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Long-Term Studies of *Puccinia coronata* f. sp. *avenae* in Australia Reveal High Pathogenic Diversity, Regional Virulence Differences, Evidence of Clonality and Rapid Emergence of Virulence Matching Deployed Host Resistance

Robert F. Park[1] | Evsey Kosman[2] | Jeremy Roake[1] | Yi Ding[1] | Bruce Winter[3] | Pamela K. Zwer[4] | Davinder Singh[1]

[1]Plant Breeding Institute, School of Life and Environmental Science, Faculty of Science, The University of Sydney, Narellan, New South Wales, Australia | [2]Institute for Cereal Crops Research, School of Plant Sciences and Food Security, Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel | [3]Department of Agriculture and Fisheries, Toowoomba, Queensland, Australia | [4]Oatz Consulting, Adelaide, South Australia, Australia

Correspondence: Robert F. Park (robert.park@sydney.edu.au)

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ABSTRACT

Long-term, detailed Australia-wide studies of pathogenic variability in *Puccinia coronata* f. sp. *avenae* (Pca) were conducted from 1 April 1998 to 31 March 2024, along with analysing crown rust resistance in oat cultivars grown to examine its potential role in shaping Pca populations. A total of 2846 identifications of 172 pathotypes were made from 1559 rust samples. Six regional populations (Queensland [Qld], northern NSW [nNSW], southern NSW [sNSW], Victoria and Tasmania [V&T], South Australia [SA], Western Australia [WA]) became more variable over time, and variability decreased along an east-to-west gradient. Evidence was obtained for migration of at least five pathotypes between eastern and western cereal belts, indicating limited gene flow between them predominantly in a west-to-east direction. Two pathotypes were isolated throughout the study in most eastern regions, providing evidence of long-term persistence of pathogen clones. Two major shifts in the composition of regional populations were documented. The first involved rapid adaptation by Pca to *Avena sterilis*-derived resistance genes following their deployment in principally grazing oat cultivars in the subtropical region spanning nNSW and Qld. The second shift involved large changes between regional populations in Qld, nNSW, sNSW and WA beginning around 2011, and in V&T and SA around 2015, which were associated with the emergence, spread and increase of pathotypes typified by virulence for *Pc64*. Because Pca spreads rapidly throughout Australia and moves freely between grazing, hay and milling oat crops, reducing the impact of this damaging disease will require the deployment of durable resistance in oats of all end-uses.

1 | Introduction

Oat is a globally important crop that is grown principally for grain and fodder. Among the foliar pathogens impacting production in the leading oat-growing regions of the world, crown rust caused by the fungal pathogen *Puccinia coronata* f. sp. *avenae* (Pca) is considered the most damaging (Park et al. 2022). Pca is a heteroecious macrocyclic fungus, with the telial stage occurring on oat and some other grass species, and the aecial stage occurring principally on species of

Rhamnus (Simons 1970). The aecial stage has been reported in many oat-growing regions in the Northern Hemisphere where rhamnaceous hosts occur near oats (Simons 1970). In other regions such as South America (Simons 1970) and Australia, the alternate host is either absent or has not been found to be infected by Pca.

Efforts to develop oat cultivars with resistance to Pca have principally targeted the use of major resistance genes that are expressed at all growth stages (All Stage Resistance, ASR) (Park et al. 2022). These efforts have been underpinned by surveys of Pca assessing pathogenic variability (virulence/avirulence) on ASR, providing information that has been used to identify the best sources of crown rust resistance for oat improvement. Where an understanding of the ASR genes present in commercial oat cultivars has been available, surveys have allowed early warnings to be issued to growers on rust resistance 'breakdown' following the emergence of virulence matching deployed ASR genes (e.g., Park 2013). A detailed understanding of virulence/avirulence in specific rust isolates has also enabled geneticists and pre-breeders to identify, characterise, and map the genes that underlie ASR to facilitate their use in resistance breeding. Pathogenicity surveys that have retained core differential genotypes over time have also provided valuable insight into rust pathogen epidemiology and long-term population dynamics and evolution (e.g., Chong and Kolmer 1993; Moreau et al. 2024).

Studies of pathogenic variability in cereal rust pathogens in Australia were initiated at the University of Sydney in 1921 and in most cases have continued uninterrupted since. These studies initially targeted *Puccinia graminis* f. sp. *tritici* (Pgt), with *P. triticina*, *P. striiformis* f. sp. *tritici* (Pst), Pca, *P. graminis* f. sp. *avenae* and *P. hordei* commencing later. Due to the geographic isolation of Australia, it has been possible to hypothesise from detailed studies that populations of Pgt, *P. triticina* and Pst have been shaped by periodic exotic incursions of founding isolates, mutation leading to clonal lineages and asexual recombination (somatic hybridisation) (Park 2015). Virulence surveys for Pca in Australia were initiated at the University of Sydney in 1925. Waterhouse (1938) detected five races (pathotypes) of Pca based on the responses of 11 differential oat genotypes (Ruakura, Green Russian, Hawkeye, Anthony, Sunrise, Green Mountain, White Tartar, Red Rustproof, Sterisel, Belar, Glabrota). Two further differentials were added later (Victoria and Bond), both with uncharacterised ASR, and the 13 differentials allowed 13 pathotypes to be identified from 567 isolates collected from 1936 to 1951, mostly from New South Wales (NSW) but also from other regions of Australia and New Zealand (Waterhouse 1952). In 1952, an International Differential Set (IDS) that was in use in the United States and Canada was adopted, comprising 10 differentials that retained Anthony, Bond and Victoria (Baker and Upadhyaya 1955). The IDS set was used in Australia until 1995, being supplemented with additional differentials that were added as new ASR resistances were discovered and on occasion used in resistance breeding. The studies by Waterhouse (1938, 1952) and Baker and Upadhyaya (1955) clearly established that Pca in Australia was highly variable in virulence and that virulence was present for many ASR genes that had not been deployed. Later studies (Brouwer and Oates 1986; Oates et al. 1983; Park et al. 2000) found very distinct differences in the structure of populations of Pca present in eastern Australia and Western Australia (WA), greater pathotypic diversity and greater virulence in pathotypes present in Queensland (Qld) and northern New South Wales (nNSW). Studies of pathogenic variability among Australian isolates of Pca collected in 1993 by Bonnett (1996) documented prevalent virulence for eight of the 10 IDS genotypes in all Australian regions surveyed except WA. Based on this, a perceived lack of relevance of many of the ASR resistances represented in the IDS to oat breeding in Australia, and parallel studies of the effectiveness and genetic basis of ASR in oat lines that included the IDS and Australian supplementary differentials (Bonnett 1996), a new set of 29 differentials was adopted in 1995 (Oates et al. 1996; Tables S1 and S3). The new set was refined to 26 differential genotypes in 1998 (Table S1).

This study presents findings from comprehensive Australia-wide surveillance of Pca from 1 April 1998 to 31 March 2024, in which the pathogenicity of field-collected isolates was assessed using a set of 26 oat crown rust differentials. Hypotheses around temporal and spatial distributions of pathotypes and virulence for specific ASR genes were examined over the 26-year period, along with studies of the resistance of oat cultivars deployed over that time and their impact on populations of Pca.

