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Variation in the *Ononis repens* group (Fabaceae): implications for species determination and conservation

Chris A. Skilbeck[1†]; Michael Wilcox[2*]

[1]Department of Life Sciences, Natural History Museum, London; [2]Bradford, UK

***Corresponding author:** michaelpw22@hotmail.com

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Abstract

Whilst going through the *Ononis* L. specimens in the British & Irish Herbarium at the Natural History Museum, London (**BM**), problems were encountered with distinguishing some specimens of *O. repens* L. from *O. spinosa* L. due to conflicting morphological characteristics. This may come as no surprise to those familiar with identifying *Ononis*, but it did prompt an interesting investigation into the herbarium specimens at **BM**, as well as a survey of relevant literature and field observations. What emerged is an interesting story in which both genetic and environmental factors appear to influence *Ononis* morphology in ways that could have implications for recording accuracy and conservation. To further explore this, the ploidy levels of putative hybrids collected in the field were assessed using flow cytometry. As expected, *O. spinosa* was confirmed to be diploid and *O. repens* tetraploid, suggesting that any F_1 hybrids should be triploid. However, all morphologically intermediate specimens analysed were diploid. It is therefore hypothesised that these diploid intermediates are a form of *O. spinosa* displaying some morphological features of *O. repens* due to phenotypic adaptation or introgression. The ecological and taxonomic implications of these findings are discussed.

Keywords: phenotypic plasticity; hybrids; ploidy; Restharrows

Introduction

The Restharrows (*Ononis* L., Fabaceae) are so-called because their tough roots were prone to stop the plough, or harrow, in its tracks. *Ononis* is a large and taxonomically complex genus comprising over 80 species of herbs and shrubs. However, in the British Isles, it may appear relatively straightforward at first glance, with only four widely recognised native taxa: *O. repens* L. (Common Restharrow), *O. spinosa* L. (Spiny Restharrow), their hybrid *O.* × *pseudohircina* Schur, and *O. reclinata* L. (Small Restharrow). Yet appearances can be deceptive.

Ononis is phenotypically plastic, with morphology dependent on growth conditions and habitat, which can potentially complicate determinations. This makes *Ononis* an intriguing case study in morphological variation and a compelling example

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[†] This paper is dedicated to the memory of Chris A. Skilbeck (1970–2025), who passed away unexpectedly after its acceptance. An active member of the BSBI and a regular contributor to *British & Irish Botany*, he will be sadly missed by all who knew him.

of the 'nature versus nurture' debate. The possible existence of ecotypes also carries significant implications for conservation. To set the stage for further discussion, the following sections summarise each taxon.

Ononis repens

Ononis repens is a rhizomatous/stoloniferous perennial, usually procumbent and trailing and found on grasslands, pastures, coastal cliffs and dunes. It favours chalky, sandy or gravelly well-drained soils. Its stems are usually green, sometimes tinged light red especially in late summer. The stems are procumbent or decumbent to ascending, often rooting and are more-or-less hairy all around their circumference with hairs *not* in two distinct rows. These hairs consist of both long eglandular trichomes and shorter glandular trichomes. The terminal leaflet has a length/width ratio of 1.6 to 2.4 (Stephens, 1978), or less than 2.5 as a rule-of-thumb. The leaflets are distinctly ovate with an obtuse and notched (emarginate) apex and distinctly wider than those of *O. spinosa*. The leaves are also glandular, often extremely so, with short glandular trichomes and are viscid or oily to the touch. *Ononis repens* has a distinctive odour that has been variously described as 'harsh' and 'sweat like' (Rose, 2006), like 'petroleum jelly' (Mabey, 1996) or 'goaty', particularly when the leaves are crushed or trodden on. The pods are shorter than the calyx, the latter enlarging in fruit, (Morton, 1956; Syme, 1866). It is usually spineless. (See descriptions by Stace, 2019; BSBI Plant Crib for *Ononis* by Wigginton & Graham, 1981). Very distinctly, the calyx is densely covered in hairs. Chromosome counts suggest that *O. repens* is tetraploid with 2n = 60, giving a base chromosome number of *x* = 15 (Morisset, 1978; Table 1).

Table 1. Ploidy levels of *Ononis* taxa native to the British Isles. The ploidy level of *O. repens* subsp. *maritima* was inferred by Kloda *et al.* (2008) using allele frequencies. Those determined by Morisset (1978) and Montgomery *et al.* (1997) are from chromosome counts.

Taxon	Ploidy level	Authority
O. spinosa	2n = 30, diploid	Morisset, 1978
O. repens	2n = 60, tetraploid	Morisset, 1978
O. repens subsp. *maritima*	2n = 60, tetraploid	Kloda *et al.*, 2008
O. reclinata	2n = 60, tetraploid	Montgomery *et al.*, 1997
O. x pseudohircina	2n = c.48, triploid	Morisset, 1964

Maritime and Spiny Forms of Ononis repens

Stace (2019) also recognizes a maritime and spiny form of *O. repens*, known as subsp. *maritima* (Dumort.) Asch. & Graebn. This taxon has been treated as a separate species by some authors. For example, Kloda *et al.* (2008), who referred to it as *O. maritima* (after *O. maritima* Dumort. = *O. spinosa* subsp. *maritima* (Dumort.) P. Fourn). Despite its spininess, it is tetraploid like other *O. repens* and not diploid as in *O. spinosa*, as inferred from allele frequencies (Kloda *et al.*, 2008, Table 1). Morisset (1978) concludes that 2n = 60 is the most likely chromosome number for British plants, as assumed by Kloda *et al.* (2008).

