1 **Cross-continental variation of herbivore resistance in a global plant invader**

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Summary

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37 ● Successful plant invasions are often explained with adaptation to novel
38   environments. However, invasive species often occupy broad niches within their
39   native and introduced ranges, and a true understanding of microevolution during
40   invasion therefore requires broad sampling of ranges, ideally with a knowledge of
41   introduction history.

42 ● We tested for genetic differentiation in herbivore resistance among 128
43   introduced (Europe, North America) and native (China, Japan) populations of the
44   invasive Japanese knotweed (*Reynoutria japonica*) in two common gardens in the
45   native range.

46 ● In both common gardens we found that resistance traits of introduced populations
47   differed from most Chinese native populations, but not from populations in Japan,
48   the putative sources of introduction. Compared to Chinese populations,
49   introduced European populations had thicker leaves with a lower C:N ratio but
50   higher flavonoids contents. In the native range, variation in herbivore resistance
51   was much more strongly associated with climate of origin than in introduced
52   populations.

53 ● Our results support the idea that founder effects played a key role in the invasion
54   of knotweed into Europe and North America, with introduction of particular
55   resistance phenotypes from Japan. Our study also demonstrates how knowledge
56   of introduction history can avoid drawing wrong conclusions from observed
57   biogeographic divergence.

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59 **Keywords:** common garden, plant defense, plant invasion, rapid evolution,
60 *Reynoutria japonica*, secondary chemistry

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3

Introduction

The number of invasive plant species has increased dramatically over the past two centuries (Seebens *et al.*, 2017), causing exceedingly negative impacts on environment (Powell *et al.*, 2013; Bellard *et al.*, 2016; Castro-Díez *et al.*, 2019) and economy (Bradshaw *et al.*, 2016; Diagne *et al.*, 2021). In the context of globalization and climate change, one-sixth of the world's land surface is expected to be highly vulnerable to invasion (Early *et al.*, 2016), and the number of new invasive species is likely to increase further (Seebens *et al.*, 2017, 2021) Thus, a comprehensive understanding of the mechanisms that underlie the successful invasion of alien species is an urgent issue in ecology and evolution (Kempel *et al.*, 2013; Pyšek *et al.*, 2020).

Many eco-evolutionary hypotheses proposed to explain increased performance of invasive populations are related to altered or novel abiotic or biotic environmental conditions in the introduced ranges (Cripps *et al.*, 2006; Mitchell *et al.*, 2006; Montti *et al.*, 2016). The enemy release hypothesis (ERH) states that plant species experience a decrease in regulation by herbivores and other natural enemies, resulting in increased performance (Keane & Crawley, 2002). Since resistance to enemies can be costly (Koricheva, 2002), shifts in enemy pressure may lead to rapid evolutionary changes in growth and defense allocation (Bossdorf *et al.*, 2005; Buswell *et al.*, 2011; Li *et al.*, 2022). The evolution of increased competitive ability (EICA) hypothesis therefore predicts that invasive populations may allocate fewer resources to herbivore defenses, and thus become more competitive than their native conspecifics (Blossey & Nötzold, 1995; Joshi & Vrieling, 2005; Lin *et al.*, 2015). However, despite strong evidence for enemy release (Keane & Crawley, 2002; Liu & Stiling, 2006; Castells *et al.*, 2013; Rotter *et al.*, 2019; Xiao et al., 2020), especially with regard to specialists, the evidence in support of EICA is so far mixed (Bossdorf *et al*., 2005; Felker-Quinn *et al*., 2013; Rotter & Holeski, 2018), with lower herbivore resistance observed in several invasive species (Siemann & Rogers, 2001; Jakobs *et al*., 2004; Huang & Ding, 2016), but similar or even higher resistance in others (Genton *et al*., 2005; Lewis *et al*., 2006; Alba *et al*., 2011; Bhattarai *et al*., 2017; Gruntman *et al*., 2017; Lin *et al*., 2019). Some of these mixed results have been attributed to the different costs of different types of resistance (Koricheva, 2002; Neilson *et al*., 2013), and to the changes in herbivore community composition in the introduced ranges of invasive plants (Müller-Schärer *et al*., 2004; SDH-shifting defence hypothesis; Cripps *et al*., 2006).

96   Not only biotic conditions, but also altered abiotic environmental conditions may

97   impose novel selection pressures and thus result in adaptive evolutionary changes in

98   introduced populations (Buswell *et al*., 2011; Alexander *et al*., 2012; Lee & Kotanen,

99   2015). For instance, climatic conditions often differ between native and introduced

100   ranges of invasive species (Early & Sax, 2014; Bocsi *et al*., 2016), and several

101   previous studies have already demonstrated climate-related trait differentiation

102   between native and introduced plant populations, suggesting rapid adaptation after

103   introduction (Etterson *et al*., 2008; Alexander, 2013; Woods & Sultan, 2022).

104   Furthermore, the existence of similar trait clines along climatic gradients in native and

105   introduced populations is often also considered as evidence for adaptive post-invasion

106   evolution (Hulme & Barrett, 2013; Bock *et al*., 2015), through multiple introductions

107   of pre-adapted genotypes (Neuffer & Hurka, 1999; Keller & Taylor, 2008) and

108   subsequent selection, i.e. 'sorting out' of standing genetic variation.

109   Climatic differences can also interact with the effects of herbivores. First, climate

110   can directly affect plant defenses through physiological constraints and altered

111   resource availability (Coley, 1985; Wright *et al*., 2004). Second, climatic conditions

112   also influence the abundance and diversity of herbivores in plant communities (Maron

113   *et al*., 2014; Anstett *et al*., 2016; Zhang *et al*., 2016a), thus indirectly driving selection

114   for particular plant traits (Loughnan & Williams, 2019). Therefore, to understand

115   invasive plant success and predict habitat vulnerability to future invasion, we need to

116   understand the impacts of both enemy release and climatic conditions on herbivore

117   resistance of invasive plant populations. Nevertheless, the relationships between

118   climate and herbivore resistance have so far rarely been compared between native and

119   introduced populations (Xiao *et al*., 2020).

120   Comparisons between native and introduced populations within common gardens

121   are often facing several challenges (van Kleunen *et al*., 2018; Woods & Sultan, 2022).

122   First, invasive species often occupy broad climatic niches both in their native and

123   introduced ranges (Brandenburger *et al*., 2019, 2020; Liu *et al*., 2020), and

124   comparisons of only few populations may lead to wrong conclusions, simply because

125   the studied populations do not represent their respective ranges well enough (Yang *et*

126   *al*.,2014). Second, when testing for evolution during invasion, the sources of

127   introduced populations are of course the appropriate reference to invasive

128   populations. Thus, a lack of knowledge of invasion history may further limit our

129   ability to correctly identify trait changes in invasive populations (Cano *et al*., 2009;

130   Bukovinszky *et al*., 2014). Third, because of widespread genotype-by-environment

131   interactions (Richards *et al*., 2006), the results of common garden studies may to

132   some extent be garden-specific. For instance, differences in abiotic and biotic

133   conditions (e.g. climate, interacting species) might influence not only absolute values

134   of growth and defense traits but also their relative differences between plant origins

135   (Maron *et al*., 2004; Moloney *et al*., 2009; Qin *et al*., 2013; Yang *et al*., 2021).

