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Rapid morphological change in UK populations of Impatiens glandulifera

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Rapid morphological change in UK populations of *Impatiens glandulifera*

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Abstract

The highly invasive Impatiens glandulifera (Himalayan balsam) is one of the most prolific and widespread invasive plants in the British isles. Introduced in the early 19th century, it has now been reported in almost every vice county across the U.K and is a fierce competitor that has adverse effects on the local community structure. Despite the negative impacts that invaders like I. glandulifera have on local communities, there have been very few studies which address the morphological changes that invasive plant populations have undergone since their initial introduction. This is the first study of its kind to investigate the morphological changes that have occurred in I. glandulifera since 50 years after its introduction. 319 herbarium specimens dating from 1890-2017 were used to measure changes in morphological traits such as leaf size, flower size and stomatal characteristics. We

found that since 1890, there has been a significant reduction in overall leaf size, a significant reduction in stomatal density and a significant increase in the overall flower size. These results highlight the importance of monitoring the evolutionary change in prolific alien species over the course of their invasion, providing useful insights into changes in competitive ability which may prove useful in managing dispersal and providing options for potential management.

Keywords: Himalayan Balsam, invasive species, phenotypic plasticity, herbarium specimens, stomata, adaptation.

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1.0 Introduction

In the last 100 years, globalization and industrialisation have exponentially increased the incidence of invasions by non-native plants (Vitousek, 1997) and there is increasing concern over the resulting harm to the environment; invasive species are now regarded as a leading cause of plant extinction, second only to habitat loss (Vila, *et al,* 2011; Bellard, *et al* 2016; Park, 2004). Species introduced to new regions may face very different environmental conditions to those experienced in their native range, and there is evidence that many successful invasive species seem to undergo rapid phenotypic changes in response to the challenges posed by the novel/new environment (Maron et al. 2004, Mackin et al. 2021).

These rapid phenotypic changes may be the result of evolution (Maron et al. 2004). Reproductively isolated from their source populations and faced with novel selection pressures, introduced populations must adapt quickly to their new environment to establish a stable population in their new range. Introduced populations often have a low propagule size and low genetic diversity (Kinziger, 2011; Rollins *et al*, 2013), which would usually reduce their evolutionary potential (Willi, 2006). However, genetic bottlenecks in such invasive species can restrict gene flow (Golani, 2007) causing a rapid divergence of the phenotypes from their native range. Individuals that are better adapted to the new environment can appear rapidly and out-compete more poorly-adapted individuals during the lag phase of the invasion (Bossdorf et al, 2005; Hänfling & Kollman, 2001), with adaptive traits evolving in 20 generations or less (Prentis, *et al,* 2008). Moreover, a lack of co-evolved parasites and herbivores means non-native species may not need some costly defence strategies (e.g. secondary chemical production). This allows for resources to be invested in other traits advantageous to their new range, which will increase their competitive ability (Siemann & Rogers, 2008, Zenni, 2016).

Rapid phenotypic change may also be attributed to phenotypic plasticity, resulting from genotypes producing different phenotypes in response to different environmental conditions (Fusco & Minelli, 2010). High phenotypic plasticity is a common trait in invasive plant species, and has been demonstrated in *I. glandulifera* (Gruntman et al. 2020). However, regardless of whether it is caused by evolution or

phenotypic plasticity, rapid phenotypic changes in response to novel environmental conditions could play a role in facilitating the spread of invasive plants (Zenni et al. 2016). Previous research that investigates phenotypic change in invasive species in response to novel environments has been based on comparisons between native and introduced populations of the same species (Bossdorf et al. 2005, Brandenburger et al. 2019). Very few studies have considered the magnitude and direction of change through time since first invasion

The present study will focus on *Impatiens glandulifera* Royle (Himalayan balsam). Native to the Himalayas, it was first introduced into the UK in 1839 as a garden ornamental. It has now become a widespread and problematic invasive species found throughout most of Europe, as well as in North America, Russia, Canada and New Zealand (Kollman & Banuelos, 2004; Beerling & Perrins 1993; Clements et al., 2008; Jones 2022). A fierce competitor, *I. glandulifera* reaches up to 2.5 m tall in the UK, has a fast growth rate, over-produces nectar, and produces up to 2500 seeds per plant (Beerling & Perrins, 1993; Chittka & Schurkens, 2001; Clements et al., 2008). Forming dense monospecific stands with foliage that swamps non-native plant species, *I. glandulifera* can reduce species richness in invaded habitats by 25% Hulme & Bremner 2006), releasing allelopathic chemicals into the environment (Diller, 2023) and promoting soil erosion in riparian habitats (Greenwood & Kuhn, 2014).

In Britain, it grows taller, has higher fecundity and a larger total leaf area than in its native range, making the non-native populations more competitive than their native counterparts (Tanner, 2014). In this study, herbarium specimens were used to investigate changes in populations of *I. glandulifera* since its introduction into the U.K. This study looked at features which would give *I. glandulifera* an adaptive advantage, including leaf length, leaf width, leaf area, stomatal density and flower length.

2.0 Materials and methods

Sampling herbarium specimens

For historical data, 319 herbarium specimens were studied, with collection dates ranging from 1890 to 2017 (Appendix, A5). Specimens studied were located in the herbaria at Amgueddfa Cymru – National Museum Wales, The Royal Botanic Garden Kew and the Natural History Museum London. Only high-quality specimens were sampled; any specimens that were badly damaged or had less than three fully intact leaves were excluded from the data analysis. In this study, herbarium specimens were sampled from 41 vice-counties across the UK, covering most of Wales and southern England (Figure 1).

Field sampling

In 2017, data was obtained from 159 field specimens collected during June-July from 11 populations across South Wales (Appendix, A6). Individuals were randomly selected from each habitat and each specimen was pressed and prepared using standard herbarium methods.

Morphological data

Digital photographs of each specimen were taken using a ruler for scale. Leaf area, length and width were all then calculated using Image J (Rasband, 1997–2016). Any leaves that were located near the growing tip or that appeared immature were discounted from the analysis. Leaf length was calculated measuring from the tip of the apex to just before the petiole attachment (Figure 2A), near the basal portion of the leaf. Leaf width was recorded as the widest portion of the leaf (Figure 2B). The surface area of the leaf was measured as the circumference around the outside of the leaf (Figure 2C).Where possible, five leaves were sampled per plant: where fewer leaves were available a minimum of three leaves were sampled.

Flower length was calculated by measuring the distance from the anterior portion of the flower to the posterior floral opening (Figure 2D). At least three flowers were sampled per plant.

Stomatal density was measured by coating the abaxial surface of the leaf with Germolene New Skin liquid plaster, leaving to dry for five to ten minutes and gently prying off the surface with a pair of fine tipped tweezers. This peel was then examined using a Nikon Labophot-2 Binocular Phase Contrast Microscope. The full procedure for this can be found at

https://www.researchgate.net/publication/324784278_Non-invasive_method_for_looking_at_stomata_epidermal_cells_of_herbaria_specimens.

