# Climatic and soil characteristics account for the genetic structure of the invasive cactus moth *Cactoblastis cactorum*, in its native range in Argentina (#90876)



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# Climatic and soil characteristics account for the genetic structure of the invasive cactus moth *Cactoblastis cactorum,* in its native range in Argentina

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**Background.** Knowledge of the physical and environmental conditions that may limit the migration of invasive species is crucial to assess the potential for expansion outside their native ranges. The cactus moth, *Cactoblastis cactorum*, is native to South America (Argentina, Paraguay, Uruguay and ~~South of~~ Brazil) and has been introduced and invaded the Caribbean and southern United States, among other regions. In North America there is an ongoing process of range expansion threatening cacti biodiversity of the genus *Opuntia* and the commercial profits of ~~the~~ domesticated *Opuntia ficus-indica.* **Methods.** To further understand what influences the distribution and genetic structure of this otherwise important threat to native and managed ecosystems, in the present study we combined ecological niche modeling and population genetic analyses to identify potential environmental barriers in the native region of Argentina. Samples were collected on the host with the wider distribution range, *O. ficus-indica*. **Results.** Significant genetic structure was detected using 10 nuclear microsatellites and 24 sampling sites. At least six genetic groups delimited by mountain ranges, salt flats and wetlands were mainly located to the west of the Dry Chaco ecoregion. Niche modeling supports that this region has high environmental suitability where the upper soil temperature and humidity, soil carbon content and precipitation were the main environmental factors that explain the presence of the moth. Environmental filters such as the upper soil layer may be critical for pupal survival and consequently for the establishment of populations in new habitats. Whereas the presence of available hosts is a necessary conditions for insect survival, upper soil and climatic characteristics will determine the opportunities for a successful establishment.

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## Summary

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**Methods.** To further understand what influences the distribution and genetic structure of this otherwise important threat to native and managed ecosystems, in the present study we combined ecological niche modeling and population genetic analyses to identify potential environmental barriers in the native region of Argentina. Samples were collected on the host with the wider distribution range, *O. ficus-indica*.

**Results.** Significant genetic structure was detected using 10 nuclear microsatellites and 24 sampling sites. At least six genetic groups delimited by mountain ranges, salt flats and wetlands were mainly located to the west of the Dry Chaco ecoregion. Niche modeling supports that this region has high environmental suitability where the upper soil temperature and humidity, soil carbon content and precipitation were the main environmental factors that explain the presence of the moth. Environmental filters such as the upper soil layer may be critical for pupal survival and consequently for the establishment of populations in new habitats. Whereas the presence of

available hosts is a necessary conditions for insect survival, upper soil and climatic characteristics will determine the opportunities for a successful establishment.

**Keywords:** biological invasions, gene flow, Lepidoptera, migration, population genetics, prickly pear cacti

## Introduction

Since Elton's book on the *Ecology of Invasions by Animals and Plants* (1958), the field of invasion biology has grown exponentially (Ricciardi & MacIsaac, 2008), but our ability to predict which physical and biotic factors will prevent the expansion of invasive species in their non-native range is still poorly developed (Richardson, 2011). So far, rates of invasion have increased during the last century despite control and management practices (Jaspers et al., 2021), suggesting that being able to predict the invasion dynamic will open new opportunities to cope this threat. A central element in predicting the potential migration of invasive species in foreign regions is the analysis of the natural barriers that define the spatial distribution in their native habitat (Sherpa et al., 2019). Thus, understanding native spatial patterns of dispersal of individuals and genes is a first line of evidence to identify potential environmental barriers as input for predictive models of invasion and population management.

The simplest hypothesis about gene flow establishes that this is mainly determined by the geographic distance that separates two or more populations (Isolation by Distance, IBD) (Wright, 1943). However, to find a pattern of IBD, it is necessary that the flow between populations is constant, that nothing interferes with the movement of genes in all directions (neither physical

nor environmental barriers), and that other evolutionary processes like drift or selection are weaker than the intensity of gene flow (Bolnick & Nosil, 2007, Epperson, 2010). Also, the IBD analysis does not provide information on whether environmental factors are interacting with evolutionary processes (Manel et al., 2003). To identify how the environment can contribute to facilitate or reduce the rates of movement of genes between different populations, tools have been developed in recent years to analyze various gene flow hypotheses (Anderson et al., 2010). Circuit theory has been used to build testable hypotheses of gene flow based on the ecology of the species and the presence of potential environmental and physical barriers (e.g., MacRae, 2009; Andraca-Gómez et al., 2015; Dickson et al., 2019). This information is used to construct resistance matrices that represent the probabilities of gene flow between all pairs of populations. In areas of low resistance, movement of genes between populations is more likely, while high-resistance areas represent geographic and environmental barriers (Cushman et al., 2006; McRae, 2006). This methodological approach is essential to test more realistic hypotheses of gene flow (Isolation by Environment, IBE) (Osrini et al., 2013, Sexton et al., 2014). However, to our knowledge, there have been few attempts to identify environmental barriers to gene flow of invasive species in their native range (Sherpa et al., 2019; Acevedo-Limón et al., 2020; Poveda-Martínez et al., 2023). This kind of evidence is essential for population management as input for invasion dynamic modeling to predict the expansion range in non-native regions (Brown et al. 2016; Aguirre-Liguori et al., 2021; Pilowsky et al., 2022).

The invasive cactus moth, *Cactoblastis cactorum* (Berg) (Pyralidae: Phycitinae), offers a unique opportunity to evaluate environmental barriers in the native range of an invasive species because inhabit a wide range of environmental conditions. *C. cactorum* is a cactophagous herbivore that feeds on the stems (cladodes) of the prickly pear cacti (*Opuntia* sp.). It is native to

South America (mainly Argentina) and was initially used as a biological control agent against *Opuntia* in Australia, South Africa, and the Caribbean (Zimmermann et al., 2007). After being introduced in the Caribbean in 1956 (Simmond & Bennett, 1966), the cactus moth was dispersed to North America via commercial transportation and hurricanes (Simonsen et al., 2008; Marsico et al., 2010; Andraca-Gómez et al., 2015, 2020), entering Florida in 1989, and since then, representing a major threat to the biodiversity and commercial production of *Opuntia* in Mexico (Soberón et al., 2001). Mexico is known to be one of the highest cacti biodiversity hotspots worldwide, as well as one of the main producers of *Opuntia*. Therefore, identifying environmental conditions that constrain the presence of *C. cactorum* in its native range will help future efforts to predict the spatial invasion dynamics before reaching the major areas of *Opuntia* diversity in North America.

