

Natural selection drives chemical resistance of *Datura stramonium* to its specialized seed-predator

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20

21 Abstract

22 Plant resistance to herbivores involves physical and chemical plant traits that affect herbivores
23 fitness and hence may promote coevolutionary arm-races between interacting species. *Datura*
24 *stramonium* possesses tropane alkaloids that prevent and/or reduce damage by leaf beetles, but it
25 is not known whether ~~the specialist weevil, *Trichobaris soror* exert selection on these these~~
26 resistance characters ~~function to reduce seed predation by the specialist weevil, *Trichobaris*~~
27 ~~*soror*, and are affected by natural selection.~~ We measured infestation by *T. soror* as well as the
28 concentration of the plants' two main tropane alkaloids to in 279 plants of *D. stramonium* plants
29 aeross belonging to 31 populations in central Mexico, ~~as well as the concentration of the~~
30 ~~plants' two main tropane alkaloids.~~ We assessed whether seed predators exerted preferences on
31 the levels of both alkaloids and whether they affect plant fitness. ~~Overall, we found great~~
32 ~~variation in infestation by *T. soror* to plants of *D. stramonium*.~~ Atropine concentration relates
33 negatively to both the proportion of infested fruits per plant, and to the intensity of infestation
34 (weevils/fruit). In contrast, scopolamine concentration correlates negatively with the number of
35 sound, remaining, seeds per plant. Infestation was higher in dry, high-altitude, as compared to
36 wet lower-altitude habitats. The detection of positive selection differential of atropine's
37 concentration in populations where the impact of seed predators is stronger indicates that higher
38 atropine concentrations are favored regardless the average level in the population. Yet, atropine is
39 selected against in many populations too. The among-populations variation of *D. stramonium* in
40 infestation by *T. soror*, in the concentrations of the two main alkaloids, atropine and
41 scopolamine, and the direction and strenght of natural selection on these, may result from a
42 coevolutionary interaction with its specialized seed predator.

Comentario [msb1]: Since you address plant fitness and not insect fitness in this paper, saying this would be confusing. I would rewrite this sentence otherwise.

Comentario [msb2]: ?

43 **Subjects.** Ecology, Evolutionary Studies

44 **Key-words:** *Trichobaris soror*, *Datura stramonium*, natural selection, plant resistance, seed

45 predator, tropane alkaloids, Selection differentials

46

47 **Introduction**

48 The coevolutionary process involves reciprocal selection-adaptation between interacting
49 species through time (Dawkins & Krebs 1979; Thompson 1999; Thompson 2005). This
50 adaptation and counter-adaptation phenomenon could result in a coevolutionary arms
51 race, a process of offense-defense (Dawkins & Krebs 1979). A coevolutionary relationship
52 between plants and herbivores may generate symmetrical or asymmetrical selective pressures
53 between interacting species. These selective pressures may be different spatially and could
54 produce a geographic structure of interactions (Forde et al. 2004; Gomulkiewicz et al. 2002;
55 Nuismer et al. 2000; Thompson 1999; Thompson 2005). In some locations the interacting species
56 exert reciprocal selection pressures to one another (coevolutionary hotspots), whereas in other
57 locations reciprocal selection is highly asymmetric (coevolutionary coldspots) (Gomulkiewicz et
58 al. 2002; Nuismer et al. 2000; Thompson 1999; Thompson 2005).

59 A coevolutionary arms-race between herbivores and plants may be favored in
60 specialized interactions as in the case of insects that detoxify specific compounds
61 (Janzen 1969; Janzen 1973; Schoonhoven et al. 2005). For instance, the aphid *Macrosiphum*
62 *albifrons* is adapted to consume *Lupinus angustifolius* with a low content of alkaloids (Philippi et
63 al. 2015); however, dietary specialist herbivore insects may also be adapted to tolerate
64 secondary metabolites of their host plants without switching to other different host
65 plants (Laukkanen et al. 2012). In *Arabidopsis thaliana* the abundance of two aphid
66 herbivore species is correlated to a genetic polymorphism of the plant's resistance locus. This
67 polymorphism is under selection due to changes in population size of the two aphid species (Züst
68 et al. 2012).

