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Growth trajectories of diploid and tetraploid trees of the *Betula pendula/B. pubescens* complex (Betulaceae): a 38-year record of trunk circumference

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Abstract

Growth in trunk circumference in a natural, uneven-aged stand of 20 trees in the *Betula pendula/B. pubescens* complex at Holme Fen, Cambridgeshire (v.c.31), UK was monitored over a period of 38 years, from 1977-2014. At the beginning of the study, their chromosome numbers were determined, and trees were aged by counting the rings in radial cores. Circumferential growth trajectories with increasing age of five diploid and 13 tetraploid trees were modelled using three-factor sigmoidal regression. As with previous morphological and molecular studies of the same trees, the tetraploids were considerably more variable in growth trajectory than the diploids; tetraploids included both the slowest- and fastest-growing individuals. Diploids behaved more coherently, having more similar trajectories. Greater variation in tetraploids probably reflects their allopolyploid origin, with subsequent unidirectional introgression. There were indications that diploids may grow faster and reach greater asymptotic circumferences than tetraploids but the differences in this small sample were not statistically significant. There was no evidence that the two cytotypes have different life expectancies.

Keywords: age structure; birch trees; population variation; radial growth; sigmoidal model

Introduction

Birch trees in Britain are mainly represented by two closely related species, the diploid *Betula pendula* Roth and the tetraploid *B. pubescens* Ehrh. (Atkinson, 1992). *B. pendula* tends to occur in drier situations, and has a generally more southerly and easterly distribution than *B. pubescens* (Amphlett, 2021). Nevertheless, the two species are largely sympatric in Britain and, in the lowlands at least, mixed stands are common. Both species are typically light-demanding, successional pioneers, with a relatively short lifespan of about a hundred years (Kinnaird, 1968; Hynynen *et al.*, 2010; Atkinson, 1992). *B. pendula* is reported to be longer lived than *B. pubescens* (Ovington & Madgwick, 1959). Natural stands regenerate readily from wind-dispersed seeds in different environments ranging from acidic heaths to fen peatlands, especially after clear-felling or the cessation of management by grazing. Although both species are of considerable silvicultural importance in northern Europe

(Nieuwenhuis & Barrett, 2002; Hynynen *et al.*, 2010, little information is available on their long-term growth rates in more natural stands.

Although distinct over much of their Eurasian range, the two species show much morphological ambiguity, particularly in secondary woodlands in East Anglia, UK (Walters, 1968) and much effort has gone into distinguishing between them (Atkinson & Codling, 1968; Wang et al., 2014; Amphlett, 2021). As part of a larger morphometric investigation of this problem, Gill & Davy (1983) determined the chromosome number of 50 trees at three East Anglian sites. The diploid trees ($2n = 28$) agreed reliably with descriptions of *P. pendula*, whereas tetraploids ($2n = 56$) showed more morphological and molecular variation, including that expected for both *B. pubescens* and *B. pendula*, with many intermediate forms (Gill & Davy, 1983; Howland *et al.*, 1995). Trunk circumferences of trees at two of the sites were measured in 1977 and their ages determined subsequently. Shortly afterwards the stand at Dersingham Heath and Bog, Norfolk was clear-felled as a result of site management and all the trees were lost; however, the stand at Holme Fen, Cambridgeshire has survived, allowing repeated measurements of trunk circumference over 38 years.

Our objective was to investigate variation in the long-term growth trajectories of these trees and how circumferential growth changed with age in a mixed-age, natural stand. We hypothesised that (1) diploids trees would grow faster and live longer than tetraploids; and (2) diploid trees would behave more coherently as a group and show less variation in growth than tetraploids, as seen with morphological and molecular variation.

Materials and Methods

Site and tree selection

Holme Fen National Nature Reserve, Cambridgeshire (v.c.31), UK (National Grid reference TL 205892) comprises an area of deep fen peat bathed by the alkaline waters of the River Nene (Gill & Davy, 1983). According to Ovington & Madgwick (1959) Holme fen was drained and reclaimed for agriculture early in the 19th century but by the turn of the century most of the area had been invaded by birch and had reverted to woodland. Subsequently patches were cleared at different times by accidental fires or wartime felling, only to be rapidly recolonised by birch. The result was stands that vary in age and, as there has been little silvicultural management, stocking densities are largely the result of natural competition processes (Ovington & Madgwick, 1959).

