Adaptive divergence in resistance to herbivores in *Datura stramonium*

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ABSTRACT

Defensive traits exhibited by plants vary widely across populations. Heritable phenotypic differentiation is likely to be produced by genetic drift and spatially restricted gene flow between populations. However, spatially variable selection exerted by herbivores may also give rise to differences among populations. To explore to what extent these factors promote the among-population differentiation of plant resistance of 13 populations of *Datura stramonium*, we compared the degree of phenotypic differentiation (P_{ST}) of leaf resistance traits (trichome density, atropine and scopolamine concentration) against neutral genetic differentiation (F_{ST}) at microsatellite loci. Results showed that phenotypic differentiation in defensive traits among-population is not consistent with divergence promoted by genetic drift and restricted gene flow alone. Phenotypic differentiation in scopolamine concentration was significantly higher than F_{ST} across the range of trait heritability values. In contrast, genetic differentiation in trichome density was different from F_{ST} only when heritability was very low. On the other hand, differentiation in atropine concentration differed from the neutral expectation when heritability was less than or equal to 0.3. In addition, we did not find a significant correlation between pair-wise neutral genetic distances and distances of phenotypic resistance traits. Our findings reinforce previous evidence that divergent natural selection exerted by herbivores has promoted the among-population phenotypic differentiation of defensive traits in *D. stramonium*.

Subjects Ecology, Evolutionary Studies
Keywords Adaptive divergence, Tropane alkaloids, Leaf trichomes, Plant defense, P_{ST}–F_{ST} comparison, *Datura stramonium*, Divergent natural selection, Genetic drift and restricted gene flow, Plant resistance

INTRODUCTION

Most species consist of a series of populations that are often phenotypically differentiated (*Rice & Jain, 1985*; *Thompson, 2005*). Heritable phenotypic differentiation in multiple

traits can be effectively produced by processes like genetic drift, mutation, founder effects or population isolation (*Gomulkiewicz et al., 2007*). However, phenotypic differentiation in traits that contribute to individuals' fitness may also have a spatial structure caused by varying selective regimes exerted by biotic and/or abiotic factors (*Holsinger & Weir, 2009*). Furthermore, stabilizing selection may promote phenotypic similarity among populations (*Merilä & Crnokrak, 2001*). Elucidating to what extent these processes promote character differentiation among populations is central if we are to fully understand the prevalence of among-population variation in the wild (*Lynch, 1990*; *Althoff & Thompson, 1999*; *Criscione, Blouin & Sunnucks, 2006*; *Kelly, 2006*; *Gomulkiewicz et al., 2007*). Here we aimed to determine if among-population variation in traits that confer resistance to herbivores in the annual plant *Datura stramonium* is consistent with a scenario of varying selection or genetic drift and restricted gene flow.

To infer whether natural selection explains the observed differentiation among populations in putatively adaptive quantitative characters (Q_{ST}), it is necessary to contrast this hypothesis against a null model of differentiation at adaptively neutral loci (F_{ST}; *Spitze, 1993*; *Martin, Chapuis & Goudet, 2008*; *Whitlock, 2008*). The detection of a significant difference between the estimators of differentiation, Q_{ST} and F_{ST}, may imply adaptive differentiation among populations. The comparison of the differentiation indices has three possible outcomes each with a unique interpretation (see Table 1 in *Merilä & Crnokrak, 2001*). When Q_{ST} and F_{ST} are statistically equal, this implies that the degree of differentiation in quantitative traits could be produced by drift alone. This does not necessarily imply that genetic drift produced the observed phenotypic differentiation but that the roles of selection and drift are indiscernible. When $Q_{ST} < F_{ST}$, it means that natural selection might be favoring the same phenotype across populations. Finally, when Q_{ST} significantly exceeds F_{ST}, it means that directional selection is favoring different phenotypes in different populations. When Q_{ST} and F_{ST} are equal, it is expected that both indices, estimated among pairs of populations of the same species, will be positively correlated, implying isolation by distance, restricted gene flow and genetic drift (although a partial role of selection could be involved also), or high recombination between molecular neutral marker loci and quantitative trait loci (*Merilä & Crnokrak, 2001*). In contrast, no correlation between both indices of differentiation among local populations may implicate a role of natural selection (see 'Discussion').

In order to explore the signals of non-neutral evolution in quantitative traits it is necessary to estimate Q_{ST} and F_{ST}. F_{ST} is commonly estimated by analyzing variance in allele frequency (*Wright, 1951*) at molecular markers, like microsatellite loci. On the other hand, to estimate Q_{ST} it is necessary to know the amount of additive genetic variance of quantitative traits in many local populations (*Spitze, 1993*). However, accomplishing the latter objective is not feasible for a large number of populations because it requires estimating the breeding values of genotypes (families) for a suite of phenotypic characters in each local population. P_{ST} (degree of phenotypic differentiation index) is an analogous index to Q_{ST} (*Leinonen et al., 2006*; *Leinonen et al., 2013*), useful for exploring if phenotypic differentiation among populations exceeds genetic differentiation in neutral

markers (*Merilä & Crnokrak, 2001*). The use of P_{ST} instead of Q_{ST} is justified when estimates of additive genetic variance are not available (*Leinonen et al., 2006*; *Leinonen et al., 2013*; *Lehtonen et al., 2009*). Estimation of additive genetic variation in traits makes it necessary to obtain the phenotypic covariance between relatives (families) with an experimental common garden and/or the use of reciprocal transplant experiments to rule out the environmental effects on phenotypes. Hence, P_{ST} can be used as a surrogate of Q_{ST}.

