The effects of island forest restoration on open habitat specialists: the endangered weevil *Hadramphus spinipennis* Broun and its host-plant *Aciphylla dieffenbachii* Kirk

Emily D Fountain, Jagoba Malumbres-Olarte, Robert H Cruickshank, Adrian M Paterson

Human alteration of islands has made restoration a key part of conservation management. As islands are restored to their original state, species interactions change and some populations may be impacted. In this study we examine the coxella weevil, (*Hadramphus spinipennis* Broun) and its host-plant Dieffenbach's speargrass (*Aciphylla dieffenbachii* Kirk), which are both open habitat specialists with populations on Mangere and Rangatira Islands, Chathams, New Zealand. Both of these islands were heavily impacted by the introduction of livestock; the majority of the forest was removed and the weevil populations declined due to the palatability of their host-plant to livestock. An intensive reforestation program was established on both islands over 50 years ago but the potential impacts of this restoration project on the already endangered *H. spinipennis* are poorly understood. We combined genetic and population data from 1995 and 2010-2011 to determine the health and status of these species on both islands. There was some genetic variation between the weevil populations on each island but little variation within the species as a whole. The interactions between the weevil and its host-plant populations appear to remain intact on Mangere, despite forest regeneration. A decline in weevils and host-plant on Rangatira does not appear to be caused by canopy regrowth. We recommend that (1) these populations be monitored for ongoing effects of long-term reforestation, (2) the cause of the decline on Rangatira be investigated, and (3) the two populations of weevils be conserved as separate evolutionarily significant units.

2   Emily D. Fountain[1,3]*, Jagoba Malumbres-Olarte[2,3], Robert H. Cruickshank[3], and Adrian M. Paterson[3]

3   [1]Department of Forestry and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI 53706,

4   USA

5   [2]Center for Macroecology, Evolution and Climate, Natural History Museum of Denmark, University of

6   Copenhagen, DK-2100 Copenhagen E, Denmark

7   [3]Department of Ecology, Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647,

8   Christchurch, New Zealand

9   *corresponding author: efountain@gmail.com, +1 7173427959

Introduction

11 Island restoration is a key focus of conservation biology. Often islands are home to endemic flora and

12 fauna which may be heavily impacted by human modification and introduced species (Hutton et al.

13 2007). Although restoring an island to a pre-human state is ideal, its restoration is not straightforward

14 (Lawton 1997) given a lack of information on species interactions and pre-human community

15 composition and ecosystem conditions. Islands are unlikely to ever be fully restored to their previous

16 'pristine' states (Atkinson 1990). The dynamic nature of colonization from the mainland (Sinclair &

17 Byrom 2006) and a relative lack of literature regarding long-term management also hinder island

18 restoration (Simberloff 1990).

19 Despite these constraints, successful restoration has been achieved on small scales (Simberloff 1990).

20 Extensive ecological research is necessary for restoration efforts to be successful, and also competent

21 genetic management of island species is pivotal for long-term success (Jamieson et al. 2006). Genetics

22 plays an important role in ecology and conservation (Frankham et al. 2002). The loss of genetic

23 variation, common among island populations with low effective population sizes (N_e), can decrease

24 fitness and lower ability to adapt to changing environments (Pertoldi et al. 2007). Island restoration

25 often involves the translocation or captive rearing of organisms that are already genetically depauperate

26 (Jamieson et al. 2006). Understanding of the genetic characteristics of species involved in island

27 restorations is vital for long-term viability of the species, and the success of a restoration project.

28 Islands within the New Zealand archipelago have been actively restored for many years, using pest

29 eradication, replanting, translocation, captive rearing and reintroductions (Towns et al. 2012). One

30 group of such islands is the Chatham Islands (Chathams), which is approximately 800 km east of New

31 Zealand's South Island and consists of two large populated islands (Chatham and Pitt) and a series of

32 smaller, uninhabited islands. The Chathams have a history of intense geological activity that resulted in

33 frequent submerging and re-emerging of the islands (Heenan et al. 2010). The most recent emergence

34 of the smaller islands, such as Mangere, is thought to have been between 3.0 and 2.0 Ma (Heenan et al.

35 2010). Two of the Chatham islands, Rangatira and Mangere, have been under restoration since 1961

36 and 1968, respectively. The two islands are home to several rare birds [e.g. *Petroica traverse* (Chatham

37 Island black robin], plants [e.g. *Myosotidium hortensia* (Chatham Island forget-me-not)] and

38 invertebrates [e.g. *Amychus* spp. (Chatham's giant click beetle)].

39 The coxella weevil (*Hadramphus spinipennis* Broun 1911) is a large, flightless weevil endemic to the

40 Chatham Islands. There are four species in the genus *Hadramphus* (Craw 1999), all endemic to New

41 Zealand, three of which are listed as threatened or vulnerable, including *H. spinipennis* (Hitchmough et

42 al. 2007). The extant populations of this species are found on Rangatira, Little Mangere and Mangere

43 Islands, but the weevil was also historically recorded on Pitt Island in 1900 (Emberson et al. 1996).

44 Surveys of Mangere and Rangatira Islands (Schöps 1998) suggested that the weevil was thriving with

45 an estimate of over 10,000 weevils found in the 1995/1996 summer on Mangere Island (Schöps 2002) .

46 The weevil is associated with the plant Dieffenbach's speargrass (*Aciphylla dieffenbachii*), which is

47 found in open habitats on the islands. However, it has also been sighted on *Pseudopanax chathamicum*

48 Kirk (Araliacene) several hundred meters away from *A. dieffenbachii* (A.Liddy, G. Taylor pers.

49 comm.; Emberson et al., 1996). Choice tests between *A. dieffenbachii* and *P. chathamicum* suggest that

50 *Pseudopanax* is most likely not a viable host plant for this weevil (Schöps et al. 1999). *Hadramphus*

51 *spinipennis* is often found during September to February, feeding and mating on its host plant on

52 warm, humid nights (Schöps et al. 1999).

53   Schöps (2000) performed behavioral and ecological studies on *H. spinipennis* and its host plant, *A.*

54   *dieffenbachii*, on Mangere Island between 1993 and 1997, and on Rangatira Island in the summer of

55   1995/6. The main driver for this study was the observation of local extinctions of the host-plant on

56   Mangere, which were thought to be caused by the weevil. Local extinctions of the host-plant was

57   caused by the weevils before relocating to a new patch of *A. dieffenbachii* (Schöps 1998). Schöps

58   (2000) recommended, as part of the conservation management strategy (Department of Conservation

59   1999), that *H. spinipennis* and *A. dieffenbachii* should be monitored every three to four years on

60   Mangere and Rangatira to ensure that the regenerating forests do not affect the population dynamics

61   and survival of the weevil and its host-plant.

62   Our study was conducted 13 years after the initial research by Schöps (2000) and has two main aims:

63   (1) to measure the genetic similarity between the two populations and the genetic diversity within the

64   species as a whole, and (2) determine if the current population dynamics are consistent with those

65   found by Schöps and whether they support her original prediction of stable metapopulation dynamics.