Previous studies in the native region (Argentina) using insect samples from seven host species of *Opuntia* revealed the presence of four genetic groups based on mitochondrial DNA (COI) (Marsico et al., 2010). Morphological differentiation of larvae was detected among the four genetic groups, which also were associated with different host usage, suggesting a possible host effect on ecotypic differences (Brooks et al., 2014). Although some degree of preference to oviposit on the exotic *O. ficus-indica* rather than on other native species was recorded, *C. cactorum* behave as a generalist with little host preference (Varone et al., 2014). Recent analyses using genome wide SNPs and niche modelling data indicated that past climatic changes during the Quaternary and shifts in host use conditioned the actual distribution of genetic variation of *C. cactorum* in Argentina (Poveda-Martínez et al., 2023). Ecological niche modelling using bioclimatic variables indicated that environmental suitability increases since the last glacial maximum (ca. 21 ky) from the west to the east, north and south of the present distribution

(Poveda-Martínez et al., 2023). During the Spanish settlement in South America, five centuries ago, *O. ficus-indica* was introduced and likely colonized by *C. cactorum* since then (Erwin, 2012). The genetic structure of *C. cactorum* estimated across seven native hosts species suggest no evidence that the introduction of *O. ficus-indica* in the native range and the subsequent human-commercial dispersal have promoted contemporary admixture between distant populations (Poveda-Martínez et al. 2023). Within Argentina, *O. ficus-indica* occupies a larger area and a wider environmental range than any of the other native *Opuntia* species (Varone et al., 2014), representing a suitable system to examine possible contemporary environmental effects on genetic variation and structure without strong historical effects nested within native hosts distribution (e.g., Poveda-Martínez et al., 2023). To control these sources of variation and to explore the contemporary environmental factors that affect the genetic structure of the species, in the present study, species-specific nuclear microsatellites were used to characterize the geographic pattern of genetic variation *C. cactorum* associated with the distribution of *O. ficus-indica*.

Genetic analyses were combined with ecological niche modelling to test the hypothesis that environmental conditions affected the genetic structure of the species. Given that the insect pupates in the upper soil layer (Zimmerman et al., 2004) and is sensitive to temperature (Legaspi & Legaspi, 2007), we estimated its niche using soil and climatic variables to identify environmental barriers to species distribution. In addition, incorporating soil information in ecological niche models is known to reduce overestimation of expected suitability (Coudum et al., 2006; Beauregard & de Blois, 2014). The predictive model was used to build the Isolation by Environment (IBE) hypothesis represented by the resistance matrix to gene flow between pairs of sampling sites. A significant correlation between resistance and genetic differentiation

133 matrices would indicate the existence of environmental barriers limiting dispersal (Hernández-
134 Leal et al., 2022).

135 In the present study, we identified geographic and environmental (bioclimatic and soil)
136 characteristics that may function as barriers for gene flow. Specifically, we (1) determined the
137 existence of a significant genetic structure within the sampled region of Argentina where *C.*
138 *cactorum* is associated with *O. ficus-indica*, (2) identified climatic and soil variables within the
139 sampled region that better explain the distribution of *C. cactorum* following a niche modeling
140 approach, and (3) combined these two pieces of evidence to test whether environmental
141 conditions explain the geographic pattern of genetic differentiation (McRae, 2009; Andraca-
142 Gómez et al., 2015; Borja-Martínez et al., 2022).

143

144 **Methods**

145 **Study species**

146 *Cactoblastis cactorum* is distributed in tropical and subtropical regions in South America,
147 between 0 and 1200 masl in Uruguay, south of Paraguay and Brazil, and in the central and
148 northern part of Argentina (Mann, 1969; McFadyen, 1985; Varone et al., 2014), comprising the
149 Chaco and Pampean biogeographical provinces (Morello et al., 2012; Oyarzábal et al., 2018,
150 Arana et al., 2021, Morrone et al., 2022). Within this area, it uses several native host species of
151 prickly pear cacti (*Opuntia anaconda*, *O. megapotamica*, *O. elata*, *O. anacantha*, *O.*
152 *bonaerensis*, *O. cardiosperma*, *O. surfurea, O. quimilo, O. rioplatensis, O. penicilligera*) and the
153 exotic *O. ficus-indica* (Marsico et al., 2010; Varone et al., 2014). The life cycle encompasses a
154 gregarious larval stage within the cladodes, a pupal stage in the soil (approximately 5-10 cm in

depth) and a free adult stage (Andraca-Gómez *personal observation*). The whole cycle lasts between 4-5 months and depends on environmental conditions (Dood, 1940; Pettey, 1948; Mann, 1969). In particular, temperature determines the percent of hatches (Legaspi & Legaspi, 2007, Marti & Carpenter, 2008).

**Data collection**

Between 2011 and 2012, 508 larvae were collected from 24 sites within the distribution range of *C. cactorum* in Argentina; mainly in the Chaco and Pampa biogeographic provinces and included three ecoregions (Sampling approved by the Servicio Nacional de de Sanidad y Calidad Agroalimentaria from Argentina) (Table 1, Fig. 1, Löwenberg-Neto, 2014)). During two consecutive years, between February and March, one larva per cladode was collected, georeferenced and deposited in 1.5 ml vials with alcohol (96%) until DNA extraction. The samples were collected in the widely distributed exotic host, *O. ficus-indica*. Sample sizes varied between 10 and 30 individuals per site (Table 1, Fig. 1). DNA extraction was performed with the DNEasy® blood & tissue kit (QIAGEN, Maryland, USA, cat.60504) and the resulting product was diluted to 20 ng/µl to warrant PCR amplification. We used microsatellites specifically developed for *C. cactorum* (Andraca-Gómez et al., 2020). The resulting PCR products were sent to the Core DNA Sequence Facility at the University of Illinois and analyzed in an Applied Biosystems sequencer (3730 xl). The GeneMarker program (version 2.20 demo) was used to genotype individual larvae.

