pollinators provides a very interesting system for the study of signals.

In general, corolla size (Campbell, 1989; Galen, 1989; Campbell et al., 1996; Caruso 2000) and nectar guides (Bolwig, 1954; Waser & Price, 1985; Medel et al., 2003) have been identified as targets for pollinator mediated selection, however, the application of signaling theory to understand the evolutionary trajectories of signal honesty in plant pollinator interactions is a much less studied subject (but see Cresswell & Galen, 1991; Blarer et al., 2002; Armbruster et al., 2005; Fenster et al., 2006; Gomez et al., 2008; Raguso, 2008; Benitez-Vieyra et al., 2010; Bolstad et al., 2010; and Pélabon et al., 2012).

1. TRAIT VARIATION

In previous studies, both the size of the corolla (CA) and the size of the nectar guide (NGA) have been established to be highly variable among populations (Medel et al., 2007). This variation was confirmed by our study, we detected high variability in CA and NGA, although, absolute values for the traits were in general somewhat smaller than the ones reported in Medel et al. (2007). This difference can be maybe attributed to the fact that we measured the traits in common garden environment, and not directly in nature.

Reward traits; i.e. nectar and pollen, nectar volume (NV) and number of pollen grains per flower were highly variable as well. Sugar concentration (SS) was the least variable trait measured among populations, SS was highly correlated to nectar volume, in populations were the amount of volume was higher, a lower SS was detected. The low variation detected for this trait could be an indication of the high environmental dependency of it. Since it was measured in a common environment, differences between populations were not as high as we would expect if SS was measured in the natural environment (Mitchell, 2004).

2. SIGNAL HONESTY

Corolla area (CA) was as a reliable signal in five out of six populations studied, and interestingly the reward associated to CA was slightly different between the populations. CA reliably predicted nectar volume and sugar and pollen in La Laguna. However in TCH, it only predicted the amount sugar in the nectar and the amount of pollen. We were not able to detect a significant negative association between this signal, CA, and ovule production and even though correlation coefficients showed a tendency to be negative in almost all populations, none of them were significant; therefore, we failed to demonstrate the Zahavi's principle acting on this signal, at least by assessing the cost of signal production through ovule production.

Even though we can not completely discard strict allometric effects for CA correlations to rewards, the decoupling observed in PJ and TCH, provides evidence that CA is not an index (i.e. a signal whose reliability is maintained by a mechanistic link (physical connection) between signal intensity and a given trait (Maynard Smith & Harper, 2003). Moreover, the nectar guide was significantly associated with nectar volume in only one population, La Laguna, and therefore, if pollinators are using these signals to guide their foraging behavior, we might argue that the signals are deceiving.

According to Szamadó (2011), a mechanism that maintains the honesty of the signals is the potential cost of cheating, since pollinators can learn and discriminate individuals that provide poor or no rewards at all, dishonest individuals might loose the pollination service, turning the cheater strategy to be highly unsuccessful.

The fact that NGA was not correlated to any reward in five out of six populations, and especially in the populations with the highest values for this trait (ET, PJ, FAR, and EY), might be an indication that NGA could be serving other functions. Recent studies have examined the functional significance of nectar guides in plant pollinator interaction, not only as a reward advertising trait, but rather as contrasting patterns that aid the pollinator foraging behavior, increasing significantly the plant fitness, and the pollinator foraging efficiency (Hansen et al., 2012; Leonard et al., 2013). Leonard et al., 2013, through an experimental approach stated that nectar guides in addition to increase pollinator foraging efficiency by decreasing flower handling times, prevented nectar robbery by bees, due the guidance provided by the contrasting patterns, an increased probability of the bees approaching the flower in a way that assures the contact of the body of the insect to the reproductive structures of the flowers was achieved, making the visual signals a form of resistant to nectar robbing.

In conclusion, the communication between *M. luteus* and its pollinators proved to be occurring through honest signals five populations studied, which implies that the selective pressures imposed by pollinators may be playing an important role in the maintenance of the honesty in communication, through the potential cost of loosing the pollination service, which might be preventing the appearance of dishonest signals. We were not able to detect a direct cost of advertising, therefoire we were not able to verify Zahavi's handicap principle as the mechanism that maintains honest signaling.

The data observed in this study strongly suggest that differential ecological conditions, to which different populations are subjected to, are playing a major role in shaping the evolution of floral phenotypes.

ACKNOWLEDGMENTS

We thank Gastón Carvallo, Gabriel Esterio, Maureen Murua, and Marcela Espinoza for their invaluable contribution to field, greenhouse and laboratory work. This research was funded and supported by Anillo - PBCT ACT-34/2006 and CONICYT 21060185; 24080048, 29090020, and 23100196 scholarships to PPS.

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CAPÍTULO IV

THE GEOGRAPHIC MOSAIC OF FLORAL HONESTY IN *MIMULUS LUTEUS* L. (PHRYMACEAE): ECOLOGICAL CONTEXT FOR SIGNAL HONESTY EVOLUTION.

ABSTRACT

Plant pollinator mutualisms are characterized by the communication between plants and pollinators through advertising signals that indicate the presence and level of rewards. Plants benefit from pollinator visits by obtaining the pollination service, whereas the pollinators rely on these signals to obtain essential elements for their reproduction and survival. Signal production is essential to maintain the interaction; however, since rewards are often concealed within the flowers, and its production implies an energetic cost, plants might be subjected to constant selection pressures to evolve dishonest signals. Deceptive pollination systems are relatively common among Angiosperms, implying that such a strategy can be successful under certain ecological conditions. The mosaic theory of co-evolution predicts different evolutionary trajectories for traits subjected to variable complex selection scenarios in different populations. We studied floral features in ten populations of *Mimulus luteus*, to determine the reliability of signals within this species, and test the prediction of the Geographic Mosaic Theory of Co-evolution (GMTC), as an attempt to increase our understanding of the evolutionary processes that are shaping the floral phenotype within this species. Our results demonstrated that honest signaling is the common strategy for *M. luteus,* however, as predicted by the GMTC, signal honesty is presented in different forms, in different populations. This variation may be explained by differential composition of pollinator assemblages within different populations; however this mechanism remains to be tested. One hypothesis evaluated was population structure due to gene flow or common descent, following an isolation by distance model, in this case for one of the signals detected, nectar guide area as a signal to indicate nectar volume, this hypothesis was rejected, however for corolla and floral tube length associations to pollen, it was not rejected, implying that, even if pollinators are using these traits as signals, the effect of these factors can not be ruled out. Our results provide evidence for signal honesty at a geographic scale; however the mechanisms by which honesty has evolved and is maintained remain to be tested.