66   We hypothesize that (1) geographic isolation inhibits gene flow between weevil populations and (2)

67   loss of open-habitat has caused a decline in the *A. dieffenbachii* populations defined by Schöps over a

68   decade ago. We performed DNA analysis on weevils from both islands and repeated the surveys

69   conducted on Mangere and Rangatira Island. Our study explores the possible negative impacts of

70   restoration on an endangered invertebrate by incorporating a genetic and ecological approach.

71   Methods

72   *Site descriptions*

73   Rangatira (South East) Island is 219 ha and was heavily farmed until its purchase by the New Zealand

74   government in 1953, after which all livestock were removed in 1961 (Department of Conservation

75   2012) (Fig. 1). Much of the original forest was destroyed for farming and many of the native plants that

76   remained were grazed by livestock. Currently, the island is mostly covered by remnant or regenerating

77   forest and *A. dieffenbachii* is limited to the coastal cliffs and rocky shores.

78   Mangere Island is 113 ha and surrounded by cliffs, with the highest cliff reaching 286 m (Fig. 1). The

79   island was once covered with native forest but 90% of the forest was burned for sheep farming and

80   many of the native vascular plants and megaherbs were suppressed by heavy grazing (Ritchie 1970).

81   Several plants, such as *A. dieffenbachii*, started to regenerate with the removal of livestock in 1968 and

82   an intensive forest planting program was implemented in 1974 (Butler & Merton 1992). *Aciphylla*

83   *dieffenbachii* grows along the steep, rocky cliffs of Mangere and in the open grasslands. The plant has

84   a patchy distribution over the whole island.

85   *Survey of* Hadramphus spinipennis *and* Aciphylla dieffenbachii

86   Rangatira (South East) Island (14-19 February 2010) and Mangere Island (17-23 February 2011) were

87   surveyed for *A. dieffenbachii* during the day. The search was restricted to coastal and open areas where

88   *A. dieffenbachii* has been documented to grow (Schöps 2000). Due to adverse weather conditions, the

89   high cliffs of both islands could not be surveyed although on Rangatira, although there had been

90   reports of a large *A. dieffenbachii* population on North Summit.

91   When plants were found, the quantity and state of flowering and a visual assessment of plant size were

92   made. Plants were searched for signs of weevil feeding and activity, in particular the characteristic

93   margin feeding of *H. spinipennis* (Schöps 2000). Although other herbivores can be found on *A.*

94   *dieffenbachii*, the feeding pattern of *H. spinipennis* is distinctly different and well documented by

95   photograph making feeding signs easily observable (Fig. 2). Each plant was photographed and the

96   surrounding area was searched for new seedlings. GPS coordinates of major plant clusters were

97 recorded (Table S1). At night, starting at 22:00, known *A. dieffenbachii* populations were visually

98 searched for *H. spinipennis* individuals. Data collection on Mangere was conducted in the same

99 manner as on Rangatira. All locations where *A. dieffenbachia* and *H. spinipennis* had previously been

100 recorded were visited and surveyed.

101 *DNA collection and PCR*

102 Due to collection restrictions of protected species by the Department of Conservation and also local

103 imi/iwi, weevils for DNA analysis were limited to 15 individuals captured per island. Adult *H.*

104 *spinipennis* range from 18-22 mm in length. Individuals were randomly selected from different

105 subpopulations on the islands; the tarsal claw and the first two segments of the tarsus were removed

106 using ethanol-cleaned scissors and the weevil was then released. Previous work has shown that a tarsal

107 clip has no known negative impacts on the weevils (Fountain et al. 2013). Clips were stored in

108 propylene glycol and, when returned to the laboratory, were washed with 95% ethanol and then stored

109 at -20°C in 95% ethanol. Each tarsal clip was cut into several pieces using a sterile scalpel blade and

110 then transferred into a 1.7mL Eppendorf tube. A QIAmp DNA Investigator Kit (Qiagen, Auckland,

111 catalog # 56504) was used for DNA extraction following the manufacturer's protocol for tissue

112 samples.

113 Two mitochondrial genes, cytochrome *c* oxidase subunit I (COI) and cytochrome b (cytb), and one

114 nuclear gene, internal transcribed spacer 2 (ITS2), were amplified by polymerase chain reaction (PCR).

115 For all PCRs, 2.5 μl of the DNA extraction was added to the following: 2.5μL of 0.25 mM of dNTPs,

116 .2μl of polymerase, 1μL of 20 μM for each primer, 2.5μL of 10x PCR buffer (*i*-taq, iNtRON

117 Biotechnologies) and deionized water to bring the total reaction volume to 25 μl.

118   COI was amplified using the primers LCO1490 and HCO2198 (656 base pair fragment) (Folmer et al.

119   1994). The PCR cycle was 94°C for 3 min followed by 35 cycles of 94°C for 45 s, 45°C for 45 s and

120   72°C for 1 min 20 s, with a final extension at 72°C for 5 min. The primers CB1 and CB2 (432 base pair

121   fragment) (Simon et al. 1994) were used to amplify cytb. The PCR cycle was 94°C for 3 min followed

122   by 40 cycles of 94°C for 30 s, 49°C for 45 s and 72°C for 1 min, with a final extension at 72°C for 5

123   min.

124   Due to the difficulty in amplifying a large fragment of ITS2 using the original primer set, a new genus-

125   specific primer set was developed for a 450 base pair fragment of ITS2 (Table 1) (See Appendix S1 for

126   primer design details). The PCR mixture and cycle for these primers were the same as those for ITS3

127   and ITS4, except that the annealing temperature was decreased to 54°C. Every PCR reaction included a

128   negative (water) control with no DNA.

129   *Molecular Data Analysis*

130   Sequence chromatograms for 30 COI, 16 cytb and 25 ITS2 sequences were visualized using FinchTV

131   1.4 (Geospiza) and forward and reverse sequences were manually aligned in Mega 5.05 (Tamura et al.

132   2011). No insertions, deletions or stop codons were found for COI or cytb. Within ITS2 a variable AT

133   short tandem repeat (STR) was found in the middle of the sequences. Short tandem repeats may have

134   higher mutation rates compared to the flanking regions which may interfere with the phylogenetic

135   signal (Selkoe & Toonen 2006) so we conducted preliminary analysis on the ITS2 data set with and

136   without the STR. Preliminary phylogenies were constructed in MEGA from a Kimura two-parameter

137   (K2P) distance matrix (Kimura 1980) using neighbor joining (NJ) with 1,000 bootstrap replicates. No

138   differences were found in the ITS2 analysis when the STR was removed; we chose to use the dataset

139 without the STR for additional analysis to be confident the STR would not interfere with the

140 phylogenetic inference.