In the **BM** collection *O. repens* subsp. *maritima* is often a small and compact plant. The flowers are typically shorter than the calyx segments. Stace (2019) gives the flower length as 7-12 mm (in Devon populations) and this was used as an additional diagnostic criterion in the **BM**. The leaflets are also generally shorter and a useful diagnostic measure is the leaf index (leaflet length/leaflet width). Indeed, Stephens (1978) stated that 'most coastal colonies [of *O. repens*] have lower leaf indices, and therefore broader terminal leaflets, than the inland colonies'. The coastal plants in Fig. 1, between Deal and Sandwich in Kent (vc15) had a leaf index of 1.51 (1.38–1.67) compared to the more typical value of 1.6 to 2.4 for inland plants of typical *O. repens*.

In general, apart from the small flowers and spines, *O. repens* subsp. *maritima* has an exaggerated *O. repens* phenotype: more prostrate, smaller leaf length / width ratio (leaf index) and is more glandular. Indeed, Širjaev (1932) cites Henslow (1895) who stated that the excessively spiny form grows on maritime sands but that: 'It is much less spiny in waste places by roadsides etc. and becomes the variety *inermis* [typical *O. repens*] elsewhere'. Thus, there would appear to be a whole spectrum of types, depending on habitat, either due to genetic gradients or phenotypic adaptation to growth conditions. In other words, we may be looking at genetic varieties or ecotypes (phenotypic adaptations to environment driven by genetic differences).

Bennett (1890) studied a population in Yarmouth Denes and grew them from seed (in a mixture of garden soil and Redhill sand) and found that they did not produce spines until their third year when they flowered. Flowering stems produced spines, though it was not verified if this is the case after the third year. Some of these specimens are in the **BM** collection. Beeby sent some of these Yarmouth specimens to Prof. Lange in Denmark, who published a monograph on *Ononis* (Lange, 1873) and Lange replied, 'It is exactly what I do mean by var. *horrida*'. Linton (1895) also noted that this taxon is 'abundant near the sand dunes by Moechras' and that only the older plants produce spines.

For the current study, a search was carried out for *O. repens* subsp. *maritima* on the East Kent coast (vc15) including Deal and Sandwich, where it had been collected more than a century before. The closest form found was a population of plants intermediate between subsp. *maritima* and the non-*maritima* form, growing at the top of shingle beaches where the shingle was partially stabilised. These were situated at intervals along almost the entire coastline between Deal and Sandwich. Typical coastal specimens of *O. repens* are shown in Fig. 1.

Nomenclatural Note

Ononis repens subsp. *maritima* was referred to by Lange (1873) as *O. repens* var. *horrida* and the (usually) spineless more familiar form of *O. repens* as *O. repens* var. *inermis*. If we are to use subspecies designations rather than varieties, then this leaves the question as to the correct subspecies name for the *inermis* variety. *Ononis repens* subsp. *arvensis* (L.) Greuter is considered an accepted name by IPNI, but rather than get entangled in nomenclature we will refer to it simply as *O. repens*, meaning in the narrow and more familiar non-*maritima* sense.

Ononis spinosa

Ononis spinosa is not rhizomatous and its stems do not root above the base. It prefers moister soils, often over base-rich clays. It is found on grassy commons, barren pastures, rough ground and roadsides (Syme, 1866; Stace, 2019). The terminal leaflet has a length/width ratio of 2.3 to 3.0 (Stephens, 1979) or over 2.5

as a rule-of-thumb. The leaflets are narrower than in *O. repens* and generally have acute tips. There is often a single dominant row of long eglandular hairs which frequently alternates from one side of the stem to another from one internode to the next, giving the stem the appearance of bearing two rows of these hairs.

The description of the indumentum requires clarification. The older, more basal, internodes usually lose most of their hairs. It proved most useful to look at the 2nd to 4th internodes from the shoot apex. In addition to the main rows of hairs described, there are often some long eglandular hairs scattered on other parts of the stem, between the rows. Shorter glandular hairs may be present on any part of the stem, especially on the first few internodes from the apex. The mature pods are longer than, or the same length as, the calyx (Morton, 1956; Syme, 1866). The calyx has distinctly fewer eglandular hairs than that of *O. repens* and these may be lacking on the calyx altogether or a few may be present on its base. The calyx does generally have some shorter glandular trichomes. The leaflets have very few glandular hairs. Thus, *O. repens* is more glandular than *O. spinosa*, especially on the leaves. Typical coastal specimens of *O. spinosa* are illustrated in Fig. 2.

Figure 1. *Ononis repens* growing in a sub-maritime habitat at the top of a shingle beach beside a trackway between Deal and Sandwich (Kent, vc15). This form had flowers (A) measuring 12-15 mm in length but also displays some characteristics of subsp. *maritima*, in particular the mean leaf index (of 5 leaves from each of 5 plants) sampled from the population was only 1.51 (range: 1.38 – 1.67) and there was a very high density of glandular hairs on the leaflets (A and B). The plants were prostrate (C, D) and strongly aromatic. Note the reddish-brown colouration on some stems (B) others being green (compare to Fig. 2c). These

plants therefore had a 'sub-maritime' phenotype. Both spiny and non-spiny specimens of *O. repens* subsp. *maritima* from this location exist in the BM collection.