69 Host-parasite systems, that exert reciprocal selection pressures, offer the opportunity to
70 assess the asymmetry in selection pressures and the potential to produce adaptation (Greischar &
71 Koskella 2007). Local adaptation may produce population differentiation as a by-product of
72 natural selection (Kawecki & Ebert 2004). This process is well illustrated by the weevil *Curculio*
73 *camelliae* (Coleoptera: Curculionidae) that parasitizes the fruits of *Camellia japonica* (Theaceae)
74 (Iseki et al. 2011; Toju 2007; Toju 2009; Toju & Sota 2006). The fruits of *C. japonica* are
75 capsules with a thick pericarp, dehiscent, with three locules and one seed per cavity (Okamoto
76 1988). Females of *C. camelliae* perforate the thick pericarp with its long *rostrum*, modified labial
77 cavity in insects, (Resh & Cardé 2009), and oviposit on the seeds (Toju 2007). A successful
78 weevil infestation, or oviposition, depends on the phenotypic match between the rostrum length
79 and pericarp thickness. These two traits that mediate the interaction vary geographically and are
80 under selection (Toju 2007; Toju 2009; Toju & Sota 2006). Some evidence, however, indicates
81 that these phenotypic characteristics may also vary according to abiotic factors, i. e., the latitude
82 (Iseki et al. 2011). Furthermore, infestation by the weevil *C. camelliae* increases at higher-
83 altitude localities and its obligated host plant decreases its resistance (Toju 2009). In the *C.*
84 *camelliae*-*C. japonica* system, natural selection acts on pericarp thickness that is a physical
85 barrier that prevents infestation by weevils (Toju 2007; Toju 2009; Toju 2011; Toju & Sota
86 2006).

87 In the annual herb *Datura stramonium* tropane alkaloids function either as resistance
88 characters preventing foliar damage by herbivores and/or as phagostimulants to them (Castillo et
89 al. 2013; Castillo et al. 2014; Shonle & Bergelson 2000). Evidence shows that alkaloid
90 concentration in *D. stramonium* varies across populations (Castillo et al. 2014). In some
91 populations, atropine concentration is selected against by dietary specialist and generalist

92 folivores, whereas scopolamine is negatively selected by the dietary specialist folivore *Lema*
93 *daturaphila* and positively selected by the generalist grasshopper *Sphenarium purpurascens*
94 (Castillo et al. 2014). Fruits of *D. stramonium* are parasitized by *Trichobaris soror* (Coleoptera:
95 Curculionidae) that reduces plant fitness by consuming the seeds (Cabrales-Vargas 1991; Cruz
96 2009; De-la-Mora et al. 2015). However, to what extent alkaloids of *D. stramonium* could affect
97 infestation by the seed predator is not known. Here, we analyzed the relationship between tropane
98 alkaloids produced by *D. stramonium* and infestation by the specialized seed predator *T. soror*
99 across multiple populations in central Mexico. We aimed to determine whether *D. stramonium*'s
100 tropane alkaloids prevent infestation by *T. soror*. Specifically, we addressed the following
101 questions. 1. Are alkaloids resistance characters that prevent/reduce infestation by weevils? 2. Do
102 seed predators exert natural selection upon plant's alkaloids concentration? and 3. Do variation of
103 both alkaloid concentration and infestation by weevils across populations is correlated to the
104 localities' environmental conditions? *v.gr.* (Toju 2009).

