



Reproductive strategy of a temperate canopy tree *Tilia cordata* Mill. (Malvaceae) is related to temperature during flowering and density of recent recruits

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Abstract

Facultative clonality is extremely common in plants, but the relative emphasis on sexual versus asexual reproduction varies both between and within species, which in turn may influence individual fitness and population persistence. *Tilia cordata* is a temperate, entomophilous canopy tree that is partially clonal. Favourably warm climatic conditions have been linked with successful sexual reproduction in the species with clonality being suggested as the reason for population persistence in colder periods. Despite this the extent, character and structure of asexual reproduction in the species have never been described, nor has its relationship with climate. Fine-scale spatial genetic structure was assessed in 23 stands across a latitudinal gradient. The proportion of individuals that are of clonal origin has a wide range with a mean of ~43%. Genetic diversity is high, with even mostly clonal stand possessing several distinct genotypes. A beta regression model shows that historic summer temperatures and density of recent recruits are predictors of the proportion of clonal recruitment. Clonal reproduction is less important in stands that experience higher temperatures during flowering while stands with more saplings have more clones. Additional factors likely affect the balance between the two reproductive modes. The climatic relationship suggests a trend towards a higher proportion of recruitment from seed in a warming climate, although factors such as herbivory may prevent this.

Keywords Environmental predictor — evolution · Fertilisation success · Fine-scale genetic structure · Genetic diversity · Population genetics · Sexual systems

Introduction

Although obligate asexual reproduction is rare in angiosperms, facultative clonality is extremely common (Barrett 2015; Eckert 2002; Harper 1977), particularly in perennial plants (Nuortila et al. 2002; Holsinger 2000). Vegetative propagation is the most common form of clonality with an estimated ~80% of all angiosperm lineages possessing some means of reproducing in this manner (Klimeš et al. 1997). Compared to exclusively sexual reproduction, a mixed reproductive strategy provides several ecological benefits. These include an increased capacity for resource

acquisition in patchy environments (light, nutrients, water), lowered chance of death for a particular genotype due to spread mortality risks, and the possibility of physiological integration and spatial division of labour (e.g. sharing of root systems) amongst shoots (Barrett 2015). Clonal reproduction may also provide a buffer against complete reproductive failure in periods of poor weather or when conditions are otherwise unsuitable for sexual reproduction (Barrett 2015).

However, over longer time scales, clonality can have negative consequences on individual fitness and population duration. It reduces genotypic diversity by definition, and if species are self-incompatible and the spatial arrangement of vegetative growth is aggregated, outcrossing success can also be reduced (Vallejo-Marín et al. 2010; Charpentier 2001). This can affect the viability of small populations due to lower sexual fecundity as a result of intraclone incompatibility (Honnay and Bossuyt 2005; Nuortila et al. 2002). This process can ultimately lead to a complete failure of sexual reproduction and if any particular clonal lineage has

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a competitive advantage, monoclonal stands (Halkett et al. 2005; Honnay and Bossuyt 2005). Reduced genotypic diversity and a lack of sexual reproduction can affect future adaptive potential by lowering the efficiency of natural selection or increase the rate at which deleterious alleles can accumulate, further decreasing reproductive capacity. Either of these factors could affect the likelihood of long-term population persistence (Holsinger 2000); hence, the necessity of understanding both how common clonal reproduction is as well as its character and spatial arrangement.

The relative emphasis on sexual versus asexual reproduction differs not just between but also within species (Silvertown 2008), with different contexts favouring one reproductive mode over the other. For instance, in widely distributed species, clonality is often more prevalent in marginal populations which are subject to less favourable environmental conditions (Brzosko et al. 2013). If these reduce the success rate of sexual reproduction or cause it to fail entirely, vegetative propagation can contribute to population persistence due to the longevity of clonal individuals and the ability to continue recruiting (Barrett 2015; de Witte and Stöcklin 2010). Specific factors that can shift the balance between sexual and asexual reproduction include disturbance regime (Morris et al. 2004), water depth in aquatic species (Li et al. 2018), individual density (Rautiainen et al. 2004) and sex ratios in dioecious species (Dering et al. 2016). Climatic factors such as water availability have also been shown experimentally to shift the balance between the two modes by increasing the relative cost of sex (Wang et al. 2018). Broadly speaking, species that exist in environmental extremes such as arctic regions, aquatic or shady habitats and nutrient poor conditions show a tendency towards clonality. This suggests a correlation between clonality and habitat quality, amongst other aspects of environmental variation (Ye et al. 2014, 2016; van Groenendael et al. 1996).

The climate is rapidly changing (IPCC 2021); this will in turn have an impact on the distribution of species; an understanding of the balance of reproductive modes in a species can be an important predictor of migration potential given the relative effectiveness of seed versus vegetative dispersal (Morris et al. 2014; Silvertown 2008). This makes an understanding of clonality potentially important information in the generation of species distribution models used to forecast responses to climatic change (as these can benefit from the inclusion of life-history traits, e.g. Matthews et al. 2011). Similarly, the impact of some plant pathogens is expected to increase (e.g. *Phytophthora* spp.) as a result of more favourable environmental conditions or an increased susceptibility of host species due to factors such as drought stress (Sturrock et al. 2011). As biotic stress tolerance generally and pathogen resistance specifically are linked to genetic diversity (or the ability to generate novel genotypes via recombination), an understanding of clonality may be

part of mitigating these impacts (Honnay and Bossuyt 2005). Finally, where genetic variation is at risk and the need for conservation has been realised, the knowledge of this aspect of an organism's life history allows appropriate management measures that best capture and maintain the species' genetic variation to be chosen (Namroud et al. 2005).

The difficulty of examining reproductive balance and determining its correlates varies between taxa. Community-level studies that look at prevalence of clonality in a particular habitat have demonstrated environmental correlates of the same (e.g. Ye et al. 2014, 2016; Silvertown 2008). The difficulty with such an approach lies in removing phylogenetic constraints (van Groenendael et al. 1996), which brings to the fore the importance of examining within-species clonal variation as a way of elucidating the role of environmental factors in inducing shifts in reproductive mode (Morris et al. 2004). The previously cited examples of shrubs and forbs show that some species can be manipulated experimentally to assess directly how a particular abiotic or biotic variable affects clonal growth. For trees however, their slow growth and size can make such experiments unfeasible, and studies must be undertaken using natural populations. A wide variety of tree species undertake some form of clonal propagation, including many *Quercus* (Fagaceae), *Populus* (Salicaceae), *Fagus* (Fagaceae) and *Tilia* species (Malvaceae) (Logan et al. 2019; Evans and Morris 2016; Guarino et al. 2015; Logan et al. 2015; Morris et al. 2014; Lone et al. 2011; Mock et al. 2008; Alfonso-Corrado et al. 2005; Bakker et al. 2001; Montalvo et al. 1997; Pigott 1991). Trees have been shown to exhibit within-species variation in the balance between sex and vegetative propagation, just as other plants do. For example, differences in clonal incidence between stands of oak have been linked with distinct disturbance regimes (Valbuena-Carabaña and Gil 2017). Previous work describes or tests for differences in the incidence of clonality between locations in distinct environmental contexts (low, high altitude) or broad categories of habitats (marginal, central) (Logan et al. 2019; Morris et al. 2004, 2014; Persson et al. 2004). To our knowledge, however, no explicit link between climate and clonality in temperate canopy trees has been demonstrated; a direct comparison of climatic variables and reproductive strategy is lacking. This represents a significant gap in the literature given the role of trees in dominating and shaping their community.

Tilia cordata Mill. (small leaved lime) is a species with a potential connection between climate and reproductive balance. It is an entomophilous canopy tree with a widespread but patchy distribution (Fig. 1). An effective vegetative propagator, it produces clonal individuals ('ramets', *sensu* Harper 1977; cf. 'genets', the clonal group as a whole) in a variety of ways. Shoots from the root collar can develop into multiple stems and aboveground connections can disappear over time, leading to separate individuals. These basal

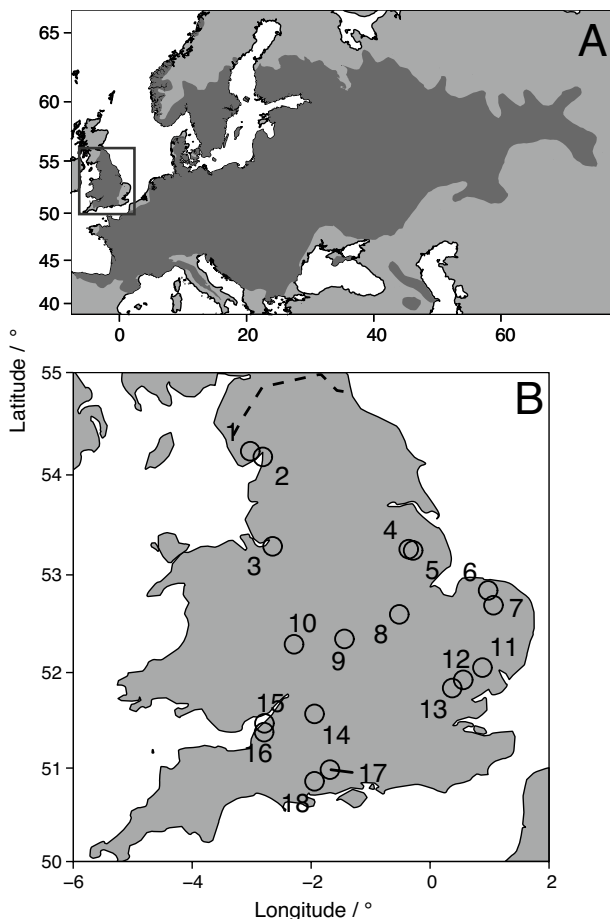


Fig. 1 **A** Overall range of *Tilia cordata* (EUFORGEN 2009) with context of sampled area indicated by a box. **B** The location of sampling sites (see Table 1 for key to numbers and exact location); the dashed line indicates the approximate location of the northern range edge in the UK, based on Pigott (1991)

