1   **Urbanization alters ecological and evolutionary interactions between Darwin's finches and**

2   ***Tribulus cistoides* on the Galápagos Islands**

3   L. Ruth Rivkin[1,2,3,*], Reagan A. Johnson[4], Jaime A. Chaves[5,6], Marc T.J. Johnson[2,3,*]

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[1]*Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada*

[2]*Department of Biology, University of Toronto Mississauga, ON, Canada*

[3]*Centre for Urban Environments, University of Toronto Mississauga, ON, Canada*

[4]*St. James Catholic Global Learning Centre, Mississauga, ON, Canada*

[5]*Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Campus Cumbayá, Quito, Ecuador*

[6]*Department of Biology, San Francisco State University, San Francisco, CA, United States*

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[*]Authors for correspondence: ruthrivkin@gmail.com and marc.johnson@utoronto.ca

5   **Abstract**

6   Emerging evidence suggests that urbanization shapes the ecology and evolution of species

7   interactions. Islands are particularly susceptible to urbanization due to the fragility of their

8   ecosystems; however, few studies have examined the effects of urbanization on species

9   interactions on islands. To address this gap, we studied the effects of urbanization on interactions

10   between Darwin's finches and its key food resource, *Tribulus cistoides,* in three towns on the

11   Galápagos Islands. We assessed the effects of urbanization on seed and mericarp removal,

12   mericarp morphology, and finch community composition using natural population surveys,

13   experimental manipulations, and finch observations. We found that both seed and fruit removal

14   rates were higher in urban compared to non-urban populations in the natural and experimental

15   populations, and that urbanization modified selection on mericarp size and defense. Urban

16   environments supported smaller and less diverse finch communities than non-urban

17   environments. Together, our results suggest that urbanization can dramatically alter ecological

18   interactions between Darwin's finches and *T. cistoides*, leading to modified selection on *T.*

19   *cistoides* populations. Our study demonstrates that urban development on islands can have

20   profound effects on the ecology and evolution of trophic interactions.

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1. Introduction

Urbanization results in substantial changes to the environment. Urban habitats are typically warmer, more polluted, and more fragmented than nearby non-urban habitats, which can lead to changes in the abundance and persistence of populations, and altered diversity and community [1–3]. Emerging evidence suggests that ecological changes associated with urbanization may alter natural selection and drive the evolution of novel adaptations [4,5]. Most examples of contemporary urban evolution occur in well-established cities, especially in Europe and North America [4,6]. However, we still have limited knowledge of how urbanization in tropical regions and particularly on islands can influence the ecology and evolution of species. Islands may be particularly sensitive to urbanization because of the unique and often fragile ecosystems they support, and even small human settlements may have large-scale effects on the ecosystem [7]. Our study addresses these gaps using the iconic Darwin's finch-*Tribulus* interaction of the Galápagos archipelago.

Urbanization can affect both the ecology and evolution of species interactions [8]. Predictions about how interactions will respond to urbanization are complex, especially for antagonistic interactions [9,10]. Antagonistic (e.g. predator-prey) interactions are inherently interconnected, and may be susceptible to urbanization through effects on one or both trophic levels. For instance, urbanization may decouple predator-prey interactions through the addition of food subsidies from anthropogenic resources [11], or may be intensify interactions when urban habitat fragmentation reduces available niche space, increasing the frequency of interactions [12,13]. These changes may lead to novel selection pressures on one or both interacting species [10]. Despite increasing work on species interactions in urban environments,

47   it remains unclear how urbanization simultaneously shapes both the ecology and evolution of

48   these interactions.

49   The Galápagos Islands of Ecuador provide an ideal system to test questions about how

50   urbanization affects the ecology and evolution of species interactions on islands. Ground finches

51   and *Tribulus cistoides* L. (Zygophyllaceae, common names puncture vine or Jamaican

52   feverplant)) on the Galápagos have a long history of study [19–21], and it is clear that these

53   species are experiencing a dynamic and ongoing co-evolutionary arms-race [17]. When resources

54   are scarce during the dry season, *T. cistoides* is an important food resource for three medium and

55   large beaked ground finch species: *Geospiza fortis*, *G. magnirostris*, and *G. conirostris* [18–20].

