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Honestidad de señales florales y selección correlacional señal-recompensa en *Mimulus luteus*

Tesis entregada a la Universidad de Chile en cumplimiento parcial de los requisitos para optar al grado de Magíster en Ciencias Biológicas

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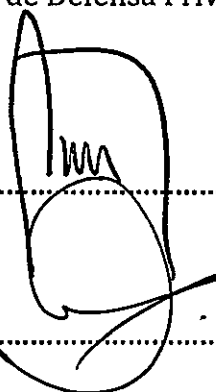
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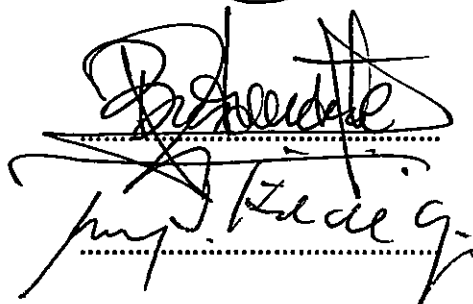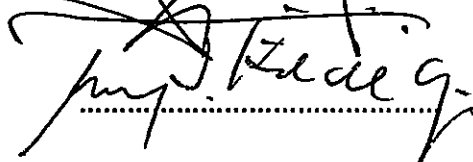Comisión de Evaluación de la Tesis

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A mis Papás,
por su apoyo incondicional.

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RESUMEN

La teoría de señales ha sido ampliamente aplicada a la biología evolutiva, especialmente en estudios de selección sexual en animales. Algunos estudios la han aplicado en las interacciones planta-polinizador para entender por qué y bajo qué condiciones las plantas pueden dar señales honestas a sus polinizadores. Sin embargo, ningún estudio ha evaluado si la selección ejercida por los polinizadores puede promover la honestidad en las poblaciones. El objetivo del presente trabajo fue examinar, en dos poblaciones de Chile central (Juncal, V región y El Yeso, RM) , si la honestidad de las señales producidas por la herbácea *Mimulus luteus* (Phrymaceae) puede ser resultado de selección correlacional mediada por polinizadores. Específicamente, se examinó la presencia de asociaciones señal-recompensa mediante un análisis de correlaciones y la ocurrencia de selección fenotípica correlacional sobre ambos tipos de caracteres. En ambas poblaciones, se midió el volumen y la concentración de néctar, el número de granos de polen por antera, el tamaño de la guía de néctar y el tamaño de la corola en 1-3 flores en 108 y 120 plantas en Juncal y El Yeso, respectivamente. Las correlaciones mostraron una asociación positiva entre tamaño de la corola y dos recompensas, el volumen de néctar y el número de granos de polen para ambas poblaciones. Los coeficientes de selección revelaron selección correlacional entre el volumen de néctar y el tamaño

de la guía de néctar para la población de El Yeso. Estos resultados indican que para la población de El Yeso, la selección favorecería la evolución de honestidad mediante la promoción intergeneracional de plantas con asociación positiva señal–recompensa. Sin embargo, la ausencia de correlación fenotípica entre las variables sugiere que la selección detectada no se traduce en una respuesta evolutiva. Es posible que ciertas restricciones a la evolución de la honestidad sean importantes en este sistema. Futuros estudios que examinen restricciones ecológicas y genéticas a la evolución de la honestidad serán de utilidad para conocer los factores que inciden en este sistema de estudio.

Palabras claves: Selección correlacional, honestidad, selección mediada por polinizadores, señales, recompensas.

ABSTRACT

Signaling theory has been largely applied in evolutionary biology, especially in studies related to sexual selection. Some studies have applied this theory to plant-pollinator interactions in order to understand why and under what conditions plant species may provide honest or dishonest signals to pollinators. However, no study has evaluated if pollinator-mediated selection promotes honesty in natural populations. The aim of this study was to examine if the honesty in the perennial herb *Mimulus luteus* (Phrymaceae) may be promoted by correlational selection of signals and rewards exerted by pollinators in two population of central Chile (Juncal, V region; El Yeso, RM). Specifically, we examined the association between signals and rewards through phenotypic correlations and correlational phenotypic selection between these traits. In both populations, we measured nectar volume and concentration, number of pollen grains, corolla size and nectar guide size in 1-3 flower in 108 and 120 plants of each population, respectively. In both sites, we found a positive association between corolla size and two rewards (nectar volume and number of pollen grains). Selection gradients revealed correlational selection between nectar volume and nectar guide size in El Yeso, favoring a positive association between these two traits. These results suggest that pollinator-mediated selection favors the evolution of honesty by promoting the signal-reward

association. However, the absence of phenotypic correlation between variables suggests that this correlational selection does not translate into evolutionary change. Therefore the constraints to the evolution of honesty may be important in our system. More studies evaluating ecological and genetic constraints to the evolution of honesty are required to determine which are the factors determining its evolution in our study system.

Key words: correlational selection, honesty, pollinator-mediated selection, signals, rewards.

INTRODUCCIÓN

La teoría de señales busca entender el comportamiento de un emisor y un receptor cuando éstos tienen acceso diferencial a la información. El componente emisor debe elegir una manera o señal para comunicar un determinado mensaje y el receptor, con las herramientas disponibles, debe ser capaz de interpretarlo (Connelly y col. 2011). Esta teoría ha sido ampliamente aplicada en biología evolutiva para entender por qué y bajo qué condiciones los emisores pueden dar señales honestas o deshonestas a los receptores. En el campo de la biología evolutiva, una señal corresponde a un acto o estructura que incrementa la adecuación biológica de un individuo (emisor) mediante el cambio de la conducta de otro individuo (receptor), lo que ocasiona la evolución de la señal ya que es un rasgo funcionalmente relacionado con la adecuación biológica del emisor (Dawkins y Krebs 1978, Krebs y Dawkins 1984, Hasson 1994, Maynard-Smith y Harper 1995, Számadó 2011). Las señales honestas, producirán entonces un incremento en la adecuación biológica tanto del emisor como del receptor, y las señales deshonestas producirán, de igual manera, un incremento en la adecuación biológica del emisor, pero un decremento en la del receptor.

Diferentes modelos teóricos, relacionados en su mayoría a estudios sobre selección sexual, han sido propuestos para explicar la evolución de las señales

honestas y deshonestas en las poblaciones. Zahavi (1975, 1977) propuso "El Principio del Handicap o Principio de la Desventaja" como un posible mecanismo para explicar cómo se mantienen las señales honestas en las poblaciones. El Principio de Zahavi indica que las señales honestas son altamente costosas para quienes las producen, debido a que el emisor no sólo debe pagar el costo de su producción, sino que también el costo en sobrevivencia asociado a estar más visibles para sus antagonistas. Estos costos por tanto, sólo pueden ser asumidos por individuos de alta calidad, lo que hace que la interacción entre emisor y receptor se vuelva estable, generando un incremento en la adecuación biológica de los interactuantes (Számadó 2011). Este principio ha sido criticado por diferentes autores, quienes proponen otros mecanismos para asegurar la honestidad (Davis y O'Donald 1976, Maynard Smith 1976, Számadó 2011). De esta manera, Maynard Smith y Harper (1995) sugieren que existen tres tipos de señales que promueven la evolución de la honestidad. Las primeras son las *señales mínimas*, las que sólo tienen el costo de transmisión y alcanzarán honestidad si el emisor y el receptor no se encuentran en conflicto de interés. Esta ausencia de conflicto se da posiblemente porque la sobrevivencia de una de las partes depende de la sobrevivencia de la otra (Maynard Smith y Harper 1991). Luego se encuentran las *señales de costo agregado*, las que presentan un mayor costo que el de su transmisión. Esta categoría coincide con el tipo de señales honestas propuestas por Zahavi (1975, 1977), ya que dentro de ella se encuentran aquellas señales altamente costosas para los emisores y que por tanto sólo pueden ser producidas y mantenidas por individuos de alta calidad. Finalmente, se encuentran los *índices*, los que alcanzan honestidad no por su costo

para el emisor, sino porque están físicamente relacionados a alguna característica del emisor que es de interés para el receptor e indicativa de su calidad. En general, las propuestas de Zahavi y Maynard Smith y Harper se basan en los costos de producción, mantención y transmisión de las señales. Sin embargo, existe una aproximación diferente sugerida por Számadó (2011), en la que establece que existe otro posible mecanismo capaz de mantener señales honestas en las poblaciones relacionado a los costos que genera al emisor dar señales falsas al receptor. En este sentido, los emisores darán señales honestas únicamente cuando el costo de la deshonestidad sea mayor al beneficio generado al engañar al receptor.