shoots can also layer themselves, while fallen trees can produce multiple vertical shoots along their length, which ultimately develop into new ramets. The capacity for vegetative propagation does not seem to reduce with stem age (Pigott 1991). By contrast, successful sexual reproduction in the species seems to rely on critical temperatures being reached during both pollination and seed development (Pigott 1981; Pigott and Huntley 1981). In the UK, flowering is typically early to mid-July in the south and mid to late July in the north (Pigott 1981, 2012; Pigott and Huntley 1981), with a 50-day difference on flowering times across the full latitudinal range (Pigott 2012). The current extent of its range was established during the Holocene climatic optimum when summer temperatures were 1–2 °C higher than currently (Birks 1989; Davis et al. 2003), and its fertility was presumably not an issue. The subsequent temperature decline means that northern marginal populations rarely set fertile seed except during the warmest summers, due to

either a failure of pollination (Pigott and Huntley 1981) or of fruit development (Pigott 1981). This is in contrast to more southern populations which frequently produce viable fruits (Pigott 1991). In concert with other potential factors such as herbivory (Pigott 1991), this ultimately leads to infrequent sexual recruitment, which may promote vegetative reproduction, or at least increase its relative importance (Silvertown 2008). As a result, clonal growth has been suggested as the reason for population persistence in marginal locations such as the north-west of England (Fig. 1) (Pigott and Huntley 1978). Recent work in Denmark, also close to the northern limit of the species, found clonal reproduction in eight of the nine study sites and all four of those that were exhaustively sampled, suggesting the clones may simply have not been detected at the ninth site (Erichsen et al. 2019). In comparison, sites in central range locations can have little to no clonal growth at all (Logan et al. 2019); however, the clonal status of the most recent recruits in northern populations has yet to be investigated so it is unclear if this may have changed over recent years.

Although it is clear that tree species modify their reproductive strategy based on environmental differences between populations, the relationship between clonality and relevant climatic variables has not been examined explicitly. *T. cordata* is an ideal model organism for testing this, given a potential mechanism in temperature-linked fertility and already observed differences between northern and southern populations in terms of clonality levels. Furthermore, its current distribution in the UK is largely the product of natural processes rather than forestry practices, being rarely planted (Peterken and Mountford 2017; Rackham 2003), in contrast with other species (Rodwell 1991). This presents a natural experiment that would allow a potential clonality-climate link to be identified and quantified. Understanding this relationship would add to our knowledge of how environmental constraints shape reproductive strategy in temperate canopy tree species, which will be an important part of assessing the evolutionary potential of species in a climate that is selectively dynamic. Here, then, we assess the levels of clonality in semi-natural populations of a canopy-dominant tree across a climatic gradient and in doing so test the hypothesis that reproductive balance is related to temperatures during flowering. Given current climatic trends, with increasingly warmer summers (IPCC 2021), the clonal status of the most recent recruits will also be established, as they may demonstrate changing strategies; this has not yet been assessed in *T. cordata*. Other factors have been linked with the frequency of asexual reproduction such as individual density and disturbance, and therefore, their relationship with clonality here will be assessed (Morris et al. 2014; Weed and Schwarzländer 2014; Valbuena-Carabaña and Gil 2013). Similarly, the consequences of clonality for population persistence depend on its character, such as the diversity

of clonal genotypes and their spatial arrangement (Vallejo-Marín et al. 2010; Honnay and Bossuyt 2005), and so these will also be determined. Taken together, a direct comparison of climate and clonality, the addition of demographic comparisons between sites and a spatially explicit sampling scheme, represents a significant advance on previous such studies in *T. cordata* (e.g. Logan et al. 2019).

Methods

Sampling method and location

Eighteen semi-natural ancient woodlands (ASNW) containing populations of *Tilia cordata* were sampled. These areas were spread across a 274 km east-west and 376 km north-south gradient, which represents a majority of the native range of the species within the UK, encompassing a spread of climatic conditions likely to be relevant to the balance between sex and clonal recruitment (Fig. 1A, Table 1). The most northern populations here are also close to the species' latitudinal range edge in Western Europe (Fig. 1B) (Pigott 1991). Populations sympatric with the other UK native *Tilia* species (*T. platyphyllos* Scop., large-leaved lime) were not sampled to reduce the possibility of hybridisation as a confounding variable, particularly as both species are similar ecologically (Barker et al. 2015; Logan et al. 2015).

In each location, areas where *T. cordata* was a canopy-dominant were identified and a single 30 × 30 m quadrat was

placed using random number generation. Leaf material was collected from all *T. cordata* individuals where possible and dried in silica gel (representative voucher specimens from each population were submitted to LIV and MANCH). To allow for an assessment of the spatial arrangement of clonality, the location of each tree was mapped using measuring tapes (Arnaud-Haond et al. 2007a). Trees often possessed multiple stems due to the sites' history of coppicing as a management strategy.

Therefore, only stems not connected aboveground and separated by a distance of more than 0.2 m were considered distinct individuals. Due to the history of coppicing as a management strategy in these populations, and the link between clonality and disturbance generally or this practice specifically, any stem part of an obvious coppice stool was noted as such. As stools gradually become separate individuals (Del Tredici 2001), the proportion of the quadrat composed of visible coppice stools should therefore serve as a proxy for time elapsed since the stand was last coppiced. This allows for an estimation of the relationship between disturbance regime, or at least that part of it caused by forestry practices, and clonal growth.

Similarly, if clonality is linked with climate, then changes in the origin of new recruits may have occurred over time due to global warming. It is important then to understand the origins of any younger individuals within quadrats, which has not been done yet in this species. Therefore, demography data was recorded. Stems were classified as adult or juvenile based on trunk diameter at 1.37 m (DBH), and overall

Table 1 Names and precise locations of sample sites, the number of individuals within each sample, and the code number used to refer to that location within the text, for the sake of brevity. Also provided are proportions of each sample with regard to demography

Code	Name	n	Demography		Latitude	Longitude
			% Juvenile	% Adult		
1	Roudsea Wood	22	0.00	100.00	54.2332	-3.0246
2	Eaves Wood	34	44.12	55.88	54.1784	-2.8128
3	Bank Rough	13	0.00	100.00	53.2878	-2.6459
4	Hardy Gang	39	23.08	76.92	53.2604	-0.3615
5	Ivy Wood	70	70.00	30.00	53.2469	-0.2855
6	Swanton Novers	53	33.96	66.04	52.8445	0.9787
7	Hockering Wood	22	45.45	54.55	52.6908	1.0640
8	Collyweston Great Wood	71	40.85	59.15	52.5972	-0.5156
9	Ryton Wood	19	15.79	84.21	52.3475	-1.4421
10	Shrawley Wood	29	24.14	75.86	52.2920	-2.2850
11	Groton Wood	48	10.42	89.58	52.0510	0.8819
12	Bovingdon Hall	49	51.02	48.98	51.9244	0.5552
13	Garnetts Wood	40	17.50	82.50	51.8383	0.3712
14	Webbs Wood	57	63.16	36.84	51.5701	-1.9428
15	Weston Big Wood	24	0.00	100.00	51.4713	-2.7846
16	Kings Wood	25	24.00	76.00	51.3806	-2.7899
17	Langley Wood	25	24.00	76.00	50.9831	-1.6823
18	Queens Wood	23	8.70	91.30	50.8582	-1.9428
Total		663				

height. Adult individuals were those with a DBH > 0.1 m, while juvenile individuals (both ‘seedlings’ and saplings) had an overall height of < 1.37 m and/or a DBH < 0.1 m (respectively).

Occasionally, leaf material was inaccessible due to its height, and in these instances, the presence and position of these individuals were noted regardless (64 *n*). A total of 663 trees were sampled across all locations (Table 1).

DNA extraction & genotyping

Sample DNA was extracted using the ‘crude extract’ procedure described in a KAPA3G Plant PCR Kit (KAPA Biosystems, London, UK). Briefly, a 6.35-mm circular leaf punch was taken from each sample and placed in 125 µl of extraction buffer (50 mM Tris-HCl, 0.1 mM EDTA•Na₂, 2% v/v β-mercaptoethanol, 1 mM TCEP) before being heated at 95 °C for 5 min. This crude extract was used as the template for all genotyping.

Individuals were genotyped at 10 microsatellite loci (Table S1) using four multiplex PCR following Phuekvilai and Wolff (2013), with minor modifications. Loci were initially developed for *T. platyphyllos* and the selection here is based on cross-amplification success and polymorphism in *T. cordata*. Amplification was carried out in 10 µl total volume containing 1× Bioline MyTaq Plant-PCR Mix (0.2 mM each dNTP, 1.5 mM Mg²⁺; Bioline Reagents Ltd., London, UK), 0.1–0.2 µM of each primer (Table S1), 0.5 mM TCEP and 1 µl of template. Forward primers were tagged with the fluorescent dyes NED, VIC (Applied Biosystems, Warrington, UK) or 6-FAM (Sigma-Aldrich, Dorset, UK). Dye colour and primer concentration can be found in Table S1. Thermal cycler conditions were as described previously (Phuekvilai and Wolff 2013).

PCR product size was determined using capillary electrophoresis on an AB3500 Genetic Analyser (Applied Biosystems, Warrington, UK). Amplicons were diluted 1:10 with nuclease-free water. One microliter of this dilution was mixed with 8.9 µl Hi-Di formamide and 0.1 µl LIZ500 size standard before running. Allele peaks were called automatically in GeneMapper 5.0 (Applied Biosystems, Warrington, UK) and then checked manually for errors. To reduce the potential for human error in the binning process, the software TANDEM, version 1.07 (Matschiner and Salzburger 2009) was utilised to produce integer allele sizes from the fractional sizing data produced by GeneMapper.

Statistical analysis

Population genetic parameters

For comparison with other studies, typical population genetic parameters were calculated using the package

hierfstat, version 0.04-22 (Goudet 2005) in the R software package, version 3.4.2 (R Core Team 2017); all other analysis was also undertaken in R. Genetic diversity of quadrats was demonstrated by calculating the percentage of polymorphic loci, mean number of alleles and observed and expected heterozygosities. In addition to this, differences in the number of individuals in quadrats meant that simple counts of alleles are not directly comparable, and therefore, rarefied allelic richness ($n = 13$) was also calculated (El Mousadik and Petit 1996). Finally, the inbreeding coefficient F_{IS} was calculated as an estimate of individual relatedness within the quadrats, and its 95% confidence interval estimated by bootstrapping (999 replicates).