136   Here, we worked with the invasive Japanese knotweed (*Reynoutria japonica*)

137   and built on a previous cross-continental field survey of the species, which provided

138   plant materials, along with important metadata, that allowed us to establish two large

139   common gardens in different climatic zones of the native range, with 55 populations

140   of origin from native range in China and Japan, and 73 populations from the

141   introduced ranges in Europe and North America. The worldwide distribution and

142   relatively clear invasion history of invasive knotweed provided an excellent model to

143   explore biogeographic divergence in herbivore resistance and resistance traits (leaf

144   traits and leaf chemistry), as well as their relationships with herbivore pressure and

145   climatic factors at the collecting sites. We expected that (1) introduced populations

146   have lower herbivore resistance than native populations, and that (2) traits of native

147   populations are more strongly associated with climates of origin than those of

148   introduced populations.

149

150   **Materials and methods**

151   *Study system*

152   *Reynoutria japonica* (Japanese knotweed) is native to eastern Asia and was introduced

153   to Europe in the early 1840s and to North America in the 1870s as an ornamental

154   (Bailey & Conolly, 2000). Along with its sister species *R. sachalinensis* it has become

155   widely naturalized in both introduced ranges (Barney, 2006; Shimoda & Yamasaki,

156   2016; Del Tredici, 2017). *R. japonica*, *R. sachalinensis*, and their hybrid *R. ×*

157   *bohemica* spread rapidly along river banks and roadsides, often forming dense stands

158   of hundreds of square meters (Bímová *et al*., 2004; Tiébré *et al*., 2008; Rouifed *et al*.,

159   2014). Knotweed invasion seriously threatens the biodiversity and integrity of native

160   ecosystems (Stoll *et al*., 2012; Mincheva *et al*., 2014), which causes substantial

161   economic damage (Reinhardt *et al*., 2003; DEFRA, 2003). As a major environmental

162   threat in Europe and North America, Japanese knotweed is listed among the 100

163   world's worst invasive alien species by IUCN (Lowe *et al*., 2000).

6

164

165 *Sample collection*

166 The plant materials we used came from a cross-latitudinal survey of 150 Japanese

167 knotweeds populations in the native range of China and the introduced ranges of

168 North America and Europe (Fig. **1**, Table S1; Irimia *et al*., 2023). In China our survey

169 ranged from the Guangdong province in the South to the Shandong province in the

170 (North-)East, in Europe from Northern Italy to Central Sweden, and in the United

171 States from Georgia to Maine (Fig. **1**, Table S1). In each range we surveyed 50

172 populations along a 2000 km transect (*c.* every 40 km). The surveys in Europe and

173 North America were done in 2019, that in China in 2020. For full details on the field

174 survey please see Irimia *et al*. (2023). Briefly, at each sampling site we confirmed the

175 knotweed taxon based on morphological characters (Bailey *et al*., 2009), laid a 30 m

176 transect for sampling, selected five knotweed stems at regular intervals along the

177 transect, and collected rhizomes from these individuals.

178 　　In addition to the main field survey, we also collected six native *R. japonica*

179 populations from around Nagasaki in Japan, the region believed to be a key source of

180 European and North American introductions (Bailey & Conolly, 2000; Del Tredici,

181 2017). A recent phylogenetic analysis based on chloroplast DNA (Zhang *et al*.,

182 unpublished data) has confirmed this hypothesis. Between 28 April and 4 May 2021,

183 we collected rhizomes from 5-8 individuals separated by at least 6 m in each Japanese

184 population. The rhizomes from Japan, Europe and North America were imported and

185 temporally grown in a quarantine glasshouse at Xishuangbanna Tropical Botanical

186 Garden, whereas the Chinese rhizomes were only stored at 4 °C for 2 weeks and then

187 planted in pots to minimize maternal effects before setting up the common gardens.

188 Because of legal restrictions, only one rhizome per North American populations could

189 be imported prior to setting up the experiment.

190

191 *Common garden experiments*

192 To test for heritable variation among knotweed plants from different ranges and

193 populations, we set up two large common garden experiments, one at Fudan

194 University in Shanghai (31°20′N, 121°30′E) and the other at Xishuangbanna Tropical

195 Botanical Garden, Chinese Academy of Sciences (21°41′N, 101°25′E) in the Yunnan

196 province. The two common garden locations differed in their climatic conditions, with

197 warmer and moister conditions in Xishuangbanna (average March-October

7

198   temperature 24.1 °C; average monthly precipitation 171 mm) compared to Shanghai

199   (average temperature 20.3 °C; monthly precipitation 116 mm) (http://www.nmic.cn/;

200   He *et al.*, 2001; Song *et al.*, 2010; Yu *et al.*, 2021).

201   Because of import restrictions and variable cultivation success during quarantine

202   conditions, we had to omit some introduced populations and ended up with a total of

203   128 *R. japonica* populations of origin: 55 populations from the native range (50 from

204   China, 5 from Japan) and 73 populations from the introduced ranges (46 from Europe,

205   27 from North America) (Fig. **1**, Table S1). Both gardens were set up in open areas on

206   flat ground covered with artificial grass mats.

207   Prior to planting the experiments, we cut rhizomes from each individual and kept

208   them at 4 °C for at least four weeks to facilitate sprouting success. We cut all

209   rhizomes to a size of 3-10 g (at least one intact node), removed the fine roots, and, as

210   a measure of initial size, determined the fresh weight of each cutting. On 11 March

211   2022, all rhizomes were treated with fungicide and planted separately into 10.8 L pots

212   filled with the same potting soil (Pindstrup substrate 0–10 mm, Pindstrup Mosebrug

213   A/S, Denmark) in both gardens. Because of limited rhizome availability, we

214   eventually planted a total of 518 *R. japonica* individuals in the Shanghai garden (27

215   individuals from 27 North Amercian populations; 218 individuals from 46 European

216   populations; 239 individuals from 50 Chines populations; 34 individuals from 5

217   Japanese populations), and 463 individuals in the Xishuangbanna garden (16

218   individuals from 16 North American populations; 190 individuals from 46 European

219   populations; 232 individuals from 50 Chinese populations; 25 individuals from 5

220   Japanese populations). In each garden, we arranged the plants in five blocks, ideally

221   with one individual from each of the populations in each block, random assignment to

222   blocks, and random order within blocks. To avoid aboveground interference, the

223   distances between pots were all at least 90 cm. To avoid nutrient depletion, we added

224   10 g Osmocote fertilizer (Osmocote plus 801, N: P: K 16:8:12, Everris International

225   B.V., Heerlen, Netherlands) to each pot at the beginning of the experiment, and once

226   again in the middle of the experiment. Throughout the experiment, we watered the

227   plants whenever the soil had become dry. To avoid losses of water and nutrients, all

228   pots were individually placed on plastic trays. The pots, soils, and fertilizers used in

229   the two common gardens were identical.

230

231   *Herbivore damage and resistance traits*

232   To quantify variation in plant herbivore resistance, we estimated the degree of

233   herbivore damage experienced by each individual in each garden, determined the

234   levels of leaf secondary metabolites (lignin, flavonoids, alkaloids), and measured

235   additional leaf traits that are often associated with palatability to herbivores (leaf

236   toughness, leaf thickness, leaf ratios C : N) (Feng *et al*., 2011; Lin *et al*., 2015). In the

237   middle of growing season (July 2022), we estimated herbivore damage as the

238   percentage of leaf area eaten on each plant individual, separately for beetle and

239   caterpillar damage (based on chewing modes and leaf notch types; Fig. S2). Two days

240   later, we sampled five fresh, fully developed leaves (leaves 1, 2, 4, 5 and 6 from the

241   top) from the tallest shoot of each plant individual, and measured the leaf thickness of

242   each plant with a digital micrometer (Digimatic Outside Micrometer, Mitutoyo,

243   Japan), and its toughness with a penetrometer (FA10, SAUTER, Balingen, Germany)

244   in the Shanghai garden, and with a mechanical testing machine (ZQ990A, Dongguan

245   Zhiqu Precision Instrument Co., Ltd, China) in the Xishuangbanna garden. For each

246   plant, we then estimated the leaf thickness and toughness as the averages of the five

247   measurements.