Work was carried out in an uneven-aged stand of trees of the *Betula pendula/pubescens* complex that had been colonised naturally (Fig. 1). Twenty trees were selected at random in an open area (100 x 45 m) where research was allowed; an area of low canopy density was selected such that leaves would be available at an accessible height for morphometric study. In 1977, the positions of the trees were mapped and the stand surveyed by levelling. Surface topography varied by only 23 cm within the stand. The depth of the water table varied but was usually 0.5-1.5 m below the peat surface.

Tree measurements

Trees were permanently labelled with plastic tags attached with wire loops to small, galvanized nails. The circumference of each trunk was measured at a fixed height of 1 m above the ground during the dormant season (November–March) on 25 annual occasions over a period of 38 years: 1977, 1980, 1986-2000, 2005-2012 and 2014. In January 1980, a radial core (4 mm in diameter) was removed from each trunk at a height of 1 m using an And. Mattson No. 3 stainless steel corer and extractor; the hole was then carefully plugged. The ages of the trees were estimated by counting annual growth rings in the cores. *Betula* wood is diffuse porous and so comparisons with tree sections of known age were used to aid in discriminating growth rings. Despite this, it was not possible to age two trees (H10 and H11) reliably from the cores obtained. The values obtained are likely to underestimate true age by *c.*2-3 years because of the height at which the cores were taken.

Figure 1. Two views of open birch woodland at Holme Fen, Cambridgeshire, representative of the stand of trees studied from 1977 to 2014. Birch trees are shown at the early leafing stage, on 21 April 2022. Images: Stephen McAdam

Chromosome counts

The chromosome number of each tree was determined from squashes of stem-apex meristems. Tips were pre-treated with 8-hydroxyquinoline, before hydrolysis with hydrochloric acid and staining with toluidine blue; finally, squashes were incubated with RNAase to improve contrast. Further details are given by Gill & Davy (1983).

Statistical analysis

The relationship between trunk circumference and age for each individual tree was modelled by a procedure similar to that used by Prévosto *et al.* (1999). Three-parameter sigmoidal regressions were fitted using SigmaPlot 11.2 (Systat Software Inc.):

$$y = a/(1+e^{-((x-c)/b)})$$

where *a* is an asymptotic circumference, *b* is a rate or growth parameter, and *c* adjusts for initial circumference. This model yielded slightly better fits to the data than the Chapman-Richards' three-factor sigmoidal model commonly used in forestry (Prévosto *et al.*, 1999). Means were compared using *t*-tests, or the Mann-Whitney rank-sum test where the assumptions of parametric statistics were not met.

Results

Tree distribution, age structure and mortality

Six of the trees were diploid (2n = 28) and were 14 tetraploid (2n = 56). The two cytotypes were spatially distributed evenly across the stand (Fig. 2). The age structure in 1980 of the 18 trees whose age could be determined is shown in Fig. 3. Ages ranged from 11 to 60 years with a modal class of 20-30 years and a mean age of 31 years. Subsequent mortality was low, with 17 trees (85%) still alive at the end of the study in 2014, by which time the oldest survivor of known age was more than 93 years old. All of the trees survived until 1999, when H13 and H17 died; H15 was dead by 2005, when measurements restarted after a short break. All of the mortalities were among the more numerous tetraploid trees.

Average annual growth rates

Over the whole period of recording the mean annual increment in circumference was 50% greater in diploid trees than tetraploid trees: 1.51 ±0.30 (SE) cm for diploids, in comparison with 1.00 ±0.18 cm for tetraploids, although this difference was not significant ($t_{5,13} = 1.505$, $P = 0.150$).

Individual trajectories with age

Circumferential growth was generally non-linear and the individual trajectories of circumference with age were well described by the three-factor sigmoidal model (Table 1; Fig.4). The regressions for all trees were highly significant ($P < 0.001$) and their individual parameters were also significant, with the conspicuous exception of H15, for which none of the parameters was significant. This was the oldest tree (60 years) at the beginning of the study, subsequently grew very slowly and then died at the age of 80. Regressions for diploid trees explained *c.*98% of variation in circumference, while those for tetraploids explained 93-99% (apart from H6, whose regression explained only 85%).