105 **Materials and methods**

106

107 *Study system*

108 The weevil *Trichobaris soror* (Coleoptera: Curculionidae) is intimately associated to the life
109 cycle of *D. stramonium* (Borbolla 2015; Cabrales-Vargas 1991). *Trichobaris soror* is
110 distributed mainly in central Mexico (Barber 1935; De-la-Mora et al. 2015); adult weevils
111 feed on leaves, calyx and floral tissues of *D. stramonium*. After mating, females oviposit at
112 the base of developing fruits. Their larvae feed exclusively on immature seeds inside the
113 developing fruit where they build tunnels with their own feces. Larvae pupate in the fruit
114 and sometimes are parasitized by wasps. The weevils hibernate inside the fruit of *D.*

115 *stramonium* until the next rainy season (Borbolla 2015; Cabrales-Vargas 1991).

116 Besides the seed predator, *D. stramonium* (Solanaceae) is preyed upon by
117 specialist leaf-beetles (*Lema trilineata* and *Epitrix parvula*, Coleoptera: Chrysomelidae).

118 This weed species has been widely studied in relation to its resistance characters
119 (alkaloids, leaf trichomes) against these leaf herbivores (Bello-Bedoy & Núñez-
120 Farfán 2011a; Cabrales-Vargas 1991; Carmona & Fornoni 2013; Castillo et al. 2013;
121 Castillo et al. 2014; Núñez-Farfán & Dirzo 1994; Shonle & Bergelson 2000; Valverde et al.
122 2001). However, it is unknown if chemical defense of *D. stramonium* prevents the
123 infestation by the weevil *T. soror*.

124

125 *Sampled populations*

126 During the reproductive season of *D. stramonium* (September-November) in 2007, we

127 sampled different populations across Central Mexico. We collected an average of 30
128 plants from 31 populations (Fig. 1, Table 1). For all plants, each mature fruit collected was
129 bagged and labeled. Before opening, the width and length of each fruit was measured to
130 calculate its volume with the equation:

Con formato: Resaltar

Comentario [msb3]: Explain whether all fruits per plant were collected

131

$$V = \left(\frac{4}{3}\right) (\pi) (length) \left(\left(\frac{width}{2}\right)^2\right)$$

132

133

134 We estimated the number of expected seeds by multiplying the volume by 0.026 (Fornoni et al.
135 2004). In the laboratory, we opened the fruits to determine the infestation, by counting the
136 weevils. Also, for each fruit we counted the number of remaining, sound, seeds after
137 predation.

138

139 *Seed predator infestation measures*

140 We measured the intensity of infestation by *T. soror* to *D. stramonium* as the number of
141 weevils divided by the number of fruits per plant (Greischar & Koskella 2007). Also, we
142 measured the infestation as the proportion of infested fruits per plant.

143

144 *Alkaloid concentration*

145 We quantified the concentration of leaf atropine and scopolamine per plant (Boros et al.
146 2010), by means of high-performance liquid chromatography (HPLC), following Castillo
147 et al. (2013). We obtained the average leaf alkaloid concentration from a sample of 8-
148 10 plants per population. Additionally, we measured alkaloid concentration of mature
149 seeds in 8-10 plants of 15 populations of *D. stramonium* in order to assess if
150 alkaloid concentration of leaves and seeds are correlated (Table S1).

Comentario [msb4]: This alkaloids in leafs and seeds were measured in the same plant individuals right?

151

152 *Characterization of environmental variables*

153 In order to control for some concomitant environmental variation, the values of mean annual
154 temperature and precipitation for the 31 studied populations of the *D. stramonium* were obtained
155 from Worldclim (Hijmans & Graham 2006). In order to characterize each population, we
156 calculated the Lang's aridity index (Rehman 2010) as the ratio of population mean annual
157 precipitation and mean annual temperature. Values between 0-20 correspond to desert, 20-40 arid
158 habitats, 40-60 wet type steppes, 60-100 wet woodland and 100-160 wet forests (Perry 1986).

159

160 *Statistical analyses*

161 The among-population variation in the intensity of infestation and the proportion of infested
162 fruits was assessed by Wilcoxon Chi-square tests, with the null hypothesis that infestation by the
163 seed predator does not vary among populations.

Comentario [msb5]: How so?

