1 **Title**

2 Enemy release mitigates inbreeding depression in native and invasive *Silene latifolia*

3 populations: experimental insight into the role of inbreeding × environment interactions in

4 invasion success

5 **Running title:**

6 I×E interactions in a plant invader

7 **Type of article**

8 Research article

9 **Authors**

10 Karin Schrieber[1,2], Sabrina Wolf[2], Catherina Wypior[2], Diana Höhlig[2], Stephen R. Keller[3],

11 Isabell Hensen[2,4], Susanne Lachmuth[2,4]

12 [1]Bielefeld University, Faculty of Biology, Department of Chemical Ecology, 33615 Bielefeld,

13 Germany

14 [2]Martin-Luther-University Halle-Wittenberg, Institute of Biology, Geobotany & Botanical

15 Garden, 06108 Halle (Saale), Germany

16 [3]University of Vermont, Department of Plant Biology, Burlington, VT 05405, U.S.A.

17 [4]German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, 04103

18 Leipzig, Germany

19 **Corresponding author**

20 Karin Schrieber

21 Bielefeld University, Faculty of Biology, Department of Chemical Ecology

22 Universitätsstraße 25

23 D-33615 Bielefeld

24   GERMANY

25   Phone: +49 (0) 521 106 67317

26   E-mail: karin.schrieber@uni-bielefeld.de

27   **No. of**

28   Words (total): 8736

29   Words (main body): 5138

30   Figures: 4

31   Tables: 1

32   References: 76

33   Supporting information: 2

34   **Provision of data**

35   All datasets that support this article will be deposited in Dryad in case the article should be

36   editorially accepted for publication.

3

37 **Abstract**

38 Inbreeding and enemy infestation are common in plants and can synergistically reduce their

39 performance. This inbreeding × environment (I×E) interaction may be of particular

40 importance for the success of plant invasions if introduced populations experience a release

41 from attack by natural enemies relative to their native conspecifics. Using native and invasive

42 plant populations, we investigate whether inbreeding affects infestation damage, whether

43 inbreeding depression in performance is mitigated by enemy release and whether genetic

44 differentiation among native and invasive plants modifies these I×E interactions. We used the

45 plant invader *Silene latifolia* and its natural enemies as a study system. We performed two

46 generations of experimental out- and inbreeding within eight native (European) and eight

47 invasive (North American) *S. latifolia* populations under controlled conditions using field-

48 collected seeds. Subsequently, we exposed the offspring to an enemy exclusion and

49 inclusion treatment in a common garden in the species' native range to assess the interactive

50 effects of population origin (range), breeding treatment and enemy treatment on infestation

51 damage as well as plant performance. Inbreeding increased flower and leaf infestation

52 damage in plants from both ranges, but had opposing effects on fruit damage in native

53 *versus* invasive plants. Both inbreeding and enemy infestation had negative effects on plant

54 performance, whereby inbreeding depression in fruit number was higher in enemy inclusions

55 than exclusions in plants from both ranges. Moreover, the magnitude of inbreeding

56 depression in fruit number was lower in invasive than native populations. Our results support

57 that inbreeding increases enemy susceptibility of *S. latifolia*, which magnifies inbreeding

58 depression in the presence of enemies. Enemy release in the invaded habitat may thus

59 increase the persistence of inbred founder populations and thereby contribute to successful

60 invasion. Moreover, our findings emphasize that genetic differentiation among native and

61 invasive plants can shape the magnitude and even the direction of inbreeding effects.

4

62   **Keywords**

63   biological invasion, genetic differentiation, genetic paradox, herbivory, purging, white

64   campion

Introduction

66 Understanding the forces that promote or prevent species range expansions remains a

67 challenging goal in ecology (Barrett, 2015). During invasion of a new range, populations can

68 be simultaneously exposed to increased inbreeding following founder effects (Schrieber &

69 Lachmuth, 2017) and to substantial alterations in the biotic and abiotic environment (Catford,

70 Jansson, & Nilsson, 2009). Inbreeding and environmental change are known to interact in

71 affecting individual fitness (Fox & Reed, 2011), population growth (Reed, Briscoe, &

72 Frankham, 2002) and colonization abilities (Hufbauer, Rutschmann, Serrate, Vermeil de

73 Conchard, & Facon, 2013). Such inbreeding × environment (I×E) interactions are

74 increasingly perceived as potential determinants of species ranges and their dynamics under

75 global change (Colautti, Alexander, Dlugosch, Keller, & Sultan, 2017; Leimu, Vergeer,

76 Angeloni, & Ouborg, 2010; Reed, Fox, Enders, & Kristensen, 2012; Schrieber & Lachmuth,

77 2017). Nevertheless, empirical studies on the environmental dependency of inbreeding

78 effects in the context of invasions are scarce (Murren & Dudash, 2012; Rosche et al., 2017),

79 despite their potential relevance for the prediction and management of invasive species.

80 Invaders can preserve high levels of genetic diversity and sufficient outcrossing rates

81 during their expansion due to e.g. mass introductions and genetic admixture (Hufbauer,

82 2008; Rius & Darling, 2014). However, numerous invasions were evidently accompanied by

83 repeated population bottlenecks during initial introduction and/or colonization at the leading

84 edge of expansion, which resulted in successive genetic depletion and severe inbreeding in

85 phases most crucial for invasion success (reviewed in Schrieber & Lachmuth, 2017).

86 Inbreeding can reduce fitness in the offspring generation (Angeloni, Ouborg, & Leimu, 2011).

87 Such inbreeding depression arises from an increase in genome-wide homozygosity, which

88 enhances the phenotypic expression of deleterious recessive mutations (dominance) and

89 reduces the expression of heterozygote advantage (over-dominance) (Charlesworth & Willis,

90 2009). Fitness reductions following inbreeding compromise the establishment and growth of

91 colonizing populations (Hufbauer et al., 2013) and are thus assumed to hamper invasions.

92   The environmental dependency of inbreeding depression provides a hitherto

93   underappreciated explanation for invasion success in the face of increased inbreeding rates.

94   Abiotic and biotic stressors induce changes in gene expression, protein and metabolite

95   synthesis, which maintain physiological homeostasis and, consequently, fitness under

96   unfavorable environmental conditions (Bundy, Davey, & Viant, 2008). Inbreeding can

97   compromise such stress responses *via* dominance and over-dominance effects, which

98   increases the magnitude of inbreeding depression in stressful environments (Fox & Reed,

99   2011; Kristensen, Pedersen, Vermeulen, & Loeschcke, 2010) while inbreeding depression

100   remains low to absent in benign environments (Enders & Nunney, 2016; Rosche et al.,

101   2017). Plant invasions are often accompanied by a release from environmental stressors

102   such as resource limitation (Blumenthal, 2006), competition (Mitchell et al., 2006) and natural

103   enemies (Keane & Crawley, 2002). Both inbreeding and stress release occur particularly

104   during the early stages of invasion and towards the leading edge of expansion (Dietz &

105   Edwards, 2006; Mitchell et al., 2006). As a consequence, inbreeding depression may be

106   mitigated in small founder populations that experience a stress release in the non-native

107   range, thus fostering invasion success (Schrieber & Lachmuth, 2017).

108   Natural enemies are especially important to I×E interactions during invasions, since they

109   have the potential to regulate long-term patterns of host plant abundance, population

110   dynamics and distribution (Maron & Crone, 2006). Moreover, there is ample evidence for the

111   dependency of plant inbreeding depression on rates of infestation by natural enemies (Bello-

112   Bedoy & Núñez-Farfán, 2011; Carr & Eubanks, 2002; Hayes, Winsor, & Stephenson, 2004).

113   Studies quantifying inbreeding depression in native and invasive plant populations in the

114   presence *versus* absence of their natural enemies may thus provide insight into the role of

115   I×E interactions in invasion success. Such studies can also yield information on how

116   phenotypic differentiation among host populations impacts the outcome of I×E interactions,

117   which may help to explain reported inconsistency in their effects on plant performance (Fox &

118   Reed, 2011; Sandner & Matthies, 2016; 2017). During invasions, plant species often evolve

119   differences in performance and defense traits (Orians & Ward, 2010; Whitney & Gabler,

120 2008). This phenotypic divergence can arise either from adaptive responses to changes in

121 the selective regime, for e.g. climate, competition and natural enemies (Agrawal et al., 2015;

122 Colautti & Barrett, 2013; Zhang & Jiang, 2006), or from neutral processes such as genetic

123 drift and founder effects (Keller & Taylor, 2008; Lachmuth, Durka, & Schurr, 2011; Travis et

124 al., 2007). Both adaptive and non-adaptive genetic differentiation likely also alter the genetic

125 architecture underlying inbreeding depression and its dependency on the environment, e.g.

126 through differences in the accumulation and purging of genetic load (Klopfstein, Currat, &

127 Excoffier, 2006; Schrieber & Lachmuth, 2017).

128 Here, we investigate the combined effects of inbreeding and enemy infestation on the

129 performance of native and invasive populations of the plant species *Silene latifolia* Poir.

130 (Caryophyllaceae). The species is native to Eurasia and has been introduced to North

131 America in the early 19th century. During its invasion of North America, *S. latifolia*

132 experienced events conducive to the expression of I×E-interactions: introduced plants

133 escaped their natural enemies (Wolfe, 2002) and experienced severe population bottlenecks

134 (Keller, Gilbert, Fields, & Taylor, 2012; Taylor & Keller, 2007) as well as high inbreeding

135 levels in founder populations (Fields & Taylor, 2014; Richards, 2000). Moreover, invasive

136 populations evolved differences in enemy susceptibility and performance (Blair & Wolfe,

137 2004; Wolfe, Elzinga & Biere, 2004; Keller, Sowell, Neimann, Wolfe & Taylor 2009; Schrieber

138 et al. 2017) making *S. latifolia* ideally suited for examining the impact of genetic

139 differentiation on the outcomes of I×E interactions. We conducted experimental inbreeding

140 and outbreeding within native and invasive *S. latifolia* populations, exposed the offspring to

141 the absence and presence of natural enemies, and measured traits related to growth,

142 reproduction and infestation damage to address the following predictions: i) Inbred plants

143 incur higher infestation damage than outbreds. ii) Plant growth and reproduction are lower in

144 inbreds than outbreds (inbreeding depression) and in the presence than absence of natural

145 enemies (stress). iii) The magnitude of inbreeding effects on growth and reproduction is

146 higher in the presence than in the absence of natural enemies (I×E interaction). iv) The

147 aforementioned individual and combined effects of inbreeding and enemy infestation are

148 modified by the distinct evolutionary histories of native and invasive populations.