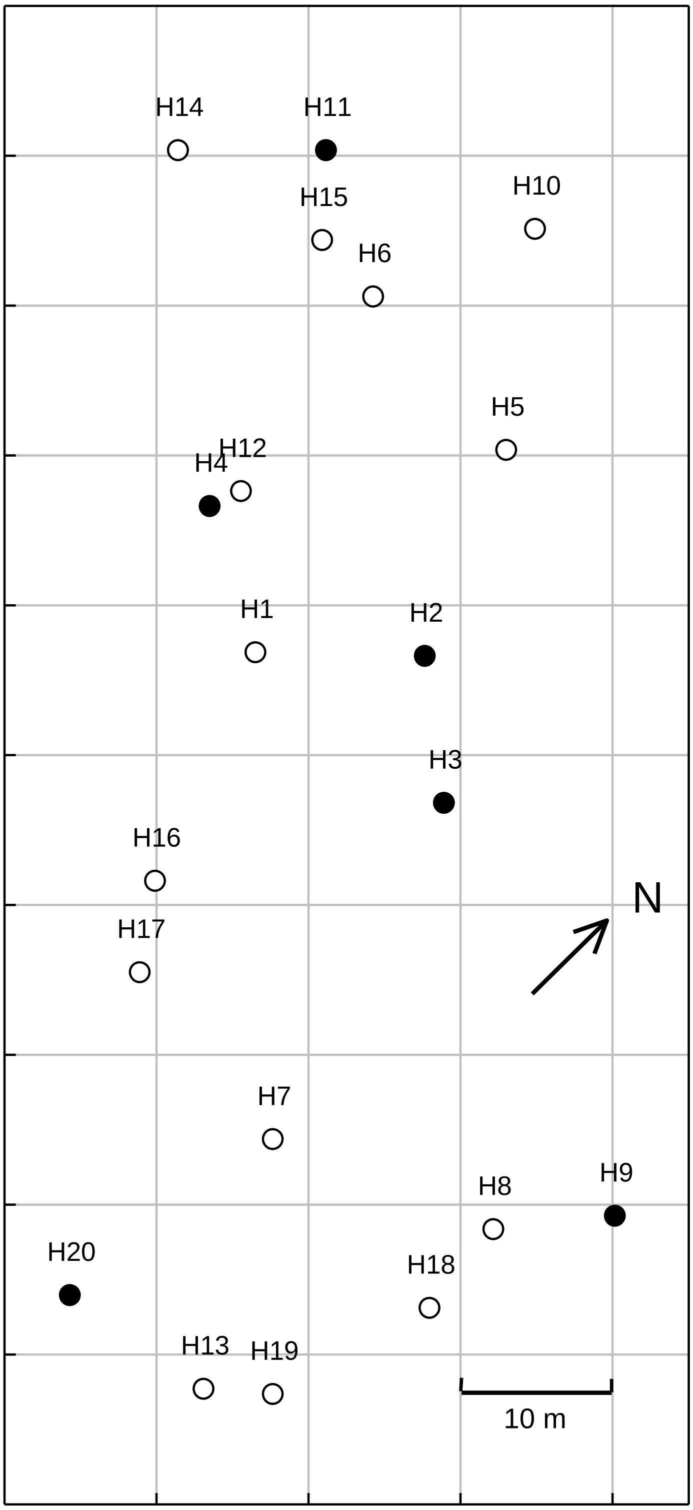

Figure 2. The distribution of 20 birch trees (*Betula pendula/B. pubescens*) in a 10 x 4.5 m plot at Holme Fen, Cambridgeshire: ●, diploid trees; ○, tetraploid trees