This should be done by generalized linear models

164 We assessed the correlation between the population average of alkaloid concentration of
165 seeds and leaves by means of Pearson's correlation. Also, we evaluated whether the intensity of
166 infestation and the proportion of infested fruits were correlated between them. In order to assess
167 whether alkaloids function as resistance traits against seed predation, we carried out regression
168 analysis of the intensity of infestation and the proportion of infested fruits, on the
169 concentration of leaf alkaloids across populations. Additionally, we tested if the concentration
170 of (1) atropine and (2) scopolamine increases plant fitness, measured as both the mean of
171 remaining sound seeds, and the number of non-infested fruits, by means of linear regression.

Comentario [msb6]: This should be done at the plant individual level, not the population unless measures have been taken from different individuals.

NOTE that in the data file it is not specified whether alkaloid values belong to leaves or seeds...Please specify

Comentario [msb7]: Why is it necessary to know this?

Comentario [msb8]: How come regression?

Did you use the population average? You should rather use the plant level measures in generalized linear mixed models in which population should be random factors

Comentario [msb9]: Again, this needs to be done at the plant individual level. Not the population level.

Comentario [msb10]: You did this at the plant level for each population as I can see in the dataset. This is correct.

This makes me think that the lines before are not properly explained and also that it was correctly analyzed. Is this right?

Comentario [msb11]: Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D, Vignieri SN, Hill CE et al. (2001) The strength of phenotypic selection in natural populations. *Am Nat* 157: 245-261.

172 Selection differentials $-S$ that account for direct and indirect selection acting on a
173 trait were calculated through Pearson correlation coefficient for each population (Lande &
174 Arnold 1983). The concentration of atropine and scopolamine was standardized ($x' = \frac{(x - \mu)^2}{\sigma}$).

175 As a proxy for plant fitness ~~was we used obtained as the expected estimated~~ number of seeds
176 per plant ~~(see above)~~, as an estimator of potential individual fitness ~~(Lande & Arnold 1983)~~ other
177 examples in Kingsolver et al. 2001. The relative fitness was defined as the ratio between
178 individual fitness and population average fitness. Selection differentials were estimated for
179 each population by correlating alkaloid concentration standardized (atropine
180 or scopolamine) with relative fitness (such in Sobral et al. 2015). Statistical analyses
181 were performed with JMP v10.0 statistical package (SAS 2012).

182 In order to explore the effect of selection exerted by the seed predation and environmental
 183 variables on ~~mean remaining sound seeds and the proportion of infested fruits~~ across populations
 184 of *D. stramonium*, we performed two linear mixed effect models, LMM; (such in Sobral et al.
 185 2013). In the first model, the number of sound seeds was the response variable while the
 186 population term is a random factor; the covariates were the concentration of each alkaloid, the
 187 selection differential of each alkaloid, the proportion of infested fruits together with latitude,
 188 longitude, altitude and Lang's index of populations. In the second model, the proportion of
 189 infested fruits was the response variable and the same covariates, except the proportion of
 190 infested fruits. We estimated the Variance Inflation Factor (VIF) before using the variables in
 191 each model. The VIF is a measure of multicollinearity that uses the proportion of the variance
 192 that is not shared between the variables studied. The variables with values of VIF > 10 indicate
 193 severe collinearity and therefore must be eliminated of the multivariate models (O'brien 2007).
 194 Previous analyses indicated that all variables had VIF < 5 (Table S2) and then used in the LMM.
 195 To obtain the best reduced LMM, the variables with a significance value less than 0.05 were
 196 retained (Zuur et al. 2009). We selected the models with lowest AIC values, namely those that
 197 explain better the relationship between the variables (Akaike 1974). LMM analyses were
 198 estimated using the function nlme in R Core Team v.3.2.2 (R 2011).

