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Biological Flora of the British Isles: Neottia cordata

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Abstract

1. This account presents information on all aspects of the biology of Neottia cordata (L.) Rich. (Lesser Twayblade; Listera cordata (L.) R. Br.) that are relevant to an understanding of its ecological characteristics and behaviour. The main topics are presented within the standard framework of the Biological Flora of the British Isles: distribution, habitat, communities, responses to biotic factors, responses to environment, structure and physiology, phenology, reproductive characteristics, herbivores, history, and conservation.

2. Neottia cordata is an inconspicuous orchid, confined to humid heathlands and woodlands rich in bryophytes in the British Isles.

3. Neottia cordata is a polycarpic, perennial herb with populations maintained predominantly by vegetative reproduction from root suckers. The main perennating organ is a short rhizome that produces two new internodes each year. The long adventitious roots are mainly colonized by mycorrhizal basidiomycetes from the Sebacinales (clade B, Serendipitaceae), but also by several other fungal groups.

4. The species flowers from mid-April to August depending on latitude and altitude. The flowers possess a sensitive rostellum that releases a viscid fluid when touched, ensuring that the pollinia are glued to the pollinator. The flowers produce nectar and are pollinated mainly by fungus gnats (Mycetophilidae and Sciaridae). Between 60 and 80% of open flowers set fruits.

5. Neottia cordata is classified as Least Concern in Great Britain. It is still locally abundant in north and west Scotland but has declined elsewhere, especially at lower altitudes, because of burning on grouse moors, drainage of swamps and cutting of wet forests. It is likely to have been under-recorded throughout its range, particularly in earlier surveys, due to its inconspicuousness.

KEYWORDS

communities, conservation, geographical and altitudinal distribution, germination, herbivory, mycorrhiza, reproductive biology, rostellum
Lesser Twayblade. Orchidaceae, subfamily Epidendroideae, tribe Neottieae. *Neottia cordata* (L.) Rich. (syn. *Listera cordata* (L.) R. Br.) is a polycarpic, rhizomatous, perennial herb. Rhizome short, growing horizontally or vertically, c. 2 mm in diameter. Adventitious roots sparse, 5-15 cm long and 0.7-1.5 mm in diameter. Stem (3)5-10(33) cm, solitary or occasionally with 2 or 3 stems from the same rhizome, erect, pale green or reddish purple, ridged towards the tip, with fine glandular hairs for a short distance above the leaves, with 1-2 membranous, brownish sheaths at the base. Leaves 2 (very rarely up to 4), shiny green above, paler below, sub-opposite, one third to halfway up the stem or at the apex of non-flowering stems, triangular-ovate, 1.5-2.5 cm long and 1-2.5 cm wide, faintly net-veined (reticulate), with a prominent midrib that terminates in a tiny projecting point (mucro), and undulating margins. Inflorescence short, relatively open, (1.5)2-3(4) cm long and 1 cm in diameter, with 3-15(20) flowers. Bracts triangular-ovate or lanceolate, 2-3 mm long and 0.8-1.1 mm wide, greenish. Flowers 3-4 mm across, variable in coloration, usually yellow-green, green, or reddish-purple, with complete or partial resupination. Outer perianth segments c. 2.5 mm, oval, with blunt tips, greenish, variably reddish in the centre and around the edges; inner perianth segments of similar length and colour, narrower and more strap-shaped, tending to be redder; outer and inner perianth segments spread widely forming a star-like pattern around the column. Labellum relatively large, 3.5-4.5 mm long, coppery or pale green, washed red, usually pendulous, divided more than halfway into two sharply pointed lobes; nectary tiny, disc-shaped at the base of labellum, just below the column, with 2 very short horn-shaped lobes on either side and a longitudinal nectar-filled groove running from the labellum base to the start of the fork. Spur absent. Short whitish column 0.5 mm across, arching over the ovate anther; stigma positioned in front of the column just below the rostellum. Leaf-like rostellum concave on the upper side, forming a clinandrium (a cavity in the apex of the column) in which the pollinia rest after they fall out of the anther at flower opening. Pollinia 2, consisting of two halves containing loosely connected, powdery pollen grains united into tetrads. Stigma reniform. Ovary spherical, pale green, 4.5-5.5 mm long and 2.2-2.7 mm in diameter, a little shorter than the reddish or greenish flower stalk, with six prominent reddish ribs.

Until very recently, the genus *Neottia* comprised entirely species lacking chlorophyll (Bird’s-nest orchids). Species with a pair of green leaves were placed in a separate genus *Listera* (Twayblades), although the close relationship between the two genera had long been recognized (Dressler, 1990). *Neottia s.s.* and *Listera*, have very similar flower structure and recent genetic research has shown that they are so closely related that they should be united into a single genus (Chase et al., 2003). *Neottia* now contains c. 60 species, including 14 non-
green fully mycoheterotrophic species (Merckx, 2013), and is widely distributed in the Northern Temperate Zone of the Old and New World, with a few species extending south to tropical mainland Asia and the adjacent islands (Pridgeon et al., 2005).

*Neottia cordata* shows little morphological variability in the Old World. Plants with three or four leaves have been assigned to *Listera cordata* f. *trifolia* (Asch. & Graebn.) Pauca & Stefur. and *Listera cordata* f. *tetraphylla* Lavoie, respectively (for more details see WCSP, 2016). The third and fourth leaves are usually smaller, more pointed and placed above main leaves. In the British Isles, f. *trifolia* is rare and has been recorded in Scotland (Harrap & Harrap, 2005). The greater variability within North American populations has led to the description of *Listera cordata* var. *nephrophylla* (Rydb.) Hultén, which has broader leaves (1.8-3.8 cm) and a more elongated labellum (5-6 mm) compared to the nominate variety, and produces only green flowers (Hultén, 1941; Luer, 1975). This variety is found in western North America, its range overlapping with var. *cordata*, and there is debate over whether the variation should be recognised at this level (Calder & Taylor, 1968; Coleman, 1995). In Japan, var. *japonica* Hara. has smaller flowers and broader leaves than European plants.

*Neottia cordata* is an inconspicuous, easily overlooked, native orchid that is confined to threatened humid habitats, such as heather (*Calluna vulgaris*) moorlands, peat bogs and wet forest. Although still widespread and locally abundant in coastal and upland regions of Scotland, it has declined from similar habitats in lowlands throughout the rest of Britain and Ireland.