2 | Materials and Methods

2.1 | Regions

Based on previous studies of pathogenic variability in Pca in Australia (Bonnett 1996; Brouwer and Oates 1986), six regions were used (Figure S1): Queensland (Qld) and northern New South Wales (nNSW) are characterised by a summer dominant rainfall pattern, which increases the likelihood for cereal rusts to over-summer; southern New South Wales (sNSW), Victoria and Tasmania (V&T) and South Australia (SA) are all characterised by a winter dominant rainfall pattern but are regarded as discrete on the basis of the different cultivars grown in each; Western Australia (WA) is isolated from the eastern Australian cereal-growing regions by more than 1000 km of arid land.

2.2 | Sample Collection, Storage, Host Differential Sets and Inoculation

Each survey period ran from 1 April until 31 March the following year. The survey periods are referred to by the 1 April to 31 December calendar year (e.g., 1 April 1998–31 March 1999 was referred to as '1998') because that is when cereal cropping occurs in Australia.

Samples of crown rust were collected from oat crops, experimental plots, or self-sown cultivated and wild oat plants by co-operators and forwarded to the University of Sydney's Plant Breeding Institute Cobbitty (PBIC) by post. Additional samples were collected by PBIC staff during regular surveys that involved random crop inspections every 20–30 km along predetermined routes. Rust samples were stored in paper envelopes and dried in a dehumidified cool room at 10°C–12°C, 12% RH. Rust isolates maintained good viability under these conditions for up to 3 weeks.

Plastic pots (95 mm diameter) were filled with a potting mixture of pine bark fines and coarse sand in a ratio of 4:1. A complete soluble fertiliser (Aquasol, 30 g/10 L of water for 200 pots) was applied to the pots and allowed to drain before sowing. Between 8 and 10 seeds of each oat genotype were sown with four or five lines planted as clumps in each pot. The seeds were covered with a 1–1.5 cm layer of potting mixture. The pots were moved to microclimate rooms at 18°C–20°C and irrigated daily. At 6–7 days after planting, Aquasol was applied a second time and seedlings were allowed to grow until the primary leaf had emerged fully.

Samples that lacked sufficient inoculum were first increased on the Australian oat cv. Swan by transferring inoculum from dried leaf samples to primary leaves with a spatula and incubating for 2 days in a chamber at 100% RH and 20°C–25°C. Field-collected samples with adequate inoculum were cut into short segments (c. 1 cm in length) and agitated in a light mineral oil (Pegasol 3440 Special; Mobil Oil) to produce a spore suspension that was applied directly to differential sets with an atomiser.

Inoculated differential sets were incubated in an insulated misting room at ambient temperatures in darkness for 18–24 h. Where accessions comprised more than one pathotype, and if it was not possible to identify the pathotypes present accurately, single pustule isolates were established from particular differential genotypes using the susceptible cv. Swan. Usually, one inoculation cycle on Swan was sufficient to generate the inoculum needed to inoculate a full differential set.

Inoculated differential sets were maintained in a greenhouse microclimate compartment with temperature controlled within the range 18°C–25°C, and with natural lighting. Results were recorded at 11–12 days post-inoculation using a 0–4 infection type (IT) scale (Nazareno et al. 2018). The infection types were: 0 = no visible symptoms, hypersensitive flecks, 1 = minute uredinia surrounded by mainly necrotic tissue, 2 = small to medium sized uredinia surrounded by chlorotic and/or necrotic tissue, 3 = medium uredinia with or without surrounding chlorosis, 4 = large uredinia without chlorosis, X = mesothetic, heterogeneous infection types similarly distributed over the leaf. The letters C and N were included to indicate greater than normal chlorosis or necrosis, respectively, and symbols − or + to indicate lower or higher infection types, respectively, than normal. Infection types of 3+ or higher were considered compatible (i.e., virulent pathogen/susceptible host). IT 3 was considered incompatible because of the presence of a host response that impeded pathogen infection. Such intermediate ITs have been reported in incompatible interactions in wheat for genes such as *Lr13*, the expression of which can vary from 1 to 3 depending on pathogen isolate, environmental conditions and host genetic background (McIntosh et al. 1995).

2.3 | Differential Genotypes

The differential stocks used in the current study were imported by staff at the University of Sydney from various researchers over many years. They were maintained through periodic seed increase and, critically, multipathotype rust testing using reference isolates of Pca of known virulence/avirulence to ensure both purity and correct identity.

Following Oates et al. (1996) and Bonnett (1996), a set of 26 differentials was used (Table S1). In the text that follows, differential lines that are reputed to carry single genes only are referred to as PC (e.g., PC36, PC38), and the resistance genes as *Pc* (e.g., *Pc36*, *Pc38*). Full virulence/avirulence formulae were determined for all pathotypes identified, and for ease of reference each unique pathotype was given a unique number. Five grazing oat cultivars that were considered resistant to crown rust when released were added to the differential set during the study (Gwydir, added in 1999; Nugene 2000; Volta 2006; Genie and Drover 2010) to monitor virulence on each.

2.4 | Multipathotype Crown Rust Tests of Australian Oat Cultivars

A total of 69 oat cultivars that were released in Australia from 1990 to 2020 (Tables 1 and S2a) were assessed for response to arrays of reference isolates of pathotypes of Pca that are maintained in liquid nitrogen at PBIC. Nine oat cultivars (Bass, Cooee, Dawson, Flinders, Galileo, Graza 80, Heritage Lordship, Mammoth, Warlock) released between 1990 and 2020 were not tested because seed was not available. The cultivars were tested in two experiments, the first comprised 38 cultivars that were tested with 19 pathotypes, and the second 31 cultivars that were tested with 23 pathotypes. Cultivars were sown and raised as outlined for pathotype testing. A set of differential lines was included for each pathotype. The cultivars and extended differential sets were inoculated using inoculum of each pathotype retrieved from long-term storage in liquid nitrogen at a rate of approximately 10–12 mg of spores/10 mL oil per 200 pots. Inoculated plants were given a dew treatment, moved to a microclimate room, and scored once rust had fully developed as described for pathotype analysis.

2.5 | Data Analysis

Analyses based on the annual Pca collections of isolates in the six Australian regions were not possible because of highly variable sample sizes and either very small numbers or no data for some regions in some years. Therefore, a preliminary analysis was performed to combine the samples from each of the six regions into a reduced number of time periods. The simple mismatch dissimilarities between virulence profiles of all 2846 Pca isolates identified were calculated and employed to generate a UPGMA dendrogram of relationships between the isolates (data not shown). Based on the isolate separation into clusters obtained in all regions and sampling details, nine time periods were assigned: 1998–1999, 2000–2001, 2002–2004, 2005–2007, 2008–2010, 2011–2014, 2015–2016, 2017–2020 and 2021–2023. Altogether, relationships and differences between 54 (6×9) region-period populations were analysed using diversity analysis tools.

Original virulence data were clone-corrected so that each group of isolates with identical virulence phenotypes was represented by one unique Pca phenotype in each annual regional population. All unique annual phenotypes were then grouped according to the nine survey periods (pools of two to four annual collections) in all regions. Each obtained population

TABLE 1 | Year and state of release, primary end use, parentage from pedigree and postulated All Stage Resistance genotypes of 69 Australian oat cultivars released between 1990 and 2020.