199

200 Results

201 The intensity of infestation as well as the proportion of infested fruits varied significantly among
 202 populations ($\chi^2 = 400.1647$, *d. f.* = 30, $P < 0.0001$; $\chi^2 = 383.2304$, *d. f.* = 30, $P < 0.0001$,
 203 respectively) (Fig. 2). The average intensity of infestation by *T. soror* varies from populations
 204 without infestation up to those with 5 weevils per fruit, per plant. Variation in the average

Comentario [msb12]: I tthis models the response variables should be the alkaloid concentration at the population level, which is the selected trait. So the one which makes sense to analyse in function of the among population selective pressures

Comentario [msb13]: Please change for the pertinent response variables (a model for scopolamine in seeds, other por leaves, other for both, other for atropine...)

If you find that selection coefficients are related to the average level of alkaloid in one or several of thiese models you can conclude that selection on this traits drive their among population variation, thus they are evolving.

Con formato: Tachado

Comentario [msb14]: Also LMM are used if the response variable is normal, in the case of seeds they are not, so ther are not the correct models, for number of seeds you need to used generalized models fitted top poisson distributions.

But note that for the correct responses to be included (average alkaloids per population) it is likely that you will be ok with LMM.

Comentario [msb15]: This should be the responses

Comentario [msb16]: This does not need to be included

Comentario [msb17]: no

205 proportion of infested fruits ranged from zero (populations Coatepec, Huitzuco and Jalapa) up to
206 90% (populations Teotihuacan and Tlaxiaca; Fig. 2).

207 The concentration of atropine in leaves and seeds are positive and significantly
208 correlated ($r = 0.6276$, $P = 0.0123$, $N = 15$). Similarly, the concentration of seed alkaloids,
209 atropine and scopolamine, showed a positive correlation between them ($r = 0.5851$, $P =$
210 0.0219 , $N = 15$). No correlation between the concentration of scopolamine in leaves and
211 seeds was detected.

Comentario [msb18]: why did not you thi at the plant level

212 We found a high and positive correlation between the proportion of infested fruits and
213 intensity of the infestation ($r = 0.8994$, $P < 0.0001$, $N = 31$). The mean of sound remaining
214 seeds showed a negative correlation to the proportion of infested fruits ($r = -0.5545$, $P =$
215 0.0012 , $N = 31$) and to the intensity of the infestation ($r = -0.5306$, $P = 0.0021$, $N = 31$). The
216 negative correlation between leaf atropine concentration and both the proportion of infested
217 fruits ($r = -0.4134$, $P = 0.0208$, $N = 31$, Fig. 3A) and the intensity of infestation ($r = -0.3270$,
218 $P = 0.0725$, $N = 31$, Fig. 3B), indicates that this alkaloid reduces infestation by the seed
219 predator. Besides, the number of uninfested fruits increases as atropine concentration
220 increases ($r = 0.4288$, $P = 0.0161$, $N = 31$, Fig. 3C), but it is uncorrelated with the mean of
221 remaining sound seeds ($r = 0.1147$, $P = 0.539$, $N = 31$). The scopolamine concentration is
222 negatively correlated with the number of remaining sound seeds ($r = -0.36$, $P = 0.0467$,
223 $N=31$, Fig. 3D).

224 In the first linear mixed effect model (LMM), the remaining sound seeds were
225 negatively related to the selection differential of atropine concentration across populations
226 and with the proportion of infested fruits (Table 2, Fig. 4A, 4B). The second LMM indicates
227 that the proportion of infested fruits was correlated negatively to Lang's index and positively

Comentario [msb19]: unfortunately, this model lacks any conceptual significance. Selection acts on the phenotypic trait (the alkaloid) and not on the fitness.

Please re-run this model again for the proper response variables.

to altitude of the localities (Table 2, Fig. 4C, 4D).