56   Predation on *T. cistoides* has led niche segregation and evolutionary changes in beak morphology

57   in these finch species [21–23]. In turn, finches influence mericarp survival, and select for

58   smaller, harder, and more defended mericarps [17]. Urbanization on the Galápagos Islands

59   influences both finches and *T. cistoides* populations [24–28]. Humans are one of the primary

60   dispersers of *T. cistoides* on the Galápagos [27], and urbanization reduces resource partitioning

61   through selection on beak morphology in *G. fortis* [25,29], likely due to the increased availability

62   of human food [25] and urban-induced behaviour modifications [30].

63   Despite clear evidence of the impacts of urbanization on ground finches and *T. cistoides*

64   individually, no work has examined the effects of urbanization on interactions between these

65   species. Our objective was to understand the effects of urbanization on the ecology and evolution

66   of interactions between ground finches and *T. cistoides*. We studied seed removal rates, selection

67   on mericarp morphology, and ground finch community composition on three islands on the

68   Galápagos archipelagos (figure 1). We used this system to ask three questions: 1) Does seed

69   removal by finches differ between urban and non-urban populations of *T. cistoides*? 2) Does

70   urbanization alter selection imposed by seed removal on mericarp size and defense? 3) Does the

71   abundance and community structure of Galápagos finch populations differ between urban and

72   non-urban sites, and how might this relate to patterns of seed removal and selection? Here, we

73   aim to identify the joint effects of how urbanization affects the ecology and evolution of

74   antagonistic species interactions in a fragile island ecosystem.

75   **2. Methods**

76   **a) Study site and system**

77   We studied seed and fruit removal rates, selection on mericarp morphology, and finch

78   community composition on three islands on the Galápagos archipelagos: Floreana, Isabela, and

79   Santa Cruz. We selected these locations because both large beaked ground finches and *T.*

80   *cistoides* are common in and around the towns. We have described our study site and study

81   system in detail in the supplemental methods.

82   **b) Study design**

83   This study was comprised of three components: a survey of natural populations of *T. cistoides* to

84   estimate seed removal rates; an experiment with mericarp defense traits artificially manipulated

85   to measure mericarp removal; and ground finch community observations. We studied each of

86   these components at the end of the dry season from January-March 2018 in urban and non-urban

87   locations on each of the three islands. For each component described below, we considered a

88   population to be urban if it occurred within a town's borders.

89   *Natural population survey*

90   To test for differences in seed removal rates and selection among urban and non-urban

91   populations, we conducted a survey of seed removal in natural *T. cistoides* populations in

92   February 2018. Natural populations provided us with a picture of natural variation in seed

93   removal between urban and non-urban habitats across islands over 8-12 months because

94   mericarps are produced in the wet season, and typically persist for many months until

95   germinating or decaying the following wet season [31]. We sampled 16 populations on Floreana

96   ($N = 9$ urban and 7 non-urban), 28 populations on Isabela ($N = 15$ urban and 13 non-urban), and

97   41 populations on Santa Cruz ($N = 22$ urban and 19 non-urban). We collected 20 mericarps from

98   each population (except for one population where we only found 19 mericarps), for a total of

99   1,699 mericarps. We counted the number of seeds eaten from each mericarp to estimate seed

100   removal rate. Following a modified protocol outlined in Carvajal et al. [17], we measured six

101   size and defense traits on each mericarp: mericarp length, width, depth, the length of the longest

102   spine, presence or absence of lower spines, and spine position (figure 1d).

103   *Fruit removal experiment*

104   At the same time as the natural population surveys, we conducted a six week-long experiment to

105   test for variation in fruit removal rates and selection in *T. cistoides*. This experiment

106   complemented our natural population surveys by allowing us to causally determine how

107   morphology affects removal and natural selection on mericarps by finches. However, the shorter-

108   term nature of the experiment meant that mericarp removal rates provided a shorter window of

109   predation pressure. We collected 800-900 intact mericarps (i.e. not attacked by finches) from

110   non-urban populations on each island. We weighed the mericarps and measured the same six

111   morphological traits measured in the natural population survey (figure 1d). We selected 20 urban

