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CHLOROPLAST DNA DIVERSITY OF *HIERACIUM PILOSELLA* (ASTERACEAE) INTRODUCED TO NEW ZEALAND: RETICULATION, HYBRIDIZATION, AND INVASION[1]

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The European hawkweed *Hieracium pilosella* is a successful invader and a troublesome weed in New Zealand. The systematics of the genus *Hieracium* is extremely complex and contentious, probably due to recent speciation, hybridization, polyploidy, and diverse reproductive strategies. In the first chloroplast DNA survey of the group, we sequenced 285 plants (including *H. pilosella* and 12 other species of subgenus *Pilosella*) from New Zealand and Europe for 900 bp of *trnL-trnF*. Eleven haplotypes were identified with much sharing among species. Three haplotypes (A, D, G) were found in seven, three, and four species, respectively, but two species (*H. lactucella* and *H. auricula*) had single, private haplotypes. Our cpDNA data for subgenus *Pilosella* are consistent with the group's having incomplete lineage sorting and/or recent reticulate evolution. Six haplotypes were identified in *H. pilosella*, four of these unique to this taxon in our sample. In New Zealand, haplotype A was common and occurred in plants of different ploidy (i.e., 4×, 5×, 6×), whereas haplotypes C, B, and M were restricted to 4×, 5×, and 6× plants, respectively. The distribution of haplotype variation suggests that some or all of the *H. pilosella* seeds accidentally introduced into New Zealand probably came from east Europe rather than the United Kingdom and that a minimum of four lineages were introduced. Within New Zealand, hybridization of *H. pilosella* with a related taxon (probably *H. praealtum*) has occurred at least three times, involving both obligate sexual tetraploids and facultative apomictic pentaploids of *H. pilosella*.

Key words: Asteraceae; cpDNA; hawkweed; *Hieracium pilosella*; hybrid; introduced; New Zealand; reticulate; variation; weed.

Invasive species threaten the conservation of regional economies and biotas and thus draw interest from ecologists (Lee, 2002). But the biology of invasive species also provides valuable, observable events that parallel and potentially illuminate prehistoric range changes. A large part of research in the field of molecular ecology has focused on determining population genetic effects of geologically recent environmental perturbations, in particular Pleistocene glaciation (Hewitt, 1996; Taberlet et al., 1998; Abbott et al., 2000). This research contributes to our understanding of how factors such as dispersal ability, reproductive behavior, and genetic diversity are implicated in the colonization of new habitat. However, interpretation of phylogeographic data is to a greater or lesser extent post hoc, because measurements are made long after the colonization event. Invasive or weedy species offer a finer degree of resolution as it is through the auspices of human introduction that potentially invasive taxa are presented with new habitats. Thus, we expect to be able to observe many of the initial ecological/evolutionary responses of novel biotic and environmental interaction. In particular, we have the opportunity to study in detail the role of genetic diversity (Neuffer and Hurka, 1999), polyploidy, and hybridization in invasion (e.g., Stebbins, 1985; Raybould et al., 1991; Abbott, 1992; O'Hanlon et al., 1999; Ellstrand and Schierenbeck, 2000), features that must apply during natural range changes.

The genus *Hieracium* (Asteraceae) is among the most species rich of plants with more described species than any other genus in Europe (Sell and West, 1976). The systematics of the hawkweed genus is extremely complex and contentious, with much disagreement among systematists even with respect to numbers of species (Gadella, 1991). The instability of the taxonomy is probably a reflection of the polyphyletic origin of many *Hieracium* taxa (Krahulcova et al., 2000), though there is little direct evidence for this (but see Shi et al., 1996). Many *Hieracium* species comprise a complex of ploidal levels, and in at least some instances, putative species may be composites of several independent hybridization events (Krahulcova et al., 2000). Interestingly, reproductive systems vary among ploidal levels even within species; for example, in *H. pilosella* even ploidal numbers (e.g., 2, 4) are generally obligate sexuals while those with odd ploidal numbers (e.g., 3, 5) are usually facultative apomicts (Gadella, 1987, 1991). In related taxa such as *H. praealtum*, this is not the case; tetraploid and pentaploid plants are known, but all are apomictic.

The *Hieracia* of New Zealand comprises a minute, and thus manageable, fraction of the full diversity of the genus. The 10 species reported from the field are all accidental introductions from Europe first reported in New Zealand over 100 years ago (Travers, 1884). These plants are generally thought to have arrived as contaminants of imported grass seed. The most likely source was the United Kingdom because this was the origin of most human and agricultural colonists. In addition, British colonialists founded acclimatization societies for the purpose of introducing plants and animals to New Zealand. Subgenus *Pilosella* is represented in South Island New Zealand by five

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species (*H. pilosella*, *H. praealtum*, *H. caespitosum*, *H. aurantiacum*, *H.* × *stoloniflorum*; Webb et al., 1988). Of these, *H. pilosella* has, perhaps with the aid of human habitat modification, become a successful and troublesome weed among high country grasslands that predominate in the South Island (Treskonova, 1991; Jenkins and Jong, 1997). Hieracium is much scarcer in North Island and, so far, of little ecological significance. *Hieracium pilosella* forms small, prostrate leaf rosettes and, like other members of the subgenus, spreads vegetatively via stolons. In New Zealand, *H. pilosella* often forms dense mats that exclude other plants, but it is not a significant weed in Europe, where it occurs as a component of mixed grassland floras.

In Europe, five cytopes of *Hieracium pilosella* have been identified in the field, ranging from diploid to heptaploid (Gadella, 1991). The range is dominated by sexual tetraploids and apomictic pentaploids, with higher ploidies occurring mostly in the north (United Kingdom, Scandinavia) and montane areas of central Europe. Morphologically too, *H. pilosella* is extremely variable. For instance, Gadella (1987) reports that Zhan (1923) recognized 624 variants of the species.

In New Zealand, initial cytological studies of *H. pilosella* indicated that populations were predominantly, if not entirely, pentaploid (Makepeace, 1981; Jenkins, 1991). However, subsequent studies have found aneuploids (Chapman and Lambie, 2000) and hybrids (Morgan-Richards et al., in press), which may reflect the presence of obligate sexuals. Tetraploid sexual plants have been confirmed in a number of populations, and these may have evolved in situ (Chapman et al., 2003) through residual sex of facultatively apomictic pentaploids (Houliston and Chapman, 2001). There is also considerable morphological variation (Chapman and Brown, 2001) and evidence of high genetic diversity (Chapman et al., 2000). Considering the recent, probable small size and apomictic reproduction of the initial introduction, *H. pilosella* in New Zealand is surprisingly variable. The degree to which this variability reflects initial introduced diversity vs. postcolonization changes remains unresolved (Chapman and Brown, 2001) and is the subject of ongoing research.