Clonal identification

Identical multi-locus genotypes (MLG) were assumed members of the same clone. This identification was confirmed by assessing the probability that identical genotypes would be encountered a second time as a result of chance recombination events (p_{sex}), based on sampled allele frequencies (Parks and Werth 1993). This was calculated using the R package poppr, version 2.5.0 (Kamvar et al. 2014, 2015). Where p_{sex} was below 0.01 (Arnaud-Haond et al. 2007b), replicate genotypes were confirmed as the product of vegetative propagation.

The corollary to this is that individuals with distinct genotypes may actually be members of the same clone. Both somatic mutations and scoring errors introduced during genotyping can generate genetic differences in otherwise identical individuals (Meirmans and Van Tienderen 2004; Douhovnikoff and Dodd 2003; Klekowski 2003). The presence of these processes was inferred by producing histograms of inter-individual genetic distance as measured by the number of shared alleles (Arnaud-Haond et al. 2007a). These plots showed bimodal distributions, with a second peak at small but non-zero distances, which suggests that at least one of these processes has occurred (Arnaud-Haond et al. 2007a; Rozenfeld et al. 2007). Therefore, any pairwise comparison between individuals that differed by only one allele was considered to indicate membership of the same clonal group (Schnittler and Eusemann 2010). This clonal identity was confirmed as before by calculating p_{sex} , but with the distinct locus temporarily removed from the data. Values of 0.01 or less were considered part of the same clonal multi-locus lineage (MLL).

Incidence and character of clonality

The relative contribution of sexual versus asexual reproduction was assessed using a proportional measure of genotypic richness, R :

$$R = \frac{(G - 1)}{(N - 1)},$$

where G is the number of distinct genotypes/lineages and N the total number of individuals. This varies from 0 when all sampled individuals are identical to 1 when all observed genotypes are distinct (Dorken and Eckert 2001). It is preferable to the often-used measure of proportion distinguishable (G/N), which can never reach zero even in populations composed of a single clone (Arnaud-Haond et al. 2007a). Summary statistics (mean and range) describing the number of ramets (N_R) within each clonal group were calculated. To describe how equitable clonal reproduction in *T. cordata* is, the complement of Simpson's index ($1 - D$, often called D^*) was calculated (Arnaud-Haond et al. 2007a). To aid interpretation of this, a stacked bar plot showing the proportion of each quadrat occupied by each genotype was produced. This illustrates whether many lineages reproduce vegetatively, or if the production of clones is dominated by a small number of genotypes.

The spatial arrangement of clonal genotypes was described by mapping individuals and denoting clonal status. Due to the number of samples, the spatial size of multi-locus lineage (MLL) was quantified by calculating the maximum linear distance between clonal individuals (D_{max}) (Jarni et al. 2015). The degree of aggregation exhibited by each MLL was described using the clonal dominance index D_c (Ohsako 2010). This is calculated for each group with ≥ 3 members:

$$D_c = \frac{(N_S - 1)}{(N_T - 1)},$$

where N_S is the group size (as number of ramets), and N_T is the total number of individuals of any genotype occurring within the spatial range of particular MLL (here, a convex hull described by the outermost members of the lineage in question). This has been used to describe whether clonal groups are aggregated or intermingled with other genets (Dering et al. 2015; Chenault et al. 2011; Ohsako 2010). If MLL totally exclude other individuals ($N_S = N_T$), then it reaches its maximum value of 1, while the value for more intermingled groups will approach 0.

Relationship of levels of clonality with climatic or demographic variables

A regression model was generated to test the hypothesis of an association between clonal reproduction and climate, and to explore whether other factors previously linked with clonality were involved. The proportional measure of genotypic richness R was used as the response variable and summaries of recent local climate during flowering were used as predictors. The WorldClim2 data set provided

mean July temperatures by day (minimum, maximum, mean), as well as wind speed, precipitation, water vapour pressure and solar radiation for each site for the period 1970–2000 (Fick and Hijmans 2017). These last four variables can be expected to affect pollinator behaviour (e.g. Lawson and Rands 2019; Tuell and Isaacs 2010; Vicens and Bosch 2009; Kilkenny and Galloway 2008; Herrera 1995; Abrol and Kapil 1986). Since *T. cordata* is primarily entomophilous (Pigott 1991; Anderson 1976), they could affect the success of sexual reproduction and therefore may be relevant when considering reproductive balance. As stands had differing numbers of trees and different demographic profiles, the density of adult and juvenile stems per square metre and the proportion visible coppice that were established trees were also included as predictors to account for any variation in clonal proportion these might generate (Cristóbal et al. 2014; Weed and Schwarzländer 2014; Valbuena-Carabaña and Gil 2013; Johansson and Lundh 1988).

The response variable is bounded to the unit interval [0, 1], and so a beta regression model was used to avoid prediction of nonsensical values (i.e. > 1 , < 0) (Simas et al. 2010; Ferrari and Cribari-Neto 2004). Calculations were performed using the R package betareg, version 3.1.0 (Cribari-Neto and Zeileis 2010). Beta regression also shares similarities with generalised linear models, in particular the use of a link function between the response and predictors. This provides additional flexibility, accounting for factors such as skew within the response (Ferrari and Cribari-Neto 2004).

Since our goal was partly descriptive and exploratory concerning other potential factors involved in generating reproductive balance, rather than predictive, model selection techniques were avoided (Mac Nally et al. 2018). A complementary log-log link function $\log_e(-\log_e[1 - \mu])$ for the mean parameter μ was found to improve model fit over a logit link, $\log_e(\mu / [1 - \mu])$, significantly increasing log-likelihood based on a Wald test ($X^2 = 0.09$, df. = 1, $p < 0.001$) and raising Akaike information criterion (AIC) markedly (Canterle and Bayer 2017; Akaike 1974).

Pairwise Pearson's correlation coefficients were used to determine whether any predictors were collinear. Mean minimum, average and maximum daily temperatures were strongly correlated with each other as well as mean rainfall ($r > 0.7$), and so, all bar mean daily maximum temperatures were removed (Pigott and Huntley 1981). The two remaining climatic variables, water vapour pressure and solar radiation, were also collinear. Therefore, only solar radiation was retained due to ease of interpretation; light levels are commonly linked with pollinator behaviour (see above). Similarly, the density of sampled stands was strongly correlated ($r > 0.7$) with demographic variables such as proportional measures of age. Since the density of adult and juvenile trees was uncorrelated, these were retained.

This was validated using typical approaches. To ensure that the assumption of homoscedasticity was not violated; residuals were examined by plotting against fitted values. Since sampled populations were clustered in geographic locations due to the nature of *T. cordata*'s scattered native distribution (Pigott 1991); independence of observations was assessed by using a Moran's *I* test to determine whether residuals exhibited spatial autocorrelation. Finally, the presence of influential observations was assessed by calculating Cook's distance for each data point.

Results

Population genetic parameters

Levels of genetic diversity were high across all quadrats (Table 2). In all but one instance (site 10), 100% of loci were polymorphic. Mean number of alleles and the rarefied equivalent were broadly similar and do not appear to vary with latitude. Heterozygosity was fairly high and again similar between quadrats. The inbreeding coefficient F_{IS} varied, but only three had a 95% confidence interval (CI) that did

not overlap zero (sites 3, 13 and 18); here, the values were negative, indicating that observed heterozygosity was lower than expected.

Clonal identification

The final number of distinct multi-locus lineages (MLL) observed was 370, for a total proportion 44.2% clones overall. All p_{sex} values for single re-encounters of the repeated genotypes were below the chosen alpha of 0.01, confirming initial clonal identification. Of the final set of repeated MLL, 32 had genotypes that differed from the other members of their clonal lineage by only one allele. After temporarily removing this locus and recalculating p_{sex} , all values were below the original threshold of 0.01, confirming this additional clonal identification.

Clonal incidence and diversity

Clonal reproduction occurred at all sites, at variable levels. The proportion of non-clonal individuals *R* was typically over half of all trees sampled (mean = 0.57, standard deviation = 0.20). *R* ranged from 0.17 in quadrat #5 to 0.96 in

Table 2 Samples and some of their observed population genetic parameters

Code	<i>A</i>	% <i>P</i>	<i>R_S</i>	<i>H_{obs}</i>	<i>H_{exp}</i>	<i>F_{IS}</i>	<i>F_{IS}</i> 95% <i>CI</i>		
1	3.9	100	3.3	0.55	0.57	0.04	−0.15	-	0.26
2	4.6	100	3.2	0.64	0.58	−0.05	−0.21	-	0.07
3	3.1	100	2.8	0.62	0.49	−0.20*	−0.36	-	−0.11
4	6.3	100	3.7	0.56	0.60	0.10	−0.08	-	0.27
5	4.9	100	3.3	0.71	0.59	−0.20	−0.44	-	0.04
6	4.7	100	3.2	0.55	0.54	−0.01	−0.18	-	0.18
7	4.3	100	3.5	0.70	0.63	−0.08	−0.26	-	0.11
8	5.6	100	3.6	0.57	0.62	0.10	0.00	-	0.21
9	3.7	100	3.2	0.60	0.54	−0.10	−0.20	-	0.08
10	4.8	90	3.5	0.57	0.57	0.06	−0.11	-	0.16
11	5.7	100	3.9	0.68	0.65	−0.03	−0.07	-	0.02
12	6.6	100	3.8	0.64	0.59	−0.03	−0.19	-	0.10
13	4.5	100	3.2	0.62	0.53	−0.14*	−0.22	-	−0.10
14	6.3	100	4.1	0.58	0.62	0.07	−0.06	-	0.26
15	4.3	100	3.6	0.58	0.61	0.06	−0.06	-	0.24
16	4.3	100	3.4	0.63	0.56	−0.12	−0.23	-	0.02
17	4.6	100	3.3	0.58	0.58	0.03	−0.11	-	0.15
18	3.9	100	3.1	0.65	0.54	−0.18*	−0.30	-	−0.08
Mean	4.78	99.44	3.43	0.61	0.58	−0.01	−0.18	-	0.12

The sites are given in order from north to south, as elsewhere. Columns two through six represent the genetic diversity within each sample: *A* is the mean number of alleles across all loci; % *P*, percentage of polymorphic loci within a sample; *R_S*, rarefied allelic richness (*n* = 13); *H_{obs}* and *H_{exp}*, observed and expected heterozygosity respectively; *F_{IS}*, the mean inbreeding coefficient across loci; finally, the estimated 95% confidence interval (CI) for this mean, calculated by bootstrapping within samples (999 replicates). *F_{IS}* values with a CI that did not overlap zero are marked with an asterisk

quadrat #17 (Fig. 2, Table 3). The number of ramets (N_R) within each genet was typically low, with an overall mean of 3.34, and only one quadrat (#5) had a clonal group with more than ten members. Both the complement of Simpson's index show D^* and stacked bar plot of genotypic proportions show that clonal reproduction is not usually dominated by any particular lineage (Fig. 3, Table 3), but evenness does vary between locations. Despite this, even those locations with large MLL and low genotypic richness such as quadrat #5 and #7 have reasonably high levels of diversity ($D^* = 0.84$ and $D^* = 0.74$, respectively), with several clonal genotypes observed.