**Genetic analyses**

177 The presence of Hardy-Weinberg equilibrium and linkage disequilibrium at each location was
178 tested with *Genepop* (web version, Rousset , 2008) while null alleles among loci were estimated
179 with *FreeNA*. Loci with more than 20% of null alleles were eliminated from the analyses
180 (Chapuis & Estoup, 2007), as well as those that were out of the Hardy-Weinberg equilibrium in
181 more than 50% of the locations. *FSTAT* (version 2.9.3.2, Goudet, 2002) was used to calculate the
182 number of alleles, the allele richness, the observed and expected heterozygosity, and
183 differentiation between all pairs of sites and genetic groups ($F_{ST}$) (Weir & Cokerman, 1996;
184 Chapuis & Estoup, 2007).

185

186 **Genetic structure**

187 First, a Bayesian grouping approximation was implemented in *GENELAND* (version 4.0)
188 (Guillot et al., 2008) in R Core Team (2023), to determine the existence of significant population
189 genetic structure. *GENELAND* identifies groups of populations based on genetic similarity and
190 geographic proximity. The analysis was performed in 10 independent runs of Monte Carlo
191 Markov Chains (MCMC) with 1,000,000 iterations each and a minimum group value ($k$) of 1 and
192 a maximum of 24. Assuming a significant genetic structure, uncorrelated allelic frequencies were
193 chosen. We also incorporated the possible genetic ambiguity (excess homozygotes) in the
194 grouping algorithm, assuming the existence of null alleles. The location of each individual in the
195 analysis was included as a geographic coordinate in decimal degrees with a minimum distance of
196 0.001 ° (approximately equivalent to 100 meters). Burning was applied to the first 10 % (200
197 chains) of the total iterations.

198 Second, to detect the presence of potential barriers to gene flow, we used the program
199 *BARRIERS* (Version 2.2; Manni et al., 2004). This applies the Monmonier and Delaunay
200 methods of triangulation of spatial coordinates of sampled sites and generates a map representing
201 the relationship between the populations and the areas where the possible barriers can be found.
202 We allowed a maximum of five barriers based on the number of genetic groups obtained by
203 *GENELAND*. Genetic groups of populations were assigned a significance value after
204 bootstrapping a set of 100 distance matrices using Nei (1972) genetic distance estimations. The
205 100 matrices required by the program were generated by resampling individuals within the
206 populations using the program *MSA* (version 4.051). A multivariate analysis of molecular
207 variance (AMOVA) was performed to decompose the total amount of genetic variation among
208 and within genetic groups (Arlequin 3.5; Excoffier & Lischer, 2010).

209

210 **Ecological niche modeling and environmental barriers**

211 To identify environmental barriers related to genetic grouping of sampled sites, niche modeling
212 and isolation by resistance ~~analyzes~~ were combined (Manthey & Moyle, 2015) (McRae & Beier,
213 2007; McRae et al., 2008). The MaxEnt algorithm executed in the ntbox package in R (Osorio-
214 Olvera et al., 2020) was used to build a niche model hypothesis for the sampled area of *C.*
215 *cactorum*. To carry out the modeling, we used 40 sites in Argentina where individuals of *C.*
216 *cactorum* were observed during sampling. To build the model, climatic and soil variables were
217 gathered from WorldClim (https://www.worldclim.org/data/bioclim.html), Soil (Biosoil)
218 (https://zenodo.org/record/4558732) (Lembrechts et al., 2021) and SoilGrids
219 (https://www.isric.org/explore/soilgrids) databases. We curated our occurrence data using
220 standard steps in ecological niche modeling literature and using the approach of Cobos et al.

(2018). We eliminated spatial duplicates by using a threshold distance of 0.04 grades (~ 2.5 km at the equator). To avoid collinearity-related problems, we estimated the correlation among each pair of predictors and kept only those with correlation values < 0.7. We ran iteratively MaxEnt models using its auto features and explored variable contribution via the Jackknife test on AUC values (area under the receiver operating characteristic (ROC) curve). After each run, we removed the least contributing variable from the list of non-correlated environmental variables. After the selection model procedure, using AUC and the ROC curve, we ended up with the six best environmental variables that had the highest contribution in most of the models. The final model prediction (suitability map) was used to construct the resistance matrix in *CIRCUITSCAPE* (version 3.5, McRae & Shah, 2009; Andraca-Gómez et al., 2020). Geographic points with low suitability delineate areas of high resistance for establishment, suggesting the presence of a geographic or environmental barrier. Multiple matrix regression with randomization (MMRR) was performed using the genetic distance matrix based on $F_{ST}$ values between pairs of sites as the response variable against the geographic distance matrix and the resistance (environmental) matrix following the niche model prediction (Wang, 2013). The distance matrix was adjusted to control for the great-circle distance (i.e., shortest distance between two points on the surface of a sphere) using the package *sf* in R (Pebesma, 2018).

## Results

*Genetic variation and structure*. After an initial study, 4 out of 14 nuclear microsatellite loci were eliminated because they had a null allele frequency greater than 20%. A total of 152 polymorphic loci were used in the final analyzes. Among the 24 locations sampled, the allele richness varied between 3.36 and 5.78 and the observed heterozygosity (Ho) between 0.36 and

0.63 (Table 2). All sites, excepting population 14 (Yuquerí), had fewer heterozygotes than expected under the Hardy-Weinberg equilibrium ($F_{IS} > 0$, Table 2). Significant paired genetic differentiation among sites ranged from $F_{ST} = 0.0228$ between locations 22 and 24 to $F_{ST} = 0.3011$ between locations 4 and 12. The mean level of genetic differentiation for the whole set of sampling sites was $F_{ST} = 0.178$. Within the sample region, the analysis of genetic structure using *GENELAND* indicated that the most probable number of genetic groups was six (Fig. 2B). Genetic groups (hereafter populations) were defined by a probability of assignment between 0.30 and 0.36 (Fig. 2A). The 15th collection site corresponds to an isolated group in the northern Yungas ecoregion, within a mountain forest near the Dry Chaco. On the east side of the distribution, within the Pampean province, there is a group of six sampling sites (green dots in Fig. 2A) corresponding to the Espinal ecoregion with humid flats between the Paraná and Uruguay rivers. On the west area of the distribution within the Dry Chaco ecoregion, there are four genetic groups: a northwestern group (yellow dots in Fig. 2A), a southwestern group (blue dots in Fig. 2A), and two groups in the middle, one on the east border (purple dots in Fig. 2A) and another on the west border (red dots in Fig. 2A). The results of AMOVA indicated that the variation within sites accounted for most of the genetic variation (81.8%) followed by the variation among sites within genetic groups (9.9%) and the variation among genetic groups (8.26%). Genetic differentiation among genetic groups was $F_{CT} = 0.078$ (Fig. 2D). Heterozygosity for each genetic group estimated using the pooled sample of sites was similar to the average $H_o$ when using each site as a replicate (Fig. 2C). The presence of potential barriers to gene flow with a probability of more than 50% existence strongly matched the clustering proposed by *GENELAND* (Fig. 2A). The barriers with higher probability delimited the four genetic groups within the west region of the distribution range, while less intense barriers

