

1           **Relative roles of genetic variation and phenotypic plasticity in the invasion of**  
2                           **monkeyflower *Erythranthe gutatta* in New Zealand**

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5 Michelle Williamson<sup>1</sup>, Daniel Gerhard<sup>2</sup>, Philip Hulme<sup>3,4</sup>, Hazel Chapman<sup>5\*</sup>

6  
7 <sup>1</sup> Institute of Environmental Science and Research ESR Christchurch, Canterbury, New  
8 Zealand

9 <sup>2</sup> School of Maths and Stats, University of Canterbury,,PB 4800 Christchurch, New Zealand

10 <sup>3</sup> Department of Pest Management and Conservation, Lincoln University, P.O. Box 85084,  
11 Lincoln 7647, New Zealand

12 <sup>4</sup> Bioprotection Aotearoa, Lincoln University, P.O. Box 85084, Lincoln 7647, New Zealand  
13 ORCID 0000-0001-5712-0474

14 <sup>5</sup> School of Biological Sciences, University of Canterbury, PB 4800, Christchurch New  
15 Zealand ORCID 0000-0001-8509-703X

16 \* [hazel.chapman@canterbury.ac.nz](mailto:hazel.chapman@canterbury.ac.nz)

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19 **Abstract**

20 Evolutionary processes which increase the probability of an introduced plant species  
21 becoming invasive include high levels of genetic diversity and phenotypic plasticity.  
22 Naturalised in New Zealand, monkeyflower, (*Erythranthe gutatta*), a clonally spreading  
23 herb of waterways and seepage areas native to the Western USA, shows marked variation in a  
24 range of vegetative, reproductive and inflorescence traits. We used two common gardens  
25 differing in elevation to explore the relative contribution of genetic versus plastic variation  
26 within nine traits among 34 monkeyflower clones from across the New Zealand South Island.  
27 We looked for evidence of clinal variation across elevation gradients and for home site  
28 advantage. We found both high genetic diversity and trait plasticity explain the observed  
29 variation, although less evidence for adaptive plasticity. Most genetic variation was observed  
30 in the lowland garden (9m a.s.l.), where the overall trend was for above ground dry weight to

31 be lower, and horizontal shoot length greater, than at the montane garden (560m a.s.l). We  
32 found no evidence of local adaptation to any of the measured environmental variables.  
33 However, we observed a pattern of higher biomass and higher plasticity at lower versus  
34 higher elevations and in clones originating from lower elevation sites.

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37 **Key Words** Phenotypic plasticity, common garden, elevational gradient, *Mimulus guttatus*,  
38 rapid evolution

39

#### 40 **Acknowledgments**

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44 work support.

45 INTRODUCTION

46 Invasive alien plants often show considerable phenotypic variation in their introduced range,  
47 explained by local adaptation, phenotypic plasticity (whereby plants are able to adjust their  
48 phenotype according to the local environment (Pigliucci et al. 1995; Geng et al. 2007;  
49 Pitchancourt and Van Klinken 2012) or a combination of both (Moroney et al. 2013; Si et al.  
50 2014). However, despite a plethora of studies ( Geng et al. 2016; Hiatt and Flory, 2020;  
51 Fakhr et al. 2022;), the relative contribution of genes and the environment to such variation  
52 remains poorly understood (Vanwallendael et al., 2018; Villellas et al. 2021; Yuan et al.  
53 2022). While the success of clonally reproducing invasive species is often attributed to  
54 plasticity (Loomis and Fishman 2009; Riis et al. 2010; Keser et al. 2014), quantitative links  
55 between plasticity and fitness are rare (Liu et al. 2016; Bufford and Hulme 2021a). Plastic  
56 traits can be neutral or even mal-adaptive, a consequence of genetic correlation or trade-offs  
57 (van Kleunen and Fischer 2004) and so evidence of plasticity does not necessarily equate to  
58 increased fitness. Of course, adaptation can play an important role in invasion success, even  
59 in clonal plants (Geng et al. 2016).

60 Given that understanding why some species become invasive but others don't is one of the  
61 main focal areas of invasion biology (Moloney et al. 2009) this facet of invasion success  
62 warrants further investigation (Ghalambor et al. 2007; Geng et al. 2016; Lee-Yaw et al. 2019;  
63 Enders et al. 2020). It is especially relevant for clonal ornamental plants given their under-  
64 representation in the literature and the predicted global increase in garden escapes with  
65 climate change (van Kleunen et al. 2018; Hulme 2020). Many ornamentals reproduce easily,  
66 often with a strong clonal component to reproduction and are predicted to be the major pool  
67 of future invasive species worldwide (van Kleunen et al. 2018).

68 Local adaptation allows for invasive success (Lee et al. 2002; Leimu and Fischer 2008;  
69 Colautti and Lau 2015 ; Molina-Montenegro et al. 2018; Lee-Yaw et al. 2019) by conferring  
70 genotypes with a 'home site advantage' (Kawecki and Ebert 2004; Maron et al. 2004; Allan

71 and Pannell 2011; Bennington et al. 2012), whereby plants grown in their home environment  
72 are more fit than plants from elsewhere (but see Lee-Yaw et al. 2019 ). In contrast  
73 phenotypic plasticity allows the same genotype to thrive in a range of environments  
74 (Bossdorf et al. 2005; Richards et al. 2006; Hulme 2007; Moloney et al. 2009, ; Bufford and  
75 Hulme 2021a; but see Palacio-Lopez and Gianoli 2011). As with local adaptation, plasticity  
76 may evolve post introduction (Lande 2015). Demonstrating the relative contribution of each  
77 evolutionary strategy is difficult for a number of reasons (Noble et al. 2019; Vilellas et al.  
78 2021) and findings have to date been equivocal (Bossdorf et al. 2005; Richards 2006; Hulme  
79 2007; Bock et al. 2015).

80 One approach to untangle the different components (genetic versus plastic) of intra- specific  
81 phenotypic variation is to use common garden experiments, where clones from a number of  
82 environmentally different populations are grown together under as near -identical conditions  
83 as possible (Allan and Pennell 2009; Cheplick 2015; Vilellas et al. 2021). If, under the  
84 same environmental conditions clones maintain their home site phenotypes, this variation is  
85 deemed genetic (Turesson 1922; Nunez-Farfan and Schlichting 2001). In contrast, if under  
86 the same conditions the clones from different locations exhibit similar phenotypes the  
87 variation is considered plastic. Comparing trait values within and among clones, analysis of  
88 variance allocates the proportion of variance within clones into genetic and plastic  
89 components. In invasion ecology common garden experiments have been used to compare  
90 provenances from native and introduced ranges in the introduced range (Bufford and Hulme  
91 2021a; b; Colautti and Barrett 2009 and references within ), alien provenances from just the  
92 introduced range (Kollmann and Baneulos 2004; Rapson and Willson 1992; Colautti and  
93 Barrett 2013; Monty and Mahy 2009) and provenances in both the native and introduced  
94 range (Maron et al. 2004; Flory et al. 2011; Shelby et al. 2016; Berend et al. 2019; Oduor et  
95 al. 2016 and references therein). However, often such experiments have been compromised  
96 by their design. For example, too few gardens or gardens with very similar environments

97 (Moloney et al. 2009; Villelas et al. 2021). Moreover, well designed experiments have  
98 shown that contradictory results in different gardens are to be expected and help to untangle  
99 genetic versus environmental influences (Maron et al. 2004; Williams et al. 2008; Moloney et  
100 al. 2009; Florey 2011; Villelas et al. 2021).