1 | GEOGRAPHICAL AND ALTITUDINAL DISTRIBUTION

*Neottia cordata* has been recorded from 1088 (27%) of the 10-km squares in the British Isles Figure 1; Table 1; Preston, Pearman & Dines, 2002; Online Atlas of the British and Irish flora 2017). It is widespread and locally abundant in suitable habitats in northern and western Scotland, including the Hebrides, Orkney and Shetland, although it is absent from much of the central lowlands (Foley & Clarke, 2005). In England, it is known from Derbyshire (where it was re-discovered in 2010; Willmot & Moyes, 2015), North Lancashire and Southwest Yorkshire northwards, with a southernmost outpost on Exmoor (Somerset/Devon) where it still occurs in small quantity (Carter, 2005; Smith et al., 2016). There were formerly also a number of other isolated occurrences of *N. cordata* in southern England (Harrap & Harrap, 2005). In 1853 and 1895 it was recorded near to Bournemouth in Hampshire; later it appeared in the New Forest near Brockenhurst in 1927-30 and was reported again there in the 1970s with a later suspected deliberate introduction near to Bratley in Hampshire in about 1980.
It was recorded at a few sites in Dorset and Shropshire in the 19th century but is now long extinct in both counties (Bowen, 2000; Lockton & Whild, 2015). More recently it has been recorded from Baldwin’s wood in Buckinghamshire in 1980 and from Gravetye Woods in East Sussex in 1975 and then again in 1989 (Hall, 1980; Harrap & Harrap, 2005). It is suspected that some of these more southerly occurrences represent accidental introduction of seeds with planted pines and rhododendrons although some may represent relict populations on southern heaths (Harrap & Harrap, 2005). In Wales, *N. cordata* occurs sporadically from mid-Wales (Brecon, Radnorshire, Ceredigion) northwards to Snowdonia, Flintshire and the Isle of Anglesey. In Ireland, the species is widespread and locally abundant in Co. Sligo, Co. Cavan and Co. Down northwards, but it is very rare in the south: in Co. Dublin and Co. Wicklow in the east, Co. Galway in the west, and from Co. Limerick and Co. Tipperary southwards (Harrap & Harrap, 2005).

*Neottia cordata* has a circumpolar distribution and is one of the few orchids to grow in the Arctic tundra (Figure 2; Table S1). The southern boundary of its Eurasian range extends from the Pyrenees in Spain, to Corsica and the Apennines in Italy (Aeschimann et al., 2004), mountainous regions in Serbia and Bulgaria, and across Turkey to the west Caucasus (Georgia). The southernmost localities occur in Central Rhodope mountains of Greece near to the Bulgarian border (Tsiftsis, Tsiripidis & Vidakis, 2012). The northern boundary of its range includes Greenland (Desmet & Brouillet, 2013), Iceland, northernmost Scandinavia, the Baltic states, European Russia, across Siberia, including Altay, to the Russian Far East, Kamchatka, Sakhalin, the Kuril Islands and Japan (Uemura, 1989; Vakhrameeva et al., 2008: map 20). In North America, it grows in the Aleutian Islands (Talbot & Talbot, 1994), Alaska (Noble, Lawrence & Streveler, 1984), the western United States, across Canada, around the Great Lakes and on the East Coast from North Carolina to Maine (Penaido, Aguirre & de la Cruz, 1998). Southern extensions of range occur in the Rocky Mountains to New Mexico and in the Appalachians to North Carolina (Magrath & Coleman, 2002).

## 2 HABITAT

### 2.1 Climatic and topographical limitations

*Neottia cordata* belongs to the Circumpolar Boreal-montane element of the British flora (Preston & Hill, 1997). This element includes Circumpolar species which are found in mainland Europe both in the Boreal zone and in the mountains to the south. In Britain, these Boreal species are widespread in Wales, northern England and Scotland. The mean January and mean July temperatures, and mean annual precipitation for the 10-km squares occupied
by *N. cordata* in Britain are 2.4 °C, 12.8 °C and 1450 mm, respectively (Hill, Preston, & Roy, 2004). Some populations experience a minimum mean January temperature as low as -1 °C (Figure 3). An Ellenberg value of 3 for light indicates a preference for less than 5% of relative illumination (Hill et al., 2004).

The altitudinal range of *N. cordata* in the British Isles is almost from sea level to 1065 m at Stob Coire Easain, Inverness-shire (Harrap & Harrap, 2005) although 80% of populations occur below 550 m altitude (Figure S2). Elsewhere in Europe, its lower altitude limit is c. sea level in the coastal areas of The Netherlands, whereas the upper limit reaches at 2300 m in the French Alps. Altitudinal ranges in individual regions vary from 600 to 1500 m in Russia (Efremov & Antosyak, 1990), from 550 to 1405 m in the Czech Republic (Rollerová, 2015), from 800 to 2300 m in France (Bournérias & Prat, 2005), up to 1550 m in Bulgaria (Jordanov, 1964), from 1600 to 1900 m in the Rhodope range in Greece (Petrou & Giannakoulias, 2011), from 600 to 2100 m in the Alps (AGEO, 2017, Ziegenspeck, 1936), and from 350 to 1870 m in Slovenia (Dakskobler, Rozman & Vreč, 2012). In North America *N. cordata* has been found at elevations from sea level up to 3500 m (Magrath & Coleman, 2002).

### 2.2 | Substratum

Despite its wide distributional range, *N. cordata* is restricted to a narrow range of abiotic conditions. It favours humid to very moist, very acidic to acidic (pH 2.8 – 5.5) nutrient-poor humus or peat (Sundermann, 1970; Hoy, 2002; Šibík, Dítě & Pukajová, 2006; Rollerová, 2015). *N. cordata* never occurs directly on neutral or alkaline soils although it occurs on limestone pavement on Moughton Fell in Yorkshire, because it grows on deep humus and moss layers under the shade of juniper (*Juniperus communis*) bushes (Foley & Clarke, 2005). A similar situation has been reported from the Slovak Republic, where *N. cordata* grows very rarely in cold and wet northern exposures of chalk or dolomite bedrock, which is, however, overlaid by a deep layer of acid humus and spruce-needle litter (Dítě, Hrivnák & Jasík, 2012). Throughout its range, it is typically found growing on or through deep cushions of moss, usually *Sphagnum* spp. or *Polytrichum commune* (Sundermann, 1970). Because of its consistent link with *Sphagnum* and other moss substrates the species has been classified as a ‘bryophile’ (Mazureiko & Khokhryakov, 1989). Ellenberg’s indicator values (modified for the UK) for edaphic characteristics at sites where *N. cordata* is found are 2 for pH (the species favours sites with strongly acid to acid reaction), 6 for moisture (wet to moist soils), 2 for nitrogen (extremely poor to poor sites) and 0 for salinity (it does not occur in saline habitats) (Hill et al., 2004). In euoceanic areas, the plant does not appear to grow on severe slopes,
exceeding 45°, where drainage is effective despite the high and continuous precipitation (Doyle & Kirby, 1987). A study analysing impact of nitrogen deposition on vascular plants in Britain found a distinct optimum for *N. cordata* at around 20 kg N ha\(^{-1}\)year\(^{-1}\), possibly reflecting its dependence on aerial deposition of nutrients for growth in nutrient-poor habitats (Henrys et al., 2011).

### 3 | COMMUNITIES

In Britain and Ireland, *Neottia cordata* occurs in two very different groups of plant communities: in humid heathlands (wet heath and blanket mire) and moist to damp woodlands. Both are rich in bryophytes and ericaceous plants. The species favours shaded conditions (Summerhayes, 1951; Foley & Clarke, 2005; Harrap & Harrap, 2005), where it is almost always concealed beneath a closed canopy of ericaceous sub-shrubs (e.g. *Calluna vulgaris*, *Erica* spp. or *Vaccinium* spp.).