Resistance traits exhibited by plants (i.e., traits that prevent/reduce damage by natural enemies) vary widely across populations (*Núñez-Farfán, Fornoni & Valverde, 2007*; *Züst et al., 2012*). Selection exerted by herbivores is a major force driving the evolution of plants' resistance traits (*Rausher, 2001*; *Anderson & Mitchell-Olds, 2011*; *Züst et al., 2012*). Thus, among-population differentiation in resistance traits is likely to be produced by spatial variation in the local selective regimes exerted by herbivores. Such spatially variable selection can be generated by among-population variation in the abundance, species composition, feeding styles, and degree of dietary specialization of herbivores to their host plants (*Falconer & Mackay, 1996*; *Charlesworth, Nordborg & Charlesworth, 1997*; *Parchman & Benkman, 2002*; *Arany et al., 2008*; *Hare, 2012*). *Datura stramonium* (Solanaceae) provides an optimal system for studying among-population differentiation in resistance traits. Because of its wide distribution (Mexico, Canada, United States, and Europe), *D. stramonium* is exposed to different environmental conditions and to a wide diversity of herbivore species (*Weaver & Warwick, 1984*; *Valverde, Fornoni & Núñez-Farfán, 2001*; *Cuevas-Arias, Vargas & Rodríguez, 2008*). Resistance against herbivores in *D. stramonium* includes leaf trichomes (*Valverde, Fornoni & Núñez-Farfán, 2001*; *Kariñho-Betancourt & Núñez-Farfán, 2015*) and tropane alkaloids (*Shonle & Bergelson, 2000*), of which atropine, hyosciamine and scopolamine are the most abundant (*Parr et al., 1990*; *Kariñho-Betancourt et al., 2015*). These secondary metabolites affect the activity of the neurotransmitter acetylcholine (*Roddick, 1991*) with negative effects on insects and vertebrate herbivores (*Hsiao & Fraenkel, 1968*; *Krug & Proksch, 1993*; *Wink, 1993*; *Shonle, 1999*; *Mithöfer & Boland, 2012*). Recent studies have found ample geographic variation in leaf trichome density and atropine and scopolamine concentration in central Mexico (*Castillo et al., 2013*; *Castillo et al., 2014*). However, it is unclear if selection by herbivores or neutral processes, among other factors, can account for the observed among-population differentiation in these resistance traits.

Here, we assessed to what extent population differentiation in resistance leaf traits (trichome density, atropine and scopolamine concentrations) of *D. stramonium* is accounted by neutral processes (genetic drift and restricted gene flow) or divergent natural selection. To do so, we compared the degree of phenotypic differentiation of resistance traits by means of P_{ST} estimated for the whole range of values of heritability, with the neutral expectation set by allelic divergence at microsatellite loci (F_{ST}). We expect that P_{ST} of each resistance character would be significantly higher than the index of population differentiation in neutral molecular markers (F_{ST}), since previous studies have detected contrasting selection exerted by herbivores on the three characters.
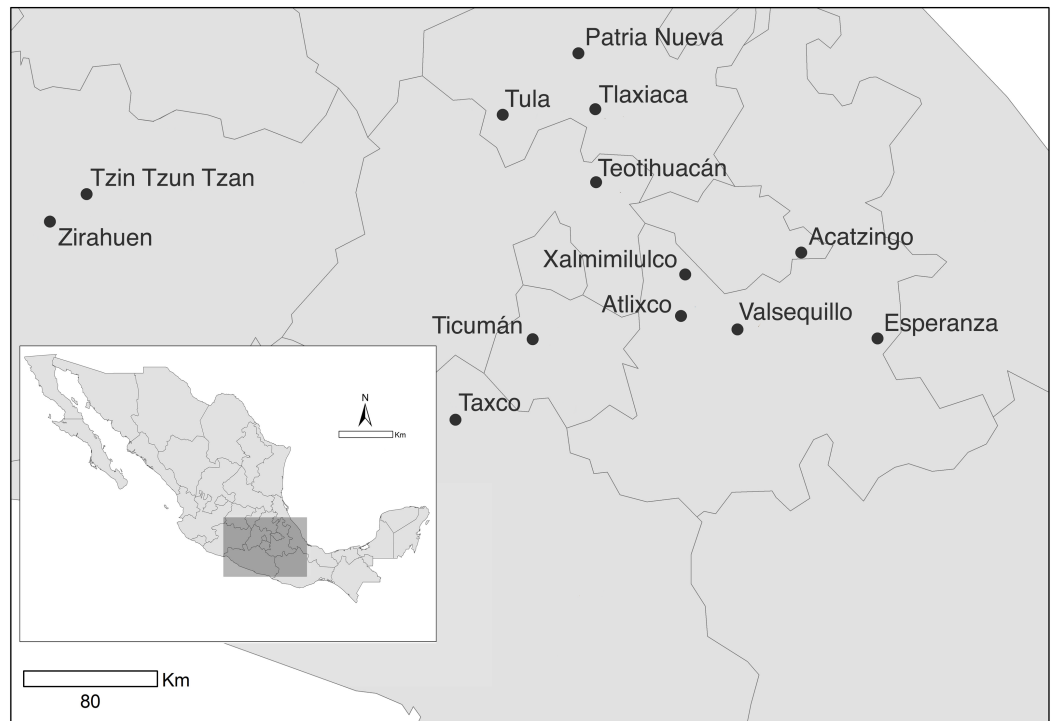
Figure 1 Sampled populations of *Datura stramonium* in central Mexico (see Table 1).

METHODS

Study system

Datura stramonium L. (Solanaceae) is an annual herb commonly found in roadsides, cultivated areas and disturbed environments in Mexico, the United States, Canada, and Europe (*Valverde, Fornoni & Núñez-Farfán, 2001*; *Weaver, Dirks & Warwick, 1985*; *Van Kleunen, Markus & Steven, 2007*). In Mexico, leaves of *D. stramonium* are consumed by a dietary specialist herbivore, the chrysomelid *Lema trilineata* (*Nuñez-Farfan & Dirzo, 1994*), the dietary oligophagous *Epitrix parvula* (Chrysomelidae), which also feeds from other members of the Solanaceae family (*Glass, 1940*), and by the dietary generalist grasshopper *Sphenarium purpurascens* (*Nuñez-Farfan & Dirzo, 1994*). *Datura stramonium* features leaf trichomes and tropane alkaloids (atropine and scopolamine) as resistance traits against herbivory. These traits have shown heritable basis (*Shonle & Bergelson, 2000*; *Valverde, Fornoni & Núñez-Farfán, 2001*; *Kariñho-Betancourt & Núñez-Farfán, 2015*), and are under selection by dietary specialist and generalist herbivores (*Castillo et al., 2014*).