101 We investigate the relative contribution of genotype versus plasticity to the widespread  
102 success in New Zealand of the introduced *Erythranthe gutatta* (D.C.) G. L. Nelson,  
103 (section *Simiolus*, Phrymaceae), previously known as *Mimulus gutattus* D.C. Lowry et al.  
104 (2019), and commonly referred to as monkeyflower. Due to its wide range of life histories  
105 and environmental tolerance, and its propensity for whole genome duplication, *E. gutatta* has  
106 become a model species in evolutionary ecology and invasion biology (Wu et al. 2008;  
107 Friedman et al. 2015; Da Re et al. 2020). *E. gutatta* is a widespread, common and rapidly  
108 spreading clonally reproducing weed in New Zealand and is of considerable interest because  
109 it shows a marked niche-shift, occupying habitats in New Zealand which lie outside of the  
110 environmental conditions found in its native range (Da Re et al. 2020). One explanation for  
111 this lack of niche conservatism is that different genotypes (different source populations)  
112 respond differently to different environments. Alternatively, the niche-shift may reflect post-  
113 introduction evolution, either of plasticity or by local adaptation (Da Re et al. 2020). While  
114 New Zealand *E. gutatta* populations undoubtedly represent bottlenecked populations and,  
115 according to (Vallejo-Marin et al. (2021) historical, rather than recent introductions, they do  
116 include both native US and bridgehead UK populations, which suggests the potential for  
117 novel genetic combinations in New Zealand. How likely this is remains speculative given the  
118 primarily clonal nature of *E. gutatta* spread.

119 We undertook a set of common garden experiments to distinguish the extent to which the  
120 considerably phenotypic variation observed in *E. gutatta* in New Zealand reflects plasticity  
121 versus contemporary evolution leading to local adaptation. The selection pressure we  
122 considered most likely to impact plants from across our sampled sites was elevation,

123 incorporating a steep cline in temperature and rainfall. We thus chose our two common  
124 gardens based on elevation; Ilam (hereafter referred to as the lowland site) and Cass  
125 (hereafter referred to as the montane site), at 9 m and 580 m respectively, to expose any GxE  
126 effect (different genotypes responding differently to the two environments). Given that our  
127 sites spanned the entire length of the South Island, we also looked for evidence of a  
128 latitudinal cline in biomass. Our specific hypotheses were that 1) we would detect clinal  
129 variation associated with elevation, so that clones from montane populations would be fittest  
130 in the montane garden and vice versa. 2) We would detect home site advantage, such that the  
131 Cass clone grown in the Cass garden would be the fittest clone in the montane garden and  
132 similarly, clones from close to the lowland Ilam garden would be the fittest clones in the  
133 lowland garden.

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135

## 136 **Methods**

### 137 **Study species**

138 *Erythranthe gutatta* (D.C.) G. L. Nelson, is the most common species in a large *Mimulus*  
139 species complex (Ritland and Ritland 1989). Widespread across the Western US (Friedman et  
140 al. 2015; Demarche et al. 2016), *E. gutatta* has become naturalised across Europe and in New  
141 Zealand. First recorded in New Zealand in 1878 (Webb et al. 1988), Vallejo-Marin et al.  
142 (2021) found that New Zealand populations have been introduced multiple times both  
143 directly from native Alaska and by way of a bridgehead from the UK.

144

### 145 **Experimental design**

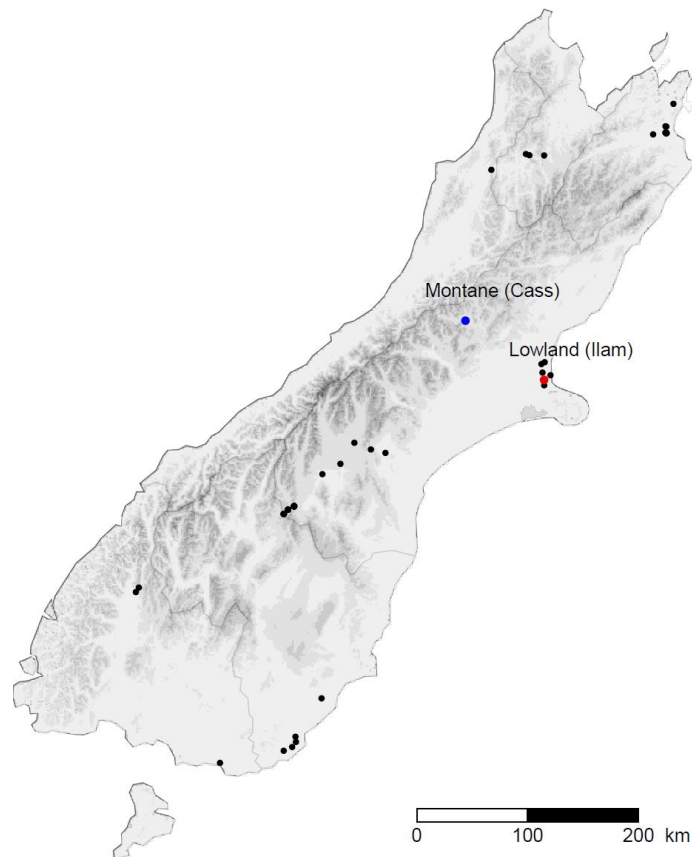
146 We sampled a single clone from each of 34 distinct locations across the South Island of New  
147 Zealand. Locations were never less than 10 km apart and were chosen to be representative of

148 environmental and altitudinal variation across the South Island and included seven  
149 geographically isolated regions (Fig 1).

150

151 **Fig 1** Sample locations of *E. gutatta* across the New Zealand South Island  
152 (<https://www.stats.govt.nz>). The sites are clustered according to geographical region.