267 separated the north and east regions (Fig. 2A). Clusters 1, 2, 3, and 5, correspond to the Dry
268 Chaco ecoregion, while cluster 6 corresponds to the Yungas ecoregion close to the Dry Chaco.
269 Cluster 4 is located within the Pampean province, in a humid flat, within the Espinal ecoregion.
270 Clusters 1, 2, 3, and 5 within the Dry Chaco are separated by mountain ranges, salt flats, and
271 wetlands in arid or semi-arid conditions. Group 1 in the north (yellow dots in Fig. 2A)
272 corresponds to forests and shrublands, to the north of Salinas Grandes and south of the wetlands
273 of the Salado river. Group 2 is located in salt flats within the Monte ecoregion surrounded by the
274 Sierra de Ancasti to the north and Salinas Grandes to the west (red dots in Fig. 2A). Group 3
275 corresponds to dry forests and shrublands in a zone of low mountains, south of Salinas Grandes
276 and west of Sierra Grande (blue dots in Fig. 2A). Group 5 is located within an area surrounded
277 by Salinas de Ambargasta (East), Sierra de Ambargasta and Sierra de Sumampa (South), Salina
278 del Saladillo (North) and delta of the Dulce River and Mar Chiquita (National Park Ansenuza
279 Lagoon (Northeast) (black dots in Fig. 2A).

280

281 *Niche modeling*. The niche model of *C. cactorum* had an AUC value of 0.875 and an omission
282 rate of zero under a five percentile threshold corresponding to a suitability value of 0.074. The
283 main environmental variables that better explained the distribution of the moth were related to
284 precipitation and temperature on the soil surface and within the upper soil layer (10 cm depth), as
285 well as the soil carbon content. These correspond to: average temperature of the driest quarter
286 (relative contribution to the model, 30%), maximum soil temperature of the warmest month
287 (relative contribution to the model, 16.1%), annual temperature range (relative contribution to
288 the model, 14.6%), precipitation seasonality (relative contribution to the model, 14.3%), mean
289 soil temperature of the wettest quarter (relative contribution to the model, 13.7%), and soil

290 organic carbon density (relative contribution to the model, 9.9%). A higher environmental
291 suitability was detected in the west region where more genetic groups were found. From the west
292 to the north and east areas of the distribution, the environmental suitability declines consistently
293 (Fig. S1).

294 *Environmental-genetic association*. The MMRR analysis showed that the environmental distance
295 matrix (based on the prediction of the niche model) was significantly related to the genetic
296 distance matrix ($\beta_E = 0.506$, $P = 0.032$) supporting the hypothesis of Isolation by Environment
297 (IBE). On the contrary, the same analysis rejected the hypothesis of Isolation by Distance (IBD)
298 ($\beta_D = 0.053$, $P = 0.793$) (that is, there is no significant association between genetic and
299 geographic distance matrices. Thus, environmental filters conditioned the genetic structure and
300 dispersal of *C. cactorum*.

301

302 **Discussion**

303 Our analyzes demonstrate the existence of a significant genetic structure of *C. cactorum* in
304 Argentina associated with the exotic host *O. ficus-indica*, introduced in this region about 500
305 years ago. While the western part of the distribution comprises more genetic diversity (four
306 genetic groups) and has higher environmental suitability, the genetic groups in the east and north
307 correspond to areas with lower environmental suitability. The environmental suitability of the
308 western region corresponds to an area with high environmental heterogeneity (Oyardazabal et al.,
309 2018) but climatically more stable during the Quaternary (Poveda-Martínez et al., 2023)
310 representing a Pleistocene refuge for biodiversity during the last glaciation (Baranzelli et al.,
311 2017; Robbiati et al., 2021). Furthermore, the suitability for *C. cactorum* in the sampled region

seems to be highly influenced by temperature and precipitation above and below ground, in combination with other soil characteristics. Genetic analyses, allowed us to identify barriers corresponding to mountain ranges, salt flats, wetlands, and the largest lagoon in central Argentina (Mar Chiquita). These barriers delimited areas with significant variation in temperature and precipitation that influenced the genetic clustering of prickly pear moth populations and may represent major environmental filters for its distribution, dispersal, and genetic variation.

The levels of genetic diversity estimated by heterozygosity showed deficiency ($F_{IS} > 0$) in most of the samples of *C. cactorum*, excepting sampling site 14 (Yuquerí). Deficiency of heterozygotes and a high proportion of null alleles (> 20%) are a common phenomenon among Lepidoptera (Malausa et al., 2007; Sinama et al., 2011; Guillemaud et al., 2015). This condition is associated with high rates of mutation in genetic regions flanking microsatellites, as well as the presence of transposable elements (Sinama et al., 2011). Other factors like gene flow, genetic drift, and the genetic structure of populations (Wahlund effect) can also account for lower-than-expected levels of heterozygotes (Haldane, 1948; Kimura, 1968). When the average heterozygosity for each genetic group was compared with the observed heterozygosity for the entire genetic group, no differences were observed. This suggests that possible Wahlund effects were not likely related to the genetic structure of populations (Waples, 2015). The heterozygosity was rather uniform among the sampling sites, suggesting that there were no strong effects of genetic drift. Furthermore, the east genetic group had the lowest $F_{IS}$ values and is less differentiated from the other groups. Despite significant paired genetic differentiation between sampling sites, the low amount of variance explained by genetic groups suggests that gene flow has been moderate. Levels of paired genetic differentiation among sampling sites (range $F_{ST}$ =

335 0.022 - 0.301) fall within the range detected using nuclear SNPs across a pooled sample of seven
336 hosts within the same region ($F_{ST}$ = 0.023 - 0.448) (Poveda-Martínez et al., 2023). Ongoing
337 genomic analysis will provide more information to explain positive $F_{IS}$ values and to unravel the
338 causes of genetic differentiation.