141 For Bayesian analysis, the COI data set was reduced to 16 sequences to match them with the cytb

142 sequences. Sequences of the two genes were then concatenated in R 2.13.2 (R Development Core

143 Team 2011). The concatenated mitochondrial sequence data were analyzed separately from the ITS2

144 data because they are independently evolving loci and also have differences in mutational rates. The

145 ITS2 analysis was performed on the full set of 25 sequences. Bayesian analyses were performed using

146 Beast 1.7.1 (Drummond et al. 2012). The best partitioning scheme and evolutionary model were found

147 with PartitionFinder (Lanfear et al. 2012) using the corrected Akaike Information Criterion (AICc) for

148 COI and cytb. The Kimura three-parameter (K81) model (Kimura 1981) was chosen with no

149 partitioning between the concatenated genes (known collectively below as mtDNA). Since ITS2 is not

150 a protein coding gene it was not considered for partitioning and the best fit evolutionary model was

151 found using the AICc with jModeltest 2.1.1 (Darriba et al. 2012), which identified the symmetrical

152 (SYM+G) model (Zharkikh 1994) with gamma distribution as the optimal model.

153 The mtDNA and ITS2 data were analyzed under a strict molecular clock with a coalescent (constant

154 population size) tree prior. For each gene, four replicate runs of a chain run for 50 million generations

155 were performed, sampling every 2000 generations. Convergence and effective sample size of each

156 parameter was assessed in Tracer 1.5 and samples from the four runs were pooled using Log Combiner

157 1.7.2. After discarding the initial 10% as burn-in, a maximum clade credibility tree was compiled in

158 TreeAnnotator 1.7.1 (Drummond et al. 2012).

159 For the mitochondrial data, two Bayesian analyses were conducted to provide estimates of divergence

160 time using (i) the geological evidence and the timing of the volcanic emergence of Mangere, 3-2

161 million years ago (Heenan et al. 2010), and (ii) the standard invertebrate mitochondrial rate of 0.0115

162 substitutions/site/million years (Brower 1994). For geological dating, priors were set to allow tree

163 calibration using direct input of a fixed date for specific nodes; the root of the tree was constrained to

164 no older than 3 million years with uniform prior. Since we aim to estimate the split between the

165 Rangatira and Mangere populations a distribution of dates was not used as Mangere did not emerge

166 until 3 million years ago at the earliest. All analyses were performed in BEAST 1.7.2 (Drummond et

167 al. 2012) using the same model and partitioning scheme employed in the previous analysis. Four

168 independent runs consisting of a chain run for 50 million generations were conducted, sampling every

169 2000 generations. Maximum clade credibility trees were generated in the same manner as in the

170 previous analysis.

171 Pairwise genetic distances for mtDNA were taken from the Bayesian maximum clade credibility tree

172 using PASSaGE 2.0 (Rosenberg & Anderson 2011). A Neighbor-Net network of ITS2 haplotypes was

173 generated using SplitsTrees 4.12.3 (Huson & Bryant 2006) to visualize conflicting patterns in the

174 phylogenetic signal. Distances for the Neighbor-Net network were calculated under the generalized

175 time reversible (GTR) model and no rate heterogeneity among sites.

176 ## Results

177 *Survey*

178 Rangatira

179 Three locations were found to have subpopulations of *A. dieffenbachii*: East Clears, West Clears, and

180 West Landing (subpopulations A, B, C in Fig. 3). Subpopulation A showed signs of heavy weevil

181 herbivory on all plants. In subpopulation B only a few plants showed signs of weevil damage, whereas

182   subpopulation C had no weevil feeding damage. Table 3 provides a detailed description of the number

183   of plants, size of plants, and flowering state for the *A. dieffenbachii* populations.

184   In subpopulation A, 29 *H. spinipennis* were found feeding and mating on the plants. The male: female

185   sex ratio of the weevils was 15:14. In subpopulation B, three out of the nine major *A. dieffenbachii*

186   groups had weevils. Group 1 had two males and two females: one pair breeding and the other two

187   feeding. Group 4 had one breeding pair, and group 9 had one male feeding.

188   West Landing was searched for two nights but no weevils were found (Fig. 3). One male *H.*

189   *spinipennis* was found on *Pseudopanax chathamicus*, approximately 250 meters from the nearest *A.*

190   *dieffenbachii* at West Landing.

191   Mangere

192   Seven subpopulations of *A. dieffenbachii* were identified on Mangere (Fig. 4). Feeding signs were

193   observed in plants from all subpopulations. In addition to the plants found in the seven subpopulations,

194   a few individual plants were also found scattered along the south-east coast of the island but had no

195   evidence of weevil feeding damage (Fig. 4). A detailed description of the *A. dieffenbachii* populations,

196   including number of plants, size of plants and flowering state, is provided in Table 4.

197   Individuals of *H. spinipennis* were found in five of the seven *A. dieffenbachii* subpopulations. The

198   observed number of weevils reached 26 in location 1, which corresponded to subpopulation 1 of *A.*

199   *dieffenbachii*. Ten specimens were counted in locations 4, 6 and 3, and only two in location 2. The

200   male: female sex ratio ranged from 19:7 in location 1, through 8:6 in location 4, to 1:1 in locations 3

201   and 2. Weevils were observed feeding and mating in all locations except for location 2, where the two

202   individuals were feeding on separate plants.

203   *Molecular analysis*

204   In total, 30 specimens of *H. spinipennis* were successfully sequenced for COI, 16 for cytb and 25 for

205   ITS2. For COI, a 656 bp fragment was obtained, which had three variable sites, two of which were

206   parsimony informative. Base frequency means for COI were unequal and AT-rich (T=35.0%,

207   C=19.6%, A=30.3% and G=15.1%); a chi-square test confirmed heterogeneity of base frequencies

208   across all taxa (d.f. =87, *p*=1.00). For cytb, a 432 bp fragment was obtained, which had one variable

209   site, which was parsimony informative. Base frequency means for cytb were unequal and AT-rich

210   (T=36.6%, C=22.2%, A=29.3%, and G=11.9%); a chi-square test confirmed heterogeneity of base

211   frequencies across all taxa (d.f. =45, *p*=1.00). The 30 COI sequences showed little variation with only

212   two individuals sharing a single nucleotide difference between themselves and the rest of the samples.

213   However, for cytb there was a clear difference at one nucleotide in all samples between individuals

214   from each of the two islands. The pairwise genetic distances in the concatenated mitochondrial genes

215   were low with a mean of 0.002 (max 0.003) substitutions/site between the two island populations. The

216   maximum P-distance within each island was 0.001 substitutions/site.

217   After the removal of the AT variable repeat, a 428 bp fragment was obtained for ITS2, which contained

218   24 variable sites, 17 of which were parsimony informative. Four indels were found in the sequences,

219   two from an individual on Rangatira and two from an individual on Mangere. The Neighbor-Net

220   network does not show strong genetic structure within or between the islands (Fig. 5). The results of

221   the Neighbor-Net network (Fig. 5) suggest that there may be a complicated signal between the two

222   islands represented by the network separating the two groups.