149 **Materials and Methods**

150 Study system

151 *Silene latifolia* is a short-lived perennial herb mainly distributed across ruderal habitats. The

152 plant is dioecious and produces sexually dimorphic flowers pollinated by insects. Females

153 develop large numbers of capsules containing several hundred seeds, which lack a specific

154 dispersal syndrome and are thus mainly dispersed passively and by human activities. Limited

155 seed dispersal and restricted pollen transfer among neighboring plants can lead to restricted

156 gene flow and the formation of kin-structured patches within populations (McCauley, 1997;

157 1994). These characteristics have been shown to result in high levels of biparental

158 inbreeding in small, isolated or recently founded *S. latifolia* populations (Fields & Taylor,

159 2014; Richards, 2000).

160 In its native range (Eurasia), *S. latifolia* is attacked by three specialist enemies: *Hadena*

161 *bicruris* Hufn. (Noctuidae) - a noctuid moth that is a specialist pollinator (adult) and a seed

162 predator (larva) at the same time; *Microbotryum violaceum* (Pers.) G. Deml & Oberw.

163 (Mycrobotryaceae) - a systemic sterilizing fungus; and *Brachycaudus lychnidis* L. (Aphididae)

164 - an aphid that causes flowers to abort due to phloem-feeding (Wolfe, 2002). Moreover,

165 native populations are attacked by various leaf- and flower feeding generalist herbivores,

166 including slugs (mainly *Arion lusitanicus* Mabille (Arionidae)), beetles, thrips, caterpillars

167 (often *Mamestra brassicae* L. (Noctuidae)) and leaf miners as well as by several generalist

168 rust and mildew fungi (Schrieber et al. 2017). In the invaded range (North America), *H.*

169 *bicruris* is completely absent (Wolfe, 2002), the occurrence of *M. violaceum* is locally

170 restricted to a small region in Virginia (Antonovics, Hood, Thrall, Abrams, & Duthie, 2003),

171 and the abundance of aphids as well as leaf and flower feeding generalists is very low

172 relative to the native range (Wolfe, 2002). Invasive *S. latifolia* populations exhibit higher

173   growth and reproduction as well as higher susceptibility to enemy infestation relative to

174   native populations, which can be attributed to both adaptive and non-adaptive evolutionary

175   processes (Blair & Wolfe 2004; Wolfe et al. 2004; Keller et al. 2009; Schrieber et al. 2017). A

176   trade-off between growth/reproduction and enemy susceptibility was not detected in this

177   species (Schrieber et al. 2017).

178   <u>Field sampling and experimental setup</u>

179   We collected open-pollinated seeds from eight native and eight invasive *S. latifolia*

180   populations (Supporting Information Fig. S1, Table S2). Sampling in the native range

181   comprised the geographic source regions of introduction (broadly, eastern and western

182   Europe), while sampling in the invasive range comprised the geographic regions of initial

183   introduction and early expansion (eastern North America), as identified by Taylor & Keller,

184   (2007) and Keller et al. (2012). Within each population, we sampled one capsule (maternal

185   family) from each of five different female plants that were equally distributed over the

186   population area and spatially separated from each other as far as possible. Using these

187   maternal families, we conducted two generations of experimental inbreeding and outbreeding

188   within all native and invasive populations under controlled greenhouse conditions. The

189   offspring were exposed to the absence and presence of natural enemies in a common

190   garden in the species' native range. Data for the outbred plants from this experiment have

191   previously been used to investigate adaptive and non-adaptive differentiation in growth,

192   reproduction and enemy susceptibility between the native and invaded range (Schrieber et

193   al., 2017).

194   <u>Experimental inbreeding and outbreeding</u>

195   For the P-generation, we germinated ten seeds from each of the five field-collected families

196   in 0.8 mM Giberellic acid in a germination chamber (16 h light at 25 °C, 8 h dark at 13 °C).

197   After six days, the seedlings were planted into pots and transferred to the greenhouse (16 h

198   light at 25 °C, 8 h dark at 13 °C) where they received weekly fertilization (Kamasol Brilliant

199   Rot, Compo Expert, Münster, GE). After seven weeks, we randomly chose one male and one

200   female plant per family for crosses. Each female received pollen from a sib male belonging to

201   the same family (inbreeding), and pollen from a male belonging to a different family within the

202   same population (outbreeding) at distinct flowers (Fig. 1). The crossing of the P-generation

203   resulted in 160 population (N = 16) × family (N = 5) × breeding treatment (N = 2)

204   combinations (PFBCs). For the second generation, we randomly chose one capsule per PFB

205   and propagated the F1-plants from its seeds as described for the P-generation. Female

206   inbred offspring received pollen from an inbred male from the same family, while female

207   outbred offspring received pollen from an outbred male from a different family with respect to

208   the relationships created in the first generation (Fig. 1). We lost seven of the 160 PFBCs due

209   to lack of germination, high mortality, lack of flowering or production of sterile flowers in both

210   inbred and outbred families during the propagation of the F1-generation. Consequently, we

211   obtained a total of 153 PFBCs for the F2-generation plants, which were used for the enemy

212   release experiment.

213   Enemy release experiment

214   We exposed native and invasive, inbred and outbred *S. latifolia* plants from the F2-

215   generation to an enemy exclusion and an enemy inclusion treatment using a fully factorial

216   experimental approach (16 populations [8 native *versus* 8 invasive] × 4-5 families × 2

217   breeding treatments [inbred *versus* outbred] × 2 enemy treatments [exclusion *versus*

218   inclusion] × 8 replicates = 1,224 plants). In early spring, we germinated eight seeds

219   originating from one capsule per PFBC and reared the F2-plants for six weeks in a common

220   garden in Halle (Saale), Germany (51.489 °N 11.959 °E alt: 88 m). After six weeks, we

221   moved the plants to the UFZ Research Station in Bad Lauchstädt, Germany (51.391°N,

222   11.878°E, alt: 116 m). The planting area was densely covered by a diverse plant community

223   of grasses and forbs including a patchy population of *S. latifolia* that was infested by all of the

224   above-mentioned specialist and generalist enemies. In the common garden, we established

225   four vegetation-free belts, which comprised four plots respectively (∑ = 16 plots) (Fig. 2).

226   Each plot included all native and invasive populations represented by two to three F2

227   maternal families each with one inbred and one outbred individual. As such, the five families

228   within each population were split between two plots (plot pair), which together comprised all

229   of the 153 PFBCs. Each plot pair was replicated an additional seven times. While

230   populations and families were planted randomly within the plots, the range and breeding

231   treatments were uniformly distributed according to a fixed scheme (Fig. 2) in order to reduce

232   confounding plot edge effects. Plots within pairs and plot pair repetitions were randomly

233   distributed across the experimental area. We experimentally excluded natural enemies in

234   eight of the plots (enemy exclusions) over a period of three months (Fig. 2). For this purpose,

235   we used slug fences coated with a gastropod deterrent (Schneckenabwehrpaste, Irka,

236   Mietingen, GE), as well as a molluscicide (Limex, Celaflor), systemic insecticides (alternating

237   between Calypso and Confidor, Bayer, Leverkusen, GE) and a systemic universal fungicide

238   (Baycor M, Bayer, Leverkusen, GE), which were applied in a two-week cycle in accordance

239   with the manufacturers instructions. The remaining eight plots (enemy inclusions) were not

240   treated with pesticides and therefore extensively colonized by specialist and generalist

241   herbivores two weeks after the experiment was set up. The removal of vegetation however

242   deterred *A. lusitanicus* from entering the inclusion plots, so we equipped them with slug

243   fences whose impassable sides were turned towards the plot interior and introduced 15 *A.*

244   *lusitanicus* individuals to each plot. We adjusted the number of slugs within each inclusion

245   plot to 15 three times a week. The infection with specialist and generalist fungi remained low

246   in all inclusion plots for the entire experimental period. All plots were weeded weekly and

247   watered when necessary during the experiment.

248   After three months of enemy treatment application, we collected data on morphological

249   defense and infestation damage for each plant in the enemy inclusion plots. We collected

250   leaves at similar stages of development to determine trichome density in a 5 × 5 mm area

251   away from the main vein and at the broadest section of the leaf. In addition, we determined

252   the proportion of flowers (including buds) damaged by tissue removal (generalist herbivores)

253   or phloem sucking (*B. lychnidis)*, the proportion of fruits predated by *H. bicruris* larvae and

254 the proportion of fully grown leaves infested by generalist herbivores (mainly *A. lusitanicus*

255 and *M. brassicae*). Data on infection rates with the specialist fungus *M. violoceaum* and other

256 generalist fungi were not included in the data analysis, as the abundance of these pathogens

257 was generally very low. Furthermore, we collected data on plant growth and reproduction in

258 both enemy inclusion and exclusion plots. We measured the corolla diameter of the biggest

259 flower and counted the number of flowers (including buds) for all male and female plants.

260 Moreover, we determined the number of fruits for all female plants. Seed number, weight and

261 germination were not assessed, since seeds resulted from uncontrolled crossings among

262 plants from all populations, both ranges and both breeding treatments. Finally, we

263 determined the dry aboveground biomass (48 h, 80 °C) for all plant individuals.

264 Statistical analysis

265 All statistical analyses were conducted with R version 3.2.3 (R Development Core Team,

266 2015). We used linear mixed-effects models (LMMs) for response variables with Gaussian

267 error distribution and generalized linear mixed-effects models (GLMMs) for response variables

268 with Poisson or binomial errors (R-package: lme4; Bates, Maechler, Bolker, & Walker, 2014).