248   Finally, we dried all leaves at 60 °C for 72 h, and used these to analyze leaf

249   chemistry. After grinding samples to the required particle size with a ball mill

250   (MM400, Retsch, Germany), we measured total C and N with an organic elemental

251   analyzer (FlashSmart™ Elemental Analyzer, Thermo-Fisher Scientific, USA) via

252   thermal combustion and TCD/IR detection of CO_2/N_2, and also measured leaf lignin,

253   alkaloids and total flavonoids using the MZS-1-G, SWJ-1-Y and LHT-1-G test kits

254   (Suzhou Comin Biotechnology Co., Ltd., Suzhou, China), respectively. In total, we

255   analysed 981 plant samples, 518 from the Shanghai garden and 463 individuals from

256   the Xishuangbanna garden.

257

258   *Statistical analyses*

259   To test for range differences in plant traits and herbivore damage, we fitted linear

260   mixed models in R version 4.2.1 (R Core Team, 2022), with range (China, Japan,

261   Europe, North America) as fixed effect, and population and block as random effects.

262   To account for variation in initial size, our models included initial rhizome weight as

263   covariates. We assessed the significance of fixed effects through Type III Wald chi-

264   squared tests using the *car* package (Fox & Weisberg, 2018). For traits that displayed

265   a significant range effect ($P < 0.05$), we then conducted Tukey post-hoc tests with the

266 *emmeans* and *pairs* functions (*emmeans* package; Lenth, 2018). Where necessary, we

267 *log*-transformed the herbivore damage data to normalize the distribution of residuals.

268   To test for associations between leaf traits and herbivore damage, we calculated

269 population-level spearman correlation coefficients for each pairwise combination of

270 resistance trait (leaf traits and leaf chemistry) and herbivore damage, separately for

271 each garden, using the *Hmisc* package (Hauke & Kossowski, 2011).

272   Finally, we tested whether the climatic conditions of population origins were

273 associated with the resistance traits of native and invasive populations. For this, we

274 obtained eight bioclimatic variables from the WorldClim database (Fick & Hijmans,

275 2017) that seemed particularly meaningful for characterising growing and

276 overwintering conditions for knotweed plants: annual temperature (bio1), maximum

277 temperature of the warmest month (bio4), minimum temperature of the coldest month

278 (bio5), temperature seasonality (bio6), mean annual precipitation (bio12),

279 precipitation of the wettest month (bio13), precipitation of the driest month (bio14),

280 and precipitation seasonality (bio15). We simplified these climate data through a

281 principal component analyses (PCA) using the *prcomp* function. The first and second

282 principal component ('climate PC1' and 'climate PC2' hereafter) explained 54% and

283 21% of the variance in the eight climatic variables across all populations, respectively,

284 with climate PC1 mainly related to mean temperature as well as cold and precipitation

285 extremes, and climate PC2 related to temperature maxima and seasonality (Fig. S1,

286 Table S2). We then performed population-level linear regression analyses with either

287 climate PC1 or climate PC2 as explanatory variables, and the common-garden

288 averages of the resistance traits of native or introduced populations as dependent

289 variables (Moreira *et al*., 2018; Galmán *et al*., 2021).

290

291 **Results**

292 We found that on average the traits of invasive populations often differed from those

293 of native populations. However, these overall patterns were largely driven by the

294 native populations from China which were distinct from European and North

295 American populations, whereas the native populations from Japan were often similar

296 to the invasive ones.

297

298 *Range differences in herbivore damage*

299 The average levels of herbivore damage differed substantially between the two

300 common gardens: in Xishuangbanna the percentages of leaf area eaten by beetles and

301 caterpillars were three and 19 times higher, respectively, than in Shanghai (Fig. **2**). In

302 Shanghai, plants from Chinese populations experienced significantly higher levels of

303 caterpillar herbivory than plants from Europe or North America (Fig. **2b**), and the

304 same was true for beetle damage in Xishuangbanna, where the Chinese populations

305 were also most strongly attacked (Fig. **2c**). Interestingly, Chinese populations

306 experienced less caterpillar damage than European populations in Xishuangbanna, but

307 we never found any significant differences between the two introduced ranges, or

308 between Japanese populations and the invasive populations, in the two gardens.

309

310 *Range differences in resistance traits*

311 Plants from native Chinese populations were also distinct in their resistance traits. In

312 both common gardens, Chinese plants had significantly thinner leaves than plants

313 from invasive populations (Fig. **3a,d**), and in the Shanghai garden they also had

314 higher leaf ratios C : N and lower leaf flavonoids than plants from both invasive

315 ranges (Figs. **3c** and **4c**). Moreover, Chinese populations had tougher leaves than

316 European populations in the Shanghai garden (Fig. **3b**) and higher leaf ratios C : N

317 than North American populations in the Xishuangbanna garden (Fig. **3f**). We did not

318 find any range differences in leaf lignin and leaf alkaloids between native and

319 invasive populations Fig. **4a,b,d,e**), and there were no significant differences in leaf

320 traits between native populations from Japan and the invasive populations (Figs. **3** and

321 **4**).

322

323 *Correlations between resistance traits and herbivore damage*

324 In the Shanghai garden, leaf damage by caterpillars was negatively correlated with

325 leaf thickness and leaf flavonoids, but positively with leaf ratios C : N (Table 1). In

326 contrast, in the Xishuangbanna garden, beetle herbivory was negatively correlated

327 with leaf thickness and flavonoids, but caterpillar herbivory was positively correlated

328 with leaf thickness (Table 1).

329

330 *Correlations between climates of origin and resistance traits*

331 Significant correlations between climates of origin and resistance traits were frequent

332 among native populations but much less common among introduced populations (10

333 vs. 3 correlations in native vs introduced plants; Table 2). In both common gardens,

334   leaf thickness was negatively correlated with climate PC1 but positively correlated

335   with climate PC2 for native but not invasive plants, and leaf ratios C : N of native

336   populations was negatively related to climate PC1. Only in the Xishuangbanna

337   garden, leaf ratios C : N of invasive populations was positively correlated to climate

338   PC2. For native populations, leaf flavonoids were positively related to climate PC1

339   but negatively to PC2 in both gardens, but for invasive populations we found a

340   positive climate PC1-flavonoid correlation only in Xishuangbanna, and a negative

341   correlation of flavonoids with climate PC2 only in Shanghai (Table 2).

342   In other words, native populations from warmer, humid sites with high

343   temperature seasonality tended to have thicker leaves with a higher ratios C : N and

344   lower flavonoids contents (Table 2), where for invasive populations climate

345   associations were rarer and more variable: in Shanghai, higher flavonoid contents of

346   invasive populations were associated with high summer temperature and low

347   seasonality whereas in Xishuangbanna lower flavonoids were associated with warm

348   and humid areas of origin (Table 2).

349

350   **Discussion**

351   Successful plant invasions are often explained by evolutionary changes in plant traits.

352   However, the power of previous common garden comparisons of native versus

353   introduced populations has often remained limited. Here, we tested for genetic

354   differentiation in herbivore resistance among 128 native and introduced populations

355   of invasive knotweed (*Reynoutria japonica*) in two common gardens in the native

356   range. We found that on average introduced populations from Europe and North

357   America differed in several resistance traits from native Asian populations, but that

358   these overall differences were driven by native populations from China, whereas the

359   resistance traits of native Japanese populations were generally similar to populations

360   from the introduced range. Our study supports – at the level of phenotypes – a

361   Japanese source of knotweed invasions, and that founder effects – rather than post-

362   invasion evolution – explains overall range differences in knotweed traits.