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162 At each sampling site we recorded 16 environmental variables and calculated the annual

163 average temperature, growing season average temperature, average minimum and maximum

164 temperature, extreme minimum and maximum temperature, annual average rainfall, humidity

165 and precipitation from data collected from local climate stations between 1971 and 2000 (Table  
 166 S 1). Populations were mostly small patches along stream sides or drainage ditches and we  
 167 collected stems of *E. gutatta* with basal runners from a single clone from each location. From  
 168 each clone we propagated twelve replicates from small internode cuttings. To reduce clonal  
 169 environmental effects as much as possible, cuttings were <2cm long and were in similar  
 170 developmental and physiological condition (Libby 1962; Weiner et al. 1997). Cuttings were  
 171 planted in separate 10 X 10 X 10 cm<sup>3</sup> pots filled with potting mix. Pots were placed on tables  
 172 in a glasshouse, with their distribution on the tables randomized weekly. Early in the 2018  
 173 growing season cuttings were translocated into individual pots (7.5L, 20.5 cm wide x 25.5 cm  
 174 diameter), lined with plastic bags and filled with a slow release fertiliser potting mix. The pots  
 175 were moved into the two common gardens. Half (six replicates per clone) went into the  
 176 lowland garden on the University of Canterbury Ilam campus (43°31'S 172°35'E and the  
 177 remainder were set up at a montane garden located at the University of Canterbury Cass field  
 178 station (43°02'S, 171°45'E). The montane garden was significantly cooler and wetter than the  
 179 lowland garden (Table 1).

180 **Table 1** Environmental measures of Ilam, Christchurch and the Cass Field Station. Averages 1971-  
 181 2000

Location	Elevation (m)	Annual Average Temperature (°C)	Average Temperature (°C)	Average Min Temperature(°C)	Extreme Min Temperature(°C)	Average Max Temperature(°C)	Extreme Max Temperature(°C)	Annual Average Rainfall (mm)	Humidity (%)	Precipitation (mm)	Max Sunlight Hours	Min Sunlight Hours
Ilam, Christchurch	9.69	12.29	14.7	9.63	0.62	19.76	32.5	131.3	75.3	10.10	15.25	8.27
Cass Field Station	578.81	8.22	10.72	4.17	-5.3	17.27	30.06	2289.6	84	192.6	15.22	9

182



183 Plants were randomly assigned positions in each common garden and their distributions  
184 randomized weekly. *E. gutatta* occurs primarily in wetlands and riparian areas and thus plants  
185 were kept moist with daily automatic watering to limit any effect of drought or precipitation  
186 on performance.

187

### 188 **Performance traits**

189 We chose nine quantitative traits which have previously been shown to exhibit marked inter-  
190 population variation in *E. gutatta* (eg Hall 2006; van Kleunen and Fischer 2008). We counted  
191 the maximum number of buds on each plant. In mid-April, towards the end of growing season,  
192 we measured the vegetative, reproductive and flower traits in both gardens: mean length and  
193 width of the largest two leaves, length of the longest horizontal shoot and length between the  
194 second and third internodes, the largest flower length, depth and width. All plants were then  
195 harvested towards the very end of the growing season in mid-April and dry weight of above  
196 ground tissue was measured after drying at 80°C for 72 h. We chose above ground dry weight  
197 as our proxy for fitness.

198

### 199 **Statistical analysis**

200 To estimate the total genetic and environmental components of the variation in vegetative,  
201 reproductive and flower traits among clones, and their genotype x environment (GxE)  
202 interactions, we used linear mixed-effects models. In the models, each of the quantitative traits  
203 were analysed separately, using a logarithmic transformation on each observation after visually  
204 inspecting model diagnostics. For the GxE investigation for each response, the expected  
205 population effect of the two common garden locations was estimated together with two  
206 variance components, quantifying the site-specific variability in the montane garden and the  
207 site-specific variability of the garden location effect (ie lowland – montane). This last term in  
208 the model allows us to predict the relative garden performance for each clone compared to the

209 population average. Posterior variance estimates were used to construct 95% confidence  
210 intervals for individual random effect predictions. We plotted reaction norms (Schlichting and  
211 Pigliucci 1998) of each clone for each of the nine traits to illustrate how the phenotypes of the  
212 different genotypes expressed in each of the two gardens.

213 To visualise the trait differences among the 34 clones and to observe how the same clonal  
214 replicates responded in each of the lowland and montane common gardens, we ran a principal  
215 component analysis (PCA). Based on the covariance between traits, the scaled and centred  
216 clone predictions were summarised in a biplot of the first two principal components. We treated  
217 all predictions as new observations, ignoring any further estimation uncertainty. To test for  
218 association between all measured traits and elevation we plotted principal component scores  
219 vs elevation. We plotted principal component scores vs all environmental variables to test for  
220 association between all measured traits and all environmental site environmental variables  
221 (Table S1). All statistical analyses were performed using the open-source program R, version  
222 4.0.5 (R Core Team 2018) and package lme4 (Bates et al. 2015).

223

## 224 **RESULTS**

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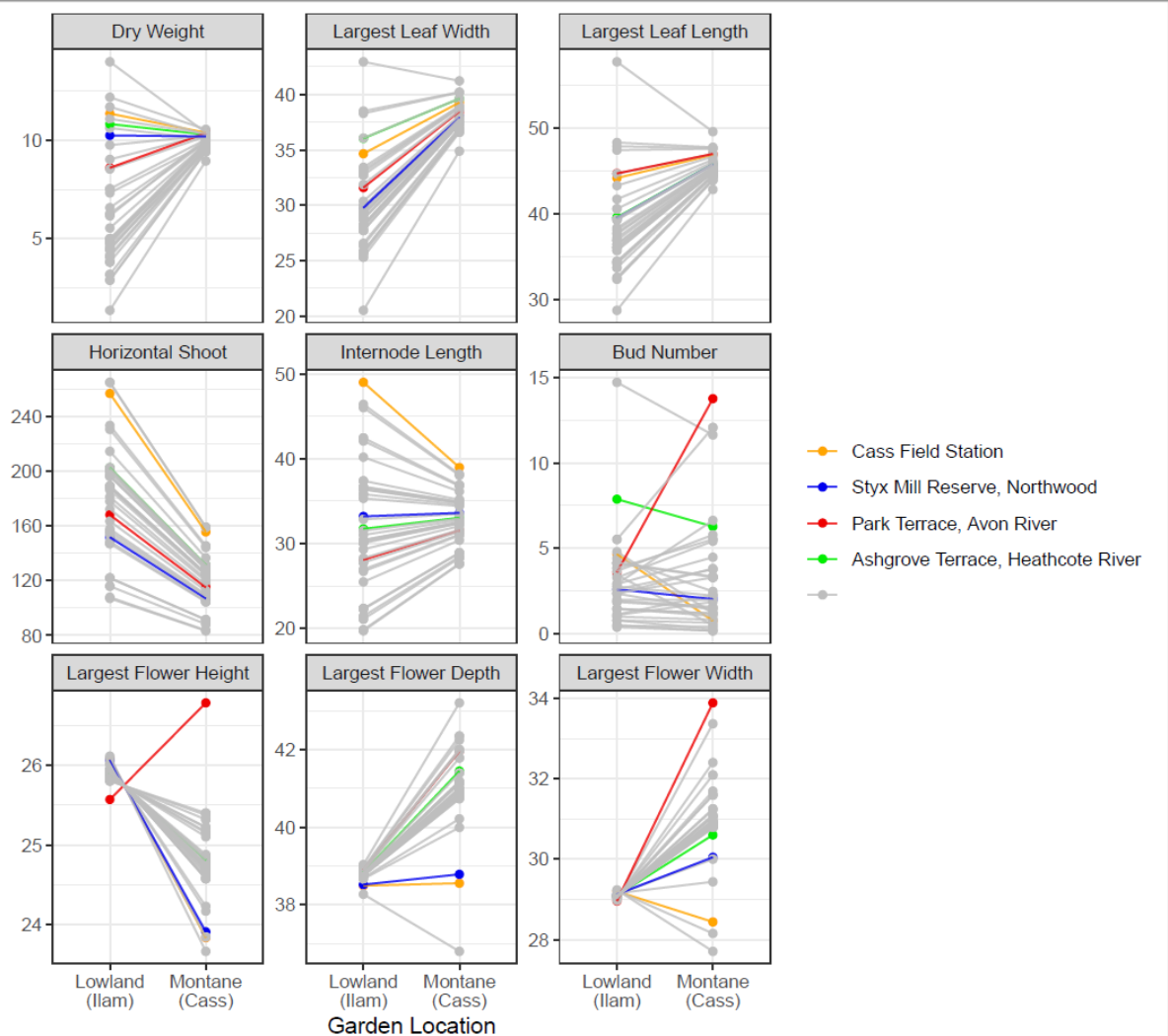
### 226 *Phenotypic variation*