Climatic data and geographic data

Mean monthly Central England Temperature (CET) records for the period 1890-2017 were obtained from the UK Meteorological Office (http://hadobs.metoffice.com/hadcet/cetml1659on.dat). These temperature series for Central England are representative of roughly a triangular area enclosed by Bristol, Lancashire and London (Parker, Legg and Folland, 1992). Croxton et al. (2006) found that monthly temperatures from stations distributed across the UK are highly correlated with the corresponding CET, indicating that its applicability extends beyond central England. Longitude and latitude were calculated using the centroid of the Watsonian vice-county for each specimen.

Statistical analyses

All statistical analyses were performed in R v. 4.0.0 (R Core Team, 2020). Multiple regression models were used to explore the relationship between traits measured (leaf area, leaf width, leaf length, flower length) and time, mean annual temperature, latitude and longitude. As time was positively correlated with mean annual temperature (*Pearson's r* > 0.7; Figure A7), different models were run for each of these two predictors to avoid issues due to multicollinearity. The package 'visreg' in R (Breheny and Burchett, 2017) was used to allow the visualization of the relationship between the response variable (partial residuals) in relation to a given predictor while holding all other variables constant.

Models were validated via diagnostic plots of model residuals to verify the assumptions of normality and homoscedasticity (Zuur et al. 2009). Log10-transformation was applied to leaf area, length and width and to flower length to meet statistical assumptions.

Results

Leaf area, length and width

Leaf area, leaf width and leaf length decreased with increasing time. In addition, leaf area and leaf width (but not leaf length) decreased also as a function of increasing mean annual temperature (Table 1, Figure 3). No significant effect of geographical location (as indicated by Latitude and Longitude) was found on leaf area, leaf width or leaf length (see Tables 1 and 3).

Stomatal Density

Stomatal density decreased significantly with increasing time, and also with increasing mean annual temperature (Table 2, Figure 4 a,b). No significant effect of geographical location (as indicated by Latitude and Longitude) was found on stomatal density (see Table 2).

Flower Length

Flower length increased significantly with increasing time and with increasing mean annual temperature (Table 2, Figures 3c, d). No significant effect of geographical location (as indicated by Latitude and Longitude) was found on flower length (see Table 2).

Discussion

Results of the present study indicate distinct morphological changes over time in all traits measured in UK populations of *I. glandulifera*: leaf area, leaf length, leaf width and stomatal density have all decreased since 1890. The most significant change is in flower length, which increased by 84 % between 1890 and 2017. This suggests that there have been microevolutionary changes in UK populations of *I. glandulifera,* with a shift towards less competitive leaf traits, but an increase in flower size. This latter trend may reflect directional selection driven by pollinators.

Leaf length, width and area

According to the Evolution of Increased Competitive Ability hypothesis (EICA) traits such as increased height, leaf area and growth rate that give a non-native species a competitive advantage appear early during biological invasions and can result in aggressive phenotypes (Whitney & Gabler, 2008; Blossey & Notzold, 1995). However, there have been inconsistent results in the literature on leaf characteristics in invasive species: some studies suggest that leaf area increased after the initial invasion (Bossdorf *et al*., 2005; Grotkopp & Rejmanwk, 2007; Gallagher *et al*., 2015) but an increasing number of studies have reported a reduction in total leaf area after invasion (Buswell *et al*., 2011; Liu, 2016; Feng, 2008). The results of the present study on *I. glandulifera* support the latter view, with leaf area, length and width all decreasing since 1890 (Figure 3). Our study also found that leaf area and leaf width decreased with increasing mean annual temperature, a trend suggested by other studies (e.g., Guerin et al. 2012). Smaller leaves appear to have better thermal regulation than larger leaves (Wright et al. 2017), but regulation of leaf size is complex and can also be strongly influenced by other components of climate, such as precipitation (McDonald et al 2003).

Although not measured in this study, a greater investment in the number of leaves produced as a result of an increase in height, could explain a reduction in leaf size as proposed by the leaf size-number trade-off theory (Kleiman & Aarssen, 2007). Indeed, in the introduced range, *I. glandulifera* has been found to be taller than plants in the native range (Skalova, 2012; Andrews, 2005; Tanner et al. 2014). Several advantages have been suggested for an increase in leafing intensity, including a greater potential for higher fecundity allocation as a result of an increase in lateral inflorescences (Kleiman & Aarssen, 2007). In fact, taller plants in *I. glandulifera* have been found to produce more seeds in UK populations (Willis & Hulme, 2004).

Stomatal Density

Decreases in stomatal density with time are consistent with previous findings in the literature and have been linked to the sensitivity of this trait in response to increasing CO_2 levels (Woodward, 1987; Woodward & Kelly, 1995; Royer, 2001). Similarly, decreases in stomatal density have also been reported in response to increasing

temperatures, as a possible mechanism to help reduce water loss by reducing the stomatal conductance of the leaf (Beerling and Chaloner, 1993).

Floral traits: flower length

I. glandulifera is known for its large, self-compatible, colourful flowers that produce copious quantities of nectar with the highest sugar content of any other annual species in Europe. This has been found to negatively affect native plants by being a successful competitor for pollinators (Chittka & Schurkens, 2001). This study found that an overall 84 % increase in the length of *I. glandulifera* flowers occurred between 1890 and 2017.

Although flowers of *I. glandulifera* are self-compatible, they are rarely self-pollinated and have a wide range of insect pollinators, mostly bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera* L.) (Valentine, 1971; Nienhuis & Stout 2009). Selection for a larger flower size in *I. glandulifera* may give this species an adaptive advantage in its non-native range, attracting a larger number and greater diversity of pollinators, as has been frequently observed in other species with larger flowers (Shohei & Hiroshi, 2017; Parachnowitsch & Kessler, 2008; Cartar, 2004). In fact, pollinators have been shown to have a substantial effect on directional selection of flower size across different plant families, selecting for larger, more showy flowers that produce more nectar (Brys, 2008; Parachnowitsch & Kessler, 2008). Possibly bumblebees and honeybees are selectively attracted to larger flowers and visit them more frequently; a study by Tsujimoto & Hiroshi (2017) demonstrated that larger flowers are more easily seen by bumblebees along their foraging route compared to small flowers, and the bumblebees actively visit them more frequently.

In this study we found a positive relationship between flower size and mean annual temperature. Elevated temperatures have been found to influence flowering traits, including pollen, nectar and flower production and also flower size (Scaven & Rafferty, 2013). However, no clear, consistent overall trends have been reported on the effects of warming on flower size, with some studies demonstrating a reduction, whilst others find an increase, pointing to species specific responses (Scaven & Rafferty, 2013). Regardless of whether the observed changes are driven by pollinators or due to increasing temperatures, it is clear that these changes may be capable of altering the interaction of *I. glandulifera* with pollinators.

Conclusions

This study demonstrates the importance of herbarium collections for research into the dynamics of vegetation change (Besnard *et al.*, 2018; Cleal *et al.*, 2021). Without the collections that were investigated here, it would have been impossible to provide the temporal / historical context for the evidence obtained from fieldwork. The work has revealed how *I. glandulifera* has undergone rapid phenotypic changes in the UK since 1890, in particular a reduction in leaf size and stomatal density, and an increase in mean flower size. These morphological changes can be linked to an increase in competitive ability of the species and can be partly predicted under the EICA hypothesis (Blossey & Notzold, 1995). The reduction in leaf size may be compensated for by an increased investment in reproductive structures. In addition, the larger *I. glandulifera* flowers may attract more and a greater diversity of pollinators. The next logical step would be to look at how biotic interactions have been shaping this change; are there are other biotic (and abiotic) conditions, not addressed in this study, that may also be shaping flower evolution in *I. glandulifera*? Future increases in mean annual temperature associated with climate change may favour these trends and promote increasing flower length.