Key words: *Mimulus luteus,* plant-pollinator communication, signal honesty, floral advertisement, rewards, geographic mosaic theory of co-evolution.

INTRODUCTION

1. PLANT POLLINATOR COMMUNICATION

The interaction between plant and pollinators is a mutualism which is defined as an inter-specific interaction in which both parties obtain benefits, i.e. the investment that each party makes on the other produces a benefit that outweighs its cost (Connor, 1995). In this interaction, pollinators obtain rewards such as nectar, pollen, waxes, scents, and other elements required for its survival and/ or reproduction. In exchange, plants obtain the pollination service, increasing its reproductive success (Waser et al., 1996).

However, this interaction is not free of conflict. On the one side, because the production of rewards represents, in general, an energetic cost for the plants (Pleasants & Chaplin, 1983; Southwick, 1984; Pyke, 1991; Ordano & Ornelas, 2005; but see Leiss et al. 2004), any strategy reducing this cost without reducing pollinators visits will be favored by natural selection (Benitez- Vieyra et al., 2010). On the other side, pollinators will benefit form visiting the most rewarding plants (Waddington & Holden, 1979; Rathcke, 1992), and therefore, selection favoring pollinators cognitive capacity to optimize the energetic expenditure for reward recollection should be expected (Chittka et al., 2001; Raine & Chittka, 2008).

Following Ruxton & Schaefer (2011) definition, in a plant-pollinator interaction, an honest signal is a flower attribute used by pollinators to guide their foraging behavior, because it provides reliable information on reward status in the flowers, because it is significantly correlated with the quality and/or quantity of reward contained in the flower. Such a signal will benefit the pollinator, which benefits from visiting flowers with high reward quality and quantity (Waddington & Holden, 1979) and the plant, by obtaining the pollination service.

The most obvious case of honest signals is when the signals are emitted directly by the rewards (e.g. Hansen et al., 2006; Wright & Schiestl, 2009). However, in many cases, the rewards can not be detected directly by pollinators, and therefore, they must rely on other floral attributes, advertising floral traits significantly associated with the rewards, to select the flowers to visit (e.g. Cresswell & Galen, 1991).

Although, in general, advertising floral traits are positively correlated to rewards, and therefore provide useful information to pollinators (Plowright, 1981, 1987; Cresswell & Galen, 1991; Duffield et al., 1993; Mitchell & Shaw, 1993; Campbell, 1996; Davis, 1997; Klinkhamer & van der Veen-van Wijk, 1999; Armbruster et al., 2005; Fenster et al., 2006; Gomez et al., 2008; Benitez- Vieyra et al., 2010), deceptive signals are also widely spread among angiosperms, and the evolution of unrewarding flowers with conspicuous displays has been described in at least 32 Angiosperms families (Renner, 2006). This poses the question on the mechanisms by which honest signaling evolves and is maintained in plant pollinator interactions.

2. THE GEOGRAPHIC MOSAIC THEORY OF CO-EVOLUTION

The response of floral phenotype to selective pressures imposed by pollinators is dependant on several factors, such as spatial and temporal consistency of pollinator assemblages, quality and quantity of deposited pollen, visiting rates, companion flora composition and structure, among others (Schemske & Horvitz, 1989; Johnston, 1991; Maad & Alexandersson, 2004; Nattero et al., 2010). To predict the output of the interaction of plants and pollinators, it is essential to understand the main selective forces driving evolution in the system, including selection regimes and ecological and genetic constrains.

The geographic mosaic theory of co-evolution proposed by Thompson (1994, 2005) provides a theoretical framework for co-evolution which states that evolutionary processes do not necessarily occur al well defined local scales, but more precisely in a complex set of inter-connected populations where the species influence each other in different ways among populations, depending on the particular environment within each population, which will result in the occurrence of complex selection environments that will vary between populations, resulting in different evolutionary outputs (Thompson, 2005).

According to the Geographic Mosaic Theory of co-evolution, three processes lead co-evolutionary dynamics: (1) geographic selection mosaics (2) intermingled co-evolutionary hot and cold spots, (3) and trait remixing (Thompson, 2005).

This theory has received empirical support in plant animal interaction systems, such as insect-plant herbivory systems (Thompson & Fernandez, 2006; Muola et al., 2010), seed - predator systems (Benkman, 1999; Rey et al., 2005; Parchman & Benkman, 2008), host plant parasitic plant systems (Medel et al., 2010) and mutualistic plant-pollinator systems (Anderson & Johnson, 2008; Gomez et al., 2009). Due to the complex and diverse scenarios that the theory of the geographic mosaic predicts, different outputs are expected regarding the reliability of signals in different populations of the same species.

Most information in coevolving interactions has been collected in specialized systems, in which inter-specific interactions are tightly fitted (Thompson, 2005; Hoeksma and Thomposn, 2007). In contrast, the study of how geographically varying evolutionary dynamics shape the phenotypic evolution of generalist organisms has been addressed less frequently (but see Gomez et al., 2009a, 2009b).

Since honesty is a trait intrinsically related to the pollinator environment, it is expected that that the honesty phenotype is shaped by the selection imposed by pollinators. Plants actually interact with pollinator assemblages that often vary geographically (Aigner, 2005; Moeller, 2005, 2006; Price et al., 2005). Since different pollinators have different morphologies, foraging behavior, and flower trait preferences, a probable consequence of this variation is the occurrence of a selection mosaic (Gomez & Zamora, 2000; Herrera et al., 2006), resulting in variable outputs in the honesty of the signals emitted to pollinators.

The aim of this study was to determine if the honesty of signals in *Mimulus luteus* behaves in the way predicted by the geographic mosaic of co-evolution theory. Assuming a selection mosaic we expect that different attributes will function as advertising traits in different populations, and also that signal honesty will vary according to pollinator environment, which will allow the exploration of different mechanisms that can help to understand the evolutionary dynamics of signal honesty in *Mimulus luteus*, considering the geographic context for this trait.