227 All plants survived in both common gardens and we found considerable variation in all  
228 measured traits across the two gardens. In the lowland garden the mean dry weight of the largest  
229 clone was almost 20 times that of the smallest, with measures of leaf size varying by more than  
230 a twofold difference. As result, there were clear morphotypes; while the majority of plants were  
231 somewhat intermediate, individual clones ranged from short, horizontally spreading plants to  
232 tall, multi-stemmed and multi flowered individuals. In contrast in the montane garden,  
233 vegetative traits among clones were markedly less variable and it was more difficult to  
234 recognise morphotypes among the clones. However, in the montane garden flower size varied  
235 among clones more than in the lowland garden (Fig. 2). The reaction norms of all clones,  
10

236 except from Park Terrace, Christchurch, responded in a similar way to the environments in the  
237 lowland and montane common gardens (Fig. 2).

238 The first two axes of the PCA together explained 66% of the total variation (Fig 3). Axis 1  
239 (38%) correlated most strongly with the following vegetative traits: above ground dry weight,  
240 leaf size (length and width), internode length and stolon length. The second axis (28%)  
241 correlated most strongly with florescence traits such as flower width and depth. Most variation  
242 among clones in the lowland garden was along axis 1, and in the montane garden along axis 2.

243 **Fig 2** Reaction norms of single genotypes - predictions of expected trait variables by clone.  
244 Lines connect the predictions for the same genotype at the two different gardens. All  
245 predictions are presented on the original scale of each measurement.

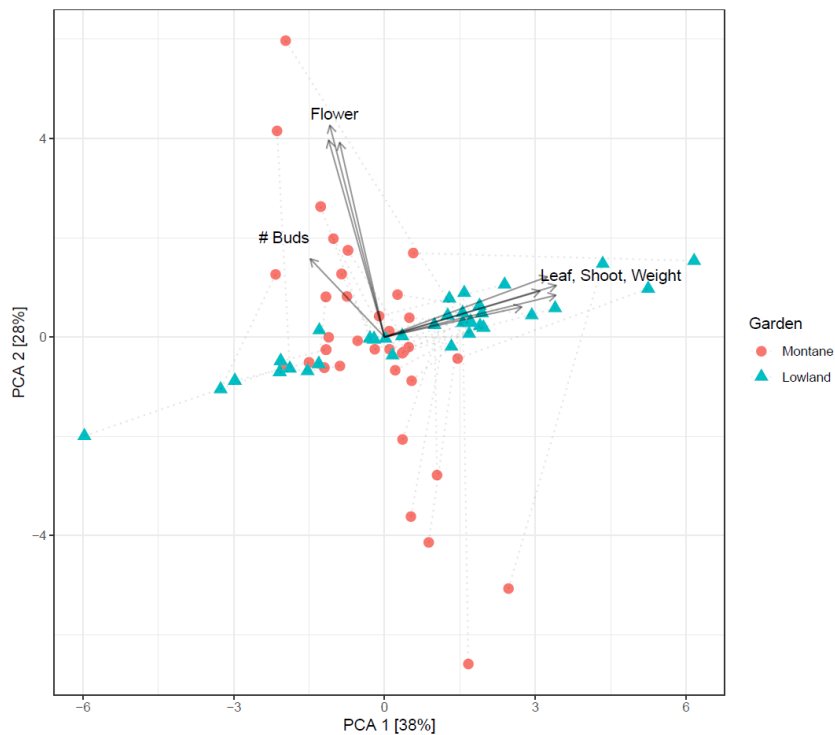


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249 **Fig 3** Biplot of the first two principal components, summarising all traits (arrows indicating the rotated  
250 coordinate system) for each population-specific prediction. The (0,0) coordinate represents the  
251 population average for all traits



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255 All clones show plasticity with variance-changing  $G \times E$ , and we found marked variation  
256 among clones in the degree of plasticity exhibited. For example, two clones from lowland  
257 populations in Christchurch showed marked differences in their reaction norms for plant dry  
258 weight: the Styx Mill clone showed no shift between elevations while the Park Terrace clone  
259 a marked decline in the montane garden (Figure 2). The direction of plasticity also differed  
260 among clones, for example despite being collected in the same region and elevation the Styx  
261 Mill clone showed a marked decline flower length with elevation while the Park Terrace clone  
262 a marked increase in the montane garden. However, while plasticity is common among clones,  
263 the rank order of clones remained the same for most traits, except for, the number of buds and  
264 flower length where the reaction norm of the Park Terrace was inconsistent with the remaining  
265 clones.

266 Evidence for home site advantage would be apparent if the clone from Cass outperformed all  
267 other clones in the montane garden and performed less well relative to lowland clones in the  
268 lowland garden. This was generally not the case, within the montane garden the Cass clone is  
269 within the top few clones in terms of vegetative traits but is outperformed by several lowland  
270 clones. It maintains its relatively high ranking in many traits in the lowland garden. Similarly,  
12

271 there was no evidence at all for the lowland Christchurch sites in the lowland garden  
272 outperforming clones from other locales (Fig. 2).

273

274 *Genetic versus plastic components to variation*

275 The GLMM analysis to determine total genetic, environmental (residual) and GxE components  
276 of the variation in vegetative and reproductive traits among clones revealed that in the lowland  
277 garden clone (genotype) explained 33%-45% of the variance in vegetative traits and almost  
278 none of the variation in inflorescence traits (Table 2). In contrast in the montane garden it was  
279 the inflorescence traits (corolla depth and width and bud number) and stolon length which  
280 showed most genetic variability among clones.