Spatial data indicates that clones are aggregated in typically exclusive groups, but that these groups are not large (Fig. 4). The mean maximum distance between members of the same clonal lineage (D_{max}) was low at 3.92 m, with an overall range of 0.33–21.6 m (Table 3). Only seven sites had observed D_{max} values greater than 10 m (e.g. Fig. 4A, B), with the least clonal sites having lower D_{max} (e.g. Fig. 4C, D, E). The aggregation of these clonal groups was described by the clonal dominance index D_c , which indicated that MLL with more than two members very rarely contained other genotypes within a convex hull described by their outermost members (mean $D_c = 0.98$). Even quadrats such as #5 (Fig. 4A) and #7, with large distances between members of the same MLL and high numbers of ramets in clonal groups, have a mean D_c of 1, showing no intermingling of genets.

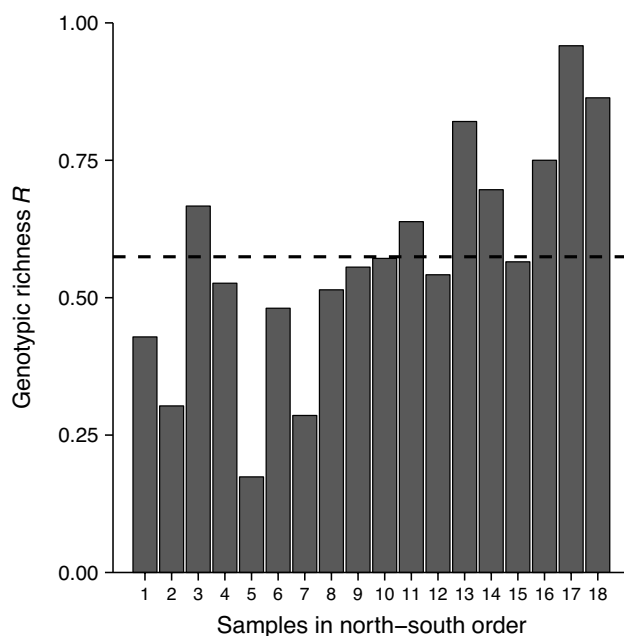


Fig. 2 Genotypic richness R (Dorken and Eckert 2001) across all samples. A score of one indicates no clonal reproduction (all genotypes are unique) whereas zero would be a monoclonal site (all genotypes identical). Mean R (0.57) is indicated by the dashed line

Relationship of clonality with climate and other factors

A beta regression model showed that only some of the examined factors are significant predictors of the proportion of clonality observed ($n = 18$, pseudo- $R^2 = 0.66$; Table 4). Namely, the density of juvenile stems in a quadrat and the mean maximum daily temperature in July for the period 1970–2000 are significant predictors of the incidence of clonality (Fig. 5). Temperature explained slightly more variance in R than the density of juveniles (Table 4). Model validation showed reasonable fit and no violation of assumptions. The lack of obvious patterns when residuals were plotted against fitted values confirmed homoscedasticity (Fig. S1A); geographically clustered sample populations did not violate independence of observations based on a Moran's I test of the residuals, which showed no spatial autocorrelation ($I = -0.05$, $p = 0.91$). Finally, no calculated Cook's distance was above 0.5, showing that no data points had undue leverage (Fig. S1B).

Discussion

Despite tree species modifying their reproductive strategy between populations based on environmental differences between locations, an explicit comparison between a climatic variable and levels of clonality has not been made previously. Therefore, we assessed levels of clonality in semi-natural populations of a temperate canopy-dominant tree, *Tilia cordata*, across a climatic gradient and compared these observations with historic temperatures during the critical period of flowering, amongst other factors. *T. cordata* was found to be partially clonal throughout its UK range and that current clonal incidence is correlated with historic July temperatures. These findings are supported by previous studies that suggest *T. cordata* is more clonal in marginal populations and that recruitment from seed is likely to be linked with temperature (Logan et al. 2019; Pigott 1991; Pigott and Huntley 1980). It contrasts with previous reports based on morphological observations which were identified northern populations as exclusively clonal (Pigott and Huntley 1981); recent recruits derived from seed were observed in one of the most northern populations (#2). Our results fit with other studies that suggest tree species modify their reproductive strategy between populations depending on environmental conditions. Such factors include altitudinal differences (Morris et al. 2004), marginal/central populations (Persson et al. 2004) and disturbance regime (Valbuena-Carabaña and Gil 2013, 2017; Morris et al. 2014).

While almost all populations exhibit both clonal and sexual reproduction, populations at more northerly latitudes with cooler summers possess a greater proportion

Table 3 Descriptive summary statistics relating to the clonal status of all samples, as identified by their code number

Code	<i>n</i>	MLL	<i>R</i>	<i>D</i> *	Density / <i>n</i> m ⁻²		<i>N_R</i>		<i>D_{max}</i> / m		<i>D_c</i>	
					Juveniles	Adults	Range	Mean	Range	Mean	Range	Mean
1	22	10	0.43	0.86	0.000	0.024	2–6	3.00	1.71–9.36	4.11	1–1	1.00
2	34	11	0.30	0.82	0.017	0.021	2–9	5.60	3.11–21.6	8.47	1–1	1.00
3	13	9	0.67	0.86	0.000	0.014	2–3	2.33	2.09–7.02	4.28	1–1	1.00
4	39	21	0.53	0.91	0.010	0.033	2–9	3.57	0.91–15.76	6.79	0.67–1	0.85
5	70	13	0.17	0.84	0.054	0.023	2–22	7.33	0.89–15.98	6.21	1–1	1.00
6	53	26	0.48	0.94	0.020	0.039	2–8	3.08	0.95–13.2	4.00	0.67–1	0.93
7	22	7	0.29	0.74	0.011	0.013	2–8	4.75	1.2–13.51	6.46	1–1	1.00
8	71	37	0.51	0.96	0.032	0.047	2–5	3.13	0.34–11.12	3.22	0.8–1	0.98
9	19	11	0.56	0.89	0.003	0.018	2–3	2.60	0.95–5.43	3.07	1–1	1.00
10	29	17	0.57	0.92	0.008	0.024	2–4	3.00	2.45–4.29	3.48	1–1	1.00
11	48	31	0.64	0.96	0.006	0.048	2–4	2.70	0.71–2.99	2.31	1–1	1.00
12	49	27	0.54	0.94	0.028	0.027	2–6	3.44	0.72–8.41	4.22	0.83–1	0.97
13	40	33	0.82	0.97	0.008	0.037	2–2	2.00	0.8–5.73	1.90	-	-
14	57	40	0.70	0.97	0.040	0.023	2–4	2.42	0.33–5	2.27	1–1	1.00
15	24	14	0.57	0.90	0.000	0.027	2–5	2.67	0.6–6	3.39	1–1	1.00
16	25	19	0.75	0.93	0.007	0.021	2–4	2.50	0.6–5.67	3.11	1–1	1.00
17	25	24	0.96	0.96	0.007	0.021	2–2	2.00	1.54–1.54	1.54	-	-
18	23	20	0.86	0.94	0.002	0.023	2–3	2.50	0.51–4.93	2.72	1–1	1.00
Mean	36.83	20.56	0.58	0.91	0.014	0.027	-	3.26	-	3.98	-	0.98

The number of individuals per sample (*n*) and distinct multi-locus lineages therein (*MLL*), a proportional measure of genotypic richness (*R*), the complement of Simpson's index describing genotypic diversity (*D**) and the number of ramets in observed *MLL* (*N_R*) all provide information regarding the extent and evenness of clonality in all sites. The density of the distinct age classes, juvenile and adult, in each sample (see text for classification criteria) gives a measure of the level of recruitment and stand dominance respectively. Both the maximum distance between members of the same *MLL* (*D_{max}*) and the clonal dominance index (*D_c*) describe the spatial arrangement of clonal groups. *D_{max}* illustrates the spatial extent of *MLL*; *D_c* demonstrates how aggregated individuals are relative to other genotypes (higher scores indicate more exclusive genets)

of individuals that are the result of vegetative propagation. As a plausible mechanistic link between temperature during flowering and successful production of fertile seed has already been demonstrated experimentally (Pigott and Huntley 1981), this relationship is likely causative. Since other climatic variables were removed as predictors due to collinearity, the role of factors such as precipitation cannot be eliminated. Similarly, the exact nature of the link, whether the critical variable is minimum, maximum or mean temperature during flowering, requires further investigation. Since month-to-month temperatures are naturally correlated, other potential candidates for predictors of clonality include August temperatures, which are important for seed development (Pigott 1981).

The balance of reproductive modes is not predictable by wind speed or solar radiation during flowering. As *T. cordata* is primarily entomophilous (Fromm 2001; Pigott 1991; Anderson 1976), this suggests that limitations associated with pollinator behaviour are not involved, given the links between insect movements and weather (Vicens and Bosch 2009; Kilkenny and Galloway 2008; Herrera 1995; Abrol and Kapil 1986). This also suggests that the level of rainfall

during flowering, which was removed as a predictor due to collinearity with temperature, is not directly implicated, as its mechanism of action would be via changes to pollinator behaviour (Lawson and Rands 2019). It is possible that precipitation during flowering could still have an effect via interaction with temperature to make fertilisation success more variable (i.e. sufficient temperatures do not always result in successful sexual reproduction if weather conditions are otherwise unfavourable). If this is the case, then there may be a longitudinal trend in variability of clonality estimates at similar latitudes, which may be something to consider in other studies of widespread clonal trees.