Fieldwork

During August–September 2007 we sampled 13 natural populations of *D. stramonium* in central Mexico (Fig. 1). Selected populations inhabit a wide range of habitat types. The geographic location and habitat characteristics are shown in Table 1. From each population we sampled 30 randomly chosen individual plants.

Table 1 Vegetation type, latitude, longitude, altitude and population means of leaf trichome density, and atropine and scopolamine concentrations of 13 populations of *Datura stramonium* in central Mexico.

	Vegetation type	Latitude	Longitude	Altitude (m a.s.l.)	Trichome density ($2.5 \times mm^2$)	Atropine (mg/g)	Scopolamine (mg/g)
1. Acatzingo	DS	−97.78	19.32	2,160	8.99	0.295	0.159
2. Atlixco	DS	−98.42	18.98	1,840	9.04	0.691	0.577
3. Esperanza	DS	−97.37	18.85	2,278	9.57	0.535	0.542
4. Patria Nueva	DS	−98.96	20.38	2,040	12.62	0.317	0.367
5. Taxco	TDF	−99.66	18.5	1,582	9.02	0.957	0.266
6. Teotihuacán	DS	−98.86	19.68	2,294	8.73	0.437	0.353
7. Ticumán	TDF	−99.2	18.86	1,210	6.6	0.938	1.889
8. Tlaxiaca	DS	−98.86	20.08	2,340	9.36	0.288	0.458
9. Tula	DS	−99.35	20.05	2,020	6.06	3.129	2.804
10. Tzin Tzun Tzan	POF	−101.58	19.63	2,050	4.29	0.994	2.995
11. Valsequillo	DS	−98.11	18.91	2,209	6.09	1.767	0.044
12. Xalmimilulco	POF	−98.38	19.2	1,200	4.66	2.688	2.513
13. Zirahuén	POF	−101.91	19.43	2,174	4.91	0.618	1.968

Notes.

DS, desert shrub; POF, Pine–Oak forest; TDF, tropical deciduous forest.

Resistance traits quantification

Following *Valverde, Fornoni & Núñez-Farfán (2001)*, we estimated leaf trichome density as the total number of trichomes in an observation field of 2.5 mm^2 located in the central basal region of the adaxial side of the leaf, using a stereoscopic microscope. Then we averaged the trichome density per plant from a random sample of 20 fully expanded leaves. We also quantified the concentration of atropine and scopolamine (two major alkaloids in *D. stramonium*) from a sample of 20 leaves per plant by means of High Precision Liquid Chromatography (HPLC). Details of the extraction method and HPLC conditions can be found elsewhere (see *Castillo et al., 2013*).

Data analysis

We estimated the neutral genetic differentiation among populations of *D. stramonium* using F_{ST} values obtained from five nuclear microsatellite markers designed specifically for *D. stramonium* as reported by *Andraca-Gómez (2009)*. F_{ST} values were calculated using FSTAT 2.9.3.1 (*Goudet, 2001*) employing approximately 30 individuals per population. In addition, we assessed the statistical power of our five microsatellites by means of Wright–Fisher simulations as implemented in the program PowSim (*Ryman & Palm, 2006*). The program requires a divergence time and effective populations sizes so we tested a number of feasible combinations.

Phenotypic divergence in resistance traits

We used the degree of among-population phenotypic divergence (P_{ST}) to explore if restricted gene flow and genetic drift (F_{ST}) alone can account for this differentiation or if there is a signal of differentiation promoted by divergent selection on resistance traits

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5/15

(*Leinonen et al., 2006*; *Pujol et al., 2008*). We estimated P_{ST} as

$$P_{ST} = \frac{\sigma^2_{GB}}{\sigma^2_{GB} + 2(h^2 \bullet \sigma^2_{GW})},$$

where σ^2_{GB} is the variance among populations, σ^2_{GW} is the variance within population, and h^2 is the trait heritability (*Leinonen et al., 2006*). Since this is not feasible for a large number of populations we used an approximation by P_{ST}.

In order to obtain P_{ST} values for resistance traits, we simulated the whole range of heritabilities ($0 \le h^2 \le 1$). To estimate P_{ST} values we fitted a linear model for each resistance trait, under the assumption that the distribution of resistance traits was normally distributed. The *population* term was considered as a random effect. To test the hypothesis that P_{ST} is higher than F_{ST}, a Monte Carlo test was carried out, approaching a sample of 10,000 deviates from both P_{ST} and F_{ST} by means of their estimated error. P_{ST} error was estimated from the likelihood errors of its components (variances among- and within-populations), while F_{ST} error was obtained by bootstrapping (*Goudet, 2001*). The 10,000 random deviates of F_{ST} and P_{ST} were compared and the *p*-value was obtained as the proportion of comparisons in which the F_{ST} was equal or higher than the P_{ST} (null hypothesis).

We further evaluated the pair-wise Pearson's correlation between F_{ST} and P_{ST} for all populations. Neutral marker variation can be used as a neutral expectation against which the phenotypic divergence of traits can be compared (*Gomulkiewicz et al., 2007*). If resistance phenotypic differentiation between populations (P_{ST}) is the result of neutral processes rather than selection, differentiation among populations in these traits should correlate positively with differentiation in selectively neutral markers (F_{ST}) (*Merilä & Crnokrak, 2001*; *Gomulkiewicz et al., 2007*; *Lehtonen et al., 2009*; *Leinonen et al., 2013*). We evaluated the pair-wise correlation between the F_{ST} and P_{ST} for different scenarios of heritability ($h^2 = 0.1, 0.25, 0.5, 0.75$ and 1.0). Statistical analyses were performed using JMP® version 9.0.0 (SAS Institute, Cary, NC, 1989–2007).