Como se mencionó anteriormente, la teoría de señales ha sido utilizada como una aproximación al estudio de algunas áreas de la biología evolutiva. La mayoría de los estudios que la aplican buscan entender el comportamiento de machos y hembras en la selección sexual (Maynard Smith 1976, Ryan y Rand 1993, Harari y col. 2011, Wenzel y col. 2013). Un menor número de estudios la ha aplicado al estudio de las interacciones planta-animal, con especial énfasis en las interacción planta-polinizador (Armbruster y col. 2005, Hansen y col. 2006, Keasar y col. 2006, Benítez-Vieyra y col. 2010, Bolstad y col. 2010, Pélabon y col. 2012, Zhang y col. 2012, Benítez-Vieyra y col. 2014). Esta interacción mutualista es un buen modelo de estudio para la teoría de señales en poblaciones naturales, ya que los polinizadores deben confiar en las señales entregadas por las flores, debido a que, en la mayoría de los casos, las recompensas están escondidas y los polinizadores no pueden percibirlas directamente para elegir que flores visitar. Es por esto, que en principio, rasgos flores, tales como olores, colores, guías de néctar, tamaño y número de flores,

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entre otros, deberían funcionar como indicadores de la cantidad y calidad de las recompensas florales (Armbruster y col. 2005, Chittka y Raine 2006, Fenster y col. 2006, Wright y Schiestl 2009).

En sistemas planta-polinizador, la honestidad debiera promoverse de manera indirecta a través de la selección correlacionada entre rasgos que actúen como señal y las recompensas ofrecidas (Bolstad y col. 2010). Algunos estudios han evaluado la selección sobre señales honestas en la interacción planta-polinizador, a través de diferentes metodologías. Uno de estos estudios es el desarrollado por Benitez-Vieyra y col. (2010), donde se evaluó la oportunidad de las plantas para engañar a sus polinizadores en *Turnera ulmifolia*. Estos autores estimaron la selección sobre la precisión de la señal, el despliegue floral y la producción de néctar, siendo la precisión de la señal una estimación de la variación intra-individual en la correlación de la señal y la recompensa, relativo a los valores poblacionales promedio. Los autores encontraron que la evolución de la deshonestidad estaría limitada por la selección sobre la precisión de la señal. De esta manera, los polinizadores estarían promoviendo, en principio, la honestidad de la población en estudio. Otro estudio, es el realizado por Armbruster y col. (2005) en un sistema con exposición directa de la recompensa, donde evaluaron la honestidad en poblaciones de *Dalechampia ipomoeifolia* y evaluaron si existía selección actuando directa e indirectamente sobre señales florales. Ellos encontraron una asociación positiva entre el largo de las brácteas (señal) y el área de la glándula (como una estimación de la recompensa), indicando que la población en estudio presentaba honestidad en las señales que entregaba a sus polinizadores. Además, a través de un análisis de

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vías, concluyen que los polinizadores prefieren señales honestas, ya que se observó selección directa sobre brácteas más largas e indirecta por glándulas más grandes. Los estudios ya mencionados evaluaron la selección actuando sobre la honestidad de las señales, pero no hay estudios enfocados en evaluar en qué medida los rasgos asociados con la honestidad pueden evolucionar de manera correlacionada con la recompensa. Esta información es crítica para el desarrollo de la teoría, ya que de otra manera no es posible asegurar que las plantas evolucionan estrategias honestas en su interacción con polinizadores a nivel poblacional. Debido a esto, este trabajo busca poner a prueba la hipótesis que la honestidad presente en las poblaciones naturales puede ser resultado de la selección correlacional ejercida por los polinizadores a nivel poblacional.

Este estudio fue realizado en dos poblaciones de la hierba perenne *Mimulus luteus*. Esta especie constituye un buen modelo para evaluar selección de honestidad en interacciones planta-polinizador, ya que en el pétalo inferior de su corola presenta una mancha roja que ha sido descrita como guía visual de néctar para sus polinizadores (Medel y col. 2003, Botto-Mahan y col. 2004). Además, las recompensas ofrecidas están escondidas dentro de la flor, por lo que los polinizadores deben confiar en algunos rasgos florales para decidir dónde forrajear. El objetivo principal de este estudio fue examinar si los rasgos de señal y recompensa de *Mimulus luteus* pueden ser seleccionados de manera correlacionada como consecuencia de la elección de los polinizadores a nivel poblacional. Por esto, se propusieron los siguientes objetivos específicos: (1) describir para *M. luteus* los ensambles de polinizadores y sus tasas de visita dos poblaciones ubicadas en Chile

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central, (2) determinar si las poblaciones en estudio son honestas en las señales producidas, (3) determinar si hay selección actuando directa o indirectamente sobre los rasgos de atracción y recompensa, con especial énfasis en los gradientes de selección correlacional. Basándose en los antecedentes propuestos y las características de la especie de estudio, se espera que la guía de néctar actúe como una señal honesta de la cantidad y/o calidad de las recompensas ofrecidas y que la asociación señal-recompensa haya evolucionado producto de la selección correlacional ejercida por los polinizadores.

HISTORIA NATURAL Y SITIOS DE ESTUDIO

Mimulus luteus L. (Phrymaceae) es una hierba perenne, auto-compatible (Pohl y col. 2006) que habita arroyos y humedales entre 0-3650 m.s.n.m. desde los 29° a los 45°S (von Bohlen 1995). Esta especie tiene una altura promedio de 53 cm (Población Los Pelambres, IV región, Medel y col. 2003) y produce 1-4 flores. Las flores de esta especie son solitarias, hermafroditas, zigomorfas y presentan corolas de color amarillo compuestas por 5 pétalos unidos en la base. En la mayoría de los casos, el pétalo inferior presenta una mancha roja, de tamaño variable, la que actúa como guía de néctar. Estudios previos indican que esta guía no refleja en el espectro UV (Botto-Mahan y col. 2004). El período de floración y fructificación se extiende desde mediados de Diciembre hasta fines de Febrero, con un máximo de floración entre mediados de Enero y Febrero. 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 y col. 2003). Si bien esta especie ha sido descrita como auto-compatible, estudios previos indican que la auto-polinización automática produce menor número de frutos por planta y un decrecimiento en la producción de semillas en comparación con plantas que han recibido polen externo. Por lo tanto, esta especie requiere de vectores para asegurar una polinización efectiva (Medel y col. 2003, Pohl y col. 2006, Carvallo y Medel 2010).

El presente estudio se realizó durante los meses de verano del año 2013 en dos poblaciones de *M. luteus*. Una de ellas se ubica en Juncal (32°51'S, 70°08'W, 2398 msnm, V Región), a 80 km al noreste de Santiago y la otra en el Embalse El Yeso (33°37'S, 70°01'W, 2460 msnm, Región Metropolitana, Chile), a 98 km al sureste de Santiago. Ambos sitios corresponden a poblaciones altoandinas que se cubren de nieve durante los meses de invierno. En Juncal, la vegetación corresponde al piso vegetacional subandino (Hoffmann y col. 1998) y en El Yeso al piso andino (Teillier y col. 2011). Se utilizó una parcela en cada población, la cual fue cercada para evitar el ingreso del ganado. La especie más abundante dentro de las parcelas fue *M. luteus* para ambos sitios (abundancia relativa obtenida a partir de 5 transectos de 8 m por sitio, Juncal: 44.75%; El Yeso: 45.17%). Otras especies encontradas dentro de las parcelas fueron, Juncal: *Brassica campestris* (Brassicaceae) (7.8%), *Chrysanthemum parthenium* (Asteraceae) (6.3%), *Veronica anagallis-aquatica* (Scrophulariacae) (4.3%) *Verbascum densiflorum* (Scrophulariaceae) (1.3%), *Solidago chilensis* (Asteraceae) (1.0%) y *Lactuca serriola* (Asteraceae) (8.8%); El Yeso: *Veronica anagallis-aquatica* (Scrophulariacae) (5.2%), *Matricaria chamomilla* (Asteraceae) (1.3%) y *Cardamine sp* (Brassicaceae) (0.9%).