223   The maximum clade credibility trees were very similar in topology to the NJ trees; due to the splitting

224   of zero-length branches in the maximum clade credibility trees we opted to display the NJ trees. The

225 mitochondrial tree shows a weakly-supported split between the two islands (Fig. 6a), and the branch

226 length separating the two islands is extremely small at 0.002 substitutions/site. The universal

227 mitochondrial rate for invertebrates of 0.0115 substitutions/site/million years places the origin of this

228 split around 1,300 BP (95% HPD 0.000-0.0139 BP) and the geological time calibration of 4 million

229 years places it around 600 BP (95% HPD 0.0001-0.0016 BP) (Fig. 6a, Table 2). The ITS2 tree shows a

230 lack of resolution and does not support any split between the two islands (Fig. 6b). There is no

231 evidence for genetic differentiation in COI and ITS2 among the subpopulations for each island

232 [subpopulations A, B, C for Rangatira and subpopulations 1-7 for Mangere (Figs. 3 and 4)].

233 Discussion

234 The genetic analysis shows a population of weevils that is highly similar across the islands with only a

235 small amount of difference between the two islands in the mitochondrial genes. A genetic difference

236 between the weevil populations of Mangere and Rangatira has been previously reported for a different

237 section of the COI gene (Goldberg & Trewick 2011). The analysis with the two concatenated

238 mitochondrial genes showed a strong support for a split between the two islands. This difference is not

239 seen in the nuclear gene ITS2; however, due to the longer coalescent times of nuclear genes compared

240 to mitochondrial genes, there may have been insufficient time for ITS2 to achieve reciprocal

241 monophyly. Although some signal interference in the ITS2 gene caused by incomplete lineage sorting

242 and recent divergence can skew the resulting phylogeny, there is still strong support for a group that

243 includes two individuals, one from each island. Further molecular work should be conducted on more

244 nuclear genes to discover a clearer signal of genetic differentiation between the two islands.

245 The difference in the mitochondrial and nuclear gene trees can be explained by the four times faster

246 coalescence time in mitochondrial genes compared to nuclear genes (Ballard & Whitlock 2004). The

247 island split in the mitochondrial gene tree may be the result of the two populations having no gene

248 flow. Given the faster mutation rate of mitochondrial genes versus the nuclear genes (Moriyama &

249 Powell 1997), reproductive isolation is likely to lead to faster differentiation of the mitochondrial

250 genes. In the future, this split may become evident in the slower evolving nuclear genes if the

251 populations remain genetically isolated. Currently the populations of the weevil and its host plant on

252 Rangatira are not as heavily monitored as they are on Mangere. If the Rangatira and Mangere

253 populations are confirmed to be genetically isolated, it is of utmost importance to take conservation

254 measures to make sure that both populations have similar chances of survival.

255 Both methods of dating the separation between individuals on the two islands using the mitochondrial

256 genes date the mean time for this split as rather recent, in the last few thousand years. This timing

257 approximately coincides with the arrival of humans and introduced mammals, particularly Polynesian

258 rats, which are known to predate *Hadramphus* weevils (Towns 2009). Calibrating the molecular clock

259 using the geological age of 4 million years as the last emergence of the islands gave a smaller 95%

260 confidence interval. Given that using a universal mitochondrial rate is fraught with problems, such as

261 variation between genes, differences depending on substitution model used and the universal rate does

262 not account for variance in coalescent times (Papadopoulou et al. 2010), geological dating can offer a

263 better estimation of the time of separation between the two islands when applying the correct

264 distribution to account for dating uncertainty (Ho 2007).

265 The lack of genetic diversity found within *H. spinipennis* may be a result of population decline in the

266 weevils. A likelihood of population decline is supported by the extinction of populations of *H.*

267 *spinipennis* from Pitt Island in the late 1800s due to the loss of habitat and habitat modification from

268 humans. Loss in genetic variation and extinction of populations points directly to a population

269 bottleneck in *H. spinipennis*.

270   The results of this study suggest that there has been no decline in the number of the *H. spinipennis* and

271   *A. dieffenbachii* populations surveyed on Mangere and population numbers have not been affected by

272   the forest regeneration. The weevil has a consumer-resource metapopulation relationship with its host

273   plant and will decimate populations of *A. dieffenbachii* before moving onto another population of

274   plants (Schöps 1998). Metapopulation theory suggests that as long as recolonization by the consumer

275   exceeds or equals the extinction rate of the resource, the metapopulation will persist (Taylor 1990). On

276   Mangere, there is evidence for subpopulations going extinct while new *A. dieffenbachii* populations

277   have arisen which suggests a dynamic system of localized host-plant exploitation, extinction, and

278   weevil dispersal.

279   On Rangatira, there has been a decline in both *H. spinipennis* and *A. dieffenbachii* populations.

280   Although two of the populations (A & B) of *A. dieffenbachii* were found in 1995 and 2010, the plants

281   were in decline in 2010 with no flowering and no large-sized plants. One population of *A. dieffenbachii*

282   that was found in 1995 was longer in existence, not even as seedlings, in 2010. The newly discovered

283   population of plants on the West Clears contained mainly small plants and seedlings, with only one

284   plant that flowered, and may not be able to support a large weevil population. The decline on Rangatira

285   does not seem to be the result of forest regeneration as the locations of all *A. dieffenbachii* populations

286   are on open grasslands and cliffs where no forest has regenerated. Modeling suggests that if the

287   distance between host-plant populations is great enough to not be easily traversed, but not so far that it

288   is unreachable by the weevils, then the consumer-resource dynamic could persist for the long-term

289   (Johst & Schöps 2003). The host-plant populations on Rangatira were separated by several hundred

290   meters and the weevil is known to travel up to 500 m to a new plant resource (Schöps 1998).

291   Therefore, distance between populations does not appear to be the cause of its decline. More intensive

292 monitoring should be conducted on Rangatira to determine the cause of the host-plant decline and

293 whether the metapopulation dynamic between *H. spinipennis* and *A. dieffenbachii* has been altered.

294 *Hadramphus spinipennis* is listed as endangered by the New Zealand Department of Conservation and

295 is considered a species of concern due to its very restricted range. Although spatial models have shown

296 that the weevil population is in equilibrium, and could possibly be classified as "common" (Kean

297 2006), combining genetics with longer-term ecological data does not completely support the notion

298 that *H. spinipennis* is common on both Mangere and Rangatira. In regards to the number of weevils

299 and host-plant populations on Mangere, the weevil seems relatively abundant; however, on Rangatira

300 lower weevil numbers were found. Genetic data indicates that both populations do not possess a large

301 amount of genetic diversity and have most likely undergone a population bottleneck. Population

302 bottlenecks are related to a loss of genetic diversity which can have significant consequences for long-

303 term viability of small populations (Grueber et al. 2008). As the forests on Mangere and Rangatira

304 Islands continue to regenerate, *A. dieffenbachii* may decline due to a loss of open habitat. Although the

305 population counts suggest no change in *H. spinipennis* on Mangere, the weevil may be unable to adapt

306 to a possible decline in its host-plant resulting from the loss of open habitat. This is particularly a

307 concern on Rangatira where a decline in weevil numbers was seen.