Figure 2. *Ononis spinosa* from the base of Tankerton Slopes near Whitstable (vc15). This is a coastal site but one with well-formed, and well-drained, soil. The flowers (A and B) were the typical size for *O. spinosa*, around 17 mm in length. Mean leaf indices of two plants were 2.5 and 3.0. Note that the leaflets are

long/narrow and acute and less hairy/glandular than in *O. repens*. Note the more upright habit and the distinctly red stems (C), the pods longer than the calyces (D) and the spines (E). Stem hairiness mostly conformed to typical *O. spinosa*, with occasional intermediacy.

Spineless forms of O. spinosa

Morisset & Boutin (1984) found that *O. spinosa* grown in shade can assume some of the morphological features associated with *O. repens*. When grown in shade it became decumbent rather than erect-ascending and unarmed rather than spiny, developed greenish instead of reddish stems and the leaflets became wide and obtuse rather than narrow and acute. Conversely, *O. repens* can develop a redder stem and some weak spines late in the season (Morisset & Boutin, 1984).

Ononis reclinata

Ononis reclinata is easily identifiable on herbarium sheets by its leaves which are trifoliate or ternate, with 3 smaller leaflets (in addition to a pair of stipules). In contrast the leaves of *O. repens* and *O. spinosa* are only sometimes trifoliate: they are generally trifoliate at the base of the stem and unifoliate towards the apex (Syme, 1866). *Ononis reclinata* is also identifiable by its distinctly smaller flowers (3 - 10 mm long). It is also identifiable by its fruit: pods which have more seeds (with 10 – 20 seeds, compared to 1 – 2 for *O. repens* and 2 (– 4) for *O. spinosa*). Its fruit and their accompanying calyxes hang downwards in a very distinctive manner: the pedicels become longer than the calyx in fruit and hooked such that the calyx droops after flowering (Syme, 1866). The calyx is also more deeply divided into narrow lobes than in either *O. repens* or *O. spinosa*. This drooping of the calyx and the form of the calyx lobes made it quite straightforward to determine this species in herbarium specimens in the British & Irish Herbarium (**BM**).

Ononis reclinata is rare in Britain and restricted to a few local populations (Stroh *et al.*, 2023). It is native on sands, cliffs and dry areas in the whole Mediterranean and has been considered an adventive in Britain, having been found, for example, at Berry Head in Devon (Širjaev, 1932). Others consider it to be native to Britain (Stace, 2019) as the British Isles are at the northern edge of its range (Preston, 2007). It is an annual with fibrous roots and branches near the base of the stem. It is tetraploid (Stace, 2019).

Aims of the current study

The chief problem we encountered in the herbarium was misidentification of *O. repens* and *O. spinosa*, due to the presence of spines in some forms of *O. repens* and the presence of intermediate forms. The authors therefore decided to carry out a careful determination of the herbarium specimens and examine the intermediate forms, including putative *O.* x *pseudohircina*, in the field. Can all intermediate forms be designated as the hybrid *O.* x *pseudohircina*? Is there any evidence for intermediates resulting from phenotypic plasticity without reticulation? Stace (2019) reports that *O.* x *pseudohircina* has a triploid chromosome count of about 48, and hence the ploidy level of morphological intermediates collected in the field will be measured by flow cytometry to assess likely hybridity.

Methods

Determination of Herbarium Specimens

To obtain a broader overview of variation in *Ononis* within the British Isles, a determination of herbarium specimens held at the British & Irish Herbarium in the Natural History Museum, London (**BM**) was carried out. Particular attention was paid to specimens intermediate in form and belonging to less-well recorded taxa, including maritime forms.

Flow Cytometry

To ascertain whether intermediate forms are hybrids, the quantity of nuclear DNA was measured (as an indication of ploidy level) by flow cytometry on samples collected fresh from the field. Specimens determined as intermediates were analysed and also reference samples possessing the characteristics of either parent species. Searches for hybrids between *O. repens* and *O. spinosa*, in the field, relied upon leaflet shape as the principal character when selecting putative hybrids for ploidy analysis.

Nuclear DNA content was estimated by propidium iodide flow cytometry using fresh leaf material. Approximately 1 cm^2 fresh, mature leaf tissue was co-chopped in a petri dish with the internal standards *Solanum lycopersicum* (L. "Stupiké polní rané" 1x = 958.44 Mb (Dolezel et al. 1992)) for *O. spinosa* and *Petroselinum crispum* ((Mill) Nyman ex A.E.Hill 'Champion Moss Curled ', 1x = 2,200 Mb (Obermeyer et al. 2002)) for *O. repens* using a new razor blade in 1 ml of General Purpose Buffer (GPB) supplemented with 3% PVP (Loureiro et al. 2007). A further 1 ml of GPB was added to the sample and the contents gently mixed. The sample was then passed through a 30 µm nylon filter, stained with 100 µl propidium iodide (1 mg/ml) and incubated on ice for 15 minutes. A sample was prepared from an individual and three replicates were run, recording up to 1,000 nuclei per fluorescence peak using a Sysmex CyFlow Space (Sysmex Europe GmbH, Norderstedt, Germany) flow cytometer fitted with a 100 mW green solid state laser. The resulting histograms were analysed with the WindowsTM-based FlowMax software (v. 2.9 2014, Sysmex GmbH) and the average of each sample was used to estimate genome size (Pellicer et al. 2021).

Results

Determination of Herbarium Specimens

With herbarium specimens, leaflet shape, calyx hairiness, stem colour, spininess and finally stem hairiness proved the most useful characters (in that order) for distinguishing *O. repens* from *O. spinosa*. Growth habit (prostrate, procumbent or decumbent versus ascending or upright) is also a good character, but this is rarely self-evident from an herbarium sheet (sometimes it is noted by the collector or suggested by sand grains adhering to the plant). Although stem hairiness is a useful character, it was frequently ambiguous (it may perhaps be easier in life when one can cut a cross-section of the stem) and may vary from internode to internode. This ambiguity may even reflect morphological intermediacy and possible hybridity, as suggested by Morton (1956). Consistent with Morton's study of hybrids along the Durham coast, this investigation found that no single character is sufficient to determine taxon identity. Only when two or more characters showed incongruent combinations was a specimen designated as an 'intermediate' form.