Honesty and signal-reward correlational selection in the Andean monkeyflower *Mimulus luteus* (Phrymaceae)

Abstract

Signaling theory has been largely applied in evolutionary biology, especially in studies related to sexual selection. Some studies have applied this theory to plant-pollinator interactions in order to understand why and under what conditions plant species may provide honest or dishonest signals to pollinators. However, no study has evaluated if pollinator-mediated selection promotes honesty in natural populations. The aim of this study was to examine if the honesty in the perennial herb *Mimulus luteus* (Phrymaceae) may be promoted by correlational selection of signals and rewards exerted by pollinators in two population of central Chile (Juncal, V region; El Yeso, RM). Specifically, we examined the association between signals and rewards through phenotypic correlations and correlational phenotypic selection between these traits. In both populations, we measured nectar volume and concentration, number of pollen grains, corolla size and nectar guide size in 1-3 flower in 108 and 120 plants of each population, respectively. In both sites, we found a positive association between corolla size and two rewards (nectar volume

and number of pollen grains). Selection gradients revealed correlational selection between nectar volume and nectar guide size in El Yeso, favoring a positive association between these two traits. These results suggest that pollinator-mediated selection favors the evolution of honesty by promoting the signal-reward association. However, the absence of phenotypic correlation between variables suggests that this correlational selection does not translate into evolutionary change. Therefore the constraints to the evolution of honesty may be important in our system. More studies evaluating ecological and genetic constraints to the evolution of honesty are required to determine which are the factors determining its evolution in our study system.

Key words: correlational selection, honesty, pollinator-mediated selection, signals, rewards.

Introduction

Signaling theory has been largely applied to evolutionary biology to understand why and under what conditions senders provide honest or dishonest signals to receivers. In this context, a signal is defined as an act or structure that increases the fitness of an individual (sender) by altering the behavior of other individual that perceives this signal (receiver) and they evolve because, on average, they increase signaler fitness (Dawkins & Krebs 1978, Krebs & Dawkins 1984, Hasson 1994, Maynard Smith & Harper 1995, Számadó 2011). As honest and dishonest signals may increase or decrease the receiver fitness, respectively, dishonest signals may lead to

a conflict of interest between both parties that destabilize their interaction. Different theoretical models have been proposed to explain the evolution of honest and dishonest signals. Zahavi (1975, 1977) proposed "The Handicap Principle" as a way to understand how honest signals are maintained in populations. This principle indicates that honest signals are costly for senders, because they have to pay the cost of producing and carrying these traits, which may affect their survival by making them more visible to harmful interactors. These costs can be afforded only by high-quality individuals, making the sender-receiver interaction more stable as honest signals increase the fitness of both interacting species (Számadó 2011). Different authors have criticized this principle, proposing other mechanisms to ensure reliability (Davis & O'Donals 1976, Maynard Smith 1976, Számadó 2011). For instance, Maynard Smith and Harper (1995) suggested that the evolution of honesty may occur through three types of signals: (1) Minimal signals: the cost of a signal involves only the transmission cost and honesty will arise when sender and receiver have no conflict of interest, probably because the survival of one of the interacting species depends strictly on the survival of the other (Maynard Smith 1991); (2) Cost-added signals: these are more costly than minimal signals because they exceed the cost required for their transmission. These types of signals coincide with the ones suggested by Zahavi (1975, 1977), and propose that under conflict of interests, only costly signals can become honest because only high-quality individuals can afford them; (3) Indexes: unlike minimal and cost-added signals, these signals do not relate to the cost they generate to the sender as they are reliable because of their close relation to some physical feature of the sender that

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relates to its quality. Both Zahavi's principle and Maynard Smith and Harper's classification based their ideas in the costs of production, transmission and maintenance of signals; however, there is a different approach suggested by Számadó (2011), which suggests that the maintenance of honest signals in populations relates to the cost of cheating. In this case, the focus does not rely on the cost of signal production for senders, but on the cost senders have to pay when they decide to cheat. In this sense, senders will provide honest signals when the potential cost of dishonesty becomes higher than the benefit of their actions.

Most studies using signaling theory in evolutionary biology have focused on mate choice and sexual selection (Maynard Smith 1976, Ryan & Rand 1993, Harari *et al.* 2011, Wenzel *et al.* 2013). A few numbers of studies have applied this theory to plant-animal interactions (Armbruster *et al.* 2005, Hansen *et al.* 2006, Keasar *et al.* 2006, Benítez-Vieyra *et al.* 2010, Bolstad *et al.* 2010, Pélabon *et al.* 2012, Zhang *et al.* 2012, Benítez-Vieyra *et al.* 2014). Plant-pollinator interactions represent an appropriate model to study signaling theory in natural populations because pollinators have to rely on the flower signals to access to the often concealed rewards. Therefore, pollinators may rely on flower number, flower size, flower color, odor, and nectar guides, as indicators of the quality and quantity of floral rewards (Armbruster *et al.* 2005, Chittka & Raine 2006, Fenster *et al.* 2006, Wright & Schiestl 2009). Under this idea, it is expected that pollinator-mediated selection on rewards might be indirect through selection on the correlation between signal and rewards (Bolstad *et al.* 2010). Some studies have measured the selection on honest signals in plant-pollinator systems using diverse methodologies. For

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example, Benítez-Vieyra *et al.* (2010) evaluated the opportunity of cheating in a population of the perennial weed *Turnera ulmifolia* by estimating the selection on signal accuracy, floral display and nectar production. Under this approach, signal accuracy is a measurement of intra-individual variation in the correlation of the signal and reward relative to the population values. The authors found that the evolution of dishonest signals may be constrained by the selection of signal accuracy, promoting honesty in the population. Armbruster *et al.* (2005) studied the honesty of a population of the tropical vine *Dalechampia ipomoeifolia*, a species that presents an exposed reward. They estimated the selection acting directly and indirectly on floral rewards, advertisements and other traits related to pollinator efficiency. They found a positive correlation among bract length (signal) and gland area (a measurement of the standing crop of resin) indicating that the population was honest in the advertisement provided to pollinators. Using path analysis, they concluded that pollinators preferred honest signals, because bees were directly selecting larger bracts and indirectly larger gland areas. These studies as a whole, have evaluated selection on honest signals, but no study has evaluated the extent to which the traits associated with honesty evolve in a correlated manner, a critical condition for the evolution of honesty in natural populations.

This study was performed in two populations of the perennial herb *Mimulus luteus* in central Chile. This species is an appropriate model to evaluate selection of honesty in plant-animal interactions because it has a conspicuous red spot, described as a nectar guide for pollinators, and the rewards (i.e., nectar and pollen) offered by this species are concealed inside flowers. In this way, pollinators have to

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rely on some floral traits to make their foraging decisions. The principal aim of this work was to evaluate the honesty of plant signals and pollinator-mediated correlational selection on advertisement and rewards.

Materials and Methods

Natural history and study sites

Mimulus luteus L. (Phrymaceae), also known as Andean monkey flower, is a perennial self-compatible herb (Pohl *et al.* 2006) that inhabits streams and wetlands from 0 – 3650 m.a.s.l. between 29° - 45°S (von Bohlen 1995). This species is 53 cm in height on average and produces one to four solitary hermaphrodite flowers (data from Los Pelambres population, VI Region, Medel *et al.* 2003). These flowers are zygomorphic and have yellow corollas composed of 5 petals. In most cases, the lower lobe presents a red spot, which acts as a nectar guide and has been described that it does not reflect in the UV (Botto-Mahan *et al.* 2004). The flowering and fruiting periods extend from mid December to late February with a peak of flowering during mid January - mid February. *M. luteus* flowers remain open for 3.6 days when they have been pollinated and 5.9 days when they have not (Medel *et al.* 2003). Even though this is a self-compatible species, previous studies performed in different populations of *M. luteus* indicate that autonomous self-pollination produces lower fruit set and decreased seed production than crossed pollination; therefore, pollen vectors are required to assure effective pollination (Medel *et al.* 2003, Pohl *et al.* 2006, Carvallo & Medel 2010).