In Scotland, where *N. cordata* is most common, a survey of the vegetation of Glen Coe in the Highlands reported it from two British plant communities: *Calluna vulgaris* — *Eriophorum vaginatum* blanket mire (H19, see Figure 4a), *Erica tetralix* sub-community (M19a), and *Calluna vulgaris* — *Vaccinium myrtillus* — *Sphagnum capillifolium* heath (H21), *Calluna vulgaris* *Pteridium aquilinum* sub-community (H21a) (Averis & Averis, 2006). The latter is confined to oceanic mountains on the north-west coast of Scotland, where the microhabitat is extremely humid and sheltered leading to the development of deep hepatic mats of bryophytes (Ratcliffe, 1968). In addition, Rodwell (1992) describes it from other sub-communities of M19 mirre and H21 heath: *Empetrum nigrum* subsp. *nigrum* (M 19b) and *Vaccinium vitis-idaea* — *Hylocomium splendens* (M19c) sub-communities, and also the *Mastigophora woodsii* — *Herbertus aduncus* subsp. *hutchinsiae* sub-community (H21b). *N. cordata* has also been reported from drier heather moorland communities managed for Red Grouse (*Lagopus lagopus* subsp. *scoticus*), namely *Calluna vulgaris* — *Vaccinium myrtillus* heath (H12, all sub-communities), *Calluna vulgaris* — *Arctostaphylos uva-ursi* heath (H16), *Pyrola media* — *Lathyrus montanus* (H16a) and *Vaccinium myrtillus* — *Vaccinium vitis-idaea* (H16b) sub-communities); at moderate to high altitudes in the Scottish Highlands it also occurs in *Vaccinium myrtillus* — *Rubus chamaemorus* heath (H22, both sub-communities) (Rodwell, 1992).

Woodland populations of *N. cordata* are much less common, largely due to the rarity of native woodlands in the uplands of the British Isles (when compared to the extent of upland heathlands). In woodland (Rodwell, 1991) it grows in *Pinus sylvestris* — *Hylocomium*
splendens woodland, with a prominent layer of bryophytes (W18; subcommunities Erica cinerea — Goodyera repens (W18a), Vaccinium myrtillus — Vaccinium vitis-idaea (W18b), Luzula pilosa (W18c) and the more-boggy Sphagnum capillifolium/quinquefarium — Erica tetralix (W18d)). The species has been also found in Juniperus communis subsp. communis — Oxalis acetosella woodland (W19; only the Vaccinium vitis-idaea — Deschampsia flexuosa (W19a) sub-community, which has open cover of Juniperus bushes and hummock-hollow topography with abundant ericoid sub-shrubs growing on the top of hummocks).

Neottia cordata occurs in humid heathland and dune slacks planted with pines at Tentsmuir NNR in Fife, Scotland (Marks, 2000) and Newborough Warren NNR in Anglesey (K.J. Walker, personal observation). It also grows on shaded pathsides and under planted conifers in Caenlochan Glen, Scotland where the conditions were relatively dry when compared to more typical habitats (Walker K.J. personal observation).

Elsewhere in western Europe, N. cordata grows on the Frisian Islands (The Netherlands) in a dune moorland on acid sand soil, under and between tall plants of Calluna vulgaris, Erica tetralix, Empetrum nigrum and Vaccinium uliginosum (Westhoff, 1959). The biotic conditions strikingly resemble those on moorland in the Scottish Highlands. It also inhabits the secondary vegetation of planted pine forests on dunes (Westhoff, 1959). In the Eastern Pyrenees, N. cordata appears to grow in forests with Pinus mugo and Rhododendron ferrugineum (Braun-Blanquet, Sissingh & Vlieger, 1939).

In Central Europe (Jersáková & Kindlmann, 2004; Šibík et al., 2006; AHO Sachsen-Anhalt, 2011), N. cordata grows in montane spruce forests (Piceion abietis: subcommunities Calamagrostio villosae — Piceetum abietis, Mastigobryo — Piceetum and Sphagno—Piceetum, see Figure 4b), bog and waterlogged spruce forests (Piceion abietis: Equiseto sylvatici — Piceetum abietis, Soldanello montanae — Piceetum abietis and Vaccinio uliginosi — Pinion sylvestris: Vaccinio uliginosi — Piceetum abietis), waterlogged broad-leaved floodplain forests with Alnus glutinosa or A. incana in the mountains (Alnion glutinosae, Alnion incanae), subalpine Pinus mugo scrubs (Pinion mugo: Adenostylo alliariae — Pinetum mugo) and open raised bogs (Sphagnion magellanici: Andromedo polifoliae — Sphagnetum magellanici). Typical tree species that accompany N. cordata in bog and waterlogged spruce forests are Picea abies, Pinus mugo agg. and Betula pubescens agg., together with small ericaceous shrubs (Vaccinium myrtillus, V. uliginosum, V. vitis-idaea, V. oxyccoccus, Empetrum nigrum and Calluna vulgaris). Dominant herbaceous plants are in the genera Eriophorum and Carex, and the herbaceous layer covers 20 to 25% of the soil surface. A moss layer is formed by Sphagnum and Polytrichum species with more than 85 % cover
(Šibík et al., 2006; Rollerová, 2015). The species typically grows on peat-moss hummocks which are drier compared to open, waterlogged peat bog.

In the Julian Alps and the eastern Karavanke mountains of Slovenia, *N. cordata* occurs in natural spruce forests and is found also in the submontane pioneer forest of *Pinus sylvestris* on dolomite bedrock (Erico carnea — Pinion sylvestris: Erico carnion — Pinetum sylvestris), in subalpine larch forests (Piceion excelsae: Rhodothamno — Laricetum deciduae) and in special forms of *Pinus mugo* stands (Pinion mugo: Rhododendron hirsutum — Pinetum mugo) on shady ridges and in gullies where poorly decomposed organic matter accumulates and snow lingers long into the spring (Dakskobler et al., 2012). The cover of the herb layer ranges from 40 to 80%, while the moss layer ranges from 10 to 40%. Typical associates of *N. cordata* include *Erica carnea*, *Homogyne alpine*, *Larix decidua*, *Lycopodium annotinum*, *Pinus mugo*, *Rhododendron hirsutum*, *Sorbus chamaemespilus*, *Vaccinium myrtillus* and *V. vitis-idaea*, and the mosses *Hylocomium splendens* and *Rhytidiadelphus triquetrus*.