Intermediates in the BM Collection

Although most specimens could be decisively determined as either *O. repens* or *O. spinosa*, a small but significant fraction were *O. repens* subsp. *maritima* and a smaller number again were marked as possible intermediates, either because they had intermediate characters, such as intermediate leaf-shape or a mixture of conflicting characters (as might occur with introgression). Some of these were later determined as close enough to either parent to be classified with them, some as spiny forms of *O. repens*, including *O. repens* subsp. *maritima* and a couple were spineless *O. spinosa*, though this was the rarest category.

Six specimens were already designated as *O. x pseudohircina* by their collectors. These specimens had a mixture of attributes and not all key characteristics were intermediate. Their spininess varied from spiny to weakly spiny to non-spiny.

Ononis repens subsp. maritima in the BM Collection

Ononis repens subsp. *maritima* was often distinguished from *O. repens* var. *horrida* by early collectors. The latter was a name given either to the spinier *O. repens* or sometimes more exclusively to very spiny maritime forms. In most cases it was clearly synonymous with *O. repens* subsp. *maritima*.

However, there are also specimens in the collection that have the distinct *maritima* characters, including small flowers, but which lack spines. Thus, *O. repens* subsp. *maritima* could be split into a spineless and a spiny form, both distinct from *O. repens*. Collectors in the past often designated any spiny form of *O. repens*, especially if coastal, as *horrida* (presumably in the sense of: *Ononis repens* f. *horrida* (Lange) Asch. & Graebn.).

About two-thirds of the *O. repens* subsp. *maritima* specimens in the **BM** collection are spiny, often very spiny, and possess the strong spines characteristic of *O. spinosa* rather than the weak spines sometimes found in *O. repens*. This form is stoloniferous and more prostrate than *O. repens* and has leaves thickly covered with glandular trichomes, making the plant extremely hairy and it is often reported by collectors as very viscous. The leaflets are often smaller and often markedly obovate but sometimes elliptical. This plant was often recorded from maritime sands.

Collection of intermediates in the field

Specimens collected and determined as intermediates (and hence putative hybrids) based mainly on leaf shape, are shown in Figs. 3a-c, compared to collected reference specimens determined as *O. repens* (Fig. 3d) and *O. spinosa* (Fig. 3e).