We undertook a study of chloroplast DNA (cpDNA) sequence variation to further our understanding of the extent and origin (pre- or postintroduction) of genetic diversity of *H. pilosella* introduced to New Zealand. Our rationale was that any variants encountered were extremely unlikely to have evolved since colonization and could not be the result of recombination (unlike nuclear markers). We surveyed all other species of *Hieracium* subgenus *Pilosella* in New Zealand to seek evidence of hybridization, introgression, or reticulate evolution since colonization. To facilitate this and to shed light on the possible European source(s) of New Zealand lineages, we examined samples representing the same and closely related species sourced directly from European populations, in the first cpDNA survey of the group.

MATERIALS AND METHODS

Sampling—In New Zealand, whole plants were collected from sites throughout South Island and maintained in the glasshouse. Sites at Drac Flat, Lyndon, and Rakaia have been the focus of a broad research program and have thus been extensively sampled. At these three sites, field sampling used a systematic transect-based approach (Chapman et al., 2003). However, our approach to sequencing DNA from plants maintained in the glasshouse was selective; we took a random subsample of pentaploid plants but examined all surviving tetraploids. Other sites were sampled much less intensively in the field, but we collected at many locations. Given that *H. pilosella* can be highly clonal, this strategy minimized the risk of resampling clones within sites and maximizes the chances of finding different lineages. At all sites, individuals (ramets) were collected at well-spaced intervals (>30 cm and usually >100 cm). In New Zealand, *H. pilosella* is most abundant in the high country (600–1000 m asl; Treskonova, 1991; Svavarsdóttir et al., 1999) where it has invaded native grasslands; these were the source of most of our material. But we also located and collected specimens from low altitude sites where populations generally consist of small and possibly ephemeral mats. We also sourced DNA from herbarium specimens in the University of Canterbury collection.

European specimens were obtained as leaf samples, dried and stored with silica gel, and in some cases additional samples were provided as DNA sequences by collaborators. Our sampling in Europe reflects the availability of collectors and the distribution of taxa (e.g., *H. praealtum* in the United Kingdom is a localized garden escape and thus not in our sample). The provenance of our specimen of *H. auricula* is not known because it has been in culture for some time; however, we retained it in the study because it was the sole representative of a haplotype that forms an internal node in our tree.

Taxonomy—The systematics and nomenclature of the *Hieracia* is far from stable and several competing systems are in current use in Europe. For plants collected in New Zealand, we have followed Webb et al. (1988) and as far as possible accommodated material from Europe within this system. To do this, we have relied on local expertise, and we acknowledge that without comparison of morphological and cytogenetic data some question as to placement of individuals may exist. However, we believe the determination of *H. pilosella* specimens is reliable and can be accommodated within all current taxonomic systems.

Ploidy—The ploidy of New Zealand specimens was determined using a combination of flow-cytometry and direct counts of chromosomes (Morgan-Richards et al., in press). For European material for which DNA was extracted from silica-gel-dried specimens or from herbarium specimens or was obtained as raw DNA, we were not able to determine ploidy.

DNA extraction—Each sample was ground using a hot (60°C) ceramic pestle and mortar with preheated extraction buffer comprising 1000 μL 2% hexadecyltrimethylammonium bromide (CTAB) buffer in 100 mmol/L Tris-HCl pH 8.0, 1.4 mol/L NaCl, 20 mmol/L EDTA, and 2 μL β-mercaptoethanol. Homogenates were incubated at 60°C for 30–60 min. An equal volume of 24 : 1 chloroform : isoamyl alcohol was added, and the mixture vigorously shaken and centrifuged at 16 060 g for 5 min. Supernatants were pipetted into fresh tubes and combined with a two-thirds volume of cold 100% isopropanol. DNA was pelleted after 15–60 min by spinning at 16 060 g for 10 min and then washed with 500 μL 70% ethanol by spinning briefly. The 70% ethanol was discarded and the pellet dried and dissolved in 30 μL of water.

DNA amplification and sequencing—We targeted noncoding regions of cpDNA as described by Taberlet et al. (1991). Polymerase chain reaction (PCR) was used to amplify the *trnL-trnF* chloroplast gene fragment (Taberlet et al., 1991). Primers c and f (Taberlet et al., 1991) were used to amplify the *trnL* intron and *trnL*/*trnF* intergenic spacer. The polymerase chain reactions were carried out in 25-μL volumes containing 2.5 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 1× PCR buffer, 0.625 units *Taq* (Roche Diagnostics, Mannheim, Germany) and 1.5 μL of diluted (1 : 20–1 : 100) DNA template. Thermal cycling conditions were: 2 min at 94°C, 35 cycles of 15 s at 94°C, 30 s at 48°C, and 90 s at 72°C, followed by 3 min at 72°C. The PCR products were purified using High Pure spin columns (Roche Diagnostics, Mannheim, Germany). Cycle sequencing utilized Dyenamic chemistry (Amersham Biosciences, Freibury, Germany) and the Taberlet et al. (1991) primers c, e, and f following the manufacturer's protocols. Cycle sequencing products were electrophoresed on an ABI automated sequencer (PE Biosystems, Foster City, California, USA). Confirmation of base calls by comparison with chromatograms and alignment of sequences was achieved by eye using SeqEd version 3.01. We constructed a minimum spanning tree of the haplotypes identified manually.

TABLE 1. Haplotype frequencies among *Hieracium* taxa collected in New Zealand and Europe.

Haplotype	*H. pilosella*	*H. praealtum*	*H. caespitosum*	4× hybrids	*H. aurantiacum*	*H. × stoloniflorum*	Others	Total
New Zealand								
A	97	22	4	9				132
B	26			1				27
C	12			8				20
D		2			6			8
G						5		5
I								
J								
L								
M	2							2
N								
	137	24	4	18	6	5		194
Europe								
A	26	3	9		1		10	49
B	18							18
C	3							3
D		1			7		1	9
G	2				1		2	5
I			2					2
J							1	1
L	3							3
M								0
N					1			1
	52	4	11		10			91

RESULTS

Haplotype diversity and frequency—We obtained cpDNA sequences from 194 *Hieracium* subgenus *Pilosella* plants from New Zealand. These comprised 137 *H. pilosella* and 57 representatives of four other species and hybrids (Table 1) collected from 44 sites (Fig. 1). Ninety-one individuals (including 52 *H. pilosella*) from ~12 species were surveyed from ~72 locations in Europe (Table 1, Appendix 1) in 36 regions (Fig. 2). In total, 285 plants were sequenced.

We identified 11 *trnL-trnF* haplotypes among these plants after alignment of 900 bp sequences (Table 2). Among the 11 haplotypes, there are 17 variable sites comprising 12 single nucleotide substitutions and 5 insertion/deletions (indels). Four of the indels are tandem repeats, the fifth is a mononucleotide repeat (six As or seven As) that appears as a single base indel in Table 2 (position 98). Sequences of two representative haplotypes (A and D) have been deposited at Genbank (accession numbers AY342314 and AY342315).