112   and 20 non-urban populations per island ($N = 40$ populations per island) and placed a petri dish

113   (100 mm diameter) in each population. Each dish contained 20 mericarps placed on top of

114   locally collected substrate (i.e. volcanic sand and gravel) for a total of 2,120 mericarps. We

115   randomly selected half of the mericarps and used wire cutters to clip off all of their spines to

116   create an "undefended" mericarp, while the other half were left with their spines intact as

117   "defended" mericarps. Although *T. cistoides* exhibits natural variation in spine number [17,18],

118   we selected mericarps that had four spines so that our manipulation simulated mericarps with

119   four (defended) versus zero (undefended) spines. We marked each mericarp with a unique

120   identifier on its dorsal surface using a black sharpie marker so that we could identify each

121   individual mericarp at the conclusion of the experiment.

122   We left the mericarps in the field for six weeks, and then collected them to score removal.

123   Using the identifying marks placed on the mericarps, we determined which mericarps had been

124   removed and which remained in the tray. If a mericarp was removed, we counted it as "eaten"

125   because ground finches often carry mericarps away from the location where they collect them to

126   crack them on a hard surface. We also counted the number of seeds removed from each mericarp

127   that was recovered, but the number of recovered mericarps with seeds was too small for analysis

128   (< 1% of the total sample), so our analyses focused on the removal rate. We carefully placed

129   petri dishes in locations where humans would not walk, thus we are confident that mericarp

130   removal was due to finch consumption and not human dispersal [27]. Some petri dishes were

131   disturbed during the experiment (three on Floreana, six on Isabela, eight on Santa Cruz), and

132   these petri dishes were excluded from the analyses.

133   *Finch community observations*

134   To determine how ground finch community composition varies with urban development, we

135   conducted surveys at urban and non-urban *T. cistoides* natural populations on each island. We

136   selected six sites per island ($N = 3$ urban and 3 non-urban) ensuring that each site had clear lines

137   of sight within 50 m of the center of the population. We surveyed each location for five minutes

138   and recorded finch sightings within 50 m during that time. Although *G. fortis* and *G.*

139   *magnirostris* are the only vertebrate seed predators of *T. cistoides* seeds on the islands we studied

140   [17], we recorded all finch species that frequently interact with *G. fortis* and *G. magnirostris* and

141   thus could influence their distribution, abundance, or behavior. We repeated the surveys four

142   times on Santa Cruz and three times on Floreana and Isabela.

143   **c) Statistical analyses**

144   We used R v 3.6.2 [32] for all analyses, details about the statistical analyses can be found in the

145   supplemental materials and methods. The R code and data files can be found in the supplemental

146   materials.

147   **3. Results**

148   **a) Natural population survey**

149   There were effects of urbanization, mericarp size, and defense on seed removal rate in *T.*

150   *cistoides* populations (Table S2). The number of seeds eaten per mericarp was 1.25% higher in

151   urban populations than in non-urban (*Urbanization*: $\chi^2_1 = 3.91$, p = 0.048). More seeds were

152   eaten from small mericarps (*Size*: $\chi^2_1 = 10.74$, p < 0.001), but on average seed removal was

153   greater in urban populations than from non-urban population (*Urbanization* × *Size*: $\chi^2_1 = 4.51$, p

154   = 0.034; figure 2a), suggesting seed removal imposed stronger selection for large mericarps in

155   urban populations. There was no main effects of island or mericarp defense, however the effect

156   of both size and defense differed among islands *(Island* × *Size*: $\chi^2_2 = 8.89$, p = 0.012; *Island* ×

157   *Defense*: $\chi^2_2 = 6.69$, p = 0.035), consistent with selection on these traits varying among islands.

158   **b) Fruit removal experiment**

159   Mericarp removal during our experiment was influenced by urbanization and defense traits

160   (Table S2). Mericarp removal rate was 43% higher in urban populations (*Urbanization*: $\chi^2_1 =$

161   4.98, p = 0.026), and 39% higher on clipped (undefended) mericarps (*Clipped*: χ^2_1 = 8.44, p =

162   0.004), although this effect did not vary with urbanization (*Urbanization × Clipped*: χ^2_1 = 0.05,

163   p = 0.823).