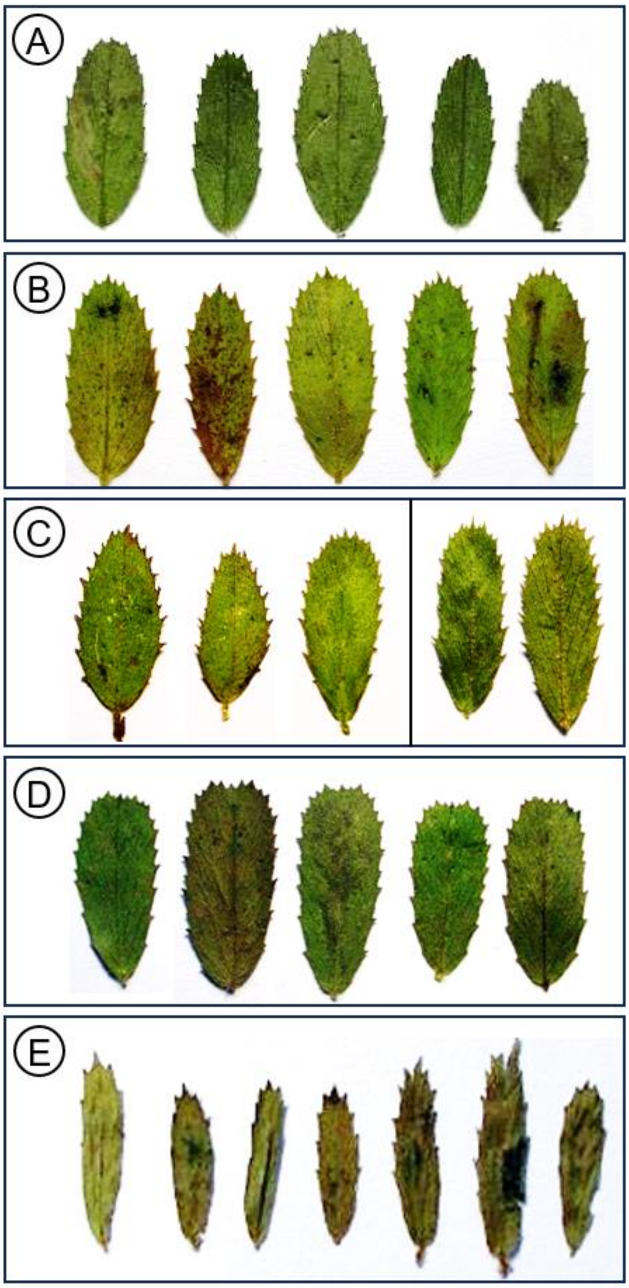

Figure 3. A: collected by C.A. Stace from TM103591, in a roadside nature reserve at Earl Stonham, leaflet length (LL) 12.5 - 18 mm, leaf index (LI) = 2.42 (*n* = 10 measurements); B: collected by J.D. Shanklin from TL36645122, Orwell Cambridgeshire, LL 9 – 12.6 mm, LI = 2.24 (*n* = 10). A and B show intermediate shape in being elliptic-oval with rounded apices and are from putative *O*. x *pseudohircina*. C: Putative *O*. x *pseudohircina* collected from Blackhall Rocks, left group NZ4701639252, LL 14.5 - 18.5 mm, LI = 2.13 (*n* = 5); right group NZ4701539252, LL 15.5 – 18 mm, LI = 2.15 (*n* = 5) with narrow teeth and a prominent apical tooth. D: *O. repens* collected from NZ4739, Blackhall Rocks, LL 11.5 – 17 mm, LI = 2.19 (*n* = 10). Note the typical *O. repens* shape of the leaflets D, with a truncate-emarginate obtuse apex and oblong-obovate contour. E: *O. spinosa* with atypically small leaves, collected by David Pearman & Ian Bennallick from SW93187769, Trebetherick Bay Cornwall, LL 6.4 – 11.5 mm, LI = 3.4 (*n* = 15).

Ploidy level of intermediates

To address the question of hybridity, samples collected from various locations were analysed by flow cytometry to ascertain nuclear DNA content. The results are shown in Table 2. All plants with intermediate leaves were diploid, as were the *O. spinosa* specimens. The *O. repens* were tetraploid, as expected.

Table 2. Flow cytometry results for *Ononis* specimens determined primarily on leaf morphology and leaf index (leaf length/width). *O.* x = *O.* x *pseudohircina* had a leaf index between 2.2 and 2.5, *O. spinosa* greater than 2.9, *O. repens* less than 2.0 (occasionally 2.2). Key: Collection IDs are as follows, BE = Boston, East from the bank of Hobhole Drain by Nunn's Bridge; CH = Cold Hanworth from the verge of Mill Lane; GBTS = Gibraltar Point, Tennyson Sands, sample A from beside a cycle track, sample B beside a path; HM = Heath's Meadows LWT NR; HW = Hetchell Wood; LP = collected by Lee Paton; PK = collected by Paul Kirby, PK1 and PK2 from coastal sand dunes at Saltfleetby, PK3 from calcareous grassland at Swaby, PK4 from Claxby St Andrew on the verge of Shaddy's Walk and PK5 from a road verge in Low Hameringham; RC = River Calder; RLH = Roach Lime Hills; TH = Townclose Hills. Units: pg = picograms, Gbp = gigabase-pairs for the 1x = haploid state.

Determined Species	Collection ID, location	1x DNA (pg)	1x DNA (Gbp)	Ploidy
O. X	LP1 NX80555192	0.73	0.71	diploid
O. X	LP2 NX80555192	0.73	0.72	diploid
O. X	LP3 NX80555192	0.71	0.69	diploid
O. X	LP4 NX80555192	0.71	0.69	diploid
O. repens	HW SE37714 42288	1.37	1.34	tetraploid
O. X	RLH1 SE41897 31362	0.70	0.68	diploid
O. X	RLH2 SE41883 31417	0.72	0.71	diploid
O. X	RLH3 SE418177 31528	0.69	0.67	diploid
O. X	RLH4 SE41880 31437	0.71	0.69	diploid
O. X	TH1 SE40537 30338	0.71	0.69	diploid
O. X	TH2 SE40513 30397	0.69	0.68	diploid
O. repens	TH3 SE40526 30355	1.37	1.34	tetraploid
O. X	RC	0.70	0.69	diploid
O. X	GBTS A TF5576558758	0.72	0.70	diploid
O. X	GBTS B TF5574258680	0.74	0.72	diploid
O. spinosa	RC SE16595 21957	0.72	0.70	diploid
O. spinosa	CH TF0271881964	0.75	0.73	diploid
O. spinosa	HM TF4853463939	0.75	0.73	diploid
O. spinosa	BE TF3673441543	0.74	0.73	diploid
O. repens	PK1 TF4682291773	1.41	1.38	tetraploid
O. repens	PK2 TF4683291796	1.43	1.40	tetraploid
O. repens	PK3 TF3932177625	1.41	1.38	tetraploid
O. repens	PK4 TF4505970978	1.40	1.37	tetraploid
O. repens	PK5 TF3006766717	1.40	1.37	tetraploid

Discussion

Are intermediate forms hybrids?

Intermediate plants, sharing a mixture of morphological characteristics of both *O. spinosa* and *O. repens* are not infrequently encountered in the British Isles. The current study found that intermediates collected fresh from five separate locations in Britain were all diploid, as determined by flow cytometry. Furthermore, plants determined as *O. spinosa* were diploids, while those determined as *O. repens* were tetraploids. If these intermediates are hybrids, then their diploidy requires explanation, for first generation hybrids are expected to be triploid. An alternative hypothesis is that the diploids are a form of *O. spinosa* showing some morphological features of *O. repens* because of phenotypic variation or adaptation to habitat. Studies on coastal populations of *O. repens* in Kent (VC15) combined with studies on maritime forms in the **BM** herbarium highlighted the ecological importance of such morphological variation in *Ononis*. The evidence for each hypothesis will now be discussed.

Morton (1956) conducted an insightful study on intermediate forms between *O. spinosa* and *O. repens* in Durham, comparing localities where both species co-occurred with pure populations. He found that mixed populations exhibited intermediate leaf indices of around 1.9 to 2.2, along with variations in stem colour, leaf apex shape, stem hairiness, growth habit, and spininess. Morton concluded that these intermediate characters were predominantly associated with hybrid plants, stating that they were not distributed throughout most individuals in any given colony. This observation raises important questions about the nature of the intermediate forms observed.

