Rapid evolution of phenology during range expansion with recent climate change

Global Change Biology

Lustenhouwer, Nicky; Wilschut, Rutger A.; Williams, Jennifer L.; van der Putten, Wim H.; Levine, Jonathan M.

https://doi.org/10.1111/gcb.13947

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed using the principles as determined in the Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. According to these principles research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact openaccess.library@wur.nl

Received: 27 June 2017 | Revised: 29 September 2017 | Accepted: 29 September 2017
DOI: 10.1111/gcb.13947

PRIMARY RESEARCH ARTICLE

WILEY Global Change Biology

Rapid evolution of phenology during range expansion with recent climate change

Nicky Lustenhouwer[1] | Rutger A. Wilschut[2,3] | Jennifer L. Williams[4] | Wim H. van der Putten[2,3] | Jonathan M. Levine[1]

[1]Institute of Integrative Biology, ETH Zurich, Zurich, Switzerland

[2]Department of Terrestrial Ecology, NIOO-KNAW, Wageningen, The Netherlands

[3]Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands

[4]Department of Geography, University of British Columbia, Vancouver, BC, Canada

Correspondence
Nicky Lustenhouwer, Institute of Integrative Biology, ETH Zurich, Universitätstrasse 16, 8092, Zurich, Switzerland.
Email: lustenhouwer@usys.ethz.ch

Funding information
Schweizerischer Nationalfonds zur Förderung der Wissenschaftlichen Forschung, Grant/Award Number: 31003A_141025; H2020 European Research Council, Grant/Award Number: 26055290 (SPECIALS)

Abstract

Although climate warming is expected to make habitat beyond species' current cold range edge suitable for future colonization, this new habitat may present an array of biotic or abiotic conditions not experienced within the current range. Species' ability to shift their range with climate change may therefore depend on how populations evolve in response to such novel environmental conditions. However, due to the recent nature of thus far observed range expansions, the role of rapid adaptation during climate change migration is only beginning to be understood. Here, we evaluated evolution during the recent native range expansion of the annual plant *Dittrichia graveolens*, which is spreading northward in Europe from the Mediterranean region. We examined genetically based differentiation between core and edge populations in their phenology, a trait that is likely under selection with shorter growing seasons and greater seasonality at northern latitudes. In parallel common garden experiments at range edges in Switzerland and the Netherlands, we grew plants from Dutch, Swiss, and central and southern French populations. Population genetic analysis following RAD-sequencing of these populations supported the hypothesized central France origins of the Swiss and Dutch range edge populations. We found that in both common gardens, northern plants flowered up to 4 weeks earlier than southern plants. This differentiation in phenology extended from the core of the range to the Netherlands, a region only reached from central France over approximately the last 50 years. Fitness decreased as plants flowered later, supporting the hypothesized benefits of earlier flowering at the range edge. Our results suggest that native range expanding populations can rapidly adapt to novel environmental conditions in the expanded range, potentially promoting their ability to spread.

KEYWORDS
adaptation, climate change, contemporary evolution, *Dittrichia graveolens*, flowering time, migration, population spread, range shift

1 | INTRODUCTION

The redistribution of species in response to climate change (Chen, Hill, Ohlemüller, Roy, & Thomas, 2011; Parmesan, 2006) has focused ecologists and evolutionary biologists on the processes determining population spread. The persistence of many species will depend on their ability to migrate (Thuiller et al., 2008), and the resulting range shifts will have broad implications for both natural systems and human welfare (Pecl et al., 2017). A central unresolved question in the study of range expansion is whether evolutionary changes that occur in contemporary time facilitate the spread of range shifting populations and thereby contribute to persistence (Hoffmann & Sgrò, 2011; Urban et al., 2016). In particular, as populations spread, they face novel environments, and thus adaptation to local conditions - or the lack thereof – could profoundly affect species' capacity to establish and therefore migrate (Garcia-Ramos & Rodriguez, 2002; Gilbert et al., 2017). Forecasting species' responses to climate change could therefore benefit from a better understanding of the role of adaptive evolution during native range expansions (Urban et al., 2016).

Even if the northward range expansion of climate change migrants is ultimately set in motion by warming conditions (Parmesan & Yohe, 2003), continued expansion may require evolution in response to other environmental variables experienced in the expanded range. For example, day length, light quality, and seasonal variation in climate all change markedly with latitude (Saikkonen et al., 2012; Taulavuori, Sarala, & Taulavuori, 2010), and may present range expanding populations with conditions not experienced within their historic range. Consequently, the cues used by many plant and animal species to time key events in their life cycle may no longer match the temperature conditions optimal for life cycle transitions (Visser, 2008), and populations may be maladapted to the environment of the expanded range, limiting further range expansion. Phenology in particular, is expected to be under strong selection as populations spread northward because timing reproductive events with the seasonal climate pattern is essential for reproductive success (Maron, Vilà, Bommarco, Elmendorf, & Beardsley, 2004; Saikkonen et al., 2012).

That range expanding populations can evolve during spread is supported by studies of species introduced to new continents (reviewed in Colautti & Lau, 2015). For example, non-native species of plants and animals have established latitudinal clines in photoperiodic response (Urbanski et al., 2012), developmental rate (While et al., 2015), and phenology (Colautti & Barrett, 2013b; Novy, Flory, & Hartman, 2013; Weber & Schmid, 1998) in their exotic range over a time period of decades to a few hundred years. However, our understanding of evolution during exotic range expansions after introduction to new continents cannot be simply extrapolated to intracontinental, native range expansions induced by climate change, because these two processes differ in several key ways. Native range-expanding populations may be under weaker selection than their exotic counterparts, because environmental change is expected to be more gradual than after introduction to a new continent (Bossdorf et al., 2005). Moreover, genetic diversity and gene flow often decrease only gradually with distance from the core within a native range (Austerlitz, Jung-Muller, Godelle, & Gouyon, 1997), whereas both are reduced abruptly upon introduction to an exotic range.

Studies of evolution during native range expansion (e.g., Buckley & Bridle, 2014; Lancaster, Dudaniec, Hansson, & Svensson, 2015) remain rare, in part because the spatial extent of most native range expansions has thus far been modest, especially relative to the migrations expected with future climate change (Parmesan & Yohe, 2003; Root et al., 2003). Moreover, due to the recent nature of these expansions, the range expansion history of many species is only beginning to be documented. Finally, demonstrating adaptive differentiation in spreading populations, native or exotic, is challenging, because they evolve through a combination of genetic drift, gene flow, and natural selection. For example, range edge populations may sometimes be preadapted to local conditions beyond the historical range limit by originating from similar environments within the historical native range (Colautti & Lau, 2015; Oduor, Leimu, & van Kleunen, 2016).