In Scandinavia, *N. cordata* is a constant species of north boreal dark coniferous forests (community Piceion excelsae) growing in association with *Betula pubescens* agg., *Cornus suecica*, *Linnaea borealis*, *Orthilia secunda*, *Picea abies*, *Sphagnum* spp. and *Vaccinium* spp., (Dierssen, 1996; Fremstad, 1997). These forest communities include widespread Scandinavian mountain birch woodlands on moist sites in higher elevations on very acid raw humus (the dwarf cornel birch forests of subcommunity Corno — Betuletum and meadow birch forests of subcommunity Geranio — Betuletum with *Calluna vulgaris*, *Dryopteris* spp., *Geranium sylvaticum*, *Juniperus communis*, and open spruce swamp forest (subcommunity Rubo chaememoro — Piceetum) (Dierssen, 1996; Fremstad, 1997). In Russia, *N. cordata* grows in communities analogous to those of Scandinavia, comprising dark, wet coniferous taiga forests on acid soils (order Piceetalia excelsa; communities: Piceion excelsae, Aconito rubicundi — Abietion sibiricae, Pino sibiricae — Abietion sibiricae, Rhododendro caucasici — Betulion litwinowii) with *Abies sibirica*, *Cornus suecica*, *Linnaea borealis*, *Larix* spp., *Lycopodium* spp., *Orthilia secunda*, *Picea abies*, *Sorbus sibirica* and *Vaccinium* spp. (Mirkin & Naumova, 2012). The species was also recorded in dwarf-shrub tundra, both in European and in Asian parts of Russia. *N. cordata* has been found in the alpine zone of the Khibiny Mountains, in small herbaceous patches of Ranunculo — Poion alpinae (Koroleva, unpublished data, pers. com.) as well as on the species-rich sloping mires of Sphagno warnstorffii — Tomentypnion nitentis (Koroleva, 2001). Moreover, it occurs in tundra on the Barents Sea shore, in herb-rich communities of the vegetation formerly recognised as Potentillo — Polygonion vivipari (now a synonym of Kobresio — Dryadion; Koroleva, 2006).
In the Far East of Russia, *N. cordata* grows in a wide range of vegetation types: the snow-bed plant communities on the slopes of the Alaid volcano on the Kuril Islands (Grishin et al., 2009) and the species-rich *Larix cajanderi* and *Betula lanata* woodlands with tall herbs such as *Aconitum ajanense*, *Angelica saxatilis*, *Aruncus dioicus*, *Atragene ochotensis*, *Calamagrostis langsdorffii* and *Veratrum oxysepalum*, on the flood-plains of Magadan Oblast (Moskaliuk & Dokuchaeva, 2014). In the treeless Commander Islands *N. cordata* can be found in meadow communities, dwarf-shrub tundra, and floodplain willow groves (Mochalova & Yakubov, 2004) and in groves of *Rhododendron camtschaticum*. Alder woods along small streams were shown as *N. cordata*-preferred habitats in Magadan Oblast and the Kuril Islands (Vyshin, 1996). In Kamchatka, the species is common in stone birch (*Betula ermanii*) woodlands as much as in spruce forests, on sedge — *Sphagnum* bogs, in communities dominated by *Empetrum nigrum*, and in grassy and mossy communities on open slopes of the mountains and volcanos (Yakubov & Chernyagina, 2003).

Japanese populations of the species are strictly linked to the sub-alpine mountain coniferous forests on Hokkaido, Honshu, and Shikoku islands (Ohwi, 1965).

Habitats of *N. cordata* in North America includes moist *Sphagnum* hummocks in northern woods and cold bogs, but it is also found in thick, rather dry moss mats on headlands and in the humus or accumulated conifer needles of deciduous and coniferous forests (for details see Hoy, 2002). Hemlock (*Tsuga canadensis*) groves in ravines may harbour it as well. Typical associates of *N. cordata* in the Northeast America are the trees *Abies balsamea*, *Acer rubrum*, *Alnus incana*, *Cornus canadensis*, *Pinus strobus*, *Picea mariana* and *Thuja occidentalis*, the shrub *Ledum groenlandicum*, the herbs *Carex* spp., *Circaea alpina*, *Galium* spp., *Linnaea borealis*, *Oxalis montana*, and the mosses *Polytrichum* spp. and *Sphagnum* spp. The southernmost populations are found in forests made up of *Betula* spp., *Fagus grandifolia*, *Picea rubens*, and *Tsuga canadensis*.

### RESPONSE TO BIOTIC FACTORS

The association of *Neottia cordata* with cushions of moss, usually *Sphagnum*, or the understorey of heather, on moorland or in shady and wet woodland (Harrap & Harrap, 2005) probably reflects its preference for very humid conditions and intolerance of competition from dense cover of herbs and grasses. It rapidly disappears after habitat disturbance and changes to vegetation structure, for example the removal of the sub-shrub canopy and moss layers due to burning of heather on grouse moors. The species survives haymaking and low-intensity pasture, but succumbs to human trampling (Bayfield & Brookes, 1979; Vakhrameeva et al.,
2008; Hegland & Rydgren, 2016). Chater (2010) noted that *N. cordata* was particularly abundant in areas where strips of heather were experimentally mown.

5 RESPONSE TO ENVIRONMENT

5.1 Gregariousness

*Neottia cordata* usually forms colonies of several dozens to hundreds of plants, often in small groups with a high density of individuals (Andrienko, 1983; Efremov & Antosyak, 1990; Zagulskii, 1994; Hoy, 2002; see Figure 4c). For example, in the Czech Republic, one population in the Šumava mountains had about 300 individuals growing in an area of 2 m² (M. Kotilínček, personal observation). Case (1987) describes stands of *N. cordata* from the Great Lakes region as "large colonies" of 25–40 plants. Weijer (1949) observed c. 10,000 individuals within an area of 20 m² on the West Frisian Island of Ameland. K.J. Walker (personal observation) recorded 103 individuals (71 flowering, 32 non-flowering) in a 10 x 5 m island of blanket mire, surrounded by burnt heathland, on moorland in Yorkshire at the extreme southern edge of the species’ range in the British Isles. Extensive populations, comprising well-scattered individuals of *N. cordata* with up to 10-20 m between individual plants, have been found in various geographic locations, including humid heathlands on the Hebridean Islands of Eigg and Colonsay (K.J. Walker, personal observation), spruce forest in Arkhangelsk Oblast, and Primorye Territory, Russia, and redwood forest in California (I. Tatarenko, unpublished data). Single individuals, with no sign of colonies, have been reported in the larch and birch woodlands in the South of Magadan Oblast (Moskaliuk & Dokuchaeva, 2014).

Established colonies may fluctuate considerably and unpredictably in size from year to year (Smith, 1993; Hoy, 2002; AHO Sachsen-Anhalt, 2011).

5.2 Performance in various habitats

The habitat variability reported from its North American range might reflect genetic diversity associated with the varieties *cordata* and *nephrophylla* (Hoy, 2002). The former is reported from mountainous regions in mossy, damp, coniferous or mixed coniferous-hardwood forests, sphagnum bogs and evergreen swamps at elevations from sea level up to 1200 m, while the latter inhabits the shaded humus of rich, well-drained, coniferous or coniferous-hardwood forest from 1500 to 3500 m (Magrath & Coleman, 2002). Vakhrameeva et al. (2008) mentioned plants in forests being larger and more robust than plants in bogs. K.J. Walker (personal observations) noticed very large plants growing on path sides and under the shade
of plantations in the Central Highlands of Scotland (Caenlochan Glen) which were much larger than those typically encountered under heather in northern England and on the north-west coast of Scotland.

5.3 | Effect of frost, drought etc
As certain populations in the British Isles experience a minimum mean January temperature of -1 °C (Figure 3) and the species can be found at altitudes up to 3500 m (see 2.1) it is likely to be resistant to frost.

No data are available related to the effect of drought but drainage of bogs, wet forests and swamps leads to changes in vegetation cover and microclimate, and rapid decomposition of peat or humus layer that may adversely affect *N. cordata* populations.

Similarly, observations in northern England and Scotland suggests that *N. cordata* is highly sensitive to burning of heather on grouse moors, with surviving populations confined to ‘islands’ of heather protected from repeated burning as a result of topography, position of flushes, mires and watercourses, etc. (Glaves et al., 2013).