363

364   *Range differences in herbivore resistance*

365   The EICA hypothesis predicts that because of a release from native herbivores

366   introduced populations might have reduced herbivore defenses (Blossey & Notzold,

367   1995). We found that introduced European and North American populations were

368   indeed significantly different in many leaf traits from native Chinese populations, but

369   that there were little differences between introduced populations and the putative

370   source populations from Japan. Our results thus demonstrate the importance of

371   accounting for invasion history when testing for evolution during invasions (Colautti

372   & Lau, 2015; Brandenburger *et al.*, 2020).

373   We found no significant differences in many herbivore resistance traits such as

374   leaf ratios C : N, leaf toughness, leaf flavonoids, lignin or alkaloids between

375   introduced European and North American and native Japanese populations in both

376   common gardens. There were also no differences in the herbivore damage of plants in

377   both gardens, which represented two different natural herbivore environments in the

378   native range. Our results thus did not support the EICA prediction that invasive

379   populations have evolved lower herbivore resistance than their native sources

380   (Blossey & Notzold, 1995). Several studies with other invasive species have shown

381   no difference or even higher resistance of invasive populations in common garden

382   experiments, e.g. with *Chromolaena odorata* (Liao *et al.*, 2014), *Brassica nigra*

383   (Oduor *et al.*, 2011), or *Verbascum thapsus* (Endriss *et al.*, 2018). We are only aware

384   of one previous common garden study with native and introduced populations of *R.*

385   *japonica* (Rouifed *et al.* 2018). It compared only five native Japanese and five

386   invasive French populations in a common garden in the introduced range, but it also

387   found that introduced populations did not differ in their composition of secondary

388   metabolites, stem stiffness and leaf thickness, but that they had even higher leaf

389   toughness than native Japanese populations. As the Japanese populations used in our

390   experiments – as well as those used in Rouifed *et al.* (2018) – were from the putative

391   source region of knotweed introductions to Europe and North America, our results

392   thus indicate that the introduction of plants with particular resistance profiles, rather

393   than post-invasion evolution, underlies the trait divergence between the native and

394   introduced range, and may have played a role in the invasion success of *R. japonica*.

395   We found that herbivore damage varied substantially between the two common

396   gardens, and related to different leaf traits. For instance, in the Shanghai garden

397   damage by caterpillars was negatively associated with leaf thickness and flavonoids,

398   but positively with leaf ratios C : N, whereas in Xishuangbanna, the same type of

399   damage was only positively associated with leaf thickness. Part of these divergent

400   results might have resulted from the different herbivore communities in the two

401   common gardens, e.g. if different herbivores have different feeding preferences (Van

402   der Meijden, 1996; Bossdorf *et al*., 2004; Yang *et al*., 2014). In Xishuangbanna, we

403   found that damage by beetles was associated with different traits than damage by

404   caterpillars: beetles preferred plants with thinner leaves and lower flavonoid levels,

405   whereas caterpillars preferred plants with thicker leaves. Besides the herbivore

406   communities, the two common gardens of course also differed in climatic conditions,

407   including temperature, precipitation and solar radiation, and since leaf traits could

408   also be related to other environmental drivers, e.g. influence drought or UV tolerance

409   (Levitt & Lovett, 1985; Strauss & Agrawal, 1999; Harborne & Williams, 2000; Peter

410   Constabel *et al*., 2014; Barton & Boege, 2017; Li *et al*., 2022), this may have further

411   contributed to divergent herbivory-leaf trait relationships. More generally, our results

412   demonstrate the environmental contingency of common garden results, including

413   when testing for variation in herbivore resistance (Maron *et al*., 2004; Qin *et al*.,

414   2013; Yang *et al*., 2014; Bossdorf *et al*., 2005; Yang *et al*., 2021), and hence the value

415   of working with multiple common gardens.

416

417   *Associations between climates of origin and herbivore resistance*

418   We found that the climatic conditions at the collecting sites were significantly

419   associated with resistance traits in both common gardens, but only for plants from the

420   native range, whereas for plants from the introduced range there were only very few

421   significant associations. Many previous studies of invasive plants have documented

422   the development of parallel clinal patterns in ecological traits among populations of

423   native and introduced ranges (Agrawal *et al*., 2015; Leger & Rice, 2007; Etterson *et*

424   *al*., 2008; Rosche *et al*., 2019; Hodgins *et al*., 2020) and have usually interpreted this

425   as evidence for rapid post-invasion evolution and adaptation. However, there are also

426   studies of successful invasive plants with unparalleled (Bhattarai *et al*., 2017; Yang *et*

427   *al*., 2021; Woods & Sultan, 2022) or no clinal patterns at all (Endriss *et al*., 2018; Sun

428   & Roderick, 2019), and our results also provide little evidence for post-invasion

429   genetic differentiation of introduced European or North American populations in

430   relation to climate.

431   A simple reason for the observed lack of genetic differentiation could be that

432   there was too little genetic variation for natural selection to act on. The majority of

433   invasive *R. japonica* populations are thought to be descendants of a single introduced

434   clone (Hollingsworth & Bailey, 2000; Richards *et al*., 2012; Gaskin *et al*., 2014;

435   Groeneveld *et al*., 2014; Zhang *et al*., 2016b). However, so far this knowledge is

14

436   based on low-resolution molecular studies of limited numbers of populations, and we

437   clearly need broader and higher-resolution population genomic analyses to evaluate

438   this explanation. Moreover, even if little genetic variation was introduced in the first

439   place, novel mutations can sometimes be common enough to result in genetic

440   variation that can be exploited for adaptation, even within a relatively short period of

441   time after an invasion (Lynch & Conery, 2000, Ossowski *et al*., 2010). In any case, an

442   alternative explanation for the lack of climate-related genetic differentiation could be

443   that adaptation did not occur *yet*. Although rapid evolutionary changes have

444   frequently been reported for invasive plants, examples from the invasion literature

445   show that geographic clines usually develop 50-150 generations after introduction

446   (Moran & Alexander, 2014), but many of these examples came from short-lived

447   plants. *Reynoutria japonica* was introduced to Europe *c.* 170 years ago and some

448   decades later to North America, but it began to expand rapidly only in the past 50-100

449   years (Bailey & Conolly, 2000), and it is a perennial, so it is a possibility that there

450   was not enough time. Again, genomic approaches will help to answer this question.

451

452   **Conclusion**

453   Our study combined replicate common gardens and a biogeographical approach with

454   samples spanning an exceptionally large climatic gradient from both introduced and

455   native ranges to understand evolution of herbivore resistance in the invasive species

456   *R. japonica*. We found that the resistance traits of introduced European and North

457   American populations differed from most Chinese native populations but were

458   generally similar to Japanese populations that are putative sources of the

459   introductions. Thus, we find little evidence for post-invasion evolution but that plants

460   with particular resistance profiles have been introduced to Europe and North America,

461   and may have played a role in the invasion success of *R. japonica*. Our study

462   demonstrates the importance of accounting for invasion history when testing for

463   evolution during invasion.

464

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476

Conflict of interest statement

478   The authors declare no conflict of interests.

479

Author contributions

481   PC, ZL, MP, OB, CLR and BL designed the experiments. PC, ZL, SW, LZ, YZ and

482   JB conducted the research. PC and ZL analyzed the data and wrote the first draft. All

483   authors contributed to the revision and improvement of the manuscript.