We found six haplotypes in *Hieracium pilosella* (A, B, C, G, L, M). Only two of these (A, G) were also found in other taxa that we sampled in the subgenus: A—widely and G—rarely. Haploytpe A was in *H. caespitosum* and *H. praealtum* from New Zealand and Europe and also in a number of other taxa sampled from Europe (Table 1, Appendix 1). Of the six haploytpes found in *H. pilosella*, three (A, B, C) were encountered in New Zealand and European samples, two (G, L) were found only in Europe (Austria and France, respectively), and one (M) was found only in New Zealand. Haplotype G was also found in New Zealand but in a different taxon, *H. × stoloniflorum*.

Only two other haplotypes (D, G) were present in more than one taxon (Table 1, Appendix 1). Most samples of haplotype D came from *H. aurantiacum* (NZ and Europe), but also from *H. glaciale* (Europe) and *H. praealtum* (Europe and NZ). The two instances of haplotype D in New Zealand *H. praealtum* were in rare pentaploid individuals (Hope Saddle, Fig. 1); other pentaploid and all tetraploid *H. praealtum* in New Zealand had haplotype A. Haplotype G was found in *H. × stoloniflorum* (NZ), *H. aurantiacum*, *H. florentinum*, and *H. pilosella* from Europe.

New Zealand Hieracium pilosella—In New Zealand, haplotype A was the most frequent (70%) in both our total sample and within *H. pilosella* alone (Table 1, Table 3). A was present in representatives of all three ploidal levels of *H. pilosella* encountered (Table 4). Conversely, the other haplotypes found in New Zealand *H. pilosella* (B, C, and M) were restricted to pentaploids, tetraploids, and hexaploids, respectively (excluding F1 hybrids, see later). C was restricted to three adjacent locations (Drac Flat, Lyndon, and Rakaia), and M was found at just one location (Twizel) (Fig. 1, Table 4). All the tetraploid individuals in our New Zealand sample came from three intensively surveyed locations (Drac Flat, Lyndon, and Rakaia), but because we sequenced all tetraploids that we found, but not all the pentaploids, the ratio in our sample is skewed. However, the dominance of one haplotype (C) at Drac Flat is informative. Across the majority of locations, only pentaploids were found and these were either haplotype A or B (Fig. 1). At seven of 20 locations where more than one individual was examined, both A and B were found (Table 3).

We sequenced 18 tetraploid interspecies hybrids (probably *H. pilosella* × *H. praealtum*; Morgan-Richards et al., in press) from three sites (Drac Flat, Lyndon, Cowans Hill). Half had haplotype A, one had B (Lyndon), and eight had C (Lyndon 1, Drac Flat 7) (Table 3, Table 4).

European taxa—Haplotype A was also encountered with high frequency in Europe, being present in 53% of all plants and 50% of *H. pilosella* examined (Table 1). Of 12 taxa sampled, eight had Haplotype A and four of these had at least one other haplotype (Appendix 1). The actual extent of haplotype sharing is almost certainly much higher than this because our sample of most taxa was very small. In Europe, rare haplo-

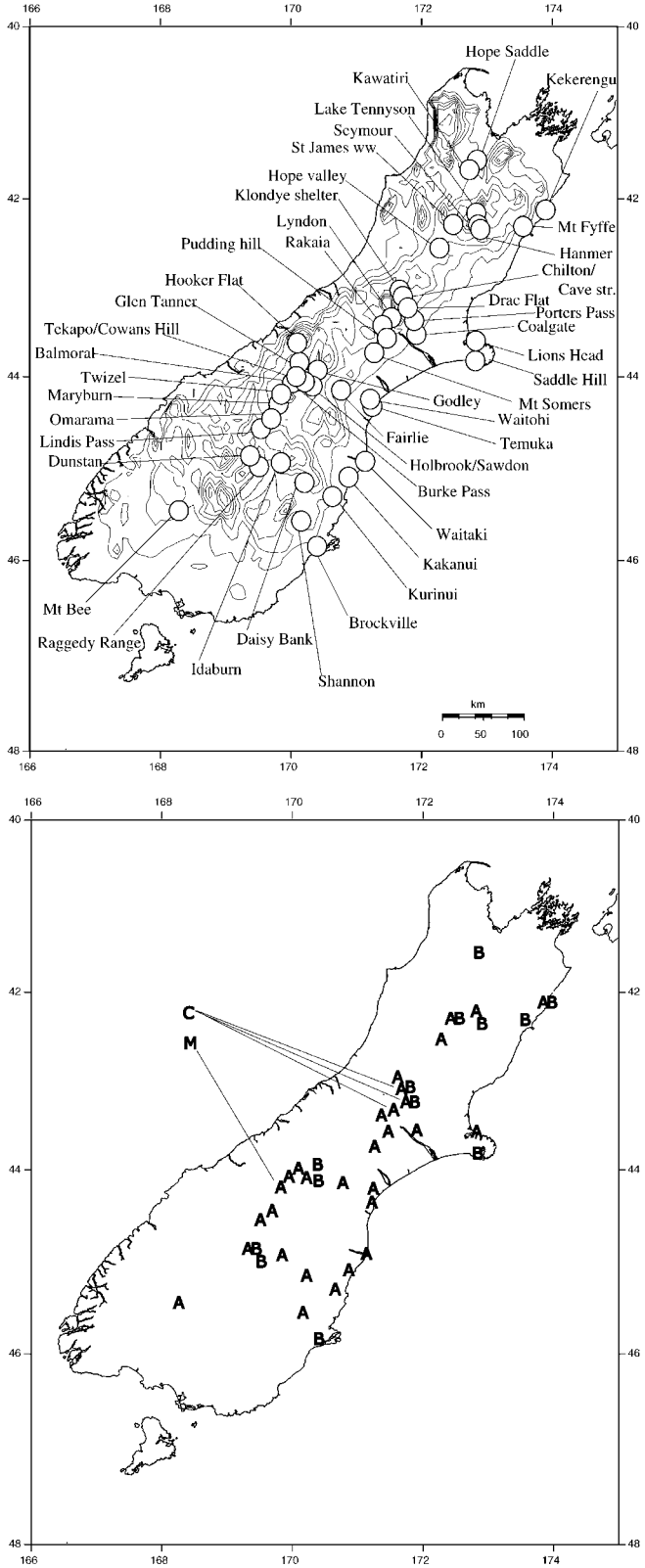

Fig. 1. Collection locations and occurrence of cpDNA haplotypes in *Hieracium pilosella* in South Island, New Zealand. This species is scarce in North Island.