Therefore, the main objective of this study was to describe the geographic mosaic of honesty in *Mimulus luteus,* to answer the following questions: (1) which is the geographic pattern of honesty variation between different populations of *M. luteus*?, and (2) is the pattern of variation in agreement with the predictions of the geographic mosaic of co-evolution theory?

To achieve this objective we selected ten populations of the hydrophilic herbaceous *Mimulus luteus*, located along the Andes between 30° and 37° SL, which covers the complete distribution range of the species, to characterize signal reward associations at a geographical scale, increasing our understanding of this complex interaction.

MATERIALS AND METHODS

1. STUDY MODEL: *MIMULUS LUTEUS* L.

Mimulus luteus L. (Phrymaceae) is hydrophilic herbaceous, perennial species that inhabits streams and wetlands in the South American Andes from 0 – 3650 m.a.s.l. between 29° -45°S (von Bohlen, 1995). In Chile, flowering and fruiting occurs from December to February. This species produces zygomorphic flowers with yellow corollas composed of five petals. In most cases, the lower lobe presents a conspicuous red spot, which acts as a nectar guide. The guide size is more variable between than within plants (Medel et al., 2003).

The flowers emit radiation in wavelengths that can be detected by pollinators (Botto-Mahan et al., 2004; Pohl et al., 2006) and produce small amounts of nectar, that accumulates during the day (von Bohlen, 1995; Medel et al., 2003; Botto-Mahan et al., 2004; Medel et al., 2007). Even though this species is described as self- compatible, previous studies performed in different populations indicated that automatic self-pollination produces significantly less seed when compared to cross pollinated plants (Medel et al., 2003; Carvallo & Medel, 2010). Therefore, the species is dependant on vectors for effective pollination (Medel et. al., 2003; Carvallo & Medel, 2010).

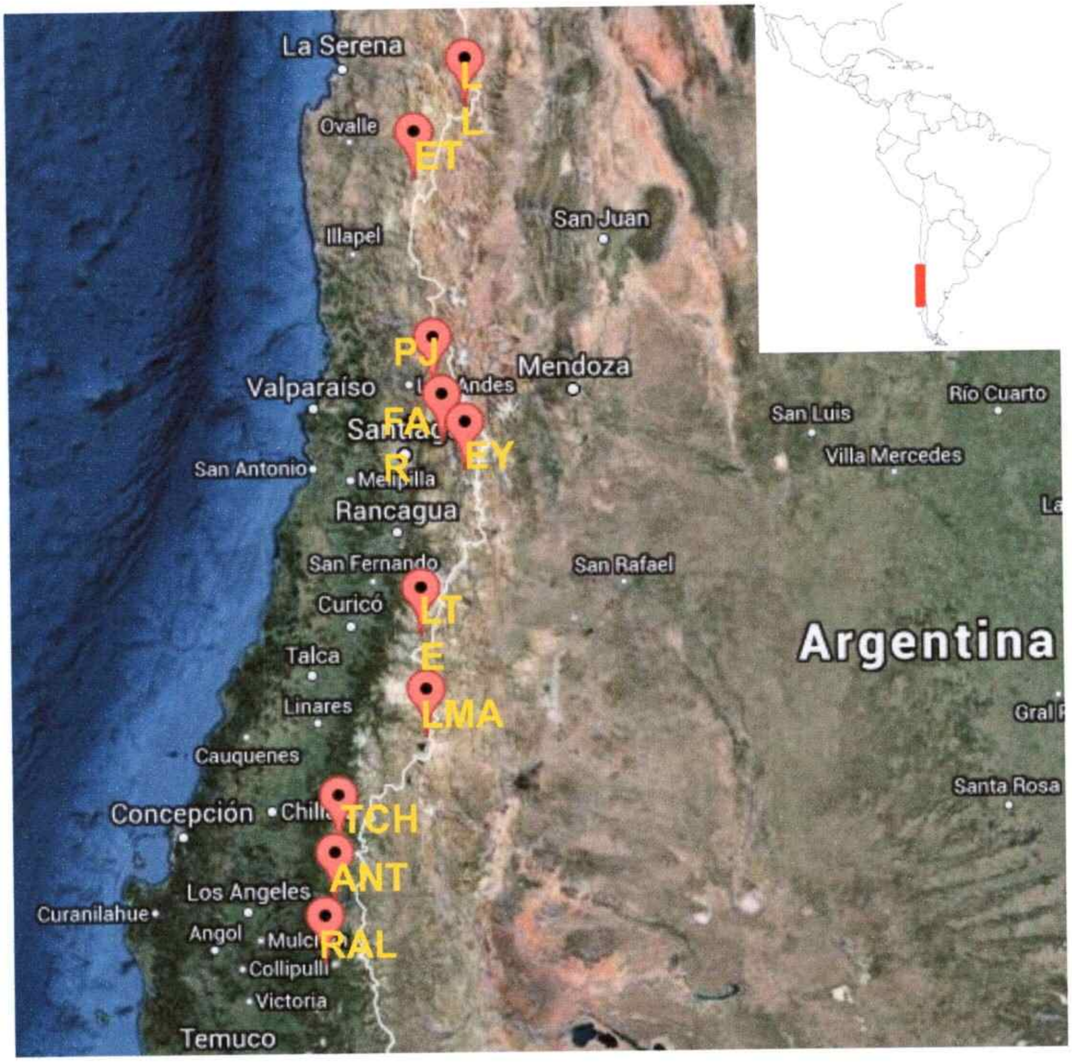
Ten populations of *Mimulus luteus* were selected for this study, located along the Andes between 30° and 38° SL, between Coquimbo and Bio-Bio Regions in Chile (Table 1, Figure 1).

During February and March 2008 the populations were visited to collect seed. One fruit per individual was collected on at least 100 individuals randomly selected per population. The seed was germinated and only one randomly selected individual per collected fruit was grown in a common garden in the Greenhouse of the Evolutionary Ecology Laboratory, at Facultad de Ciencias at Universidad de Chile in Santiago, Metropolitan Region, Chile, obtaining between 41 and 99 individuals per population (Table 1). The plants were grown in individual pots, distributed in the greenhouse in a complete randomized design, during August 2008 to June 2009, in homogeneous environmental conditions in terms of substrate, irrigation, pest and diseases management, temperature and luminosity.