269 The models for the responses trichome density (LMM, Gaussian, square-root transformed),

270 leaf damage (GLMM, binomial), flower damage (GLMM, binomial) and fruit damage (GLMM,

271 binomial) (all assessed in enemy inclusion plots only) comprised the fixed effects of range and

272 breeding treatment as well as an interaction among both factors. The models for the

273 responses biomass (LMM, Gaussian, square-root transformed), corolla size (LMM, Gaussian),

274 number of flowers (GLMM, Poisson) and number of fruits (GLMM, Poisson) (all assessed in

275 both enemy exclusions and inclusions) comprised the fixed effects of range, breeding

276 treatment and enemy treatment as well as all possible interactions among these factors. All of

277 the described models additionally involved the latitudinal coordinates of the population of origin

278 (centered and scaled) and plant sex (except for fruit damage and number of fruits) as

279 covariates. Moreover, all models included the random effects of plot, population and paternal

13

280 plant in P-generation nested within population as well as maternal plant in P-generation nested

281 within population.

282 All models were fitted with a maximum likelihood approach. GLMMs were then tested for

283 under- and overdispersion (R-package: blemco, Korner-Nievergelt et al. 2015). The GLMMs

284 for leaf damage and number of flowers were overdispersed and consequently complemented

285 by an observational level random factor in order to improve the model fit and avoid biased

286 parameter estimates (Harrison, 2014; 2015). After assuring that (G)LMMs exhibit variance

287 homogeneity and normal distribution of residuals by means of visual inspection (Zuur, Ieno,

288 Walker, Saviliev, & Smith, 2009), we applied step-wise backward model selection to obtain the

289 minimal adequate models. Here, we removed fixed effect terms with $p > 0.05$ based on

290 likelihood ratio tests (Venables & Ripley, 2000). For illustration of the interactive effects of

291 range, breeding treatment and enemy treatment on plant performance responses, we

292 extracted least square means with standard errors from the respective full mixed effects

293 models (Lenth, 2016). In contrast to raw data means and their standard errors, these model

294 estimates account for the specific error distribution of the responses, for the effects of

295 covariates as well as for random effects.

296 **Results**

297 Interactive effects of range and breeding treatment on morphological plant defense and

298 infestation damage

299 The density of leaf trichomes was not significantly influenced by range, breeding treatment, the

300 interaction range × breeding treatment or one of the covariates (Table 1, Fig. 3a). The

301 proportion of damaged leaves was significantly related to range and breeding treatment (Table

302 1). Invasive plants experienced more leaf damage compared to native plants ($p < 0.05$, $\chi^2 =$

303 5.4) and inbred plants from both distribution ranges suffered stronger from leaf infestation

304 compared to outbreds ($p < 0.001$, $\chi^2 = 41.7$) (Fig. 3b). The proportion of damaged flowers

305 depended significantly on range, breeding treatment and the covariate sex (Table 1). Flower

306   infestation was higher for invasive than native ($p < 0.01$, $\chi^2 = 6.8$), inbred than outbred ($p <$

307   0.001, $\chi^2 = 41.0$) (Fig. 3c) and male than female plants ($p < 0.05$, $\chi^2 = 5.2$). The proportion of

308   damaged fruits was significantly influenced by the interaction range $\times$ breeding treatment ($p <$

309   0.05, $\chi^2 = 4.1$). Here, invasive plants received generally more fruit damage than native plants

310   and fruit infestation was higher on inbred than outbred native plants but lower on inbred than

311   outbred invasive plants (Fig. 3d).

312   Interactive effects of range, breeding treatment and enemy treatment on plant growth and

313   reproduction

314   The aboveground biomass of experimental plants was significantly related to the interaction

315   range $\times$ enemy treatment, to breeding treatment and to plant sex (Table 1, Fig. 4a). Plants

316   exhibited reduced biomass in enemy inclusions relative to exclusions, whereby this effect

317   was stronger in invasive than native populations ($p < 0.05$, $\chi^2 = 4.8$). Inbred plants produced

318   significantly less biomass compared to outbred plants ($p < 0.001$, $\chi^2 = 116.6$) and female

319   plants had higher biomass than males ($p < 0.001$, $\chi^2 = 44.5$). Range, breeding treatment and

320   enemy treatment had no significant interactive effects on the corolla diameter of *S. latifolia*

321   plants (Table 1). Instead, corolla size was generally lower for inbred than outbred ($p < 0.001$,

322    $\chi^2 = 54.5$) (Fig. 4b) and female than male plants ($p < 0.001$, $\chi^2 = 41.4$). The number of flowers

323   per plant individual was distinctively lower for inbred than outbred ($p < 0.001$, $\chi^2 = 24.5$) (Fig.

324   4c) and female than male plants ($p < 0.001$, $\chi^2 = 133.5$). The number of fruits produced by

325   female plants depended significantly on the two-way interactions range $\times$ breeding treatment

326   and breeding treatment $\times$ enemy treatment (Table 1, Fig. 4d). Invasive plants produced more

327   fruits than native plants in both breeding and enemy treatments. Moreover, inbred plants had

328   less fruits than outbred plants in both enemy treatments and in populations from both

329   distribution ranges. This inbreeding depression was less intense in invasive than native

330   populations ($p < 0.05$, $\chi^2 = 5.9$) and stronger in enemy inclusions than exclusions ($p < 0.05$,

331    $\chi^2 = 4.1$).

Discussion

Our study provides support that I×E interactions can contribute to successful plant invasion and that these interactions are shaped by the evolutionary histories of plant populations. Here, we discuss a) that inbreeding increases enemy infestation damage, which in turn magnifies inbreeding depression in *S. latifolia* plants from both distribution ranges; and b) that some of the inbreeding effects on infestation damage and reproductive traits differ in their magnitude and even in their direction among native and invasive plants as a result of non- adaptive/ adaptive evolutionary processes.

Enemy release mitigates inbreeding depression in native and invasive *S. latifolia* plants

In accordance with our hypothesis, inbred *S. latifolia* plants from both distribution ranges for the most part incurred higher infestation damage from natural enemies in the common garden than outbreds (Fig. 3b, c; but see Fig. 3d and discussion in next section). Plants often exhibit increased susceptibility to enemies following inbreeding (Bello-Bedoy & Núñez-Farfán, 2011; Campbell, Thaler, & Kessler, 2013; Kariyat, Mena-Alí, et al., 2012), since dominance and over-dominance can either affect gene-loci that contribute directly to plant resistance against enemies (Kariyat, Mena-Alí, et al., 2012) or induce general stress responses that trade-off against responses to environmental stressors such as natural enemies (Kristensen et al., 2010). Using the same inbred and outbred families of native and invasive *S. latifolia* populations investigated in the present study, Schrieber, Kröner, Schweiger and Müller (in press) demonstrated that inbreeding significantly compromises the plants' chemical responses to insect herbivory. That study also indicated that higher infestation damage on inbred *S. latifolia* individuals can result from compensatory feeding triggered by poor host plant quality. Previous studies on other plant species also demonstrated that inbreeding reduces the concentration of chemicals mediating direct (Campbell et al., 2013) and indirect (Kariyat, Mauck, De Moraes, Stephenson, & Mescher, 2012) plant defense as well as host plant quality (Leimu, Kloss, & Fischer, 2008).

359   In line with our expectation, both inbreeding and enemy infestation reduced the growth and

360   reproduction of *S. latifolia* in native and invasive populations, whereby inbreeding had a

361   pronounced impact while the effect of enemy infestation was more moderate (Fig. 4). As

362   hypothesized, the effects of breeding and enemy treatment were not purely additive. While the

363   magnitude of inbreeding depression was independent of the enemy treatment for biomass,

364   corolla diameter and flower number (Fig 4a, b, c), it was significantly lower in enemy

365   exclusions than inclusions for fruit number in both distribution ranges (Fig. 4d). The

366   observation that inbreeding depression in traits closely linked to individual fitness increases

367   under stress, while morphological traits that are only indirectly related to reproductive success

368   (i.e., biomass, corolla diameter) are not significantly affected by I×E interactions, has also

369   been made in previous studies (Bello-Bedoy & Núñez-Farfán, 2011; Schou, Loeschcke, &

370   Kristensen, 2015). This difference can indeed be expected, since the investment in

371   reproduction by the end of a growing season is highly dependent on an individual's cumulative

372   performance and thus cumulative (interactive) effects of inbreeding and herbivory on

373   performance throughout the season (Orr, 2009). Moreover, in contrast to flower number, fruit

374   number in experimental *S. latifolia* plants was affected by all three types of infestation damage

375   (leaf, flower and fruit infestation) and thus pressure by natural enemies was highest for this

376   fitness related trait. Our finding indicates that the enemy release in the invaded habitat may

377   mitigate detrimental inbreeding effects on reproductive output in colonizing *S. latifolia*

378   populations. The I×E interactions detected for native populations under experimental

379   conditions may be representative of a scenario of initial population founding and establishment

380   during early invasion phases, in which plants are naive to the novel environment. I×E

381   interactions in the investigated invasive plants, in turn, may represent a scenario of population

382   founding at the leading edge, where populations have already undergone evolutionary

383   responses to the environment of the introduced range.

384   Our findings of lower inbreeding depression in benign *versus* harsh habitats in a plant

385   invader go along with those of two previous studies on *Mimulus guttatus* DC. (Murren &

386   Dudash, 2012) and *Centaurea stoebe* L. (Rosche et al., 2017), which together emphasize the

387   relevance of I×E interactions for species expansions and the need for further research under

388   more realistic field conditions. The transplantation of inbred and outbred plants to native as

389   well as invasive field habitats is necessary to assess the net effect of multiple stressors

390   occurring in both environments on the magnitude of inbreeding depression. Ideally, such

391   approaches should involve the quantification of demographic rates in order to parameterize

392   models that estimate population growth and/or spread rates (Normand, Zimmermann, Schurr,

393   & Lischke, 2014; Schultz, Eckberg, Berg, Louda, & Miller, 2017). Studies of this kind could

394   further elaborate whether and to what extent I×E interactions add to several other mechanisms

395   (e.g., genetic admixture, mass introductions, Estoup et al., 2016; Roman & Darling, 2007) that

396   can explain the successful spread of invaders in the face of genetic bottlenecks, i.e. the so-

397   called genetic paradox of biological invasions (Schrieber & Lachmuth, 2017).