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This work was performed during the summer season of 2012-2013 in two populations of *M. luteus*. The first is located at Juncal (32°51'S, 70°08'W, 2398 m.a.s.l., V región, Chile), which is 80 km northeast of Santiago and the other at El Yeso (33°37'S, 70°01'W, 2460 m.a.s.l., Región Metropolitana, Chile) about 98 km southeast of Santiago (Fig. 1a). Both sites are high Andean populations that are entirely covered by snow during at least 3 months a year. The vegetation type of Juncal corresponds to associations of the subalpine belt (Hoffmann *et al.* 1998) and the El Yeso to the Andean belt (Teillier *et al.* 2011) (more information about sites available in Supplementary material Figure 1S). In each site, we used a fenced plot to exclude the cattle present in the area (e.g., goats, horses and cows) (Fig. 1b and 1c). In both sites, the most abundant plant species inside the plot was *M. luteus* (Phrymaceae)(relative abundance from five 8 m transects per site, Juncal: 44.75%; El Yeso: 45.17%). The species following in abundance inside the plot were: Juncal: *Brassica campestris* (Brassicaceae) (7.8%), *Chrysanthemum parthenium* (Asteraceae) (6.3%), *Veronica anagallis-aquatica* (Scrophulariacae) (4.3%) *Verbascum densiflorum* (Scrophulariaceae) (1.3%), *Solidago chilensis* (Asteraceae) (1.0%), and *Lactuca serriola* (Asteraceae) (8.8%): El Yeso: *Veronica anagallis-aquatica* (Scrophulariacae) (5.2%), *Matricaria chamomilla* (Asteraceae) (1.3%), and *Cardamine sp* (Brassicaceae) (0.9%).

Pollinator assemblages and visitation rate

We recorded the identity and number of visits of each pollinator during 57 h per site (Juncal: December 26-31 and January 23-24; El Yeso: January 30-February 2). Six

well-trained observers recorded the visits by one-hour focal observations on sunny days since 08:00 am until 12:00 pm approximately. A visit was considered valid when pollinators entered to the floral tubes. We estimated the visitation rate per flower per hour for each one-hour focal observation and then calculated the average visitation rate per species per site. Additionally, we compared the pollinator assemblages of both sites using the proportional similarity index (Schemske & Bradshaw 1999, Kay & Schemske 2003):

$$PS = 1 - \frac{1}{2}\sum_{i=1}^{n} |Pai - Pbi|$$

where, P_{ai} and P_{bi} represent the proportion of total visitation rate performed by the pollinator species i in the population a and b, respectively. The index ranges from 0 to 1, where zero indicates low similarity between sites and one high similarity. In order to have an estimation of the variability of the index, we calculated the confidence interval using 5000 bootstrap iterations. We compared the visitation rates of each species between sites using a paired t-test.

Signal honesty

To evaluate the relation between rewards and floral traits, we randomly selected 108 and 120 plants in Juncal and El Yeso, respectively. To ensure that flowers measured were first-day flowers, we removed the corollas of every flower in anthesis the evening before, leaving only flower buds in the patch. On each plant, we tagged and harvested 1-3 recently opened flowers per plant depending on the availability. On each flower, we measured nectar volume (µl), nectar concentration

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($^{\circ}$Bx), corolla size (mm^2), nectar guide size (mm^2), and the number of pollen grains per anther. We chose the corolla and nectar guide size as signals, because they have been previously described as relevant traits for insect and bird pollinators (Medel *et al.* 2003). Flowers suffering florivory or nectar robbing were not included in the analyses. 10-μl capillary tubes were used to obtain the nectar available in the flower. To determine the nectar volume, the amount of nectar in the capillary was measured using a digital caliper (precision 0.01 mm) and the measure was extrapolated to μl. When nectar volume was greater than zero, we used a hand-held refractometer to determine the sugar content on each sample. Both measurements, nectar volume and concentration, were recorded before pollinator arrival. To estimate the corolla and nectar guide size, we took perpendicular pictures to the plane of the flower. Each picture included a graded ruler as a scale in order to convert a linear measurement in pixels and then obtain the areas using the program ImageJ (available in http://rsb.info.nih.gov/ij/ download.html). To determine the number of pollen grains per anther, we collected two anthers from each flower in ethanol 70% to be analyzed later in the laboratory. To determine the number of pollen grains on each sample, we used a coulter counter Beckman Z1. The samples were spun at 1300 rpm for 2 min in order to separate the pollen grains from the liquid. Then, pellets were suspended in 10 ml of NaCl 1% for measurement. The analysis consists in determining the number of particles in 0.5 ml of the prior dilution. Dilution factors were accounted when calculating the number of pollen grains per sample. All samples were collected before pollinator arrival to the site.

All flower traits measured were more variable between than within plants (see Supplementary material Table 1S)

The presence of phenotypic correlations between signals and rewards was examined through Pearson's correlation coefficients using the mean trait per plant. The significance of each correlation coefficient was assessed using permutation with 5000 iterations, as variables did not fulfill assumptions of parametric statistics. A population was considered honest for a pair of traits (signal-reward) when the correlation coefficient was significant regardless the sign of the association.

Phenotypic selection analysis

In order to evaluate if honesty could be explained by correlational pollinator-mediated selection on advertisement and rewards on each population, we used the same plants of the correlation analysis to estimate selection coefficients. The predictor variables included in the analysis were: nectar volume, corolla size, nectar guide size, and number of pollen grains per anther. We excluded nectar concentration from the analysis because when nectar volume was zero, we could not estimate the concentration. This problem greatly reduced the sample size of the analysis, because 14% and 60% of the plants of Juncal and El Yeso did not produce nectar in any flower that was evaluated. Each predictor variable (values were mean per plant) was standardized to mean = 0 and variance = 1, in order to express the phenotypic change in standard deviation units and make the results comparable between populations. The response variable of our analysis was relative female fitness. At the end of the fruiting period, we collected capsules and counted the

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number of seeds per capsule. The fitness estimation per plant was relativized using the population mean female fitness ($w = w_i/w_{population\ mean}$). The variance in relative fitness is an indicative of the opportunity of selection (I) in the populations. This coefficient is intended to estimate the chance of a trait being selected in a population (Jones 2009). The total selection acting on each trait (i.e., the selection acting directly on the trait plus the selection acting indirectly on correlated traits) was estimated using the directional selection differentials (S'). This measurement was calculated as the covariance between each standardized character and the relative fitness, $S_i = COV\ (z_i, w)$ and its significance through correlation by permutations using 5000 iterations. Then, we estimated the direct and indirect selection acting on traits through linear and nonlinear gradients of selection. The linear selection gradients were calculated using the multivariate linear model proposed by Lande and Arnold (1983):

$$w = \alpha + \sum_{i=1}^{n} \beta_i z_i + \varepsilon$$

where w is the relative fitness, α is the intercept, β_i represents the slope of the partial relation between the trait z_i and w, and ε is an error term. The value of β_i indicates the magnitude and direction of the directional selection acting on each trait. The nonlinear selection gradients describe the type of nonlinear selection acting on each trait (i.e., disruptive or stabilizing selection) and on the correlation between two traits. These gradients (γ_{ii} and γ_{ij}), were estimated from the second order coefficients in:

$$w = \alpha + \sum_{i=1}^{n} \beta_i \, z_i + \frac{1}{2} \sum_{i=1}^{n} \gamma_{ii} \, z_i^2 + \sum_{i=1}^{n} \sum_{i \neq j}^{n} \gamma_{ij} \, z_i z_j$$

where γ_{ii} corresponds to the curvature of the relation between the trait i and w. Positive values indicate upward concavity and consequently represent disruptive selection. Negative values results from downward concavity and represent stabilizing selection. The term γ_{ij} represents the selection acting on the correlation of the trait i and j. Linear selection gradients were estimated only through the multivariate linear model because when character departed from a multivariate normal distribution, z_i and z_i^2 in the quadratic model might be intercorrelated (Lande & Arnold 1983). Selection models were tested for multicolinearity through the estimation of the variance inflation factor (VIFs). There was no multicolinearity in any of the variables of the selection model (see Hair *et al.* 1992). The statistical significance of the linear and nonlinear gradients was obtained from the p-values of the regression analysis when the assumption of normality was met. We performed Bonferroni sequential correction to reduce the type I error due to multiple comparisons. When the distribution of the model departed from normality, we estimated the significance of the selection gradients using bootstrapping methods with 5000 iterations to estimate the confidence interval (CI). In such circumstances, when CI does not overlap zero, the gradient was considered significant. All the analyses were performed in R program (R Development Core Team 2014).