Table 1: Geographic location of seed collection sites, and sample sizes obtained for all ten populations of *Mimulus luteus* included in this study.

Population	**Abbreviation**	**N**	**Latitude**	**Longitude**	**Altitude (m.a.s.l.)**	**Region**
La Laguna	**LL**	64	30° 16' S	69° 57' O	3.341	IV
El Toro	**ET**	41	30° 57' S	70° 31' O	1.740	IV
Juncal	**PJ**	89	32° 51' S	70° 80' O	2.284	V
Farellones	**FAR**	85	33° 21' S	70° 15' O	2.657	RM
El Yeso	**EY**	92	33° 37' S	70° 01' O	2.580	RM
Laguna Teno	**LTE**	94	35° 05' S	70° 29' O	1.453	VII
Laguna Maule	**LMA**	80	35° 59' S	70° 27' O	2.262	VII
Termas de Chillán	**TCH**	99	36° 54' S	71° 24' O	1.805	VIII
Antuco	**ANT**	74	37° 23' S	71° 26' O	932	VIII
Ralco	**RAL**	83	37° 55' S	71° 33' O	603	VIII

Figure: 1: Geographic location of seed collection sites as pointed by the red mark. Populations were abbreviated as: La Laguna(LL), El Toro (ET), Juncal (PJ), Farellones (FAR), El Yeso (EY), Laguna Teno (LTE), Laguna del Maule (LMA), Termas de Chillán (TCH), Antuco (ANT), and Ralco (RAL).

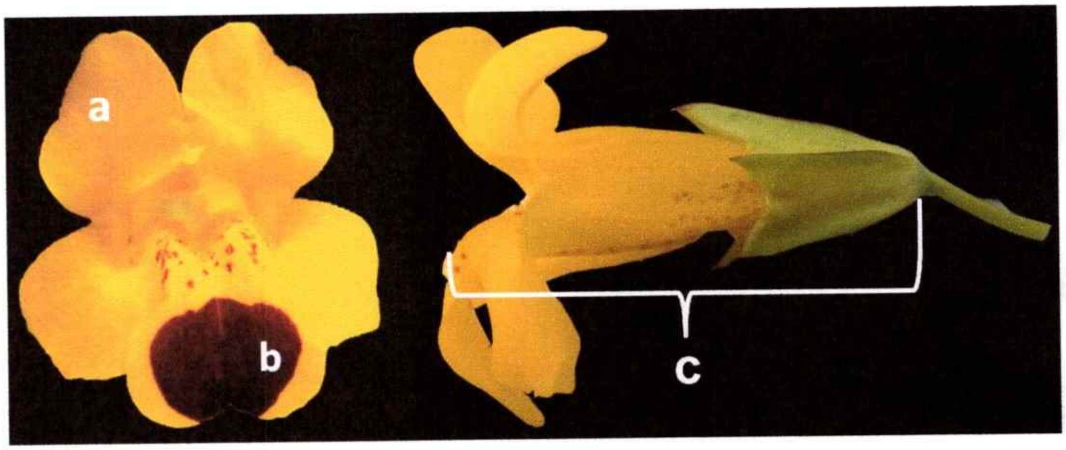


2. SIGNAL HONESTY IN *MIMULUS LUTEUS*

To characterize signal reliability in this system, different floral traits related to signal emission, i.e. floral tubes, corollas, nectar guides, nectar, and pollen were measured in a common garden environment. To measure floral tubes, corolla and nectar guide sizes, lateral and frontal photographs were taken in three to five flowers per individual on the first day of anthesis. Each photograph included a graded scale, allowing the measurement of tube length in mm and the conversion of a linear measurement in pixels, to obtain the areas of corollas and nectar guides in mm^2, using the software Image J (available at http://rsb.info.nih.gov/ij/download.html (Figure 2).

Nectar volume was measured using calibrated 5μl glass capillary tubes (Drummond Scientific, Broomall, PA USA). In every sample extracted, nectar volume was registered by measuring the height of the nectar column in the micro-cap, using a digital caliper (Mitutoyo America Corporation, Aurora, IL, USA), this number was transformed afterwards to micro-liters (μl). Soluble solids concentration was measured using a temperature-calibrated handheld Brix refractometer (PAL-1, ATAGO CO., LTD., Tokyo, Japan). To estimate the number of pollen grains, three flower buds were collected on the day previous to anthesis, to avoid pollen losses at flower opening. The flower buds were fixed in Formalin-Acetic-Alcohol (FAA) solution for 24 hours, and then storage in ethanol 70% until processed in the laboratory. Pollen grains were counted using a particle counter (Beckman Coulter Z1, GMI, Inc.).

Figure 2: Floral traits measured as putative signals emitted to pollinators. (a) Corolla area (CA), which includes the nectar guide area, (b) Nectar guide area (NGA) (to calculate the total NGA area all the red spots within the flower "throat" were added to the main red area), and (c) Floral Tube Length (FTL).



3. FLORAL TRAIT VARIATION

To determine if the trait values were different between populations, analyses of variance (ANOVAs) were performed for each trait separately, using population as the fixed factor. Trait values were calculated as an average value per trait per individual, 3 to 5 flowers per individual were measured for every trait. The number of individuals measured per population ranged between 41 and 99 (Table 1). All the analyses were performed using JMP (JMP 10, SAS Institute Inc. North Carolina U.S.A.).

4. SIGNAL HONESTY ESTIMATION

Reward traits (i.e. nectar volume (NV), soluble solids content in the nectar (SS) and number of pollen grains (POL)) correlations with signaling traits (i.e. floral tube length (FTL) corolla area (CA), and nectar guide area (NGA) were examined to determine the reliability of the signals.

A significant correlation coefficient was considered a reliable signal, whereas non significant correlations were considered dishonest. Signal honesty in each population was examined through Pearson's correlations. *P*-values for the pairwise correlations were set to 0.05 and sequential Bonferroni correction for multiple tests (Rice, 1989) was used to test for statistical significance.