Other climatic variables may have a role: for instance, the action of freeze-thawing during winter induces sprouting in *Fagus grandifolia* and this is therefore hypothesised to increase clonal growth (Morris et al. 2014). However, this is unlikely to be important in the sampled populations, given the typical mildness of winters in the oceanic climate of the UK. In more eastern populations at similar latitudes such as those in Scandinavia or Russia, the effects of sub-zero temperatures may be involved in the promotion of vegetative propagation.

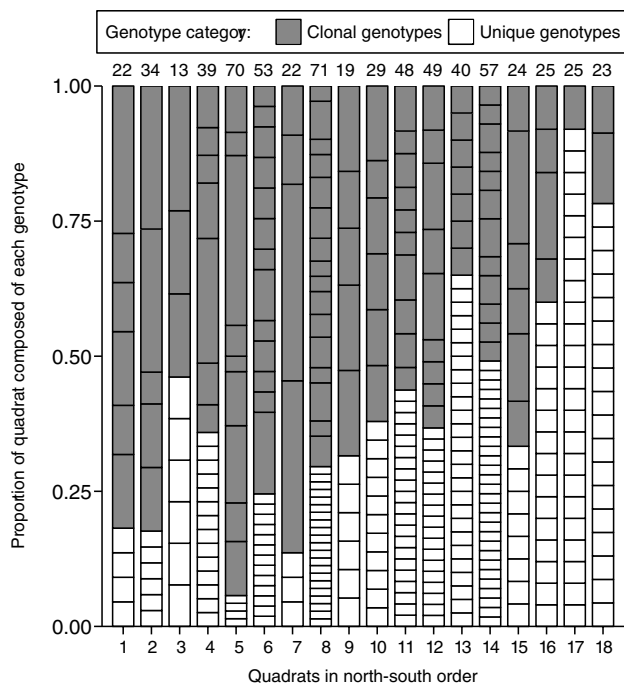


Fig. 3 A stacked barplot summarising genotypic diversity and evenness across all samples. Samples are provided in north-south order from left to right. Each stacked bar represents all genotypes for a given stand, and the relative proportion of each within that quadrat. The number above each bar represents the number of individuals in that quadrat. Filled segments are clonal (repeated) genotypes, while those unfilled are unique and likely the product of recruitment from seed. Most quadrats have several filled segments of similar size even where the proportion of individuals recruited from seed is low, showing that clonal reproduction in *T. cordata* still maintains appreciable genetic diversity. Notable exceptions include site 5 and site 7

The only other factor examined with a significant predictive relationship to clonal incidence was juvenile density. Quadrats with a higher number of saplings exhibited greater levels of clonality, suggesting that recent recruitment in the examined populations has been mostly vegetative in origin. This may be actual density-dependence or might simply reflect different levels of recruitment via both methods. Higher mortality rates for sexually recruited individuals compared with clones might be related to herbivory. Seedlings of *T.* suffer heavily from browsing by herbivores such as deer and voles (Pigott 1991). In this context, ramets may benefit from the provision of additional resources via below-ground integration with the mother tree (Liu et al. 2016). Thus, the increase in the relative importance of vegetative propagation for recruitment may simply be the result of a failure to recruit from seed, rather than any expenditure of additional energy towards one method or another (Silvertown 2008).

It is important to note that adult density was not a predictor of clonal incidence here. As all locations had a complete canopy, adult density in this instance is likely acting as a

proxy for the dominance of *T. cordata* within quadrats (i.e. the presence/absence of other species). Therefore, interspecific competition does not seem to affect reproductive balance in the species.

As the regression model generated here has a relatively low pseudo- R^2 value (0.66), it is clear that other unexamined factors are influencing reproductive balance. However, coppicing is not one of the factors, which is unexpected. Other species exhibit higher relative importance of vegetative propagation when under higher intensity disturbance regimes (Morris et al. 2014; Valbuena-Carabaña and Gil 2013), and *T. americana* has been observed directly to generate more sprouts when suffering from increased levels of herbivory (Evans and Morris 2016).

Measures of genetic diversity are in line with previous estimates (e.g. Logan et al. 2015, 2019), suggesting that clonality has not impacted upon this. Indeed, negative F_{IS} in two locations shows that it has potentially increased heterozygosity due to clonal reproduction of heterozygous individuals, similarly higher levels of heterozygosity have been reported in other populations, including higher heterozygosity amongst clone genotypes compared with non-clone genotypes within the same population (Erichsen et al. 2019). The patterns of genetic diversity are not supported by the post-glacial spread from the south: the ‘southern richness, northern purity’ paradigm (Hewitt 2000). If a reduction in genetic diversity followed northward post-glacial expansion, then we should observe a reduction in other parameters such as polymorphism or heterozygosity, but that is not the case here.

T. cordata is more clonal than its congeners *T. platyphyllos* and *T. americana*, although observed R appears to be similar when occurring in sympatry with the former species. Similarly, marginal populations of *T. platyphyllos* are also more clonal than central ones, but the degree of difference is less than in *T. cordata*. This suggests that similar environmental constraints, disturbance events or population histories may be affecting both species, but to a lesser degree in *T. platyphyllos*.

In a broader context, the proportion of unique genotypes observed here is very similar to those reported for a variety of sprouting tree species such as *Populus euphratica* Oliv. (Euphrates poplar), *Ulmus minor* Mill. (field elm) and *Quercus pyrenaica* Willd. (Pyrenean oak) (Valbuena-Carabaña and Gil 2017; Buiteveld et al. 2016; Schnittler and Eusemann 2010).

The character of clonal growth within populations was diverse, with many genotypes reproducing clonally and most genets occurring as small groups with restricted spatial extent. This suggests that even marginal *T. cordata* populations are more dynamic than might be expected, and must be recruiting from seed, albeit infrequently from a human perspective. The low frequency of members and

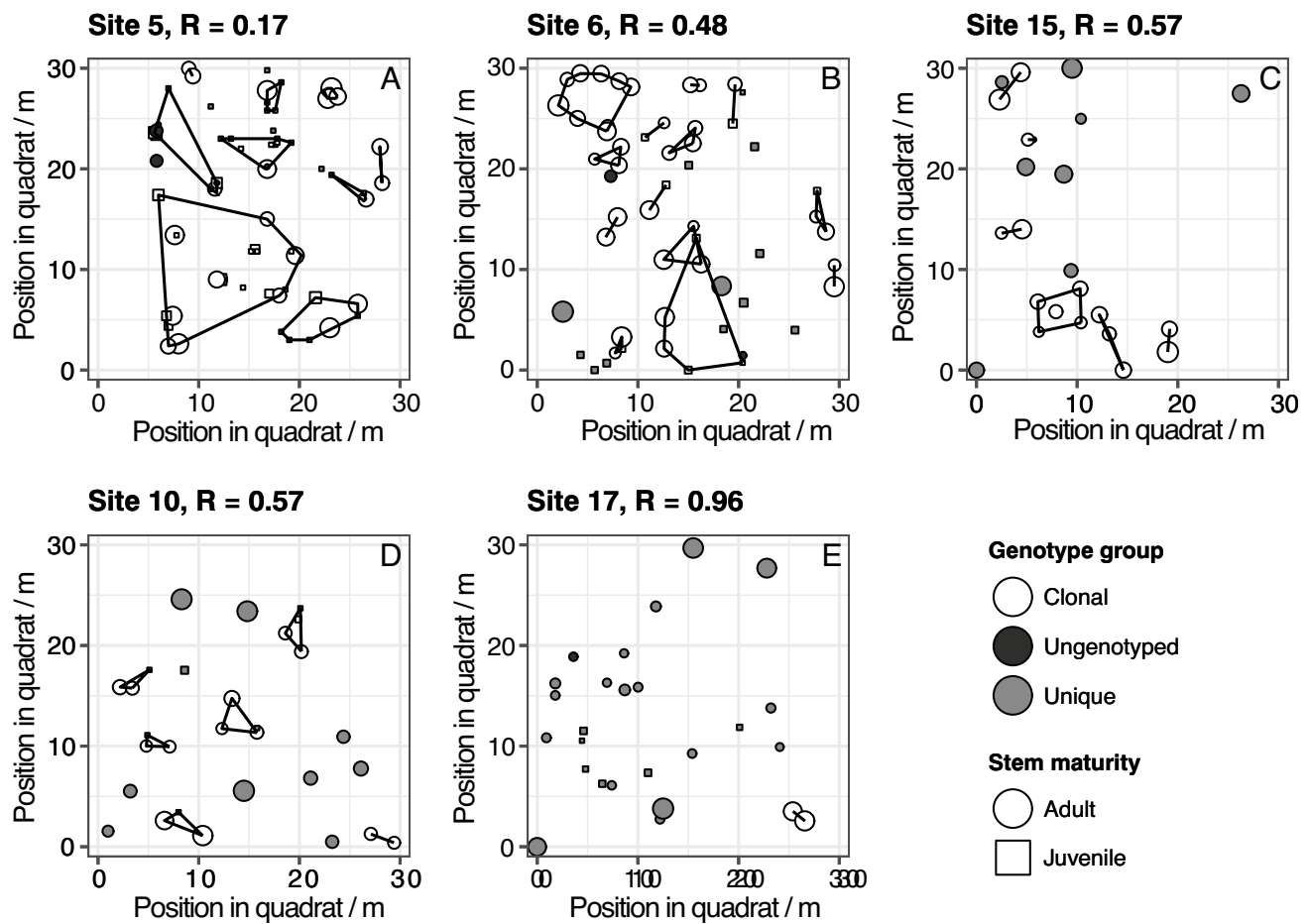


Fig. 4 The spatial arrangement of example quadrats, showing the tight aggregation of clonal groups. As well as individual location, the age class of stems and the clonal status of each are provided. Point shape indicates stem maturity, with squares being juvenile, while circles are adult, based primarily on diameter at breast height (DBH). Point size gives an indication of relative DBH, but is not to scale. Point fill indicates clonal status: open symbols are part of clonal

groups; dark grey symbols are not genotyped due to inaccessible leaves; light grey symbols indicate unique genotypes. Memberships of distinct clonal groups are circumscribed by a convex hull indicated by black lines. **A** minimum observed genotypic richness index R (0.17, sample #5); **B** 1st quartile R (0.48, sample #6); **C** median R (0.57, sample #15); **D** 3rd quartile R (0.57, sample #10); **E** maximum R (0.96, sample #17)