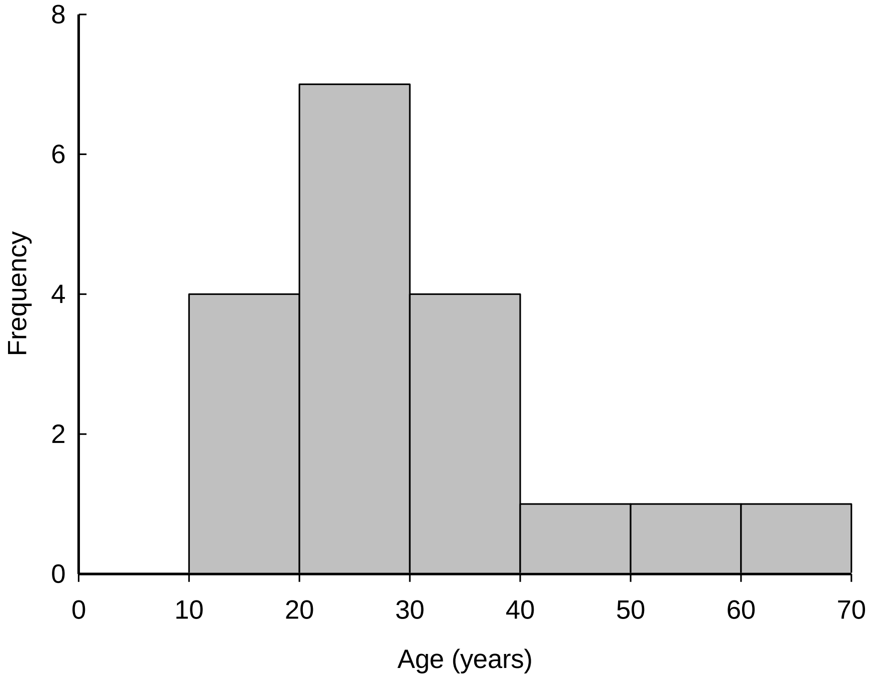

Figure 3. Age structure in 1980 of 18 birch trees (*Betula pendula/B. pubescens*), whose age could be determined by coring, within a 10 x 4.5 m plot at Holme Fen, Cambridgeshire.

Growth in the diploid trees, although encompassing five different individual age ranges was sufficiently uniform overall as to be seen as a nearly common trajectory with age over some 65 years (Fig. 4a). In contrast, the variation in the trajectories of tetraploid trees was much greater (Fig. 4b). Some tetraploid trees, including some relatively young ones, virtually ceased circumferential growth in the early stages of the study but nevertheless survived for many years. Others, including some relatively old ones, grew steadily over the whole 38-year period of the study. At the other extreme, the tree with greatest circumference throughout the study (H14) had shown no decline in its rate of growth by the end of the period of recording, by when it had reached the age of 63.

Diploid trees tended to grow faster and produce larger boles than tetraploids (Table 1; Fig. 4). The mean growth rate parameter (*b*) for diploids (15.8) was 34% greater than in tetraploids (11.8) although the difference did not attain statistical significance (Mann-Whitney $U_{5,13} = 23$, $P = 0.127$). Similarly, the mean asymptotic circumference was 37% greater in diploids (122 cm) than in tetraploids (89 cm) but again this was not significant (Mann-Whitney $U_{5,13} = 16$, $P = 0.155$).

Table 1. Parameters for three-factor sigmoidal regressions of trunk circumference on age for five diploid and 13 tetraploid birch trees at Holme Fen, Cambridgeshire. $y = a/(1+e^{-((x-c)/b)})$. ns, parameter not significant, $P > 0.05$

	r^2	Parameter			*n*
		a	*b*	*c*	
Diploid trees					
H2	0.984	165.3	21.4	33.4	25
H3	0.986	156.5	15.3	36.5	25
H4	0.976	95.2	20.4	11.4	25
H9	0.986	109.8	9.46	32.8	25
H20	0.985	85.3	12.54	21.2	25
Mean		122.4	15.8	27.1	
Tetraploid trees					
H1	0.965	90.5	10.6	19.4	25
H5	0.967	73.5	14.9	18.6	25
H6	0.847	112.2	16.8	19.2	25
H7	0.934	104.8	8.1	26.6	25
H8	0.940	32.3	3.64	28.6	24
H12	0.900	122.8	18.9	40.6	25
H13	0.962	40.5	5.6	20.1	16
H14	0.994	269	34.5	39.7	25
H15	0.957	ns	ns	ns	17
H16	0.961	40.4	3.63	14.8	25
H17	0.955	27.1	5.56	7.78	16
H18	0.987	107.6	9.85	31.4	25
H19	0.984	48.9	10.0	8.45	25
Mean		89.1	11.8	22.9	

Discussion

Most of what we know about birch demography and growth is derived from silviculture and thus relates to even-aged, heavily managed stands (Nieuwenhuis & Barrett, 2002; Hynynen *et al.*, 2010). Naturally colonised and self-thinned stands, such as that at Holme Fen, would be expected to show more variation between individuals. This is evident in the age range recorded in 1980 (11-60 years), which was similar to the span of mean ages in a series of differently aged plots (6-55 years) at the same site, described nearly two decades earlier by Ovington & Madgwick (1959). By hardwood standards birches are short-lived (Kinnaird, 1968; Hynynen *et al.*, 2010) and our data reflect this. The three individual mortalities we observed were at the ages of 33, 46 and 80, the last of these having been the oldest tree at the start of the study; thus, by the end of the study the oldest tree was 93.