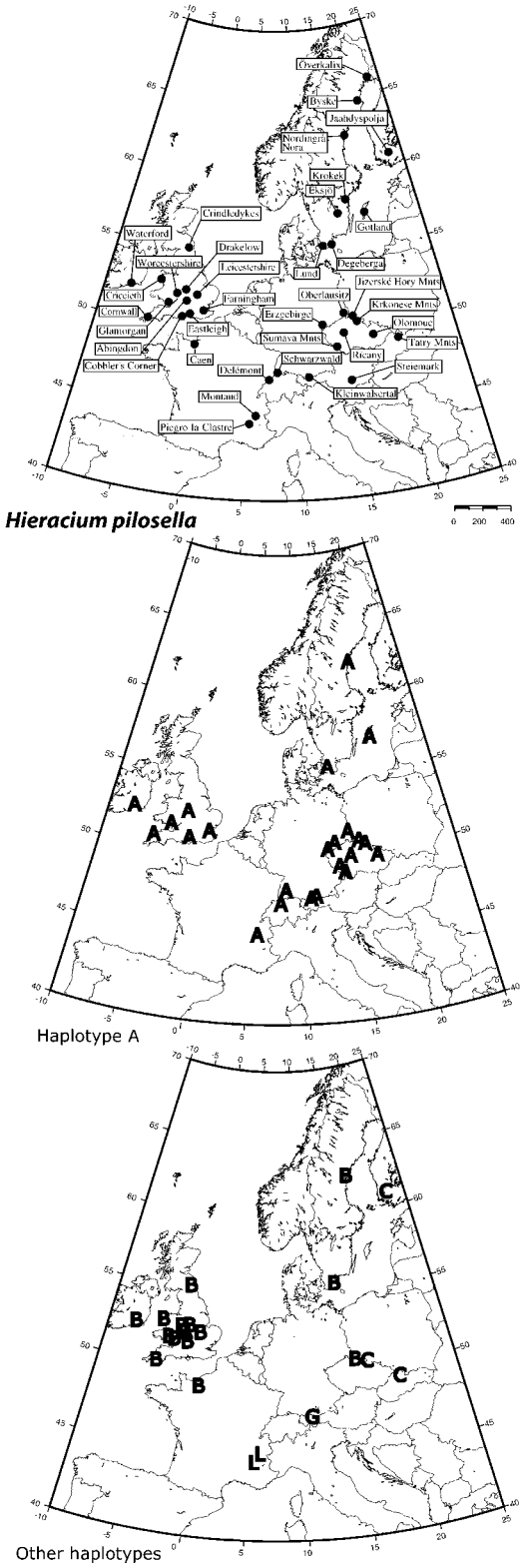

Fig. 2. Collection locations and occurrence of cpDNA haplotypes in *Hieracium pilosella* in Europe.

TABLE 2. Summary of variation encountered among chloroplast DNA sequences for the *trnL-trnF* region. Numbers indicate position of variable sites among 900 bp of 11 aligned *Hieracium* subgenus *Pilosella* haplotype sequences.

Haplotype	98	117	241	250	300	394	406	520	533	569	597	622	666	690	755	839	869
A	A	A	G	A	G	A	C	C	-----	C	T	TATTCT	A	----------	T	AATGAG	A
B	A	A	A	A	T	A	C	C	-----	C	T	TATTCT	A	----------	T	AATGAG	A
C	A	A	G	A	G	A	C	C	-----	C	T	------	A	----------	T	AATGAG	G
D	-	A	G	G	G	A	C	C	TTATC	C	G	TATTCT	A	CAAGGAATCCCCA	G	------	A
G	-	C	G	A	G	G	C	C	-----	A	G	TATTCT	A	----------	G	AATGAG	A
H	-	A	G	A	G	A	C	C	-----	C	G	TATTCT	A	CAAGGAATCCCCA	G	------	A
I	A	A	G	A	G	A	T	C	-----	C	T	TATTCT	A	----------	T	AATGAG	A
J	-	C	G	A	G	G	C	C	-----	A	G	TATTCT	G	----------	G	AATGAG	A
L	-	C	G	A	G	A	C	C	-----	C	G	TATTCT	A	----------	G	AATGAG	A
M	-	A	G	A	G	A	C	C	-----	C	T	TATTCT	A	----------	T	AATGAG	A
N	-	C	G	A	G	G	C	A	-----	A	G	TATTCT	A	----------	G	AATGAG	A

types were found in a number of taxa: I in two *H. caespitosum* (of eight), J in a single *H. lactucella*, and H in a single *H. auricula*.

Phylogeny—The relationship among these haplotypes was inferred using a fully resolved parsimony spanning tree. Many of the haplotypes we encountered appear as internal nodes in our tree, consistent with shallow or recent splitting (Fig. 3a). Interestingly, four of the five internal nodes consisted of haplotypes found in *H. pilosella*.

DISCUSSION

Considering the number of taxa surveyed, we found a high level of haplotype diversity, but we also found a high level of haplotype sharing among taxa. This might reflect two distinct processes: incomplete lineage sorting or interspecies hybridization (Comes and Abbott, 2001). Both are consistent with recent speciation. Our data do not allow us to study the phylogenetics nor the systematics of species, but our data do demonstrate the complex recent history of the group and help explain taxonomic difficulties. *Hieracium pilosella* introduced to New Zealand brought with it much of the European chloroplast variation. It has not increased cpDNA diversity via hybridization since introduction, but hybridization may nevertheless have a significant impact on invasiveness in New Zealand.

Reticulate evolution—We found six haplotypes within *H. pilosella* and extensive sharing among ploidal levels and sharing among species. Despite the predominance of apomixis the sharing of haplotypes can be explained by reticulate evolution. In New Zealand *Hieracium pilosella*, a low level of residual sex has been demonstrated in pentaploid apomicts (Houliston and Chapman, 2001) and a breakdown in microspecies boundaries has been observed (Chapman and Brown, 2001). Furthermore, sex between pentaploid apomicts not only results in pentaploids of mixed parentage but can also yield tetraploids, some of which are obligate sexuals (Chapman and Bicknell, 2000; Chapman et al., 2003). These and the sexual progeny of interspecies hybrids (Morgan-Richards et al., in press; see *Invasion* later) may further contribute to reticulation. Nuclear markers have revealed genetic variation in apomictic microspecies of *Hieracium* (hawkweeds, Shi et al., 1996; Chapman et al., 2000) and *Taraxacum* (dandelions). In contrast, Wittzel (1999) found no cpDNA variation within apomictic species of *Taraxacum*, the expected result for true clonal lineages (Richards, 1996). The presence of multiple haplotypes (or other genetic variation) within apparently apomictic species could result from the failure to correctly identify microspecies (i.e., different lineages) as Wittzel (1999) suggested for the *Taraxacum* data of King (1993). However, failure to correctly identify microspecies could be attributed to morphological instability, which would be likely if microspecies/species hybridized. It has become increasingly apparent that many (and perhaps all) apomicts are not in fact obligate apomicts but have some potential for sexual reproduction (Nogler, 1984; Bayer et al., 1990; Briggs and Walters, 1997). Gene flow in predominantly selfing polyploids could result in exchange of chloroplast and other markers among lineages (microspecies, e.g., Doyle et al., 1999) and thus inconsistent morphology.