339 Among plant-natural enemy interactions, environmental conditions and host species are
340 known to affect the distribution of the genetic variation of consumers (Mopper & Strauss, 1994;
341 Whitham et al., 2003; Wand & Bradburd, 2014; Wang et al., 2017). Disentangling the effect of
342 these sources of variation is particularly challenging when consumers interact with various hosts
343 inhabiting different environmental conditions (Wang et al., 2017). For this reason, in the present
344 study, the host species with the wider environmental range was selected to increase the power of
345 molecular markers to examine environmental effects upon genetic variation. The results indicate
346 the presence of a significant genetic structure of the cactus moth on the exotic *O. ficus-indica*, a
347 species introduced about five centuries ago during the Spanish arrival to South America (Ervin,
348 2012). The west sampled region (within the Dry Chaco) contained the highest genetic diversity
349 and suitability represented by four genetic groups (1, 2, 3, 5), which are delimited by geographic
350 barriers. This finding mirror previous research indicating that Dry Chaco corresponded to a
351 biodiversity refuge during the Quaternary climate changes (Poveda-Martínez et al., 2023), and
352 suggest an association between genetic diversity and environmental suitability (Ochoa-Zavala et
353 al., 2022). Colonization of *C. cactorum* to *O. ficus indica* followed an historical phylogeographic
354 pattern seen in other species, promoted by more recent environmental conditions. This is
355 supported by two previous findings: (1) the generalist feeding habit of the cactus moth (Varone
356 et al., 2014) that likely allowed the colonization of *O. ficus-indica* since its introduction, and (2)
357 the absence of human-mediated dispersal of *O. ficus-indica* related to agroindustry that promote

admixture among distant populations (Poveda-Martínez et al., 2023). Since its introduction in the Dutch Antilles in 1956 (Simmonds & Bennett, 1966), a similar pattern was found in the invaded region of North America (Florida) and the Caribbean (Andraca-Gómez et al., 2020), where the moth followed the phylogeographic pattern recorded for other native species of turtles, birds, crabs, and beetles (Avise, 2000). Thus, the presence of *C. cactorum* on *O. ficus-indica* in Argentina represent a useful natural setup to better understand how contemporary environmental conditions affect the distribution of genetic variation and environmental barriers to gene flow.

Ecological niche models have become a central tool for identifying environmental filters and barriers to migration (Razgour et al., 2013; Goudarzi et al., 2019). In turn, environmental filters can provide useful information to identify relevant life stages and traits related to environmental tolerance (Renault et al., 2018). Our results indicate that temperature (above and below ground), precipitation (seasonality), and soil organic carbon content can be the most relevant variables to predict the distribution of the cactus moth in the sampled region. Our results add to previous results of niche modeling for *C. cactorum* in North (Soberón et al., 2001) and South America (Poveda-Martínez et al., 2023) using only bioclimatic variables as soil characteristics significantly contributed to the model prediction. Since the moth pupates approximately in the top 10 cm of soil, temperature below the growth level, moisture and organic carbon content probably play a major role in pupal survival. Other species of lepidopteran have a high mortality rate during the pupal stage when soil humidity increases (Wang et al., 2017; Shi et al., 2021; Thian et al., 2021), but a low content can also affect pupal survival and emergence (Wang et al., 2017). Experimental studies and demographic analyses in different populations of *C. cactorum* in South Africa and under experimental conditions in Florida, found a lower development of larvae at <18°C and > 34°C (Zimmermann & Moran, 1991; Legaspi & Legaspi,

2007). In the present study, the greater environmental suitability in the drier western region suggests that pupae are probably more vulnerable to high soil moisture during the summer as precipitation is drastically reduced from the eastern plains of the Pampean region to the semi-arid shrublands and dry forests of Dry Chaco (Oyarzabal et al., 2018). The lower number of populations and the environmental suitability of the eastern group support the expectation that this region is under less benign conditions for moth development on *O. ficus-indica*. Ecological niche theory proposes that more populations will be found at the center of the ecological niche (Martínez-Meyer et al., 2013; Osorio-Olvera et al., 2020), corresponding to the area with optimal conditions for survival, growth, and reproduction (Lira-Noriega & Manthey, 2014; Osorio-Olvera et al., 2016). Our results support this expectation, as the region with higher environmental suitability following the niche model also corresponds to the region where *C. cactorum* was more abundant and where more genetic groups were detected. As environmental suitability is not homogeneously distributed within the sampled region, patterns of dispersal and genetic differentiation would be affected by environmental filters (e.g., Acevedo-Limón et al., 2020; Valdez et al., 2020; Hernandez-Leal et al., 2022).

In particular, the isolation by environment hypothesis (IBE) following the principles of electric resistance has helped to identify potential environmental barriers to species distribution and gene flow (MacRae, 2006; Wang & Bradburd, 2014). This approximation has increased the predictive power to account for the spatial distribution of genetic variation (McRae & Shah, 2009; McRae & Beier, 2007; Wang & Bradburd, 2014; Andraca-Gómez et al., 2015). Whereas the IBE hypothesis can be constructed using natural history information, niche models can provide a quantitative more precise estimation of environmental suitability (see Andraca-Gómez et al., 2015 and Poveda-Martínez et al., 2023). The significant effect of the environment on the

distribution of genetic variation allowed us to successfully identify important geographic and environmental barriers for gene flow and/or genetic differentiation in *C. cactorum*. Our results extend previous findings that the central Dry Chaco region comprises the ancestral genetic lineage (Poveda-Martínez et al., 2023), indicating that this area also present high diversity of genetic groups and the presence of significant environmental barriers. One of the strongest barrier separated the westerns groups within the Dry Chaco from sites located in the Pampean province (e.g., Poveda-Martínez et al., 2023). Barriers represented by mountain ranges, salt flats, wetlands, and soil conditions translate to different combinations of humidity and temperature of the upper soil layer where the moth pupates. Therefore, this stage of the life cycle seems to be critical for the environmental tolerance of the moth. Although the presence of a suitable host is a prerequisite for survival, it is not a sufficient condition for the presence of *C. cactorum*. In fact, during sampling, the moth was not detected at several sites where *O. ficus-indica* was present (Andraca-Gómez, unpublished data). Given the climatic and soil differences among the genetic groups, phenological asynchrony is expected, reducing the opportunities for effective gene flow (Zimmer & Emlen, 2013) and probably a higher heterogeneity in the life history traits of the cactus moth. This may explain the presence of at least four genetic groups within the western region. Overall, our results provide a new piece of evidence to understand the relevance of contemporary environmental conditions on the genetic structuring of this invasive species within its native range.