Since significant correlations might be explained by allometric associations between traits, rather than adaptation, or alternatively in response to abiotic factors, multiple regression analyses were used to examine simultaneously the effects and the relative importance of abiotic predictors on reward attributes.

Abiotic variables considered were latitude, longitude and altitude, as measured by GPS. All the analyses were performed using JMP (JMP 10, SAS Institute Inc. North Carolina U.S.A.).

These analyses assume that trait values result from independent evolution of populations, and not from populations structured by gene flow or common descent, therefore, to test the hypothesis of geographic structure we followed the methodology proposed by Anderson & Johnson (2008), using Mantel tests (9.999 permutations) implemented in R package "ade4" (Dray & Dufour, 2007).

A positive relationship between paired geographic distances and paired differences between traits would imply that trait values in each population are influenced more by gene flow or common descent. Alternatively, if no relation is detected, this would suggest that trait values are more influenced by local evolutionary processes than by gene flow or common descent (Anderson & Johnson, 2008).

RESULTS

1. FLORAL TRAIT VARIATION

High significant variation among populations was detected for all traits studied (Figure 3).

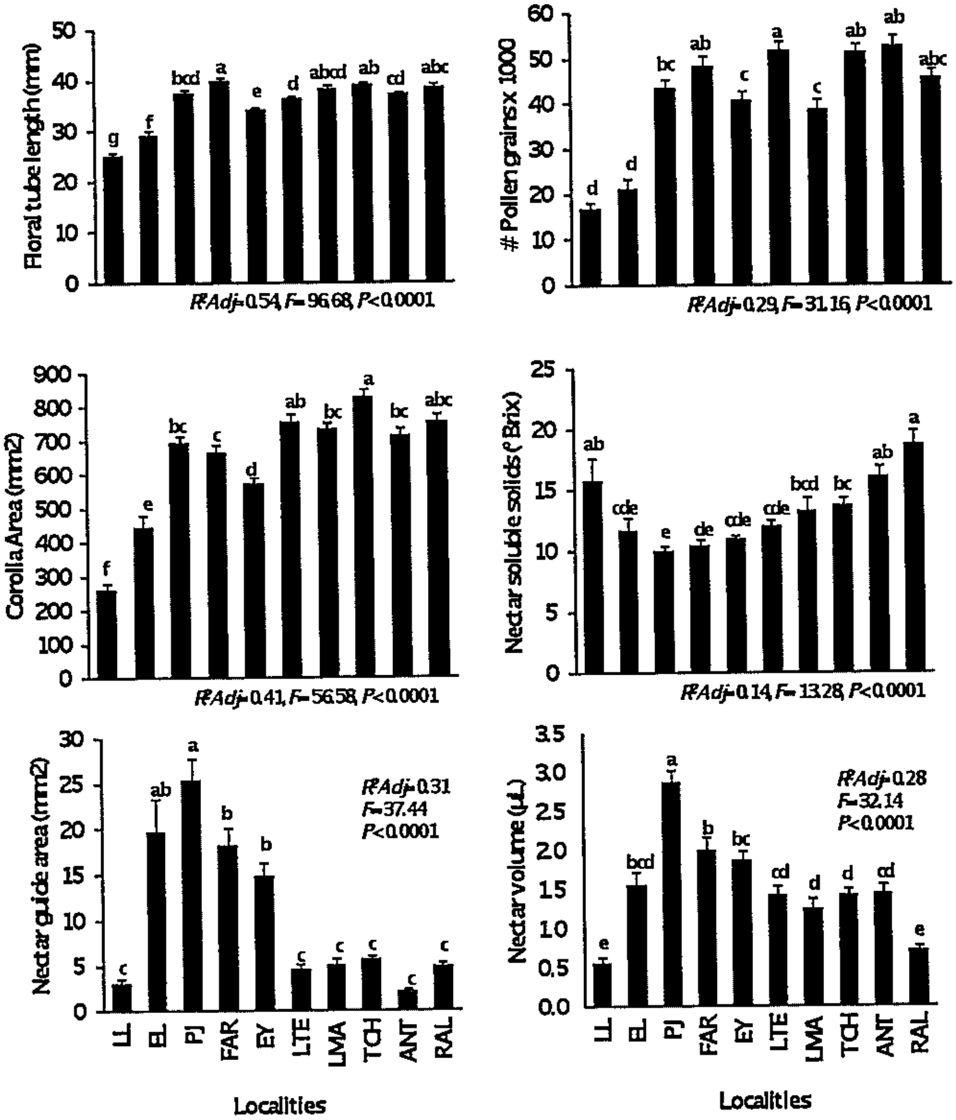
Floral tube length (FTL) varied between 40mm in FAR and 25 mm in LL, whereas corolla areas (CA) varied between 833 mm^2 in TCH to 264mm^2 in LL. The biggest nectar guide areas were observed in PJ, and the smaller ones, not statistically different between them, in LL, LTE, LMA, TCH, ANT, and RAL, ranging from 5.75mm^2 in TCH to 2.21 in ANT.

For nectar volume (NV), the highest volume was measured in PJ, 2.89µL per flower on average; whereas the lowest volumes were detected for LL, with only 0.57µL per flower on average.

Sugar content (SS), measured as dissolved solids in the nectar, presented the highest value in LL (16°Brix), and the lowest in PJ (10 °Brix).

Regarding the number of pollen grains per flower, the highest value was observed in LTE, 51,806 grains per flower on average, and the minimum in LL and ET, with 16,952 and 21,246 pollen grains per flower on average.

Figure 3: Average floral traits in ten populations of *Mimulus luteus*. Floral tube length (FTL), Corolla Area (CA), Nectar Guide Area (NGA), Nectar Volume (NV), Soluble Solids (SS), and number of pollen grains (POL). Bars above columns are 1 SE. Populations are arranged along the x-axis from left to right in the north- south gradient. Significant groupings are indicated by the lowercase letters (α= 0.05, Tukey's HSD).