Origins of New Zealand Hieracium—*Hieracium pilosella* and presumably the other species of the genus found today in

TABLE 3. Locations in the South Island, New Zealand, sampled for *Hieracium* subgenus *Pilosella*, with occurrence of haplotypes indicated by location, taxon, and ploidy. Frequency of haplotypes in *H. pilosella* are shown as *n*.

		H. pilosella				*H. praealtum*		*H. caespitosum*	*H. × stoloniflorum*	*aurantiacum*
Location	*n*	4×	5×	6×	?	4×	5×	4×	6×	4×
Brockville	6		B							
Burke Pass	2,1		AB				A			
Chilton	2,2		AB							
Coalgate	1		A							
Cowans Hill	2		A			A				
Daisy Bank	1		A			A				
Drac Flat	9,13,1	C	AB			A	A	A	G	
Dunstan Range	1,1		AB							
Fairlie dom.	1,1		A	A						
Glen Tanner						A				
Godley	1		B			A				
Hanmer										D
Holbrook	1		B							
Hooker Flat						A				
Hope Saddle						A	D			
Hope Saddle top	1		B							
Idaburn	1		A							
Kakanui	3		A							
Kawatiri	1		B							
Kekerengu	2,2		AB							
Klondye shelter	2		A							
Kurinui	5		A			A				
Lake Tennyson										D
Lindis Pass	2		A			A				
Lions Head	6		A							
Lyndon	5,1,10,2	AC	AB			A				
Maryburn								A		
Mt. Bee	1		A							
Mt. Fyffe	2		B							
Mt. Somers	1		A							
Omarama	1		A							
Porters Pass										D
Pudding Hill	1		A							
Raggedy Range	1		B							
Rakaia	7,2,6	AC	A							
Saddle Hill	1		B							
Sawdon							A			
Seymour	1		A							
Shannon	2		A							
St. James ww	3,1		AB							
Tekapo										D
Temuka dom.	8		A							
Twizel dom.	1,2			AM						
Waitaki	1		A							
Waitohi memorial	1		A							
Balmoral herb.	1				A					
Cave Stream herb.	1				B					
Hanmer herb.	1				B					
Hope Valley herb.					A					
Rakaia herb.	1,1				AB					
Tekapo herb.	1				A					
Temuka dom. herb.	1				B					

TABLE 4. Frequency of three haplotypes among tetraploid and pentaploid *Hieracium pilosella* and tetraploid putative hybrids, at three sites in South Island, New Zealand.

	4×			5×			6×		4× hybrids			
Site	A	B	C	A	B	C	A	M	A	B	C	Total
Drac Flat			9	13	1				2		7	32
Lyndon	5		1	10	2				3	1	1	23
Rakaia	7		2	6								15
Other				54	23		2	2	4			85
Total	12		12	83	26		2	2	9	1	8	155

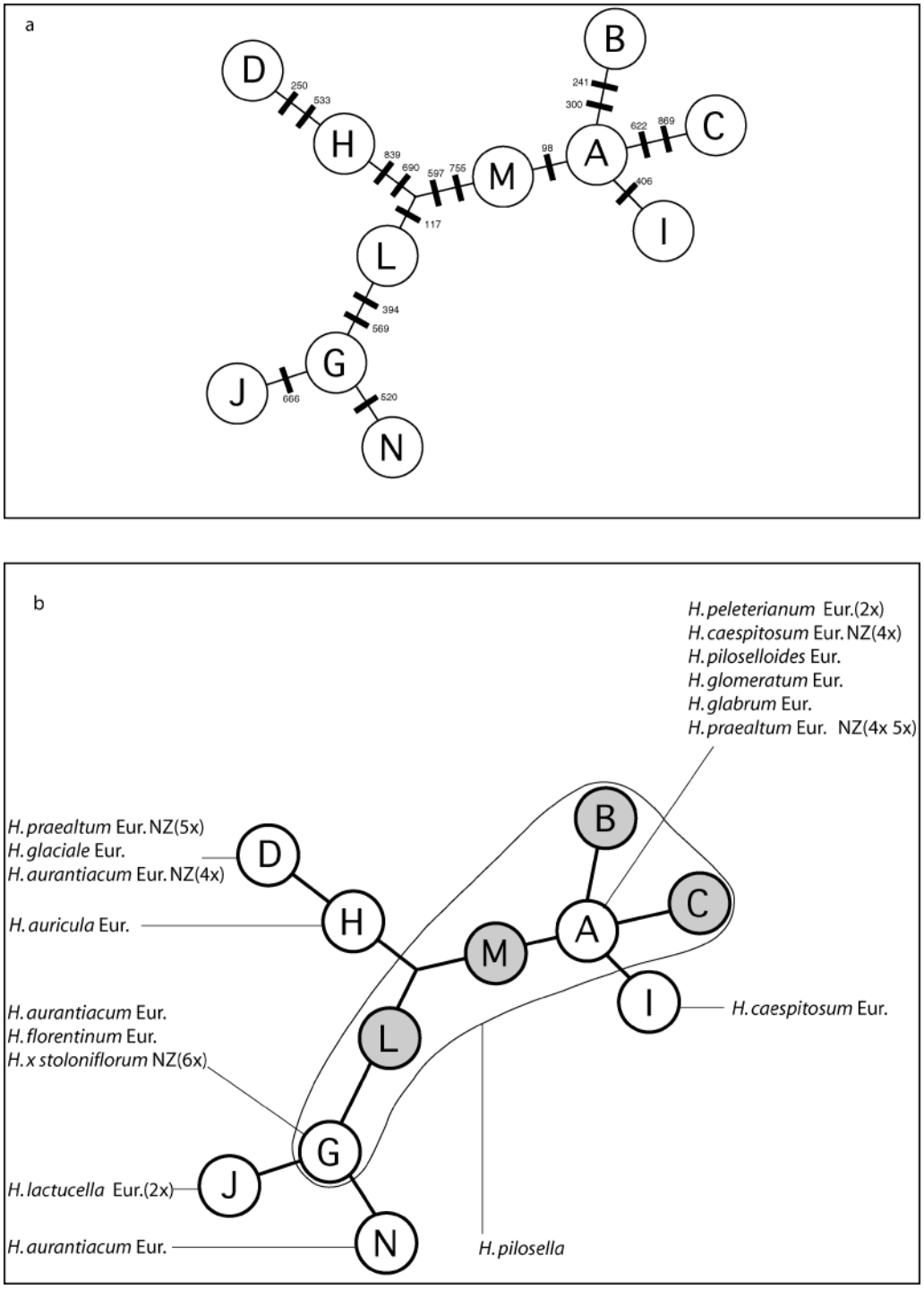

Fig. 3. Minimum spanning tree of 11 *Hieracium* subgenus *Pilosella* cpDNA haplotypes, with point mutations and tandem repeats scored equally as single events. (a) Bars indicate character state changes at 17 variable sites, numbered according to position in aligned sequence (see Table 1). (b) The species, in which the haplotypes were found, and their ploidy (where known). Grey fill indicates haplotypes found only in *H. pilosella*.