State of release and cultivar	Year of release/ virulence first detected[a]	Primary end use	Parentage from pedigree[b]	Postulated ASR genes
Queensland				
Amby	1991	Grazing	Cortez, Pendek, ME1563, C75-12, Coker227, Coker234, TAM0312, TAM0301, CI9221	*Pc61*[c]
Culgoa	1991	Grazing	Cortez, Pendek, C5-2, ME1563, TAM0312, Coker227, TAM0301, CI9221	*Pc39/55/71*, *Pc58/59*[c]
Panfive	1991	Grazing	Lodi, PI 267989	*Mortlock*[d]
Cleanleaf	1992	Grazing	ND78A211, ND78D316	*Pc38*, *Pc39/55/71*, *Pc52*[c]
Riel	1993	Grazing	RL3057, Otana	*Pc38*, *Pc39/55/71*[c]
Condamine	1994	Grazing	Coker227, Cortez, Pendek, ME1563, Coronado, SR cpx	*Pc61*[d]
Graza 51	1994	Grazing	Froker, RL3038, Hudson, Porter	*Pc68*[d]
Graza 70	1994	Grazing	Not available	*Pc38*, *Pc39/55/71*[c]
Nobby	1994	Grazing	Coker234, Coker227, TAM0301, TAM0312	*Pc58/59*, *Pc61*[c]
Barcoo	1996/2005	Grazing	Bordenave, Kenya SR res line 8025, 8014, Barrow, SR cpx, 1563 CR cpx	*Pc39*, *Pc58/59*, *Pc61*, *PcBett*[c]
Graza 68	1998/1999	Grazing	OT212, RL364	*Pc68*[c]
Moolah	1998	Grazing	PC68, Dumont	*Pc68*[c]
Gwydir	1999/2003	Grazing	Panfive, Q18994	*Pc36+*[c]
Warrego	1999/1998	Grazing	IL81-2570, ND840871	*Pc38*, *Pc61*[c]
Nugene	2000/2001	Grazing	ND881673 = R805065-5, ND880909 = ND830775, Dumont	*Pc48*[d]
Taipan	2001	Grazing	ND879845, ND890358	*Pc48*[c]
Volta	2003/2008	Grazing	37–9, Guiba Line	*Pc50*[e]
Drover	2006/2012	Grazing	ND90141, ND900118	*Pc91*[d]
Qantom	2006	Grazing	Not available	*Pc50*[d]
Graza 50	2007	Grazing	91RAT20, AC Medallion	*Pc38*, *Mortlock*[c]
Genie	2008/2011	Grazing	Not available	*Pc48*, *Pc56*[d]
Aladdin	2012	Grazing	Not available	*Pc91+* (*+Pc50*?)[d]
Comet	2014/2018	Grazing	Not available	*Pc91+* (*+PcWIX or Pc48*?)[d]
Wizard	2016	Grazing	Not available	*Pc50*[d]
New South Wales				
Bimbil	1993	Grazing	Cooba, TAM0301	*Pc58/59*[d]

(Continues)

TABLE 1 | (Continued)

State of release and cultivar	Year of release/ virulence first detected[a]	Primary end use	Parentage from pedigree[b]	Postulated ASR genes
Enterprise	1993	Grazing	W78-137, Omihi	*Pc39/55/71*[c]
Eurabbie	1998	Grazing	Echidna, Avon, Fulmark, Ballidu, Kent, Cooba	*Ruakura*[d]
Yiddah	2001	Hay/feed	Quaker82-238, Cooba, Yarran, Mortlock	Unknown[d]
Outback	2005	Grazing	Not available	*Pc38*, *Pc58/59*[d]
Mannus	2006	Feed grain	MA5103, TAMO386	Unknown[d]
Victoria and Tasmania				
Cluan	1990	Feed grain	Shaw, Ogle	*Pc39/55/71*[c]
Quamby	1990	Feed grain	OT52, RL3060	*Landhafer*, *Ukraine*, *Pc39/55/71*[c]
Targa	1999	Grazing	Quamby, Nile	*Pc1*[c]
South Australia				
Bandicoot	1991	Feed grain	Terra, West, CI4492, OX77, 119–3-2	*Landhafer*, *Santa Fe*, *Ukraine*[d]
Potoroo	1991	Feed grain	OX79, 119–20, OX80, 266-2H, Echidna	*Bond*, *Santa Fe*[c]
Bettong	1992	Hay	Coronado, Cortez, Pendek, ME1563	*Trispernia*, *Pc45*, *PcBett*[c]
Euro	1994	Milling	Mortlock, Echidna	*Mortlock*[c]
Glider	1998	Hay	ME1554, Crcpx, C7512, Srcpx	*Pc39/55/71*, *Pc58/59*[c]
Numbat	1998	Feed grain	Bandicoot, OX82, 059–78 (= Mortlock/Echidna)	*Landhafer*, *Ukraine*, *Trispernia*[c]
Quoll	1998	Feed grain	MIOLRP-86-3, Bandicoot	*Pc61*[c]
Possum	2002	Milling	ND863468, OX82059-58-10, Carrolu	*Pc48*[d]
Wintaroo	2002	Hay	MIOLRP-86-3, Echidna, Wallaroo	*Ukraine*[d]
Brusher	2003	Hay	Dumont, Wallaroo, Bandicoot	*Pc39/55/71*[d]
Kangaroo	2005	Hay	88,123-104, 84Q406, 86,153–101	*Ukraine+*[d]
Mitika	2005	Milling	87,072-13, 87,080–1, 88,045–12	*Pc39/55/71*[d]
Mulgara	2009	Hay	89,030-26, Quaker 93–112	Unknown[d]
Yallara	2009	Milling	Euro, ND931075 or ND9308572	*Pc38*[d]
Tungoo	2010	Hay	Not available	*Pc39/55/71*[d]
Dunnart	2012	Milling	Toodyay, 91,165–3, 92,029–42	*Landhafer*, *Santa Fe*, *Ukraine*[d]

(Continues)

TABLE 1 | (Continued)

State of release and cultivar	Year of release/ virulence first detected[a]	Primary end use	Parentage from pedigree[b]	Postulated ASR genes
Forester	2012	Hay	OT285, OX92, 056–4	*Pc38*, *Pc39/55/71*[d]
Tammar	2012	Hay	Not available	*Pc58/59* +[d]
Wombat	2012	Milling	Possum, 82,059–58-10, Bettong, Potoroo	*Landhafer*, *Santa Fe*, *Ukraine*[d]
Kowari	2017	Milling	Mitika, WAOAT2099	*Pc39/55/71*[d]
Bilby	2019	Milling	98,011-6, 98,240–19	*Landhafer*, *Santa Fe*, *Ukraine*[d]
Koorabup	2019	Hay	96Q791-17-M32, 96Q780-34-M33	*Landhafer*, *Santa Fe*, *Ukraine*[d]
Western Australia				
Yilgarn	1991	Feed grain	Unknown, Swan, Nora	*Pc1*, *Trispernia*, *Mortlock*[c]
Carrolup	1993	Milling	Mortlock, Kent, Ballidu, Curt, Cortez, TAMO312, West	*Mortlock*[c]
Pallinup	1994	Milling	M127	*Landhafer*, *Ukraine*, *Mortlock*[c]
Coomallo	1996	Milling	Brooks, Lort, Mortlock	*Mortlock*[c]
Toodyay	1996	Milling	Mortlock, Fulmark, Newton, Swan, XBVT183, IORN78.45, Dwarf, Paletine, M127, Curt	*Pc38*, *Mortlock*[c]
Hay	1998	Hay	OT207, Swan	*Ukraine*[c]
Hotham	1998	Milling	West, Spear, Mortlock	*Landhafer*, *Trispernia*, *Mortlock*[c]
Needilup	1998	Feed grain	Avon, Swan, Jaycee, OT207, Moregrain, West	*Pc1*[c]
Vasse	1998	Hay	IORN-82-47, 75Q:198 (OT207, Swan fixed)	*Mortlock*+[d]
Wandering	1999	Milling	Mortlock, Echidna, OT207, Swan	*Pc1*[d]
Kojonup	2006	Milling	CI, CC, OT207, Mortlock, Coomallo	*Pc1*[d]
Bannister	2012	Milling	Dumont, Echidna, Mortlock, 75Q:198, Swan, Fulmark, Newton	*Pc39/55/71*[d]
Williams	2013	Milling	90Q209-21-34, 93Q496-13	Unknown[d]
Durack	2016	Milling	Not available	Unknown[d]