Table 4 Coefficients from a fixed dispersion beta regression model examining which variables are predictors of genotypic richness, which is a proportional measure of the amount of clonality within a sample ($n = 18$; pseudo- $R^2 = 0.66$)

Link function	Estimate	SE	z	p	Parameter
$\log(-\log(1-\mu))$	-0.17	0.08	-2.04	0.04	Intercept
	0.12	0.10	1.15	0.25	Proportion of sample part of visible coppice stool
	-0.01	0.10	-0.13	0.90	Density of adults/stems m^{-2}
	-0.24	0.10	-2.37	0.02	Density of juveniles stems/stems m^{-2}
	0.28	0.11	2.55	0.01	Mean daily maximum temperature during July, 1970–2000/ $^{\circ}C$
	0.16	0.10	1.59	0.11	Mean daily solar radiation during July, 1970–2000/ $kJ\ m^{-2}\ d^{-1}$
	-0.14	0.11	-1.31	0.19	Mean daily wind speed during July, 1970–2000/ $m\ s^{-1}$
ϕ	14.95	4.85	3.08	0.002	Precision

Statistically significant coefficients are highlighted in bold. Only temperature and the density of juvenile individuals in a sample are predictors of its clonal incidence

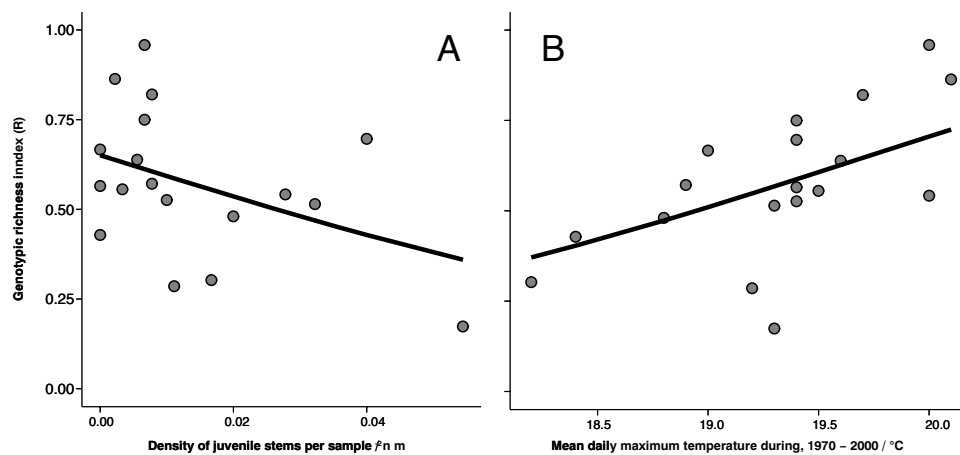


Fig. 5 A beta regression model (pseudo- $R^2 = 0.66$, $n = 18$), examining the incidence of clonality and its covariates. A) Scatterplot of juvenile stem density in sampled stands against the proportional measure of genotypic richness R , showing that the number of recently recruited stems within the sample quadrat is a significant predictor of clonality. B) Scatterplot of mean daily maximum temperature dur-

ing July plotted against the same response, showing that temperature during the flowering period is a significant predictor of clonality. In both instances, the line of best fit is calculated from the model's complementary log-log link function with all other covariates set to their mean values. Only significant covariates are plotted here; the full model is specified in Table 4

small spatial spread of genets is to be expected, as basal sprouting is the usual method of vegetative propagation. Occasional observations of longer distance dispersal events can be explained by opportunistic layering and windthrow producing ramets separated by relatively large distances. Individual ramets typically excluded other genets with only four quadrats (seven out of 663 individuals overall) containing any individuals that were either a product of seed or from another genet. This suggests that large clonal groups tend to out-compete other genotypes within their immediate surroundings.

If any particular clonal lineage has a competitive advantage, over long timescale monoclonal stands can occur (Halkett et al. 2005; Honnay and Bossuyt 2005). This did not occur in the populations studied here. Monoclonal stands are observed in other tree species with mixed reproductive systems (e.g. Buiteveld et al. 2016; Evans and Morris 2016; Fuentes-Utrilla et al. 2014; Morris et al. 2014).

If future projections of responses to climate change in these or other taxa are to be made, then it is important to consider how these findings might influence this. The spatial arrangement of ramets and the overall number of individuals in genets have implications for the likelihood of out-crossing success, which might be important for population persistence and is relevant when considering the efficacy of selective responses (Barrett 2015; Vallejo-Marín et al. 2010). Although clonal groups were highly aggregated, their small size (in terms of both space and frequency) should not increase inbreeding and accelerate genetic diversity loss. Further evidence of this can be found in observations of pollen movements within the species, which are much higher on average than the largest clone area occurring here (Fromm

2001). In addition the diversity of clones in a population will act as a buffer to genetic diversity loss during periods of unsuitable conditions for sexual recruitment, before future climatic conditions allow for successful recruitment from seed. This of course depends on the presence of suitable conditions in all other regards, and as noted above, unexamined factors are likely involved in shifting reproductive balance.

These results suggest several avenues for further examining the role of asexual reproduction in *T. cordata*. While clonal recruitment disappears completely in locations where with summer temperatures allow consistent annual seed production, such as continental Europe (Logan et al. 2019; Pigott and Huntley 1981). Marginal populations are likely to retain vegetative reproduction. Habitats may however be marginal for distinct reasons; the southern and eastern range limits of *T. cordata* are a result of inadequate moisture (Pigott and Pigott 1993; Pigott 1991). Climatic changes in these regions are forecast to lead to extinction unless the species are able to make altitudinal range shifts (Attorre et al. 2011). A movement towards vegetative propagation under adverse conditions would impose limitations on dispersal. Therefore, it should be assessed whether reproductive balance is affected by these distinct environmental constraints (e.g. drought-related mortality rates in seedlings could exceed that of ramets).

The role of asexual reproduction in responding to disturbance should be examined more closely by assessing sprouting frequency and its relation to herbivory, controlling for the confounding influence of climate; a link has already been demonstrated in the congeneric species *T. americana* (Evans and Morris 2016). More broadly, this study demonstrates that natural populations provide a

suitable context for detecting a relationship between incidence of clonality and its climatic constraints in trees with mixed reproductive systems. A similar approach should therefore be applied in other taxa (e.g. Valbuena-Carabaña and Gil 2017; Persson et al. 2004), given the contribution of reproductive mechanism to population persistence in a changing climate (Dodd and Douhovnikoff 2016; Sturrock et al. 2011).

In summary, this study shows that *T. cordata* has a variable reproductive strategy that shifts alongside typical summer temperatures. Together with previous work demonstrating a mechanistic relationship between successful pollination and ambient temperature, this demonstrates that environmental constraints are able to modify, directly or indirectly, the relative importance clonal growth versus recruitment from seed. Although other studies suggest similar links, this shows by direct comparison that canopy tree species behave in this respect as do other plant groups (e.g. Ma et al. 2013; Hautier et al. 2009). For those forest taxa with a mixed reproductive strategy, it is important to consider how this might shift because of climate change. Shifts in one direction or the other likely have major implications for long-term forecasts of responses, e.g. the likelihood of local adaptation. This demonstrates the importance of generating a better understanding of how environmental constraints interact with reproductive strategy in tree species. It also shows the value and feasibility of direct comparisons between clonal incidence and ecologically relevant variables of interest in the context of a natural experiment, using taxa with low experimental tractability, which might otherwise be ignored when studying clonality in plant species.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval The authors confirm that they follow the rules of good scientific practice and all ethical standards requested by the journal. Field collection was carried out in compliance with all local laws and with appropriate permissions/permits.

Conflict of interest The authors declare no competing interests.

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References

- Abrol DP, Kapil R (1986) Factors affecting pollination activity of *Megachile lanata lepel*. *Proc Anim Sci* 95:757–769
- Akaike H (1974) A new look at the statistical model identification. *IEEE Trans Autom Control* 19:716–723. <https://doi.org/10.1109/TAC.1974.1100705>
- Alfonso-Corrado C, Esteban-Jiménez R, Clark-Tapia R, Piñero D, Campos JE, Mendoza A (2005) Clonal and genetic structure of two Mexican oaks: *Quercus eduardii* and *Quercus potosina* (Fagaceae). *Evol Ecol* 18:585–599. <https://doi.org/10.4236/nr.2014.59041>
- Anderson GJ (1976) The pollination biology of *Tilia*. *Am J Bot* 63:1203
- Arnaud-Haond S, Duarte CM, Alberto F, Serrão EA (2007a) Standardizing methods to address clonality in population studies. *Mol Ecol* 16:5115–5139. <https://doi.org/10.1111/j.1365-294X.2007.03535.x>
- Arnaud-Haond S, Migliaccio M, Diaz-Almela E, Teixeira S, van de Vliet MS, Alberto F, Procaccini G, Duarte CM, Serrão EA (2007b) Vicariance patterns in the Mediterranean Sea: east-west cleavage and low dispersal in the endemic seagrass *Posidonia oceanica*. *J Biogeogr* 34:963–976. <https://doi.org/10.1111/j.1365-2699.2006.01671.x>
- Attorre F, Alfò M, De Sanctis M, Francesconi F, Valenti R, Vitale M, Bruno F (2011) Evaluating the effects of climate change on tree species abundance and distribution in the Italian peninsula. *Appl Veg Sci* 14:242–255. <https://doi.org/10.1111/j.1654-109X.2010.01114.x>
- Bakker EG, Dam BC, Eck HJ, Jacobsen E (2001) The description of clones of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. with microsatellites and AFLP in an ancient woodland. *Plant Biol* 3:616–621. <https://doi.org/10.1055/s-2001-19365>
- Barker C, Ashton M, Ashton PA (2015) Segregation of *Tilia cordata* and *T. platyphyllos* (Malvaceae) along environmental gradients. *New J Bot* 5:157–163. <https://doi.org/10.1080/20423489.2015.1123968>
- Barrett SCH (2015) Influences of clonality on plant sexual reproduction. *Proc Natl Acad Sci* 112:8859–8866. <https://doi.org/10.1073/pnas.1501712112>
- Birks HJB (1989) Holocene isochrone maps and patterns of tree-spreading in the British Isles. *J Biogeogr* 16:503–540. <https://doi.org/10.2307/2845208>
- Brzosko E, Wróblewska A, Jermakowicz E, Hermaniuk A (2013) High level of genetic variation within clonal orchid *Goodyera repens*. *Plant Syst Evol* 8:1537–1548. <https://doi.org/10.1007/s00606-013-0817-7>
- Buiteveld J, Broeck AV, Cox K, Collin E (2016) Human impact on the genetic diversity of Dutch field elm (*Ulmus minor*) populations in the Netherlands: implications for conservation. *Plant Ecol Evol* 149:165–176. <https://doi.org/10.5091/plecevo.2016.977>