RESULTS

Among-population variation in resistance traits

A multivariate analysis of variance (MANOVA) detected significant multivariate differences in the studied resistance traits of 13 populations of *D. stramonium* (Wilks' $\lambda = 0.091$, $F_{36,331.64} = 11.51$, $P < 0.0001$). After the subsequent univariate ANOVAs were applied, we found significant differences in trichome density ($F_{12,126} = 5.10$, $P < 0.0001$), atropine ($F_{12,126} = 7.85$, $P < 0.0001$), and scopolamine concentration ($F_{12,126} = 23.33$, $P < 0.0001$). Mean leaf trichome density and mean atropine and scopolamine concentration per population are shown in Fig. 2 and Table 1.

Genetic differentiation between populations of *D. stramonium*

Genetic differentiation as estimated by differences in allele frequency at microsatellite loci was moderate. F_{ST} was 0.228 (S.E. = 0.039), which is well above the minimum detectable value ($F_{ST} = 0.01$) that our sample and markers allowed with a statistical power of 0.94.
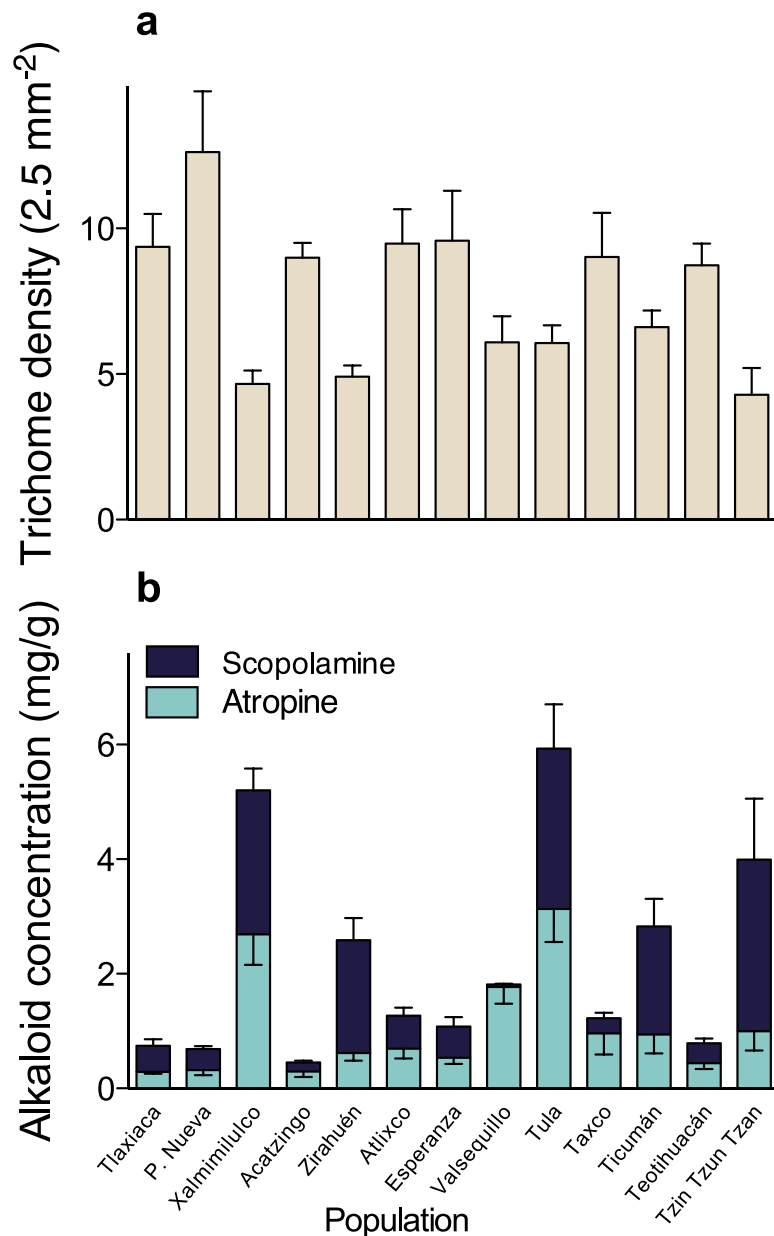
Figure 2 Among-populations variation in leaf trichome density (A), and atropine and scopolamine concentration (B) in 13 populations of *Datura stramonium* in central Mexico. Bars represent average value +1 SE.

Phenotypic divergence in resistance traits

Comparison of phenotypic (P_{ST}) and neutral genetic marker divergence (F_{ST}) showed that P_{ST} values for scopolamine concentration were significantly higher than the F_{ST} in all values of h^2 (Fig. 3). However, P_{ST} for atropine concentration was significantly higher than F_{ST} when $0 \geq h^2 \leq 0.3$ (Fig. 2), whereas P_{ST} of leaf trichome density significantly exceeded F_{ST} only when $h^2 \leq 0.1$ (Fig. 2).
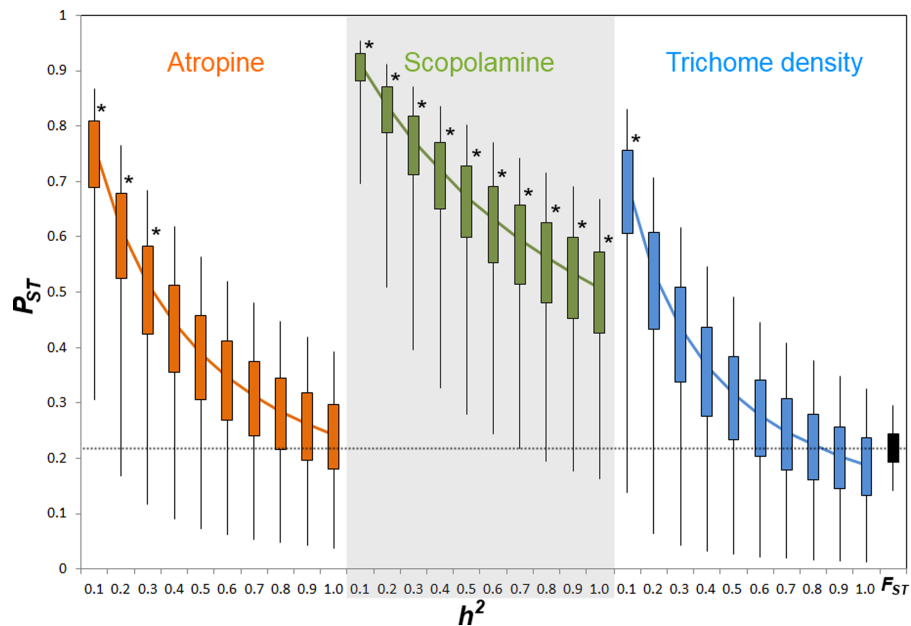
Figure 3 P_{ST} **values of putative defensive traits of** *Datura stramonium* **as a function of their genetic variance** (h^2) **among populations.** Confidence intervals of 50% and 95% are indicated by bars and lines, respectively. * Represents overall P_{ST} values that differ significantly from F_{ST} (the black bar at the right end) after a Monte Carlo test (10,000 deviates from both P_{ST} and F_{ST}; see 'Methods').