[a]Nine examples are provided of first detections of virulence matching resistance in a cultivar.
[b]Full pedigrees where available are provided in Table S2a.
[c]Data for ASR gene postulation provided in Table S2c.
[d]Data for ASR gene postulation provided in Table S2b.
[e]Data for ASR gene postulation provided in Table S2d.

could include identical phenotypes if they appeared in corresponding annual collections. In other words, virulence data were clone-corrected annually, and each unique phenotype in each time period was considered with its annual abundance (number of years it was detected in). Variability within and among the Pca populations was further analysed using such clone-corrected data.

Structure and relationships between the 54 regional Pca populations in the nine survey periods were analysed using the assignment-based approach applied to the simple mismatch dissimilarities between virulence phenotypes. The corresponding *KW* dispersion within, and *KB* distance between, populations were calculated (Kosman 1996, 2014; Kosman and Leonard 2007). The *KB* distance between populations can be interpreted as a normalised minimum total number of 'mutations' (interchange virulence-avirulence) that are needed to transform all individual virulence phenotypes of one population into the phenotypes of all individuals of the second population. The *KW* dispersion within a population can be understood as a normalised maximum total number of 'mutations' that are needed to convert each individual phenotype into another phenotype in the population so that the population remains the same. Both metrics *KB* and *KW* are obtained by an optimal one-to-one assignment between individuals (with no matching an individual to itself in the case of *KW*). Differentiation among populations was estimated with the permutation test (1000 random partitions) for differentiation statistics dif_{KW} (Czajowski et al. 2021) based on the *KW* dispersion (see also Kosman et al. 2014; eqn 13 and Kosman et al. 2014).

The number of effectively different phenotypes within a population ${}^1D(T, KW)$ was estimated with the metric of functional trait dispersion (eqns 5, 6 in Scheiner et al. (2017), for $M = KW$). The effective number ranges from 1 (all phenotypes are identical) to an actual number of phenotypes when they are absolutely different. The normalised version of this indicator ${}^1nD(T, KW)$ (Kosman et al. 2019, corrected eqn 5; Sun et al. 2020, eqn 3) expresses an extent of variability within a population independently of sample size (actual number of phenotypes in a given population).

The effective number of different Pca populations ${}^1D(ADW_{KB})$ was calculated based on the *KB* distances between populations (only polymorphic virulence loci were included) and the $ADW = ADW_{KB}$ dispersion (*Average Distance* between populations *Within* a given set of populations) with regard to the *KB* distance according to Kosman et al. (2024). The effective number ranges from 1 (all populations are identical) to an actual number of populations when they are absolutely different.

UPGMA dendrograms of relationships among the Pca isolates and virulence phenotypes with regard to the simple mismatch dissimilarity and among populations with regard to the *KB* distance between them were generated using the SAHN program of the NTSYSpc package v. 2.2 (Exeter Software). Other above-mentioned calculations were performed with VIRULENCE ANALYSIS TOOL (VAT) software (Kosman et al. 2008; Schachtel et al. 2012) and FUNCTIONAL DIVERSITY ANALYSIS TOOLS (FDAT) software. Both packages are available at https://en-lifesci.tau.ac.il/profile/kosman.

3 | Results

3.1 | Differential Genotypes and Australian Oat Cultivars

Previous studies with most of the 26 differential stocks used in the present study reported a very close phenotypic association of virulence of the sources used for *Pc58* (TAM 0–301), *Pc59* (TAM 0–312) and *Pc61* (Coker 234), with only one possible example of an isolate differing in virulence on the two TAM lines used (Bonnett 1996). All isolates examined in the present study generated identical responses on TAM 0–301 and TAM 0–312, suggesting that the sources used for these two differentials are identical. Consequently, the data for only one of these (TAM 0–312) was used in the data analyses, meaning that 25 differentials were used to define pathotypes. Given the identical responses of the two TAM lines, it was not possible to discriminate between *Pc58* and *Pc59* in gene postulation studies, and so wherever the TAM resistance specificity was observed, it was postulated as *Pc58/59*.

The Australian oat cv. Swan (Kent/Ballidu) was released in 1967 in WA. It was used as a crown rust susceptible check in studies by Bonnett (1996) and included in the differential set introduced in 1995 as a susceptible control (Oates et al. 1996). Of the 543 isolates of pathotypes identified in 1998, 15 originating from nNSW, sNSW, V&T and SA produced an incompatible level 2 infection type on Swan, demonstrating the presence of an uncharacterised resistance of intermediate effect. Subsequent studies using isolates of contrasting virulence on Swan showed that its responses were the same as those for Red Rustproof, which carries *Pc1*, suggesting that the resistance gene in Swan is *Pc1* (Table S2b).

Sixty-nine Australian oat cultivars released in Australia between 1990 and 2020 were tested for seedling-stage crown rust response using either 23 (31 cultivars) or 19 (38 cultivars) pathotypes for postulation of the ASR (major) resistance genes in each. Where available, pedigrees of the oat cultivars were used to assist in resistance gene identification (Table S2a). Crown rust resistance of five cultivars (Durack, Mannus, Mulgara, Williams, Yiddah) could not be postulated due to heterogeneity in rust response (Tables 1 and S2b). Resistance specificity for *Pc39*, *Pc55* and *Pc71* could not be discriminated with the pathotypes used and was therefore referred to as *Pc39/55/71*.

Twenty-nine cultivars were grazing oats released and grown principally in Qld and nNSW, of which all but three (Eurabbie [Ruakura resistance], Panfive [Mortlock resistance], Targa [*Pc1*]) were postulated to carry one or more of the *Avena sterilis*-derived resistance genes *Pc38*, *Pc39/55/71*, *Pc45*, *Pc48*, *Pc50*, *Pc52*, *Pc56*, *Pc58/59*, *Pc61*, *Pc68* or *Pc91* (Table 1). Although five of these genes were postulated in cultivars of other end-use classes (viz., *Pc38*, *Pc39/55/71*, *Pc48*, *Pc58/59*, *Pc61*), nearly all were first deployed in Australia in grazing oat cultivars. At