Here, we evaluate rapid evolution during the recent native range expansion of *Dittrichia graveolens* (L.) Greuter (hereafter *Dittrichia*), an annual plant species in the Asteraceae with a native distribution in the Mediterranean (Brullo & De Marco, 2000). Consistent with climate warming over the last 50 years, *Dittrichia* has rapidly expanded its range northward over this period (Rameau, 2008), spreading from France into Germany (Brandes, 2009; Jäger, 2017) and then into the Netherlands (Stouthamer, 2007). This northward expansion subjects the plants to a reduction in light availability, especially toward the end of the year (Appendix S1), which may be particularly important because the species flowers in late summer and fall in the core of its range in France (Rouy, 1903). For many plant species, growth is limited by low temperatures, and particularly frost, at the end of the growing season (Larcher & Bauer, 1981). In addition to its northward range expansion, *Dittrichia* has also expanded its range eastward into Switzerland (Ciardo & Delarze, 2005; Lauber, Wagner, & Gygax, 2012), which is located at a similar latitude and altitude as the historical northern range edge (Figure 1). The recent nature, large spatial extent, and detailed historical record of the spread of *Dittrichia* provide a unique opportunity to study the role of rapid adaptation in native range expansions. Moreover, *Dittrichia*'s annual life cycle allows for meaningful evolutionary change to occur within a few decades.

We tested the hypothesis that rapid evolution in response to novel environmental conditions promotes native range expansion with climate change. For *Dittrichia*, we specifically expected the evolution of earlier flowering time with its northward range expansion. To test this hypothesis, we examined differentiation in phenology between populations from the range core and edge, in common garden experiments situated at the Dutch and Swiss range edges. With this approach, we asked (i) How have core and range edge populations of *Dittrichia* diverged in phenology? and (ii) How does selection shape phenology under the conditions experienced at the range edge? Finally, we used RAD-sequencing to characterize population structure and genetic diversity across the native and expanded range, allowing us to place the observed differentiation in phenology in the context of the range expansion history.

2 | MATERIALS AND METHODS

2.1 | Study system

Dittrichia occurs in ruderal, open habitat such as abandoned fields, stony hillsides and river banks, roadsides, and industrial areas (Brullo

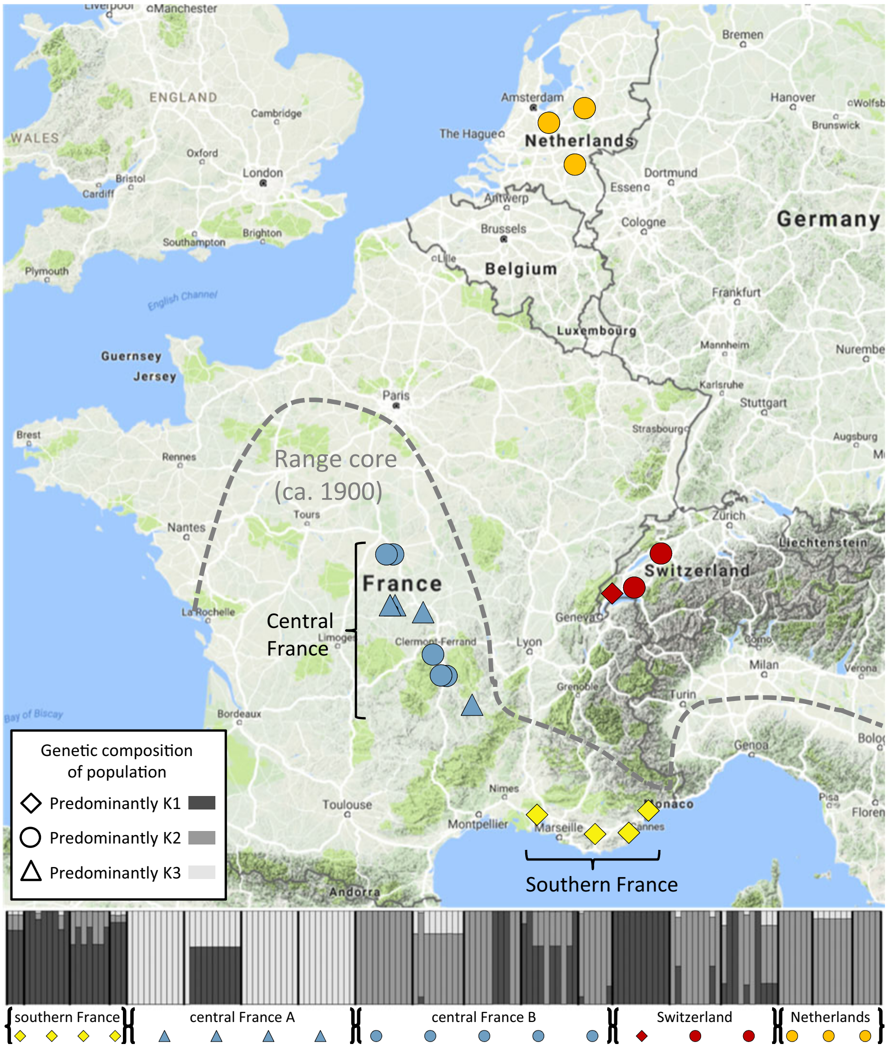

FIGURE 1 Sample locations and population structure. Top: sampled populations in the historical range (indicated by the dashed line) and expanded range of *Dittrichia*. Each symbol represents one population, located in one of four geographic regions compared in this study: southern France, central France, Switzerland, and the Netherlands. Shapes correspond to the genetic cluster most prevalent in the population (K1, diamonds; K2, circles; K3, triangles). Bottom: STRUCTURE output based on all 13,582 single nucleotide polymorphisms. Each bar represents one individual, and thick lines separate populations. Gray tones within bars indicate the degree to which an individual was assigned to each genetic cluster (K1, dark gray; K2, medium gray; K3, light gray). Central France consists of two genetically differentiated groups, with the Dutch and most Swiss populations belonging to the second group shown by circles. Map data ©2017 GeoBasis-DE/BKG (©2009), Google, Inst Geogr. Nacional

& De Marco, 2000; Rameau, 2008; Rouy, 1903). Plants are 20–50 cm high (Brullo & De Marco, 2000) and produce a few hundred to several thousand flower heads, with approximately 30 seeds per head (N. Lustenhouwer, personal observation). Fruits consist of an achene (containing the seed) and a bristled pappus, which forms a plume assisting in wind dispersal (Rameau, 2008). Flowers are insect-pollinated (Rameau, 2008) or self-fertilized. Floras of France report a flowering time between August and November (Rouy, 1903; Tison, Jauzein, & Michaud, 2014), but do not distinguish between regions. Our own field observations indicated that phenology was more advanced in central France than in southern France in early fall.

The historic range of *Dittrichia* in France around 1900 extended from the Mediterranean basin to the Atlantic coast up to Paris, excluding the east and the north of the country (Coste & Flahault, 1937; Rouy, 1903; Figure 1). Current evidence is not conclusive about whether climate change is the only cause of the recent range expansion of *Dittrichia* in Europe, but the timing and northward shift coincide with climate warming in Europe in the late 20th century (Lenoir, Gégout, Marquet, de Ruffray, & Brisse, 2008). In the 1980s, *Dittrichia* was reported on roadsides in south-west Germany (Garve & Garve, 2000; Nowack, 1993) and the northwestern Ruhr district (Dettmar & Sukopp, 1991), and began spreading along highways from there around 10 years later (Garve & Garve, 2000; Nowack, 1993; Radkowitsch, 1996). In the Netherlands, it has been expanding its range along highways since approximately 2005 (NDFF, 2015; Sparrius & Van Strien, 2014; Stouthamer, 2007). In addition to its northward range expansion, the species also recently extended its distribution eastward in France (Antonetti, Kessler, Brugel, Barbe, & Tort, 2006), and spread into Switzerland along highways (Lauber et al., 2012). By 2003, it was widely distributed around Lake Geneva (Ciardo & Delarze, 2005). In this study, we examine plant performance at the Dutch and Swiss range edges (Figure 1).