Leaf index is a particularly useful character when taken with leaflet shape, though delimiting each taxon on leaf index alone proves difficult. We found some diploid *O. spinosa* exhibited a leaf index exceeding 2.9, while Stephens (1979) recorded a range of 2.3 to 2.9 for this species. In contrast, the tetraploid *O. repens* typically had a leaf index below 2.0 (2.2), aligning with findings by Stephens (1978) and Morton (1956). Specimens displaying intermediate leaflet shapes showed leaf indices ranging from 2.2 to 2.5. Flow cytometric analyses confirmed these specimens were diploid, suggesting they are either *O. spinosa* or a distinct diploid taxon. Stace's (2019) criterion of a leaf index < 3 for *O. repens* and > 3 for *O. spinosa* might inadvertently include many *O. spinosa* specimens as *O.* x *pseudohircina*, which raises concerns about the over-recording of intermediates.

Subsequent studies, such as that by Morisset (1964), supported Morton's claims, identifying hybrids in a Cambridgeshire population with an intermediate chromosome number of approximately 2n = 48 (Stace *et al*., 2015; Morisset, 1964). These hybrids are designated *O.* x *pseudohircina* Schur. The variability of leaf morphology among the specimens from Blackhall Rocks, featured in Morton's research, highlights the significance of this area as a study site; further collection and flow cytometry analyses here would provide additional insights.

We concur with Morton's assertion that there is overlap in individual characters between *O. repens* and *O. spinosa*. For instance, while stem hairiness and leaf shape may produce ambiguous specimens, traits such as stem colour, growth habit, and calyx hairiness tend to be more consistent indicators. Therefore, specimens demonstrating two or more conflicting characters likely warrant classification as intermediate forms.

In a relevant study of British *Ononis*, Kloda *et al*. (2008) identified intermediates primarily classified as diploid, referring to them as *O. intermedia* C.A.Mey. ex Rouy (which is generally synonymous with *O.* × *pseudohircina* Schur). Their findings indicate limited gene transfer between diploids (*O. spinosa* and *O. intermedia*) and tetraploids (*O. repens* and *O. repens* subsp. *maritima*). The potential existence of *O. intermedia* derived from *O.* × *pseudohircina* necessitates a mechanism where hybrids with an intermediate number of chromosomes could trend towards diploidy in subsequent generations, possibly through backcrossing or chromosome elimination.

Following polyploidisation, flowering plants may undergo diploidization. This may involve the loss of functionality of alleles from one parent (Li *et al*., 2021) or a reduction in chromosome number by chromosome fusion (Mandáková & Lysak, 2018) or chromosome elimination (Gernand *et al*., 2005). The latter may result in partial or complete loss of one set of chromosomes, often the paternal set (Polgári *et al*., 2019). However, loss of chromosomes is typically gradual and can produce organisms with different chromosome numbers (Rodionov *et al*. 2019). It is more likely to occur when the parents are genetically distant.

Perhaps a more plausible mechanism for complete conversion of F1 triploids into diploids in *Ononis* is through the production of gametes with reduced ploidy. A proportion of gametes of triploids may contain x, $2x$ and $3x$ chromosomes instead of the expected $3x/2$ (Ramsey & Schemske, 1998). Due to the large numbers of pollen grains produced this is probably more frequent in male gametes. A reduced gamete of *O*. x *pseudohircina* with x chromosomes could perhaps backcross with a normal haploid gamete of either parent to give a diploid intermediate. An interesting case occurs in crosses between tetraploid *Petunia* x *hybrida* (Hook.) Regel (= *Petunia* × *atkinsiana* (Sweet) D.Don ex W.H.Baxter) and the diploid *P. axillaris* (Lam.) Britton, Sterns & Poggenb. Crosses between the tetraploid female and diploid male produced triploids, whilst those between diploid females and tetraploid males produced diploids which nevertheless possess characteristics of both parental species (Steere, 1932).

As expected, F1 hybrids have been recorded as approximately triploid with chromosome counts around 48. However, an initial misclassification by Morisset (1964) identified erroneous counts of $2n = 64$ for *O. repens* and $2n = 32$ for *O. spinosa*, which were later corrected to the accepted values of 60 and 30, respectively (Morisset, 1978). Notably, Stace *et al*. (2015) indicate that hybrids typically do not produce viable seeds, suggesting that while hybridization occurs, these hybrids exhibit low fertility. Despite Stace (2019) stating that these hybrids are fertile, it appears their fertility is minimal, potentially allowing for limited backcrossing with *O. spinosa* and the resultant production of diploid intermediates.

Given this evidence, we propose that most morphological intermediates may be more appropriately grouped with *O. spinosa* due to the opportunity for gene flow between these diploids; contrasted with the ploidy barrier between *O. spinosa* and *O. repens.* Kloda *et al.* (2008) recognized the intermediates as *O. intermedia*, a distinct diploid taxon, exchanging genes with *O. spinosa*. In cases where doubt arises regarding intermediates, this interpretation that the intermediates are a separate diploid taxon, has some merit, particularly if both parent species are not present to imply recent hybridization. There is little basis, however, for classifying intermediates as a distinct species, but rather perhaps as a form or variety of *O.*

spinosa. Breeding experiments are needed to confirm whether the differences are heritable. If they are and the intermediate forms occupy a different habitat or geographic range then they could be designated as a subspecies (such as *O. spinosa* subsp. *intermedia* (Rouy) P. Fourn.). In the absence of such knowledge the best default position seems to be to regard the intermediates as a form of *O. spinosa*.