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**Legends**

**Table 1.** List of 24 sampling sites of *Cactoblastis cactorum* in Argentina.

**Table 2.** Statistics of genetic diversity of *Cactoblastis cactorum* in 24 sampling sites from Argentina for 10 microsatellite loci: number of alleles (NA), allelic richness (AR) (estimated from 9 diploid individuals), expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), inbreeding coefficient (($F_{IS}$ (*non-significant values)).

**Figure 1.** Geographic location of the 24 sampling sites of *Cactoblastis cactorum* used for genetic analyses. Samples are distributed in two biogeographic provinces (Löwenberg-Neto, 2014). The numbers correspond to those of Table 1.

**Figure 2. A**. Representation of the six genetic groups defined by *GENELAND* for the 24 sampling sites of *Cactoblastis cactorum* in Argentina. The color of the dots represents the genetic group that was assigned by the *GENELAND* program. Sampling sites: 9, 11, 12, 13, 14, 16 (green dots), 1, 6, 18, 20, 22, 23, 24 (yellow dots), 7, 8, 10 (blue dots), 17, 21 (purple dots), 2, 3, 4, 5 (red dots), Letters correspond to Salinas Grandes, **SG**, Salinas de Ambargasta, **SA**, Laguna Mar Chiquita, **LA**. Brown lines indicate the geographic location of the barriers proposed by the *BARRIERS* program (the barriers depicted are those with a percentage of existence greater than 70). **B.** Density distribution of the number of clusters along the chain with a burning period of 200 iterations and 1,000,000 steps of MCMC. **C.** Observed heterozygosity ($H_O \pm$ std) for each genetic group calculated as the average Ho for the 10 loci within each group (colored bars) and as the average Ho of sampling site within a given genetic group (white bars). **D.** Matrix of paired genetic distances between genetic groups (all values are significant). The numbers and colors in figures A, C and D are equivalent and represent the six genetic groups.

704 **Suplementary Material**

705 **Table S1.** Genetic differentiation values ($F_{ST}$) between pairs of sampling sites.

706 **Figure S1.** Suitability map for *Cactoblastis cactorum* as predicted by the consensus niche model.
707 Colors indicate the model predicted suitability within the sampled region. Regions with high
708 suitability indicate a higher probability of detecting *C. cactorum* in *Opuntia ficus-indica*.

709 **Database.**

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## Table 1(on next page)

Table 1. List of 24 sampling sites of *Cactoblastis cactorum* in Argentina.

**Table 1.** List of 24 sampling sites of *Cactoblastis cactorum* in Argentina.

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| Sampling sites | Biogeographic province | Ecoregion | Coordinates | Number of individuals |
|---|---|---|---|---|
| 1. Huasapampa | Chacoan | Dry Chaco | 27°54.839'S 65°33.805'O | 30 |
| 2. Icaño | Chacoan | Dry Chaco | 28°55.996'S 65°17.955'O | 27 |
| 3. Hipódromo las Rosas | Chacoan | Dry Chaco | 28°33.111'S 65°44.912'O | 20 |
| 4. Recreo | Chacoan | Dry Chaco | 29°16.346'S 65°04.230'O | 24 |
| 5. San Martín | Chacoan | Dry Chaco | 29°13.239'S 65°46.299'O | 18 |
| 6. El Talar | Chacoan | Dry Chaco | 28°05.028'S 65°18.595'O | 30 |
| 7. San Isidro | Chacoan | Dry Chaco | 32°08.933'S 65°06.292'O | 19 |
| 8. Cruz del Eje | Chacoan | Dry Chaco | 30°42.304'S 64°48.602'O | 30 |
| 9. El Fortín | Pampean | Espinal | 31°57.88'S 62°19.721'O | 21 |
| 10. Quilino | Chacoan | Dry Chaco | 30°13.655'S 64°28.928'O | 30 |
| 11. Las Varillas | Pampean | Espinal | 31°51.463'S 62°43.197'O | 19 |
| 12. Ayuí | Pampean | Espinal | 31°11.727'S 58°02.797'O | 15 |
| 13. Federal | Pampean | Espinal | 30°55.835'S 58°46.396'O | 16 |
| 14. Yuquerí | Pampean | Espinal | 31°22.917'S 58°07.718'O | 15 |
| 15. El Carmen | Chacoan | Yungas | 24°19.764'S 65°14.988'O | 30 |
| 16. Sastre | Pampean | Espinal | 31°44.344'S 61°50.193'O | 10 |
| 17. El Cuarenta y Nueve | Chacoan | Dry Chaco | 29°02.934'S 63°57.510'O | 30 |
| 18. Hock | Chacoan | Dry Chaco | 28°21.299'S 64°19.046'O | 30 |
| 19. La Banda | Chacoan | Dry Chaco | 27°44.937'S 64°12.232'O | 11 |
| 20. La Puerta | Chacoan | Dry Chaco | 27°37.915'S 64°37.281'O | 26 |
| 21. Pozo Escondido | Chacoan | Dry Chaco | 29°28.253'S 63°39.135'O | 30 |
| 22. Ruta Nueve | Chacoan | Dry Chaco | 27°45.027'S 64°23.532'O | 12 |
| 23. Vilmer | Chacoan | Dry Chaco | 27°45.982'S 64°09.632'O | 15 |
| 24. Tucumán | Chacoan | Dry Chaco | 27°07.269'S 64°55.704'O | 26 |

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## Table 2(on next page)

Table 2. Genetic diversity estimates for 24 sampling sites for Cactoblastic cactorum in Argentina.