New Zealand arrived sometime in the mid-1800s (Travers, 1884). Introduction probably resulted from seed accidentally harvested with European grass seed used for development of New Zealand pastureland. It is assumed that the United Kingdom was the source of this seed but this is not supported by our data. We found more cpDNA haplotypes in New Zealand *H. pilosella* than in the United Kingdom. The two haplotypes in the United Kingdom (A, B) were ubiquitous throughout Europe. In particular, we did not find haplotype C in the United Kingdom, despite sampling from several populations. However, haplotype C was present in our smaller sample from eastern/northern Europe (Czech Republic, Finland). Also in our central European sample were *H. pilosella* and *H. aurantiacum* with haplotype G (Austria). Although G was not found in ei-

ther of these species sampled in New Zealand, it was present in samples of the hybrid taxon derived from these parentals (*H.* × *stoloniflorum*). We note, too, that New Zealand *H.* × *stoloniflorum*, which are evidently derived from introduced hybrids rather than arising here, are hexaploid (Jenkins and Jong, 1997; Morgan-Richards et al., in press), and this ploidy is reported from Bavaria (Germany) and the Czech Republic but not the United Kingdom (Krahulcova et al., 2000). It is significant too that all of the subgenus *Pilosella* taxa in New Zealand, except *H. pilosella*, exist only as garden escapes in the United Kingdom (Stace, 1997). The combined evidence unexpectedly indicates that some, if not all, extant New Zealand *Hieracium* taxa came from central Europe rather than the United Kingdom.

Invasion—Why some introduced taxa succeed in founding persistent populations is not well understood. Acclimatization societies in New Zealand actively introduced foreign organisms and thus supplemented the large number that arrived accidentally (Travers, 1884). A study of bird introductions to New Zealand revealed that colonization success might have as much to do with management as ecological characteristics of individual species (Veltman et al., 1996). In the case of *Hieracium pilosella*, it cannot be known which, and in what relative frequencies, lineages (genotypes) were initially introduced. Initial studies of *H. pilosella* ploidy in New Zealand indicated most plants were pentaploids and tetraploids were absent (Makepeace, 1981; Jenkins and Jong, 1997). Sexual tetraploids have since been found (Chapman et al., 2003), but so has evidence that apomict pentaploids are able to generate other ploidies through residual sex (Chapman and Bicknell, 2000; Houliston and Chapman, 2001). It has been inferred that tetraploid sexuals may have evolved in New Zealand (Chapman et al., 2003).

The present study indicates that at least one sexual tetraploid lineage (4× C) was introduced directly from Europe. To have evolved in New Zealand would require a population of haplotype C pentaploids and given that the level of residual sex in apomicts is low (up to 2.3%; Houliston and Chapman, 2001), a rather large population would be predicted. No haplotype C pentaploids have been found in New Zealand. All *H. pilosella* with haplotype C come from a small area in central South Island, and most individuals come from <25 m^2 of one site (Drac Flat). It is not surprising therefore that earlier surveys did not encounter these tetraploids. This scenario might also apply to haplotype A tetraploids, and it is interesting to note that all sexual tetraploids found so far come from sites in the vicinity of the earliest record of *Hieracium* in New Zealand (Travers, 1884). From our cpDNA and ploidy data, we can conservatively estimate that at least four different lineages (A, B, C, M) were introduced to New Zealand. There would have been a minimum of six lineages if shifts in ploidal level observed in plants with haplotype A occurred prior to colonization.

Although tetraploid *H. pilosella* are rather abundant in Europe and may have been relatively abundant in the introduced seed, it is likely that many features of pentaploids (e.g., facultative apomixis, more and longer stolons, more seeds; Gadella, 1987) gave them advantages as colonizers. Pentaploids have the potential to colonize from single seeds, whereas isolated tetraploids might soon fail as they are self-incompatible (Krahulcova et al., 2000). A rapid spread of pentaploids and localization of tetraploid sexuals can easily be visualized.

However, sexual tetraploids (whether introduced or derived) may be having a greater impact than previously realized. In New Zealand, *H. pilosella* unlike other related species (*H. caespitosum* and *H. praealtum*) appears to have rapidly increased in abundance since the 1960s (Connor, 1991; Treskonova, 1991; Scott, 1993a, b; Rose et al., 1995; Svavarsdóttir et al., 1999). Evidence from flow cytometry and crossing experiments indicate that this may be the result of interspecies hybridization (Morgan-Richards et al., in press). We have observed tetraploid interspecies hybrids (probably *H. pilosella* × *H. praealtum*) at five different sites, and sequences indicate that three different cpDNA lineages have been involved in the formation of *H. pilosella* hybrids (Table 4). Many of the hybrids are sexual and able to back cross to parentals, and morphological and flow cytometric data indicate that many putative pentaploid *H. pilosella* individuals throughout New Zealand are probably pentaploid hybrids (Morgan-Richards et al., in press). It is possible that interspecies hybridization has resulted in especially invasive lineages (Doyle et al., 1999; Ellstrand and Schierenbeck, 2000).

LITERATURE CITED

ABBOTT, R. J. 1992. Plant invasions, interspecific hybridization and the evolution of new plant taxa. *Trends in Ecology and Evolution* 7: 401–405.

ABBOTT, R. J., L. C. SMITH, R. I. MILNE, R. M. M. CRAWFORD, K. WOLFF, AND J. BALFOUR. 2000. Molecular analysis of plant migration and refugia in the Arctic. *Science* 289: 1343–1346.

BAYER, R. J., K. RITLAND, AND B. G. PURDY. 1990. Evidence of partial apomixis in *Antennaria media* (Asteraceae: Inuleae) detected by segregation of genetic markers. *American Journal of Botany* 77: 1078–1083.

BRIGGS, D., AND S. M. WALTERS. 1997. Plant variation and evolution, 3rd ed. Cambridge University Press, Cambridge, UK.

CHAPMAN, H. M., AND R. BICKNELL. 2000. Recovery of a sexual and an apomictic hybrid from crosses between facultative apomicts *Hieracium caespitosum* and *H. praealtum*. *New Zealand Journal of Ecology* 24: 81–85.

CHAPMAN, H. M., AND J. BROWN. 2001. 'Thawing' of 'frozen' variation in an adventive, facultatively apomictic, clonal weed. *Plant Species Biology* 16: 107–118.

CHAPMAN, H. M., AND S. C. LAMBIE. 2000. Chromosome numbers in New Zealand populations of *Pilosella officinarum* F. W. Shultz. & Sch. Bip. *International Organzation for Plant Biosystematics Newsletter* 31: 12.