Although all the mortalities happened to be tetraploids there was no clear evidence that the diploids (*B. pendula*) live longer, as suggested by Ovington & Madgwick (1959) and this hypothesis must be rejected; in fact, the mean ages of surviving diploids and tetraploids at the end of the study were nearly identical at 64.6 and 64.3 years, respectively.

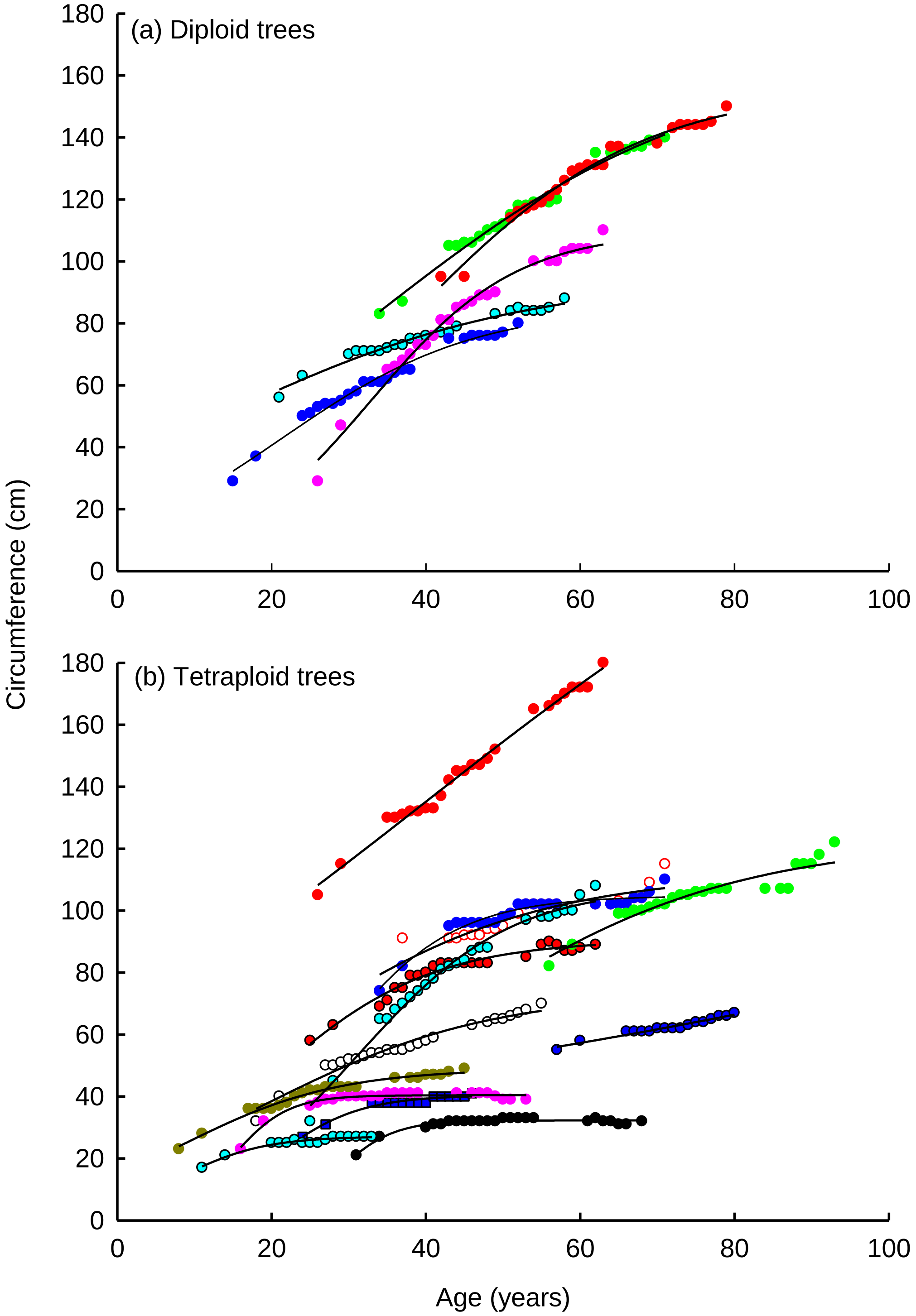

Figure 4. Trajectories in the growth of trunk circumference with age for (a) diploid and (b) tetraploid trees between 1977 and 2014 at Holme Fen,