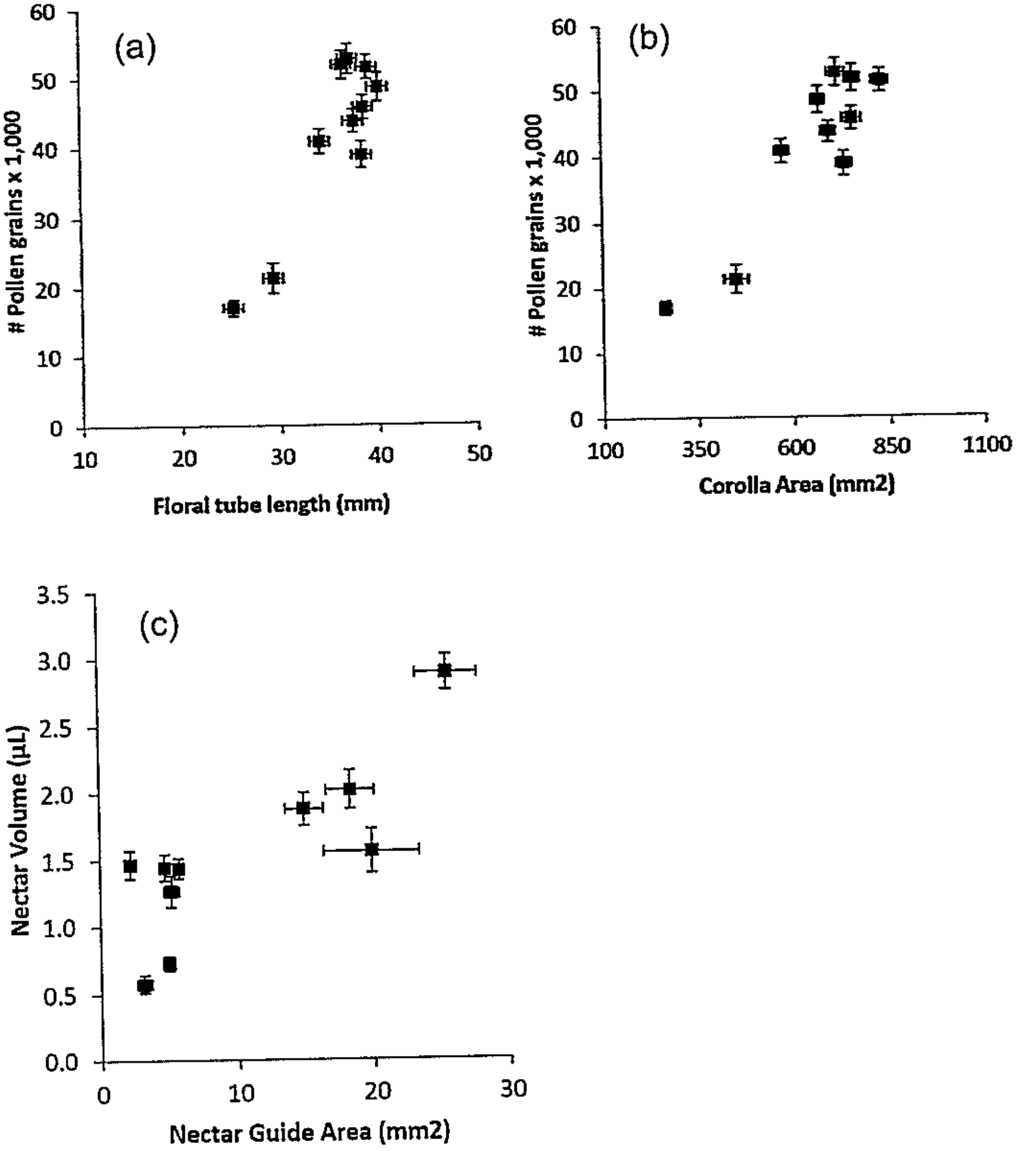
2. SIGNAL HONESTY

Pearson's correlation coefficients were examined to determine geographic patterns of association between floral traits (Table 2). Highly significant correlations were detected for the corolla area and the floral tube length with pollen and for the nectar guide area with nectar volume (Figure 4).

Table 2: Pearson's correlation coefficients (r) for all traits evaluated in ten populations of *Mimulus luteus*. Floral tube length (FTL), Corolla Area (CA), Nectar Guide Area (NGA), Nectar Volume (NV), Soluble Solids (SS), and number of pollen grains (POL). Bold numbers indicate significant correlations at *p<0.05* after sequential Bonferroni correction (Rice, 1989).

	FTL	CA	NGA	NV	SS	POL
FTL	-					
CA	**0.94**	-				
NGA	0.03	-0.13	-			
NV	0.37	0.27	**0.82**	-		
SS	0.59	0.70	-0.28	-0.02	-	
POL	**0.90**	**0.91**	-0.14	0.32	0.68	-

Figure 4: Significant Pearson's correlations between (a) Floral tube length and number of pollen grains; (b) Corolla area and number of pollen grains; and (c) Nectar guide area and nectar volume, in ten populations of *Mimulus luteus*. Each symbol represents the average trait values ±1 SE per population.



Multiple regression analyses were used to test for abiotic effects in the patterns observed, including Latitude and Altitude as environmental variables predictors added to the signaling traits, to examine the associations between signal and rewards that yielded significant Pearson's correlation coefficients (Table 3). Floral tube length (FTL) and Longitude were not included in the models, due to their extremely high correlation to Corolla Area (CA) and Latitude, respectively. Nectar guide area (NGA) remained as a significant predictor to Nectar Volume (NV). Similarly, CA remained significant as a predictor for pollen quantity, after including the abiotic predictor variables (Table 4)

Table 3: Multiple regression models for significant signal rewards associations in Mimulus luteus. Predictor variables include signaling traits, i.e. Corolla Area (CA) and Nectar Guide Area (NGA), and environmental predictor variables (Latitude and Altitude). Response variables include reward traits, i.e. Nectar Volume (NV), Soluble Solids (SS), and number of pollen grains per flower (POL). Significant standardized partial coefficients are denoted by asterisks: $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***).