Table 2 **Correlation** (r) **between pair-wise** P_{ST} **of three resistance traits and pair-wise** F_{ST} **for all populations of** *Datura stramonium*, **under different scenarios of heritability** ($h^2 = 0.1, 0.25, 0.5, 0.75$ **and 1.0**).

Resistance trait	r				
	$h^2 = 0.1$	$h^2 = 0.25$	$h^2 = 0.5$	$h^2 = 0.75$	$h^2 = 1.0$
Atropine	-0.0644	-0.0671	-0.0655	-0.0642	-0.0637
Scopolamine	0.0264	0.0344	0.0348	0.0316	0.0278
Trichome density	-0.135	-0.1218	-0.1053	-0.0939	-0.0855

Pair-wise correlation between F_{ST} and P_{ST}

We found no significant correlations between pair-wise F_{ST} and P_{ST} values among populations for any of the three resistance characters (Table 2). Most correlation values were small (i.e., $-0.135 \leq r \leq 0.034$).

DISCUSSION

Results showed that phenotypic differentiation in resistance traits among population of *D. stramonium* is not consistent with divergence promoted by genetic drift and restricted gene flow alone (*Pujol et al., 2008*; *Lehtonen et al., 2009*). Phenotypic differentiation in scopolamine concentration was significantly higher than F_{ST} across the range of h^2. In contrast, genetic differentiation in trichome density was different from F_{ST} only when heritability was very low, and most phenotypic variation could be related to major environmental factors, like annual mean precipitation and temperature. Likewise,

differentiation in atropine concentration seems to differ from the neutral expectation only at low values of h^2. Furthermore, we did not find a correlation between pair-wise neutral genetic distances and phenotypic distances of any of the three resistance traits. Taken together, results suggest that natural selection could be involved in phenotypic divergence on resistance traits among populations of *D. stramonium*.

Results indicate that populations of *D. stramonium* are differentiated in both phenotypic and neutral molecular markers. We found a moderate amount of differentiation among populations at microsatellite loci ($F_{ST} = 0.228$). Using this F_{ST} value, the indirect estimate of gene flow (Nm) is 0.846, suggesting restricted gene flow among populations of *D. stramonium*, and not sufficient to prevent differentiation by genetic drift (*Hedrick, 2000*). This contrasts with differentiation at neutral loci reported for other organisms where F_{ST} is generally lower than 0.228 (but see *Merilä & Crnokrak, 2001*). P_{ST} index values statistically not different from this value of F_{ST} imply that quantitative phenotypic characters follow a pattern of drift-induced divergence (*Leinonen et al., 2006*). Here, we found that the P_{ST} index of scopolamine was significantly higher than F_{ST} for all values of heritability considered (cf. Fig. 3). This result strongly suggests that phenotypic differentiation among populations in scopolamine concentration is congruent with a scenario of divergent selection exerted by herbivores among populations. However, P_{ST} of atropine and leaf trichome density was higher than F_{ST} only when heritability was ≤ 0.3 and ≤ 0.1, respectively. This implies that the proportion of genetic variance among populations from total genetic variance is high for these characters (*Lehtonen et al., 2009*; *Leinonen et al., 2013*). When genetic variance within populations is low, as implied by low values of heritability, there is a high opportunity to detect a significant P_{ST} given that the among-population genetic variance component has a relevant weight in the total phenotypic variance. Inversely, when heritability is high, the within-population genetic component accounts for a high fraction of total genetic variance rendering P_{ST} very small. These considerations may explain why P_{ST} of trichome density and atropine are different from F_{ST} only at very low heritability.

Although P_{ST} is used as an analog of Q_{ST} (genetic differentiation in quantitative characters) when it is not possible to obtain the amount of additive genetic variation (variance among families, within populations) (*Merilä & Crnokrak, 2001*), conclusions derived from these estimations must be interpreted with caution since this index can be biased by all environmental variation due to abiotic conditions among localities as well as environmental deviations within populations, and non-additive genetic variation (v.gr., epistatic interactions, dominance, linkage disequilibrium), among others (*Pujol et al., 2008*). Thus is relevant to ask whether P_{ST} index obtained for the resistance traits in *D. stramonium* posses genetic variance. *Datura stramonium* displays a great variation among populations in trichome density and tropane alkaloids' concentration in central Mexico (*Castillo et al., 2013*). Phenotypic variation in alkaloid concentration, like other quantitative traits, is governed by environmental physical factors and genetic variation (*Castillo et al., 2013*). Previous studies in this species have detected narrow-sense h^2 of general resistance to herbivores of 0.49 and 0.41 in two natural populations of *D. stramonium* (*Fornoni, Valverde & Núñez-Farfán, 2003*; note that general resistance may include physical and chemical

defenses). In addition, broad-sense h^2 of general resistance and trichome density has been estimated in 0.25 and 0.64, respectively (*Kariñho-Betancourt & Núñez-Farfán, 2015*). Also, genetic variance in trichome density among-populations (*Valverde, Fornoni & Núñez-Farfán, 2001*) and general resistance (*Valverde, Fornoni & Núñez-Farfán, 2003*; *Carmona & Fornoni, 2013*) has been detected in *D. stramonium*. Finally, genetic variance in alkaloid concentration (hyosciamine and scopolamine, and their ratio) has been detected previously by *Shonle & Bergelson (2000)*. Thus, there is ample evidence of genetic basis of phenotypic variation in resistance of *D. stramonium* to support our estimation of P_{ST} values.