398   <u>Evolutionary history modifies the magnitude and direction of inbreeding effects on plant</u>

399   <u>interactions with natural enemies</u>

400   We detected evolutionary differentiation in plant susceptibility to enemy infestation (Fig. 3b, c,

401   d) and plant performance among native and invasive populations of *S. latifolia* (Fig. 4d). This

402   observation has also been made in previous studies of *S. latifolia* (Blair & Wolfe, 2004; Keller

403   et al., 2009; Schrieber et al., 2017; Wolfe et al., 2004), where it has been discussed in detail

404   with regard to the driving evolutionary forces (i.e. adaptive *versus* non-adaptive evolution,

405   potential selective agents, trade-offs).

406   However, new to our study is the finding that the magnitude and even the direction of

407   inbreeding effects on some metrics of infestation damage and reproductive traits differed

408   among native and invasive *S. latifolia* populations. While inbreeding slightly increased fruit

409   damage in native plants, the proportion of fruits infested by *H. bicruris* was considerably lower

410   on inbreds than outbreds for invasive plants. At the same time, fruit infestation was generally

411   higher on invasive plants (Fig. 3d). This finding may be attributed to the combined effects of

412   genetic differentiation and inbreeding on host plant attractivity. Previous studies elaborated

413   that higher fruit infestation by *H. bicruris* on invasive *S. latifolia* does not result from higher

414   larva performance, but from increased oviposition rates (Elzinga & Bernasconi, 2009). Adult *H.*

415   *bicruris* females are attracted to oviposition on *S. latifolia* by a specific floral volatile blend,

416   whereby invasive plants emit larger total amounts of these volatiles (Dötterl, Jürgens, Wolfe, &

417   Biere, 2009). Based on studies using other plant species (Delphia, Rohr, Stephenson,

418   De Moraes, & Mescher, 2009; Ferrari, Stephenson, Mescher, & Moraes, 2006), one possibility

419   is that inbreeding reduced the total floral volatile production in *S. latifolia* either directly by

420   impairing the synthesis of volatiles or by reducing flower number (Fig 4c, enemy exclusions),

421   which may have made inbred plants less attractive hosts for oviposition. This potential

422   inbreeding effect on host plant attractivity may have been only apparent in invasive

423   populations due to a specific volatile concentration threshold for the plant's apparency to *H.*

424   *bicruris*. Further studies on the inbreeding effects on the composition and concentration of

425   floral volatiles are necessary to test this assumption.

426   In addition, we found that inbreeding depression for fruit number was less pronounced in

427   introduced relative to native populations (Fig. 4d). Differences in the magnitude of

428   experimental inbreeding depression among populations have often been related to the history

429   of inbreeding within natural source populations (Angeloni et al., 2011). Inbreeding depression

430   is assumed to be lower in populations that have experienced high levels of natural inbreeding,

431   as a result of exposing deleterious recessive mutations to negative selection. As a

432   consequence, the frequency of these mutations within populations can rapidly decrease (i.e.

433   purging of genetic load; Crnokrak & Barrett, 2002), which reduces dominance effects resulting

434   from experimental inbreeding. Invasive populations of *S. latifolia* indeed experienced

435   increased inbreeding levels during the colonization of North America, as evinced by inter- and

436   intra population crossing experiments (Richards, 2000), enhanced genetic structure in recently

437   founded compared to longer established populations (McCauley, Raveill, & Antonovics, 1995)

438   and the occurrence of severe demographic bottlenecks during initial founding (Keller et al.,

439   2012; Taylor & Keller, 2007). Since reproductive traits such as fruit production are crucial for

440   invasion success (Burns, Ashman, Steets, Harmon-Threatt, & Knight, 2011; Phillips, Brown, &

441 Shine, 2010), strong negative selection against genetic load may have induced highly efficient

442 purging under demographic conditions of colonization and invasion.

443 **Conclusions**

444 Our findings indicate that stress release during invasions may mitigate inbreeding depression

445 in founding populations, which potentially contributes to the successful establishment and

446 expansion of introduced populations. On the other hand, I×E interactions may hamper the

447 colonization of novel habitats that exhibit increased stress levels relative to a species' native

448 source habitat (Hufbauer et al., 2013). Furthermore, our data illustrate that the inbreeding

449 effects on an organism's interaction with its environment are likely shaped by the evolutionary

450 histories of populations. As the native and invaded range of a species can differ systematically

451 in the stress regimes they experience, ongoing invasions provide ideal study systems for

452 investigating the effects of evolutionary differentiation on the outcomes of I×E interactions, and

453 how, in turn, the different outcomes may alter the evolutionary trajectories of invasive

454 populations. Studies addressing these issues hold implications that extend far beyond invasive

455 model species. I×E interactions may potentially shape the dynamics of natural populations

456 whenever they are simultaneously exposed to habitat change and increased inbreeding rates

457 following founder effects or population size reductions. These conditions occur not only during

458 species range expansions, but also during range shifts and retractions in the course of global

459 change (Colautti et al., 2017).

470   **Author contributions**

471   SL and KS conceptualized the research and designed the study. KS conducted the field

472   sampling, the experimental crossings and the enemy release experiment and collected the

473   data with assistance of CW, DH and SW. KS analyzed the data. KS and SL interpreted the

474   data. KS wrote the first version of the manuscript and SL, SK and IH contributed to the final

475   version.

476   **References**

477   Agrawal, A. A., Hastings, A. P., Bradburd, G. S., Woods, E. C., Züst, T., Harvey, J. A., &

478   Bukovinszky, T. (2015). Evolution of plant growth and defense in a continental

479   introduction. *The American Naturalist*, *186*(1), E1–E15.

480   doi:http://doi.org/10.1086/681622

481   Angeloni, F., Ouborg, N. J., & Leimu, R. (2011). Meta-analysis on the association of

482   population size and life history with inbreeding depression in plants. *Biological*

483   *Conservation*, *144*(1), 35–43. doi:10.1016/j.biocon.2010.08.016

484   Antonovics, J., Hood, M. E., Thrall, P. H., Abrams, J. Y., & Duthie, G. M. (2003). Herbarium

485   studies on the distribution of anther-smut fungus (*Microbotryum violaceum*) and

486   *Silene* species (Caryophyllaceae) in the eastern United States. *American Journal of*

487   *Botany*, *90*(10), 1522–1531. doi:10.3732/ajb.90.10.1522

21

488   Barrett, S. C. H. (2015). Foundations of invasion genetics: the Baker and Stebbins legacy.

489   *Molecular Ecology*, *24*(9), 1927–1941. doi:10.1111/mec.13014

490   Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). lme4: Linear mixed-effects models

491   using Eigen and S4. R package version 1.1-7. Retrieved from URL: http://CRAN. R-

492   project. org/package= lme4 (2014).

493   Bello-Bedoy, R., & Núñez-Farfán, J. (2011). The effect of inbreeding on defence against

494   multiple enemies in *Datura stramonium*. *Journal of Evolutionary Biology*, *24*(3), 518–

495   530. doi:10.1111/j.1420-9101.2010.02185.x

496   Blair, A. C., & Wolfe, L. M. (2004). The evolution of an invasive plant: an experimental study

497   with *Silene latifolia*. *Ecology*, *85*(11), 3035–3042. doi:10.1890/04-0341

498   Blumenthal, D. M. (2006). Interactions between resource availability and enemy release in

499   plant invasion. *Ecology Letters*, *9*(7), 887–895. doi:10.1111/j.1461-

500   0248.2006.00934.x

501   Bundy, J. G., Davey, M. P., & Viant, M. R. (2008). Environmental metabolomics: a critical

502   review and future perspectives. *Metabolomics*, *5*(1), 3–21. doi:10.1007/s11306-008-

503   0152-0

504   Burns, J. H., Ashman, T.-L., Steets, J. A., Harmon-Threatt, A., & Knight, T. M. (2011). A

505   phylogenetically controlled analysis of the roles of reproductive traits in plant

506   invasions. *Oecologia*, *166*(4), 1009–1017. doi:10.1007/s00442-011-1929-9

507   Campbell, S. A., Thaler, J. S., & Kessler, A. (2013). Plant chemistry underlies herbivore-

508   mediated inbreeding depression in nature. *Ecology Letters*, *16*(2), 252–260.

509   doi:10.1111/ele.12036

510   Carr, D. E., & Eubanks, M. D. (2002). Inbreeding alters resistance to insect herbivory and

511   host plant quality in *Mimulus guttatus* (Scrophulariaceae). *Evolution*, *56*(1), 22–30.

512   doi:10.1554/0014-3820(2002)056[0022:IARTIH]2.0.CO

513   Catford, J. A., Jansson, R., & Nilsson, C. (2009). Reducing redundancy in invasion ecology

514   by integrating hypotheses into a single theoretical framework. *Diversity and*

515   *Distributions*, *15*(1), 22–40. doi:10.1111/j.1472-4642.2008.00521.x

516   Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature*

517   *Reviews Genetics*, *10*(11), 783–796. doi:10.1038/nrg2664

518   Colautti, R. I., Alexander, J. M., Dlugosch, K. M., Keller, S. R., & Sultan, S. E. (2017).

519   Invasions and extinctions through the looking glass of evolutionary ecology.

520   *Philosophical Transaction of the Royal Society B: Biological Sciences, 372*(1712),

521   20160031. doi:10.1098/rstb.2016.0031

522   Colautti, R. I., & Barrett, S. C. H. (2013). Rapid adaptation to climate facilitates range

523   expansion of an invasive plant. *Science*, *342*(6156), 364–366.