484

485

Data availability statement

487   Data available from the Dryad Digital Repository (private for peer review):

488   https://datadryad.org/stash/share/EYcQ6ThtOzgWS6oC2dS3Xlj7ZhylK9veRFDySOd

489   EZQ0, DOI is 10.5061/dryad.qbzkh18r4.

490

Table 1 Spearman's rank correlations (r_s) between leaf traits and leaf herbivory, tested across 128 native and introduced populations of Japanese knotweed, when grown in two different common gardens in Shanghai and Xishuangbanna

	Thickness	Toughness	Ratios C : N	Lignin	Alkaloids	Flavonoids
Shanghai						
% Leaf area eaten by beetles	0.13	-0.06	-0.11	-0.11	0.10	-0.07
% Leaf area eaten by caterpillars	**-0.40***	0.12	**0.34***	-0.14	0.05	**-0.35***
Xishuangbanna						
% Leaf area eaten by beetles	**-0.30**	-0.13	-0.06	0.06	0.00	**-0.31***
% Leaf area eaten by caterpillars	**0.19***	-0.08	-0.03	0.15	0.03	0.11

Significant correlations are in bold. Significance levels: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Table 2 Associations between climates of origin (first two PCs from a PCA of eight bioclimatic variables, see Table S2) and variation in leaf traits across native (N; China and Japan) and introduced (I; Europe and North America) Japanese knotweed populations, when grown in two common gardens in Shanghai and Xishuangbanna in China

		Climate PC1				Climate PC2		
		β	P	R^2		β	P	R^2
Shanghai								
Thickness	N:	**-0.01**	**0.013**	**0.09**		**0.02**	**0.001**	**0.18**
	I:	0.00	0.855	0.00		0.00	0.747	0.00
Ratios C : N	N:	**-1.29**	**0.022**	**0.08**		0.79	0.170	0.02
	I:	-0.40	0.157	0.01		-0.30	0.286	0.00
Flavonoids	N:	**1.57**	**0.002**	**0.16**		**-1.86**	**0.000**	**0.23**
	I:	-0.35	0.187	0.01		**-0.64**	**0.015**	**0.07**
Xishuangbanna								
Thickness	N:	**-0.01**	**0.016**	**0.09**		**0.02**	**0.000**	**0.22**
	I:	0.00	0.641	0.00		0.00	0.984	0.00
Ratios C : N	N:	**-2.00**	**0.000**	**0.28**		0.89	0.075	0.04
	I:	0.56	0.189	0.01		**1.38**	**0.001**	**0.16**
Flavonoids	N:	**2.73**	**0.000**	**0.30**		**-2.61**	**0.000**	**0.27**
	I:	**1.19**	**0.002**	**0.14**		0.41	0.298	0.00

The values are regression coefficients and their significance levels (*P* values < 0.05 in bold), and adjusted model R^2 from linear regressions.
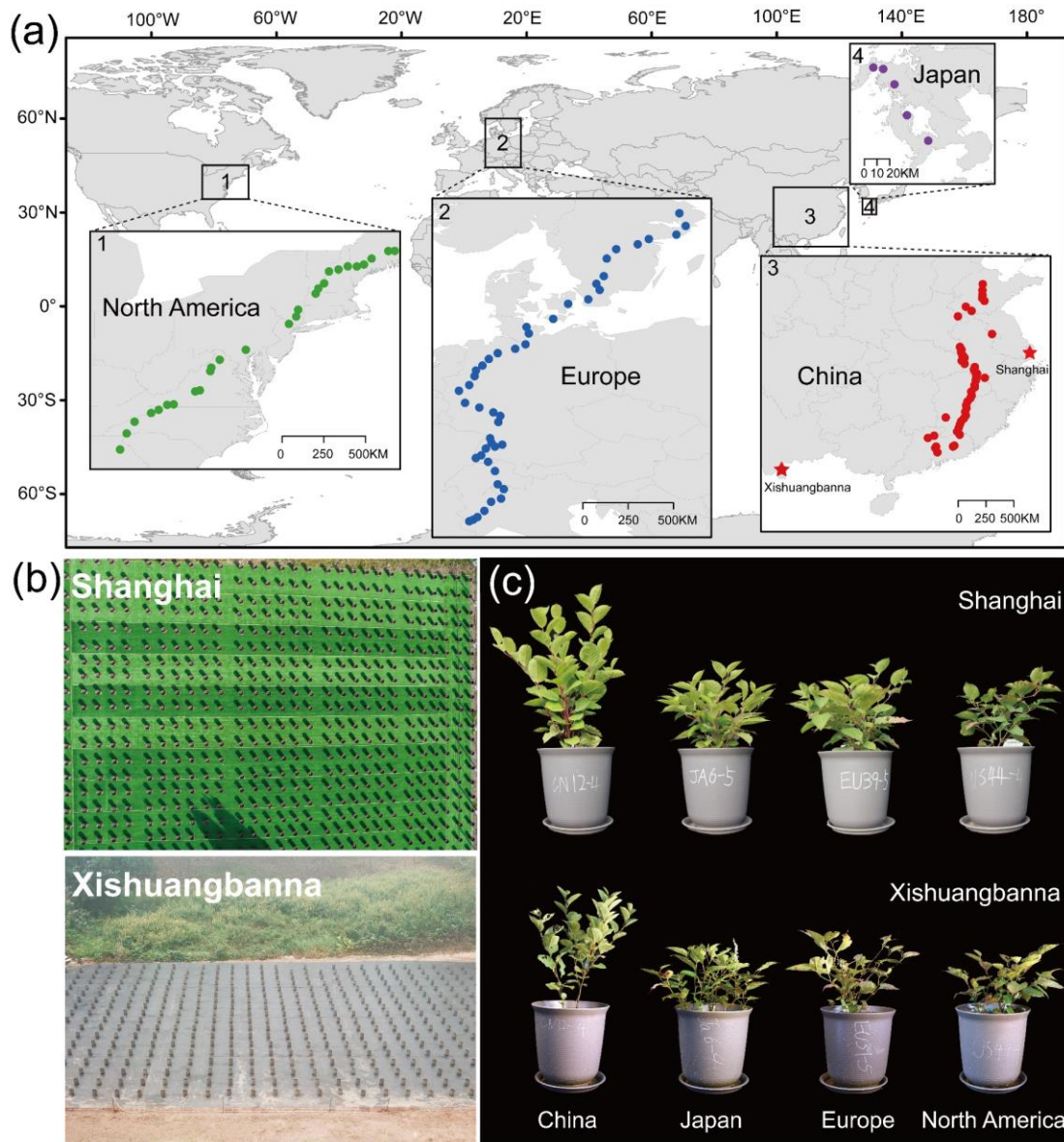
Fig. 1. Geographic origins of the studied knotweed (*Reynoutria japonica*) clones, the two common gardens, and performance variation of different knotweed origins. (a) The geographic locations where rhizomes of *R. japonica* had been collected in the introduced ranges of North America (1; *n* = 27 populations) and Europe (2; *n* = 46), and in the native ranges of China (3; *n* = 50) and Japan (4; *n* = 5), and stars represent the locations of Shanghai (Fudan University Campus) and Xishuangbanna (Xishuangbanna Tropical Botanical Garden, Yunnan) common gardens. (b) Aerial photos of the two common garden experiments in Shanghai and Xishuangbanna. (c) Representative photos illustrating average growth differences between the two gardens and among differential ranges of origin in mid-July.