Because a P_{ST} index higher than F_{ST} means that divergent selection might be involved in population differentiation of resistance traits, at least for scopolamine, it is relevant to ask to what extent natural selection by herbivores is responsible for population differentiation in this character. In *D. stramonium*, several lines of evidence strongly suggest that differentiation in resistance is accounted by for herbivores. Differential and contrasting selection gradients on resistance to herbivores were detected between two populations of this species in a reciprocal transplant experiment (*Fornoni, Valverde & Núñez-Farfán, 2004*). Likewise, *Shonle & Bergelson (2000)* detected stabilizing selection on hyosciamine and directional selection to reduce scopolamine concentration in *D. stramonium*. In a recent study of eight populations of *D. stramonium*, *Castillo et al. (2014)* found that atropine is selected against by the dietary specialist herbivores *Epitrix parvula* (in one population) and *Lema daturaphila* (in two populations). In contrast, scopolamine was positively selected in one population where the specialist *Lema daturaphila* was the main herbivore, whereas trichome density was positively selected in two populations (one with *L. daturaphila* and one with the generalist grasshopper *Sphenarium puprurascens*), and negatively selected in one population with the *E. parvula* (*Castillo et al., 2014*). Thus, although genetic drift and restricted gene flow could produce phenotypic variation in plant resistance among populations, the available evidence of spatially variable selection on resistance traits in *D. stramonium* and data presented here suggests that population differentiation can be potentially adaptive.

Furthermore, we did not detect any significant correlation between the pair-wise P_{ST} and F_{ST} among population across the whole range of heritability, suggesting that differentiation at quantitative traits and neutral molecular loci is decoupled. Theoretically, if the pace of differentiation is dictated by genetic drift only, it is expected that differentiation indices will be perfectly and positively correlated ($r = 1$, $P_{ST} = F_{ST}$; Fig. 4). If the correlation is positive but lower than 1, then genetic drift has a role but does not explain all differentiation in quantitative traits. In the region above the diagonal in Fig. 4, where $P_{ST} > F_{ST}$, any positive pair-wise correlation across populations, depicts a scenario where differentiation in quantitative traits exceeds the neutral expectation and suggests divergent selection (Fig. 4). On the other hand, in the region below the diagonal, where $P_{ST} < F_{ST}$, any positive pair-wise correlation across populations, portrays a scenario where differentiation at neutral molecular loci surpasses that of quantitative characters suggesting a strong effect of genetic drift; however at moderate values of F_{ST} stabilizing selection might be favoring the same phenotype across populations (Fig. 4). When $P_{ST} > F_{ST}$ and are uncorrelated (dotted line in Fig. 4) it shows another interesting
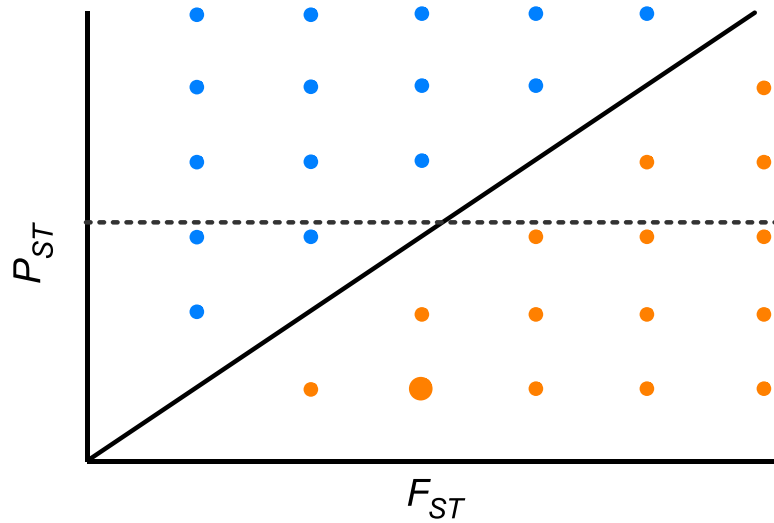
Figure 4 Theoretical relationship between pair-wise P_{ST} and pair-wise F_{ST} across populations of a species. The solid diagonal line indicates a perfect and positive correlation between both indices ($r = 1$, $P_{ST} = F_{ST}$). Above the diagonal, blue points are pairs of populations where $P_{ST} > F_{ST}$. Below the diagonal, orange points are pairs of populations where $P_{ST} < F_{ST}$. Dotted line indicates one possible scenario where both indices are uncorrelated. At moderate values of F_{ST} stabilizing selection might be promoting low phenotypic differentiation between a given pair of populations (big orange point).

scenario, as found here. This implies that genetic drift and restricted gene flow alone cannot explain (*Pujol et al., 2008*) the pattern of differentiation among populations in resistance traits in *D. stramonium*. Under this scenario there is opportunity for divergence driven by selection in resistance traits. Our results suggest that the higher P_{ST} than F_{ST} for scopolamine, together with spatial variation in resistance traits and the existence of a selection mosaic detected previously by *Castillo et al. (2014)* are consistent with outcomes predicted by the geographic mosaic of coevolution (*Thompson, 2005*).