least 13 of these cultivars were released in Qld or nNSW from direct selections from oat lines bred in Canada (viz., Graza 50 [aka Valley; *Pc38*, *Pc39*/*55*/*71*, *Pc58*/*Pc59*], Graza 51 [*Pc38*, *Pc39*/*55*/*71*, *Pc68*], Graza 68 [aka AC Assiniboia, *Pc38*, *Pc39*, *Pc68*; McCallum et al. (2007)], Moola [aka AC Medallion, *Pc38*, *Pc39*, *Pc68*; McCallum et al. (2007)], Panfive [Mortlock resistance], Riel [*Pc38*, *Pc39*]) or the United States (Amby II [*Pc61*], Barcoo [complex, including *Pc39*/*55*/*71* and *Pc61*], Cleanleaf [*Pc38*, *Pc39*/*55*/*71*, *Pc52*], Condamine [*Pc61*+], Culgoa II [*Pc39*, *Pc58*/*59*], Nobby [*Pc58*/*59*, *Pc60*, *Pc61*], Warrego [*Pc38*, *Pc61*]). An uncharacterised resistance gene *PcBett* (Bonnett 1996) was postulated in both Bettong and Barcoo, along with additional known or unknown resistance genes (Table 1). About half of the non-grazing oat cultivars were postulated to lack *A. sterilis*-derived resistances and to carry *A. sativa*-derived crown rust resistance genes that were first deployed prior to 1990 in Australia (Table 1). Some grazing oats may also carry these older resistances, as pathotypes virulent on *A. sterilis*-derived genes were also virulent on these pre-1990 resistances.

Cultivar Volta (37-9/Guiaba line) was postulated to carry *Pc50* (Table 1). The seed used in these tests was obtained in 2004 from the breeder of Volta, Dr. Leonard Song, who also provided a parental seed source of Volta (9715A-1) and sources of the two parents of Volta, Guiaba line (female) and 37–9 (male). Initial multipathotype tests of all four lines conducted in 2004 showed that while the pure seed accession carried *Pc50*, 9715A-1 carried *Pc91* and the two parental lines Guiaba line and 37–9 lacked either gene (Table S2d).

Adult plant crown rust field ratings were obtained for 67 of the 69 cultivars from annual extension bulletins released by agricultural agencies in Australia (Table S2e). Sixty cultivars were rated as moderately susceptible to very susceptible, two as moderately resistant to moderately susceptible, two as moderately resistant, and three as resistant but with a warning that a pathotype with virulence matching the resistance in these cultivars had been detected but was rare in the pathogen population.

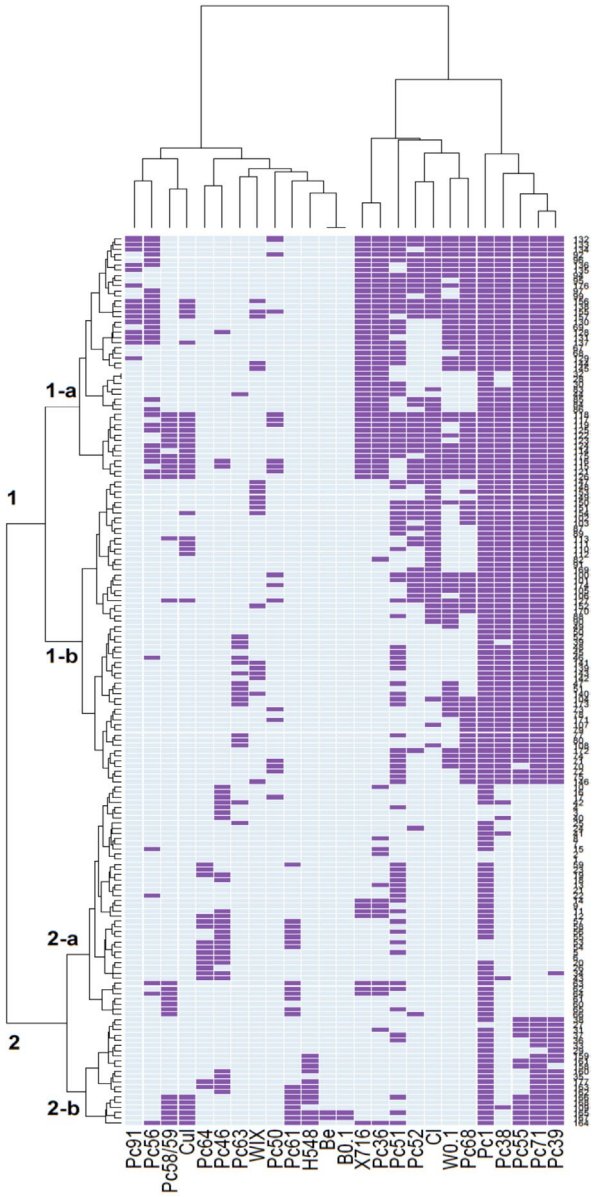

FIGURE 1 | Heatmap showing the virulence (purple)/avirulence (grey) of 172 Australian pathotypes of *Puccinia coronata* f. sp. *avenae* isolated between 1998 and 2023. Dendrograms reflect hierarchical clustering of pathotypes with similar virulence (rows) and differential responses (columns).

3.2 | Rust Samples

A total of 1850 samples were received for pathotype analysis, of which 291 failed to yield a viable isolate. Of the remaining 1559 samples, 773 comprised crown rusted cultivated oat, 768 crown rusted wild oat and 18 crown rust on an unidentified grass species. The number of samples received in each year varied greatly (maximum 299, 1998; minimum nine, 2019), principally due to environmental conditions and the incidence of crown rust on wild oats and in oat crops. A total of 2846 identifications of 172 pathotypes (Table S3) were made from the 1559 samples that yielded a viable isolate.

3.3 | Pathotypes Detected

Many of the 172 pathotypes identified were isolated from more than one region and over multiple years. The four most common pathotypes were pt. 7 (627 isolates, present in all regions in most years); pt. 61 (379 isolates, from all regions but principally Qld, nNSW and sNSW in 1998–2023); pt. 91 (176 isolates, from Qld, nNSW, sNSW and V&T in 1998–2001); pt. 3 (161 isolates, from all regions but most common in V&T and SA prior to 2015) (Tables S3 and S4a–f). Of the remaining 168 pathotypes, 60 were isolated once only, 17 twice, 67 from 3 to 20 times, 13 from 21 to 40 times and 11 from 41 to 64 times (Table S3).

The least virulent pathotype was pt. 1, which was avirulent on all 25 differential lines, and the most virulent was pt. 155, which was virulent on 17 of the 25 differential lines (Table S3). The 172 pathotypes clustered into two major groups based on virulence/avirulence on the 25 differentials, within which two further subgroups were apparent (Figure 1). The 106 pathotypes within Cluster 1 were characterised as being virulent on more differentials than those in Cluster 2 (Figure 1). Virulence for genes *Pc38*, *Pc39*, *Pc55*, *Pc56* and *Pc71* was very common among these