308 Conclusions

309 A pattern of genetic structure separating a species by island haplotype is something that has been

310 reported in other flightless beetles (Sequeira et al. 2012; Stroscio et al. 2011). Between the two islands

311 there are some genetic differences, which should be preserved if possible, as the *H. spinipennis* on

312 Rangatira and Mangere Islands are shown to be genetically isolated from each other. The unique

313 haplotypes from each island adds variation and allows for the possibility of cross-introduction of

314   weevils to preserve genetic diversity. Our results strongly suggest that forest restoration on Rangatira

315   and Mangere Islands has not had a negative impact on *H. spinipennis* or its host plant; however, a long-

316   term survey, in particular accessing the area on Rangatira that could not be searched in this study, is

317   recommended to confirm small population sizes. Although continued monitoring of the weevil

318   populations is recommended, forest restoration should continue and is not impacting on the

319   conservation of an open habitat specialist.

320   Acknowledgements

321   We thank the Chatham Islands Conservation Board, the Chatham Island imi/iwi, and the Department of

322   Conservation (DoC) for the permit (WE-26391-RES) to collect and for access to the island, housing,

323   and general assistance.

324   References

325   Atkinson IAE. 1990. Ecological restoration on islands: Prerequisites for success. In: Towns DR,

326   Daugherty CH, and Atkinson IAE, eds. *Ecological restoration of New Zealand islands*.

327   Wellington: Department of Conservation, 73-90.

328   Ballard JWO, and Whitlock MC. 2004. The incomplete natural history of mitochondria. *Molecular*

329   *Ecology* 13:729-744.

330   Brower A. 1994. Rapid morphological radiation and convergence among races of the butterfly

331   *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the*

332   *National Academy of Science* 91:6491-6495.

333   Butler D, and Merton D. 1992. *The Black Robin- Saving the world's most endangered bird*. Auckland:

334   Oxford University Press.

335   Craw RC. 1999. *Fauna of New Zealand: Molytini Number 39*. Lincoln, Canterbury: Manaaki Whenua

336   Press.

337   Darriba D, Taboada GL, Doallo R, and Posada D. 2012. jModel Test 2: More models, new heuristics

338   and parallel computing. *Nature Methods* 9:772.

339   Department of Conservation. 1999. Chatham Islands Conservation Management Strategy. Wellington

340   Conservancy Conservation Management Planning Series No.8.

341   Department of Conservation. 2012. Rangatira (South East Island)2012).

342   Drummond AJ, Suchard MA, Xie D, and Rambaut A. 2012. Bayesian phylogenetics with BEAUti and

343   the BEAST 1.7. *Molecular Biology and Evolution* 29:1969-1973.

344   Emberson RM, Early JW, Marris JWM, and Syrett P. 1996. Research into the status and distribution of

345   Chatham Islands endangered invertebrates. *Science for Conservation*:1-28.

346   Folmer O, Black M, Hoeh W, Lutz R, and Vrijenhoek R. 1994. DNA primers for amplification of

347   mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular

348   Marine Biology and Biotechnology* 3:294-299.

349   Fountain E, Wiseman B, Cruickshank R, and Paterson A. 2013. The ecology and conservation of

350   Hadramphus tuberculatus (Pascoe 1877) (Coleoptera: Curculionidae: Molytinae). *Journal of

351   Insect Conservation* 17:737-745.

352   Frankham R, Ballou JD, and Briscoe DA. 2002. *Introduction to conservation genetics*. Cambridge:

353   Cambridge University Press.

354   Goldberg J, and Trewick SA. 2011. Exploring Phylogeographic Congruence in a Continental Island

355   System. *Insects* 2:369-399.

356   Grueber CE, Wallis GP, and Jamieson IG. 2008. Heterozygosity–fitness correlations and their

357   relevance to studies on inbreeding depression in threatened species. *Molecular Ecology*

358   17:3978-3984.

359   Heenan PB, Mitchell AD, de Lange PJ, Keeling J, and Paterson AM. 2010. Late-Cenozoic origin and

360   diversification of Chatham Islands endemic plant species revealed by analyses of DNA

361   sequence data. *New Zealand Journal of Botany* 48:83-136.

362   Hitchmough R, Bull L, Cromarty P, and (compilers). 2007. New Zealand threat classification system

363   lists. *Threatened Species Occasional Publication, Department of Conservation, Wellington*.

364   Ho SYM. 2007. Calibrating molecular estimates of substitution rates and divergence times in birds.

365   *Journal of Avian Biology* 38:409-414.

366   Huson DH, and Bryant D. 2006. Application of phylogenetic networks in evolutionary studies.

367   *Molecular Biology and Evolution* 23:254-267.

368   Hutton I, Parkes JP, and Sinclair ARE. 2007. Reassembling island ecosystems: the case of Lord Howe

369   Island. *Animal Conservation* 10:22-29.

370   Jamieson IG, Wallis GP, and Briskie JV. 2006. Inbreeding and Endangered Species Management: Is

371   New Zealand Out of Step with the Rest of the World? *Conservation Biology* 20:38-47.

372   Johst K, and Schöps K. 2003. Persistence and conservation of a consumer–resource metapopulation

373   with local overexploitation of resources. *Biological Conservation* 109:57-65.

374   Kean JM. 2006. Testing models for equilibrium distribution and abundance of insects. *New Zealand*

375   *Journal of Ecology* 30:53-60.

376   Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through

377   comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.

378   Kimura M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences.

379   *Proceedings of the National Academy of Science* 78:454-458.

380   Lanfear R, Calcott B, Ho SYW, and Guindon S. 2012. PartitionFinder: Combined Selection of

381   Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology*

382   *and Evolution*.

383   Lawton JH. 1997. The Science and Non-Science of Conservation Biology. *Oikos* 79:3-5.

384   Moriyama E, and Powell J. 1997. Synonymous substitution rates in *Drosophila*: Mitochondrial versus

385   nuclear genes. *Journal of Molecular Evolution* 45:378-391.

386   Papadopoulou A, Anastasiou I, and Vogler AP. 2010. Revisiting the Insect Mitochondrial Molecular

387   Clock: The Mid-Aegean Trench Calibration. *Molecular Biology and Evolution* 27:1659-1672.

388   Pertoldi C, Bijlsma R, and Loeschcke V. 2007. Conservation genetics in a globally changing

389   environment: present problems, paradoxes and future challenges. *Biodiversity and*

390   *Conservation* 16:4147-4163.

391   R Development Core Team. 2011. R: A Language and Environment for Statistical Computing. Vienna,

392   Austria.

393   Ritchie IM. 1970. A preliminary report on a recent botanical survey of the Chatham Islands.

394   *Proceedings of the New Zealand Ecological Society* 17:52-56.