Cambridgeshire, UK. Growth curves are three-factor sigmoidal regressions (see Table 1). Coloured symbols differentiate individual trees but have no other significance.

The initial range of circumferences recorded (17-105 cm) was broader than that reported by Ovington & Madgwick (1959) previously (19-66 cm), probably not least because they presented means for differently aged plots, as opposed to our use of differently aged individual trees within a common plot. A further possible complication is the slightly different heights at which measurements were made: 1 m in our study, rather than at breast height by Ovington & Madgwick (1959).

The most striking aspect of our findings is in the trajectories of circumferential growth with age of individual trees, revealed by repeated measurements over a span of 38 years. This approach was designed to be non-destructive: growth trajectories in forestry are more commonly determined by comparing stands of different ages (space-for-time substitution) or by the measurement of annual growth rings after felling. The three-factor sigmoidal models are theoretically appropriate (Prévosto *et al.*, 1999) and provided excellent fits to changes in circumference with age, despite the absence of data for the early growth and, in many cases, the senescent growth periods. Taken overall, the variation between trees was considerable, with neither age at initial sampling nor initial circumference proving to be a good indicator of subsequent growth. However, comparison of diploid and tetraploid trees reveals that most of this variation in trajectory is in the tetraploids, with the diploids behaving more coherently with age; the tetraploids included both the fastest and slowest growing individuals. This is consistent with the patterns of variation shown previously by these same trees, whether they relate to leaf and catkin morphology, stem and bole characteristics, stomatal guard cell length, pollen diameter or rDNA polymorphism (Gill & Davy, 1983; Howland *et al.*, 1995). In each case the tetraploids have proved considerably more variable than diploids. The hypothesis that tetraploids would also be more variable in their growth trajectories is clearly supported.

Although there are indications that diploid trees may on average grow faster and achieve greater asymptotic circumferences than tetraploids, the small sample size and high variability among tetraploids limit the statistical power of this study. Consequently, the hypothesis that they behave differently must be rejected, possibly pending further investigation.

Environmental conditions, particularly site wetness, are major determinants of growth and morphological variation in birch (Davy & Gill, 1984). In this case topography and hydrology across the experimental plot in this plot were practically uniform. However, this does not exclude an environmental influence as a component of the variation in trajectories seen, because of genotype-environment interactions. Gill & Davy (1984) transplanted the seedling progeny of *B. pendula*-like and *B. pubescens*-like tetraploid trees between heath and bog environments at a different site and found responses that involved both phenotypic plasticity and heritable, genotypic effects. It is likely that the diversity of growth trajectories in tetraploids reflects their greater range of genotypes (Howland *et al.*, 1995) that have differing fitness in the heath environment. A discriminant function based on leaf shape has been used to distinguish between diploids (*B. pendula*) and tetraploids (*B. pubescens*) with a high degree of accuracy in larger samples and over a much wider geographical range than ours but even this shows continuous variation between the two species, whether they are defined by chromosome number (Atkinson & Codling, 1986) or microsatellite markers (Wang *et al*., 2014). It is highly likely that *B. pubescens* originated from the hybridisation of *B. pendula* with an Asian diploid progenitor, *B. platyphylla* Sukaczev (Wang *et al*., 2021) and such allopolyploidy could harbour greater variation. Furthermore, our previous suggestion that the greater genetic diversity in our tetraploids has also involved historical introgression between *B. pendula* and *B. pubescens*, in areas where they are sympatric and have no clear habitat differentiation (Howland *et al*., 1995), has been supported by recent evidence of unidirectional introgression from diploids (*B. pendula* and *B. nana*) into tetraploids (*B. pubescens*) in Britain (Zohren *et al*., 2016).

Acknowledgements

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