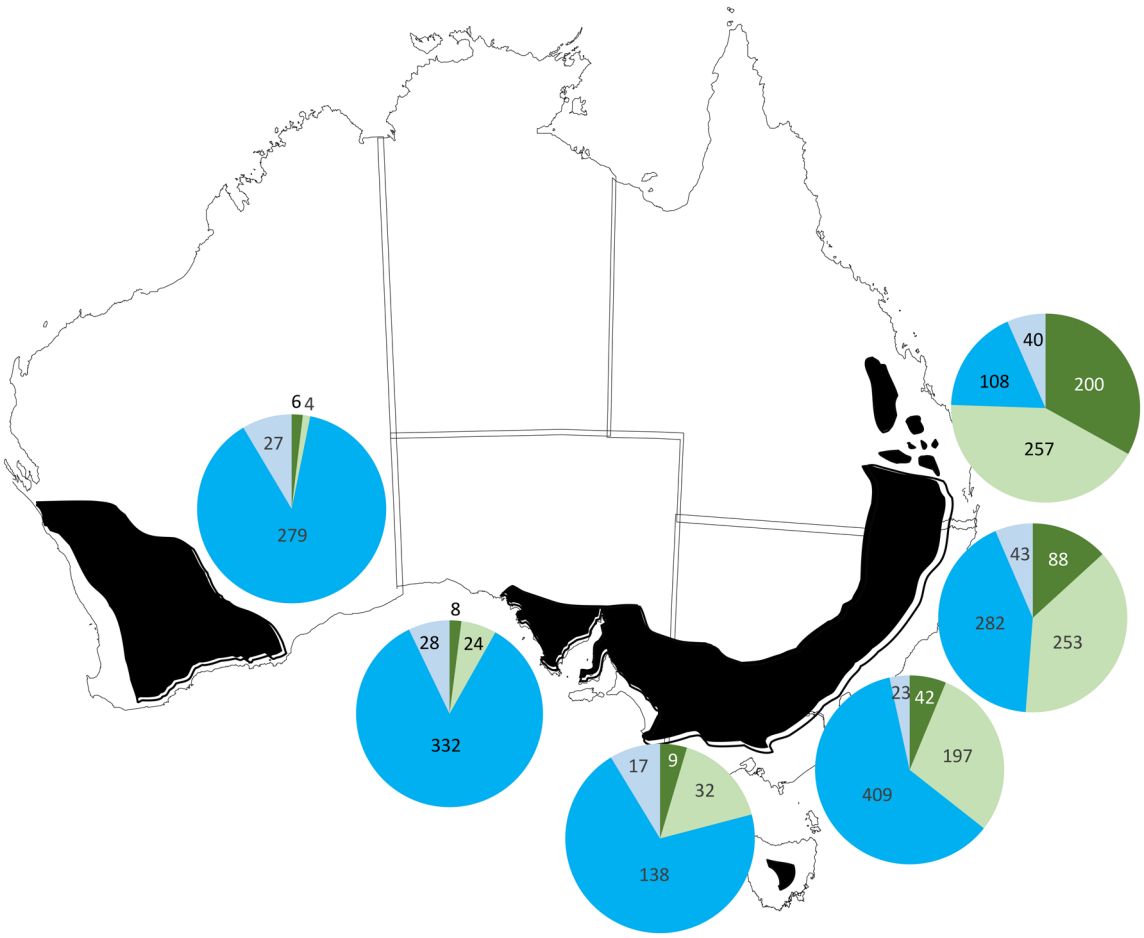

FIGURE 2 | Frequency of isolates of pathotypes of *Puccinia coronata* f. sp. *avenae* in Cluster 1 (Cluster 1-a, dark green; Cluster 1-b light green) and Cluster 2 (Cluster 2-a dark blue; Cluster 2-b light blue) in six regions of Australia (Queensland, northern New South Wales, southern New South Wales, Victoria and Tasmania, South Australia, Western Australia) from 1998 to 2023. The number of isolates in each cluster is also presented.

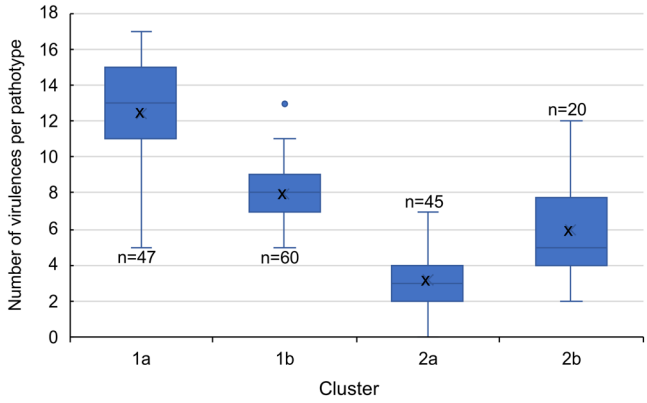

FIGURE 3 | Number of virulences on 25 differential lines for 172 pathotypes of *Puccinia coronata* f. sp. *avenae* in four clusters from Figure 1. The lower and upper quartiles are displayed as whiskers above and below the blue box, which represents the range within which 50% of the data fall. The × in each blue box is the mean value.

pathotypes, and virulence for genes *Pc50*, *Pc68*, *Pc91*, and the resistances in WIX4361-9, Cleanleaf and Warrego was exclusive to these pathotypes. Cluster 1 pathotypes dominated populations in Qld (76% of isolates; Figure 2), comprised about half of all isolates identified from nNSW, were common in sNSW, less common in V&T, and were isolated rarely from SA and WA (Figure 2). Cluster 2 comprised 66 pathotypes, members of which dominated populations in SA and WA and were common in the other regions (Figure 2). These pathotypes were in general less virulent than those in Cluster 1 (Figure 3), and included avirulences on Swan, and virulences on *Pc46*, *Pc61* and *Pc64* plus uncharacterised resistances in H548, Bettong and Barcoo.

3.4 | Virulence

Virulence was detected at least once for all 25 differentials (Table S3). Virulences for H548, WIX4361-9, and *Pc63* were present but uncommon in all regions in 1998–2023 (mean virulence over the nine periods, 4.1%–6.6%, 0.8%–5.1% and 0.8%–8.1%, respectively) (Table S5a). Virulence for cv. Swan was very common in all regions (69.2%–99.4%). Virulence on cvs Bettong and Barcoo was completely associated, being detected in two pathotypes (pts. 165 and 167) that differed only in virulence/avirulence for *Pc51* (Table S3). It was first detected in Qld in 2001, and although one or both pathotypes were isolated from all regions except WA, they were rare and found mostly in Qld and nNSW (Table S4a–f).

Virulence on the *Pc61* differential Coker 234 was detected in all regions but varied between regions and over time (Table S5a). It was common in Qld, nNSW and sNSW from 1998, principally due to the presence of pts. 61–66 and 164–168. Virulence on *Pc61* was rare in WA until 2015–2016, when it increased rapidly and remained common until 2023 (Figure 4; Table S5b). This was associated with the emergence and increase of pts. 53–59, of which pts. 57 and 58 were later detected in SA and then in V&T where they also increased in frequency (Tables S4e, S4d, respectively).