Adaptations of *I. glandulifera* that promote its spread and competitive success over native species have serious ecological and financial consequences. Jones (2022) suggests that the cost of control in the UK is around £1,000,000 annually. Understanding both the ecology of an invasive species, such as *I. glandulifera* and, the full range of its effects on the invaded ecosystem is essential in the formulation of effective control strategies and rehabilitation of invaded habitats (Coakley & Petti, 2021). The main methods of management include labour-intensive pulling, cutting, herbicide treatment with glyphosate or grazing (Jones 2022), while other studies have highlighted the effectiveness of using strains of rust originating from the native range of the species (Ellison et al. 2020,). The evolution of *I. glandulifera* plants with smaller leaves may influence the choice of management technique, for example, by reducing the quantity of herbicide necessary for control.

The long-term consequences of the evolution of larger flowers are uncertain. Tanner (2017) has pointed out that *I. glandulifera* is favoured by beekeepers because it has an extended flowering time, coupled with high rates of sugar production, highlighting

the potential use this species to support pollinating insects. Chittka and Schürkens (2001) suggest that the species has the potential to decrease genetic diversity in native plants as it lures pollinators away from natives. In contrast, Coakley & Petti (2021) suggest that an increase in species richness, visitor abundance and flower visitation was reported for plots invaded by *I. glandulifera* in multiple studies, resulting in a facilitated increase in pollinator visits for native species. Further research is clearly needed to determine the impact of invasion by *I. glandulifera* on pollination of native species and to consider how this is affected by changes in adaptive traits highlighted by this research.

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Author contribution

A.L.W, H.S.P and J.S.V participated in the field work to collect 11 new populations of *I. glandulifera* from around the South Wales area. A.L.W visited the Natural History Museum (London) and Royal Botanical Gardens (Kew) to study historical collections of *I. glandulifera* from across the U.K. J.S.V and A.L.W undertook the statistical analyses. All authors contributed to the design of the project, the interpretation of the data and the writing of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author, (ALW) upon reasonable request.

Conflict of interest statement

We have no conflicts of interest to disclose. All authors declare that they have no conflicts of interest.

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330 **Competing Interests**

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

332 **References**

333 Andrews M, Harnish G, Hodge S, Cherril A, Raven JA. 2009. Seed dormancy,
334 nitrogen and nutrition and shade acclimation in Impatiens glandulifera: implications
335 for successful invasion of deciduous woodland. Plant Ecology & Diversity 2(2): 145-
336 153.

337 Andrews M, Maule HG, Raven JA, Mistry A. 2005. Extension growth of Impatiens
338 glandulifera at low irradiance: importance of nitrate and potassium accumulation.
339 Annuals of Botany 95(4): 641-648.

340 Beerling DJ, Chaloner WG. 1993. The impact of atmospheric CO_2 and temperature
341 changes on stomatal density: observation from Quercus robur Lammas leaves.
342 Annals of Botany 71 (3): 231-235.

343 Beerling, D.J., Perrins, J.M. (1993). Impatiens glandulifera Royle (Impatiens roylei
344 Walp.). The Journal of Ecology, 81(2), 367-382. DOI:
345 https://doi.org/10.2307/2261507

346 Bellard, C., Cassey, P., Blackbun, T.M. (2016). Alien species as a driver of recent
347 extinctions. Biology Letters, 12 (2). DOI: http://dx.doi.org/10.1098/rsbl.2015.0623

348 Besnard G, Gaudeul M, Lavergne S, Muller S, Rouhan G, Sukhorukov AP,
349 Vanderpoorten A, Jabbour F. 2018. Herbarium-based science in the twenty-first
350 century, Botany Letters 165(3-4): 323-327,

351 Blossey B, Notzold R. 1995. Evolution of increased competitive ability in invasive
352 non-indigenous plants: a hypothesis. The Journal of Ecology 83(5): 887-889.

353 Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E., Prati, D. (2005).
354 Phenotypic and genetic differentiation between native and introduced plant
355 populations. Oecologia, 114 (1), 1-11. DOI: https://doi.org/10.1007/s00442-005-
356 0070-z

Brandenburger, C.R., Sherwin, W.B., Creer, S.M., Buitenwerf, R., Poore, A.G.B., Frankham, R., Finnerty, P.B., Moles, A.T. (2019). Rapid reshaping: the evolution of morphological changes in an introduced beach daisy. Proc. R. Soc. B 286: 20181713. http://dx.doi.org/10.1098/rspb.2018.1713

Breheny P, Burchett W. 2017. Visualization of regression models using visreg. R Journal 9:2, 56–71.

Briton JR, Gozlan RE. 2013. How many founders for a biological invasion? Predicting introduction outcomes from propagule pressure. Ecology 94(11): 2558-2566.

Brys R, Jacquemyn H, Hermy M. 2008. Pollination efficiency and reproductive patterns in relation to local plant density, population size, and floral display in the rewarding Listera ovata (Orchidaceae). Botanical Journal of the Linnean Society 157(4): 713–72.

Buswell JM, Moles AT, Hartley S. 2011. Is rapid evolution common in introduced plant species? Journal of Ecology 99(1): 214-224.

Cartar RV. 2004. Resource tracking by bumble bees: responses to plant-level differences in quality. Ecology 85: 2764-2771.

Chittka, L., Schurkens, S. (2001). Successful invasion of a floral market- an exotic Asian plant has moved in on Europe's river-banks by bribing pollinators. Nature 411 (6838), 653. DOI: 10.1038/35079676

Cleal CJ, Pardoe HS, Slade K, Whyman S, Tangney RS, Jüttner I. 2021. The Welsh National Herbarium. Botany Letters: DOI: 10.1080/23818107.2021.1977178.

Clements, D.R., Feenstra, K.R., Jones, K., Staniforth, R. (2008). The Biology of Invasive Alien Plants in Canada. 9. Impatiens glandulifera Royle. The Canadian Journal of Plant Science, 88: 403-417. DOI: 10.4141/CJPS06040

Clements DR, Jones VL .2021. Rapid Evolution of Invasive Weeds Under Climate Change: Present Evidence and Future Research Needs. Frontiers in Agronomy 3:664034. doi: 10.3389/fagro.2021.664034

385 Coakley, S, Petti, C. 2021. Impacts of the Invasive Impatiens glandulifera: Lessons
386 Learned from One of Europe's Top Invasive Species Biology 10, 619.
387 https://doi.org/10.3390/biology10070619.
388 Conner J, Rush J. 1996. Effects of flower size and number on pollinator visitation to
389 wild radish, Raphanus raphanistrum. Oecologia 105(4): 509-516.