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Guillermo Castillo conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Pedro L. Valverde analyzed the data, wrote the paper, reviewed drafts of the paper.
- Laura L. Cruz, Guadalupe Andraca-Gómez and Juan Fornoni performed the experiments.
- Johnattan Hernández-Cumplido performed the experiments, reviewed drafts of the paper.
- Edson Sandoval-Castellanos analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
- Erika Olmedo-Vicente analyzed the data.
- César M. Flores-Ortiz performed the experiments, contributed reagents/materials/analysis tools.
- Juan Núñez-Farfán conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

Data Availability

The following information was supplied regarding data availability:
    The research in this article did not generate any raw data.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.1411#supplemental-information.

REFERENCES

Althoff DM, Thompson JN. 1999. Comparative geographic structures of two Parasitoid-Host interactions. *Evolution* **53**:818–825 DOI 10.2307/2640721.

Anderson JT, Mitchell-Olds T. 2011. Ecological genetics and genomics of plant defences: evidence and approaches. *Functional Ecology* **25**:312–324 DOI 10.1111/j.1365-2435.2010.01785.x.

Andraca-Gómez G. 2009. Genética de poblaciones comparada entre *Datura stramonium* y su herbívoro especialista *Lema trilineata*. MSc dissertation, Universidad Nacional Autónoma de México (UNAM), Mexico City.

Arany A, De Jong T, Kim H, Van Dam N, Choi Y, Verpoorte R, Van der Meijden E. 2008. Glucosinolates and other metabolites in the leaves of *Arabidopsis thaliana* from natural populations and their effects on a generalist and a specialist herbivore. *Chemoecology* **18**:65–71 DOI 10.1007/s00049-007-0394-8.

Carmona D, Fornoni J. 2013. Herbivores can select for mixed defensive strategies in plants. *New Phytologist* **197**:576–585 DOI 10.1111/nph.12023.

Castillo G, Cruz LL, Hernández-Cumplido J, Oyama K, Flores-Ortiz CM, Fornoni J, Valverde PL, Núñez-Farfán J. 2013. Geographic association and temporal variation of defensive traits and leaf damage in *Datura stramonium*. *Ecological Research* **28**:663–672 DOI 10.1007/s11284-013-1059-4.

Castillo G, Cruz LL, Tapia-López R, Olmedo-Vicente E, Carmona D, Anaya-Lang AL, Fornoni J, Andraca-Gómez G, Valverde PL, Núñez-Farfán J. 2014. Selection mosaic exerted by specialist and generalist herbivores on chemical and physical defense of *Datura stramonium*. *PLoS ONE* **9**:e102478 DOI 10.1371/journal.pone.0102478.

Charlesworth B, Nordborg M, Charlesworth D. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetical Research* **70**:155–174 DOI 10.1017/S0016672397002954.

Criscione CD, Blouin MS, Sunnucks P. 2006. Minimal selfing, few clones, and no amog-host genetic structure in a hermaphroditic parasite with asexual propagation. *Evolution* **60**:553–562 DOI 10.1111/j.0014-3820.2006.tb01136.x.

Cuevas-Arias CT, Vargas O, Rodríguez A. 2008. Solanaceae diversity in the state of Jalisco, Mexico. *Revista Mexicana de Biodiversidad* **79**:67–79.

Falconer DS, Mackay T. 1996. *Introduction to quantitative genetics*. 4th edition. Harlow: Addison-Wesley Longman.

Fornoni J, Valverde PL, Núñez-Farfán J. 2003. Quantitative genetics of plant tolerance and resistance against natural enemies of two natural populations of *Datura stramonium*. *Evolutionary Ecology Research* **5**:1049–1065.

Fornoni J, Valverde PL, Núñez-Farfán J. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. *Evolution* **58**:1696–1704 DOI 10.1111/j.0014-3820.2004.tb00455.x.

Glass EH. 1940. Host plants of the tobacco flea beetle. *Journal of Economic Entomology* **33**:467–470 DOI 10.1093/jee/33.3.467.

Gomulkiewicz R, Drown DM, Dybdahl MF, Godsoe W, Nuismer SL, Pepin KM, Ridenhour BJ, Smith CI, Yoder JB. 2007. Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity* **98**:249–258 DOI 10.1038/sj.hdy.6800949.

Goudet J. 2001. *FSTAT, a program to estimate and test gene diversities and fixation indices*. version 2.9.31. Lausanne: Institut d'Ecologie, Batiment de Biologie, Universite de Lausanne. *Available at http://www2.unil.ch/popgen/softwares/fstat.htm.*

Hare JD. 2012. How Insect herbivores drive the evolution of plants. *Science* **338**:50–51 DOI 10.1126/science.1228893.

Hedrick PW. 2000. *Genetics of populations*. 2nd edition. Sudbury: Jonesand Bartlett Publishers.

Holsinger KE, Weir BS. 2009. Genetics in geographically structured populations: defining, estimating and interpreting F_{ST}. *Nature Reviews Genetics* **10**:639–650 DOI 10.1038/nrg2611.

Hsiao TH, Fraenkel G. 1968. The role of secondary plant substances in the food specificity of the Colorado potato beetle. *Annals of the Entomological Society of America* **61**:485–503 DOI 10.1093/aesa/61.2.485.

Kariñho-Betancourt E, Agrawal AA, Halitschke R, Núñez-Farfán J. 2015. Phylogenetic correlations among chemical and physical plant defenses change with ontogeny. *New Phytologist* **206**:796–806 DOI 10.1111/nph.13300.

Kariñho-Betancourt E, Núñez-Farfán J. 2015. Evolution of resistance and tolerance to herbivores: testing the trade-off hypothesis. *PeerJ* **3**:e789 DOI 10.7717/peerj.789.

Kelly JK. 2006. Geographical variation in selection, from phenotypes to molecules. *The American Naturalist* **167**:481–495 DOI 10.1086/501167.

Krug E, Proksch P. 1993. Influence of dietary alkaloids on survival and growth of *Spodoptera littoralis*. *Biochemical and Systematic Ecology* **21**:749–756 DOI 10.1016/0305-1978(93)90087-8.