524   doi:10.1126/science.1242121

525   Crnokrak, P., & Barrett, S. C. (2002). Perspective: purging the genetic load: a review of the

526   experimental evidence. *Evolution, 56*(12), 2347–2358. doi:10.1111/j.0014-

527   3820.2002.tb00160.x

528   Delphia, C. M., Rohr, J. R., Stephenson, A. G., De Moraes, C. M., & Mescher, M. C. (2009).

529   Effects of genetic variation and inbreeding on volatile production in a field population

530   of horsenettle. *International Journal of Plant Sciences*, *170*(1), 12–20.

531   doi:10.1086/593039

532   Dietz, H., & Edwards, P. J. (2006). Recognition that causal processes change during plant

533   invasion helps explain conflicts in evidence. *Ecology*, *87*(6), 1359–1367.

534   doi:10.1890/0012-9658(2006)87[1359:RTCPCD]2.0.CO;2

535   Dötterl, S., Jürgens, A., Wolfe, L., & Biere, A. (2009). Disease status and population origin

536   effects on floral scent: potential consequences for oviposition and fruit predation in a

537   complex interaction between a plant, fungus, and noctuid moth. *Journal of Chemical*

538   *Ecology, 35*(3), 307–319. doi:10.1007/s10886-009-9601-0

539   Elzinga, J. A., & Bernasconi, G. (2009). Enhanced frugivory on invasive *Silene latifolia* in its

540   native range due to increased oviposition. *Journal of Ecology*, *97*(5), 1010–1019.

541   doi:10.1111/j.1365-2745.2009.01534.x

542   Enders, L. S., & Nunney, L. (2016). Reduction in the cumulative effect of stress-induced

543   inbreeding depression due to intragenerational purging in *Drosophila melanogaster*.

544   *Heredity*, *116*(3), 304–313. doi:10.1038/hdy.2015.103

545   Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is There a

546   Genetic Paradox of Biological Invasion? *Annual Review of Ecology, Evolution, and*

547   *Systematics*, *47*(1), 51–72. doi:10.1146/annurev-ecolsys-121415-032116

548   Ferrari, M. J., Stephenson, A. G., Mescher, M. C., & Moraes, C. M. D. (2006). Inbreeding

549   effects on blossom volatiles in *Cucurbita pepo* subsp. texana (Cucurbitaceae).

550   *American Journal of Botany*, *93*(12), 1768–1774. doi:10.3732/ajb.93.12.1768

551   Fields, P. D., & Taylor, D. R. (2014). Determinants of genetic structure in a nonequilibrium

552   metapopulation of the plant *Silene latifolia*. *PLOS ONE*, *9*(9), e104575.

553   doi:10.1371/journal.pone.0104575

554   Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental

555   stress: an experimental study and meta-analysis. *Evolution*, *65*(1), 246–258.

556   doi:10.1111/j.1558-5646.2010.01108.x

557   Harrison, X. A. (2014). Using observation-level random effects to model overdispersion in

558   count data in ecology and evolution. *PeerJ*, *2*, e616. doi:10.7717/peerj.616

559   Harrison, X. A. (2015). A comparison of observation-level random effect and Beta-Binomial

560   models for modelling overdispersion in binomial data in ecology & evolution. *PeerJ*, *3*,

561   e1114. doi:10.7717/peerj.1114

562   Hayes, C. N., Winsor, J. A., & Stephenson, A. G. (2004). Inbreeding influences herbivory in

563   *Cucurbita pepo* ssp. texana (Cucurbitaceae). *Oecologia*, *140*(4), 601–608.

564   doi:10.1007/s00442-004-1623-2

565   Hufbauer, R. A., Rutschmann, A., Serrate, B., Vermeil de Conchard, H., & Facon, B. (2013).

566   Role of propagule pressure in colonization success: disentangling the relative

567   importance of demographic, genetic and habitat effects. *Journal of Evolutionary*

568   *Biology*, *26*(8), 1691–1699. doi:10.1111/jeb.12167

24

569   Hufbauer, R. A. (2008). Biological invasions: paradox lost and paradise gained. *Current*

570   *Biology*, *18*(6), R246–R247. doi:10.1016/j.cub.2008.01.038

571   Kariyat, R. R., Mauck, K. E., De Moraes, C. M., Stephenson, A. G., & Mescher, M. C. (2012).

572   Inbreeding alters volatile signalling phenotypes and influences tri-trophic interactions

573   in horsenettle (*Solanum carolinense* L.). *Ecology Letters*, *15*(4), 301–309.

574   doi:10.1111/j.1461-0248.2011.01738.x

575   Kariyat, R. R., Mena-Alí, J., Forry, B., Mescher, M. C., Moraes, C. M., & Stephenson, A. G.

576   (2012). Inbreeding, herbivory, and the transcriptome of *Solanum carolinense*.

577   *Entomologia Experimentalis et Applicata*, *144*(1), 134–144. doi:10.1111/j.1570-

578   7458.2012.01269.x

579   Keane, R. M., & Crawley, M. J. (2002). Exotic plant invasions and the enemy release

580   hypothesis. *Trends in Ecology & Evolution*, *17*(4), 164–170. doi:10.1016/S0169-

581   5347(02)02499-0

582   Keller, S. R., Gilbert, K. J., Fields, P. D., & Taylor, D. R. (2012). Bayesian inference of a

583   complex invasion history revealed by nuclear and chloroplast genetic diversity in the

584   colonizing plant *Silene latifolia. Molecular Ecology*, *21*(19), 4721–4734.

585   doi:10.1111/j.1365-294X.2012.05751.x

586   Keller, S. R., Sowell, D. R., Neiman, M., Wolfe, L. M., & Taylor, D. R. (2009). Adaptation and

587   colonization history affect the evolution of clines in two introduced species. *New*

588   *Phytologist*, *183*(3), 678–690. doi:10.1111/j.1469-8137.2009.02892.x

589   Keller, S. R., & Taylor, D. R. (2008). History, chance and adaptation during biological

590   invasion: separating stochastic phenotypic evolution from response to selection.

591   *Ecology Letters*, *11*(8), 852–866. doi:10.1111/j.1461-0248.2008.01188.x

592   Klopfstein, S., Currat, M., & Excoffier, L. (2006). The fate of mutations surfing on the wave of

593   a range expansion. *Molecular Biology and Evolution*, *23*(3), 482–490.

594   doi:10.1093/molbev/msj057

25

595   Korner-Nievergelt, F., Roth, T., Felten, S., Guelat, J., Almasi, B., & Korner-Nievergelt, P.

596   (2015). Bayesian data analysis in ecology using linear models with R. *Elsevier*.

597   Retrieved from https://www.R-project.org/package=blmeco

598   Kristensen, T. N., Pedersen, K. S., Vermeulen, C. J., & Loeschcke, V. (2010). Research on

599   inbreeding in the 'omic' era. *Trends in Ecology & Evolution*, *25*(1), 44–52.

600   doi:10.1016/j.tree.2009.06.014

601   Lachmuth, S., Durka, W., & Schurr, F. M. (2011). Differentiation of reproductive and

602   competitive ability in the invaded range of *Senecio inaequidens*: the role of genetic

603   Allee effects, adaptive and nonadaptive evolution. *New Phytologist*, *192*(2), 529–541.

604   doi:10.1111/j.1469-8137.2011.03808.x

605   Leimu, R., Kloss, L., & Fischer, M. (2008). Effects of experimental inbreeding on herbivore

606   resistance and plant fitness: the role of history of inbreeding, herbivory and abiotic

607   factors. *Ecology Letters*, *11*(10), 1101–1110. doi:10.1111/j.1461-0248.2008.01222.x

608   Leimu, R., Vergeer, P., Angeloni, F., & Ouborg, N. J. (2010). Habitat fragmentation, climate

609   change, and inbreeding in plants. *Annals of the New York Academy of Sciences*,

610   *1195*(1), 84–98. doi:10.1111/j.1749-6632.2010.05450.x

611   Lenth, R. V. (2016). Least-squares means: the R package lsmeans. *Journal of Statistical

612   Software*, *69*(1), 1–33. doi:10.18637/jss.v069.i01

613   Maron, J. L., & Crone, E. (2006). Herbivory: effects on plant abundance, distribution and

614   population growth. *Proceedings of the Royal Society of London B: Biological

615   Sciences*, *273*(1601), 2575–2584. doi:10.1098/rspb.2006.3587

616   McCauley, D. E. (1997). The relative contributions of seed and pollen movement to the local

617   genetic structure of *Silene alba*. *Journal of Heredity*, *88*(4), 257–263. doi:88/4/257.1.x

618   McCauley, D. E. (1994). Contrasting the distribution of chloroplast DNA and allozyme

619   polymorphism among local populations of *Silene alba*: implications for studies of gene

620   flow in plants. *Proceedings of the National Academy of Sciences*, *91*(17), 8127–8131.

621   McCauley, D. E., Raveill, J., & Antonovics, J. (1995). Local founding events as determinants

622   of genetic structure in a plant metapopulation. *Heredity*, *75*(6), 630–636.

623   doi:doi:10.1038/hdy.1995.182

624   Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N.,

625   … Vázquez, D. P. (2006). Biotic interactions and plant invasions. *Ecology Letters*,

626   *9*(6), 726–740. doi:10.1111/j.1461-0248.2006.00908.x

627   Murren, C. J., & Dudash, M. R. (2012). Variation in inbreeding depression and plasticity

628   across native and non-native field environments. *Annals of Botany*, *109*(3), 621–632.