Fig. 2. Average levels of herbivores damage observed in *R. japonica* plants from China (CN), Japan (JA), North America (NA) and Europe (EU) when grown in common gardens in Shanghai (a, b) and Xishuangbanna (c, d). The values are adjusted means and SEs from ANOVA, with different letters above error bars indicating significant group differences based on Tukey's HSD post-hoc tests. *Chi-square* statistics and significance level of regression models are also shown. ns, not significant.
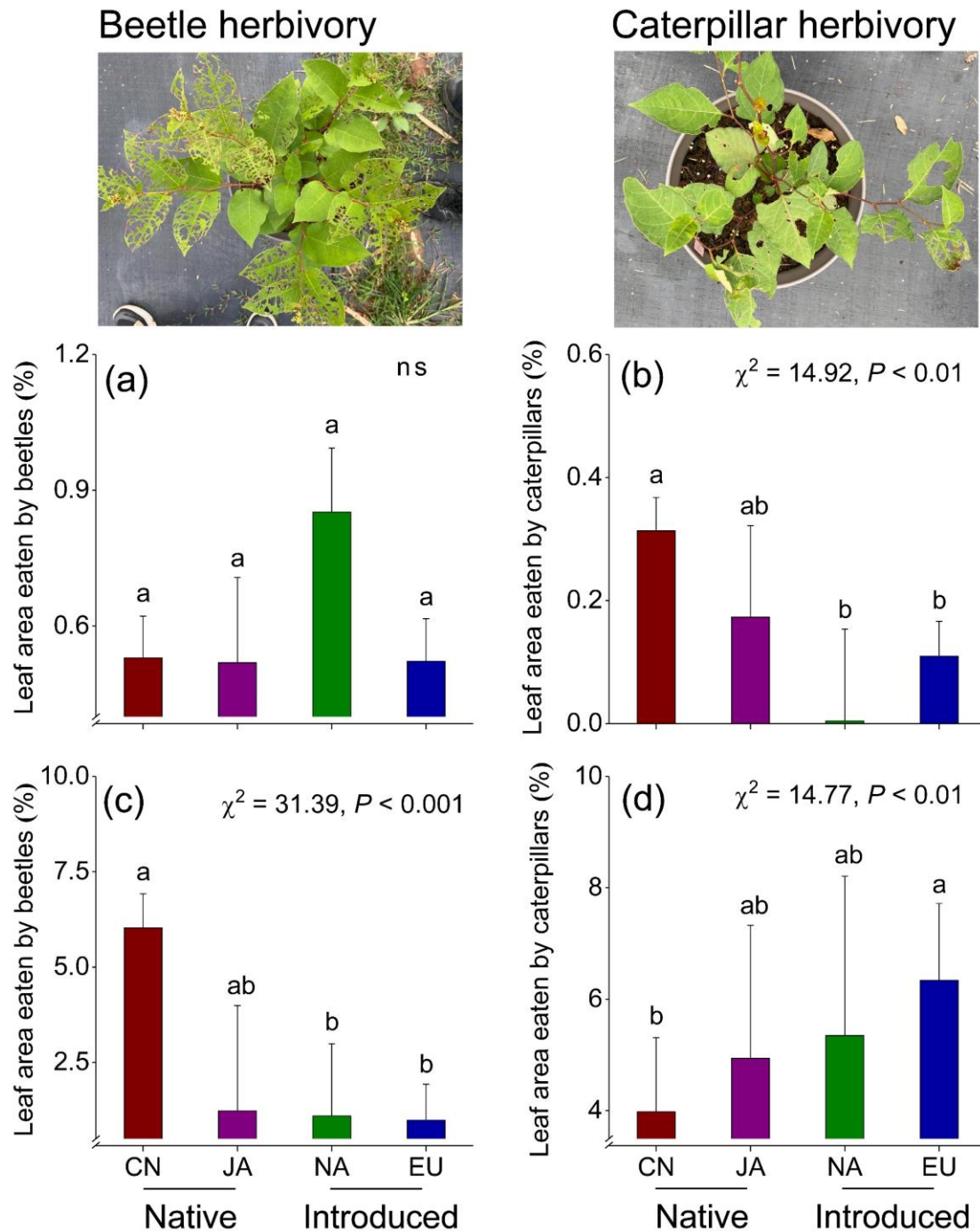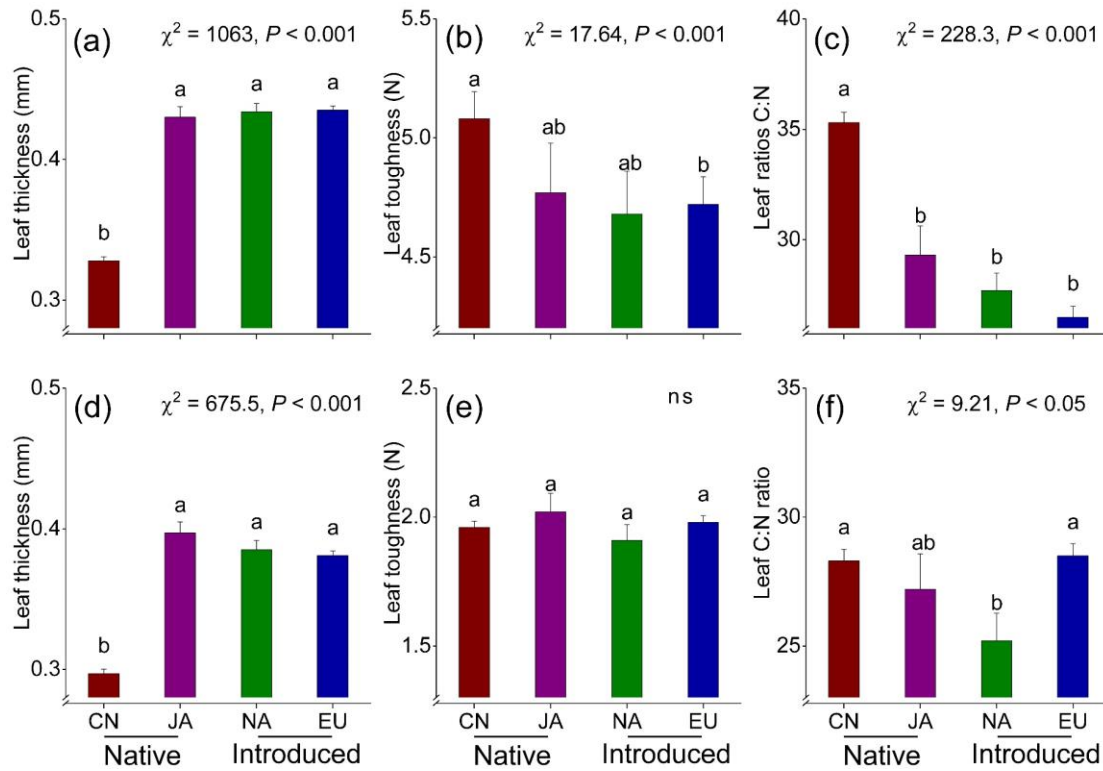
Fig. 3. Average levels of leaf traits observed in *R. japonica* plants from China (CN), Japan (JA), North America (NA) and Europe (EU) when grown in common gardens in Shanghai (a-c) and Xishuangbanna (d-f). The values are adjusted means and SEs from ANOVAs, with different letters above error bars indicating significant group differences based on Tukey's HSD post-hoc tests. *Chi-square* statistics and significance level of regression models are also shown. ns, not significant.