Lehtonen PK, Laaksonen T, Artemyev AV, Belskii E, Both C, Bureš S, Bushuev AV, Krams I, Moreno J, Mägi M. 2009. Geographic patterns of genetic differentiation and plumage colour variation are different in the pied flycatcher (*Ficedula hypoleuca*). *Molecular Ecology* **18**:4463–4476 DOI 10.1111/j.1365-294X.2009.04364.x.

Leinonen T, Cano J, Mäkinen H, Merilä J. 2006. Contrasting patterns of body shape and neutral genetic divergence in marine and lake populations of threespine sticklebacks. *Journal of Evolutionary Biology* **19**:1803–1812 DOI 10.1111/j.1420-9101.2006.01182.x.

Leinonen T, McCairns RS, O'Hara RB, Merilä J. 2013. Q_{ST}–F_{ST} comparisons: evolutionary and ecological insights from genomic heterogeneity. *Nature Reviews Genetics* **14**:179–190 DOI 10.1038/nrg3395.

Lynch M. 1990. The rate of morphological evolution in mammals from the standpoint of the neutral expectation. *The American Naturalist* **136**:727–741 DOI 10.1086/285128.

Martin G, Chapuis E, Goudet J. 2008. Multivariate Q_{ST}—F_{ST} comparisons: a neutrality test for the evolution of the G matrix in structured populations. *Genetics* **180**:2135–2149 DOI 10.1534/genetics.107.080820.

Merilä J, Crnokrak P. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* **14**:892–903 DOI 10.1046/j.1420-9101.2001.00348.x.

Mithöfer A, Boland W. 2012. Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology* **63**:431–450 DOI 10.1146/annurev-arplant-042110-103854.

Nuñez-Farfan J, Dirzo R. 1994. Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. *Evolution* **48**:423–436 DOI 10.2307/2410102.

Núñez-Farfán J, Fornoni J, Valverde PL. 2007. The evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics* **38**:541–566 DOI 10.1146/annurev.ecolsys.38.091206.095822.

Parchman TL, Benkman CW. 2002. Diversifying coevolution between crossbills and black spruce on Newfoundland. *Evolution* **56**:1663–1672 DOI 10.1111/j.0014-3820.2002.tb01478.x.

Parr A, Payne J, Eagles J, Chapman B, Robins R, Rhodes M. 1990. Variation in tropane alkaloid accumulation within the solanaceae and strategies for its exploitation. *Phytochemistry* **29**:2545–2550 DOI 10.1016/0031-9422(90)85185-I.

Pujol B, Wilson AJ, Ross RIC, Pannell JR. 2008. Are Q_{ST}–F_{ST} comparisons for natural populations meaningful? *Molecular Ecology* **17**:4782–4785 DOI 10.1111/j.1365-294X.2008.03958.x.

Rausher MD. 2001. Co-evolution and plant resistance to natural enemies. *Nature* **411**:857–864 DOI 10.1038/35081193.

Rice K, Jain S. 1985. Plant population genetics and evolution in disturbed environments. In: Pickett STA, White PS, eds. *The ecology of natural disturbance and patch dynamics*. New York: Academic Press, 287–303.

Roddick J. 1991. The importance of the solanaceae in medicine and drug therapy. In: Hawkes JG, Lester RN, Nee M, Estrada N, eds. *Solanaceae. III. Taxonomy, chemistry, evolution*. Kew: Royal Botanic Garden Press, 7–23.

Ryman N, Palm S. 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* **6**:600–602 DOI 10.1111/j.1471-8286.2006.01378.x.

Shonle I. 1999. Evolutionary ecology of tropane alkaloids. PhD dissertation, University of Chicago, Chicago, IL.

Shonle I, Bergelson J. 2000. Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). *Evolution* **54**:778–788 DOI 10.1111/j.0014-3820.2000.tb00079.x.

Spitze K. 1993. Population structure in Daphnia obtusa: quantitative genetic and allozymatic variation. *Genetics* **135**:367–374.

Thompson J. 2005. *The geographic mosaic of coevolution*. Chicago: University of Chicago Press.

Valverde PL, Fornoni J, Núñez-Farfán J. 2001. Defensive role of leaf trichomes in resistance to herbivorous insects in *Datura stramonium*. *Journal of Evolutionary Biology* **14**:424–432 DOI 10.1046/j.1420-9101.2001.00295.x.

Valverde PL, Fornoni J, Núñez-Farfán J. 2003. Evolutionary ecology of *Datura stramonium*: equal plant fitness benefits of growth and resistance against herbivory. *Journal of Evolutionary Biology* **16**:127–137 DOI 10.1046/j.1420-9101.2003.00482.x.

Van Kleunen M, Markus F, Steven DJ. 2007. Reproductive assurance through self-fertilization does not vary with population size in the alien invasive plant *Datura stramonium*. *Oikos* **116**:1400–1412 DOI 10.1111/j.0030-1299.2007.16004.x.

Weaver SE, Dirks V, Warwick S. 1985. Variation and climatic adaptation in northern populations of *Datura stramonium*. *Canadian Journal of Botany* **63**:1303–1308 DOI 10.1139/b85-181.

Weaver SE, Warwick SI. 1984. The biology of Canadian weeds: 64. *Datura stramonium* L. *Canadian Journal of Plant Science* **64**:979–991 DOI 10.4141/cjps84-132.

Whitlock MC. 2008. Evolutionary inference from Q_{ST}. *Molecular Ecology* **17**:1885–1896 DOI 10.1111/j.1365-294X.2008.03712.x.

Wink M. 1993. Allelochemical properties or the raison d'etre of alkaloids. In: Cordell GA, ed. *The alkaloids*, vol. 43. New York: Academic Press, 1–118.

Wright S. 1951. The genetical structure of populations. *Annals of Eugenics* **15**:323–354 DOI 10.1111/j.1469-1809.1949.tb02451.x.

Züst T, Heichinger C, Grossniklaus U, Harrington R, Kliebenstein DJ, Turnbull LA. 2012. Natural enemies drive geographic variation in plant defenses. *Science* **338**:116–119 DOI 10.1126/science.1226397.