Mating system and pollen limitation

In order to explain potential differences between sites in the phenotypic selection analysis, we performed the following four treatments on 50 flowers of different plants per site to determine the breeding system and pollen limitation on each population: (1) Automatic self-pollination (ASP): unmanipulated buds were bagged to avoid pollinator entrance to the flower. (2) Hand self-pollination (HSP): opened flowers were hand pollinated using their own pollen and were bagged to prevent pollination with foreign pollen. This treatment gives us information about the self-compatibility level of each population. (3) Saturation (S): opened flower were hand pollinated until saturation with a mix of pollen from flowers of different plants that were at least 10 m apart from the focal plant. (4) Control (C): opened flower were left unmanipulated to be naturally pollinated. We used 50 flowers from different plants of the selection analysis for the fourth treatment. After the fruiting period all tagged capsules were collected for subsequent seed counting in the laboratory. The average seed production per flower on each treatment was compared per population using a Kruskal-Wallis test. Additionally, treatments were used to estimate three indexes: (1) Self-incompatibility index (ISI): it is typically calculated dividing the average number of seeds per flower of HSP by hand cross-pollination treatment. A score of 1 represents self-compatible species; values ≤ 0.2 are indicative of self-incompatibility and scores between 0.2 and 1 indicate incomplete self-compatibility (Ruiz & Arroyo 1978). In this study, we calculated this index dividing the HSP by S, since we did not have a hand cross-pollination treatment. (2) Automatic self-pollination index (IAS): this index results from the division of ASP

and HSP. This index has been used only in self-compatible species to determine the degree of autogamy in plant populations. Values between 0 and 1 are typical of partially autogamous species and a score of 1 corresponds to fully autogamous ones (Ruiz & Arroyo 1978). Based on the fact that *M. luteus* has been described as a self-compatible species (Pohl *et al.* 2006), the reproductive success estimated as the number of seeds produced per plant is determined by both the automatic self-pollination and pollinator-mediated pollination. Therefore, the ISI and IAS indexes allow determining the level of self-incompatibility and the contribution of self-pollination to plant seed production on each population. (2) Pollen limitation index: $L = 1-(C/S)$. Values close to zero represent no pollen limitation in the populations (Larson & Barrett 2000). Previous studies have described a positive relation between the level of pollen limitation and the strength of phenotypic selection, which suggests the attraction to pollinators might be more important when plants are pollen limited (Totland 2001, Hansen & Totland 2006). Therefore, potential differences in the levels of pollen limitation may explain differences in the selective regimes in the populations under study.

Results

Mating system and pollen limitation

The results for each treatment are shown in Figure 2. We found differences between treatments in both populations (Juncal: Kruskal-Wallis chi-square = 73.65, df = 3, *P* < 0.0001; El Yeso: Kruskal-Wallis chi-square = 50.86, df = 3, *P* < 0.0001). The results of the self-incompatible index indicate that this species showed incomplete self-

compatibility in both populations (ISI_{Juncal} = 0.60, ISI_{ElYeso} = 0.79). The scores for the automatic self-pollination index were close to zero in both populations, suggesting that even though this species is self-compatible there are mechanisms that probably prevent self-pollination. Additionally, the similarity in the results of the populations indicates that the contribution of the self-pollination to the total seed production is similar in the populations (IAS_{Juncal} = 0.26, IAS_{ElYeso} = 0.21). The pollen limitation index shows that the population of Juncal exhibits more pollen limitation than El Yeso (L_{Juncal} = 0.82, L_{ElYeso} = 0.38). Therefore, it is expected to find higher selection strength in Juncal.

Pollinator assemblages and visitation rate

The composition of the assemblages was similar in both study sites, with 83% of the pollinator species shared. In Juncal we recorded six different species, the bees *Bombus terrestris*, *Centris nigerrima*, *Centris chilensis*, *Megachile saulcyi* and *Megachile semirufa*, and the hummingbird *Oreotrochilus leucopleurus*. In El Yeso we only found the five bee species described above for Juncal. The visitation rates per species per sites are shown in Table 1. In spite of the fact that the pollinator with the highest visitation rate differed between sites (Juncal: *C. nigerrima*, El Yeso: *M. saulcyi*), we did not find differences in the overall visitation rate comparing all the species among sites (paired t-test, t = -0.4, df= 5, P = 0.71). Moreover, we obtained a proportional similarity index of 0.61 (CI: 0.36-0.83), indicating a high similarity between sites.

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Signal honesty

The mean (± SD) of each trait per site are reported in Table 2. The mean values for all traits differed between sites (MANOVA: F = 45.47, df = 1, P < 0.001). The nectar volume of samples from Juncal was one order of magnitude higher than the ones from El Yeso, but it was half the concentration. The flowers of Juncal showed larger corollas and nectar guides than the flowers from El Yeso, but the pollen production per anther was similar in both sites (Table 2). Regarding phenotypic correlation analysis similar results were obtained in both populations (Table 3). In both sites, there was a positive and significant correlation between corolla size and the rewards nectar volume and number of pollen grains. Additionally, we found a positive correlation between the floral signals evaluated (nectar guide size and corolla size) (Table 3). Only for the population of El Yeso, we found a positive correlation between two rewards, number of pollen grains and nectar volume. Nectar guide size and nectar concentration were not associated with any of the variables evaluated.

Phenotypic selection analysis

We tagged 108 and 120 plants at Juncal and El Yeso, respectively; however, during the recollection not all the capsules were found. In the population of Juncal we marked a total of 324 flowers from 108 plants but we collected 187 capsules from 95 individuals. In the case of El Yeso, 263 flowers from 120 plants were tagged, but 177 capsules of 102 plants were collected. Since we worked with the mean values per plant, the reduction of sample sizes did not affect the analysis.

The mean seed production per plant was 159.8 ± 199.1 seeds in Juncal (mean ± SD), ranging from 0 to 847 seeds per plant. The population of El Yeso had a larger seed production of 830.4 ± 437.7, ranging from 0 to 2304 seeds per plant. The opportunity of selection differed between sites (I_{Juncal} = 1.55, $I_{El\ Yeso}$ = 0.27, F Bartlett's test = 65.36, P < 0.001), suggesting that the chance of populations to evolve under pollinator-mediated selection is different. Standardized selection differentials and linear and nonlinear gradients are reported in Table 4. Selection differentials revealed that selection favored large-sized corollas in both sites. We did not find significant differentials for any of the rewards evaluated. The evaluation of normality indicated that the only model meeting this assumption was the linear one from the population of Juncal; therefore, the significance of the coefficients in the other models was estimated through bootstrapping methods (see Materials and Methods). No character was under directional (β_i') and quadratic selection (γ_{ii}'), indicating that directional, disruptive and stabilizing selection were not significant forces acting on the populations under study. Regarding correlational selection gradients, we detected only one significant coefficient, corresponding to a positive correlational gradient between nectar volume and nectar guide size in El Yeso (Table 4). This gradient indicates that pollinators favored the combination of flowers with large nectar guides and high nectar volumes, promoting the evolution of honest relation between signals and rewards (Fig. 3).

Discussion

In this study, we evaluated the association between visual signals and rewards of *Mimulus luteus* as a measurement of the honesty of the signals that plants provide to pollinators. Moreover, we examined if honesty could be explained by correlational pollinator-mediated selection on advertisements and rewards. To this end, we first described the pollinator assemblages in the populations under study. We found a large similarity of pollinator assemblages between sites, with 83% of the species being shared. The only pollinator species that was not shared was the Andean hummingbird *Oreotrochilus leucopleurus*, only recorded in Juncal. However, this species has been recorded in the population of El Yeso in previous studies on *M. luteus* (Pohl *et al.* 2006, Medel *et al.* 2007, R. Medel personal observations 2014). In consequence, visitation rates did not differ substantially between sites and the assemblages had a high similarity index. After the analysis of pollinator assemblages, we evaluated the honesty of two signals previously described to be important cues for pollinators of *M. luteus*, namely corolla and nectar guide sizes (Medel *et al.* 2003). These signals were positively correlated in the two populations, indicating that flowers with large corollas have large nectar guides. In a similar way, Medel *et al.* (2007) have previously found high positive correlations between corolla and nectar guide sizes in four out of seven *M. luteus* populations. Despite this correlation between signals, our results showed that only corolla size was associated with some of the rewards evaluated. More specifically, corolla size was consistently correlated with nectar volume and number of pollen grains per anther in the populations (Table 3). Nectar guide size was not associated with any of the

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rewards evaluated. These results would suggest that corolla size is an honest signal in the populations under study, as flowers with larger corollas tend to have larger amounts of nectar (Kaczorowski *et al.* 2005, Fenster *et al.* 2006, Kaczorowski *et al.* 2008). In our system, nectar guide size did not provide any information about the quantity and quality of the rewards. Therefore, as described before, the main role of the nectar guide may be to indicate pollinators the way to reach the rewards and an orientations to pollinators how to enter to flowers rather than a quantitative signal of the amount and quality of the recompenses (Kandori & Ohsaki 1998, Owen & Bradshaw 2011, Hansen *et al.* 2012).