6 | STRUCTURE AND PHYSIOLOGY
6.1 | Morphology
*Neottia cordata* has an aerial rosette usually with two, rarely three or four, opposite leaves positioned at the top of the vegetative shoot or in the middle part of a generative shoot (see Figure 4d). The plant has a short, thin perennial rhizome with 1 or 2 long, cord-like and densely haired adventitious roots growing from node of each annual growth increment (see Figure 4d; Ziegenspeck, 1936; Tatarenko, 2015). Horizontal and vertical rhizomes show sympodial growth, unlike seedlings which have monopodial growth. The roots spread horizontally immediately under the moss cushions or carpets, amongst their rhizoids, to contact the soil. Roots also penetrate peat layers to a depth of 2-5 cm.

6.2 | Mycorrhiza
Mycorrhizal associations between *Neottia cordata* and fungi have been reported for both seedlings and adult plants. In adults, hyphal coils (pelotons) have been found in roots (Fig. 4a), while the rhizomes are free of mycorrhizal colonization (Rasmussen, 1995). The roots become infected when they reach 5-10 cm in length. The roots have an epidermis with root hairs and the fungus is distributed unevenly in the root and colonisation intensity is low, i.e. 7-
45% of cells of roots sections were occupied by fungi (Nieuwdorp, 1972; Selivanov, 1981; Tatarenko, 1995). Molecular identification of the fungi associated with adults in meadow and woodland habitats across Europe revealed Sebacinales Clade B (Serendipitaceae) as the dominant mycorrhizal symbionts, and their ability to form pelotons was confirmed by electron microscopy (Figure 5b, Těšitelová et al., 2015; Yagame et al., 2016). Other symbionts that have been found less frequently include rhizoctonias from Ceratobasidiaceae and Tulasnellaceae, ectomycorrhizal fungi from Russulaceae, Atheliaceae (Tylospora), Thelephoraceae, and numerous presumably endophytic ascomycetes and basidiomycetes (Těšitelová et al., 2015; Yagame et al., 2016; Schiebold et al., 2017). These findings partly correspond with an earlier cultivation-based study (Chodat & Lendner, 1896), where a wide range of fungi were isolated, including soil fungi probably unrelated to orchid mycorrhiza, as well as fungi likely to be from the rhizoctonia group (based on the morphology of isolates displayed in the figures in Rasmussen, 1995).

Carbon and nitrogen stable isotope content of *N. cordata* leaves varied between values typical of autotrophic plants and slightly enriched $^{13}$C and $^{15}$N values typical of partially mycoheterotrophic species (Hynson, Bidartondo & Read, 2015; Těšitelová et al., 2015; Schiebold et al., 2017). Similarly, hydrogen stable isotope composition corresponded to partially mycoheterotrophic plants (Schiebold et al., 2017). This indicates that *N. cordata* could be partly mycoheterotrophic at adulthood, and additional carbon could be obtained from fungi that are mainly non-ectomycorrhizal (Těšitelová et al., 2015, Schiebold et al., 2017).

### 6.3 Perennation: reproduction

*Neottia cordata* is a perennial plant with a total ontogeny lasting for only a few years (Smith, 1993). Tatarenko (1996) estimated the total life expectancy (without taking into account vegetative spread) of 8-13 years (Tatarenko, 1996). In contrast, individuals of *N. ovata* have an estimated half-life of c. 70 years (Tamm, 1991).

Vegetative propagation appears to be the primary means of reproduction; long roots that do not penetrate deeply into the substratum can act as runners (Figure 4d). The root-tip meristem can transform directly into a shoot meristem. It sheds its root cap after a shoot meristem with leaf primordium forms beneath it and the apical meristem then develops into a shoot (Rasmussen, 1986). In the first year, the bud develops into three internodes with scales. The first mycorrhizal root grows out of the first node. The bud hidden under scales on a third internode develops into two small leaflets in the following year. Under suitable conditions, the new plant can flower in the third year of development (Ziegenspeck, 1936). According to
Vinogradova (1996), the root-suckers originate only on the roots formed in the previous year, more often in injured maternal plants, but they may also originate on the roots of the suckers of previous years. The root-connection with the mother plant disappears at that time and the new plant is indistinguishable from individuals originating from seeds (Ziegenspeck, 1936).

Nieuwdorp (1972) and Ziegenspeck (1936) reported the occurrence of rhizomes with multiple stems. Similarly, Hoy (2002) reported from herbarium specimens a plant with three stems (two in bloom) on one rhizome. Branching of the rhizome, leading to appearance of several stems on one plant, was observed in one out of 35 adult plants surveyed by Vinogradova (1996) in Murmansk Oblast, Russia. In some populations, e.g., on Kunashir, Kuril Islands (Tatarenko, 1996), plants did not form root buds, and vegetative propagation was absent. In Arkhangelsk Oblast, North of European Russia, we observed populations of both kinds: with and without the formation of root buds (I. Tatarenko, unpublished data). Vegetative reproduction is more frequent in young plants, occurring even in protocorms, and allows the establishment of numerous individuals within a small area (Vakhrameeva et al., 2008; Figure 4c).

In any one year, non-flowering individuals usually outnumber flowering individuals in most populations. For example, Rollerová (2015) followed one population in the National Nature Reserve of Praděd in the Jeseníky mountains of the Czech Republic over 3 years. In 2010, the population consisted of 1641 individuals of which 53% were non-flowering; in 2011, there were 1513 individuals with 77% of non-flowering plants; and in 2013 1714 individuals with 84% of non-flowering plants. Šibík et al. (2006) also reported a prevalence of non-flowering plants in populations (sometimes there were only non-flowering plants).

6.4 | Chromosomes

The chromosome number determined for *Neottia cordata* varies from $2n = 36$ to $2n = 44$; $2n = 36$ (Love & Love, 1966; Simon, 1968), $2n = 38$ (Sokolovskaja & Strelkova, 1940; Sokolovskaya, 1960; Skalinska, Sokolowska-Kulczynska & Alii, 1961; Taylor & Mulligan, 1968; Murin, 1978; Yokota, 1987; Semerenko, 1989; D’Emerico, Pignone & Scrugli, 2000), $2n = 40$ (Gadella & Kliophuis, 1963; Kliophuis, 1963; Knaben & Engelskjön, 1967; Ivanova, 1987; Ruiz, 1995), $2n = 42$ (Harding in Tischler, 1931; Blackburn in Maude, 1939; Laane, 1969; Arohonka, 1982), $2n = 38, 40$ (Engelskjón, 1979), $2n = 39-42, 44$ (Love & Love, 1981; Averyanov, Averyanova & Lavrenko, 1982), $2n = 40, 42$ (Dmitrieva, 2000). As in its close relative *N. ovata* (Vosa, 1983), these differences can be caused by variation in B-chromosomes. The chromosome number without B-chromosomes was counted as $2n = 34$. 

14
(Májovský, 1978; Averyanov, 1979; Averyanov et al., 1982); however, authors have determined different number of B-chromosomes: 0-4 (Averyanov, 1979), 4 (Májovský, 1978) or 6 (Averyanov et al., 1982).

6.5 | Physiological data
The species is photosynthetic in adulthood, but it likely obtain additional carbon from its mycorrhizal fungi and thus combine autotrophic and mycoheterotrophic nutrition (see 6.2).

6.6 | Biochemical data
No data available.