395   Rosenberg M, and Anderson C. 2011. PASSaGE: Pattern analysis, spatial statistics and geographic

396   exegesis. Version 2. *Methods in Ecology and Evolution* 2:229-232.

397   Schöps K. 1998. Metapopulation dynamics and behaviour of the endangered weevil, *Hadramphus*

398   *spinipennis* in relation to its host plant *Aciphylla dieffenbachii* on the Chatham Islands, New

399   Zealand Doctor of Philosophy. Lincoln University.

400   Schöps K. 2000. Metapopulation dynamics of the coxella weevil. *Science for Conservation* 134:1-37.

401   Schöps K. 2002. Local and regional dynamics of a specialist herbivore: overexploitation of a patchily

402   distributed host plant. *Oecologia* 132:256-263.

403   Schöps K, Wratten SD, and Emberson RM. 1999. Life cycle, behaviour and conservation of the large

404   endemic weevil, *Hadramphus spinipennis* on the Chatham Islands, New Zealand. *New Zealand*

405   *Journal of Zoology* 26:55-66.

406   Selkoe KA, and Toonen RJ. 2006. Microsatellites for ecologists: a practical guide to using and

407   evaluating microsatellite markers. *Ecology Letters* 9:615-629.

408   Sequeira AS, Stepien CC, Sijapati M, and Roque Albelo L. 2012. Comparative Genetic Structure and

409   Demographic History in Endemic Galápagos Weevils. *Journal of Heredity* 103:206-220.

410   Simberloff D. 1990. Reconstructing the ambiguous: Can island ecosystems be restored? In: Towns DR,

411   Daugherty CH, and Atkinson IAE, eds. *Ecological restoration of New Zealand islands*.

412   Wellington: Department of Conservation, 37-51.

413   Simon C, Frati F, Beckenbach A, Crespi B, Liu H, and Flook P. 1994. Evolution, weighting, and

414   phylogenetic utility of mitochondrial gene sequences and a compilation of conserved

415   polymerase chain reaction primers. *Annals of the Entomological Society of America* 87:651-

416   701.

417   Sinclair ARE, and Byrom AE. 2006. Understanding ecosystem dynamics for conservation of biota.

418   *Journal of Animal Ecology* 75:64-79.

419   Stroscio S, Baviera C, Frati F, Lo Paro G, and Nardi F. 2011. Colonization of the Aeolian Islands by

420   *Pimelia rugulosa rugulosa* Germar, 1824 (Coleoptera: Tenebrionidae) inferred from the genetic

421   structure of populations: geological and environmental relations. *Biological Journal of the*

422   *Linnean Society* 104:29-37.

423   Tamura K, Peterson D, Peterson N, Stecher G, Nei M, and Kumar S. 2011. MEGA5: Molecular

424   Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and

425   maximum parsimony methods. *Molecular Biology and Evolution* 28:2731-2739.

426   Taylor AD. 1990. Metapopulations, dispersal, and predator-prey dynamics: an overview. *Ecology*

427   71:429-433.

428   Towns D. 2009. Eradications as reverse invasions: lessons from Pacific rat (*Rattus exulans*) removals

429   on New Zealand islands. *Biological Invasions* 11:1719-1733.

430   Towns DR, Bellingham PJ, Mulder CPH, and Lyver POB. 2012. A research strategy for biodiversity

431   conservation on New Zealand's offshore islands. *New Zealand Journal of Ecology* 36:1-20.

432   Zharkikh A. 1994. Estimation of evolutionary distances between nucleotide sequences. *Journal of*

433   *Molecular Evolution* 39:315-329.

ITS2 primers

Primers developed to amplify a region of the ITS2 gene in *H. spinipennis.*

Primer Name	Primer Sequence 5' to 3'
Had ITS2 For	ATT CTG TTC CCG GAC CAC TCC TGG CTG A
Had ITS2 Rev	GCG CGC ACC GTT ACR ATC KGA CGY C

Divergence times for the mitochondrial maximum clade credibility tree

Mean divergence times (Ma) and 95% confidence interval for the two different dating schemes used to date nodes in the mitochondrial maximum clade credibility tree. Dates were estimated in BEAST using a strict molecular clock with fixed mean rate of 0.0115 substitutions/site/my, and a strict molecular clock with a 3 million year age constraint on the tree root.

	Divergence Times (Ma)	
	Fixed mean rate	Geological date
Mean	0.0013	0.0006
95% confidence interval	0.0000-0.0139	0.0001-0.0016

Aciphylla dieffenbachii populations on Rangatira

Aciphylla dieffenbachii populations found on Rangatira including estimated population size, approximate size of the plants found in the population and whether the plants were in flower.

Population	A	B	C
Number of plants	10	3 to 6[a]	2 to 7 and 55[a]
Plant size	medium	Small / medium	Small to large / seedlings
Flowering	No	No	Yes[b]

[a]Population B had nine groups of plants spread across a cliff face; each group consisted of 3 to 6 plants. Population C had five groups of plants patchily distributed with 2 to 7 plants in each group. A total of 55 seedlings were spread throughout the area. [b]Only two female plants were flowering.

Aciphylla dieffenbachii populations on Mangere

Aciphylla dieffenbachii populations found on Mangere including estimated population size, approximate size of the plants found in the population and whether the plants were in flower.

Population	1	2	3	4	5	6	7
Number of plants	410	100	180	330	20	150	17
Plant Size	Small / medium	Small to large	Small to large	Small / medium	Small	Small / medium	Small
Flowering	Yes	Yes	Yes	Yes	Yes	Yes	No