Results from phenotypic selection analysis revealed positive and significant selection differentials for corolla size in both populations, implying that a change in mean corolla size is expected to occur as a consequence of direct selection and indirect selection through correlated characters. However, these effects disappeared after controlling for correlated traits. Regarding correlational selection, a significant correlation gradient for nectar volume and nectar guide size was detected (Fig. 3), indicating that pollinators favor plants showing a positive advertisement–reward association.

In our study, the traits showing significant phenotypic correlations are different from the ones under correlational selection, suggesting that the variation of the traits evaluated in this study is, to a large degree, caused by environmental factors. Ashman and Majetic (2006) summarized the information from a large number of publications, in several species, to evaluate the heritability of floral traits to determine which are more capable of responding to natural selection. They found

that heritability was high for corolla, male and mating system traits and lower for rewards and female traits. Their results suggest that traits related to signals and rewards may have different additive genetic variance and potential to evolve under natural selection, possibly explaining why we did not find consistency in our results. It is known that correlational selection may create a phenotypic correlation between traits with the same sign of the correlational gradient (Roff & Fairbairn 2012). Genetic correlations between two traits generated by pleotropy or by strong linkage disequilibrium may constrain the evolution of a favorable combination of traits (Conner 2002). However, when there is multivariate selection acting on two heritable traits, correlacional selection may also generate a linkage desequilibrum between the loci responsible of the traits under selection, producing a genetic and phenotypic correlation between them. However, a strong and stable correlational selection across generations is needed to maintain this genetic correlation as recombination and segregation should reduced the linkage disequilibrium between the traits under selection in a few generations (Lande 1984, Sinervo & Svensson 2002). Under this idea, it is possible that a temporal variation in the phenotypic selection in our study systems constraints the possibility of correlational selection to generate a phenotypic correlation between the signal-reward traits that were under correlational selection (Kelly 1992, Caruso *et al.* 2003, Benítez-Vieyra *et al.* 2009, Reynolds *et al.* 2010).

Flower signals are supposed to be essential cues to pollinators because nectar is not visible for them. However, some studies have demonstrated that social bumblebees and honeybees can make decisions based on their individual or colony

previous experience. For example, *Bombus terristris* individuals obtain information of flower quality through observing the choices made by other individuals of the colony (Avargues-Weber & Chittka 2014). Likewise, honeybees communicate information about the location of food to their nestmates through a dance language. In the case of hummingbirds, it has been described that *Sephanoides sephaniodes* and *Selasphorus rufus* have cognitive abilities to remember the spatial location of the flowers that offer more profitable nectar sources (Hurly 1996, González-Gómez & Vásquez 2006). Overall this evidence suggests that pollinators may use other sources of information to make their foraging decisions, possibly by complementing the use of visual cues. Moreover, insects and hummingbirds seemingly use different flower cues to choose which flowers visit in *M. luteus* (Medel *et al.* 2003). While insects preferred larger corollas and nectar guides, hummingbirds are more likely to visit flowers with small corollas and nectar guides. More studies are needed to evaluate pollinator preferences by separating the contribution of each pollinator to the total selective force acting on flower phenotypes.

Another possible explanation of our results relates to the estimation of the opportunity of selection. This estimation indicated that this opportunity differed between populations, with Juncal having more chances to evolve under pollinator-mediated selection than El Yeso. This result is not consistent with the analysis of selection as the only significant selection gradient detected was from the population of El Yeso. Since the fit of the models used in the phenotypic selection analysis was low (data not shown), it is possible that the measured variables were not the best predictors of the total opportunity for selection in the populations under study

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(Moorad & Wade 2013). Since this result does not provide insights about the differences between populations, other explanations need to be invoked to understand them. First, when studying a particular plant population and its relation with pollinators, the results usually are not only dependent on its own interaction. For example, changes in the community context may play an important role in accounting for variation in selection among populations such as variation in plant composition, pollinator behavior, pollen limitation, and abiotic factors, among others (Caruso 2000, Totland 2001, Fishman & Willis 2008, Liao *et al.* 2011). In this sense, when co-flowering plant species share pollinators, there may be a reduction in the conspecific pollen arriving to the stigma, influencing the seed production per plant and in consequence the strength and magnitude of the pollinator-mediated selection acting on floral traits (Caruso 2001, Bell *et al.* 2005, Mitchell *et al.* 2009). Juncal had higher plant species richness than El Yeso (Juncal: 27 plant species; El Yeso: 15 plant species), sharing 13.5% of the plant species. We found more pollen limitation in Juncal than in the population of El Yeso. This difference may have contributed to variation in the selection surface between populations. Some studies have demonstrated that there is positive correlation between pollen limitation and the strength of selection in natural populations (Totland 2001, Hansen & Totland 2006). However, our results do not support this idea. It is possible that estimations of pollen limitation on the whole-plant level would provide a better approximation to study the relation between pollinator-mediated selection and pollen limitation as some abiotic factors may affect plan reproductive success (Totland 2001). Furthermore, in addition to variation in plant community composition and pollen

limitation, the presence of other plant-animal interaction may also influence the magnitude of pollinator-mediated selection. For instance, herbivory and nectar robbing may impact plant reproductive success and pollinator visitation to flowers in non-additive ways, which turns difficult to predict the selective impact of interactions separately (Herrera *et al.* 2002, Gómez 2003, Pohl *et al.* 2006, Strauss & Whittall 2006, Schiestl *et al.* 2014). In a study on *M. luteus* performed at El Yeso, Pohl *et al.* (2006) suggested that in patches where floral herbivores were present, hummingbirds preferred to forage on undamaged flowers rather than damaged ones, suggesting that pollination and floral herbivory may have an important role in the reproductive success of the studied species. We did not directly evaluate the presence of any type of herbivores in our study sites; therefore we cannot rule out a potential influence of floral herbivores and nectar robbers on our results. Finally, as indicated by Rausher (1992), although the phenotypic covariance between relative fitness and floral traits may be produced by selection, environmental factors often not considered in the selection models proposed by Lande and Arnold (1983) may create a spurious association between variables. Even though Juncal and El Yeso are both high Andean population differing in ca. 60 m in elevation, it is likely that soil characteristics differ between sites, hence creating different scenarios for plant-pollinator interactions. We cannot rule out variation in abiotic factors as a potential cause of variation in phenotypic selection (Totland 1999, 2001, Caruso *et al.* 2005, Rivas *et al.* 2009).

Overall our results indicate that honesty in natural populations is not a consequence of correlational pollinator-mediated selection. There could be some

genetic constraints determining our results. As described above, it is possible that the heritability of the trait evaluated in this study is too low to generate an evolutionary change through selection. Moreover, genetic correlation of the trait evaluated with other trait unrelated to our study may also affect the evolutionary response of natural selection. Finally, temporal variation in the selection forces could limit the possibility of correlational selection to generate genetic and phenotypic correlation. Additionally, community context, pollen limitation, biotic interaction and environmental factor may affect the pollinator behavior and the phenotypic selection in natural populations. Further studies are needed to estimate the heritability of the signals and rewards on *Mimulus luteus* to corroborate our suggestion and determine which are the possible ecological and genetic constrains in the evolution of honesty.

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Figure Captions

Figure 1. Description of the study sites. a) Map of the location of the populations under study, b) population of Juncal (32°51'S, 70°08'O, 2398 m.a.s.l.), c) population of El Yeso (33°37'S 70°01'O, 2460 m.a.s.l.). b) and c) fences constructed to exclude large herbivores.

Figure 2. Average number of seed per flower for each treatment. ASP = automatic self-pollination; HSP = Hand self-pollination; S = Saturation; C = Control. White bars represent the results from the population of Juncal, and black bars from El Yeso. Different letters denote significant differences between treatments. The comparisons were made per site.