164   However, urbanization interacted with natural variation in mericarp defenses

165   (*Urbanization × Defense*: χ^2_1 = 4.24, p = 0.039). Well-defended mericarps were removed more

166   often from urban populations compared to non-urban populations (figure 2b), suggesting that

167   fruit removal by finches imposes selection for poorly defended mericarps in urban populations.

168   In contrast, mericarps that were poorly defended experienced a higher removal rate in non-urban

169   populations than in urban populations, indicating that the defensive function of spines switched

170   between urban and non-urban habitats (figure 2b). To better understand which traits were

171   causing this effect, we ran additional analyses that included individual defense traits as

172   covariates, instead of the composite defense trait (supplemental methods). We found that

173   mericarps with longer spines were more likely to be removed in urban populations than non-

174   urban populations (*Urbanization × Spine length*: χ^2_1 = 4.95, p = 0.027; Table S2), but no effect

175   of lower spine or spine position.

176   **c) Finch community composition**

177   We observed five species of ground finches across sites: two *G. magnirostris*, 171 *G. fortis*, 268

178   *G. fuliginosa*, 54 *G. scandens*, and 11 *Platyspiza crassirostris*. We found no change in the total

179   or relative abundances of *G. fortis* in the finch communities among urban and non-urban sites

180   (*Urbanization*: χ^2_1 = 1.10, p = 0.294) or among islands (*Islands*: χ^2_2 = 1.22, p = 0.544). We were

181   unable to evaluate differences in abundance of *G. magnirostris* among populations because only

182   two individuals were observed.

183   The effect of urbanization on the abundance and diversity of the finch community

184   differed among islands (abundance: *Urbanization × Island*: $\chi^2_1 = 6.94$, p = 0.031; diversity:

185   *Urbanization × Island*: $\chi^2_1 = 8.591$, p = 0.001; Table S3). Finches were 55% and 18% more

186   abundant in non-urban locations relative to urban populations on Isabela and Santa Cruz,

187   respectively, whereas there was no difference in abundance between urban and non-urban

188   locations on Floreana. We observed a similar trend for diversity (Table S3).

189   **4. Discussion**

190   We found that urban environments modify the ecology and evolution of interactions between

191   Darwin's finches and *T. cistoides*. We measured the effect of urbanization on these interactions

192   using a combination of natural population surveys, field experiments, and finch community

193   observations. Seed and fruit removal rates were higher in urban populations in both natural and

194   experimental populations (Q1), and urbanization modified selection on mericarp morphology

195   (Q2). In natural populations, seed removal imposed stronger selection for large mericarps in

196   urban populations than in non-urban populations, while in experimental populations, fruit

197   removal imposed selection for poorly defended mericarps in urban populations. Lastly, while we

198   found no difference in the abundance of *G. fortis* and *G. magnirostris*, urban environments

199   supported smaller and less diverse ground finch communities than non-urban environments (Q3).

200   Together, our results suggest urbanization can dramatically alter ecological interactions between

201   finches and *T. cistoides*, leading to modified selection on *T. cistoides* populations.

202   **a) Mericarp predation in urban environments**

203   We observed direct effects of urbanization on seed and mericarp removal. We found that

204   removal was higher in urban populations than in non-urban populations in both the natural and

205   experimental populations. Increased mericarp removal in urban habitats is consistent with the

206 hypothesis that urbanization intensifies interactions between finches and *T. cistoides*. These

207 interactions may have been intensified because *T. cistoides* is more abundant in urban areas on

208 the Galápagos islands (MTJJ and RAJ, personal observation). Humans are the primary dispersers

209 of *T. cistoides* on the Galápagos [27] and *T. cistoides* populations are most likely to be

210 established in and around towns. If ground finches exhibit a functional response to *T. cistoides*,

211 then their consumption of *T. cistoides* seeds may be correlated with the plant's abundance in a

212 habitat [33]. Such a functional response would explain why urban *T. cistoides* populations

213 experienced greater predation pressure from foraging finches, despite lower finch abundances in

214 these environments.