Phenotypic Plasticity and its Implications

Could intermediates be the result of phenotypic plasticity? Morisset & Boutin (1984) observed that juvenile shoots of *O. spinosa* initially resembled the adult morphology of *O. repens*, particularly in the shape of the terminal leaflet, which matured to exhibit classic *O. spinosa* characteristics as flowering approached. Furthermore, they noted that when grown in low light, *O. spinosa* retained certain morphological traits typical of *O. repens*, including a decumbent growth form instead of an erect-ascending habit, unarmed instead of spiny, greenish instead of reddish stems, and wide obtuse leaflets. These modifications highlight the influences of environmental factors on morphological development. However, the plants eventually flowered with distinct *O. spinosa* features, characterized by fewer eglandular hairs on the calyx and pod lengths equal to or longer than the calyx itself. This indicates that while environmental conditions can influence vegetative traits significantly, reproductive traits may remain more stable. In practice this reported plasticity had little impact on the determination of herbarium species and most specimens could be confidently determined to species level. Only a few problematic intermediates remained.

Glandulosity was a useful character. However, Širjaev (1932) highlighted a historical observation by Henslow (1895) regarding *O. spinosa* grown under rich soils and high moisture conditions, noting that they gradually lost their spines and adopted the characteristic "goaty" odour of *O. repens*, gaining larger flowers typically associated with that species. This transition is intriguing, as it may be linked to an increase in glandular trichome density, although the specific glands responsible for producing odoriferous volatiles remain unverified. When returned to typical conditions, *O. spinosa* resumes its spiny morphology, reinforcing the idea that morphological traits can be highly plastic. This could cause potential confusion in species determination.

Interestingly, no noticeable differences were noted in stem glandulosity among specimens held in the British and Irish Herbarium (**BM**). However, *Ononis repens* typically exhibited more eglandular hairs and glandular trichomes on the calyx than *O. spinosa*, which contained few glandular hairs, as did some intermediates. This suggests that based on morphology alone, distinguishing hybrids or intermediates from *O. spinosa* grown in moist conditions can prove challenging. Some specimens classified as hybrids possess traits that could align them more closely with *O. spinosa*. Taken together, the phenotypic plasticity of *O. spinosa* supports the hypothesis that the diploid intermediates are a form of *O. spinosa*, perhaps resulting from local growth conditions.

Neoteny in Ononis repens?

Morisset & Boutin (1984) also noted an interesting seasonal polymorphism within *Ononis spinosa* subsp. *leiosperma* (Boiss.) Šir. In summer, this subspecies exhibits typical characteristics of *O. spinosa*, such as erect stems and narrow leaves adorned with spines. Nonetheless, in winter, it loses these spines and develops 'larger' (broader?) leaves with less erect growth. Such plastic responses to seasonal variations further illustrate the adaptability of the *Ononis* genus in response to changing conditions. *Ononis spinosa* typically exhibits spiny morphology in early summer, while *O. repens* may show spines occasionally in the autumn, particularly post-flowering. These phenomena suggest that *O. repens* characteristics may be the more juvenile.

Morisset & Boutin (1984) noted that the leaflet length-to-width ratio of *O. spinosa* tends to increase towards the apex, with the basal leaves resembling those of *O. repens*. Juvenile leaves of *O. spinosa* evidently resemble the adult leaves of *O. repens*. Thus, evidence for leaf-shape plasticity further suggests that *O. spinosa* possesses a more "adult" vegetative morphology compared to *O. repens*. Morisset & Boutin propose that *O. repens* represents a neotenic form of *O. spinosa*, where neoteny describes the retention of juvenile characteristics into adulthood while achieving sexual maturity. They hypothesise that this juvenile morphology may be a consequence of polyploidy, though that remains to be tested by genetic analysis. Nevertheless, these observations suggest that the diploid intermediates found in our study could be a more juvenile form of *O. spinosa*.

Morisset & Boutin (1984) also noted that the greener stems of *O. repens* may redden in late summer and hence resemble in colour the usually reddish stems of *O. spinosa*. Our herbarium observations, however, indicated that even when reddened, *O. repens* stems usually appear lighter than the darker, more vivid-red stems of *O. spinosa*. Stem colour hence proved a very useful diagnostic character in herbarium specimens and in the field.

Ononis repens subsp. maritima

Specimens designated as *O. repens* subsp. *maritima* in the **BM** collection are characterized by highly glandular leaves, short obovate leaflets, and smaller flower sizes. Although habit may be difficult to ascertain from herbarium specimens, many appear small and compact, often with sand grains adhered to them, indicative of a prostrate habit, as sometimes noted by collectors. It is worth observing that more than half of these specimens exhibit spiny features.

Occasionally, we reclassified specimens labelled as *O. repens* subsp. *maritima* (or var. *horrida*) as either *O. spinosa* or spiny *O. repens*. Collectors frequently assigned any spiny form of *O. repens* to var. *horrida*, while coastal specimens were often classified as subsp. *maritima*. However, not all coastal *O. repens* exhibit the defining characteristics of subsp. *maritima*; thus, our classification prioritized morphological traits over habitat context.

Historical cultivation of Yarmouth specimens of *O. repens* subsp. *maritima* by Bennett (1889) illustrated a loss of typical maritime characteristics, despite developing spines in the third year of growth. This observation suggests that spininess of this taxon might be a genetically hard-wired trait, independent of environmental influences, establishing genetic distinctions between *O. repens* subsp. *maritima* and other forms of *O. repens*. Meanwhile, the dense covering of glandular hairs on coastal plants may signify a phenotypic plastic response to high light levels or saline conditions, thereby differentiating them from *O. spinosa*. These traits should, under most circumstances, facilitate accurate identification of the maritime form from other species.