390 Croxton, P. J., Huber, K., Collinson, N., Sparks, T. H., 2006: How well do the Central
391 England temperature and the England and Wales precipitation series represent the
392 climate of the UK? Int J Climatol, 26, 2287-2292.

393 Diller, J.G.P., Hüftlein, F., Lücker, D. (2023).. Allelochemical run-off from the invasive
394 terrestrial plant Impatiens glandulifera decreases defensibility in Daphnia. Sci Rep
395 13, 1207 (2023). https://doi.org/10.1038/s41598-023-27667-4

396 Dlugosch KM, Parker LM. 2008. Founding events in species invasions: genetic
397 variation, adaptive evolution, and the role of multiple introductions. Molecular
398 Ecology 17(1): 432-449.

399 Ellison, C.A., Pollard, K.M., Varia, S. (2020) Potential of a coevolved rust fungus for
400 the management of Himalayan balsam in the British Isles: first field results. Weed
401 Research. in press.

402 Emer C, Vaughan IP, Hiscock S, Memmott J. 2015. The impact of invasive alien
403 plant, Impatiens glandulifera, on pollen transfer networks. PLoS ONE 10(12).

404 Feng YL. 2008. Photosynthesis, nitrogen allocation and specific leaf area in
405 invasive Eupatorium adenophorum and native Eupatorium japonicum grown at
406 different irradiances. Physiologia plantarum 133(2): 318-326.

407 Franks PJ, Beerling DJ. 2009. CO2 forced evolution of plant gas exchange capacity
408 and water use efficiency over the Phanerozoic. Geobiology 7(2): 227-236.

409 Fusco G, Minelli A. 2010. Phenotypic plasticity in development and evolution: facts
410 and concepts. Introduction. Philosophical transactions of the Royal Society of
411 London. Series B, Biological sciences 365 (1540): 547-556.
412 DOI:10.1098/rstb.2009.0267

413 Gallagher RV, Randall RP, Leishman MR. 2015. Trait differences between
414 naturalized and invasive plant species independent of residence time and phylogeny.
415 Conservation Biology 29(2): 360-369.

416 Ghazoul J. 2006. Floral diversity and the facilitation of pollination. Journal of Ecology
417 94(2): 295-304.

418 Golani, D., Azzurro, E., Corsini-Foka, M., Falautano, M., Andaloro, F., Bernardi, G.
419 (2007). Genetic bottlenecks and successful biological invasions: the case of a recent
420 Lessepsian migrant. Biological Letters, 3 (5): 541-545. DOI:
421 https://doi.org/10.1098/rsbl.2007.0308

422 Greenwood, P., Kuhn, N.J. (2014) Does the invasive plant, Impatiens glandulifera,
423 promote soil erosion along the riparian zone? An investigation on a small
424 watercourse in northwest Switzerland. J Soils Sediments, 14, 637–650. https://doi-
425 org.abc.cardiff.ac.uk/10.1007/s11368-013-0825-9

426 Grotkopp E, Rejmanwk M. 2007. High seedling relative growth rate and specific leaf
427 area are traits of invasive species: phylogenetically independent contrasts of woody
428 angiosperms. American Journal of Botany 94(4): 526-532.

429 Gruntman, M., Segev, U., Tielbörger, K. (2020). Shade-induced plasticity in invasive
430 Impatiens glandulifera populations. Weed Research, 60 (1), 16-25. DOI:
431 https://doi.org/10.1111/wre.12394

432

433 Guerin G R, Wen H, Lowe A J. 2012. Leaf morphology shift linked to climate change.
434 Biology Letters 8: 882–886

435 Hänfling, B., Kollman, J. (2002). An evolutionary perspective on biological invasions.
436 Trends in Ecology and Evolution, 17(12), 545-546. DOI:
437 https://doi.org/10.1016/S0169-5347(02)02644-7

438 Hulme, P.E., Bremner, E.T. (2006). Assessing the impact of Impatiens glandulifera in
439 riparian habitats: partitioning diversity components following species removal.
440 Journal of Applied Ecology, 43 (1), 43-50. DOI: https://doi.org/10.1111/j.1365-
441 2664.2005.01102.x

James S, Bell DT. 2000. Influence of light availability on leaf structure and growth of two Eucalyptus globulus ssp. Tree Physiology 20: 1007-1018.

Jones, DJ. 2022 Public information on invasive species in Wales 1 Himalayan balsam. Technical Report. Welsh Government.

Kessler, D., Gase, K., Baldwin, I,T. 2008. Field experiments with transformed plants reveal the sense of floral scents. Science. 321(5859).

Kinziger, A.P., Nakamoto, R.J., Anderson, E.C., Harvey, B.C. (2011). Small founding number and low genetic diversity in an introduced species exhibiting limited invasion success (speckled dace, Rhinichthys osculus). Ecology and Evolution, 1 (1), 73-84. DOI: 10.1002/ece3.8

Kleiman D, Aarssen LW. 2007. The leaf size/number trade-off in trees. Journal of Ecology, 95, 376– 382.

Kollman, J., Bañuelos, M.J. (2004). Latitudinal trends in growth and phenology of the invasive plant Impatiens glandulifera (Balsaminaceae). Diversity and Distributions, 10 (5-6), 377-385. DOI: https://doi.org/10.1111/j.1366-9516.2004.00126.x

Lee CE. 2002. Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17(8): 336-342.

Leishman MR, Cooke J, Ricardson DM, Newman J. 2014. Evidence for shifts to faster growth strategies in the new ranges of invasive alien plants. Journal of Ecology 102(6): 1451-1461.

Liu YL, Dawndon W, Prati D, Haeuser E, Feng Y, van Kleunen M. 2016. Does greater specific leaf area plasticity help plants to maintain a high performance when shaded. Annals of Botany 118(7): 1329-1336.

Mackin, C.R., Peña, J.F., Blanco, M.A., Balfour, N.J., Castellanos, M.C. 2021. Rapid evolution of a floral trait following acquisition of novel pollinators. Journal of Ecology. 109 (5), p. 2234-2246. DOI: https://doi.org/10.1111/1365-2745.13636

Maron, J.L., Vila, M., Bommarco, R., Elmendorf, S., Beardsley, P. (2004). Rapid evolution of an invasive plant. Ecological Monographs 74 (2), 261–280. DOI: https://doi.org/10.1890/03-4027

McDonald PG, Fonseca CR, Overton JM, Westoby M. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades?. Functional Ecology 17: 50-57. https://doi.org/10.1046/j.1365-2435.2003.00698.x

Mei D, jianzhong L, Wenju W, Jiakuan C, Bo L. 2006. Canada goldenrod (Solidago canadensis): an invasive alien weed rapidly spreading through China. Acta Phytotaxonomica Sinica 44(1): 72-85.

Met Office 2018. Weather station data. National Meteorological Library and Archive. [online]. Available at https://www.metoffice.gov.uk/public/weather/climate-historic/#?tab=climateHistoric. [Accessed 27/08/2018]

Monty A, Bizoux J-P, Escarré J, Mahy G. 2013. Rapid plant invasion in distinct climates involves different sources of phenotypic variation. PLoS ONE 8 (1): e55627. doi:10.1371/journal.pone.0055627

Nienhuis CM, Stout JC. 2009. Effectiveness of native bumblebees as pollinators of the alien invasive plant Impatiens glandulifera. Journal of Pollination Ecology 1(1): 1-11.