281

282 **Table 2** Estimates for site-specific variances by garden location and residual variances together with  
283 proportions of explained variance by population [95% confidence intervals]. All estimates are based on  
284 logarithmic transformed scales. The total variance among the 34 clones in the Lowland and  
285 Montane garden is presented for each measured trait, The residual is the total variance in each  
286 measured trait among the 34 clones not explained by garden. The GxE component for each  
287 garden is the percentage of variance for each measured trait within each garden

Trait	$\sigma^2$ Montane	$\sigma^2$ Lowland	$\sigma^2$ Residual	GxE Montane	GxE Lowland
Dry Weight	0.01	0.80	0.99	0.01 [0.00; 0.16]	0.45 [0.30; 0.58]
Flower Length	0.01	0.00	0.05	0.10 [0.00; 0.45]	0.00 [0.00; 0.29]
Flower Depth	0.01	0.00	0.02	0.21 [0.00; 0.55]	0.00 [0.00; 0.28]
Flower Width	0.01	0.00	0.04	0.19 [0.00; 0.53]	0.00 [0.00; 0.28]
Leaf Width	0.00	0.06	0.08	0.04 [0.00; 0.20]	0.44 [0.28; 0.57]
Leaf Length	0.00	0.05	0.07	0.03 [0.00; 0.15]	0.41 [0.25; 0.54]
Horizontal Shoot	0.06	0.13	0.24	0.21 [0.06; 0.40]	0.34 [0.20; 0.48]
Internode Length	0.02	0.13	0.28	0.07 [0.00; 0.23]	0.33 [0.18; 0.46]

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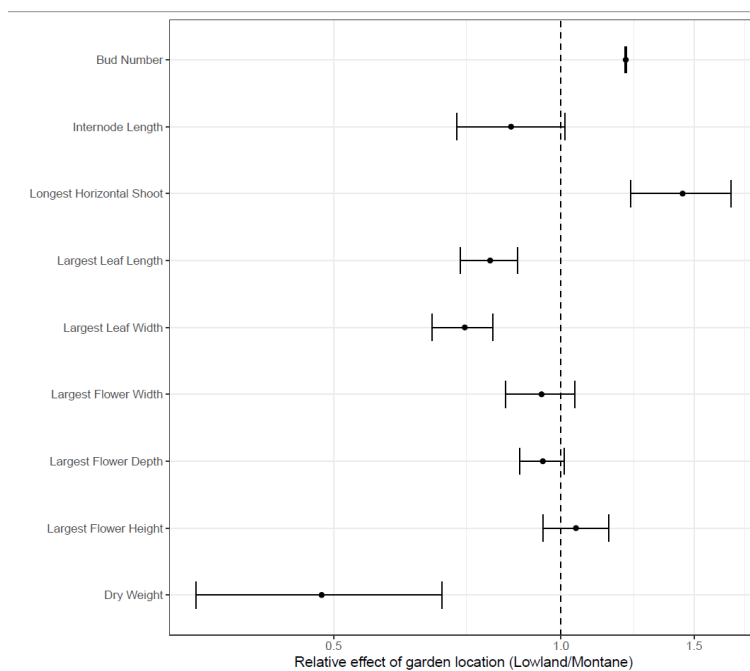
295 *Plastic variation*

296 For all of the measured traits except for stolon length and number of buds, the environmental  
297 effect of garden was greatest in the montane garden, where almost all of the clones have higher  
298 above-ground dry weight, greater leaf areas and longer internode lengths than in the lowland  
299 garden (Fig. 4). This environmental component is large, ranging from ~ 20 to 50% difference  
300 between gardens, depending on trait.

301

302 **Fig 4** Estimated relative effect of garden location Lowland/Montane and 95% confidence intervals. An  
303 estimate of 1 indicates the same expected response at both garden locations, Estimates > 1 correspond  
304 to a larger average response in the lowland compared to the montane garden and estimates of <1 indicate  
305 a greater average response in the montane, compared to the lowland garden.

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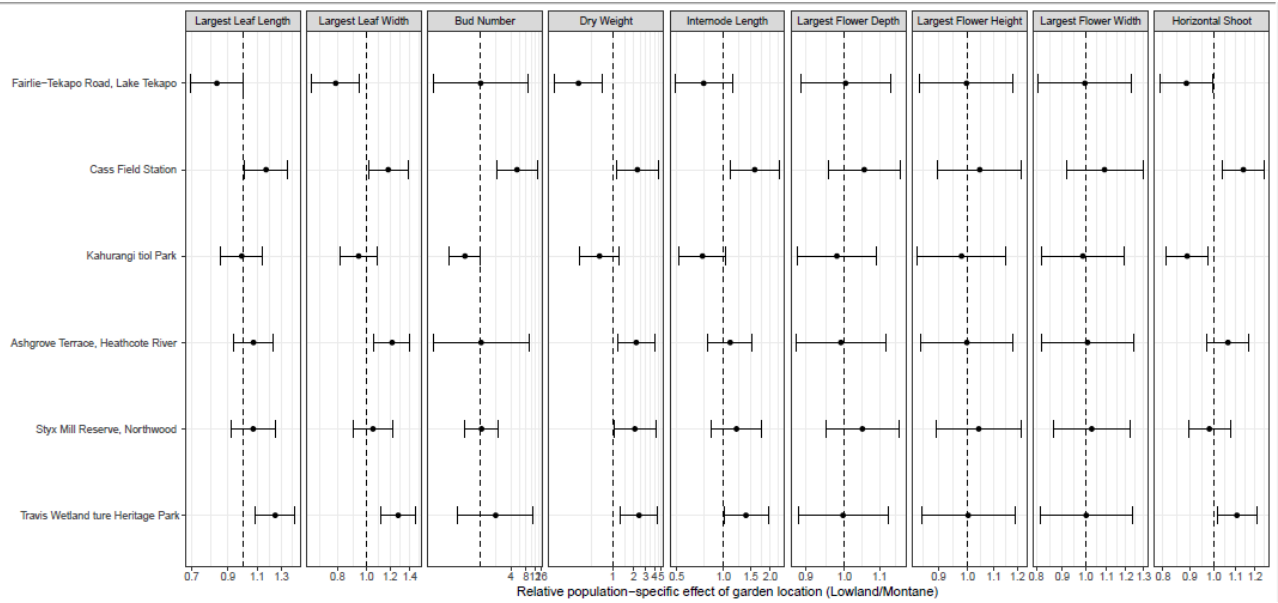
309 The importance of an environmental component to variance is also illustrated by the patterns  
310 for a selection of individual clones (Fig. 5, the individual response of each trait in all clones to  
311 the two garden environments is presented in Fig 1 Supplementary Information). For example,  
312 the clone originating from the montane (Cass) garden site showed one of the strongest and most  
313 consistent plastic responses across all traits when moved to the lowland garden, producing

314 longer horizontal shoots and internodes, and more flowers than in its home montane  
315 environment (Fig. 5). In contrast, the clones from the lowland Travis Wetland and Ashgrove  
316 Terrace sites close to the lowland common garden showed a similar, if less pronounced  
317 response to the two garden environments (Fig 5). In the lowland environment stolon length is  
318 markedly longer relative to the montane garden in all clones, although the degree of between-  
319 garden differences (plasticity) varies among clones. In contrast to the other traits, number of  
320 buds are fairly similar in both gardens for most clones, although where there are plastic  
321 response these vary in direction among clones. A few clones e.g., Ashgrove Terrace clone, do  
322 show a strong environmental response (Fig 5). As expected, we found far more within-clone  
323 variation in vegetative and reproductive (length of horizontal shoot and bud number) than floral  
324 traits.