7 | PHENOLOGY
Leafing generally starts in late April and early May. The leaves are fully developed throughout the time of flowering and fruit production. The time of flowering depends on a combination of factors, most notably latitude and altitude. The species flowers from beginning of May to August and the first capsules start to release seeds while the upper flowers are still apparently functional (Stoutamire, 1964). In California, it was observed flowering in mid-April (I. Tatarenko, unpublished data). Flowers remain fresh looking, apparently still functional, while their ovaries ripen (Stoutamire, 1964). The capsules mature fully within three weeks after pollination and seeds are rapidly shaken out of the capsules by wind (M. Kotilíněk & J. Jersáková, unpublished data). The leaves of both flowering and non-flowering plants die back after the period of seed shedding. The bud of a new shoot for the next season is usually already present during the flowering period (Figure 4d) and grows 1–1.5 cm tall (Reddoch & Reddoch, 1997). This renewal bud contains a fully formed inflorescence with flower buds, leaves, and the apices of two buds one of which will become renewal bud in next season. From shoot apex initiation, it takes at least two calendar years to complete the shoot development (I. Tatarenko, unpublished data). Vinogradova (1996) found that new shoot development starts when the main part of the parent plant dies.

8 | FLORAL AND SEED CHARACTERS
8.1 | Floral biology
Stems bear a variable number of flowers, usually more than 3 and up to 20. Melendez-Ackerman and Ackerman (2001) reported an average of 4.2-4.4 flowers per inflorescence in the Rocky Mountains (USA). The flowers within the inflorescence open acropetally and age
sequentially (Darwin, 1862). Individual flowers persist for 10 to 25 days (Ackerman & Mesler, 1979). The sepals and petals form a loose hood and the lip is bent like a knee to the base. Nectar can be found at the base of the lip and also in the shallow groove running down the lip (Claessens & Kleynen, 2011; Figure 4c).

Insect visitors usually spend a long time on an inflorescence crawling from flower to flower, thereby increasing the likelihood of self-pollination were it not for the sophisticated mechanisms promoting cross-pollination (Darwin, 1862; Ackerman & Mesler, 1979). The nectar in the groove of the lip stimulates the insect to start feeding. This nectar trail ensures that the insect head comes into contact with the protruding rostellum, which is touch-sensitive in the genus Neottia (Claessens & Kleynen, 2011). The tongue-shaped rostellum has an apex with three sensitive outgrowths that function as a lever to set off an explosive mechanism (Figure 5a). The upper layer of cells is filled with viscid fluid, which is expelled when the sensitive cells are touched, and the pollinia become glued to the pollinator. After that the rostellum bends towards the stigma, thereby ensuring that freshly removed pollinia cannot be deposited within the same flower. After a time, which varies from one to two days, the rostellum lifts until it stands perpendicular to the ovary axis, thus enabling access to the stigma (Ackerman & Mesler, 1979). The flower is protandrous because of this mechanism. The pollinium is not deposited on the stigma as a whole, but fragments, thereby allowing several flowers to be pollinated by a single pollinium.

In North America, N. cordata is mainly pollinated by fungus gnats (Mycetophilidae and Sciaridae) and occasionally (<0.1% visits out of the total number of visits to the flowers) crane flies (Tipulidae) and parasitoid wasps (Braconidae and Ichneumonidae) (Ackerman & Mesler, 1979). In Europe, N. cordata is pollinated by fungus gnats and occasionally by beetles (Summerhayes, 1951, Claessens & Kleynen, 2011). Mesler, Ackerman and Lu (1980) tested the relative frequency of geitonogamous and xenogamous pollen flow by comparing fruit set of emasculated plants with intact ones. The results showed that interplant movement of pollen is common and fungus gnats can be effective pollen vectors.

Many species of Neottia have foetid-smelling nectar (Brackley, 1985), including N. cordata. Often foul odours indicate sapromyophily (pollination by flies that oviposit on faeces, decaying fungi, or dead animals), and the plants provide no food (oviposition mimicry). Neottia cordata does not seem to fit this pattern: during observation in California (Ackerman & Mesler, 1979), pollinators never appeared to be trying to oviposit or to do anything but eat the nectar the flowers produce. Both male and female insects visited flowers, and no eggs or larvae were found on the flowers (Hoy, 2002).
8.2 | Hybrids
No inter- or intrageneric hybrids are known.

8.3 | Seed production and dispersal
*Neottia cordata* produces microscopic seeds 597 µm long (SE ±13.45, \(n = 38\)) and 165 µm in diameter (SE ±4.96, \(n = 38\)) (M. Kotilínek, unpublished data). The number of seeds per capsule has been estimated at 376 (Stoutamire, 1964). Natural fruiting rates (the percent of flowers developing into capsules) are generally high, at between 60 and 80% (Ackerman & Mesler, 1979; Mesler et al., 1980; Vakhrameeva, 1988, 1991). Claessens and Kleynen (2011) reported 24.2 and 90.8% for two German populations; Melendez-Ackerman and Ackerman (2001) found a fruiting rate 20.7% in the Rocky Mountains. A hand-pollination experiment showed that both self- and cross-pollinated flowers set capsules (Meléndez-Ackerman & Ackerman, 2001). Cross-pollinations produced significantly higher numbers of seeds per capsule than self-pollinations (181.2 ±26.64 (SE), range 13-348, v. 90.5 ±17.6, range 9-312, respectively; \(n = 14\) capsules for both treatments) and higher percentages of fertilized ovules (93.5% ±2.9, range 61.2-99.4 v. 86% ±2.7, range 63.8-97.8, respectively). Kotilínek (2012) reported 89.6% ±2.8 (range 78.8-98.2), of seeds with a developed embryo from 5 natural populations in the Czech Republic. In addition, an experiment with natural plant densities in established plots showed that density of *N. cordata* had no effect on the fruit set and seed set of plants except for reduced seed set in extremely isolated plants (nearest neighbour distance 3 m; Meléndez-Ackerman & Ackerman, 2001). The seeds are dispersed from the capsules by wind three weeks after pollination. The distances over which *N. cordata* seeds are dispersed have not yet been studied.

8.4 | Viability of seeds: germination
In general, *Neottia cordata* shows very low germination success. Asymbiotic germination experiments have been unsuccessful (Downie, 1941) or showed poor germination (Stoutamire, 1964). Asymbiotically germinated *N. cordata* were still tiny protocorms at 15 months, with no chlorophyll and approximately 1 mm long (Stoutamire, 1964). Similarly, symbiotic germination of seeds in field conditions has been rather unsuccessful (< 0.2% of seeds had an imbibed embryo with a ruptured testa, and developed rhizoids) and no protocorm formation was observed (Těšitelová et al., 2015). In addition, *N. cordata* seeds showed very high mortality: more than 98% of seeds were dead after one year of incubation in soil and
none was found alive after three years (Kotilínek, 2012; Těšitelová et al., 2015). Seeds deposited in the Millennium Seed Bank showed c. 50% seed viability according to staining with fluorescein diacetate solution (K.J. Walker, personal communication).

8.5 | Seedling morphology

Seeds germinate in humid substrata after contact with a suitable mycorrhizal fungus. The mycorrhizal seedling (protocorm) has an elongated, slightly curved, whitish, spindle-shaped body, c. 2 mm long and 0.8 mm wide, with a bud and a root apex that are close to each other (for details see Vinogradova, 1996, Figure 5d). The protocorm is covered with numerous long hairs (more than 0.3 cm) that may harbour fungal hyphae. The meristematic swollen zone represents the growing point (apex) of the primary shoot. The first cap-like leaf of the seedling consists of 3-4 cell layers containing starch. The roots start elongating before the shoot, reaching 3-4 cm in length while the shoot is 0.5 cm long. The first green leaf appears after 3-4 years of underground development (Vinogradova, 1996).