2.2 | Seed collection

To establish the common gardens and provide plant material for molecular analysis of population structure, we collected seeds from the core of the range in France, and from the expanding range edges in Switzerland and the Netherlands. We collected seeds from four populations in southern France, representing the Mediterranean conditions in much of the native range, and from nine populations in central France, along a latitudinal gradient toward the historic northern range edge. From the range edge in Switzerland, which is located at a similar latitude and altitude as the populations in central France, we collected seeds from three populations. Finally, we collected seeds from three populations in the Netherlands, representing the most novel northern latitude populations (Figure 1, Table 1). The Netherlands receives markedly less photosynthetically active radiation as compared to Switzerland, central or southern France (Appendix S1, Fig. S1), meaning that if daylight is limiting, Dutch plants must complete their life cycle earlier in the year to receive comparable photosynthetically active radiation as found in the more southern locations. Outside of winter, mean daily temperatures at our sampling locations do not differ between the Netherlands and Switzerland or central France, though are markedly lower in these regions than found in southern France (Appendix S1, Fig. S1).

The Swiss and Dutch populations all occurred close to highways, whereas the French populations were located in various disturbed habitat such as roadsides, abandoned fields and industrial areas. For each population, we collected seeds from at least 10 individual plants if available, and stored them by maternal family in paper bags at room temperature. Seeds from Switzerland and France were collected in the field in October-November 2013, and seeds from the Netherlands in October 2015.

2.3 | DNA extraction and RAD-sequencing

To identify the genetic relationships among our populations, and provide background on the spread history of *Dittrichia*, we genotyped individuals grown from the field-collected seeds from all populations of origin. If possible, we grew one seedling from each of 10 maternal families per population, adding extra half-siblings where necessary to achieve a total of 190 individuals from 19 populations. We freeze-dried the plant material in a lyophilizer for 48 hr and extracted DNA using Qiagen DNeasy® Plant Mini Kits (Ref. 69104). We then genotyped all individuals using double digest, single-end RAD-sequencing, following the protocol by Peterson, Weber, Kay, Fisher, and Hoekstra (2012) with restriction enzymes EcoR1 and Taq1 (New England Biolabs). The insert size of the library was around 600 bp. The four pools with 48 indexes were then pooled in equimolar ratios and 15% standard DNA library was added to increase complexity. All individuals were then sequenced in two lanes of an Illumina HiSeq 2,500 sequencer using a 125 bp single-end protocol at the Functional Genomics Center Zurich. DNA extraction, RAD library preparation and bioinformatics analyses were carried out in collaboration with the Genetic Diversity Center, ETH Zurich. Keygene N.V. owns patents and patent applications protecting its Sequence Based Genotyping technologies.

2.4 | Common garden experiments

To examine the reproductive timing and fitness of plants from all regions of origin under range-edge conditions, we set up parallel common garden experiments in Wageningen, the Netherlands, and Zurich, Switzerland. The gardens were established in 2016 and located at the range edges because these are the locations at which understanding how phenology has evolved and influences fitness is most relevant to future range expansion. To reduce the impact of maternal effects across all regions of origin and produce seeds for use in our main common garden experiments, we first grew plants from seed in a common environment for one (Dutch seeds) or two (Swiss and French seeds) generations of self-fertilization. Maternal families in our study descend from a single field plant (Appendix S2). Ultimately, we used seeds from three Dutch, three Swiss, six central French and three southern French populations to establish the common gardens (Table 1). Three populations from central France were excluded because they were located very close to another population within the same city, and one population from southern France was excluded due to limited seed availability. For each of the 15 populations, we grew three individuals from four maternal families, comprising 12 plants per population. This design was replicated in the two gardens, using the same seed sources (Appendix S2).

TABLE 1 Sampled populations in France, Switzerland, and the Netherlands

Population location	Latitude	Longitude	Elevation (m)
Southern France			
Fréjus	43.450	6.697	26
Aire du Merle nord	43.640	4.986	63
Pégomas	43.588	6.935	15
la Roquebrussanne†	43.373	5.974	390
Central France			
Montluçon (roadside)*	46.375	2.592	197
Montluçon (field)	46.378	2.590	196
Montmarault	46.323	2.970	475
Le-Puy-en-Velay	45.057	3.933	705
Bourges (industrial area)	47.099	2.447	138
Bourges (highway)*	47.046	2.345	131
Cournon-d'Auvergne	45.751	3.160	357
Issoire (highway)*	45.525	3.257	379
Issoire (industrial area)	45.556	3.250	383
Switzerland			
Gare de Lonay-Préverenges	46.523	6.519	388
Münsingen highway exit	46.879	7.542	525
Chemin d'Ogoz, Saint-Saphorin	46.475	6.805	564
The Netherlands			
Cuijk	51.755	5.845	14
't Harde	52.406	5.887	13
Laren	52.239	5.228	5

The order of populations matches the order (left to right) in the STRUCTURE plot in Figure 1. Populations with an asterisk (*) were not included in the common garden experiments, because another population was sampled within the same town. In such cases, we excluded roadside populations and kept the population located within the town. Population la Roquebrussanne (†) was not included in the common garden experiments due to limited seed availability.

To produce the seedlings that would eventually be planted into these gardens, on June 13th (2016), seeds were placed on moist germination paper (Zurich) or gamma-sterilized soil (Wageningen) in transparent boxes in growth cabinets set to 28°C with a 16 hr day/8 hr night cycle. After approximately 4 days, seeds began germinating and were transferred onto soil in seedling trays in a greenhouse. On July 11th, when the seedlings were strong enough, they were transplanted into 6 L plastic pots (21.2 cm in diameter and 16.8 cm high) filled with a common sterilized sandy loam soil collected from a former agricultural field in the Netherlands (Beneden-Leeuwen; 51.89°N, 5.56°E). In Wageningen, the pots were placed on a tarp in an open field (51.99°N, 5.67°E, 13 m); in Zurich, in wooden beds on a roof terrace (47.38°N, 8.55°E, 460 m). We used a randomized block design, with each of three blocks containing one plant from each maternal family. For the duration of the experiment, we provided extra water when plants were at risk of drying out due to hot or dry weather conditions.

To quantify phenology, we conducted a census twice a week on fixed days, and recorded the date each plant first produced flowers (at least one yellow floret visible). The day of first flowering (which is more accurately measured than budding or fruiting date) was our measure of phenology, quantified as days since plants were placed in the common garden. Plants were harvested on December 1st and 2nd, when development had slowed and plants started to senesce. For each plant, we counted the number of fruiting heads (those with pappus-bearing achenes) on the plant. We defined plant fitness as the number of fruiting heads multiplied by seed viability, the methods for which are explained next. To evaluate the possible fate of plants after our harvesting date, we left 11 of the latest-flowering plants in the common garden until early February, but only three of these 11 produced any viable seeds.