325

326 **Fig 5** Predicted clone-specific relative effects of garden location Lowland/Montane and 95%  
327 confidence intervals. Clones are ordered from highest (990m) to lowest (0m) elevation of the  
328 sampling location. A value of 1 indicates the same expected population average at both  
329 locations, Estimates  $> 1$  correspond to a larger average response for a clone in the lowland  
330 garden compared to montane garden.

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332

### 333 *Evidence for adaptation*

334 When the PC scores were plotted against each of the source environmental variables

335 independently, for each clone in each of the lowland and montane garden, elevation

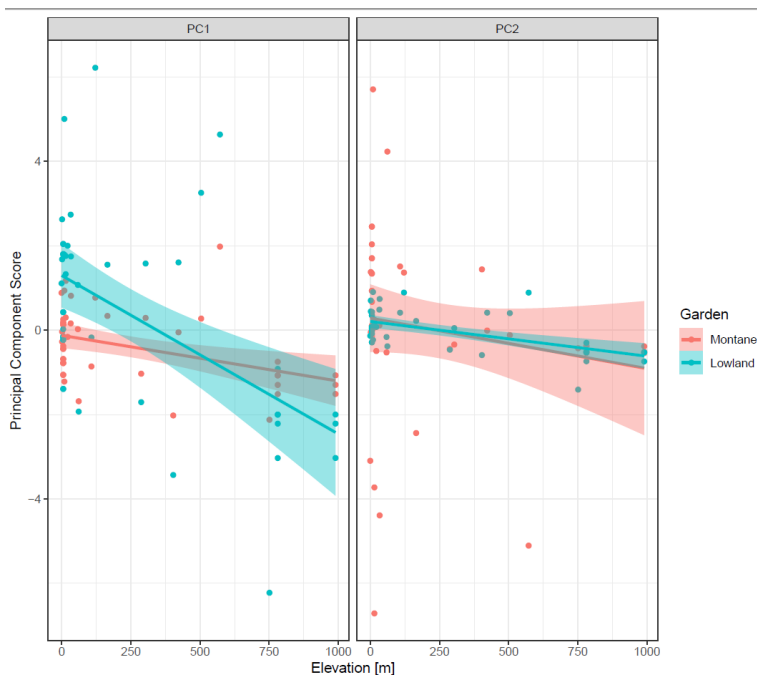
336 explained some of the variation in vegetative traits. This was most obvious in the clones

337 growing in the lowland garden, where the clones from lower elevation source sites were

338 overall markedly larger in comparison to clones from higher elevation source sites (Fig 6).

339 **Fig 6** Dependency between Principal Component scores and elevation of sample locations

340 separate for the two garden locations. The predictions of a linear regression model are added.



341



342

343 Clones from lower elevation sites were more plastic than those from higher elevations (Fig. 7).

344 This influence of source elevation on individual clone responses to garden locations is most

345 obvious for above ground dry weight, stolon length and internode length. In contrast, elevation

346 at source location had no marked effect on relative garden response in bud number or flower

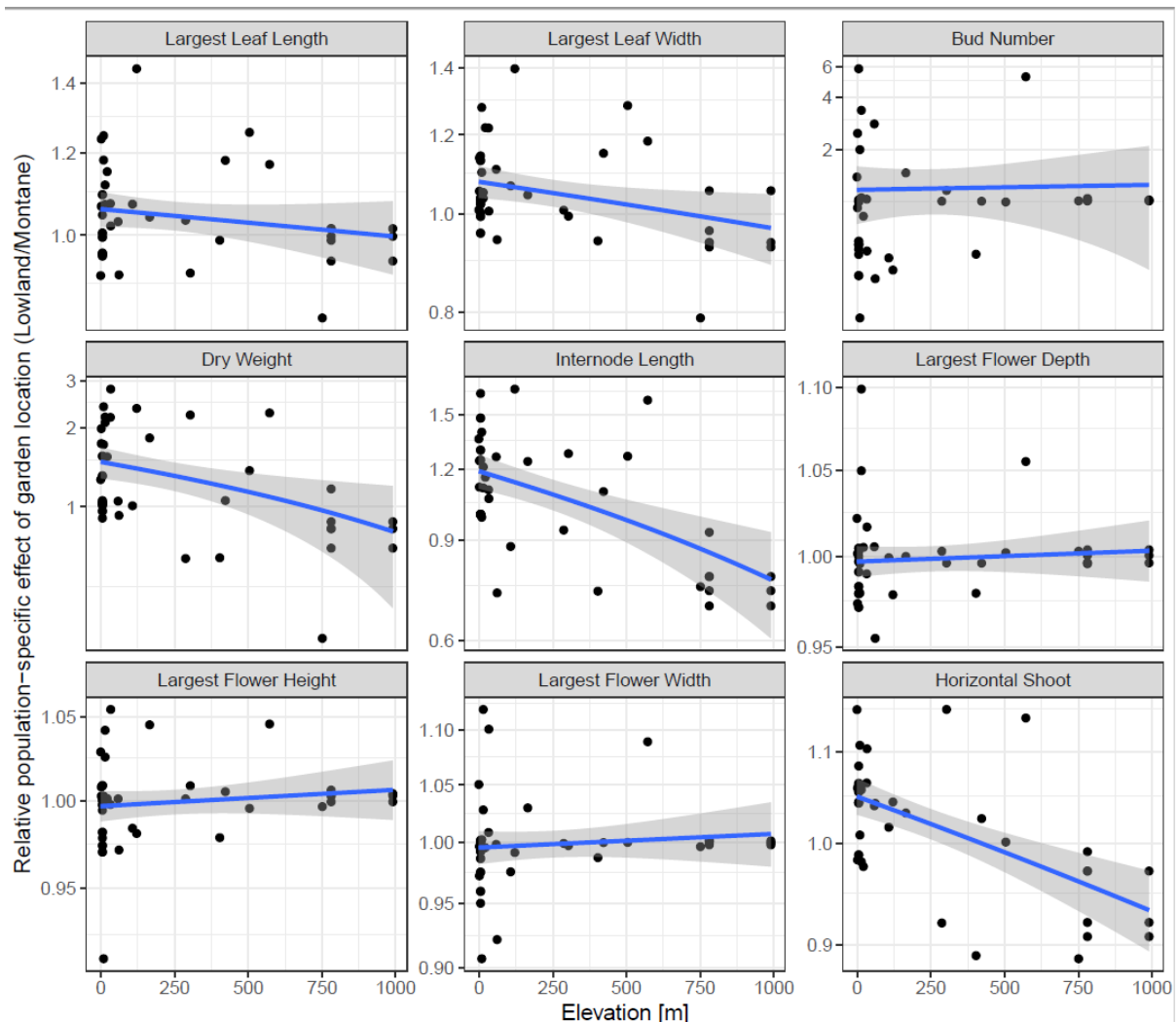
347 characteristics (Figure 7).