**Table 2.** Statistics of genetic diversity of *Cactoblastis cactorum* in 24 sampling sites from Argentina for 10 microsatellite loci: number of alleles (NA), allelic richness (AR) (estimated from 9 diploid individuals), expected heterozygosity ($H_E$), observed heterozygosity ($H_O$), inbreeding coefficient (($F_{IS}$ (*non-significant values)).

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| Sampling sites | NA | AR | $H_S$ | $H_O$ | $F_{IS}$ |
|---|---|---|---|---|---|
| 1. Huasapampa | 6 (2.494) | 4.736 (1.799) | 0.684 (0.215) | 0.491 (0.222) | 0.282 |
| 2. Icaño | 5.7 (1.16) | 4.503 (0.992) | 0.626 (0.194) | 0.444 (0.188) | 0.291 |
| 3. Hipódromo las Rosas | 4.9 (2.132) | 3.940 (1.458) | 0.560 (0.238) | 0.41 (0.251) | 0.272 |
| 4. El Recreo | 4.8 (2.251) | 3.834 (1.646) | 0.538 (0.240) | 0.365 (0.180) | 0.323 |
| 5. San Martín | 4.1 (1.524) | 3.773 (1.170) | 0.578 (0.188) | 0.448 (0.218) | 0.231 |
| 6. El Talar | 5.9 (2.685) | 4.789 (1.841) | 0.655 (0.250) | 0.427 (0.191) | 0.348 |
| 7. San Isidro | 5.4 (2.413) | 4.556 (1.879) | 0.628 (0.270) | 0.377 (0.274) | 0.399 |
| 8. Cruz del eje | 7.2 (2.57) | 5.508 (1.870) | 0.708 (0.219) | 0.487 (0.161) | 0.313 |
| 9. El Fortín | 5.9 (1.595) | 4.742 (1.247) | 0.670 (0.184) | 0.551 (0.177) | 0.177 |
| 10. Quilino | 6.3 (2.058) | 5.133 (1.548) | 0.695 (0.209) | 0.458 (0.214) | 0.207 |
| 11. Las Varillas | 6.8 (1.814) | 4.934 (1.100) | 0.681 (0.098) | 0.515 (0.140) | 0.247 |
| 12. Ayuí | 3.8 (1.687) | 3.365 (1.349) | 0.533 (0.235) | 0.441 (0.244) | 0.171 |
| 13. Federal | 5.4 (1.578) | 4.649 (1.301) | 0.664 (0.187) | 0.526 (0.233) | 0.207 |
| 14. Yuquerí | 4.2 (1.619) | 3.732 (1.237) | 0.586 (0.243) | 0.560 (0.278) | 0.052* |
| 15. El Carmen | 6.8 (1.814) | 5.008 (1.371) | 0.653 (0.188) | 0.508 (0.189) | 0.225 |
| 16. Sastre | 5.5 (1.581) | 5.333 (1.597) | 0.645 (0.232) | 0.474 (0.210) | 0.275 |
| 17. El Cuarenta y nueve | 6.7 (2.003) | 5.468 (1.611) | 0.722 (0.174) | 0.417 (0.190) | 0.423 |
| 18. Hock | 6.2 (1.687) | 4.967 (1.182) | 0.730 (0.133) | 0.440 (0.194) | 0.402 |
| 19. La Banda | 4.4 (1.174) | 4.219 (1.157) | 0.608 (0.135) | 0.427 (0.268) | 0.307 |
| 20. La Puerta | 6.1 (1.912) | 4.914 (1.310) | 0.703 (0.134) | 0.582 (0.148) | 0.175 |
| 21. Pozo Escondido | 7.1 (2.685) | 5.502 (1.611) | 0.719 (0.176) | 0.459 (0.251) | 0.365 |
| 22. Ruta 9 | 6 (2.211) | 5.577 (1.986) | 0.725 (0.213) | 0.633 (0.261) | 0.131 |
| 23. Vilmer | 5.4 (1.713) | 4.720 (1.399) | 0.692 (0.150) | 0.477 (0.229) | 0.319 |
| 24. Tucumán | 6.8 (2.15) | 5.458 (1.483) | 0.719 (0.132) | 0.541 (0.223) | 0.251 |

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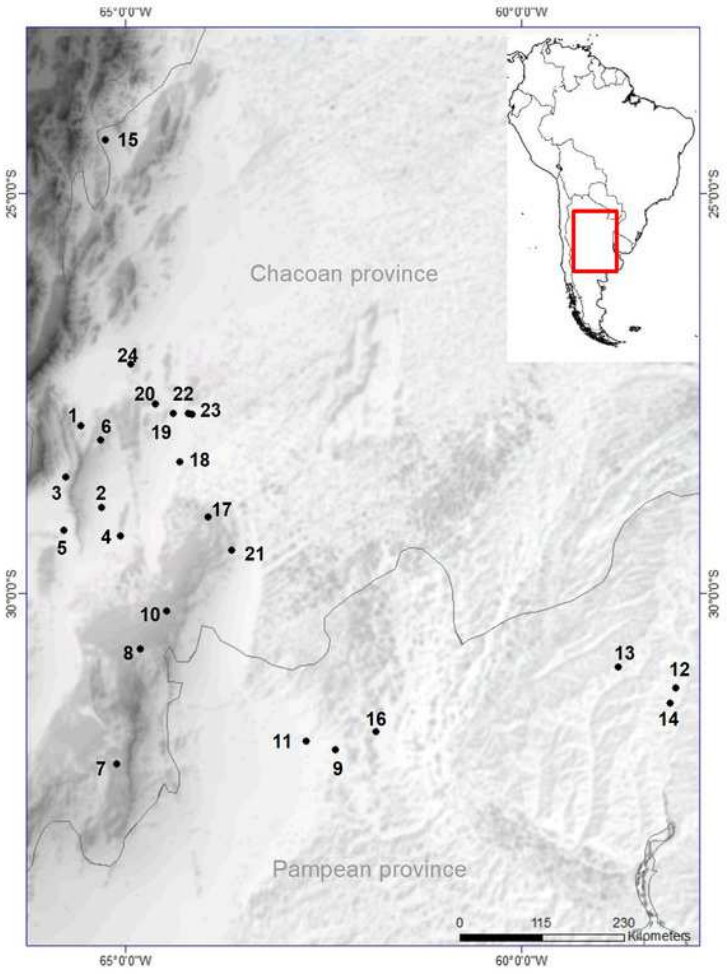
# Figure 1