After harvesting the experiment, we evaluated seed viability. For each plant, 30 seeds were selected haphazardly from fruiting heads that were collected at the time of harvest. These fruiting heads were open-pollinated, so influence from paternal individuals from different populations is possible. Nonetheless, seed development is strongly linked to the flowering phenology and maternal investment of the mother plant. We first tested the germination fraction of the seeds on moist germination paper in transparent boxes in growth cabinets set to 28°C with a 16 hr day/8 hr night cycle. Germinated seeds were counted and removed after 15–16 days. The remaining seeds were covered in a 250 mg/L gibberellic acid (GA_3) solution and further germinants were counted after 24 hr. We lastly estimated the viability of the still nongerminated seeds at the region of origin level (Netherlands, Switzerland, central France and southern France) for each garden. To do so, we selected one seed at random from 30 individuals from each region, cut the seeds in half and stained them with a 0.25% tetrazolium solution. Viable embryos were counted after 2.5 hr. The final viability rate per individual plant was thus the number of germinated seeds at the plant level plus the projected number of viable, nongerminated seeds (based on 30 seeds from the plant's region of origin and garden), divided by the total number of seeds tested.

2.5 | Data analysis

2.5.1 | Population structure and genetic diversity

Bioinformatics analysis of the RAD sequencing data (details provided in Appendix S3) yielded 13,582 single nucleotide polymorphisms (SNPs) across 190 individuals. We evaluated population structure using STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). To prevent bias in the clustering algorithm, we used the haplotype data and a single individual per maternal family (153 in total) for our analysis, selecting the half-sib with the least amount of missing data. The analysis was conducted with two to eight genetic clusters (K), and replicated 10 times using the admixture model with correlated allele frequencies. We visualized the output using Structure Harvester (Earl & vonHoldt, 2012) and determined the optimal K using the Evanno, Regnaut, and Goudet (2005) method. In range expanding populations, we may expect many loci not to be in Hardy–Weinberg equilibrium (HWE). We therefore tested the robustness of our results to this assumption by rerunning the STRUCTURE analysis using only the SNPs in HWE, and by computing a neighbor-joining tree, which does not depend on a population model (details in Appendix S3).

To infer changes in genetic diversity during range expansion, we examined the genetic diversity within three genetically distinct groups in the core of the range (identified in the population structure analysis) and at the two expanding range edges. To ensure equal numbers of individuals within these five groups, we selected 18 individuals at random per group, distributed uniformly across the available populations. Nucleotide diversity π was calculated for each SNP using VCFtools (Danecek et al., 2011), averaged across the loci and divided by the mean fragment length of 121 bp.

2.5.2 | Differentiation in phenology and adaptation to range edge conditions

All statistical analyses were conducted in R v3.4.0 (R Core Team, 2017). We analyzed phenological differences between geographic origins using a mixed-effects Cox proportional hazards model with a Gaussian distribution of random effects (coxme package, Therneau, 2015). In our analyses, we compared four distinct regions of origin: central and southern France in the core of the range, and the two range edges in Switzerland and the Netherlands. Population and maternal family (nested in population) were treated as random effects. We first fit a global model with the day of first flowering as the response variable and region of origin, common garden location, and their interaction as fixed effects. Main and interaction effects were evaluated using Type II partial-likelihood-ratio tests (car package, Fox & Weisberg, 2011). Because there was a significant interaction between origin and garden, we then fit separate models per garden and analyzed pairwise differences between regions of origin using Tukey contrasts (multcomp package, Hothorn, Bretz, & Westfall, 2008).

To estimate selection on phenology in each garden, we assumed flowering day to be a quantitative trait and standardized it to zero mean and unit variance, separately for each garden. Relative fitness was calculated by dividing each plant's fitness by the average fitness of each garden. We then estimated natural selection on phenology at the garden level using fitness splines (Schluter, 1988), fitting a nonlinear fitness function to all individuals using generalized additive models in R (R code based on Colautti & Barrett, 2013a; package mgcv, Wood, 2011). Additionally, we estimated selection on flowering day separately for individuals from each region of origin, because these regions differed in their range of phenology. Here, we used linear regression as in a classic selection analysis (Lande & Arnold, 1983).

To analyze the effects of origin, garden, and their interaction on fitness, we fit a mixed-effects negative binomial model using maximum likelihood (function glmer.nb in package lme4, Bates, Maechler, Bolker, & Walker, 2015), which is appropriate for overdispersed count data. We tested the main and interaction effects of origin and garden on fitness using likelihood ratio tests, and removed the interaction since it was not statistically significant. We then tested pairwise differences between regions using Tukey contrasts in an additive model with garden and origin as fixed effects.

3 | RESULTS

3.1 | Spread history

Population genetic analysis revealed three distinct genetic clusters of individuals (STRUCTURE analysis based on all SNPs; Figure 1). The first cluster (K1) included all plants from southern France and a few individuals from central-French populations. Within central France, we found two different clusters, one of which (K2) included all of the Dutch populations and half of the individuals from Switzerland. The other Swiss plants were more closely related to individuals from southern France (K1), or consisted of a mix of the two clusters (Figure 1). It is therefore likely that the Dutch populations originate from central France, whereas multiple introductions of mixed genetic composition occurred in Switzerland. The last cluster (K3) was common in four populations in central France, but otherwise rare. F_{ST} analysis including populations from southern France, the two groups within central France, and the range edges in Switzerland and the Netherlands supported these results (Appendix S3). Finally, the STRUCTURE analysis with only the SNPs in HWE and neighbor-joining tree produced qualitatively similar results to our analyses including all SNPs (Appendix S3).

Neutral genetic diversity was lower at the range edge in the Netherlands (nucleotide diversity $\pi = 0.0014$) than in central France populations belonging to the same genetic cluster ($\pi = 0.0023$) or in populations from southern France ($\pi = 0.0023$). In contrast, genetic diversity was not lower in the Swiss populations ($\pi = 0.0024$) compared to the central France populations, consistent with their mixed genetic composition. The genetic cluster found in central France but rare elsewhere (K3) had low genetic diversity ($\pi = 0.0013$).

3.2 | Phenological changes with spread

Plants in both common gardens showed wide variation in flowering phenology, with its onset spanning from early September to early November (Figure 2). There was a significant interactive effect of region of origin and common garden location on flowering day ($\chi^2(3) = 25.4$, $p < .001$). However, plants from different regions of origin were strongly differentiated in their phenology in both gardens (Wageningen: $\chi^2(3) = 234$, Zurich: $\chi^2(3) = 230$, $p < .001$). Plants from central France flowered 3 weeks earlier on average than plants from southern France ($z = -5.8$ and -4.3, $p < .001$). Within the expanded range, plants from the Netherlands flowered even 1 week earlier than those from central France (Wageningen garden: $z = -5.7$, $p < .001$; Zurich garden: $z = -2.3$, $p = .09$ (marginally significant); Figure 2), despite only having reached their more northern latitude over the last 20–50 years. Swiss plants had similar phenology to plants from central France, which matches their comparable latitude and altitude.