348

349 **Fig 7** Dependency between the relative clone-specific effect of garden locations and elevation

350 of sample location. The predictions of a (log)-linear model for each of the measured variables

351 is added.



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355 DISCUSSION

356 The aim of our study was to describe some of the phenotypic variation in *E. gutatta* clones  
357 from across New Zealand and to partition this variation into genetic and plastic components.  
358 We hoped to untangle the relative importance of genetic versus plastic effects in the success  
359 of *E. gutatta* as an invasive clonal plant, something which might shed light on its modelled  
360 niche shift in New Zealand (Da Re et al. 2021). Specifically we tested hypotheses of  
361 adaptation to elevation and home-site advantage. While we acknowledge that one clone per  
362 site excludes any measure of intra-population variation, we are confident that most sites  
363 comprised one or a very few clones.

364

365 *Phenotypic variation*

366 Results from our common garden experiment showed that as expected, there were both  
367 genetic and environmental components to the variance in all traits measured. However,  
368 sorting out the relative contribution of each component and answers about local adaptation  
369 became possible only because we used two common gardens and ensured that these gardens  
370 were located towards each of the two elevational extremes of source populations (Allan and  
371 Pennall 2009; Scheepens et al. 2010; Berend et al. 2019;). We found markedly different  
372 results between gardens; in the lowland garden a major proportion of variation in the five  
373 measured vegetative traits (above ground dry weight, leaf width, leaf length, horizontal shoot  
374 length and internode length) was explained by genetic differences among the clones, while in  
375 the montane garden variation in above ground dry weight, leaf width and leaf length was  
376 almost entirely masked by plastic responses. In contrast, among-clone genetic differences in  
377 flower traits was visible in the montane garden, but hidden in the lowland garden. However,  
378 caution is necessary here as conclusions may depend on which fitness measures are used  
379 (Villegas et al. 2021). Moreover, plastic responses, some passive and non-adaptive (Liu et al.

380 2016) especially in vegetative traits, may mask genetic traits under favourable conditions  
381 (Villellas et al. 2021).  
382 Previous studies using common garden experiments to explore variation within introduced  
383 species in their new range (Williams et al. 2008; Flory et al. 2011), or to investigate home site  
384 advantage (Allan and Pennal 2009) have also found marked differences in trait expression  
385 among gardens. The more similar the garden environment to the native range, or home site, the  
386 better adapted and therefore fitter the plants should theoretically be. We found limited support  
387 for a home-site advantage, with plants collected in the environs of each common garden not  
388 beings consistently superior to clones from other locations.

389

#### 390 *Genotypic variation*

391 In the lowland garden, plants ranged from tall, multiple stemmed, large-leaved individuals with  
392 abundant flowers through to low growing clones with long horizontal shoots to small  
393 individuals with few flowers. Our model predicts that up to 45% of the variance in plant dry  
394 weight and 34 % of the variance in length of horizontal shoots was genetically determined.  
395 Taken together, the variance in vegetative, and reproductive traits we observed in the lowland  
396 garden suggests that across New Zealand there is extensive genetic variation among *E. gutatta*  
397 clones. Results from the montane garden tell a different story; at Cass genetic variation among  
398 clones explains only 1% of the variation in dry weight and only 21% of the variation in  
399 horizontal shoot length, but more genetic variation in flower traits than was observed in the  
400 lowland garden. Basing our understanding on data from the montane garden only would  
401 suggest that *E. gutatta* clones across New Zealand show little genetic variation in vegetative  
402 traits but some variation in flower traits.

403

404

#### 405 *Fitness differences across environments*

406 Bearing in mind that our proxy for fitness was above ground dry weight, our plots of the PC1  
407 scores against source elevation suggest adaptation to elevation; the plot strongly indicated  
408 higher biomass in clones from low elevation compared with high elevation source sites in the  
409 lowland garden. Numerous studies globally have demonstrated the adaptive strategy of low  
410 plant height and low biomass at high elevations (Körner 2003). Moreover Olsson and Agren  
411 (2002) and Monty et al. (2009) have demonstrated this adaptation in introduced plant species  
412 in the US and Europe respectively. However, while adaptation is a credible explanation for  
413 our finding that clones from lowland sites are fitter in the lowland than in the montane  
414 garden, alternative explanations such as genetic drift through founder effects (Monty et al.  
415 2009; McGoey et al. 2020) cannot be dismissed.

416 The weak signal of advantage in clones from lowland sites in the high elevation Cass garden  
417 is in accordance with DeMarche et al. (2016) who found that low elevation *E. gutatta*  
418 ecotypes outperformed montane ecotypes in their montane common garden. However, the  
419 authors agree their conclusions are tenuous for several reasons, including inappropriate  
420 measures of fitness. In our New Zealand study, we did not measure below ground biomass,  
421 which may have conferred fitness at higher elevations. Moreover, our results are from one  
422 year only so we may have missed inter-annual variation in fitness. Alternatively, it is  
423 conceivable that genotypes better adapted to higher elevations in New Zealand have not yet  
424 reached high elevations environments.

425

#### 426 *Home site advantage*

427 Despite the wide range of genetic variation among our clones in vegetative traits we found little  
428 evidence of local adaptation, instead the ranking of clones in terms of relative trait values stayed  
429 the same across most traits in both gardens. While the Cass clone, growing in the Cass montane  
430 garden was always in the top few ranking clones for all vegetative and reproductive traits we  
431 measured, it was only top in internode length and was outperformed by clones from lowland

432 sites in length of longest horizontal shoots- stolons. Similarly, in the Ilam lowland garden while  
433 clones from low elevation sites around Christchurch relatively close to the lowland garden  
434 performed relatively well they were outperformed by several clones from other regions.  
435 Multiple introductions, small populations and founder effects (McGoey et al.), and possibly  
436 conflicting selection pressures (Barrett et al. 2008) may explain this lack of evidence for home  
437 site advantage. Strong gene flow among populations (Zhao et al. 2013) seems an unlikely  
438 explanation given the clonal nature of the spread of *E. gutatta* (Truscott et al. 2008). Multiple  
439 introductions into different catchments and founder effects seem the most likely explanation;  
440 frequent removal (sometimes annual) of *E. gutatta* to clear waterways followed by re-  
441 introduction through fragments of different provenances could well explain lack of ecotypic  
442 variation. While absence of ecotypic variation despite strong genotype variation in invasive  
443 species has been reported previously (Lord 1992; Ebeling et al. 2011; Pahl et al. 2013; Herden  
444 et al. 2019) there is always the possibility that adaptation is being missed because of  
445 inappropriate fitness measures. For example, Lord (1992) points out that while Rapson and  
446 Wilson (1992) found no evidence for home site advantage among New Zealand *Agrostis*  
447 *capillaris* populations in growth and floral morphology, they did find evidence of local  
448 adaption to soil water availability and soil nitrogen and phosphorus. Identifying the key traits  
449 is therefore essential (Williams et al. 2008; Bufford and Hulme 2021).