Discussion

The concentration of the alkaloid atropine varied across populations of *D. stramonium* and it was negatively related to both the intensity of infestation and the proportion of infested fruits by the specialist seed predator *T. soror*. We found that the concentration of atropine in seeds and leaves was positively associated across populations. Although such pattern was not detected for scopolamine, the concentration of both alkaloids in seeds shows a positive relationship across populations. Our major finding was that atropine concentration reduces infestation by *T. soror* weevils, functioning as a chemical resistance trait against the seed predator. It has not been previously reported that atropine is linked to resistance to seed predation in *D. stramonium*. The results also showed that mean plant fitness, as estimated by uninfested fruits, increases at higher concentration of atropine across populations. Moreover, we detected positive selection differentials in populations where the negative impact of infestation was more severe (i. e., the low values of remaining seeds). Thus, natural selection may favor to increase the concentration of atropine. Such populations may constitute hot spots where an asymmetric arms race process is taking place.

Previous studies have shown that atropine plays a role in the interaction between *D. stramonium* and its main folivore insects in central Mexico. For instance, populations of *D. stramonium* with higher concentration of atropine were more damaged by dietary specialist and generalist folivore insects, acting as a chemical clue that attract them and/or as a phagostimulant (Castillo et al. 2013; Castillo et al. 2014). In this study we found the opposite pattern: populations of *D. stramonium* with higher atropine concentration had a higher number of uninfested fruits. This suggests that atropine deters the seed predator *T. soror*. A similar trend has been found in

Comentario [msb20]: I HAVE JUST TAKEN THE LICENCE TO PERFORM THESE MODELS IN THE CORRECT DIRECTION, USING the dataset in your submission and the good news is that there is a very clear effect of the selection over scopolamine on the scopolamine population average, in the case of atropine there is also a effect although less strong. And this even when controlling for environmental variables which also have an effect on both models.

I would recommend to do this also separating scopolamine from seed s and leaves etc

Note that actually the scopolamine concentrations at the population level are not normal, so please try with different distributions in a generalized linear models and choose the best model (aicc)

When distributions have a poisson shape but variables use decimal data, a nice trick is to eliminate decimals and difit to a poisson distribution (which only accept natural numbers), in order to get a good fit

Comentario [msb21]: Same than in previous comment

252 the hemiparasitic plant *Castilleja indivisa* (Adler 2000), where the alkaloid lupanine, obtained
253 from its host plant, *Lupinus texensis*, reduces damage to its flowers by herbivores and increases
254 visitation by pollinators thus enhancing plant fitness, measured as the number of seeds.

255 In contrast, we found that populations of *D. stramonium* with higher scopolamine
256 concentrations had a lower number of sound seeds. This suggests that scopolamine acts as a
257 phagostimulant to *T. soror*. Selection to reduce scopolamine concentration was previously
258 demonstrated by Shonle & Bergelson (2000) in one experimental population of *D. stramonium*
259 attacked by folivore insects. Thus, the resistance role of tropane alkaloids of *D. stramonium* vary
260 according to the type of herbivore and the tissue they consume. While scopolamine is positively
261 selected by specialist folivores in some populations of *D. stramonium* from central Mexico,
262 atropine is selected against (Castillo et al. 2014). In the case of *T. soror*, atropine is positively
263 selected while populations with higher concentration of scopolamine had lowest values of
264 remaining sound seeds. It seems that the level of the chemical resistance traits in *D. stramonium*
265 may be determined, in part, by the differential or even opposed, selection pressures exerted by
266 herbivores that consume different plant tissues.

267 Females and males of *T. soror* feed on *D. stramonium* leaves, producing small holes on
268 theses. Also courtship and mating occurs on the plants (J. Núñez-Farfán, *personal observation*).
269 It is possible that *T. soror*'s females select plants to oviposit while feeding,
270 "assessing" the chemical resistance level of the plants (i.e., atropine/scopolamine
271 concentration in leaves). Some evidence in this line shows a close relationship between
272 *Trichobaris* and *Datura*. For instance, *Trichobaris bridwelli* oviposits preferentially on *D.*
273 *stramonium* rather than on tobacco plants (*Nicotiana tabacum*) (Cuda & Burke 1991),
274 since *T. bridwelli* does not tolerate the pyridine alkaloids of tobacco (Cuda & Burke 1991).