629   doi:10.1093/aob/mcr325

630   Normand S., Zimmermann N. E., Schurr F. M., & Lischke H. (2014). Demography as the

631   basis for understanding and predicting range dynamics. *Ecography*, *37*(12), 1149–

632   1154. doi:10.1111/ecog.01490

633   Orians, C. M., & Ward, D. (2010). Evolution of plant defenses in nonindigenous

634   environments. *Annual Review of Entomology*, *55*(1), 439–459. doi:10.1146/annurev-

635   ento-112408-085333

636   Orr, H. A. (2009). Fitness and its role in evolutionary genetics. *Nature Reviews Genetics*,

637   *10*(8), 531–539. doi:10.1038/nrg2603

638   Phillips, B. L., Brown, G. P., & Shine, R. (2010). Life-history evolution in range-shifting

639   populations. *Ecology*, *91*(6), 1617–1627. doi:10.1890/09-0910.1

640   R Development Core Team. (2015). R: a language and environment for statistical computing.

641   *R Foundation for Statistical Computing*. Retrieved from https://www.R-project.org/

642   Reed, D. H., Briscoe, D. A., & Frankham, R. (2002). Inbreeding and extinction: the effect of

643   environmental stress and lineage. *Conservation Genetics*, *3*(3), 301–307.

644   doi:10.1023/A:1019948130263

645   Reed, D. H., Fox, C. W., Enders, L. S., & Kristensen, T. N. (2012). Inbreeding–stress

646   interactions: evolutionary and conservation consequences. *Annals of the New York

647   Academy of Sciences*, *1256*(1), 33–48. doi:10.1111/j.1749-6632.2012.06548.x

648   Richards, C. M. (2000). Inbreeding depression and genetic rescue in a plant metapopulation.

649   *The American Naturalist*, *155*(3), 383–394. doi:10.1086/303324

650   Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the

651   success of colonising populations? *Trends in Ecology & Evolution*, *29*(4), 233–242.

652   doi:10.1016/j.tree.2014.02.003

653   Roman, J., & Darling, J. (2007). Paradox lost: genetic diversity and the success of aquatic

654   invasions. *Trends in Ecology & Evolution*, *22*(9), 454–464.

655   doi:10.1016/j.tree.2007.07.002

656   Rosche, C., Hensen, I., & Lachmuth, S. (2017). Local pre-adaptation to disturbance and

657   inbreeding–environment interactions affect colonisation abilities of diploid and

658   tetraploid *Centaurea stoebe*. *Plant Biology*. doi:10.1111/plb.12628

659   Sandner, T. M., & Matthies, D. (2016). The effects of stress intensity and stress type on

660   inbreeding depression in *Silene vulgaris*. *Evolution*, *70*(6), 1225–1238.

661   doi:10.1111/evo.12929

662   Sandner, T. M., & Matthies, D. (2017). Interactions of inbreeding and stress by poor host

663   quality in a root hemiparasite. *Annals of Botany*, *119*(1), 143–150.

664   doi:10.1093/aob/mcw190

665   Schou, M. F., Loeschcke, V., & Kristensen, T. N. (2015). Inbreeding depression across a

666   nutritional stress continuum. *Heredity*, *115*(1), 56–62. doi:10.1038/hdy.2015.16

667   Schrieber, K., Kröner, L., Schweiger, R. & Müller, C. (accepted for publication in Journal of

668   Ecology): Inbreeding diminishes herbivore-induced metabolic responses in native and

669   invasive plant populations.

670   Schrieber, K., & Lachmuth, S. (2017). The Genetic Paradox of Invasions revisited: the

671   potential role of inbreeding × environment interactions in invasion success. *Biological*

672   *Reviews*, *92*(2), 939–952. doi:10.1111/brv.12263

673   Schrieber, K., Wolf, S., Wypior, C., Höhlig, D., Hensen, I., & Lachmuth, S. (2017). Adaptive

674   and non-adaptive evolution of trait means and genetic trait correlations for herbivory

675   resistance and performance in an invasive plant. *Oikos*, *126*(4), 572–582.

676   doi:10.1111/oik.03781

677   Schultz, E. L., Eckberg, J. O., Berg, S. S., Louda, S. M., & Miller, T. E. X. (2017). Native

678   insect herbivory overwhelms context dependence to limit complex invasion dynamics

679   of exotic weeds. *Ecology Letters*, *20*(11), 1374–1384. doi:10.1111/ele.12833

680   Taylor, D. R., & Keller, S. R. (2007). Historical range expansion determines the phylogenetic

681   diversity introduced during contemporary species invasion. *Evolution*, *61*(2), 334–

682   345. doi:10.1111/j.1558-5646.2007.00037.x

683   Travis, J. M. J., Münkemüller, T., Burton, O. J., Best, A., Dytham, C., & Johst, K. (2007).

684   Deleterious mutations can surf to high densities on the wave front of an expanding

685   population. *Molecular Biology and Evolution*, *24*(10), 2334–2343.

686   doi:10.1093/molbev/msm167

687   Venables, W. N., & Ripley, B. D. (2000). *Modern Applied Statistics with S. Fourth Edition.*

688   New York, USA: Springer.

689   Whitney, K. D., & Gabler, C. A. (2008). Rapid evolution in introduced species, 'invasive traits'

690   and recipient communities: challenges for predicting invasive potential. *Diversity and*

691   *Distributions*, *14*(4), 569–580. doi:10.1111/j.1472-4642.2008.00473.x

692   Wolfe, L. M. (2002). Why alien invaders succeed: support for the escape-from-enemy

693   hypothesis. *The American Naturalist*, *160*(6), 705–711. doi:10.1086/343872

694   Wolfe, L. M., Elzinga, J. A., & Biere, A. (2004). Increased susceptibility to enemies following

695   introduction in the invasive plant *Silene latifolia*. *Ecology Letters*, *7*(9), 813–820.

696   doi:10.1111/j.1461-0248.2004.00649.x

697   Zhang, D.-Y., & Jiang, X.-H. (2006). Interactive effects of habitat productivity and herbivore

698   pressure on the evolution of anti-herbivore defense in invasive plant populations.

699   *Journal of Theoretical Biology*, *242*(4), 935–940. doi:10.1016/j.jtbi.2006.05.016

700   Zuur, R. A. F., Ieno, E. N., Walker, N. J., Saviliev, A. A., & Smith, G. M. (2009). *Mixed Effects*

701   *Models and Extensions in Ecology*. New York: Springer.

702 **Supporting Information**

703 **Supporting Information 1, Fig. S1**

704 Map of the geographic locations of the sampled native and invasive *Silene latifolia*

705 populations.

706 **Supporting Information 2, Table S2**

707 Overview of the geographic locations and sizes of the sampled native and invasive *Silene*

708 *latifolia* populations.

Figures:

Fig. 1: Overview of the two generations of experimental breeding within each of the 16 *Silene latifolia* populations. The crossings were performed with five families (numbered circles). In the P-generation females (orange plants) were fertilized with pollen from males (green plants) from the same family for inbreeding (dashed arrows), and with pollen from males from a different family for outbreeding (solid arrows). In the P-generation, inbreeding and outbreeding were performed at distinct flowers of the same female individual. In the F1-generation, inbreeding was performed with individuals from inbred families and outbreeding with individuals from outbred families from the P-generation. Numbers for the F1-generation families correspond to the maternal/paternal plant of the breedings in the P-generation.
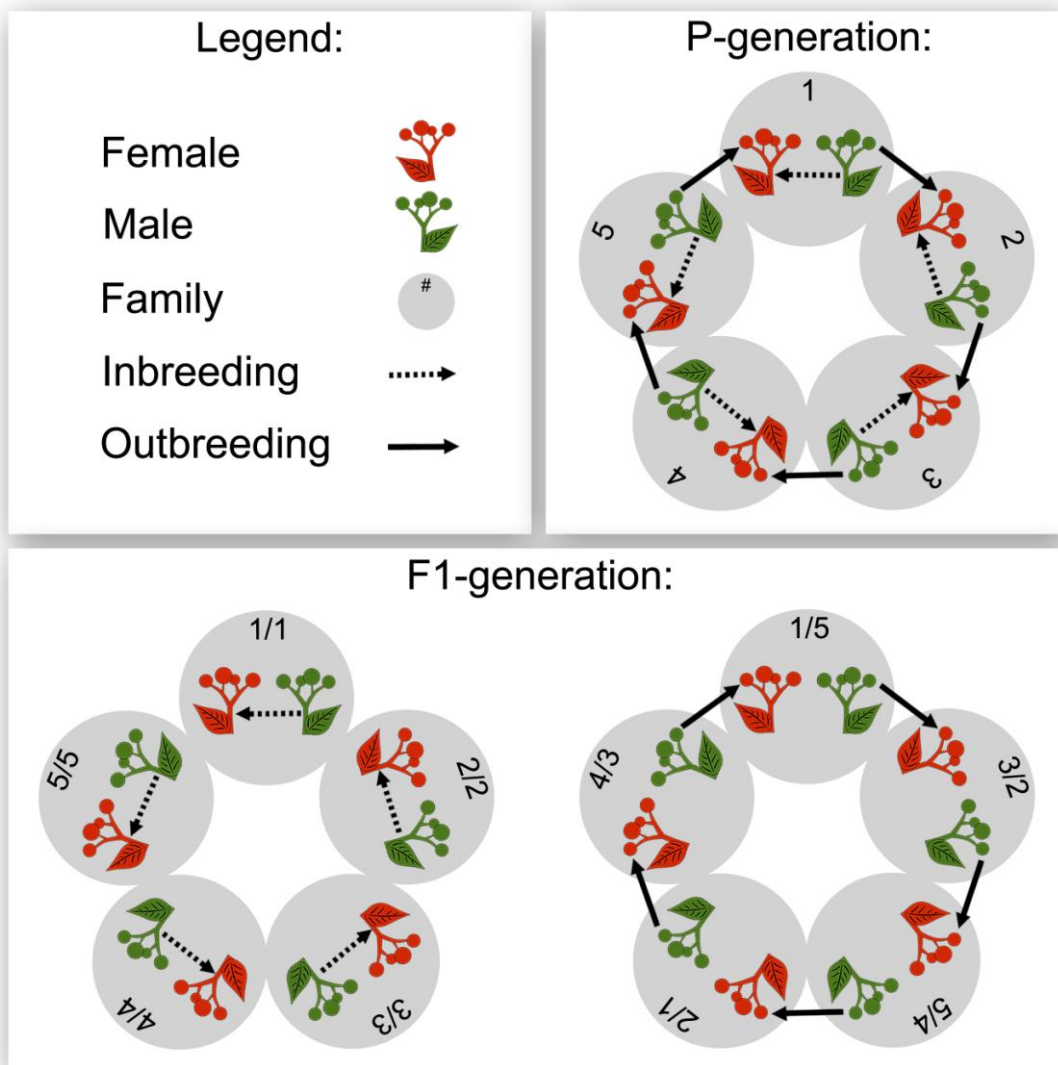
719 **Fig. 2:** Overview of the experimental manipulation of enemy infestation. The figure illustrates