Figure 3. Representation of the bivariate selection surface on nectar guide size (mm^2) and nectar volume (µl). Traits are shown on a standardized scale and fitness was made relative to the population average.

Supplementary material

Figure 1S. Average precipitation (mm) and temperature (ºC) during the summer season for both populations. Data were obtained from WorldClim data base (available at: http://www.worldclim.org/)
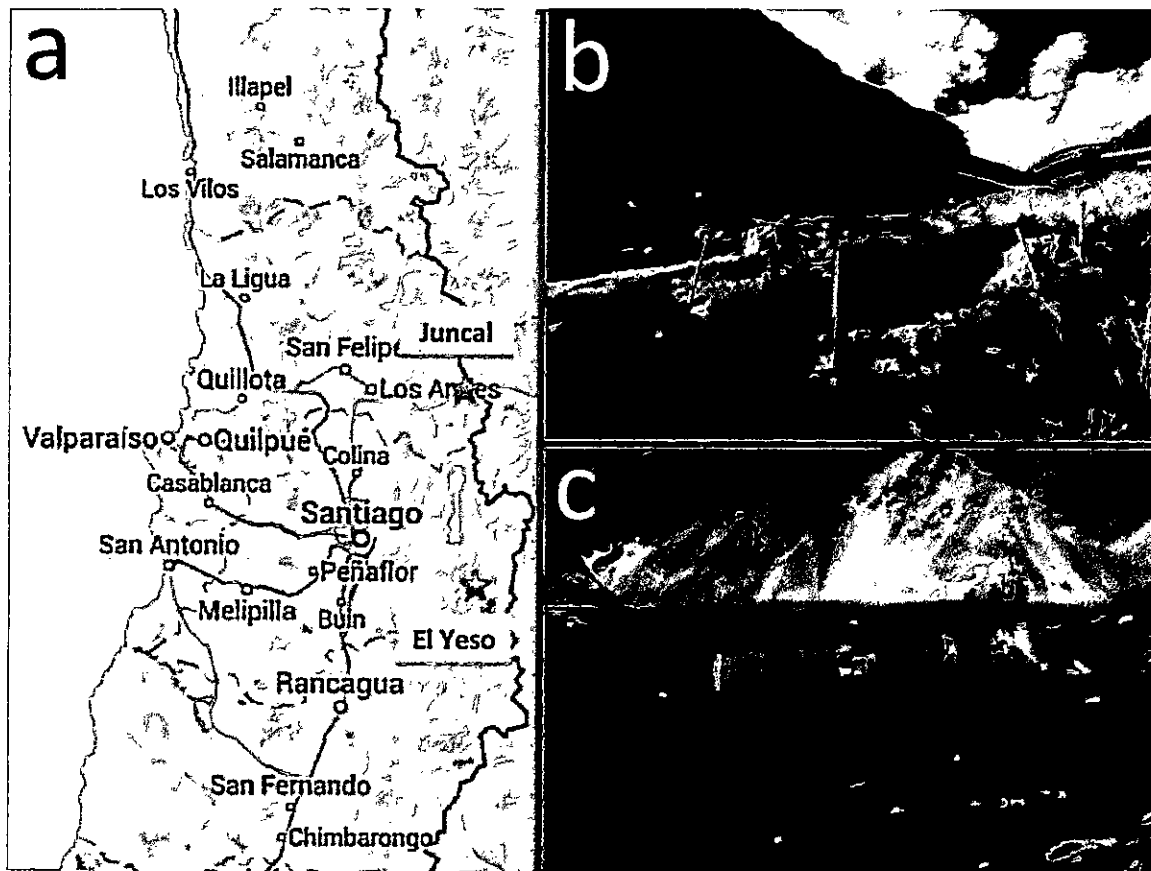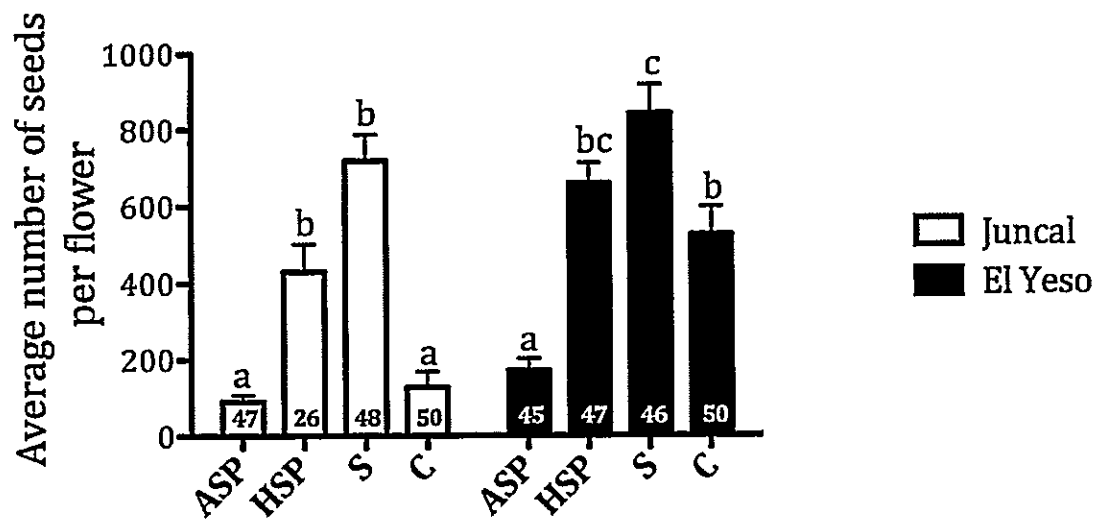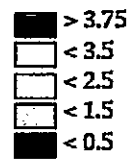
Fig. 1

Fig. 2
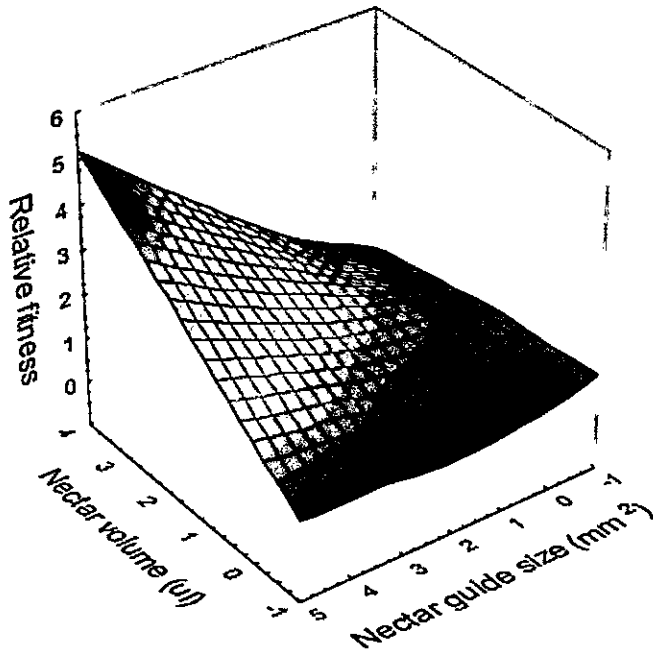
Fig. 3

Tables

Table 1. Visitation rates per species per sites.

	Visitation rate (nº visits flower^{-1}h^{-1})		% Visits	
	Juncal	El Yeso	Juncal	El Yeso
Bombus terrestris	0.0002	0.0096	0.86	28.75
Centris chilensis	0.0051	0.0046	18.47	13.77
Centris nigerrima	0.0106	0.0042	37.92	12.45
Megachile saulcyi	0.0078	0.0130	27.96	38.78
Megachile semirufa	0.0016	0.0021	5.62	6.24
Oreotrochilus leucopleurus	0.0026	0.0000	9.18	0.00

Table 2. Mean ± SD for each trait per site. Values in parentheses are sample sizes.

Traits	JUNCAL	EL YESO
Nectar volume (µl)	0.34 ± 0.38 (108)	0.03 ± 0.05 (120)
Nectar concentration (ºBx)	18.75 ± 33.59 (93)	35.95 ± 27.80 (48)
Nectar guide size (mm^2)	44.31 ± 29.82 (108)	13.95 ± 18.64 (120)
Corolla size (mm^2)	720.78 ± 155.82 (108)	537.85 ± 104.37 (120)
Nº of pollen grains	24624 ± 3591(108)	21587 ± 3620 (120)

Table 3. Correlation coefficients of signals and rewards. Values above the diagonal are from the population of Juncal (N = 108) and under the diagonal from El Yeso (N = 120). Correlations related to nectar concentration have lower sample sizes (Juncal: N = 93, El Yeso: N = 48).