To further explore the importance of early phenology for plant fitness, we estimated phenotypic selection on flowering day. In both common gardens, fitness declined as plants flowered later,

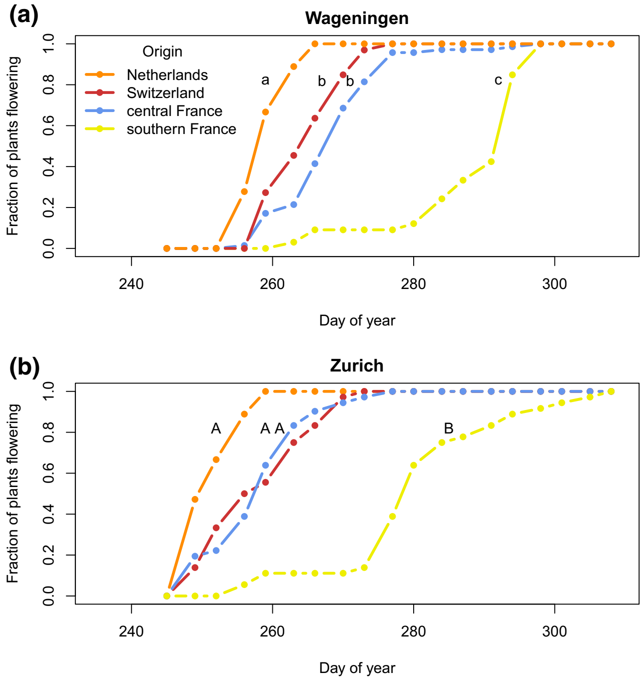

FIGURE 2 Cumulative flowering phenology of plants of Dutch ($n = 36$), Swiss ($n = 33$ in Wageningen, $n = 36$ in Zurich), central French ($n = 70$ in Wageningen, $n = 72$ in Zurich) and southern French ($n = 33$ in Wageningen, $n = 36$ in Zurich) origins in the common gardens in (a) Wageningen and (b) Zurich. We used the day each individual first produced flowers as the response variable (quantified as days since placed in the common garden), recorded in twice-weekly surveys (points). For illustration purposes, here we present the day of year, ranging from 245 (September 1st) to 308 (November 3rd). Curves that do not share letters are significantly different at $\alpha = 0.05$, tested individually for each garden

approaching zero for plants flowering from early October onwards (Figure 3). Although plants from southern France, which flowered last, had the lowest fitness, we also found selection for earlier phenology at the range edges among plants from central France, the putative region of origin of the populations spreading at both range edges (Figure 3). Statistically significant selection on earlier flowering time was found in nearly all populations placed at the range edge (Figure 3).

In both common gardens, plants of Dutch, Swiss, and central French origin had comparable fitness, whereas the fitness of plants from southern France was much lower and often zero (pairwise difference with all other regions: $p < .001$; Figure 4). Consistent with expectations based on their phenology, the fitness of Dutch plants in the common garden in Wageningen (the Netherlands) was slightly higher than the fitness of central French plants (Figure 4, left), although there was no statistical support for this result due to the limited number of sampled populations. Overall, plants in the Wageningen garden had almost three times higher fitness than plants in the Zurich garden ($\chi^2(1) = 169$, $p < .001$; Figure 4).

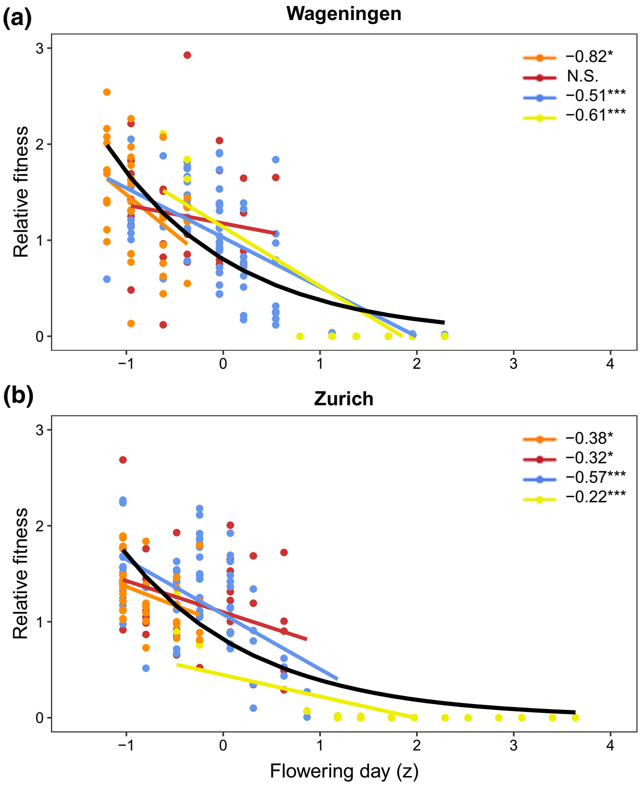

FIGURE 3 Selection on phenology in (a) Wageningen and (b) Zurich. Colors indicate regions of origin as in Figure 2. Flowering day was standardized separately for each garden; $z = 0$ corresponds to September 26th and 18th in panels (a) and (b) respectively, with a z-score of 1 equaling 12–13 days. Curved lines are nonlinear fitness splines estimated over all individuals per garden. Straight lines are linear regressions for each individual region, with estimates of the slope (i.e. the standardized selection differential). Significance codes: *<.05, **<.01, ***<.001, N.S. not significant)

4 | DISCUSSION

As species expand their ranges northward in response to climate warming, the timing of key events in their life cycle may be maladapted to the photoperiod and seasonality they encounter in the expanded range, limiting further spread. Our results suggest that as *Dittrichia* has migrated northward over the last several decades, rapid evolution has shifted its phenology to earlier in the season. We found genetic variation in flowering time associated with latitude of origin, extending from the core of the range in southern France to the expanding northern range edge in the Netherlands. Moreover, we observed a fitness advantage of early-flowering plants at both range edges, suggesting that rapid phenology evolution could promote the range expansion of *Dittrichia*. Thus, as hypothesized, even if the northward range expansion of climate change migrants is precipitated by warming conditions, continued expansion may benefit from evolution in response to other environmental variables experienced in the expanded range.

The early flowering time of the Dutch populations is striking because the species did not occur at this latitude previously, suggesting that evolution in response to novel environmental conditions in the Netherlands occurred in less than 50 years (NDFF, 2015; Stouthamer, 2007). Given our genetic results suggesting that the Dutch populations likely originate from central France (Figure 1), the phenology of the Dutch populations represents a 1-week advance in average flowering time relative to the source population (Figure 2). Swiss populations flowered around the same time as the central French populations, which would be expected based on their similar latitude and altitude. However, given the mixed genetic composition of the Swiss populations (Figure 1), only a fraction of the individuals introduced to Switzerland were of similar genetic composition as

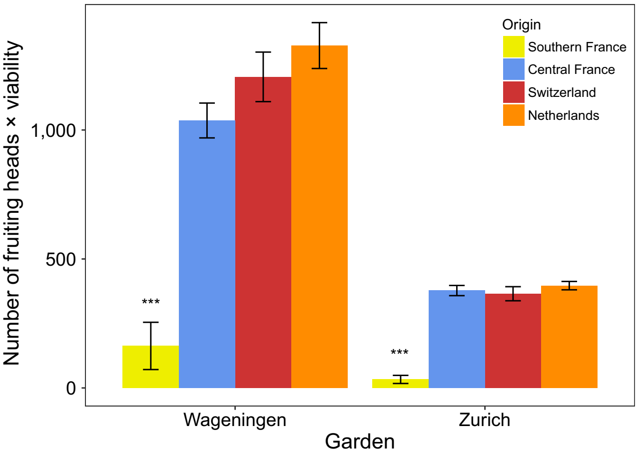

FIGURE 4 Fitness in Wageningen (left) and Zurich (right), defined as the number of fruiting heads multiplied by the proportion of seed viability. Bars display the mean ± one standard error of the raw data across all individuals per region of origin. The *** indicates that plants from southern France had lower fitness than plants from all other regions of origin ($p < .001$) in both gardens

those in central France (genetic cluster K2), and therefore likely preadapted to Swiss environmental conditions. Other Swiss populations, which more likely originated from southern France (genetic cluster K1), may have recently evolved earlier phenology as they spread north.