Parachnowitsch AL, Kessler A. 2010. Pollinators exert natural selection on flower size and floral display in Penstemon digitalis. New Phytologist 188(2): 393-402.

Park, K. 2004. Assessment and management of invasive alien predators. Ecology and Society, 9 (2): 12. DOI: http://www.ecologyandsociety.org/vol9/iss2/art12/print.pdf

Parker, D.E., Legg, T.P., and Rolland, C.K. (1992). A new daily Central England temperature series, 1772-1991. Int. J. Climatol.12, 317–342.

Pattison RR, Goldstein G, Ares A. 1998. Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. Oecologia 117(4): 449-459.

Prentis, P.J., Wilson, J.R.U., Richardson, D.M., Lowe, A.J. (2008). Adaptive Evolution in Invasive Species. Trends in Plant Science, 13(6): 288-294.7 DOI: 10.1016/j.tplants.2008.03.004

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rasband WS. 1997-2016. ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA. https://imagej.nih.gov/ij/.

Rollins, L., Moles, A.T., Lam, S., Buitenwerf, R., Buswell, J., Brandenburger, C.R., Flores-Moreno, H., Nielson, K.B., Couchman, E., Brown, G.S., Thompson, F.J., Hemmings, F., Frankham, R., Sherwin, W. (2013). High genetic diversity is not essential for biological invasion. Ecology & Evolution, 3 (13), 4501-4517. DOI: 10.1002/ece3.824.

Royer DL. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO_2 concentration. Review of Palaeobotany and Palynology 114(1-2): 1-28.

Scaven VL & Rafferty N E. 2013. Physiological effects of climate warming on flowering plants and insect pollinators and potential consequences for their interactions. Current Zoology 59(3): 418–426. https://doi.org/10.1093/czoolo/59.3.418

Shohei T, Hiroshi I. 2017. Effect of flower perceptibility on spatial-reward associative learning of bumblebees. Behavioural Ecology 71(7): 1-11.

Siemann, E., Rogers, W.E. (2001). Genetic differences in growth of an invasive tree species. Ecology Letters, 4(6), 514-518. DOI: https://doi.org/10.1046/j.1461-0248.2001.00274.x

523 Skalova H, Havlickova V, Pysek P. 2012. Seedling traits, plasticity and local
524 differentiation as strategies of invasive species Impatiens in central Europe. Annuals
525 of Botany 110: 1429-1438.

526 Stastny M, Sargent RD. 2017. Evidence for rapid evolutionary change in an invasive
527 plant in response to biological control. Journal of Evolutionary Biology 30(5): 1042-
528 1052.

529 Tanner, R.A., Jin, L., Shaw, R. et al. An ecological comparison of Impatiens
530 glandulifera Royle in the native and introduced range. (2014) Plant Ecol 215, 833–
531 843. https://doi-org.abc.cardiff.ac.uk/10.1007/s11258-014-0335-x

532 Tanner, R Gange AC. 2020. Himalayan balsam, Impatiens glandulifera: its ecology,
533 invasion and management. Weed Research 60(1):4-7 DOI: 10.1111/wre.12401

534 Tanner, R. 2017. Information on measures and related costs in relation to species
535 included on the Union list: Impatiens glandulifera. Technical note prepared by IUCN
536 for the European Commission. EPPO

537 Tsujimoto SG, Hiroshi SI. 2017. Effect of flower perceptibility on spatial-reward
538 associative learning by bumble bees. Behavioural Ecology and Socio-biology
539 71(105): 105-106.

540 Valentine DH. 1971. Flower-colour polymorphism in Impatiens glandulifera Royle.
541 Boissiera 19: 339-343.

542 Vila, M., Espinar, J.J., Hedja, M., Hulme, P.E., Jarosik, V., Maron, J.J., Pergl, J.,
543 Schaffner, U., Sun, Y., Pysek, P. (2011). Ecological impacts of invasive alien plants:
544 a meta-analysis of their effects on species, communities and eco-systems. Ecology
545 Letters, 14 (7), 702-708. DOI: 10.1111/j.1461-0248.2011.01628.x

546 Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Rejmanek, M., Westbrooks, R. (1997).
547 Introduced species: A significant component of human-caused global change. New
548 Zealand Journal of Ecology, 21 (1), 1-16.

549 Rimington, W.R., Pressel, S., Duckett, J.G., & Bidartondo, M.I. (2015). Fungal associations of
550 basal vascular plants: reopening a closed book? New Phytologist, 205 (4), 1394–1398.

551 Ward SM, Gaskin JF, Wilson LM. 2007. Ecological genetics of plant invasion: what
552 do we know? Invasive Plant Science and Management 1(1): 98-109.

553 Whitney KD, Gabler CA. 2008. Rapid evolution in introduced species, 'invasive traits'
554 and recipient communities: challenges for predicting invasive potential. Diversity and
555 Distributions 4(2): 569-580.

556 Willis AJ, Thomas MB, Lawton JH. 1999. Is the increased vigour of invasive weeds
557 explained by a trade-off between growth and herbivore resistance? Oecologia
558 120(4): 632-640.

559 Willis , S.G., Hulme, P.E. 2004. Environmental severity and variation in the
560 reproductive traits of Impatiens glandulifera. Functional Ecology. 18(6): p. p. 887-
561 898.

562 Willi, Y., van Buskirk, J., Hoffman, A.A. (2006). Limits to the adaptive potential of
563 small populations. Annual Review of Ecology, Evolution and Systematics. 92(2):
564 433-458. DOI: https://doi.org/10.1146/annurev.ecolsys.37.091305.110145

565 Woodward FI, Kelly CK. 1995. The influence of CO_2 concentration on stomatal
566 density. New Phytologist 131, 311–327.

567 Woodward, F. 1987. Stomatal numbers are sensitive to increases in CO_2 from pre-
568 industrial levels. Nature 327, 617–618. https://doi.org/10.1038/327617a0

569 Wright IJ, Dong N, Maire V, et al. 2017 Global climatic drivers of leaf size. Science
570 357:917–21

571 Zenni, R.D., Da Cunha, W.L., Sena, G. (2016). Rapid increase in growth and
572 productivity can aid invasions by a non-native tree. AOB plants, 8. DOI:
573 https://doi.org/10.1093/aobpla/plw048

574 Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M. 2009. Mixed Effects
575 Models and Extensions in Ecology with R. New York: Springer Science

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578 Figure Legends:

579 Table 1. Estimated regression parameters, standard errors, t-values and P-values for
580 the models performed on the leaf traits measured in *I. glandulifera*. P-values for the
581 estimated regression parameters are marked in bold to denote statistical significance
582 at $P<0.05$. The adjusted R^2, F and P-values for the fitted models are given in italics.