450

#### 451 *Plasticity*

452 All clones showed considerable plasticity in all the traits we measured, which was  
453 unsurprising, especially for the vegetative traits given this is a perennial, weedy herb (Bazzaz  
454 1996). We found above ground dry weight, our proxy for fitness, was almost universally  
455 higher in the montane relative to the lowland garden; dry weight was on average 50% higher  
456 across all clones at Cass than at Ilam, with little variance in dry weight among clones at Cass.  
457 While this reduced inter-clonal variance in dry weight at montane garden reflected varying

458 steepness of the reaction norm for above ground dry weight among the clones, rank order did  
459 not change. Likewise, horizontal shoot length was on average 5% shorter in the montane  
460 than in the lowland garden, but with no crossing-over of reaction norms. Consistent reactions  
461 norms among clones suggests past adaptation and may be indicative of less potential for local  
462 adaptation in new environments than if clones had shown variable reaction norms to different  
463 environments (Cheplick 2015). One interpretation of these results therefore is that the  
464 montane environment is more similar than lowland Ilam to the environment in the native  
465 range of *E. gutatta* populations; plasticity permits clones to maximize their fitness under  
466 optimal conditions (Hendry 2016). However, based on the environmental suitability models  
467 Da Re et al. (2020), neither the lowland or montane garden are especially suitable areas for *E.*  
468 *gutatta*. It may well be that much of the plasticity we observe across most of the traits we  
469 measured is passive, a side effect of adaptations or other plastic responses (De Witt et al.  
470 1998; Funk 2008; Hulme 2008; Auld et al. 2010; Bufford and Hulme 2021a), limiting  
471 species ability to respond to changing environments (Valladares et al. 2014). Bufford and  
472 Hulme (2021a) stress the need for future experimental studies linking trait plasticity to fitness  
473 along manipulated environmental gradients.

474 The direction of the reaction norm for internode length did vary among clones, with some  
475 clones showing longer internode lengths in the montane environment relative to the lowland  
476 garden and others shorter. This may indicate adaptive plasticity; certainly foraging behaviour  
477 via horizontal and vertical growth as observed in *E. gutatta* should be adaptive by allowing  
478 the colonisation of new niches (Clements et al. 2021). van Kleunen and Fischer (2021) tested  
479 this hypothesis on the clonally reproducing *Ranunculus reptans* and found genetic variation  
480 in plasticity for internode and stolon length, evidence supporting adaptive plasticity. The  
481 importance in our study of these results is that they show genetic variation among clones for a  
482 plastic trait which may support invasiveness (Clements et al. 2021).

483 Further evidence for adaptive (active) plasticity was found in bud number. For this trait  
484 reaction norm slopes crossed in some of the clones, and different clones responded differently  
485 to the two gardens. Again, this illustrates the potential for adaptive evolution across our *E.*  
486 *gutatta* clones.

487 In contrast to the other traits, the flower length, depth and width showed considerable among  
488 clone genetic variation in the montane environment but elicited a similar plastic response to  
489 the lowland garden, where flowers from all clones were essentially similar in size. We  
490 caution too much interpretation of these results because of low flower numbers, but plasticity  
491 of *E. gutatta* flowers has been reported before, a response to drought (Kelly et al. 2008).  
492 That clones from lower elevation source sites showed greater plasticity in vegetative traits  
493 than clones from high elevation source sites is important. Plasticity is selected for in changing  
494 environments (Oostra et al. 2018) and lowland sites may be more varied environments than  
495 montane ones, experiencing more fluctuation in nutrient levels and more anthropogenic  
496 disturbance. Whatever the reason for this elevational variation the finding is notable because  
497 the distribution of plastic traits across species ranges may impact how species respond to new  
498 environments and how they affect range shifts (Bufford and Hulme 2021a).

#### 499 *Future work*

500 Our study illustrates the potential opportunity in using *E. gutatta* to further understand the  
501 mechanisms of successful plant invasions into new niches. Key to taking this further is  
502 experimentation using multiple clones from each population in reciprocal transplants, both  
503 within New Zealand and between New Zealand and native US *E. gutatta* populations.  
504 Comparing home and away fitness traits and home and away plasticity (Bufford and Hulme  
505 2021b), especially in traits associated with clonal spread will be important. Trade-offs (or  
506 not) associated with greater horizontal shoot length and *e.g.* flower production and seed set  
507 should be explored. To what extent plasticity may be aiding or confounding future adaptation  
508 and spread across New Zealand needs further investigation. Future fitness trait measures

509 should include flowering phenology, well known to influence invasion success in plants and  
510 physiological traits which may be more closely associated with fitness than the traits included  
511 in this study.

512

### 513 *Conclusions*

514 Our study has shown the presence of considerable genetic variation among *E. gutatta* clones  
515 from across the South Island of New Zealand; while we found scant evidence for local  
516 adaptation (home site advantage) we found a strong signal for higher fitness in all vegetative  
517 traits across both garden environments in clones from lowland, as compared with higher  
518 elevation sites. Clones from lowland sites were more plastic relative to clones from higher  
519 elevation sites, including two key traits facilitating the spread of clonal plants, horizontal  
520 shoot length and internode length. Having variation for plasticity in essential traits such as  
521 these suggests that *E. gutatta* is well adapted to move into new environments (Gratani 2014)  
522 and that plasticity may contribute substantially to its successful niche expansion within New  
523 Zealand as compared with its native range. To what extent this reflects a trade-off or lack  
524 thereof with other reproductive traits (Bufford and Hulme 2021b) is a major focus for future  
525 investigation.

526

### 527 Data Availability Statement

528 All data are available on request: [hazel.chapman@canterbury.ac.nz](mailto:hazel.chapman@canterbury.ac.nz)

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### 860 **Competing Interests**

861 *The authors have no relevant financial or non-financial interests to disclose*

862 Please refer to the “Competing Interests” section below for more information on how to  
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### 864 **Author Contributions**

865 All authors contributed to the study conception and design. Material preparation, data  
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