Fig. 4. Average levels of leaf chemistry observed in *R. japonica* plants from China (CN), Japan (JA), North America (NA) and Europe (EU) when grown in common gardens in Shanghai (a-c) and Xishuangbanna (d-f). The values are adjusted means and SEs from ANOVAs, with different letters anove error bars indicate significant group differences based on Tukey's HSD post-hoc tests. *Chi-square* statistics and significance level of regression models are also shown. ns, not significant.
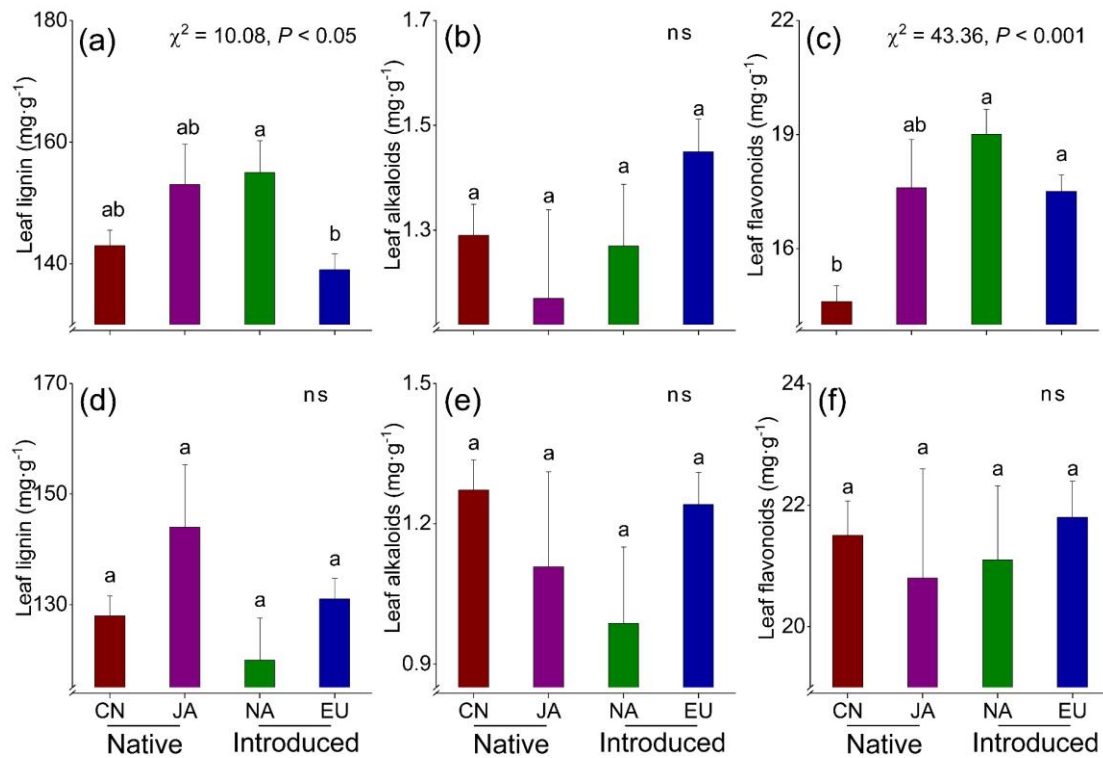
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Supporting Information

Table S1 Geographic origins of the 128 studied *Reynoutria japonica* populations and their uses in the Shanghai (SH) and Xishuangbanna (XSBN) common gardens.

Population ID	Site	Latitude	Longitude	Garden
China (Native)				
CN01	Guangdong	23.29	114.01	SH / XSBN
CN02	Guangdong	23.64	113.84	SH / XSBN
CN03	Guangdong	23.74	113.91	SH / XSBN
CN04	Guangdong	23.75	115.27	SH / XSBN
CN05	Guangdong	23.82	115.38	SH / XSBN
CN06	Guangdong	24.44	113.25	SH / XSBN
CN07	Guangdong	24.62	113.76	SH / XSBN
CN08	Guangdong	24.71	115.82	SH / XSBN
CN09	Jiangxi	24.96	115.61	SH / XSBN
CN10	Jiangxi	25.24	115.73	SH / XSBN
CN11	Jiangxi	25.53	115.84	SH / XSBN
CN12	Jiangxi	25.80	115.94	SH / XSBN
CN13	Jiangxi	26.03	116.16	SH / XSBN
CN14	Jiangxi	26.10	114.69	SH / XSBN
CN15	Jiangxi	26.27	116.32	SH / XSBN
CN16	Jiangxi	26.55	116.27	SH / XSBN
CN17	Jiangxi	26.85	116.37	SH / XSBN
CN18	Jiangxi	27.08	116.34	SH / XSBN
CN19	Jiangxi	27.38	116.45	SH / XSBN
CN20	Jiangxi	27.66	116.68	SH / XSBN
CN21	Jiangxi	27.87	116.78	SH / XSBN
CN22	Jiangxi	28.19	116.76	SH / XSBN
CN23	Jiangxi	28.54	117.05	SH / XSBN
CN24	Jiangxi	28.71	117.02	SH / XSBN
CN25	Jiangxi	29.10	117.12	SH / XSBN
CN26	Jiangxi	29.21	117.15	SH / XSBN
CN27	Jiangxi	29.31	117.85	SH / XSBN
CN28	Jiangxi	29.43	117.16	SH / XSBN

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CN29	Jiangxi	29.64	117.22	SH / XSBN
CN30	Anhui	29.81	117.05	SH / XSBN
CN31	Anhui	29.98	116.97	SH / XSBN
CN32	Anhui	30.18	117.04	SH / XSBN
CN33	Anhui	30.44	116.23	SH / XSBN
CN34	Anhui	30.69	115.98	SH / XSBN
CN35	Anhui	30.82	116.05	SH / XSBN
CN36	Anhui	30.92	116.21	SH / XSBN
CN37	Anhui	31.09	116.10	SH / XSBN
CN38	Anhui	31.21	116.02	SH / XSBN
CN39	Anhui	31.40	115.93	SH / XSBN
CN40	Anhui	31.58	115.97	SH / XSBN
CN41	Henan	31.81	115.85	SH / XSBN
CN42	Jiangsu	32.85	118.44	SH / XSBN
CN43	Henan	34.27	115.68	SH / XSBN
CN44	Jiangsu	34.72	116.79	SH / XSBN
CN45	Shandong	35.06	116.31	SH / XSBN
CN46	Shandong	35.52	117.82	SH / XSBN
CN47	Shandong	35.74	117.64	SH / XSBN
CN48	Shandong	36.00	117.65	SH / XSBN
CN49	Shandong	36.38	117.66	SH / XSBN
CN50	Shandong	36.87	117.68	SH / XSBN
Japan (Native)				
JA01	Unzen	32.81	129.92	SH / XSBN
JA02	Saikai	32.99	129.77	SH / XSBN
JA03	Sasebo	33.21	129.68	SH / XSBN
JA04	Hirado	33.32	129.60	SH / XSBN
JA05	Hirado	33.33	129.53	SH / XSBN
North America (Introduced)				
NA01	Georgia	34.24	-83.46	SH / XSBN
NA02	NorthCarolina	35.10	-83.10	SH / XSBN
NA03	NorthCarolina	35.74	-82.68	SH / XSBN