720 the non-vegetated areas (light gray faces) with the experimental plots (white faces) and the

721 vegetated areas (structured, dark gray faces) from which natural enemies colonized the

722 plots. Either the enemy exclusion (bold black frames) or the enemy inclusion (thin black

723 frames) treatment was applied to each eight uniformly distributed plots. Within each plot,

724 plants were equally distributed with respect to range (native = black, invasive = gray) and

725 breeding treatment (filled = outcrossed, open = inbred).
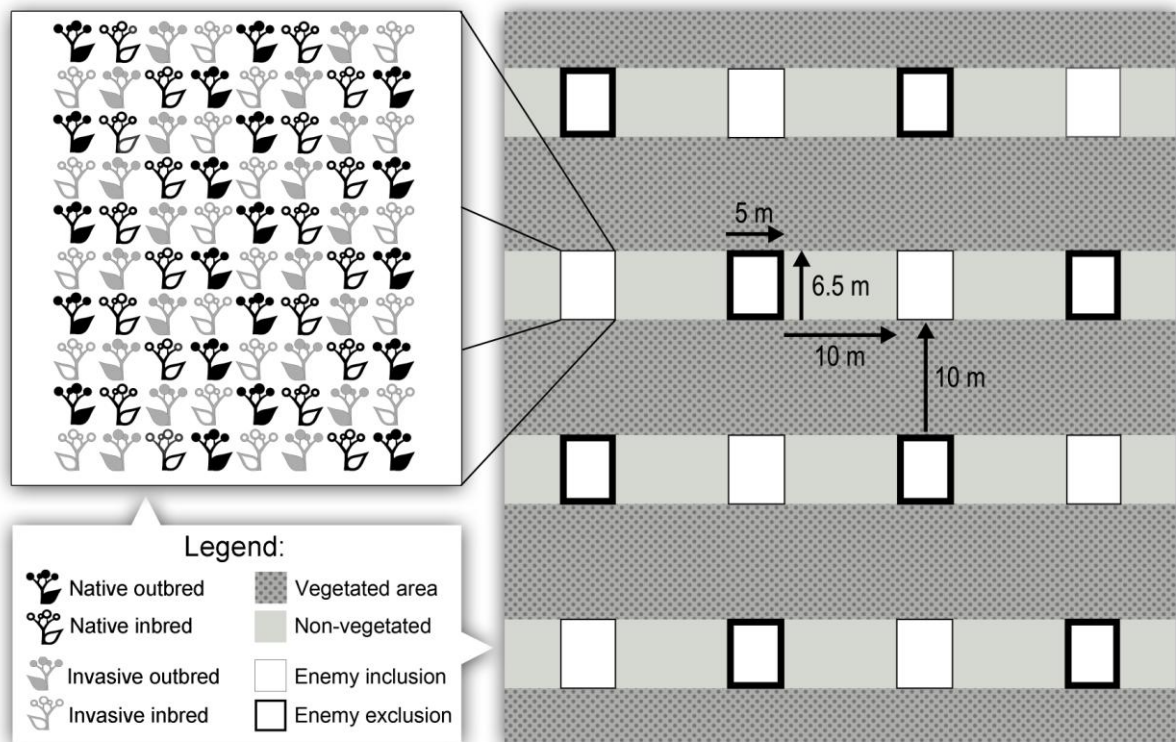
Fig. 3: Combined effects of range (native [black] *versus* invasive [gray]) and breeding treatment (outbred [filled] *versus* inbred [open]) on **a)** the density of leaf trichomes, **b)** the proportion of damaged leaves, **c)** the proportion of damaged flowers and **d)** the proportion of damaged fruits in *Silene latifolia*, which were acquired in enemy inclusions. The circles and arrows represent least square means with their standard errors extracted from the full (G)LMMs. The significance levels for fixed effects terms maintained in the minimal adequate (G)LMMs (determined with likelihood ratio tests) are denoted at the top of each plot (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Connecting lines between means of inbreds and outbreds mark significant range × breeding treatment interactions.
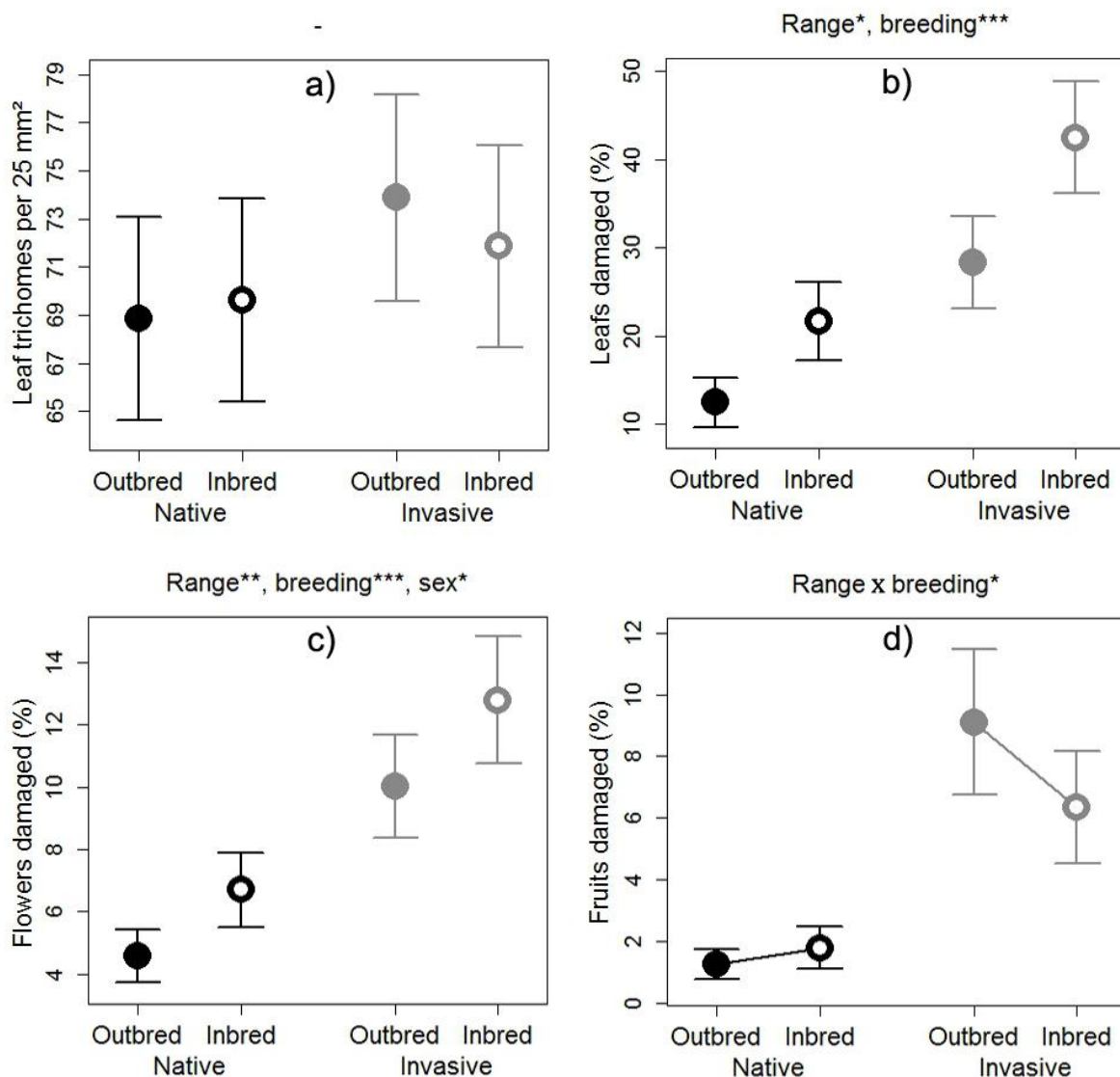
735   **Fig. 4:** Combined effects of range (native [black] *versus* invasive [gray]), breeding treatment

736   (outbred [filled] *versus* inbred [open]) and enemy treatment (exclusion [circles] *versus*

737   inclusion [squares]) on the **a)** aboveground biomass, **b)** corolla diameter, **c)** number of

738   flowers, and **d)** number of fruits of *Silene latifolia*. The circles and arrows represent least

739   square means with their standard errors extracted from the full (G)LMMs. The significance

740   levels for fixed effects terms maintained in the minimal adequate (G)LMMs (determined with

741   likelihood ratio tests) are denoted at the top of each plot (*: $p < 0.05$, **: $p < 0.01$, ***: $p <$

742   0.001). Connecting lines between means of inbreds and outbreds mark significant two-way