215   **b) Selection on mericarp morphology**

216 We found that urbanization alters selection on mericarp morphology. In urban environments,

217 consumption by finches imposed selection for large but poorly defended mericarps. In the natural

218 populations, we observed higher seed removal from small mericarps in urban and non-urban

219 habitats, but this effect was strongest in urban populations. Small mericarps may be more

220 energetically efficient to open [22,34], leading finches to choose small mericarps over large ones.

221 Combined with greater abundances of *T. cistoides* in urban areas, preferential consumption of

222 small mericarps may intensify selection for large mericarps in urban areas. Our results contrast

223 with a previous study which found that finches impose selection for smaller mericarps in natural

224 populations of *T. cistoides* [17]. The differences in findings may be the result of yearly variation

225 in climate that contributes to differences in resource availability. We conducted our study at the

226 end of the dry season following a relatively dry year, but annual variation in precipitation can

227 lead to differences in resource availability [35], and thus differences in the intensity of mericarp

228 consumption by finches. Repeating this study across wet and dry years would help separate out

229 the effects of urbanization from those of climate on the evolutionary ecology of interactions

230 between Darwin's finches and *T. cistoides*.

231   In the experimental populations, we observed increased removal of well-defended

232 mericarps from urban populations, whereas in non-urban populations we observed greater

233 removal of mericarps with fewer defences. This result suggests the surprising result that

234 urbanization is associated with finches preferring better defended mericarps. It is presently

235 unclear why urbanization modifies the direction of selection on mericarp defense. Mericarp

236 spines are expected to deter predators from accessing the seeds, an expectation that is consistent

237 with our findings from the non-urban populations and those from Carvajal et al. [17]. However,

238 we found that finches preferred mericarps with longer spines in urban populations. Finches may

239 prefer mericarps with longer spines because they were easier to pick up and manipulate.

240 Alternatively, longer spines can facilitate dispersal by humans [27], but we deliberately placed

241 the dishes in locations where humans were unlikely to disturb them so that mericarp removal

242 would be the result of finch consumption. Further experiments that include finch observations at

243 each experimental site are needed to distinguish between these possibilities.

244   While our study of natural populations was complementary to our experiments, these two

245 components of our study also differed in several important ways. First, natural populations were

246 available to finches for a longer period of time than the experimental populations, leading to

247 variation in seed removal unrelated to mericarp morphology. Natural populations may have been

248 more likely to experience seed removal because the finches would already have known where to

249 find them. In contrast, finches had to first locate the novel experimental populations before

250 removing the mericarps. Second, because they were exposed longer, the natural populations

251 experienced a greater range of climatic variation, potentially affecting the strength of selection

252   they experienced [35]. Lastly, we were able to track how many seeds were removed from each

253   mericarp in the natural populations, but not from the experimental populations. Seed removal

254   gives a more precise estimate of the fitness effects experienced by the plants and could have

255   resulted in different patterns of selection than mericarp removal.

256   **c) Urban finch communities**

257   Differences in the urban and non-urban ground finch communities may have contributed to

258   patterns of predation and selection on *T. cistoides*. Finch abundance differed between urban and

259   non-urban sites, although the direction of this difference varied among islands. We observed

260   lower abundance and diversity in urban sites than in non-urban sites on Santa Cruz and Isabela,

261   the largest two islands. This result is consistent with many other studies that find that bird

262   communities are often negatively affected by urbanization (reviewed in [8]). In contrast, there

263   were no significant differences in abundance or diversity between urban and non-urban sites on

264   Floreana, the smallest island, suggesting that town size might play a role in shaping finch

265   communities. Our study tracked how consumption by finches affects the ecology and evolution

266   of *T. cistoides*. To determine if an evolutionary response of *T. cistoides* populations feedbacks to

267   affect the ecology or evolution of finches, studies that combine yearly resampling to track

268   changes in mericarp morphology, beak shape and finch behaviour through time would be

269   necessary.

270   **d) Conclusion**

271   Together, our results suggest that urbanization modifies the ecology and evolution of interactions

272   between finches and *T. cistoides*. Our study examined the effects of urbanization in an island

273   ecosystem, which are predicted to be particularly sensitive to disturbance. Because of this

274   fragility, perturbations to the landscape through human development and the introduction of

275   invasive species may have large-scale effects on the ecology and evolution of native island

276   species [7]. Our study suggests that urbanization alters the evolutionary ecology of species on

277   islands and identifies trophic interactions as a key mediator of species interactions in the urban

278   island ecosystem.