9 | HERBIVORY AND DISEASE

9.1 | Animal feeders or parasites

There are no known herbivores specific to *Neottia cordata*. However, two insect herbivores, the flies *Delina nigrita* Fallén (Scatophagidae, Diptera) and *Chyliza vittata* Meigen (Psilidae, Diptera), are reported for the genus *Neottia* (Ellis 2017). Only *C. vittata* has been recorded on *Neottia* species in British Isles (Robbins, 1991; Pitkin et al., 2017). Larvae of *D. nigrita* mine into the rootstock, stem and leaves (Hering, 1957; Ball, 2007). Similarly, larvae of *C. vittata* mine the leaf (blade and sheath) and stem, and can cause the inflorescence of infested plants to wilt (Hering, 1957). Both species pupate in the underground parts of the plant (Hering, 1957).

In Continental Europe, populations are sometimes damaged by wild boars (*Sus scrofa* L.) (AHO Sachsen-Anhalt, 2011). Grazing by deer can damage *N. cordata* populations, as experimental exclosure of red deer (*Cervus elaphus* L.) from sampling plots in *Pinus sylvestris* forest in Western Norway increased *N. cordata* abundance (Hegland & Rydgren, 2016).

9.2 | Plant parasites and diseases

No data available.
Neottia cordata was known to pre-Linnean authors. It was described as Ophrys minima by Bauhin (1620) and described and illustrated as Bifolium minimum by Bauhin & Cherler (1651). Linnaeus, who knew it from the woods of Lappland (Linnaeus 1737), gave it the binomial Ophrys cordata in Species plantarum (1753). Subsequent authors moved the species into various genera e.g. Epipactis (Crantz, 1769), Helleborine (Schmidt, 1793), Malaxis (Bernhardi, 1800), Listera (Brown in Aiton, 1813), Serapias (Steudel, 1821), Distomaea (Spencer, 1825), Neottia (Bluff & Fingerhut, 1838), Pollinirhiza (Dulac, 1867), Dilphryllum (Kuntze, 1891) and Bifolium (Nieuwland, 1913). Until very recently, the name Listera cordata was used. The genus Listera was described in 1813 by British botanist Robert Brown (1773-1858) and was named in memory of Martin Lister (1638-1712), a pre-Linnean naturalist, a Fellow of the Royal Society and one of Queen Anne’s Physicians. Szlachetko (1995) included the genus Listera in Neottia, but his classification was ignored. Recent studies suggested merging the genera on the basis of nuclear ribosomal DNA (ITS) (Chase et al., 2003; Pridgeon et al., 2005). Their genetic similarity is supported by identical column morphology and by having powdery pollen grains united into tetrads. The touch-sensitive rostellum is also a unique shared feature. Neottia has taxonomic priority.

The specific epithet cordata refers to the heart-shaped (cordate) base of the leaves, while the common generic name Twayblade (similarly Tvåblad in Scandinavian and Zweiblatt in German) reflects the two-leaved character of these plants and was established by Henry Lyte in A Niewe herbal, or Historie of Plantes (Dodoens & Lyte, 1578) and presumably represents a translation of the herbalist’s name Bifolium. Similarly, other colloquial names refer to the shape or size of leaves – Dédhuilleog bheag in Irish, Kleines Zweiblatt in German, Listère en coeur in French and Listera minore in Italian.

The first British record was recorded as Bifolium minimum in Christopher Merrett’s Pinax Rerum naturalium Britannicarum, published in 1666. The place was located ‘near the Beacon on Pendle Hill in Lancashire’. The habitat here is a typical one for the plant - heather moorland over peat on acidic gritstone – the plant, however, has not been found there in recent years (Foley & Clarke, 2005).

Neottia cordata is classified as of Least Concern in Great Britain (Cheffings & Farrell, 2005), England (Stroh et al., 2014), Wales (Dines, 2008) and Ireland (Wyse-Jackson et al., 2016) although these assessments ignore significant ‘historic declines’ that took place prior to
1930 (see below; Stroh et al., 2014). Across its distribution range, the species is listed as Critically Endangered in the Czech Republic (Grulich, 2012), Endangered in Slovak Republic (Feráková, Maglocký & Marhold, 2001), regionally Endangered in Austria (Niklfeld, 1999; Hohla et al., 2009), Vulnerable in Bulgaria (Petrova & Vladimirov, 2009), Greece (Phitos, Constantinidis & Kamari, 2009), Poland (Kaźmierczak et al., 2016), Slovenia (Skoberne, 2007), Estonia (Nature Conservation Committee of Estonian Academy of Sciences 2008), Germany (Korneck, Schnittler & Vollmer, 1996), Lithuania (Rašomavičius, 2007), Ukraine (Didukh, 2009), Near Threatened in Switzerland (BAFU, 2016), Netherlands (SoortenBank 2017) and Slovak Republic (Eliáš et al., 2014), of Least Concern in France (UICN France, MNHN, FCBN & SFO 2009), and Sweden (Aronsson et al., 2010). The species is also included in the Red Data Books of Byelorussia and 33 regions of Russia (Vakhrameeva et al., 2008; Vakhrameeva, Varlygina & Tatarenko, 2014).

In the British Isles, Neottia cordata has disappeared from many sites in the lowlands and in the uplands on the periphery of its range in northern England, where the drainage and reclamation of bogs and heaths caused many losses in the 19th century (Harrap & Harrap, 2005). The species is now thought to be extinct in Angelsey, Cheshire, Dorset, Hampshire, Southeast and Southwest Yorkshire, Shropshire and Sussex (based on BSBI records and county floras). Kull & Hutchings (2006) calculated a 45% decline in the number of occupied hectares of the United Kingdom between 1930-1969 and 1987-1999, and a similar decline (42%) for Estonia. However, other authors point out that botanical recording data used to assess changes in the frequencies of plant species over time may overestimate the number of lost sites, as they are subject to marked variations in recording activity. Rich & Karran (2006) used N. cordata as an example of how simply looking at a distribution map separating pre- and post-1987 records may indicate nothing about the real change. When they plotted records of N. cordata on a conventional map that separates pre- and post-1987 records, there were 27 historic (i.e. lost) and 48 recent records, indicating a 36% ‘decline’ out of total 75 records. To test whether the failure to re-record in some of the squares during 1987–88 in which it had been recorded previously was likely to be a result of sampling or real change, the authors asked botanists to search for N. cordata in eight squares; it was re-found immediately in six squares, and where it could not be re-found it was commented that it was still likely to be present. Braithwaite, Ellis & Preston (2006) compared surveys of occurrences in tetrads (2 × 2 km grid squares) of the British National grid during periods 1987-1988 and 2003-2004. From the numbers of newly gained, lost and re-found records, they calculated a relative change in proportion to recorded range adjusted for over-recording. For N. cordata they found 38 gains,
11 losses and 17 re-finds and showed a positive relative change in its distribution (+0.41, note that relative change +0.5 would mean that half of the records in the second survey are new; 90% confidence interval was ± 0.24). Fay (2015) attributes this to the inconspicuousness of the species which has probably led to it being under-recorded in some areas, resulting in a high turnover in the number of sites when different time periods are compared. Unlike many orchids, the species is inconspicuous enough not to suffer from over-collection. Big fluctuations in population dynamics make it hard to assess population trends (AHO Sachsen-Anhalt, 2011).