1

Map of Chatham Islands

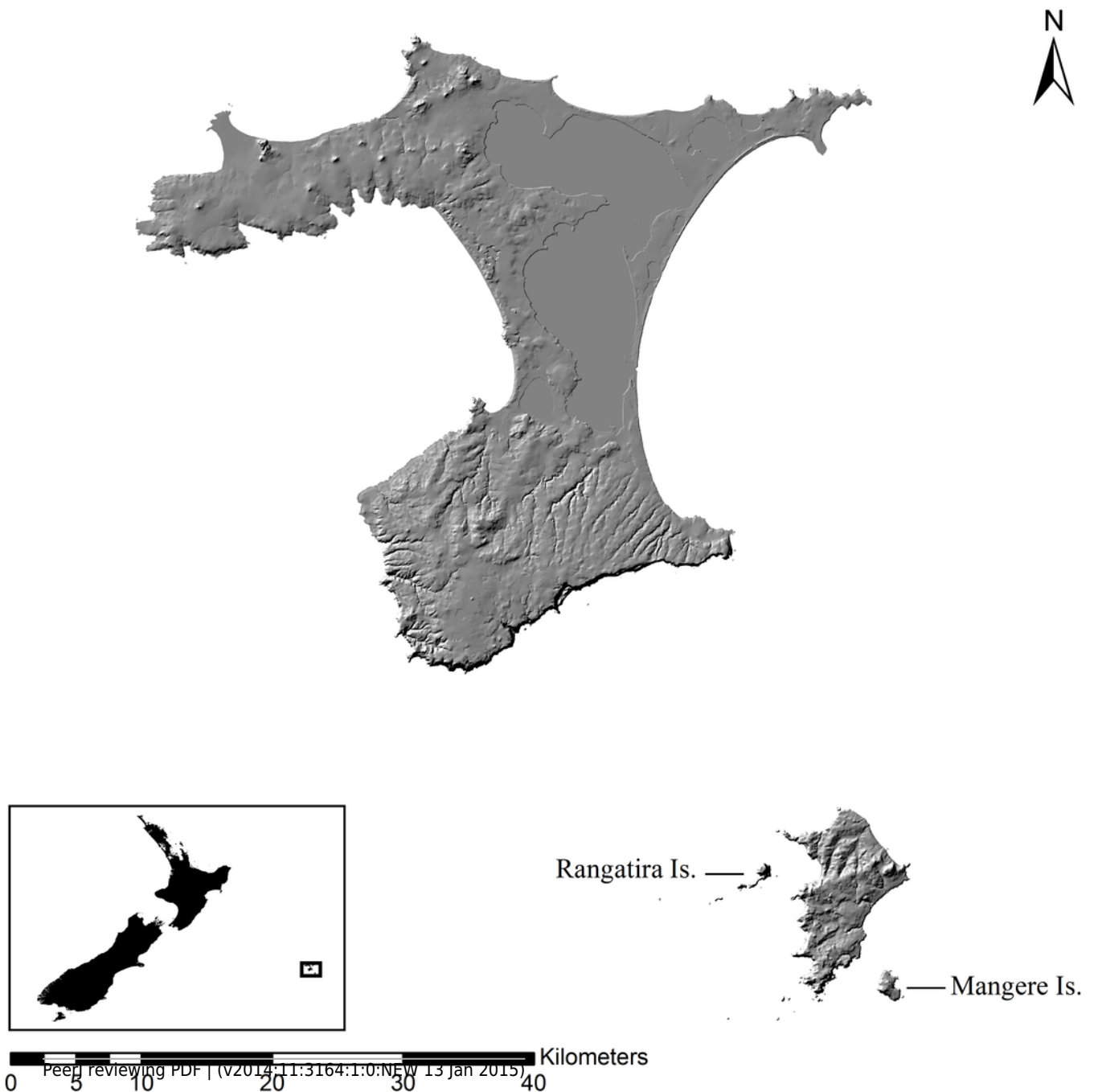
Map (1:500,000 scale) of the Chatham Islands with Rangatira (South East) and Mangere Islands labeled. Insert map shows the location of the Chatham Islands in relation to New Zealand.

N

Rangatira Is. ——

Mangere Is.

Kilometers

0   5   10   20   30   40

2

Weevil marginal feeding pattern

A photograph depicting the weevil feeding pattern, which is identified by marginal notches.

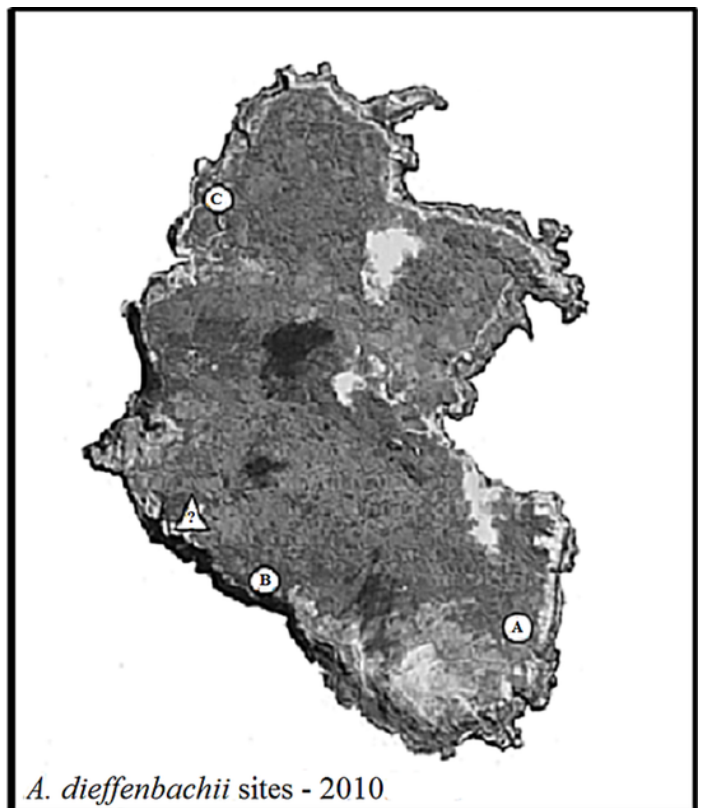
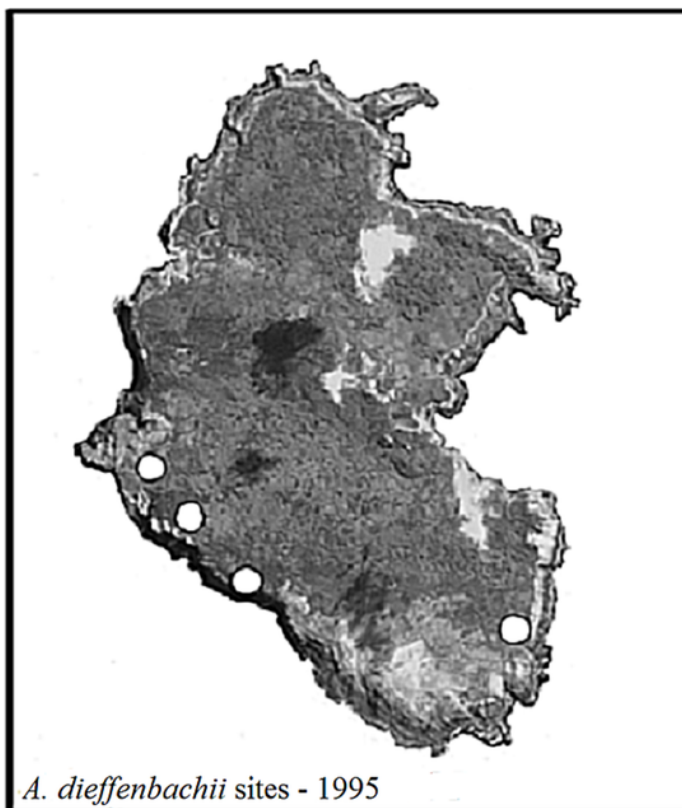
Photograph by: Jagoba Malumbres-Olarte.