- Canterle DR, Bayer FM (2017) Variable dispersion beta regressions with parametric link functions. *Stat Pap* 60:1–27. <https://doi.org/10.1007/s00362-017-0885-9>
- Charpentier A (2001) Consequences of clonal growth for plant mating. *Evol Ecol* 15:521–530. <https://doi.org/10.1023/A:1016057503722>
- Chenault N, Arnaud-Haond S, Juteau M, Valade R, Almeida JL, Villar M, Bastien C, Dowkiw A (2011) SSR-based analysis of clonality, spatial genetic structure and introgression from the Lombardy poplar into a natural population of *Populus nigra* L. along the Loire River. *Tree Genet Genomes* 7:1249–1262. <https://doi.org/10.1007/s11295-011-0410-6>
- Cribari-Neto F, Zeileis A (2010) Beta Regression in R. *J Stat Softw* 34(2):1–24. <https://doi.org/10.18637/jss.v034.i02>
- Cristóbal D, Martínez-Zurimendi P, Villamediana I, Ciriza J, Villar J, Nanos N, Sierra-de-Grado R (2014) Clonal structure and dynamics of peripheral *Populus tremula* L. populations. *iForest* 7:140–149. <https://doi.org/10.3832/for0971-007>
- Davis BAS, Brewer S, Stevenson AC, Guiot J, Contributors D (2003) The temperature of Europe during the Holocene reconstructed from pollen data. *Quat Sci Rev* 22:1701–1716. [https://doi.org/10.1016/S0277-3791\(03\)00173-2](https://doi.org/10.1016/S0277-3791(03)00173-2)
- Del Tredici P (2001) Sprouting in temperate trees: a morphological and ecological review. *Bot Rev* 67:121–140. <https://doi.org/10.1007/BF02858075>
- Dering M, Chybicki IJ, Rączka G (2015) Clonality as a driver of spatial genetic structure in populations of clonal tree species. *J Plant Res* 128:731–745. <https://doi.org/10.14214/sf.9987>
- Dering M, Rączka G, Szymt J (2016) Sex-specific pattern of spatial genetic structure in dioecious and clonal tree species, *Populus alba* L. *Tree Genet Genomes* 12:70. <https://doi.org/10.1007/s11295-016-1028-5>
- Dodd RS, Douhovnikoff V (2016) Adjusting to global change through clonal growth and epigenetic variation. *Front Ecol Evol* 4:1–6. <https://doi.org/10.3389/fevo.2016.00086>
- Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *J Ecol* 89:339–350. <https://doi.org/10.1046/j.1365-2745.2001.00558.x>
- Douhovnikoff V, Dodd RS (2003) Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. *Theor Appl Genet* 106:1307–1315. <https://doi.org/10.1007/s00122-003-1200-9>
- Eckert CG (2002) The loss of sex in clonal plants. *Evol Ecol* 15:501–520. https://doi.org/10.1007/978-94-017-1345-0_15
- El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to Morocco. *Theor Appl Genet* 92:832–839. <https://doi.org/10.1007/BF00221895>
- Erichsen EO, Wolff K, Hansen OK (2019) Genetic and clonal structures in the tree species *Tilia cordata* mill. In remnants of ancient forests in Denmark. *Popul Ecol* 61:243–255. <https://doi.org/10.1002/1438-390X.12002>
- Espinheira PL, Ferrari SLP, Cribari-Neto F (2008) On beta regression residuals. *J Appl Stat* 35:407–419. <https://doi.org/10.1080/02664760701834931>
- EUFORGEN (2009) Distribution map of Lime (*Tilia cordata*) [WWW Document]. URL www.euforgen.org
- Evans JP, Morris AB (2016) Isolated coastal populations of *Tilia americana* var. *caroliniana* persist long-term through vegetative growth. *Am J Bot* 103:1687–1693. <https://doi.org/10.3732/ajb.1600233>
- Ferrari S, Cribari-Neto F (2004) Beta regression for modelling rates and proportions. *J Appl Stat* 31:799–815. <https://doi.org/10.1080/0266476042000214501>
- Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *Int J Climatol* 37(12):4302–4315. <https://doi.org/10.1002/joc.5086>
- Fromm M (2001) Reproduktion einer entomophilen Baumart in geringer Populationsdichte – Das Beispiel der Winterlinde (*Tilia cordata* Mill.). Unpublished PhD Thesis, Universität Göttingen
- Fuentes-Utrilla P, Valbuena-Carabaña M, Ennos R, Gil L (2014) Population clustering and clonal structure evidence the relict state of *Ulmus minor* Mill. in the Balearic Islands. *Heredity* 113:21–31. <https://doi.org/10.1038/hdy.2014.21>
- Goudet J (2005) Hierfstat, a package for R to compute and test variance components and F-statistics. *Mol Ecol Notes* 5:1933–1940. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>
- van Groenendael JM, Klimeš L, Klimešová J, Hendriks RJJ (1996) Comparative ecology of clonal plants. *Philos Trans Biol Sci* 351:1331–1339. <https://doi.org/10.1098/rstb.1996.0116>
- Guarino F, Cicatelli A, Brundu G, Heinze B, Castiglione S (2015) Epigenetic diversity of clonal white poplar (*Populus alba* L.) populations: could methylation support the success of vegetative reproduction strategy? *PLoS One* 10:e0131480. <https://doi.org/10.1371/journal.pone.0131480>
- Halkett F, Simon J-C, Balloux F (2005) Tackling the population genetics of clonal and partially clonal organisms. *Trends Ecol Evol* 20:194–201. <https://doi.org/10.1016/j.tree.2005.01.001>
- Harper JL (1977) *Population biology of plants*. Academic Press, London, UK
- Hautier Y, Randin CF, Stöcklin J, Guisan A (2009) Changes in reproductive investment with altitude in an alpine plant. *J Plant Ecol* 2:125–134. <https://doi.org/10.1093/jpe/rtp011>
- Herrera CM (1995) Microclimate and individual variation in pollinators: flowering plants are more than their flowers. *Ecology* 76:1516–1524. <https://doi.org/10.2307/1938153>
- Hewitt G (2000) The genetic legacy of the quaternary ice ages. *Nature* 405:907–913. <https://doi.org/10.1038/35016000>
- Holsinger KE (2000) Reproductive systems and evolution in vascular plants. *Proc Natl Acad Sci U S A* 97:7037–7042. <https://doi.org/10.1073/pnas.97.13.7037>
- Honnay O, Bossuyt B (2005) Prolonged clonal growth: escape route or route to extinction? *Oikos* 2:427–432. <https://doi.org/10.1111/j.0030-1299.2005.13569.x>
- IPCC (2021) *Climate change 2021: the physical science basis. contribution of working group i to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge University Press, Cambridge, UK
- Jarni K, Jakše J, Brus R (2015) Vegetative propagation: linear barriers and somatic mutation affect the genetic structure of a *Prunus avium* L. stand. *Forestry* 88:612–621. <https://doi.org/10.1093/forestry/cpv029>
- Johansson T, Lundh J-E (1988) Sucker production from root cuttings of *Populus tremula* in relation to growing conditions. *Scand J For Res* 3:75–82. <https://doi.org/10.1080/02827588809382497>
- Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281. <https://doi.org/10.7717/peerj.281>
- Kamvar ZN, Brooks JC, Grünwald NJ (2015) Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. *Front Genet* 6:208. <https://doi.org/10.3389/fgene.2015.00208>
- Kilkenny FF, Galloway LF (2008) Reproductive success in varying light environments: direct and indirect effects of light on plants and pollinators. *Oecologia* 155:247–255. <https://doi.org/10.1007/s00442-007-0903-z>