Variables and fitted models	Estimate	Standard Error	t	P-value	Adjusted R^2	F
Log_{10} Leaf Area						
Model 1				*<0.001*	*0.048*	*6.32*
Intercept	5.6500	1.2464	4.533	**<0.001**		
Year Collected	-0.0016	0.0004	-4.112	**<0.001**		
Latitude	-0.0220	0.0177	-1.239	0.216		
Longitude	0.0026	0.0140	0.185	0.854		
Model 2				*0.039*	*0.017*	*2.83*
Intercept	2.8973	0.9718	2.981	**0.003**		
Mean Annual Temperature	-0.0616	0.0241	-2.552	**0.011**		
Latitude	-0.0170	0.0179	-0.947	0.344		
Longitude	0.0072	0.0142	0.506	0.613		
Log_{10} Leaf Width						
Model 1				*0.002*	*0.037*	*5.08*
Intercept	2.4705	0.6388	3.867	**<0.001**		

Year Collected	-0.0008	0.0002	-3.866	**<0.001**		
Latitude	-0.0085	0.0091	-0.934	0.351		
Longitude	-0.0057	0.0072	-0.795	0.427		
Model 2				*0.062*	*0.014*	*2.47*
Intercept	1.1824	0.4961	2.383	**0.0177**		
Mean Annual Temperature	-0.0329	0.0123	-2.670	**0.008**		
Latitude	-0.0062	0.0092	-0.680	0.497		
Longitude	-0.0039	0.0073	-0.540	0.589		
Log_{10} Leaf Length						
Model 1				*0.008*	*0.028*	*4.02*
(Intercept)	2.6614	0.6552	4.062	**<0.001**		
Year Collected	-0.0006	0.0002	-2.934	**0.004**		
Latitude	-0.0092	0.0093	-0.989	0.324		
Longitude	0.0073	0.0073	0.995	0.320		
Model 2				*0.123*	*0.009*	*1.94*
(Intercept)	1.5873	0.5077	3.127	**0.002**		
Mean Annual Temperature	-0.0196	0.0126	-1.557	0.120		
Latitude	-0.0072	0.0094	-0.767	0.443		
Longitude	0.0095	0.0074	1.282	0.201		

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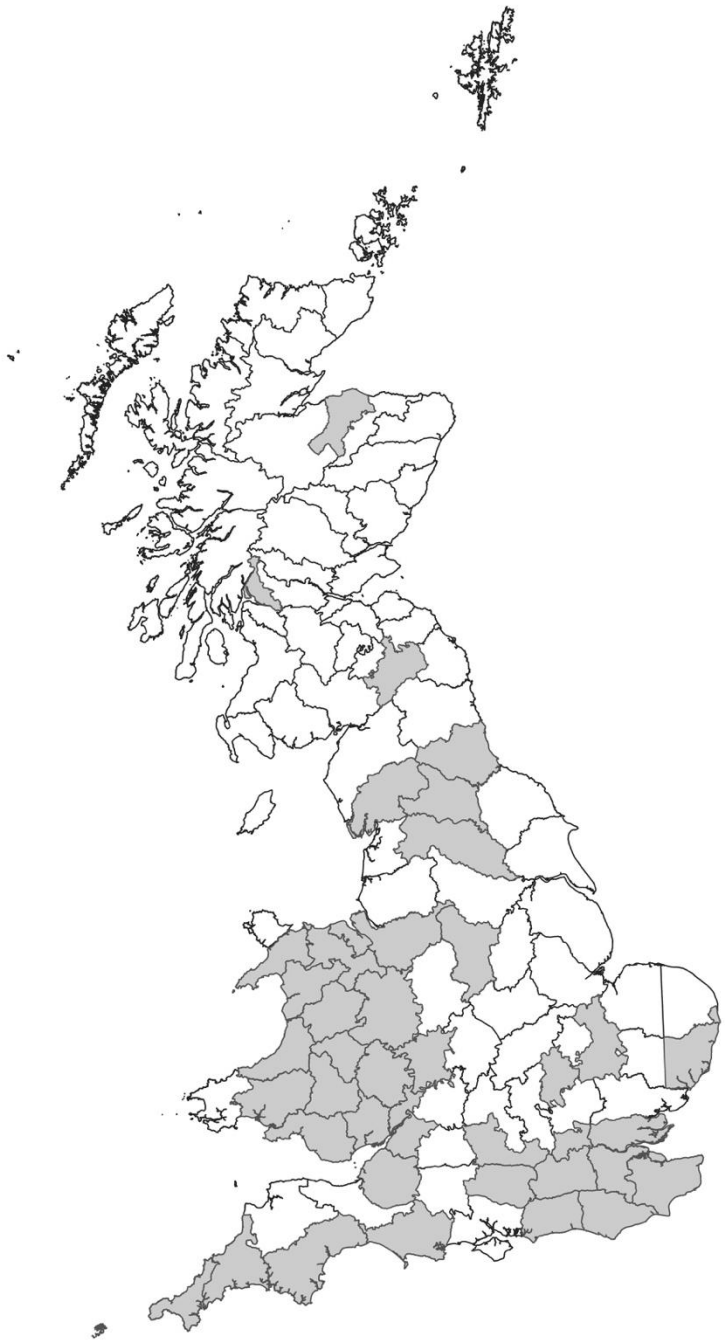
586 Table 2. Estimated regression parameters, standard errors, t-values and P-values for
587 the models performed on stomatal density and flower length (cm) in *I. glandulifera*.
588 P-values for the estimated regression parameters are marked in bold to denote
589 statistical significance at $P<0.05$. The adjusted R^2, F and P-values for the fitted
590 models are given in italics.

Variables and Fitted models	Estimate	Std. Error	t	P-value	Adjusted R^2	F
Stomatal density (number/mm^2)						
Model 1				*<0.001*	*0.088*	*10.44*
(Intercept)	421.3571	119.2098	3.535	**<0.001**		
Year Collected	-0.1866	0.0342	-5.458	**<0.001**		
Latitude	0.2038	1.7841	0.114	0.909		
Longitude	-0.2994	1.2613	-0.237	0.812		
Model 2				*<0.001*	*0.141*	*17.14*
(Intercept)	171.6800	92.1261	1.864	0.063		
Mean Annual Temperature	-14.3264	2.0301	-7.057	**<0.001**		
Latitude	0.6496	1.7242	0.377	0.707		
Longitude	-0.6564	1.2221	-0.537	0.592		

Flower length (cm)						
Model 1				*0.005*	*0.0394*	*4.42*
(Intercept)	-1.0068	0.5649	-1.782	0.076		
Year Collected	0.0006	0.0002	3.518	**<0.001**		
Latitude	0.0024	0.0079	0.303	0.762		
Longitude	-0.0002	0.0061	-0.035	0.972		
Model 2				*0.088*	*0.0143*	*2.261*
(Intercept)	0.0421	0.4347	0.097	0.923		
Mean Annual Temperature	0.0261	0.0109	2.398	**0.017**		
Latitude	0.0009	0.0080	0.109	0.914		
Longitude	-0.0018	0.0062	-0.288	0.774		