3

Maps of Rangatira survey sites

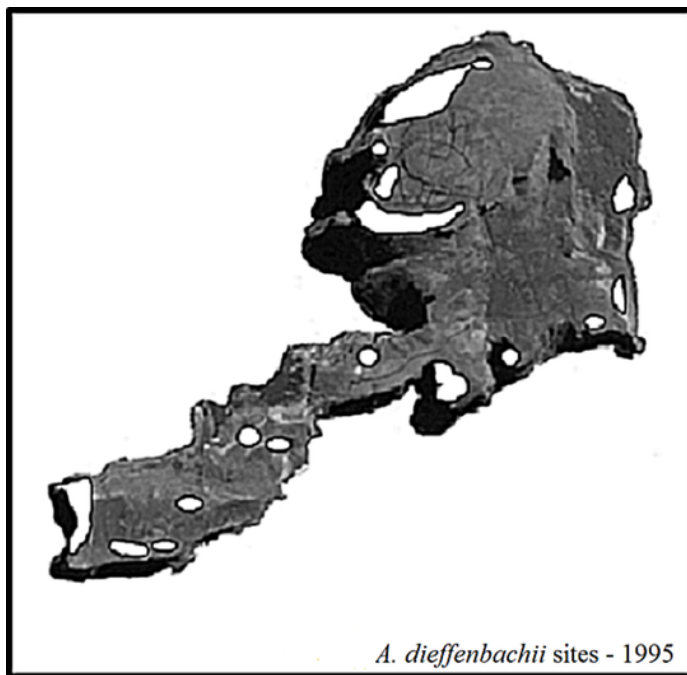
Rangatira Island with the populations of *A. dieffenbachii* that were recorded in the 1995 survey by Schöps (2000) (white circles) and the *A. dieffenbachii* populations found in the 2010 survey (white circles with letters). Letters were assigned to each site as they were found. The triangle with a question mark on the 2010 map represents the one area that could not be surveyed due to adverse weather conditions. Map image was obtained from Google Earth 2013 image and the same image was used for both maps.

A. dieffenbachii sites - 1995

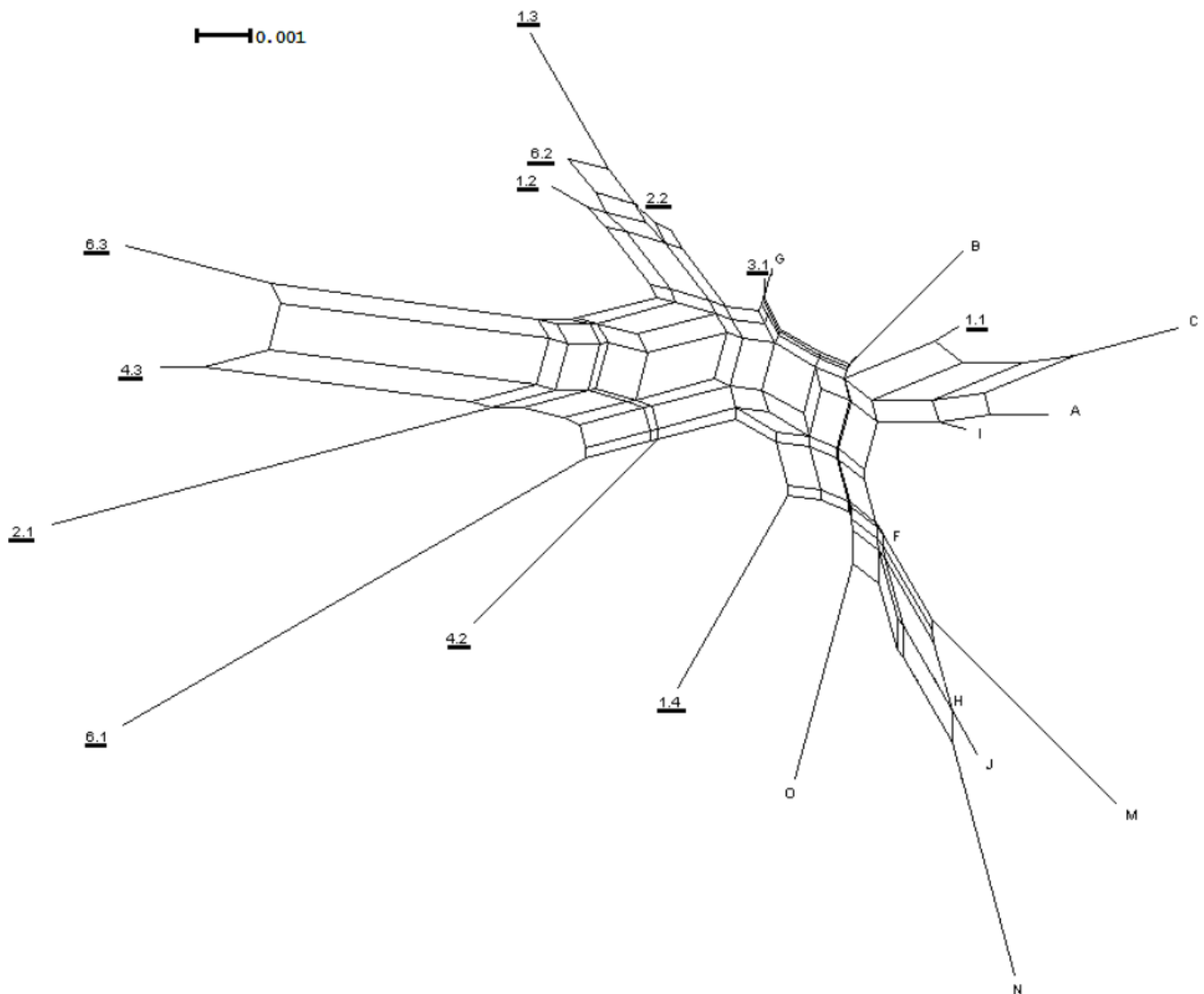
A. dieffenbachii sites - 2010

4

Map of Mangere survey sites

Mangere Island with the *A. dieffenbachii* sites that were recorded in the 1995 survey by Schöps (2000) (white areas) and the *A. dieffenbachii* populations found in the 2011 survey (white areas with numbers representing the larger patches of *A. dieffenbachii*). Numbers were assigned to each site as they were found. The entire island was surveyed visually for *A. dieffenbachii*, but some plant patches could not be reached for hand-searching for weevils. Map image was obtained from Google Earth 2013 image and the same image was used for both maps.

A. dieffenbachii sites - 1995   *A. dieffenbachii* sites - 2011

5

ITS2 Neighbor-Net network

Neighbor-Net network generated from ITS2 distances. The underlined numbers are individuals from Mangere and the letters are individuals from Rangatira.

6

Neighbor joining trees for mitochondrial genes and ITS2

(A) Neighbor joining (NJ) tree for the concatenated mitochondrial genes COI and cytb. (B)
Neighbor joining tree for ITS2. For both trees, individuals from Mangere are underlined (first
number of weevil ID is subpopulation and the second number is ID to denote different
weevils). The maximum clade credibility tree posterior probabilities higher than 95% are
labeled below the branch node and the bootstrap value for the NJ tree are above the branch
node. The root that was estimated in Beast for the maximum clade credibility tree and it is
represented by the grey root line. The scale bar is in substitutions/site.