Ecotypes, Recording, and Conservation

The findings of Kloda *et al.* (2008) that there is ready gene flow within each ploidy group but restricted gene flow between ploidy groups, support classifying the tetraploids as a single species, allowing *O. repens* and *O. repens* subsp. *maritima* to remain valid infraspecific taxa. It appears that *O. repens* subsp. *maritima* consists of both spiny and spineless variants, with convincing specimens lacking spines present in the **BM** collection. The emergence of spines reportedly occurs only after the third year, at least in cultivation. This continuum of forms, ranging from non-maritime to maritime types, suggests adaptability depending on habitat conditions.

Turrill (1946) highlighted that different ecotypes may occupy various ranges along an ecocline—a gradual variation of traits across a species' distribution, which can correspond with observable gradients. In *O. repens*, for instance, a clear ecocline may exist between inland populations and coastal sand dune forms. A similar pattern could exist along the gradient from *O. spinosa* to *O.* x *pseudohircina*.

The placement of *Ononis reclinata* within this framework remains intriguing and both the authors and Chris Dixon at the **BM** have pondered the following possibilities. Although rare in Britain, its maritime presence raises questions concerning potential hybridization with *O. repens*, possibly contributing to the origin of *O. repens* subsp. *maritima*. Notably, *O. reclinata*, like *O. repens*, is a tetraploid (2n = 60), suggesting that hybridization might occur freely.

Within *O. repens*, we propose considering subsp. *maritima* as an evident ecotype adapted to maritime environments, predominantly due to phenotypic plasticity. While some characteristics may manifest in cultivation, others appear habitat-specific. The form captured in Fig. 1 references a 'sub-maritime' variation found on shingle deposits, embodying features that may be potentially reversible in cultivation.

Additionally, Harding (1960) identified a distinct dune form from Ireland, Lincolnshire, and Norfolk that produced significantly larger seeds and exhibited a leaf index intermediate between *O. spinosa* and *O. repens*, while otherwise resembling *O. repens* in stem pubescence. A specific specimen catalogued from sand dunes in vc28 (West Norfolk) aligns with these vegetative traits, showing a leaf index of 2.5. Further investigation into these populations could yield valuable data.

Adaptations witnessed in the maritime and sub-maritime forms of *O. repens* encompass a dense accumulation of glandular hairs, a prostrate growth habit, and a reduction in leaflet length-to-width ratios and spininess. Such glandular hairs potentially help reflect and scatter UV radiation—critical in open coastal habitats with minimal cover (see Karabourniotis *et al.*, 2021). The reddening of stems, attributable to anthocyanin production, may serve to protect the plants from UV damage in stressful environments. The prostrate habit further aids in mitigating desiccation from coastal winds and facilitates colonization of unstable substrates. Consequently, it is apparent that *O. repens* exhibits a variety of forms and ecotypes along a continuum, with some variations explicable by phenotypic adaptations to differing growth conditions.

The existence of these ecotypes emphasizes the importance of precise recording. Some researchers advocate for the merging of infraspecific taxa, while others favour more distinct segmentation. Striking a balance is necessary, as evidenced by the scarcity of *O. repens* subsp. *maritima* records in the BSBI database. This record deficiency poses potential risks for conservation. For example, specimens of this taxon have been collected from sand hills in Sandwich, Kent (VC15), which have since become part of popular golf course habitats along the coast. It is uncertain if this form persists amongst these coastal populations or if introgression has occurred with other coastal *O. repens*. Such uncertainties necessitate the careful consideration and potential conservation of what may be a rare and unique taxon.

Detailed records of the precise habitat and morphology of *O. repens* populations can furnish valuable ecological insights regarding adaptation. However, the interplay of phenotypic plasticity complicates our understanding. We should regard populations exhibiting adaptations specific to a defined ecological cline as distinct ecotypes, regardless of whether these adaptations stem from stable genetic differences or reversible changes in gene expression.

The classification of intermediate forms, whether as *O.* x *pseudohircina* or *O. spinosa* subsp. *intermedia* (Rouy) P. Fourn, prompts ongoing discussion. Further genetic studies could clarify these designations. Kloda *et al*. (2008) suggested grouping diploid intermediates with *O. spinosa* based on ploidy levels and gene flow. In the absence of clear indicators of recent hybridity, such as a zone of intermediates where both parental populations meet or indications of triploidy, the simplest solution is to place the intermediates as a form or variety of *O. spinosa*. If an intermediate exhibiting low fertility occurs in an area where both parent species are present, it is more plausible that it is an F1 hybrid and presumably triploid. Conversely, if testing confirms a diploid nature, it leans towards classification as *O. spinosa* subsp. intermedia or a form/variety of *O. spinosa*. Our findings suggest that most morphological hybrids are likely diploid and thus should be classified alongside *O. spinosa* in ambiguous cases. Interestingly, Morton (1956) reported that intermediates at Marsden and Blackhall tended more towards the morphology of *O. spinosa* than *O. repens*, indicating a trend that warrants further exploration.

The precise origins of diploid intermediates remain elusive. Given the hybrids' low fertility, while it is plausible that infrequent backcrossing with *O. spinosa* could produce diploid intermediates, the occurrence of such events likely remains low. Continued research will be essential in unravelling these complex interactions within the *Ononis* genus, ultimately contributing to our understanding of plant ecology, evolution, and conservation strategies.

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