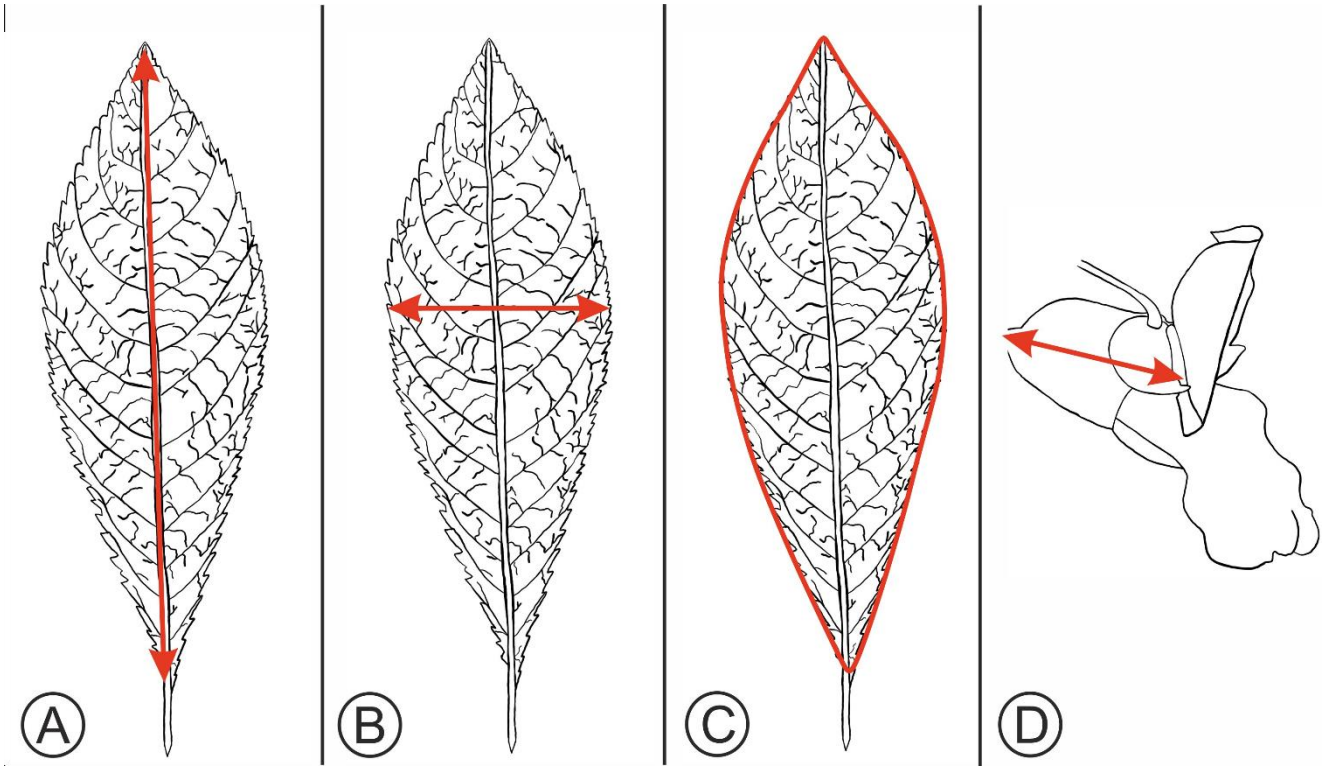
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593 Figure 1. A GIS generated map showing the source of herbarium samples (i.e., vice-
594 counties shaded in grey).

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597 Figure 2. Morphology of *I. glandulifera* leaf and flower with illustrations of how each
598 of the morphological measurements in this study were taken. A. *I. glandulifera* leaf
599 with a red line illustrating how the leaf length was calculated. B. *I. glandulifera* leaf
600 with a red line illustrating how the leaf width was calculated. C. *I. glandulifera* leaf
601 with a red line illustrating how the leaf area was calculated. D. *I. glandulifera* leaf with
602 a red line illustrating how the length of the flower was calculated.