The major threats identified are forest harvesting (Dumont, Hágsater & Pridgeon, 1996; Hoy, 2002), changes in hydrology (Vakhrameeva et al., 2008), air pollution, burning of moorlands and impact of global climate changes (Hoy, 2002). Harvesting the canopy of wet forests affects Neottia cordata habitat in several ways. Increased light availability stimulates herbaceous layer to outcompete Neottia cordata, dries out the habitat and makes it too warm, and the decaying roots of trees increase concentrations of nutrients. Driving heavy logging machines through a soft moist area or swamp seriously alters water flow and drainage by creating microdams and channels and compacting the substrate (Hoy, 2002).

Drainage of peatbogs and mires and channelling of riverbeds both cause serious changes in hydrological regime (Kull & Hutchings, 2006; AHO Sachsen-Anhalt, 2011). Consequently, it accelerates decomposition of soil organic matter, which leads to changes in plant species composition by encouraging plant species with higher nutrient demands such as shrubs and trees. The sites are then typically invaded by trees (such as pine, birch, larch, and spruce) and common reed (Phragmites australis). Sphagnum cushions disappear, while ericoid bushes increase considerably (Landry & Rochefort, 2012). Neottia cordata likely suffers in drained areas from loss of humidity, high temperatures, and increased competition. New roads can obstruct water flow and road salt and artificial impoundments can affect the habitat. Any major activity up-slope from a seep or swamp is likely to affect water quality and quantity. Groundwater that feeds the swamps can also be altered, primarily by overuse by humans (Hoy, 2002). The species does not survive peat cutting (Cooper, Mccann & Hamill, 2001).

Besides drainage and disturbance of wet forests, excessive nitrogen deposition from the air causes eutrophication of the originally nutrient poor habitats (the species has its optimum around 20 kg N ha⁻¹ year⁻¹, Henrys et al., 2011). Some 19th century losses in northern England are likely to have been associated with industrial pollution (Lightowler, 1988).

Neottia cordata is sensitive to repeated burning of moorland for driven grouse shooting (Elliot, 1953) and the recovery of populations following its cessation (Lee et al., 2013).
Global climate change is likely to affect precipitation, temperature, and storm severity and frequency. Climate change may affect *N. cordata* through the arrival of new competitors, warming of groundwater and thus microclimate, and changes in number and type of pollinators and herbivores (Hoy, 2002). The species may respond to climate change by shifting its northern (as well as southern) range margins, as has been already noted on Greenland (Daniëls & de Molenaar, 2011). Cultivation in the Botanical Garden of St. Petersburg (Russia) has highlighted potential difficulties in reproduction (Vakhrameeva et al., 2008).

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Supporting Information

Additional supporting information may be found in the online version of this article:

FIGURE S1 The altitudinal distribution of *Neottia cordata* in the British Isles.

TABLE S1 List of publications that indicate the geographical distribution of *Neottia cordata*. 
Table 1. Total number of hectads in which *Neottia cordata* has been recorded in the British Isles (derived from BSBI Distribution Database on 17<sup>th</sup> August 2017; kindly supplied by K.J. Walker of the Botanical Society of Britain and Ireland)

<table>
<thead>
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<th>Country</th>
<th>Present</th>
<th>Total</th>
<th>%</th>
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<td>741</td>
<td>1119</td>
<td>66</td>
</tr>
<tr>
<td>England</td>
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<td>1499</td>
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<tr>
<td>total</td>
<td>1088</td>
<td>1816</td>
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</tr>
</tbody>
</table>
FIGURE 1 The distribution of *Neottia cordata* in the British Isles. Each dot represents at least one record in a 10-km square of the National Grid: (●) 1970 onwards; (○) before 1970; Mapped by Colin Harrower, using Dr. A. Morton’s DMAP software, Biological Records Centre, Centre for Ecology & Hydrology, Wallingford, mainly from data collected by members of the Botanical Society of Britain and Ireland.
FIGURE 3 The climate space graphs for *Neottia cordata* based on Hill et al. (2004). Climatic values for plants were calculated as the mean climate of the 10-km squares where they occur in Britain, Ireland and the Channel Islands, averaging over the squares enumerated for the counts. Climate data for 10-km squares were taken from the baseline climate summaries of the UK Climate Impacts Programme. The baseline summaries were constructed by interpolation of daily weather measurements from individual met stations averaged over the 30-year period 1961-1990. Graphs provided by K.J. Walker from Botanical Society of Britain.
and Ireland.
FIGURE 4 (a) Typical driven-grouse moor habitat of *Neottia cordata* in Midwest Yorkshire within blanket mire. *Neottia* occurs in the ‘island of heather’ protected from burning by the
surrounding mire (with *Eriophorum vaginatum*) (photo K.J. Walker); (b) waterlogged spruce forest with small clump of *N. cordata* in foreground (photo I. Bufková); (c) flowering plants of *N. cordata* (photo I. Bufková); (d) Adult plant (A) of *N. cordata* with short rhizome (rh) still connected via root (ro) with a daughter plant (D). Both plants have a visible renewal bud (b) (photo J. Jersáková)
FIGURE 5 (a) Cross-section of an adventitious root showing brownish coils of fungal hyphae (pelotons) visible within the cells (photo M. Kotilínek); (b) Transmission electron images of hyphae with the typical structure of a Sebacinales dolipore (D) with parenthesomes (P), surrounded by plant plasma membranes (PM) (photo J. Jersáková); (c) Detail of a *Neottia cordata* flower, showing nectar at base of the lip (n), yellow pollinia resting on the rostellum (p), and stigma positioned just below the rostellum (s) (photo J. Claessens); (d) Seedling development from stage of pear-shaped protocorm (1), seedlings with developing shoot and roots (2) to a 1-year old seedling (3) (adapted from Vinogradova 1996).
Neottia cordata is an inconspicuous orchid of moist, acidic, humic soils. In the British Isles, it occurs mainly in sites rich with mosses, such as moors and bogs, or damp woodlands. It has a circumpolar distribution including North America, Europe and Asia. It reproduces by seeds and vegetatively by root suckers. The major pollinators are fungus gnats. Neottia cordata is threatened mainly by drainage and destruction of wet habitats and it is listed as Vulnerable or Near Threatened in most European Red lists.
Table S1 List of publications that indicate the geographical distribution of Neottia cordata.

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<thead>
<tr>
<th>Country</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Ireland</td>
<td>Curtis, T.G.F &amp; Thompson, R. (2009) <em>The Orchids of Ireland</em>. Northern</td>
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<td>Country</td>
<td>Sources</td>
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<td>Ireland</td>
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<td>Japan</td>
<td><a href="http://herbaria.plants.ox.ac.uk/bol/florajapan">http://herbaria.plants.ox.ac.uk/bol/florajapan</a> (Accessed 1.3.2017)</td>
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The species is absent in:

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<th>Country</th>
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FIGURE S1 Altitudinal distribution of *Neottia cordata* in the British Isles derived from the records held by the BSBI. The graph is based on occurrences of *N. cordata* in tetrads (2x2 km squares) in Britain and Ireland (including Isle of Man and Channel Islands). Graph provided by K.J. Walker, Botanical Society of Britain and Ireland.