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386 **Figures**
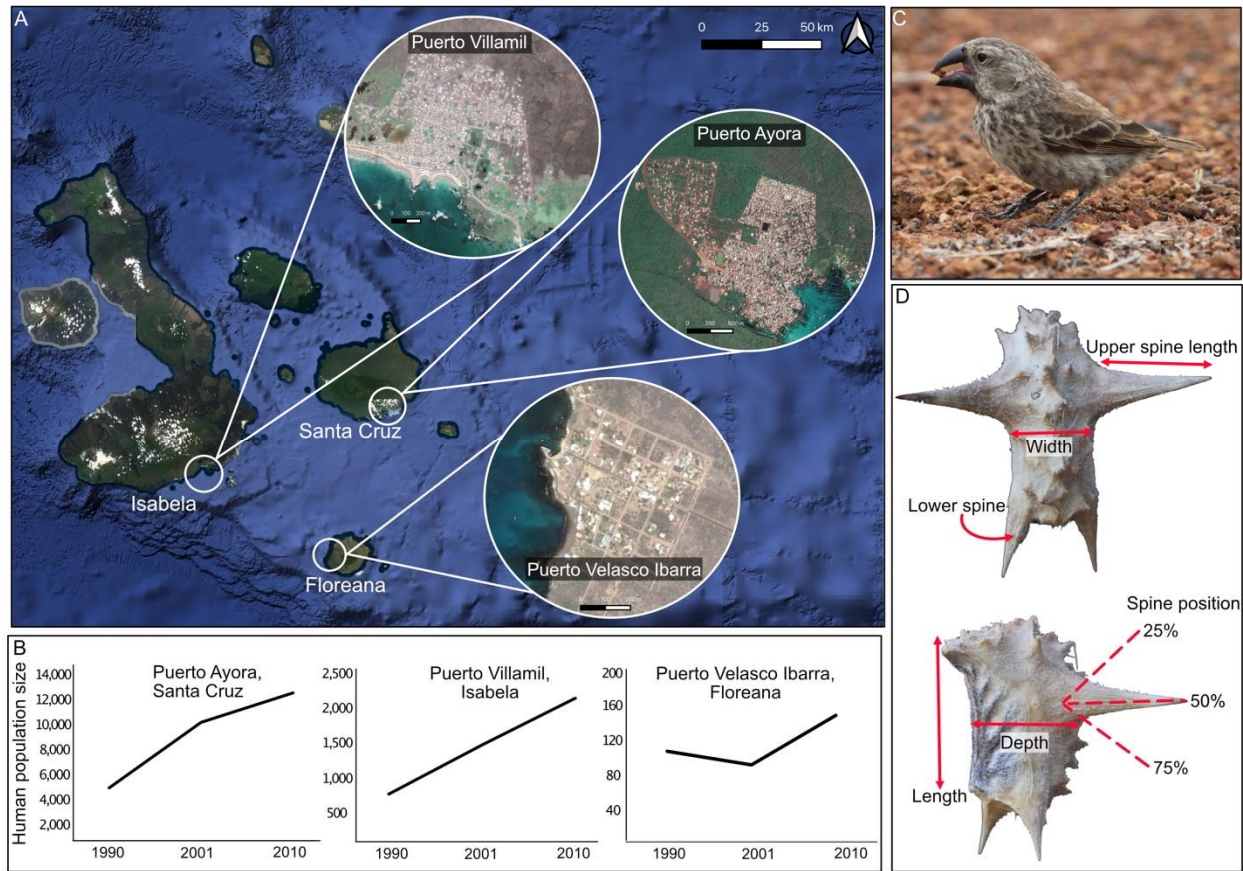
387

388 Figure 1. a) Map of the Galápagos Islands, with the three islands sampled and their principle

389 towns. Maps were taken from Google Satellite dating from 2018. B) Change in population size