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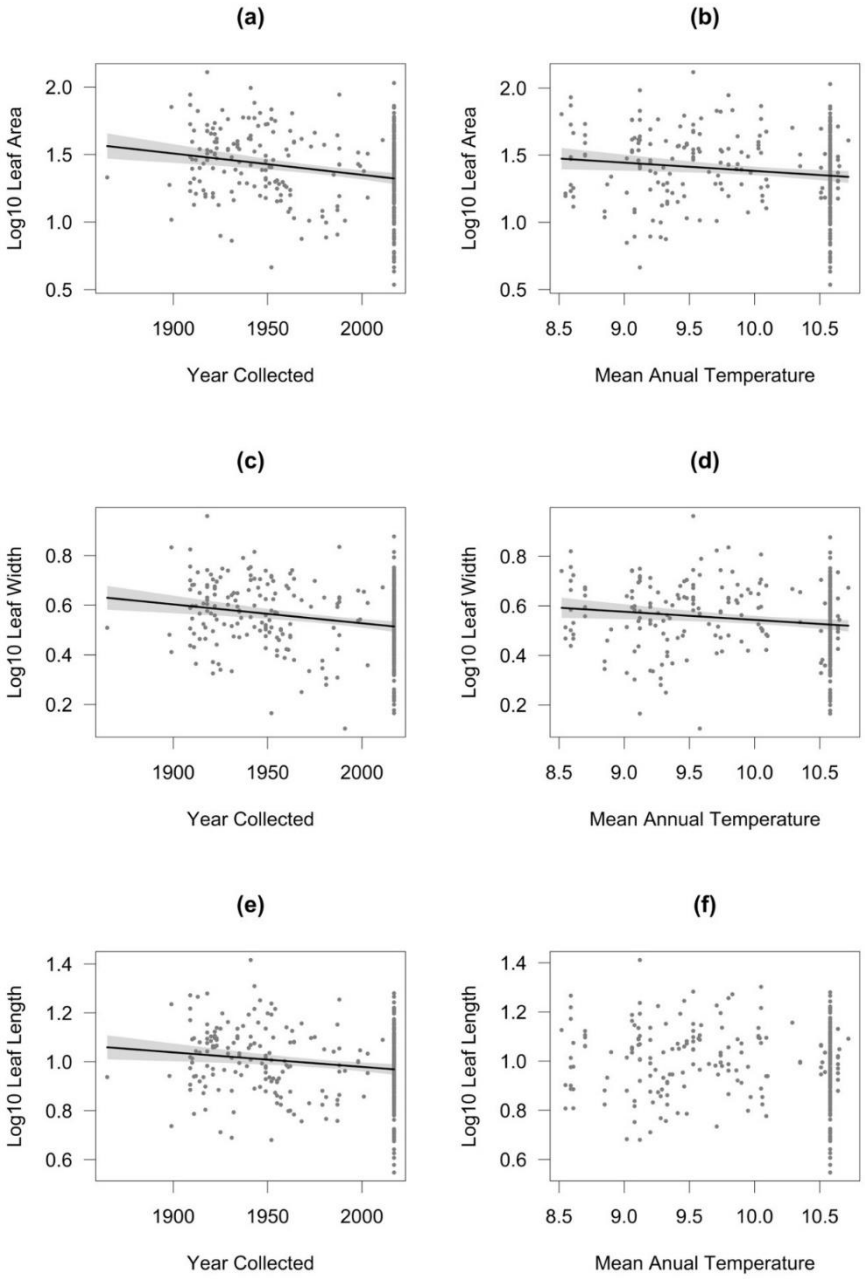
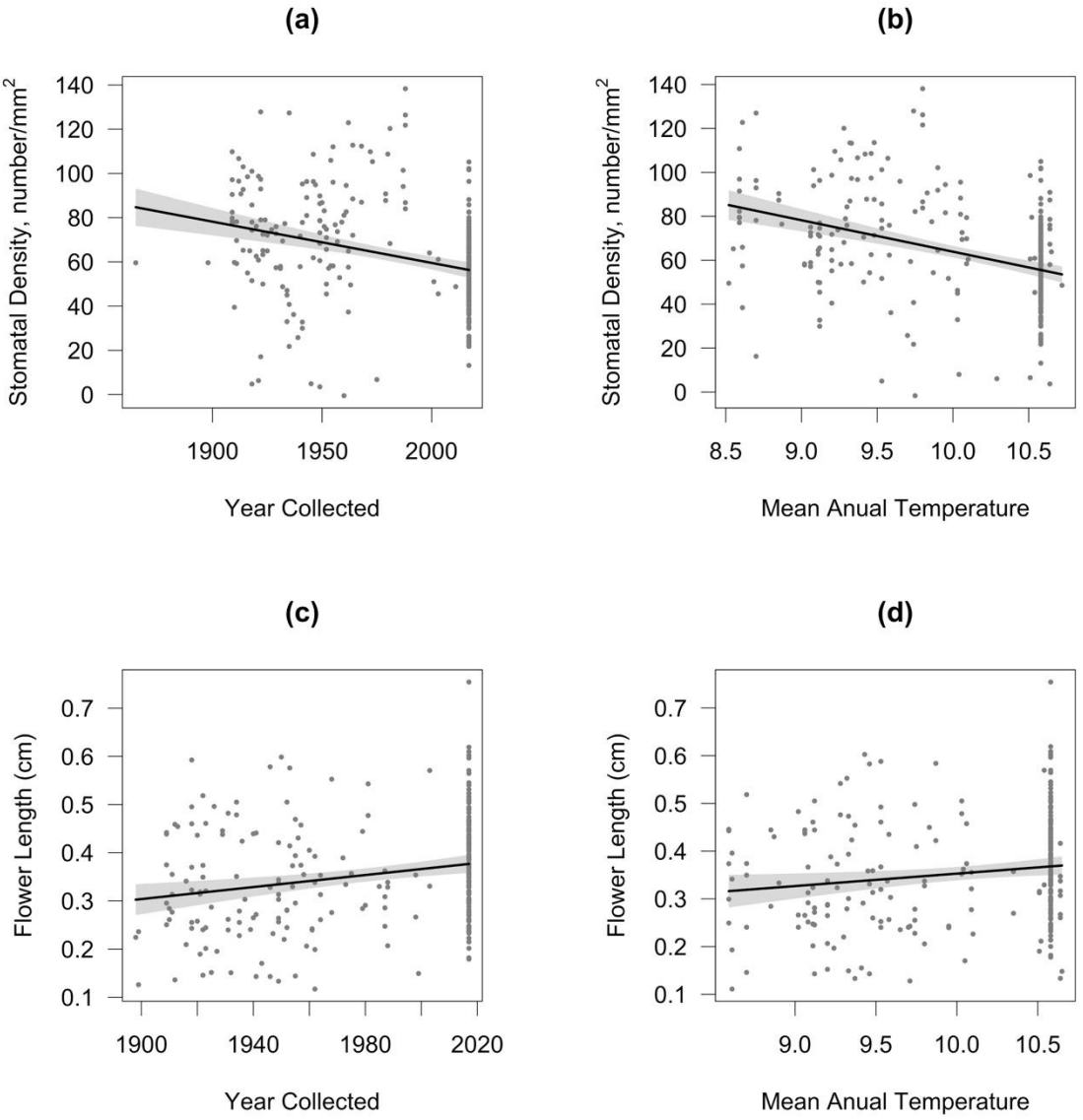


Figure 3. Partial regression plots of the effects of date of collection (Year Collected) and Mean Annual Temperature on Leaf Area (a and b, respectively), Leaf Width (c and d, respectively) and Leaf Length (e and f, respectively). Lines represent model predictions of statistically supported effects; grey bands indicate 95% confidence bands. The adjusted R^2, F and P-values for the fitted models are given in Table 1.



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630 Figure 4. Partial regression plots of the effects of date of collection (Year Collected)
631 and Mean Annual Temperature on Stomatal Density (a and b respectively) and on
632 Flower Length (c and d respectively). Lines represent model predictions of
633 statistically supported effects; grey bands indicate 95% confidence bands. The
634 adjusted R^2, F and P-values for the fitted models are given in Table 2.

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638 Table A5. A table to show the number of herbarium and field collected specimens
639 originating in different vice-counties

Number of specimens	**Vice county**
1	Bedfordshire
1	Berkshire
2	Breconshire
1	Bristol
4	Caernarvonshire
1	Cambridgeshire
1	Cardiganshire
1	Carmarthenshire
2	Cheshire
4	Denbighshire
3	Derbyshire
2	Dorset
2	Dunbartonshire
1	Durham
4	East Cornwall
1	East Kent
1	East Suffolk
1	East Sussex
1	Essex
1	Flintshire
99	Glamorgan
8	Herefordshire

7	Merionethshire
7	Middlesex
1	Mid-West Yorkshire
100	Monmouthshire
1	Montgomeryshire
2	Moray
1	North Hampshire
2	North Somerset
3	North-West Yorkshire
3	Radnorshire
1	Roxburghshire
1	Shropshire
14	South Devon
3	South Essex
16	Surrey
1	Sussex
4	West Cornwall
3	West Gloucestershire
2	West Kent
1	West Morland
2	West Sussex
1	Worcestershire

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641

642 Table A6. Field collection locations, the number of collected specimens and the
643 coordinates of the collection localities for specimens of *Impatiens glandulifera*
644 collected in 2017.

Locality name	Number of collected specimens	Coordinates
Tredegar Park 1 – Ebbw river	12	51°34'6.70"N, 3° 1'11.77"W
Tredegar Park 2- Towards Bassaleg	20	51°34'23.43"N, 3° 2'14.78"W
Bettws lake	10	51°36'18.31"N, 3° 0'41.30"W
Bute Park 1	20	51°29'11.08"N, 3°11'17.68"W
Bute Park 2	20	51°29'4.86"N, 3°11'6.19"W
Bute Park 3	10	51°28'59.99"N, 3°10'57.38"W
Caerleon - Usk Road	20	51°36'54.73"N, 2°57'2.14"W
Llantarnam Lake	8	51°38'24.45"N, 3° 0'22.55"W
Roath Park	20	51°30'14.24"N, 3°10'27.11"W
Taff River - Pontcanna	9	51°29'47.19"N, 3°12'26.86"W
Taff River - Taly	9	51°29'44.12"N, 3°11'42.12"W

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Figure A7. Correlation matrix plot displaying the Pearson correlation coefficients between paired explanatory variables: Year of collection, mean annual temperature, longitude and latitude for each of the samples of *I. glandulifera* used in the study. The bivariate scatter plots with a fitted red line are presented on the bottom of the diagonal, the values of the correlations are displayed. on the top of the diagonal. This plot was generated by using the function 'pairs' in R program.