	Standardized partial regression coefficients				Model		
Response Variable	CA	NGA	Latitude	Altitude	R^2 *(Adj)*	*F*	*P*
NV	-	**0.99****	0.12	0.00	0.80	7.88	0.0167
SS	-	**-0.53***	-0.04	0.00	0.53	4.37	0.0593
POL	**0.78***	-	0.02	0.00	0.83	10.12	0.0092

Finally, we used Mantel to test the hypothesis of geographic structure in the patterns observed, comparing pairwise geographic distances and pairwise distances between the values of signaling (FTL, CA, and NGA) and reward traits (NV; SS; and POL). Significant positive slopes were obtained for FTL, CA, POL and SS, but not for NGA and NV (Table 4), which implies that NGA and NV are not significantly structured by geographical distance.

Table 4: Mantel Test Correlations and p-values for pairwise geographical and floral traits distances in *Mimulus luteus*. Floral traits include: Floral Tube Length (FTL), Corolla Area (CA), Nectar Guide Area (NGA), Nectar Volume (NV), Soluble Solids in the nectar (SS), and Number of pollen grains per flower (POL). Significant coefficients are denoted by asterisks: $P < 0.05$ (*); $P < 0.01$ (**); $P < 0.001$ (***).

Floral trait	R	p-value:
FTL	**0.55****	0.0057
CA	**0.63*****	0.0009
NGA	0.21	0.11
NV	0.09	0.2909
SS	**0.65***	0.0462
POL	**0.57****	0.0044

DISCUSSION

The objectives of this study were to examine the geographical patterns of the variation in signal honesty across populations of *Mimulus luteus*, and to determine if this variation agrees with the expectation of the Geographic Mosaic Theory of Coevolution (GMTC) (Thompson, 2005). The GMTC hypothesizes that coevolutionary hot and cold spots, selection mosaics, and traits remixing are the primary drivers of coevolutionary dynamics (Thompson, 2005).

Our results demonstrate significant variation between populations in all traits evaluated, including advertising (floral tube length, corolla area, and nectar guide area) and rewarding (nectar volume, soluble solids in the nectar and pollen) traits, which are consistent with the results reported in Medel et al. (2007). Medel et al. (2007) evaluated the variation of corolla area and nectar guide area in seven populations of *Mimulus luteus*. They also examined the pollinator assemblages the populations, and concluded that mean corolla size increased with the proportion of bees and decreased with the proportion of lepidopterans, and that nectar guide size increased with the proportion of hummingbirds in the pollinator assemblages (Medel et al., 2007). This patterns suggest that pollinator mediated selection might explain the observed variation in this study; however this hypothesis remains to be tested.

Correlation between advertising traits and rewards was tested in our study to investigate the geographical patterns of signal reliability in *Mimulus luteus.*

In general, we found significant associations between corolla area and floral tube length, which were correlated with the amount of pollen grains and between nectar guide area and nectar volume, demonstrating that these advertising traits can be used by pollinators as reliable signals to guide their foraging behavior.

These geographical patterns of correlations along with the variation detected among populations may be an indication that these traits are under differential selection regimes, which is one of the predictions of the GMTC, and therefore it will be important to test this hypothesis in the future. The association between nectar guide area and nectar volume is particularly interesting, because it remained significant after testing for alternative hypotheses, such as abiotic gradients, or the isolation by distance model, providing some evidence that this association may be maintained through pollinator mediated selection. As opposed to the association between corolla area, floral tube length and pollen amount, where the geographic structure or pure allometric effects were not ruled out.

This suggestion is consistent with the results of Medel et al. (2003), were phenotypic selection over nectar guide area and corolla area was detected in one population located to the northern part of the distribution range. Gonzalez-Browne (2014) also detected significant phenotypic selection acting upon corolla area in two populations of *Mimulus luteus.* However, to fully support the hypothesis of the selection mosaic of the GMTC, reciprocal selection must be detected, which has not been done yet.

Carvallo & Medel (2010) examined the pollination environment in one population of *Mimulus luteus* in two consecutive years, reporting that pollinators were almost absent in this population. They also detected significant phenotypic selection against anther- stigma separation; which may contribute to autogamous reproduction. The scarcity of pollinators is unusual in other populations of *Mimulus luteus* (Medel et al., 2003; Medel et al., 2007; Gonzalez-Browne 2014).this situation provides some evidence for the existence of hot and cold coevolutionary spots, which allows the study of the evolutionary processes proposed by the GMTC.

In conclusión, *Mimulus luteus* provides honest signals to its pollinators. The analysis of the geographic patterns demonstrate that there is a clear association between the nectar guide area and the nectar volume, across populations, also a high variability between populations in the signaling traits. These results along with the evidence of diverse pollination environments (Medel et al., 2007), population lacking pollinators (Carvallo & Medel., 2010) and the phenotypic selection detected upon signaling traits (Medel et al., 2003; Gonzalez-Browne, 2014), provide evidence that makes this system an interesting model for the study of the processes predicted by the GMTC (Gomulkiewiczet al., 2007).

ACKNOWLEDGMENTS

We thank Gastón Carvallo, Gabriel Esterio, Maureen Murúa, and Marcela Espinoza for their invaluable contribution to field, greenhouses and laboratory work. This research was funded and supported by Anillo - PBCT ACT-34/2006 and CONICYT 21060185; 24080048, 29090020, and 23100196 scholarships to PS.

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CAPÍTULO V

CONCLUSIÓN GENERAL

El estudio de la comunicación, y en particular de los sistemas de señalización en diferentes interacciones ha captado la atención de los investigadores en distintas disciplinas desde hace ya varias décadas (e.g. Zahavi 1975, 1977; Grafen, 1990; Vehrencamp, 2000; Hurd & Enquist, 2005; Searcy & Nowicki, 2005; Maynard Smith & Harper, 2003; Connelly et al., 2011; Szamadó 2011; Higham, 2014). La principal pregunta que aborda la teoría de señales se refiere a los mecanismos que mantienen la honestidad en interacciones en las que hay asimetría en la información y existe un conflicto de interés (Connelly et al., 2011; Searcy & Nowicki, 2005). Si bien el marco teórico se ha estudiado y desarrollado ampliamente en el contexto de la comunicación animal (ver revisiones en Maynard Smith & Harper, 2003; Searcy & Nowicki, 2005), los modelos de comunicación planta- animal, no han recibido la misma atención, a pesar de que presentan características interesantes para testear diferentes hipótesis relacionadas con interacciones de comunicacción (Schaefer et al., 2004).