Several lines of evidence support our hypothesis that the differentiation in phenology among central France, Swiss, and Dutch populations is the result of selection for earlier flowering time over the fifty years of range expansion. Since de novo mutations are unlikely to play a large role on this timescale, sufficient genetic variation in phenology would be required within the central France populations, providing the material on which natural selection could act (Barrett & Schluter, 2008). Indeed, the phenology variation among the central France plants was such that the earliest individuals were comparable in timing to those of the Dutch and Swiss populations (Figure 3). Moreover, flowering time is highly heritable in this species ($h^2 = 0.86$; Appendix S4). Finally, we found selection for early phenology in both range edge common gardens (Figure 3). The decline in fitness as plants flowered later matches our expectation that early flowering is beneficial at northern latitudes, and is consistent with the observed differentiation in flowering time between populations from southern and central France within the range core (Figure 2). Taken together with the short generation time of *Dittrichia*, the observed genetic variation in flowering time, high heritability of flowering phenology, and selection for earlier flowering allow for the rapid evolution of phenology during the range expansion.

4.1 | Caveats and open questions

Although our results strongly support the hypothesis that *Dittrichia* evolved to flower earlier in the year over the course of range expansion, several of our results are surprising in light of the observed differentiation in flowering time between northern and southern populations. First, plants of a given origin tended to flower 1 week later in the northern range edge garden in Wageningen than in Zurich (Figure 2). This may reflect nonadaptive plasticity (Ghalambor, McKay, Carroll, & Reznick, 2007; Ghalambor et al., 2015), where growing conditions in the Netherlands delay the onset of flowering despite the adaptive benefits of flowering earlier at the northern range edge. Second, the strength of selection on flowering time was similar in both common gardens (Figure 3), even though stronger selection for earlier phenology might be expected in the north based on the evolution of earlier flowering in these locations. This result may be an artifact of the specific year and location of study. Although 2016 weather conditions in Zurich and Wageningen did not deviate markedly from long term average conditions in these locations (Appendix S1, Fig. S2), it may be that earlier flowering time in the north is selected for by infrequent weather events not experienced during the year of the study. Indeed, for plants with an annual life history and limited seed bank, 1 year of failed recruitment due to poorly timed reproduction can be catastrophic.

Third, given the evidence for the evolution of earlier average flowering time in the Dutch origin populations, and the implication that this occurred in response to northward range expansion, it is surprising that these plants overall had similar, rather than higher fitness than central French and Swiss plants at the common garden in Wageningen (Figure 4). However, the fitness of range edge Dutch populations could have been reduced by other evolutionary forces that change mean fitness during range expansion, including life history trade-offs and the accumulation of deleterious mutations over the course of spread. The latter, known as the expansion load (Peischl, Dupanloup, Kirkpatrick, & Excoffier, 2013) is supported by the lower genetic diversity in Dutch than in range core populations. We therefore believe that the evolution of earlier flowering enhanced the fitness of northern populations relative to the lower fitness they would have otherwise had due to expansion load and other factors. Finally, the overall higher fitness we found in Wageningen compared to Zurich (Figure 4) is surprising given its more northern location and the later flowering time of the plants in this garden. The most likely explanation for this result is that experimental growing conditions in each garden were influenced by microclimate factors unrepresentative of the regions as a whole, related to the field and rooftop locations of the two gardens. This may also explain why plants flowered later in the Wageningen than Zurich garden.

Although our results indicate that *Dittrichia* evolved earlier flowering time over the course of range expansion, the agents of selection and physiological and genetic trade-offs contributing to this evolutionary trajectory require further study. Previous work has identified trade-offs between flowering time and size at reproduction for *Solidago* spp., *Lythrum salicaria*, and *Microstegium vimineum*, all invasive plant species exhibiting similar latitudinal clines in phenology across their exotic range in the northern temperate zone. In the north, plant growth is constrained by shorter growing seasons, whereas in the south, later flowering allows greater investment into reproduction (Colautti & Barrett, 2013b; Colautti, Eckert, & Barrett, 2010; Montague, Barrett, & Eckert, 2008; Novy et al., 2013; Weber & Schmid, 1998). To evaluate whether late-flowering *Dittrichia* in our study had a higher reproductive potential than their earlier flowering counterparts, we examined the total number of reproductive structures plants produced, regardless of whether those seeds matured. At the garden in the Netherlands, the late-flowering plants originating from southern France produced 40% fewer heads than the plants from other populations (Appendix S5), indicating that earlier flowering does not seem to come at the expense of total reproduction at the northern range edge. In the Swiss garden, plants from southern France made as many heads as their more northern counterparts (Appendix S5), indicating equal reproductive potential. Although these results suggest that late flowering may be less detrimental to reproductive potential at the more southern latitude of Switzerland, a third common garden in southern France would be necessary to evaluate whether late flowering may in fact be advantageous under the warm climate in the Mediterranean core of the range.

A second topic for further study are the specific environmental factors favoring the evolution of earlier phenology in the Netherlands. Moving north from the core of the range, the main shift in climate occurs from southern France to central France and

Switzerland. By contrast, the amount of received photosynthetically active radiation is comparable from southern France through to central France and Switzerland, and only decreases strongly once plants spread from these central latitudes to the Netherlands (Appendix S1, Fig. S1). Although low temperatures, and frost in particular, limit reproduction of *Dittrichia* at the end of the growing season (Parsons & Cuthbertson, 2001), temperature differences do not independently explain the earlier phenology of populations from the Netherlands relative to those from central France or Switzerland, given their comparable climates. Rather, the increasing seasonality in day length in more northern latitudes and the associated reduction in light availability seem a more likely constraint on flowering time in the Netherlands (Saikkonen et al., 2012). Nonetheless, climate and photoperiod may constrain flowering time in different parts of the range, and even interact (McKown et al., 2014), an important topic of study for future work.

4.2 | Implications for the spread of climate change migrants

The evolution of earlier flowering time as *Dittrichia* spread north likely contributed to the velocity of range expansion. In the northern range edge garden (Figure 3a), late-flowering individuals from central France, the putative source region of the Dutch populations, produce less than half the seeds of the earliest-flowering individuals, illustrating the demographic costs of maladapted phenology. Such costs would likely affect how fast those populations advance (Gilbert et al., 2017), given the central role of low density growth rate in determining the invasion speed (Kot, Lewis, & van den Driessche, 1996; Lewis & Kareiva, 1993).