275 On the other hand, Diezel et al. (2011) have experimentally demonstrated that *T. mucorea*,
276 a species that burrows into the stems of *Nicotiana attenuata*, increases infestation on
277 transgenic plants of *N. attenuata* where the chemical defenses (jasmonic acid and nicotine)
278 were silenced.

279 We found evidence of natural selection on chemical resistance of *D. stramonium* since
280 higher atropine concentrations drove down seed predator infestation. The first LLM analysis
281 revealed that remaining sound seeds are negatively related to selection differentials on atropine,
282 implying that in populations where “virulence” of *T. soror* is very high, selection differentials are
283 positive (cf. Fig. 4A). In these population the negative effect of infestation promotes increases in
284 atropine concentration. Thus, in populations of *D. stramonium* where infestation is low, selection
285 acts to reduce atropine concentration (left side of Fig. 4A). Thus populations of *D. stramonium*
286 vary in the direction and strenght of selection on chemical defense, an expectation of the
287 geographic mosaic of coevolution (i.e., hotspots and coldspots; (Thompson 2005).

288 The second LMM analysis indicated that infestation by *T. soror* increases in populations at
289 higher altitudes and lower Lang’s index vales. That is to say, populations from high and dry
290 environments suffer more fruit infestation (cf. Fig. 4C, D; Castillo et al. 2013). Similar results
291 were found in other studies in *D. stramonium* (Borbolla 2015; Hernández-Cumplido 2009).
292 Likewise, in the interaction between *Curculio camelliae*-*Camellia japonica*, infestation by the
293 weevil was higher at high altitudes, where the fruits of *C. japonica* trees have thinner pericarps
294 (Toju 2009). In *D. stramonium* alkaloid concentration can be lower in dry and high altitude
295 locations. In this sense, it has been reported alkaloid production may be water limited, decreasing
296 in concentration in dry enviroments (v.gr., populations Actopan, Teotihuacan and Valsequillo;

297 Fig. 2) (Baricevic et al. 1999). Thus, the evolution of resistance to herbivores in *D. stramonium*
298 can be environmentally conditioned.

299

300 **Conclusions.**

301 In summary, the results show that the lowest proportion of infested fruits as well as the intensity
302 of infestation by *T. soror* are related the highest concentration of atropine in *D. stramonium*.
303 Selection differentials of atropine were high and positive in populations where seed predation is
304 more intense. Therefore, such populations may represent coevolutionary hotspots. Finally, the
305 higher proportion of infested fruits occurred in population inhabiting high altitude and dry
306 environments pointing that plant resistance also interacts with physical factors of the environment
307 (Castillo et al. 2013).

308

309 **Acknowledgements**

310 We thank to Luis Barbo for helping us during HPLC quantification, and the members of
311 Laboratorio de Genética Ecológica y Evolución for their logistical support and field assistance.
312 This paper constitutes a partial fulfillment of A. M-P 's Doctoral dissertation in Programa de
313 Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM).

314

315 **Funding**

316 A. M-P acknowledges the scholarship for graduate studies (251620) granted by the National
317 Council of Science and Technology (CONACyT). This project was funded by a CONACyT grant
318 to J. N-F.

319 **Grant Disclosures**

320 The following grant information was disclosed by the authors:

321 CONACyT grant: 251620.

322 **Competing interests**

323 The authors declare no competing interests.

324 **Author Contributions**

325 • Adán Miranda-Pérez conceived and designed the experiments, performed the experiments,
326 analyzed the data, wrote the paper, prepared figures and/or tables.

327 • Guillermo Castillo, Johnattan Hernández-Cumplido, María Borbolla, Laura L. Cruz,
328 Rosalinda Tapia-López, Juan Fornoni and César M. Flores-Ortiz, conceived and performed
329 the experiments, and revised the manuscript.

330 • Pedro L. Valverde, analyzed the data, wrote the paper.

331 • Juan Núñez-Farfán, conceived and designed the study, analyzed the data, wrote and revised the
332 paper,.

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