	Nectar volume	Nectar concentration	Nectar guide size	Corolla size	Nº of pollen grains
Nectar volume (µl)	-	-0.058	0.130	0.164*	-0.091
Nectar concentration (ºBx)	-0.107	-	-0.121	-0.039	-0.047
Nectar guide size (mm^2)	0.079	0.147	-	0.246**	0.003
Corolla size (mm^2)	0.328***	0.067	0.219*	-	0.182*
Nº of pollen grains	0.185*	0.103	0.001	0.325***	-

*$p<0.05$; **$p<0.01$; ***$p<0.001$

Table 4. Phenotypic selection analyses on four traits of *Mimulus luteus*. Standardized selection differentials (s_i'), linear (β_i') and nonlinear ($\gamma_{ii}', \gamma_{ij}'$) gradients are reported. Standard errors are presented in parentheses. Bolded values are significant coefficients through bootstrapping methods.

	S_i'	β_i'	γ_{ii}'	Nectar guide size γ_{ij}'	Corolla size γ_{ij}'	Pollen grains γ_{ij}'
JUNCAL (N = 95)						
Nectar volumen	0.054	-0.01(0.131)	0.044(0.082)	-0.182(0.199)	0.08(0.199)	-0.309(0.199)
Nectar guide size	0.179	0.121(0.132)	-0.008(0.105)	-	0.161(0.168)	0.197(0.190)
Corolla size	**0.256***	0.234(0.135)	0.142(0.120)		-	0.034(0.165)
Nº of pollen grains	-0.022	-0.06(0.129)	-0.133(0.103)			-
EL YESO (N = 102)						
Nectar volumen	0.071	0.044(0.055)	0.005(0.057)	**0.199(0.076)**	0.029(0.062)	-0.126(0.075)
Nectar guide size	0.019	0.008(0.053)	0.049(0.041)	-	-0.048(0.082)	0.077(0.062)
Corolla size	**0.103***	0.078(0.059)	0.076(0.059)		-	-0.070(0.074)
Nº of pollen grains	0.067	0.032(0.056)	-0.005(0.047)			-

*p<0.05; **p<0.01; ***p<0.001

Supplementary Material
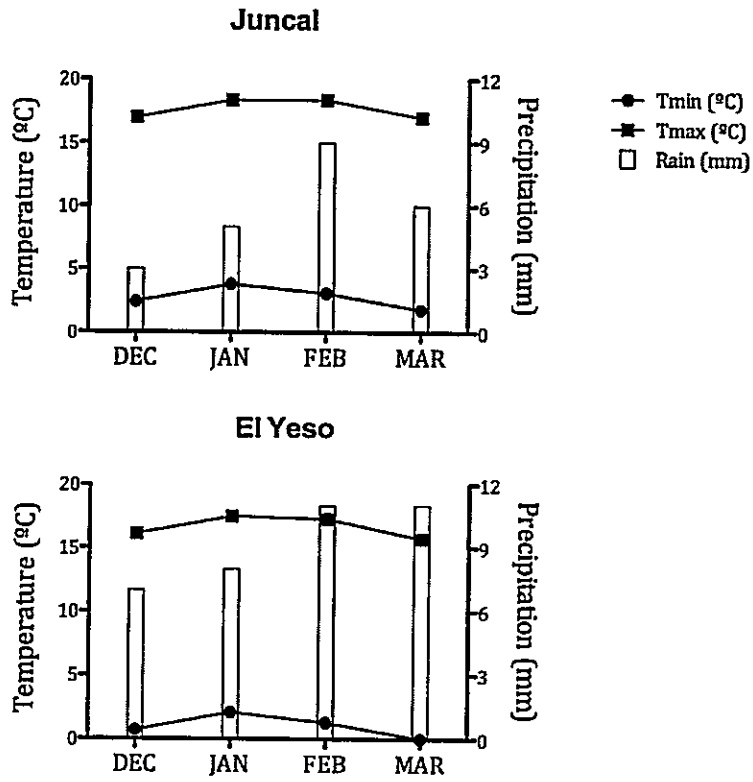
Juncal

El Yeso

Fig. 1S

Table 1S. Between and within plant variation of floral traits through one-way ANOVA per site. Plants with only one flower were removed from analyses. As a large number of flowers did not produce nectar, the significance of tests for nectar volume was estimated using permutations (1000 iterations). Significant P-levels indicate that between plants variation is larger that within plant variation.

	Juncal		El Yeso	
	F	P	F	P
Nectar volume	$F_{107,214} = 2.41$	0.0009	$F_{116,141} = 2.97$	0.0009
Nectar concentration	$F_{71,103} = 1.62$	0.0128	$F_{10,11} = 1.41$	0.289*
Nectar guide size	$F_{107,215} = 10.48$	<0.0001	$F_{117,142} = 14.35$	<0.0001
Corolla size	$F_{107,215} = 3.05$	<0.0001	$F_{117,142} = 2.84$	<0.0001
Nº pollen grains	$F_{107,208} = 2.26$	<0.0001	$F_{112,135} = 1.71$	0.0015

*The variation between plants was greater that within plant (see F-value). However, the sample size for this analysis was too reduced to find significant differences.

CONCLUSIONES

El presente trabajo tuvo como principal objetivo examinar si los rasgos de señal y recompensa de *Mimulus luteus* pueden ser seleccionados de manera correlacionada como consecuencia de la elección de los polinizadores a nivel poblacional. De los resultados obtenidos se desprenden las siguientes conclusiones. En primer lugar, las poblaciones en estudio son honestas para la señal tamaño de la corola, pero no para la guía de néctar. Esto quiere decir que existe una asociación, en este caso positiva, entre la señal tamaño de la corola y las recompensas ofrecidas a los polinizadores. Sin embargo, la honestidad encontrada no puede ser explicada por selección correlacionada mediada por polinizadores. Esto es, debido a que sólo se encontró selección correlacionada significativa para la combinación de rasgos tamaño de la guía de néctar y volumen de néctar en una de las poblaciones en estudio. Esta selección no estaría generando una correlación fenotípica de los rasgos en estudio, posiblemente porque existe restricciones genéticas y ecológicas a las evolución de la honestidad.

El trabajo de campo se realizó en dos poblaciones, con el fin de entender si la honestidad y la selección encontrada son fenómenos población-dependientes. Los resultados obtenidos indican que ambas poblaciones presentan honestidad para los

mismos rasgos, sin embargo, el análisis de selección permite interpretar que sólo

una de estás poblaciones tendría el potencial para evolucionar honestidad a través

de selección correlacionada. Si bien no se obtuvieron gradientes lineales y

cuadráticos significativas para ninguna de las poblaciones, en la población de El

Yeso los polinizadores promueven una relación positiva entre el tamaño de la guía

de néctar y el volumen de néctar. Estas diferencias entre las poblaciones en estudio,

no pudieron ser explicadas por la descripción de los ensambles de polinizadores, los

niveles de autogamia o de limitación de polen. Por lo tanto otros fenómenos no

evaluados en este estudio, debieran ser los responsables de los resultados

encontrados. Por ejemplo, es posible que la variación en el contexto comunitario de

plantas modifique la competencia entre polinizadores y la competencia entre

plantas por el servicio de polinización. Asimismo, la presencia de otra interacciones

biológicas como la herbivoría floral o el robo de néctar que pueden ser factores que

afectan el comportamiento de los polinizadores y el éxito reproductivo de las

plantas. Finalmente, condiciones abióticas tales como aspectos edáficos pueden

modificar la asignación de energía de las plantas a la formación de gametos, creando

variación en la oportunidad para la selección entre poblaciones.

Finalmente, es importante destacar que se necesitan más estudios en las

poblaciones evaluadas para determinar si existe una variación temporal en los

resultados encontrado. Además, futuros estudios son necesarios para determinar las

bases genéticas de los rasgos florales con el fin de determinar la contribución

ambiental y genética sobre la variabilidad fenotípica observada y de esta manera

estimar en qué medida la selección generada por los polinizadores puede traducirse

en una respuesta evolutiva significativa entre generaciones.

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