In combination with recent findings of rapid evolution during the native range expansion of insects (Buckley & Bridle, 2014; Lancaster et al., 2015) and birds (Gunnarsson, Sutherland, Alves, Potts, & Gill, 2011), we conclude that rapid adaptation to novel environments is not only a feature of biological invasions, but may also promote native range expansions with climate change. Though our study focused on adaptation to seasonality, a factor predictably changing with northward expansion, rapid evolution may also promote the expansion of species facing other types of novel environmental conditions. On the biotic side, range expanding populations may experience novel interactions with enemies (Doorduin & Vrieling, 2011) or mutualists. On the abiotic side, resources that were present in the native range may not be available in the expanded range, such as certain soil types or host plants (Buckley & Bridle, 2014).

Our results support growing calls for models predicting the eventual range limits and spread velocity of climate change migrants (e.g., Kearney, Porter, Williams, Ritchie, & Hoffmann, 2009) to incorporate evolutionary change (Urban et al., 2016). Given that both spreading and adapting (cf. Berg et al., 2010) may ultimately prove the most effective response of native species threatened by global warming, models properly accounting for rapid adaptation during range expansion may predict greater species persistence (Bush et al., 2016) than is typical from more traditional modeling approaches (Thomas et al., 2004). Ultimately, understanding the feedbacks between the ecology of spreading populations and their evolution in response to novel environments is key to forecasting species' responses to global change.

ACKNOWLEDGEMENTS

We thank Renato Guidon and Annabelle Valtat for carrying out the DNA extractions, Claudia Michel for the RAD library preparation, and Niklaus Zemp for his help with the genetic data analysis. We also thank everyone who assisted with seed collection and experiments at ETH and NIOO-KNAW. Members of the Plant Ecology group provided helpful comments on the manuscript, and Bas Crezee assisted in obtaining the climate and light availability data. N.L., J.L.W., and J.M.L. acknowledge support from Swiss National Science Foundation grant 31003A_141025, and R.A.W. and W.H.v.d.P. from ERC Advanced grant 26055290 (SPECIALS) to W.H.v.d.P.

ORCID

Nicky Lustenhouwer http://orcid.org/0000-0002-5157-857X

REFERENCES

Antonetti, P., Kessler, F., Brugel, É., Barbe, J.-P., & Tort, M. (2006). *Atlas de la flore d'Auvergne*. Chavaniac-Lafayette, France: Conservatoire botanique national du Massif central.

Austerlitz, F., Jung-Muller, B., Godelle, B., & Gouyon, P.-H. (1997). Evolution of coalescence times, genetic diversity and structure during colonization. *Theoretical Population Biology*, *51*, 148–164.

Barrett, R. D. H., & Schluter, D. (2008). Adaptation from standing genetic variation. *Trends in Ecology & Evolution*, *23*, 38–44.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48.

Berg, M. P., Kiers, E. T., Driessen, G., Van der Heijden, M., Kooi, B. W., Kuenen, F., ... Ellers, J. (2010). Adapt or disperse: Understanding species persistence in a changing world. *Global Change Biology*, *16*, 587–598.

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, *144*, 1–11.

Brandes, D. (2009). Autobahnen als Wuchsorte und Ausbreitungswege von Ruderal- und Adventivpflanzen. *Braunschweiger Naturkundliche Schriften*, *8*, 373–394.

Brullo, S., & De Marco, G. (2000). Taxonomical revision of the genus *Dittrichia* (Asteraceae). *Portugaliae Acta Biologica*, *19*, 341–354.

Buckley, J., & Bridle, J. R. (2014). Loss of adaptive variation during evolutionary responses to climate change. *Ecology Letters*, *17*, 1316–1325.

Bush, A., Mokany, K., Catullo, R., Hoffmann, A., Kellermann, V., Sgrò, C., ... Ferrier, S. (2016). Incorporating evolutionary adaptation in species distribution modelling reduces projected vulnerability to climate change. *Ecology Letters*, *19*, 1468–1478.

Chen, I.-C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*, 1024–1026.

Ciardo, F., & Delarze, R. (2005). Observations floristiques sur l'autoroute lémanique: Espèces nouvelles, halophiles et rares. *Saussurea; Traveaux de La Société Botanique de Genève*, *35*, 83–89.

Colautti, R. I., & Barrett, S. C. H. (2013a). Data from: Rapid adaptation to climate facilitates range expansion of an invasive plant. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.878m3

Colautti, R. I., & Barrett, S. C. H. (2013b). Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science*, *342*, 364–366.

Colautti, R. I., Eckert, C. G., & Barrett, S. C. H. (2010). Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. *Proceedings of the Royal Society of London B: Biological Sciences*, *277*, 1799–1806.

Colautti, R. I., & Lau, J. A. (2015). Contemporary evolution during invasion: Evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology*, *24*, 1999–2017.

Coste, H., & Flahault, C. H. (1937). *Flore descriptive et illustrée de la France de la Corse et des contrées limitrophes* (2. tirage., Vols. 1–3). Paris: Librairie des sciences et des arts.

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., … Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*, 2156–2158.

Dettmar, J., & Sukopp, H. (1991). Vorkommen und Gesellschaftsanschluss von *Chenopodium botrys* L. und *Inula graveolens* (L.) DESF. im Ruhrgebiet (Westdeutschland) sowie im regionalen Vergleich. *Tuexenia*, *11*, 49–65.

Doorduin, L. J., & Vrieling, K. (2011). A review of the phytochemical support for the shifting defence hypothesis. *Phytochemistry Reviews*, *10*, 99–106.

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*, 2611–2620.

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*, 2nd ed. Sage, CA: Thousand Oaks.

Garcia-Ramos, G., & Rodriguez, D. (2002). Evolutionary speed of species invasions. *Evolution*, *56*, 661–668.

Garve, E., & Garve, V. (2000). Halophytes at potash mine dumps in Germany and France (Alsace). *Tuexenia*, *20*, 375–417.

Ghalambor, C. K., Hoke, K. L., Ruell, E. W., Fischer, E. K., Reznick, D. N., & Hughes, K. A. (2015). Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature*, *525*, 372–375.

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*, 394–407.

Gilbert, K. J., Sharp, N. P., Angert, A. L., Conte, G. L., Draghi, J. A., Guillaume, F., … Whitlock, M. C. (2017). Local adaptation interacts with expansion load during range expansion: Maladaptation reduces expansion load. *The American Naturalist*, *189*, 368–380.

Gunnarsson, T. G., Sutherland, W. J., Alves, J. A., Potts, P. M., & Gill, J. A. (2011). Rapid changes in phenotype distribution during range expansion in a migratory bird. *Proceedings of the Royal Society of London B: Biological Sciences*, *279*, 411–416.

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*, 479–485.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal*, *50*, 346–363.

Jäger, E. J. (Ed.). (2017). *Rothmaler - Exkursionsflora von Deutschland. Gefäßpflanzen: Grundband* (21. Aufl. 2017, Vol. [Band 2], Ed. 21). Berlin Heidelberg: Springer.

Kearney, M., Porter, W. P., Williams, C., Ritchie, S., & Hoffmann, A. A. (2009). Integrating biophysical models and evolutionary theory to predict climatic impacts on species' ranges: The dengue mosquito *Aedes aegypti* in Australia. *Functional Ecology*, *23*, 528–538.

Kot, M., Lewis, M. A., & van den Driessche, P. (1996). Dispersal data and the spread of invading organisms. *Ecology*, *77*, 2027.