743   interactions in which breeding treatment is involved.
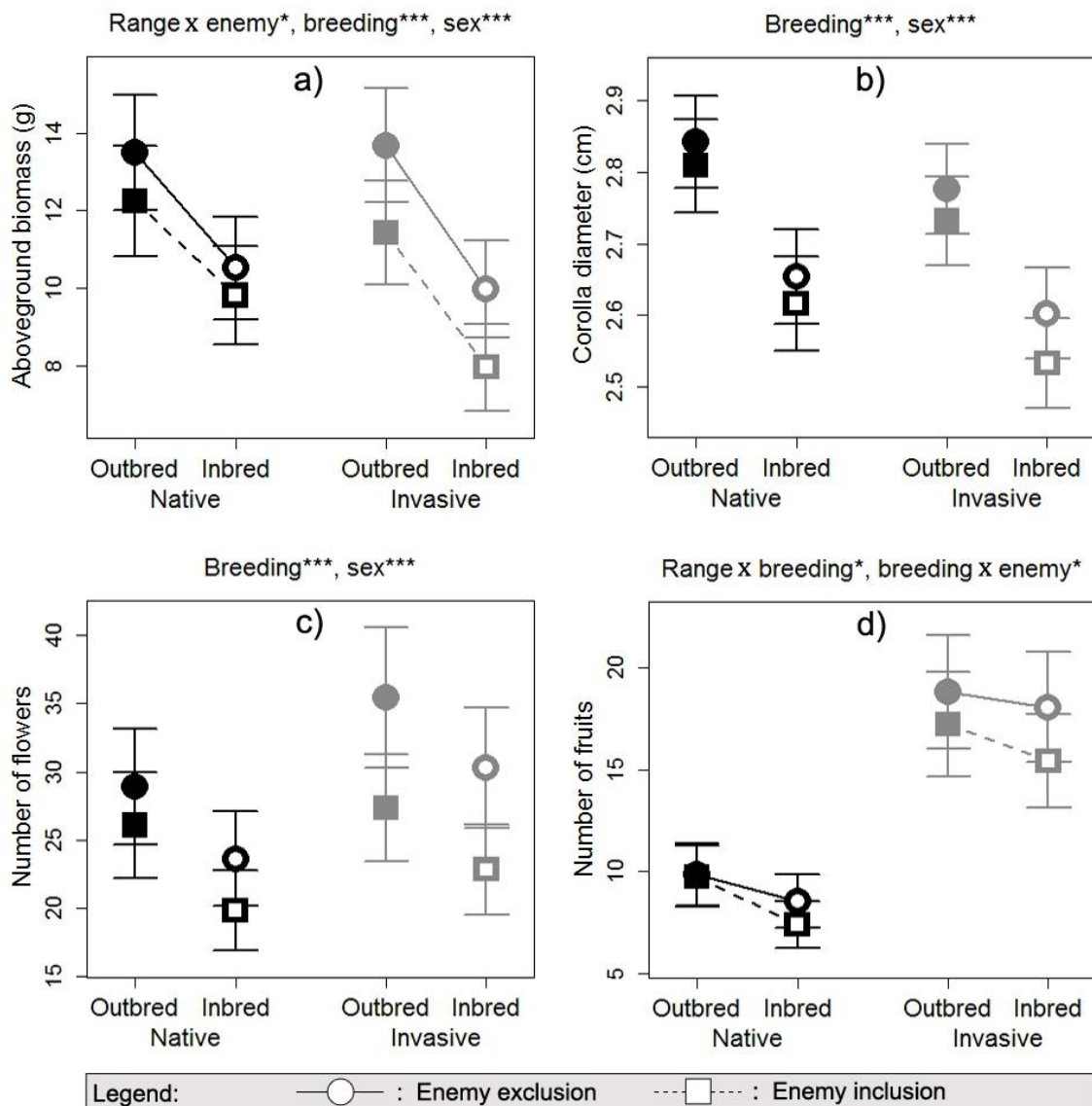
744 **Table 1:** Overview and results of analyses evaluating the interactive effects of range, breeding treatment and enemy treatment as well as the effects of
745 covariates (sex, latitudinal origin of population) on the performance of *Silene latifolia*. Responses are presented with their error distribution and the applied model
746 type (LMM, linear mixed effects model; GLMM, generalized linear mixed effects model). The table presents χ^2 and *p*-values obtained during stepwise backward
747 model selection based on likelihood ratio tests for each fixed effect term as well as the number of groups within random effects and the number of observations
748 for each response; ii: fixed effect term in significant interaction, -: fixed or random effect not tested.

Responses:	Leaf trichomes LMM (Gaussian)		Leaf damage GLMM (binomial)		Flower damage GLMM (binomial)		Fruit damage GLMM (binomial)		Biomass LMM (Gaussian)		Corolla diameter LMM (Gaussian)		Number flowers GLMM (Poisson)		Number fruits GLMM (Poisson)	
Fixed effects:	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*	χ^2	*p*
Range	0.345	0.557	**5.391**	**0.020**	**6.795**	**0.009**	i.i.	i.i.	i.i.	i.i.	1.674	0.196	1.643	0.200	i.i.	i.i.
Breeding	0.213	0.644	**41.693**	**<0.001**	**40.982**	**<0.001**	i.i.	i.i.	**116.629**	**<0.001**	**54.468**	**<0.001**	**24.516**	**<0.001**	i.i.	i.i.
Enemy	-	-	-	-	-	-	-	-	i.i.	i.i.	1.752	0.186	2.914	0.088	i.i.	i.i.
Range × breeding	0.758	0.384	0.031	0.860	1.478	0.224	**4.124**	**0.042**	3.039	0.081	0.005	0.941	0.740	0.389	**5.871**	**0.015**
Range × enemy	-	-	-	-	-	-	-	-	**4.772**	**0.029**	0.191	0.663	2.651	0.103	0.301	1.070
Breeding × enemy	-	-	-	-	-	-	-	-	0.058	0.810	0.091	0.763	0.340	0.557	**4.150**	**0.042**
Range x breeding × enemy	-	-	-	-	-	-	-	-	0.239	0.625	0.036	0.849	0.100	0.745	0.476	0.490
Sex	0.196	0.658	0.144	0.704	**5.225**	**0.022**	-	-	**44.511**	**<0.001**	**41.416**	**<0.001**	**133.510**	**<0.001**	-	-
Latitudinal origin Population	1.034	0.309	1.993	0.158	1.332	0.248	2.862	0.091	0.007	0.933	0.002	0.969	0.000	0.962	1.304	0.253
Random effects:	Groups		Groups		Groups		Groups		Groups		Groups		Groups		Groups	
Plot	8		8		8		8		16		16		16		16	
Population	16		16		16		16		16		16		16		16	
Population/mother in P-generation	76		76		76		73		76		76		76		76	
Population/father in P-generation	79		79		79		75		79		79		79		79	
Population/observation	-		592		-		-		-		-		1192		-	
Observations	551		592		571		282		1192		1128		1192		579	

Supporting Information 1

Fig. S1: Map of the geographic locations of the sampled native (left) and invasive (right) *Silene latifolia* populations.
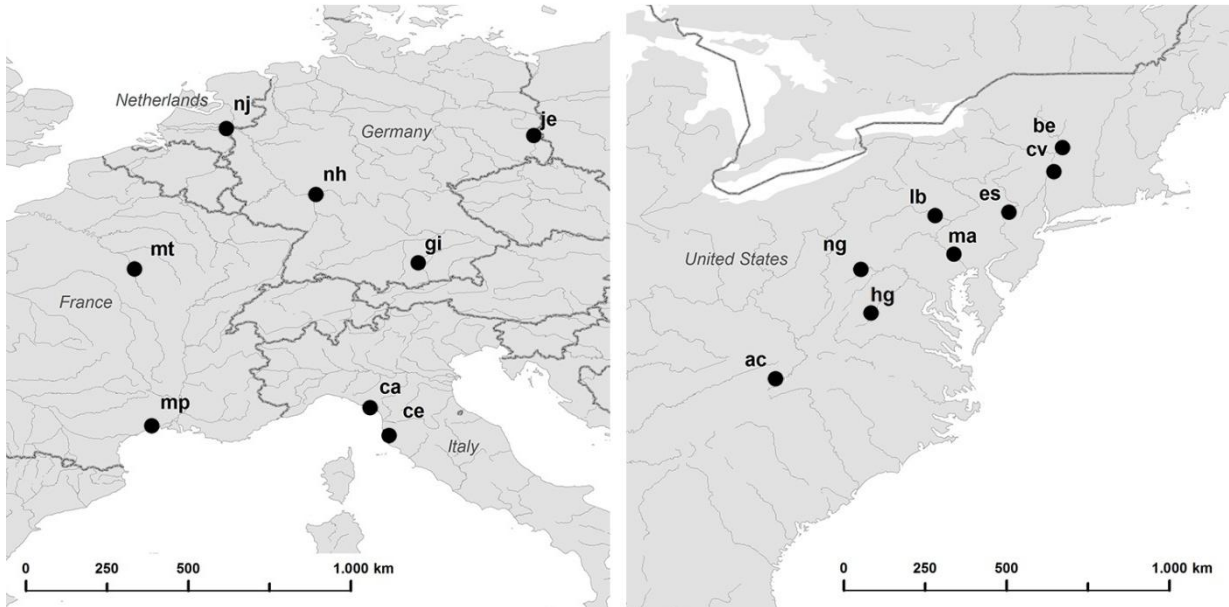
Supporting Information 2

Table S2: Overview of the geographic locations and sizes of the sampled native and invasive *Silene latifolia* populations.

Range	City (state)	ID	°N	°E	Population size
Native	Caresana (IT)	ca	44.07702	9.96384	262
Native	Cecina (IT)	ce	43.313769	10.51195	149
Native	Gilching (GE)	gi	48.105634	11.253771	35
Native	Jethe (GE)	je	51.6899	14.59683	485
Native	Montpellier (FR)	mp	43.653567	3.893247	341
Native	Monteneau (FR)	mt	47.848117	3.552508	19
Native	Nackenheim (GE)	nh	49.925119	8.342758	132
Native	Nijmengen (GE)	nj	51.883074	5.85185	46
Invasive	Crumpler (NC)	ac	36.52576	-81.41558	60
Invasive	Bennington (VT)	be	42.894717	-73.294919	31
Invasive	Hillsdale (NY)	cv	42.234825	-73.506433	350
Invasive	Bushkill (PA)	es	41.14009	-74.9294	900
Invasive	Harrisonburg (VA)	hg	38.49079	-78.97762	70
Invasive	Lewisburg (PA)	lb	40.98179	-76.93041	184
Invasive	Washington Boro (PA)	ma	39.99635	-76.47243	1100
Invasive	Grantsville (MD)	ng	39.637308	-79.099481	1000