- Klekowski EJ (2003) Plant clonality, mutation, diplontic selection and mutational meltdown. *Biol J Linn Soc* 79:61–67. <https://doi.org/10.1046/j.1095-8312.2003.00183.x>
- Klimeš L, Klimešová J, Hendriks RJJ, van Groenendael J (1997) Clonal plant architecture: a comparative analysis of form and function. In: de Kroon H, van Groenendael J (eds) *The Ecology and Evolution of Clonal Plants*. Backhuys Publishers, Leiden, NL, pp 1–29
- Lawson DA, Rands SA (2019) The effects of rainfall on plant–pollinator interactions. *Arthropod Plant Interact* 13:561–569. <https://doi.org/10.1007/s11829-019-09686-z>
- Li L, Lan Z, Chen J, Song Z (2018) Allocation to clonal and sexual reproduction and its plasticity in *Vallisneria spirulosa* along a water-depth gradient. *Ecosphere* 9:e02070. <https://doi.org/10.1002/ecs2.2070>
- Liu F, Liu J, Dong M (2016) Ecological consequences of clonal integration in plants. *Front Plant Sci* 7:770. <https://doi.org/10.3389/fpls.2016.00770>
- Logan SA, Phuekvilai P, Wolff K (2015) Ancient woodlands in the limelight: delineation and genetic structure of ancient woodland species *Tilia cordata* and *Tilia platyphyllos* (Tiliaceae) in the UK. *Tree Genet Genomes* 11:52. <https://doi.org/10.1007/s11295-015-0872-z>
- Logan SA, Phuekvilai P, Sanderson R, Wolff K (2019) Reproductive and population genetic characteristics of leading-edge and central populations of two temperate forest tree species and implications for range expansion. *For Ecol Manag* 433:475–486. <https://doi.org/10.1016/j.foreco.2018.11.024>
- Lone A, Bashir A, Tewari SK, Majeed M (2011) Characterization and identification of leaf morphology of *Populus deltoides* Bartr. clones. *For Stud China* 13:270–273. <https://doi.org/10.15740/HAS/IJFCI/8.1/24-29>
- Ma CC, Zhang JH, Guo HY, Li QF, Xie LN, Gao YB (2013) Alterations in canopy size and reproduction of *Caragana stenophylla* along a climate gradient on the Inner Mongolian Plateau. *Flora: Morphology. Distrib Funct Ecol Plants* 208:97–103. <https://doi.org/10.1016/j.flora.2013.02.002>
- Mac Nally R, Duncan RP, Thomson JR, Yen JDL (2018) Model selection using information criteria, but is the “best” model any good? *J Appl Ecol* 55:1441–1444. <https://doi.org/10.1111/1365-2664.13060>
- Matschiner M, Salzburger W (2009) TANDEM: integrating automated allele binning into genetics and genomics workflows. *Bioinformatics* 25:1982–1983. <https://doi.org/10.1093/bioinformatics/btp303>
- Matthews SN, Iverson LR, Prasad AM, Peters MP, Rodewald PG (2011) Modifying climate change habitat models using tree species-specific assessments of model uncertainty and life history-factors. *For Ecol Manag* 262:1460–1472. <https://doi.org/10.1016/j.foreco.2011.06.047>
- Meirmans PG, Van Tienderen PH (2004) genotype and genodive: two programs for the analysis of genetic diversity of asexual organisms. *Mol Ecol Notes* 4:792–794. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>
- Mock KE, Rowe CA, Hooten MB, Dewoody J, Hipkins VD (2008) Clonal dynamics in western North American aspen (*Populus tremuloides*). *Mol Ecol* 17:4827–4844. <https://doi.org/10.1111/j.1365-294X.2008.03963.x>
- Montalvo AM, Cnard SG, Conkle MT, Hodgskiss P, Conard SSG, Thompson CM (1997) Population structure, genetic diversity, and clone formation in *Quercus chrysolepis* (Fagaceae). *Am J Bot* 84:1553–1564. <https://doi.org/10.2307/2446617>
- Morris AB, Small RL, Cruzan MB (2004) Variation in frequency of clonal reproduction among populations of *Fagus grandifolia* Ehrh. in response to disturbance. *Castanea* 69:38–51. [https://doi.org/10.2179/0008-7475\(2004\)069<0038:VIFOCR>2.0.CO;2](https://doi.org/10.2179/0008-7475(2004)069<0038:VIFOCR>2.0.CO;2)
- Morris AB, Millsaps NK, Mason KQ, Howell JS, Speer JH (2014) Mature beech trees (*Fagus grandifolia*; Fagaceae) are persistently clonal in coves and beech gaps in the Great Smoky Mountains. *Am J Bot* 101:381–388. <https://doi.org/10.3732/ajb.1300161>
- Namroud M-C, Park A, Tremblay F, Bergeron Y (2005) Clonal and spatial genetic structures of aspen (*Populus tremuloides* Michx.). *Mol Ecol* 14:2969–2980. <https://doi.org/10.1111/j.1365-294X.2005.02653.x>
- Nuortila C, Tuomi J, Laine K (2002) Inter-parent distance affects reproductive success in two clonal dwarf shrubs, *Vaccinium myrtillus* and *Vaccinium vitis-idaea* (Ericaceae). *Can J Bot* 884:875–884. <https://doi.org/10.1139/b02-079>
- Ohsako T (2010) Clonal and spatial genetic structure within populations of a coastal plant, *Carex kobomugi* (Cyperaceae). *Am J Bot* 97:458–470. <https://doi.org/10.3732/ajb.0900262>
- Parks JC, Werth CR (1993) A study of spatial features of clones in a population of bracken fern, *Pteridium aquilinum* (Dennstaedtiaceae). *Am J Bot* 80:537–544. <https://doi.org/10.1002/j.1537-2197.1993.tb13837.x>
- Persson H, Widén B, Andersson S, Svensson L (2004) Allozyme diversity and genetic structure of marginal and central populations of *Corylus avellana* L. (Betulaceae) in Europe. *Plant Syst Evol* 244:157–179. <https://doi.org/10.1007/s00606-003-0073-3>
- Peterken GF, Mountford EP (2017) Woodland development: a long-term study of lady park wood. CABI, Wallingford, UK
- Phuekvilai P, Wolff K (2013) Characterization of microsatellite loci in *Tilia platyphyllos* (Malvaceae) and cross-amplification in related species. *Appl Plant Sci* 1:1200386. <https://doi.org/10.3732/apps.1200386>
- Pigott CD (1981) Nature of seed sterility and natural regeneration of *Tilia cordata* near its northern limit in Finland. *Ann Bot Fenn* 18:255–263
- Pigott CD (1991) *Tilia cordata* Miller. *J Ecol* 79:1147–1207. <https://doi.org/10.2307/2261105>
- Pigott D (2012) *Lime-trees and basswoods: a biological monograph of the genus Tilia*. Cambridge University Press, Cambridge. <https://doi.org/10.1017/CBO9781139033275>
- Pigott CD, Huntley JP (1978) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. I. Distribution in North-West England. *New Phytol* 81:429–441
- Pigott CD, Huntley JP (1980) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. II. History in north-west England. *New Phytol* 84:145–164
- Pigott CD, Huntley JP (1981) Factors controlling the distribution of *Tilia cordata* at the northern limits of its geographical range. III. Nature and causes of seed sterility. *New Phytol* 84:817–839
- Pigott CD, Pigott S (1993) Water as a determinant of the distribution of trees at the boundary of the Mediterranean zone. *J Ecol* 81:557–566. <https://doi.org/10.2307/2261534>
- R Core Team (2017) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: <https://www.R-project.org/>
- Rackham O (2003) The limewoods. In: *Ancient woodland: its history, vegetation and uses in England*, 2nd edn. Castlepoint Press, Colvend, UK, p 244
- Rautiainen P, Koivula K, Hyvärinen M (2004) The effect of within-genet and between-genet competition on sexual reproduction and vegetative spread in *Potentilla anserina* ssp. *egedii*. *J Ecol* 92:505–511. <https://doi.org/10.1111/j.0022-0477.2004.00878.x>
- Rodwell JS (ed) (1991) British plant communities Vol. 1. Woodlands and Scrub, 1st edn. Cambridge University Press, Cambridge, UK
- Rozenfeld AF, Arnaud-Haond S, Hernández-García E, Eguíluz VM, Matías MA, Serrão E, Duarte CM (2007) Spectrum of genetic diversity and networks of clonal organisms. *J R Soc Interface* 4:1093–1102. <https://doi.org/10.1098/rsif.2007.0230>

- Schnittler M, Eusemann P (2010) Consequences of genotyping errors for estimation of clonality: a case study on *Populus euphratica* Oliv. (Salicaceae). *Evol Ecol* 24:1417–1432. <https://doi.org/10.1007/s10682-010-9389-y>
- Silvertown J (2008) The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. *Int J Plant Sci* 169:157–168. <https://doi.org/10.1086/523357>
- Simas AB, Barreto-Souza W, Rocha AV (2010) Improved estimators for a general class of beta regression models. *Comput Stat Data Anal* 54:348–366. <https://doi.org/10.1016/j.csda.2009.08.017>
- Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, Lewis KJ, Worrall JJ, Woods AJ (2011) Climate change and forest diseases. *Plant Pathol* 60:133–149. <https://doi.org/10.1111/j.1365-3059.2010.02406.x>
- Tuell JK, Isaacs R (2010) Weather during bloom affects pollination and yield of highbush blueberry. *J Econ Entomol* 103:557–562. <https://doi.org/10.1603/EC09387>
- Valbuena-Carabaña M, Gil L (2013) Genetic resilience in a historically profited root sprouting oak (*Quercus pyrenaica* Willd.) at its southern boundary. *Tree Genet Genomes* 9:1129–1142. <https://doi.org/10.1007/s11295-013-0614-z>
- Valbuena-Carabaña M, Gil L (2017) Centenary coppicing maintains high levels of genetic diversity in a root resprouting oak (*Quercus pyrenaica* Willd.). *Tree Genet Genomes* 13:28. <https://doi.org/10.1007/s11295-017-1105-4>
- Vallejo-Marín M, Dorken ME, Barrett SCH (2010) The ecological and evolutionary consequences of clonality for plant mating. *Annu Rev Ecol Evol Syst* 41:193–213. <https://doi.org/10.1146/annurev.ecolsys.110308.120258>
- Vicens N, Bosch J (2009) Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). *Environ Entomol* 29:413–420. <https://doi.org/10.1603/0046-225X-29.3.413>
- Wang Z, Xie L, Prather CM, Guo H, Han G, Ma C (2018) What drives the shift between sexual and clonal reproduction of *Caragana stenophylla* along a climatic aridity gradient? *BMC Plant Biol* 18:91. <https://doi.org/10.1186/s12870-018-1313-6>
- Weed AS, Schwarzländer M (2014) Density dependence, precipitation and biological control agent herbivory influence landscape-scale dynamics of the invasive Eurasian plant *Linaria dalmatica*. *J Appl Ecol* 51:825–834. <https://doi.org/10.1111/1365-2664.12226>
- de Witte LC, Stöcklin J (2010) Longevity of clonal plants: why it matters and how to measure it. *Ann Bot* 106:859–870. <https://doi.org/10.1093/aob/mcq191>
- Ye D, Hu Y, Song M, Pan X, Xie X, Liu G, Ye X, Dong M (2014) Clonality-climate relationships along latitudinal gradient across china: adaptation of clonality to environments. *PLoS One* 9:e94009. <https://doi.org/10.1371/journal.pone.0094009>
- Ye D, Liu G, Song Y-B, Cornwell WK, Dong M, Cornelissen JHC (2016) Strong but diverging clonality - climate relationships of different plant clades explain weak overall pattern across China. *Sci Rep* 6:26850. <https://doi.org/10.1038/srep26850>

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