Lancaster, L. T., Dudaniec, R. Y., Hansson, B., & Svensson, E. I. (2015). Latitudinal shift in thermal niche breadth results from thermal release during a climate-mediated range expansion. *Journal of Biogeography*, *42*, 1953–1963.

Lande, R., & Arnold, S. J. (1983). The measurement of selection on correlated characters. *Evolution*, *37*, 1210–1226.

Larcher, W., & Bauer, H. (1981). Ecological significance of resistance to low temperature. In O. L. Lange, P. S. Nobel, C. B. Osmond & H. Ziegler (Eds.), *Physiological Plant Ecology I* (pp. 403–437). Berlin Heidelberg: Springer. https://doi.org/10.1007/978-3-642-68090-8

Lauber, K., Wagner, G., & Gygax, A. (2012). *Flora helvetica*, 5th ed. Bern: Haupt.

Lenoir, J., Gégout, J. C., Marquet, P. A., de Ruffray, P., & Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. *Science*, *320*, 1768–1771.

Lewis, M. A., & Kareiva, P. (1993). Allee dynamics and the spread of invading organisms. *Theoretical Population Biology*, *43*, 141–158.

Maron, J. L., Vilà, M., Bommarco, R., Elmendorf, S., & Beardsley, P. (2004). Rapid evolution of an invasive plant. *Ecological Monographs*, *74*, 261–280.

McKown, A. D., Guy, R. D., Klápště, J., Geraldes, A., Friedmann, M., Cronk, Q. C. B., … Douglas, C. J. (2014). Geographical and environmental gradients shape phenotypic trait variation and genetic structure in *Populus trichocarpa*. *New Phytologist*, *201*, 1263–1276.

Montague, J. L., Barrett, S. C. H., & Eckert, C. G. (2008). Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *Journal of Evolutionary Biology*, *21*, 234–245.

NDFF. (2015). *NDFF Verspreidingsatlas*. Retrieved from http://verspreidingsatlas.nl

Novy, A., Flory, S. l., & Hartman, J. M. (2013). Evidence for rapid evolution of phenology in an invasive grass. *Journal of Evolutionary Biology*, *26*, 443–450.

Nowack, R. (1993). Massenvorkommen von *Dittrichia graveolens* (L.) Greuter (Klebriger Alant) an Autobahnen in Süddeutschland. *Floristische Rundbriefe*, *27*, 38–40.

Oduor, A. M. O., Leimu, R., & van Kleunen, M. (2016). Invasive plant species are locally adapted just as frequently and at least as strongly as native plant species. *Journal of Ecology*, *104*, 957–968.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 637–669.

Parmesan, C., & Yohe, G. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, *421*, 37–42.

Parsons, W. T., & Cuthbertson, E. G. (Eds.) (2001). Stinkwort. In *Noxious weeds of Australia* (2nd ed., pp. 281–283). Collingwood, Australia: Csiro Publishing.

Pecl, G. T., Araújo, M. B., Bell, J. D., Blanchard, J., Bonebrake, T. C., Chen, I.-C., … Williams, S. E. (2017). Biodiversity redistribution under climate change: Impacts on ecosystems and human well-being. *Science*, *355*, eaai9214. https://doi.org/10.1126/science.aai9214

Peischl, S., Dupanloup, I., Kirkpatrick, M., & Excoffier, L. (2013). On the accumulation of deleterious mutations during range expansions. *Molecular Ecology*, *24*, 5972–5982.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, *7*, e37135.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Radkowitsch, A. (1996). Der Klebrige Alant—*Dittrichia graveolens* (L.) Desf. Aktueller Stand der Ausbreitung in Bayern. *Hoppea: Denkschriften Der Regensburgischen Botanischen Gesellschaft*, *57*, 473–482.

Rameau, J.-C. (2008). *Flore forestière française: Région méditerranéenne*, Vol. 3. Paris: Institut pour le développement forestier.

Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., & Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. *Nature*, *421*, 57–60.

Rouy, G. (1903). XLIV. Rubiacées - XLVIII. Composées. In G. Rouy & J. Foucaud, *Flore de France, ou description des plantes qui croissent spontanément en France, en Corse et en Alsace-Lorraine* Vol. 8, (pp. 205–206). Tours: Deslis.

Saikkonen, K., Taulavuori, K., Hyvönen, T., Gundel, P. E., Hamilton, C. E., Vänninen, I., . . . Helander, M. (2012). Climate change-driven species' range shifts filtered by photoperiodism. *Nature Climate Change*, *2*, 239–242.

Schluter, D. (1988). Estimating the form of natural selection on a quantitative trait. *Evolution*, *42*, 849–861.

Sparrius, L. B., & Van Strien, A. J. (2014). Het berekenen van jaarlijkse trends van planten op basis van verspreidingsgegevens. *Gorteria*, *37*, 31–40.

Stouthamer, W. (2007). Kamferalant verovert de snelwegen. *FLORON Nieuws*, *7*, 1.

Taulavuori, K., Sarala, M., & Taulavuori, E. (2010). Growth responses of trees to arctic light environment. In U. Lüttge, W. Beyschlag, B. Büdel, & D. Francis (Eds.), *Progress in botany 71* (pp. 157–168). Berlin Heidelberg: Springer.

Therneau, T. M. (2015). *coxme: Mixed effects cox models (Version R package version 2.2-5)*. Retrieved from https://CRAN.R-project.org/package=coxme

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., . . . Williams, S. E. (2004). Extinction risk from climate change. *Nature*, *427*, 145–148.

Thuiller, W., Albert, C., Araujo, M. B., Berry, P. M., Cabeza, M., Guisan, A., . . . Zimmermann, N. E. (2008). Predicting global change impacts on plant species' distributions: Future challenges. *Perspectives in Plant Ecology Evolution and Systematics*, *9*, 137–152.

Tison, J.-M., Jauzein, P., & Michaud, H. (2014). *Flore de la France méditerranéenne continentale*. Turriers: Naturalia Publications.

Urban, M. C., Bocedi, G., Hendry, A. P., Mihoub, J.-B., Pe'er, G., Singer, A., . . . Travis, J. M. J. (2016). Improving the forecast for biodiversity under climate change. *Science*, *353*, aad8466.

Urbanski, J., Mogi, M., O'Donnell, D., DeCotiis, M., Toma, T., Armbruster, P., . . . Shaw, E. R. G. (2012). Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. *The American Naturalist*, *179*, 490–500.

Visser, M. E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B: Biological Sciences*, *275*, 649–659.

Weber, E., & Schmid, B. (1998). Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. *American Journal of Botany*, *85*, 1110–1121.

While, G. M., Williamson, J., Prescott, G., Horváthová, T., Fresnillo, B., Beeton, N. J., . . . Uller, T. (2015). Adaptive responses to cool climate promotes persistence of a non-native lizard. *Proceedings of the Royal Society of London B: Biological Sciences*, *282*, 20142638.

Wood, S. N. (2011). Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, *73*, 3–36.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Lustenhouwer N, Wilschut RA, Williams JL, van der Putten WH, Levine JM. Rapid evolution of phenology during range expansion with recent climate change. *Glob Change Biol*. 2017;00:1–11. https://doi.org/10.1111/gcb.13947