CHAPMAN, H. M., D. PARH, AND H. ORAGUZIE. 2000. Genetic structure and colonizing success of a clonal, weedy species, *Pilosella officinarum* (Asteraceae). *Heredity* 84: 401–409.

CHAPMAN, H. M., B. ROBSON, AND I. ILENE. 2003. A case of reversal: the evolution and maintenance of sexuals from parthenogenetic clones in *Hieracium pilosella*. *International Journal of Plant Sciences* 164: 719–728.

COMES, H. P., AND R. J. ABBOTT. 2001. Molecular phylogeography, reticulation, and lineage sorting in Mediterranean *Senecio* sect. *Senecio* (Asteraceae). *Evolution* 55: 1943–1962.

CONNOR, H. E. 1991. Hawkweeds, *Hieracium* spp., in tussock grasslands of Canterbury, New Zealand, in the 1960s. *New Zealand Journal of Botany* 30: 219–282.

DOYLE, J. J., J. L. DOYLE, AND H. D. BROWN. 1999. Origins, colonization, and lineage recombination in a widespread perennial soybean polyploidy complex. *Proceedings of the National Academy of Sciences, USA* 96: 10 741–10 745.

ELLSTRAND, N. C., AND K. A. SCHIERENBECK. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences, USA* 97: 7043–7050.

GADELLA, T. W. J. 1987. Sexual tetraploid and apomictic pentaploid populations of *Hieracium pilosella*, Compositae. *Plant Systematics and Evolution* 157: 219–246.

GADELLA, T. W. J. 1991. Variation, hybridization and reproductive biology of *Hieracium pilosella* L. *Proceedings, Koninklijke Nederlandse Akadamie van Wetenschappen, Series C* 94: 455–488.

HEWITT, G. 1996. Some genetic consequences of ice ages, and their role in

divergence and speciation. *Biological Journal of the Linnean Society* 58: 247–276.

HOULISTON, G. J., AND H. M. CHAPMAN. 2001. Sexual reproduction in field populations of the facultative apomict, *Hieracium pilosella*. *New Zealand Journal of Botany* 39: 141–146.

JENKINS, T. A. 1991. A review of characteristics of mouse-ear hawkweed (*Hieracium pilosella*). *New Zealand Ecological Society Occasional Publication* 2: 15–23.

JENKINS, T. A., AND K. JONG. 1997. Significance of polyploid variation in New Zealand *Pilosella* and *Hieracium* (Asteraceae). *Botanical Journal of Scotland* 49: 75–87.

KING, L. M. 1993. Origins of genotypic variation in North American dandelions inferred from ribosomal DNA and chloroplast DNA restriction enzyme analysis. *Evolution* 47: 136–151.

KRAHULCOVA, A., F. KRAHULEC, AND H. M. CHAPMAN. 2000. Variation in *Hieracium* subgen. *Pilosella* (Asteraceae): what do we know about its sources? *Folia Geobotanica and Phytotaxonomica* 35: 319–338.

LEE, C. E. 2002. Evolutionary genetics of invasive species. *Trends in Ecology and Evolution* 17: 386–391.

MAKEPEACE, W. 1981. Polymorphism and chromosomal number in *Hieracium pilosella* L. in New Zealand. *New Zealand Journal of Botany* 19: 255–257.

MORGAN-RICHARDS, M., S. A. TREWICK, H. M. CHAPMAN, AND A. KRAHULCOVA. In press. Interspecific hybridization among *Hieracium* species in New Zealand: evidence from flow cytometry. *Heredity*.

NEUFFER, B., AND H. HURKA. 1999. Colonization history and introduction dynamics of *Capsella bursa-pastoris* (Brassicaceae) in North America: isozymes and quantitative traits. *Molecular Ecology* 8: 1667–1681.

NOGLER, G. A. 1984. Gametophytic apomixis. *In* B. M. Johri [ed.], Embryology of angiosperms, 475–518. Springer-Verlag, Berlin, Germany.

O'HANLON, P. C., R. PEAKALL, AND D. T. BRIESE. 1999. Amplified fragment length polymorphism (AFLP) reveals introgression in weedy *Onopordum* thistles: hybridization and invasion. *Molecular Ecology* 8: 1239–1246.

RAYBOULD, A. F., A. J. GRAY, M. J. LAWRENCE, AND D. F. MARSHALL. 1991. The evolution of *Spartina anglica* C. E. Hubbard (Gramineae): origin and genetic variability. *Biological Journal of the Linnean Society* 43: 111–126.

RICHARDS, A. J. 1996. Breeding systems in flowering plants and the control of variability. *Folia Geobotanica and Phytotaxonomica* 31: 283–293.

ROSE, A. B., S. K. WISER, K. H. PLATT, AND C. M. FRAMPTON. 1995. Vegetation change over 25 years in a New Zealand short-tussock grassland: effects of sheep grazing and exotic invasion. *New Zealand Journal of Ecology* 19: 163–174.

SCOTT, D. 1993a. Response of *Hieracium* in two long term manipulative agricultural trials. *New Zealand Journal of Ecology* 17: 41–46.

SCOTT, D. 1993b. Time segment analysis of permanent quadrat data: changes in *Hieracium* cover in the Waimakariri in 35 years. *New Zealand Journal of Ecology* 17: 53–57.

SELL, P. D., AND C. WEST. 1976. *Hieracium* L. *In* T. G. Tutin, V. H. Hewood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb [eds.], Flora Europaea, vol. 4, Plantaginaceae to Compositae (and Rubiacieae), 358–410. Cambridge University Press, Cambridge, UK.

SHI, Y., R. J. GORNALL, J. DRAPER, AND C. A. STACE. 1996. Intraspecific molecular variation in *Hieracium* sect. *Alpina* (Asterasceae), an apomictic group. *Folia Geobotanica and Phytotaxonomica* 31: 305–313.

STACE, C. 1997. New flora of the British Isles, 2nd ed. Cambridge University Press, Cambridge, UK.

STEBBINS, G. L. 1985. Polyploidy, hybridization, and the invasion of new habitats. *Annals of the Missouri Botanical Gardens* 72: 824–832.

SVAVARSDÓTTIR, K., J. G. PALMER, AND J. J. H. WHITE. 1999. Distribution of three *Hieracium* species in the Mt Possession area, mid Canterbury, New Zealand. *New Zealand Journal of Botany* 37: 469–477.

TABERLET, P., L. FUMAGALLI, A.-G. WUST-SAUCY, AND J.-F. COSSONS. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453–464.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.

TRAVERS, W. T. L. 1884. Acclimatisation in Canterbury. *New Zealand Country Journal* 8: 408–412 (and continued in vol. 8: 496–500, vol. 9: 259–264, vol. 10: 131–136).

TRESKONOVA, M. 1991. Changes in the structure of tall tussock grasslands and infestation by species of *Hieracium* in the MacKenzie country, New Zealand. *New Zealand Journal of Ecology* 15: 65–78.