Figure 1. Geographic location of the 24 sampling sites of *Cactoblastis cactorum.*

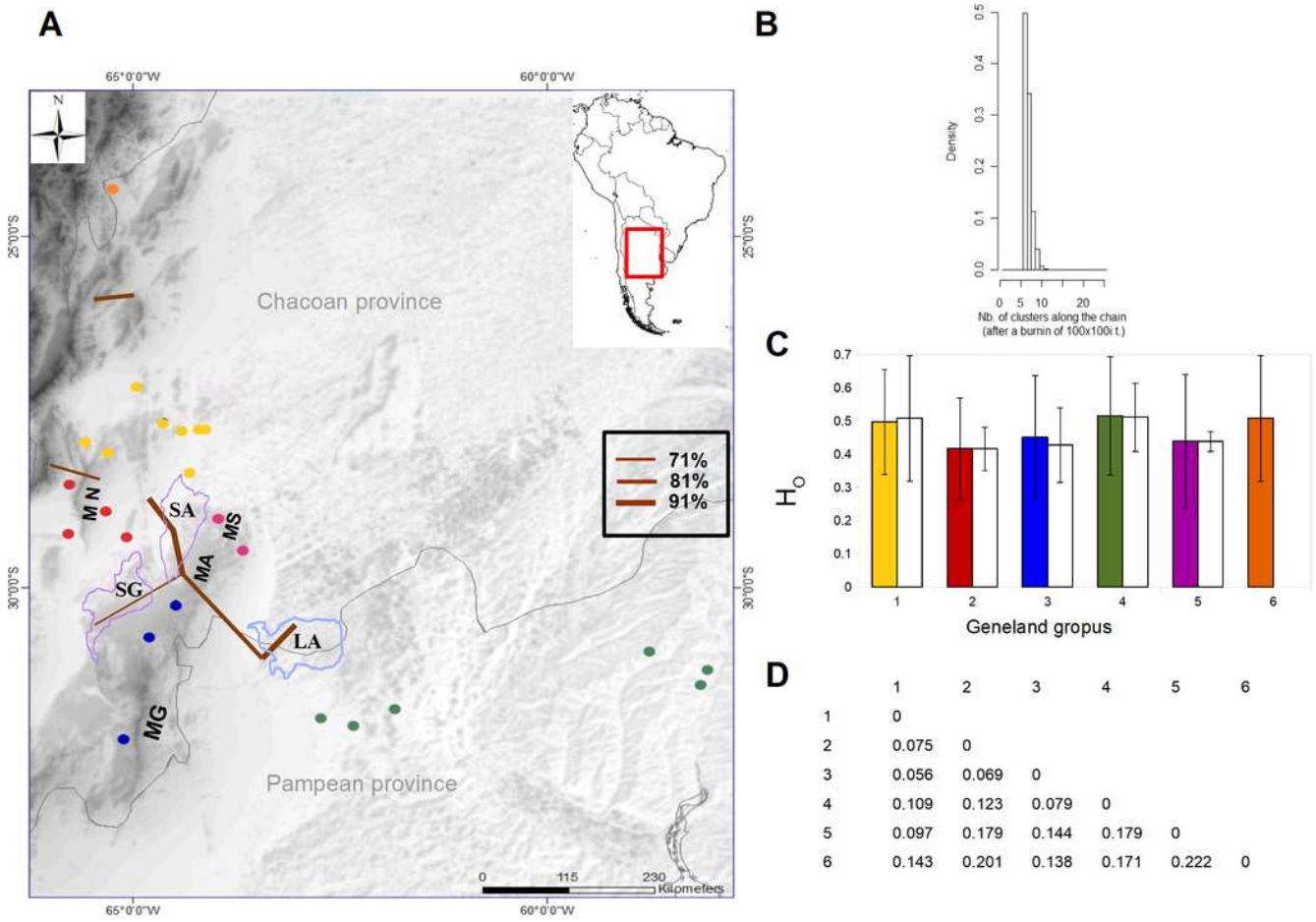
**Figure 1.** Geographic location of the 24 sampling sites of *Cactoblastis cactorum* used for genetic analyses. Samples are distributed in two biogeographic provinces (Löwenberg-Neto, 2014). The numbers correspond to those of Table 1.



# Figure 2

Figure 2. A. Results from GENELAND analysis of Cactoblastis cactorum within the sampled region of Argentina, number of genetic cluster, heterozigocity and paired genetic differentiation among groups.

**Figure 2. A**. Representation of the six genetic groups defined by *GENELAND* for the 24 sampling sites of *Cactoblastis cactorum* in Argentina. The color of the dots represents the genetic group that was assigned by the *GENELAND* program. Sampling sites: 9, 11, 12, 13, 14, 16 (green dots), 1, 6, 18, 20, 22, 23, 24 (yellow dots), 7, 8, 10 (blue dots), 17, 21 (purple dots), 2, 3, 4, 5 (red dots), Letters correspond to Salinas Grandes, **SG**, Salinas de Ambargasta, **SA**, Laguna Mar Chiquita, **LA**. Brown lines indicate the geographic location of the barriers proposed by the *BARRIERS* program (the barriers depicted are those with a percentage of existence greater than 70). **B.** Density distribution of the number of clusters along the chain with a burning period of 200 iterations and 1,000,000 steps of MCMC. **C.** Observed heterozygosity ($H_o \pm$ std) for each genetic group calculated as the average Ho for the 10 loci within each group (colored bars) and as the average Ho of sampling site within a given genetic group (white bars). **D.** Matrix of paired genetic distances between genetic groups (all values are significant). The numbers and colors in figures A, C and D are equivalent and represent the six genetic groups.




| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 0 | | | | | |
| 2 | 0.075 | 0 | | | | |
| 3 | 0.056 | 0.069 | 0 | | | |
| 4 | 0.109 | 0.123 | 0.079 | 0 | | |
| 5 | 0.097 | 0.179 | 0.144 | 0.179 | 0 | |
| 6 | 0.143 | 0.201 | 0.138 | 0.171 | 0.222 | 0 |