NA04	NorthCarolina	36.21	-81.78	SH / XSBN
NA05	NorthCarolina	36.38	-81.38	SH / XSBN
NA06	Virginia	36.65	-80.92	SH / XSBN
NA07	Virginia	36.67	-80.57	SH
NA08	Virginia	37.37	-79.41	SH
NA09	Virginia	37.43	-79.16	SH
NA10	Virginia	38.46	-78.59	SH / XSBN
NA11	Virginia	38.65	-78.54	SH
NA12	Virginia	39.08	-78.09	SH / XSBN
NA13	Maryland	39.61	-76.68	SH / XSBN
NA14	NewJersey	41.01	-74.35	SH / XSBN
NA15	NewYork	41.41	-73.96	SH / XSBN
NA16	NewYork	41.77	-73.86	SH
NA17	Massachusetts	42.64	-72.91	SH / XSBN
NA18	Vermont	42.92	-72.77	SH / XSBN
NA19	Vermont	43.20	-72.45	SH / XSBN
NA20	Vermont	43.84	-72.19	SH
NA21	NewHampshire	43.94	-71.68	SH / XSBN
NA22	NewHampshire	44.12	-71.18	SH / XSBN
NA23	Maine	44.11	-70.69	SH
NA24	Maine	44.21	-70.31	SH
NA25	Maine	44.53	-69.89	SH
NA26	Maine	44.94	-68.99	SH
NA27	Maine	44.95	-68.64	SH
Europe (Introduced)				
EU01	Italy	44.67	7.29	SH / XSBN
EU02	Italy	44.75	7.48	SH / XSBN
EU03	Italy	44.88	7.69	SH / XSBN
EU04	Italy	45.19	8.04	SH / XSBN
EU05	Italy	45.64	8.37	SH / XSBN
EU06	Italy	45.80	8.87	SH / XSBN
EU07	Switzerland	46.27	9.00	SH / XSBN
EU08	Switzerland	46.51	8.7	SH / XSBN

EU09	Switzerland	47.16	8.56	SH / XSBN
EU10	Germany	47.62	8.22	SH / XSBN
EU11	Germany	47.81	7.61	SH / XSBN
EU12	Germany	47.94	7.88	SH / XSBN
EU13	Germany	48.28	8.11	SH / XSBN
EU14	Germany	48.37	8.56	SH / XSBN
EU15	Germany	48.47	8.92	SH / XSBN
EU16	Germany	48.56	8.39	SH / XSBN
EU17	Germany	48.79	8.32	SH / XSBN
EU18	Germany	49.59	8.73	SH / XSBN
EU19	Germany	49.90	8.83	SH / XSBN
EU20	Germany	50.07	8.48	SH / XSBN
EU21	Germany	50.31	7.79	SH / XSBN
EU22	Germany	50.54	7.08	SH / XSBN
EU23	Germany	51.14	6.78	SH / XSBN
EU24	Germany	51.43	7.29	SH / XSBN
EU25	Germany	51.87	7.55	SH / XSBN
EU26	Germany	52.15	7.62	SH / XSBN
EU27	Germany	52.39	7.94	SH / XSBN
EU28	Germany	52.72	8.26	SH / XSBN
EU29	Germany	53.01	8.70	SH / XSBN
EU30	Germany	53.22	9.57	SH / XSBN
EU31	Germany	53.45	10.08	SH / XSBN
EU32	Germany	53.99	10.25	SH / XSBN
EU33	Germany	54.31	10.13	SH / XSBN
EU34	Denmark	54.71	11.45	SH / XSBN
EU35	Denmark	55.46	12.19	SH / XSBN
EU36	Sweden	55.68	13.19	SH / XSBN
EU37	Sweden	56.14	13.76	SH / XSBN
EU38	Sweden	56.45	13.60	SH / XSBN
EU39	Sweden	56.83	13.96	SH / XSBN
EU40	Sweden	57.70	14.11	SH / XSBN
EU41	Sweden	58.17	14.58	SH / XSBN

EU42	Sweden	58.41	15.65	SH / XSBN
EU43	Sweden	58.67	16.20	SH / XSBN
EU44	Sweden	58.89	17.56	SH / XSBN
EU45	Sweden	59.32	18.02	SH / XSBN
EU46	Sweden	59.95	17.71	SH / XSBN

Table S2 Summary of PCA of eight climate variables. The first and second components explained 54% and 21% of climatic variation among native and introduced ranges' localities, respectively. Climate variables with stronger loadings (> 0.40) on climate PCs are shown in bold.

|	PC1 climate	PC2 climate
Eigenvalues	4.378	1.731
% variance	54.7 %	21.6 %
Loadings		
Mean annual temperature (bio1)	**-0.461**	0.091
Maximum temperature of warmest month (bio4)	-0.063	**-0.728**
Minimum temperature of coldest month (bio5)	**-0.404**	-0.265
Temperature seasonality (bio6)	-0.323	**0.515**
Mean annual precipitation (bio12)	-0.360	0.188
Precipitation of the wettest month (bio13)	**-0.458**	0.002
Precipitation of the driest month (bio14)	0.170	0.176
Precipitation seasonality (bio15)	-0.385	-0.245
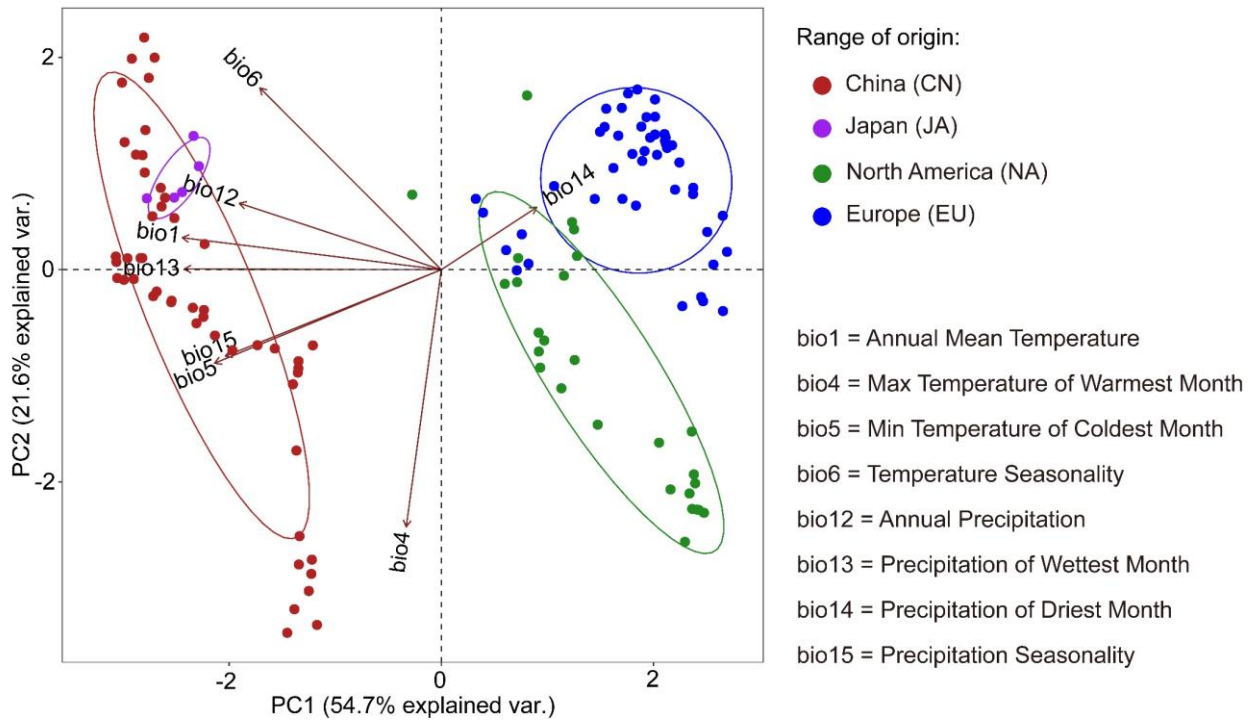
Fig. S1 Principal component analysis (PCA) for climate variables at sample collecting sites of *R. japonica* in common gardens. Colored dots indicate sample collecting sites. Percentages on the X- and Y-axes indicate the variation explained by each principal component.