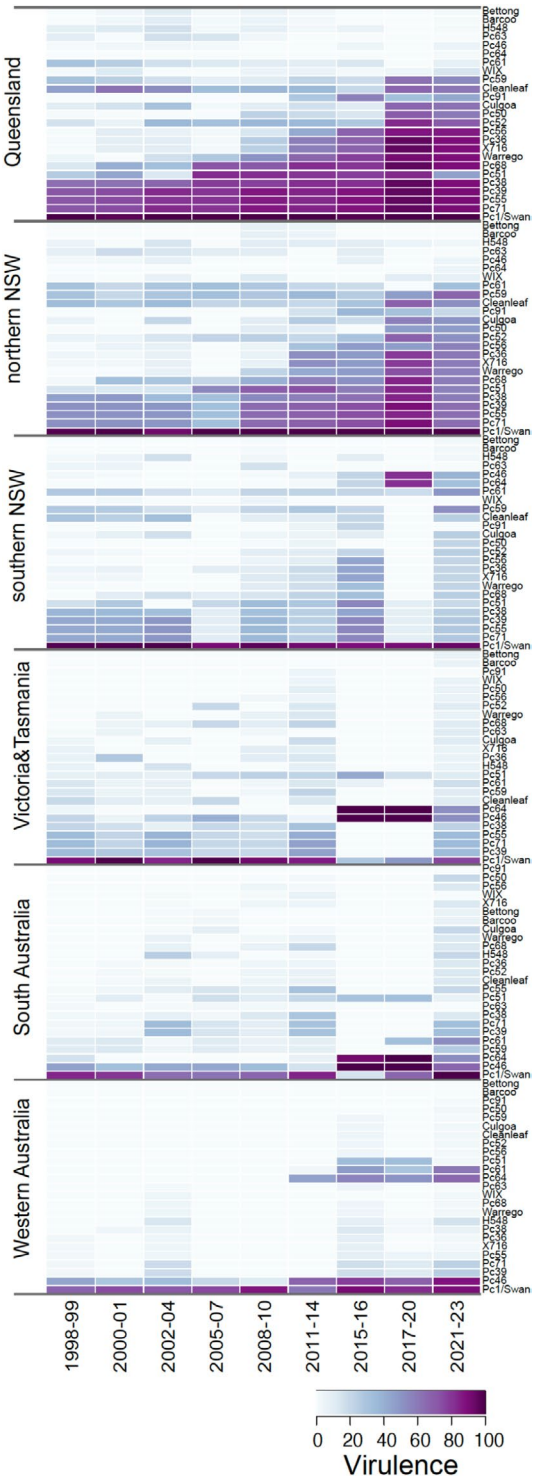

FIGURE 4 | Virulence frequencies for 25 oat crown rust differential lines in six regions of Australia from 1998 to 2023 (further details are provided in Table S5a).

Large regional differences were apparent in virulence for the remaining 18 differentials (Figure 4; Table S5a,b). Virulence for 16 differentials (PC36, PC38, PC39, PC50, PC51, PC52, PC55, PC56, PC58/59, PC68, PC71, PC91, Culgoa, Cleanleaf, Warrego and X716) was more frequent in Qld, nNSW and sNSW than in V&T, SA and WA, with the reverse for PC46 and PC64, with virulence being more common in V&T, SA and WA than it was in Qld and nNSW (Figure 4; Table S5a,b). Most virulences that were more frequent in Qld and nNSW were confined to pathotypes in Cluster 1 (Figures 1 and 2). These included virulences for *Pc68* and *Pc91*, which were detected for the first time following the first deployment of these genes (*Pc91* in cv. Drover, Figure S2a,b; *Pc68* in cv. Graza 68 and Moola), and virulences for *Pc36* and *Pc50*, which were present at low frequencies but increased rapidly following first deployment (*Pc36* in cv. Gwydir, Figure S2c,d; *Pc50* in cv. Volta, Figure S2e,f). Virulence matching the resistances in cvs Warrego (Figure S2g), Nugene (Figure S2h) and Genie (Figure S2i) were also either low or absent at the time of cultivar release but increased afterwards. The average number of years for virulence to be detected following the release of these cultivars, plus Barcoo and Comet, ranged from −1 (Warrego, virulence was detected in a seed increase block pre-release) to 9 (Barcoo) (Table 1), with an average of 3.4 years.

3.5 | Virulence Complexity

Mean virulence (i.e., average of the number of the 25 differentials rendered susceptible by each pathotype) over the 26-year period was highest in Qld (9.4, range 4.0–16.0), decreasing across the remaining regions with WA being the lowest (viz., nNSW 7.9, range 1.0–15.8; sNSW 5.1, range 1.0–9.4; V&T 3.7, range 1.0–8.0; SA 3.2, range 1.0–6.5; WA 2.7, range 1.0–6.9).

Clear and significant ($p < 0.001$) trends of increasing mean virulence over time were apparent in Qld and nNSW (Figure 5), and although smaller increases were apparent in sNSW, SA and WA, only that of WA was significant ($p < 0.05$). There was evidence of sequential mutational acquisition of virulence for specific resistance genes among pathotypes in Cluster 1 (Figure 1), which paralleled the deployment of these genes in agriculture. Table S6 provides examples of five pathotypes presumed to be stepwise mutational derivatives of pt. 50, with sequential virulence gains for five resistance genes that were first detected in Qld in 1998 (pt. 169, *Pc52*; Park et al. 2000), 2003 (pt. 106, *Pc68*; pt. 105, *Pc61*) and 2008 (pt. 174, *Pc50*; pt. 100, *Pc51*). Pt. 155, first detected in Qld and nNSW in 2018, appears most likely to be a mutational descendant of pt. 100 (first detected Qld, 2008; Table S4a) with six further additional virulences (*PcWIX*, *PcCul*, *Pc91*, *Pc36*, *Pc56*, X716).

3.6 | Variability Between Populations

Structural variability and relationships of the regional and annual populations of Pca were analysed using assignment-based