VELTMAN, C. J., S. NEE, AND M. J. CRAWLEY. 1996. Correlates of introduction success in exotic New Zealand birds. *American Naturalist* 147: 542–557.

WEBB, C. J., W. R. SYKES, AND P. J. GARNOCK-JONES. 1988. Flora of New Zealand, vol. IV, Naturalised pteridophytes, gymnosperms, dicotyledons. Botany Division, Department of Scientific and Industrial Research, Christchurch, New Zealand.

WITTZELL, H. 1999. Chloroplast DNA variation and reticulate evolution in sexual and apomictic sections of dandelions. *Molecular Ecology* 8: 2023–2035.

ZHAN, K. H. 1923. *Hieracium*. *In* A. Engler [ed.], Das Pflanzenreich IV/280: 1147–1705.

APPENDIX 1. Locations in Europe sampled for *Hieracium* subgenus *Pilosella*, with details of incidence of haplotypes among locations and taxa. Initials of collectors are given and refer to the following: Ian Bennallick (IB), John Bailey (JB), Ross Bicknell (RB), Simon Brown (SB), Alisa Burns (AB), Isabel Christian (ICh), Judith Ferher (JF), Paul Green (PG), Gitta Grosskopf (GG), Quentin Kay (QK), John Killick (JK), Geoffrey Kitchener (GK), Frantisec Krahulek (FK), Anna Krahulková (AK), Roger Maskew (RM), Brigitte Morendo-Faure (NM-F), Tim Rich (TR), John Richards (JR), Peter Selby (PS), Clive Stace (CS), Tyler Törbjorn (TT), Ron Trewick (RT).

Country	Region	Location	Collector/ID	*pilosella*	*peleterianum*	*lactucella*
		Haplotypes per taxon—Europe		ABCGL	A	J
		Haplotypes per taxon—NZ		ABCM	—	—
Austria	Kleinwalsertal	Fellhorn	SB	A		
Austria	Kleinwalsertal	Riezlern-Gundkopf	HC	A		
Austria	Kleinwalsertal	near Muttelberg-Scharle	FK			
Austria	Kleinwalsertal	near Muttelberg-Scharle	HC	G G		
Austria		Steiermark	RB			
Austria			RB			
Czech Republic		Ricany	ICh	A		
Czech Republic		near Pilsen	FK	A		
Czech Republic		Olomouc	AK	A		
Czech Republic		Praha-Vysocany	AK			
Czech Republic		Roudnice	AK			
Czech Republic	Krkonese Mnts.	Jonaboden	JF	A C		
Czech Republic	Krkonese Mnts.	Spindlerbaud	JF			
Czech Republic	Krkonese Mnts.	Schwarzenbach	JF			
Czech Republic	Krkonese Mnts.	Nova Paka	FK	B		
Czech Republic	Krkonese Mnts.	Obribouda	FK	A		
Czech Republic	Krkonese Mnts.	Zadni Rennerovsky	FK			
Czech Republic	Krkonese Mnts.		AK, FK	A A		J
Czech Republic	Sumava Mnts.		AK	A		
Czech Republic			RB	A		
Iceland			RB		A	
Ireland	Waterford	Churchtown	CS	A		
Ireland	Waterford	Villierstown	CS	B		
Finland	Jaahdysploja		RB	C		
France		Caen	RB	B		
France		Piegro-la-Clastre	RT	L L		
France		Montaud	BM-F	A L		
France		Dijon (Botanical Garden)	RB			
Germany	Erzgebirge	Altenberg	JF			
Germany	Erzgebirge	Borna	JF			
Germany	Erzgebirge	Hermsdorf	JF	A		
Germany	Erzgebirge	Hirstein	JF			
Germany	Erzgebirge	Johanngeorgenstadt	JF			
Germany	Erzgebirge	Reitzenhain	JF			
Germany	Erzgebirge	Zweibach	JF			
Germany	Oberlausitz	Berzdorf	JF			
Germany	Oberlausitz	Goerlitz	JF			
Germany	Oberlausitz	Olbersdorf	JF			
Germany	Oberlausitz	Tauchritz	JF			
Germany	Schwarzwald	Belchen	GG	A		
Germany	Schwarzwald	Bernau	GG	A		
Poland		Jizerské Hory Mnts.	AK			
Slovakia		Tatry Mnts.	AK	C		
Sweden		Byske	TT			
Sweden		Degeberga	TT	B	A	
Sweden		Eksjö	TT			
Sweden		Lund	TT	A		
Sweden		Nora	TT	B		
Sweden		Nordingrå	TT	A		
Sweden		Överkalix	TT			
Sweden	Gotland	Fleringe	TT	A	A	
Sweden	Gotland	Hejnum	TT			
Sweden	Gotland	Rute	TT			
Switzerland		Delémont	GG	A		
Switzerland			RB			
United Kingdom		Abingdon	CS	B		
United Kingdom		Cobbler's Corner	CS	A A		
United Kingdom		Crindledyes Quarry	CS	B		
United Kingdom		Drakelow	CS	B		
United Kingdom		Eastleigh	CS			
United Kingdom		Farningham	CS	A		
United Kingdom	Leicestershire	Leicester	CS	B B		
United Kingdom	Leicestershire	Lutterworth	CS			

APPENDIX 1. Extended.

praealtum	*caespitosum*	*aurantiacum*	*pilosselloides*	*glomeratum*	*glabrum*	*glaciale*	*florentinum*	*auricula*	*stoioniflorum*
AD	AI	ADGN	A	A	A	D	G	H	—
AD	A	D	—	—	—	—	—	—	G
		G							
			A						
						D			
A									
A									
		D							
				A					
		N							
		D		A	A				
		D							
	A							H	
		D							
	I								
	A								
	I								
	A								
A			A						
			A						
		D							
	A			A					
		A							
	A								
	A								
							G		
D									
							G		
		D							
		D							
	A								

APPENDIX 1. Continued.

Country	Region	Location	Collector/ID	*pilosella*	*peleterianum*	*lactucella*
United Kingdom	Cornwall	Nare Head	CS	B		
United Kingdom	Cornwall	New Down Head	CS	A		
United Kingdom	Glamorgan	Cilibion	CS	A		
United Kingdom	Glamorgan	Connelly	CS	B		
United Kingdom	Glamorgan	Cwm Ivy Tor	CS	B		
United Kingdom	Glamorgan	Maesteg	CS	B		
United Kingdom	Gwyned	Criccieth	HC	B		
United Kingdom	Worcestershire	Coombe Green Common	CS	B		
United Kingdom	Worcestershire	Knightwick-on-Teme	CS	A		
United Kingdom	Worcestershire	Windmill Hill	CS	B		
United Kingdom	Worcestershire	Worcester Beacon	CS	B		

APPENDIX 1. Extended continued.

praealtum	*caespitosum*	*aurantiacum*	*pilosselloides*	*glomeratum*	*glabrum*	*glaciale*	*florentinum*	*auricula*	*stoioniflorum*