390 in each town from 1990-2010 [41], ordered from largest (Santa Cruz) to smallest (Floreana);

391 note, the human population has continued to grow rapidly but censuses are carried out only every

392 10 years. c) A female medium ground finch (*Geospiza fortis*) holding a *Tribulus cistoides*

393 mericarp in its beak. d) Dorsal and lateral images of a *T. cistoides* mericarp, with each of the six

394 morphological traits measured. All images taken by MTJJ.
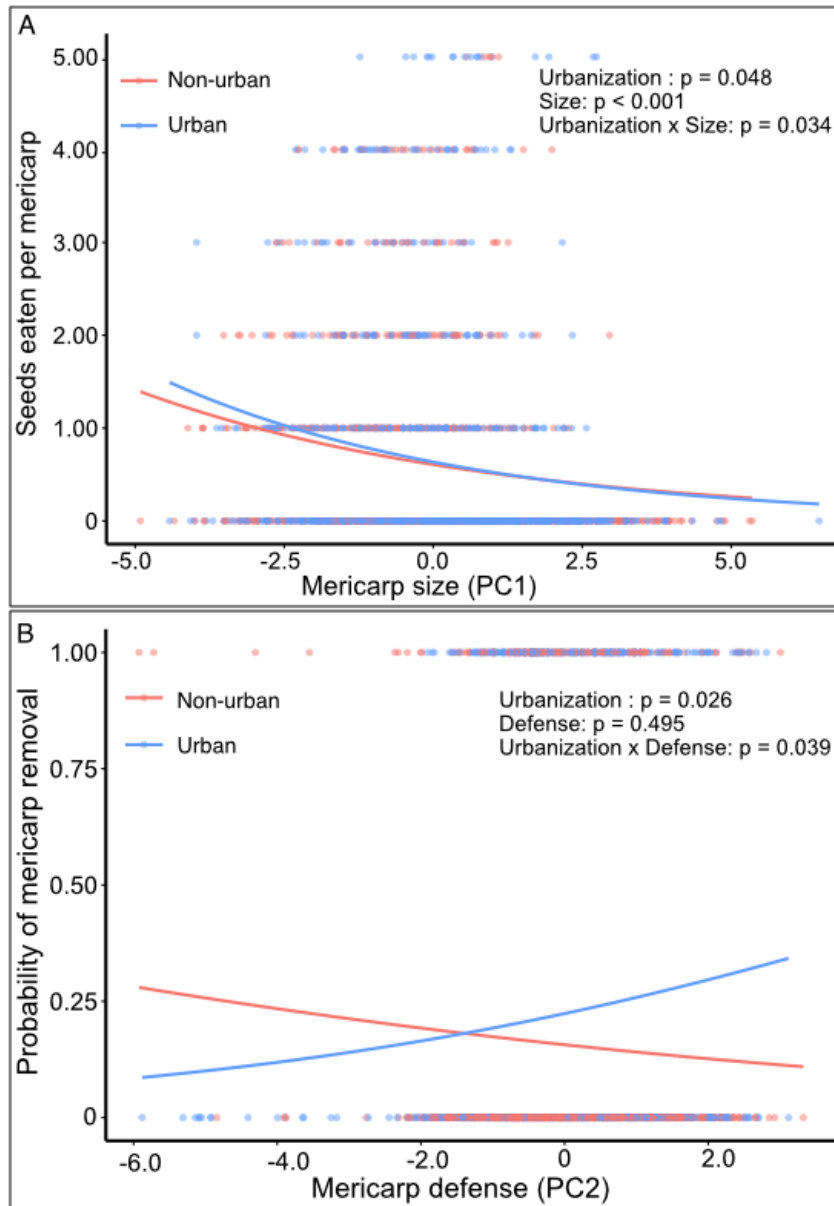
Figure 2. Seed and fruit removal by finches in natural and experimental populations of *Tribulus cistoides*. a) The number of seeds eaten per mericarp declined with cumulative mericarp size (PC1) in natural populations of *T. cistoides*, with small mericarps being eaten more in urban areas than in non-urban areas. b) The probability of mericarp removal from experimental populations increased with mericarp defense (PC2) in urban populations but declined in non-urban populations.