El principal objetivo de este trabajo era investigar la dinámica evolutiva de la honestidad en la comunicación entre plantas y polinizadores. Para eso se utilizó como modelo a la especie *Mimulus luteus* L., que posee ciertas características que la hacen un buen modelo para testear esta hipótesis. *Mimulus luteus,* a pesar de ser autocompatible, depende de los polinizadores para aumentar su éxito reproductivo (Medel et al., 2003; Pohl et al., 2006; Carvallo & Medel, 2010), posee atributos de señalización, y produce recompensas para los polinizadores, siendo estos atributos altamente variables a nivel inter-poblacional (Medel et al., 2007). Además, se ha reportado que los ensambles de polinizadores en las distintas poblaciones también varían (Medel et al., 2007; Pohl et al., 2006; Carvallo & Medel, 2010), lo que permite explorar los mecanismos que influyen en la evolución y mantención de estrategias de señalización honestas o deshonestas, en función de la interacción entre plantas y polinizadores.

De esta forma, este trabajo tuvo como primer objetivo investigar la base genética de los caracteres involucrados en el sistema de señalización. Dado que la honestidad se presenta como un rasgo adaptativo, es importante entender cual es el potencial evolutivo de los rasgos involucrados en la señalización (señales y recompensas), para determinar si existen restricciones genéticas a la dinámica evolutiva en respuesta a las presiones selectivas que imponen sobre los atributos los polinizadores u otros factores de selección presentes en el ambiente (Lande & Arnold, 1983; Falconer & Mackay, 1996; Lynch & Walsh, 1998; Conner, 2002). El estudio genético se hizo sobre una población en la que se encontraron correlaciones fenotípicas significativas entre señales y recompensas, sin embargo, no se detectaron correlaciones genéticas significativas entre los atributos, lo que permite descartar que la honestidad de las señales se mantenga por una limitación física, es decir, la presencia de índices en la señalización. Más bien se propone que la honestidad de las señales se mantiene por el potencial costo de engañar (Számadó, 2011). Este mecanismo se puede confirmar mediante el estudio de las presiones de selección impuestas por los polinizadores. Si bien en este trabajo no se evaluó la presencia de selección fenotípica sobre los atributos, y dado que esta es una pieza fundamental para entender la dinámica evolutiva de los atributos, solo se concluye acerca de las restricciones que tendría este sistema para evolucionar en presencia de selección fenotípica significativa. Se detectó heredabilidad significativa solamente para dos de los atributos de señalización, lo que restringe la evolución de honestidad, puesto que los atributos de recompensa están limitados al cambio evolutivo. Ante presiones de selección que favorezcan la evolución hacia, por ejemplo, señales más grandes, no existe variación genética aditiva para que se produzca el cambio evolutivo en todos los atributos.

Como segundo objetivo se examinó la variación en los niveles de honestidad de los rasgos florares de atracción respecto de las recompensas ofrecidas (néctar y polen), en seis poblaciones de *Mimulus luteus,* para poner a prueba el "Principio de la señal desventajosa" (Zahavi 1975, 1977, Grafen 1990), como un potencial mecanismo para la evolución y mantención de señalización honesta. El "Principio de la señal desventajosa" plantea que la honestidad de las señales se mantiene por el costo estratégico de la producción de señales, que solo los individuos honestos son capaces de pagar (Zahavi 1975, 1977, Grafen 1990). Para poner a prueba esta hipótesis, se evaluó como componente de costo la producción de óvulos, buscando correlaciones negativas con el nivel de señalización. Sin embargo, a pesar de que se detectaron señales honestas no fue posible detectar costos, por lo tanto se propone como hipótesis alternativa el mecanismo argumentado por Számadó (2011), quien propone que la señalización honesta se mantiene, no por el costo pagado por los señalizadores honestos, si no más bien por el potencial costo de engañar, que se generaría en este caso, por la perdida del servicio de polinización. Evidencia indirecta para apoyar esta hipótesis proviene de trabajos anteriores en los que se ha detectado selección fenotípica significativa mediada por polinizadores sobre las señales (Medel et al., 2003). Además, existe evidencia de que es servicio de polinización aumenta el éxito reproductivo en *Mimulus luteus* (Medel et al., 2003; Pohl et al., 2006; Carvallo & Medel, 2010). Sin embargo, para obtener evidencia directa para este mecanismo será necesario el uso de una aproximación experimental, en la que se manipulen los niveles de recompensa y señalización para determinar como esto afecta la respuesta de los polinizadores (e.g. Knauer & Schiestl, 2014).

Por ultimo, como tercer objetivo se examinó la honestidad de la señalización en 10 poblaciones que cubren el rango de distribución de *Mimulus luteus*, para hacer una aproximación al estudio de la Teoría del Mosaico Geográfico de la Coevolución. Si bien, con la información disponible no es posible poner a prueba los mecanismos que propone la teoría, se discute en base a evidencia de estudios anteriores las implicancias que tiene las estructuración geográfica detectada. De acuerdo a los datos obtenidos en este estudio, la especie *Mimulus luteus* se comunica con sus polinizadores a través de señales honestas. El análisis geográfico demuestra que existe una asociación honesta entre la guía de néctar y el volumen de néctar a través de las poblaciones. Esto sumado a la evidencia de la diversidad de ensambles de polinizadores (Medel et al., 2007), poblaciones que carecen de polinizadores (Carvallo & Medel., 2010) y la selección fenotípica detectada sobre los atributos de señalización (Medel et al., 2003), nos indican que este es un sistema interesante, que proporciona los elementos necesarios para servir como modelo de estudio para la teoría del mosaico geográfico de la coevolución (Gomulkiewiczet al., 2007).

Los resultados indican que la comunicación entre *M. luteus* y sus polinizadores ocurre a través de señales honestas, cuya honestidad se mantiene, probablemente por el potencial costo de engañar, es decir la perdida del servicio de polinización. A escala geográfica se observa una gran variación en los rasgos estudiados, con claras asociaciones entre la guía de néctar y el néctar como recompensa, esta asociación se mantiene probablemente por la selección mediada por los polinizadores, sin embargo es necesario seguir estudiando este sistema para confirmar estos mecanismos.

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