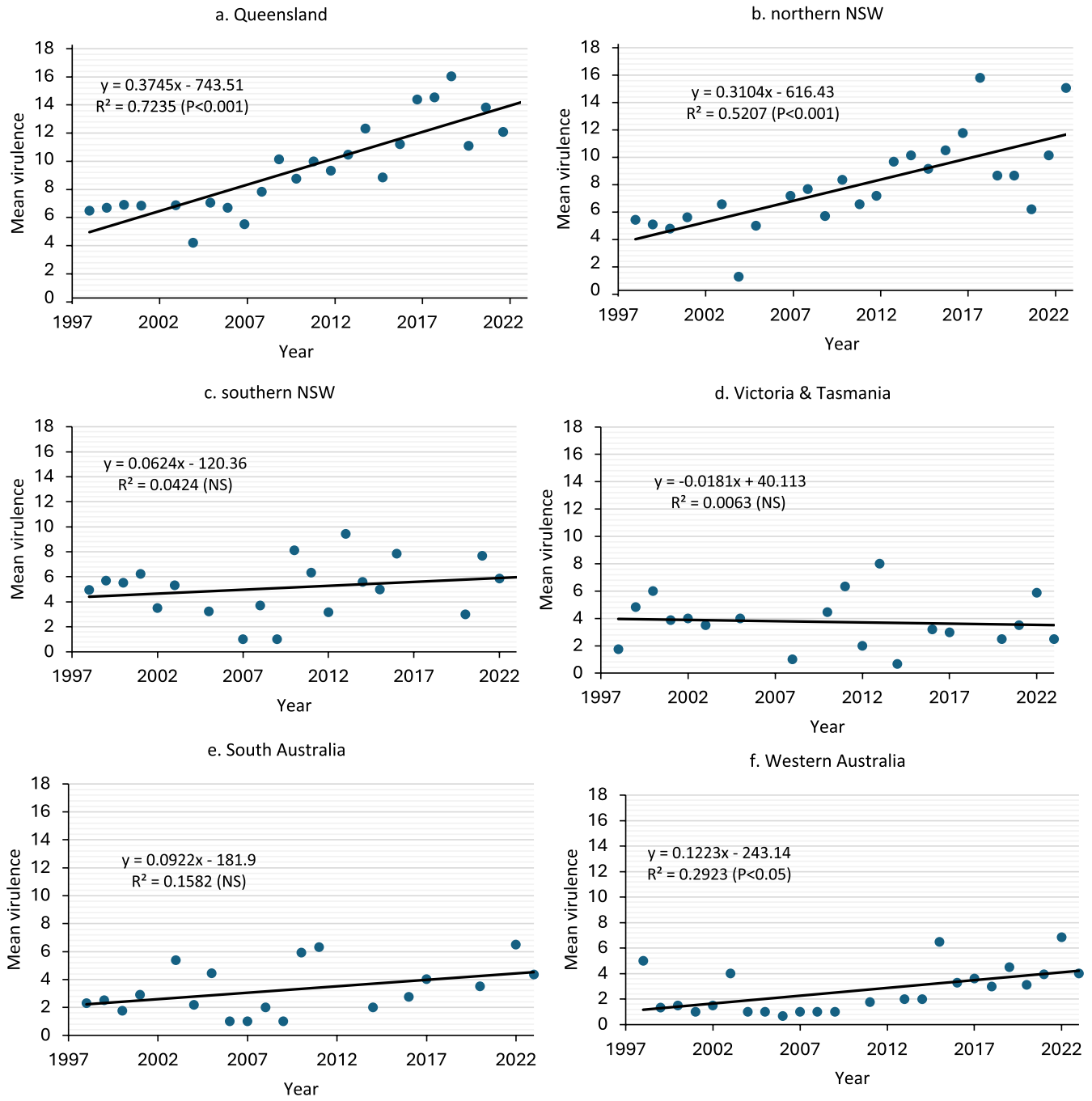

FIGURE 5 | Mean virulence of pathotypes of *Puccinia coronata* f. sp. *avenae* identified using 25 differential genotypes annually from 1998 to 2023 in six regions of Australia: (a) Queensland, (b) northern New South Wales (NSW), (c) southern NSW, (d) Victoria and Tasmania, (e) South Australia and (f) Western Australia.

metrics and approaches for virulence data. Figure 6 shows an unweighted pair-group method with arithmetic mean (UPGMA) dendrogram displaying relationships among 54 populations of Pca in Australia between 1998 and 2023. This dendrogram, based on the KB_m distance between populations and simple mismatch dissimilarity m between binary clone-corrected virulence phenotypes of isolates in each population, groups populations by proximity (overall similarity of pathotypes), providing an initial representation of the putative structure of the Australian Pca populations and extent of separation between different clusters. Overall, the dendrogram demonstrated a clear split of the Pca populations both in time and space.

Populations in Qld and nNSW from 2011 to 2023 (Cluster B, Figure 6) differed clearly from all other Pca populations (Cluster A, Figure 6). At a finer resolution, differences were apparent between populations in Qld and nNSW from 2011 to 2016 and from those of 2017–2023. The larger difference between all regions versus Qld and nNSW during 2011–2023 was due to changes in the composition of Pca pathotypes caused by the emergence, spread, and increase in frequency of a group of pathotypes

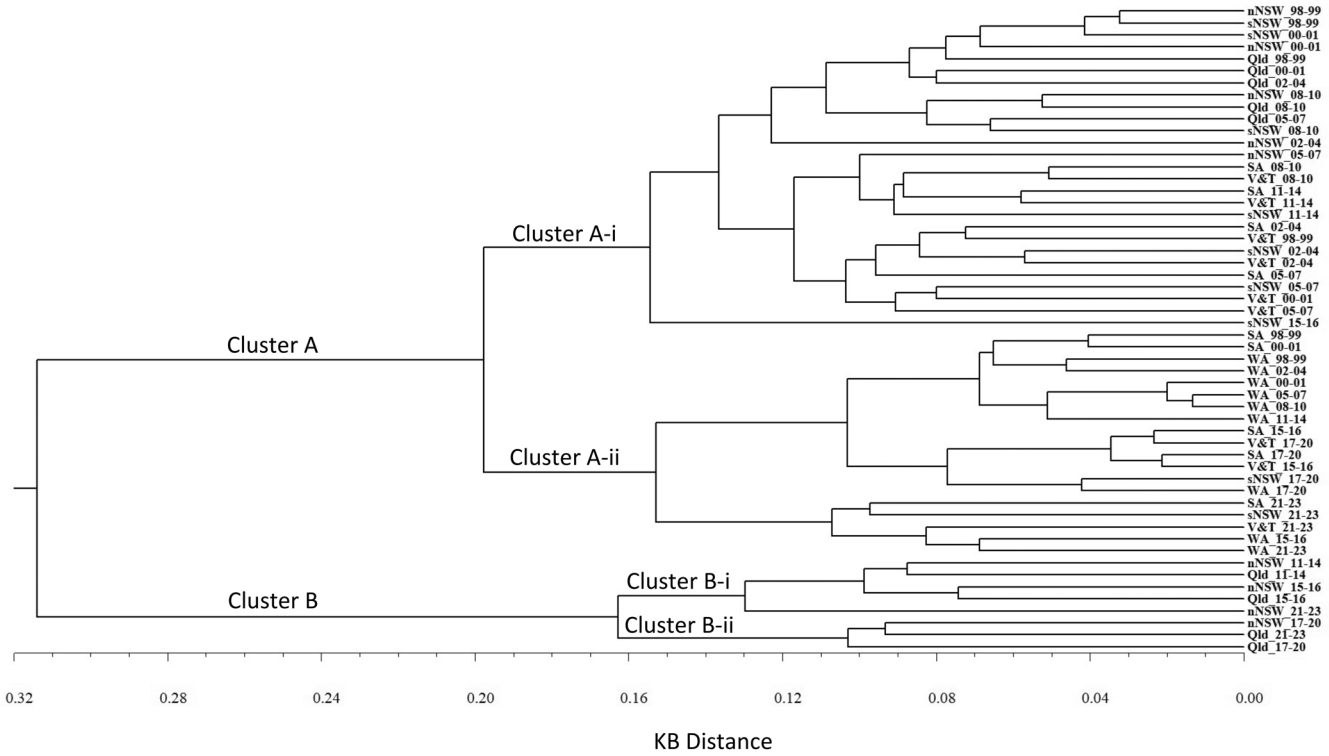

FIGURE 6 | UPGMA dendrogram of relationships between 54 populations of *Puccinia coronata* f. sp. *avenae* based on six geographic regions and nine periods between 1998 and 2023 in Australia. The dendrogram was generated based on the KB_m distance between populations with regard to the simple mismatch dissimilarity *m* between binary virulence phenotypes of clone-corrected isolates. nNSW, northern New South Wales (NSW); Qld, Queensland; SA, South Australia; sNSW, southern NSW; V&T, Victoria and Tasmania; WA Western Australia.

characterised by virulence for *Pc64* in all regions except Qld and nNSW (Table S5b). Virulence for *Pc64* was not detected in Qld and nNSW until 2022, and even then, it was uncommon (1.8% [Table S4a] and 5.7% [Table S4b], respectively).

Further partitioning of the populations within Cluster A was apparent, reflecting differences in the structure and relationships between them. One subgrouping (Cluster A-ii, Figure 6) comprised all nine populations (1998–2023) for WA, plus two populations for sNSW (2017–2023), three for V&T (2015–2023) and five for SA (1998–2001 and 2015–2023). This cluster did not include any Qld and nNSW populations. The second subgrouping (Cluster A-i, Figure 6) comprised five populations in each of Qld and nNSW (1998–2010), seven in sNSW (1998–2016), six in V&T (1998–2014) and four in SA (2002–2014). The dendrogram raised several hypotheses about spatial (regional) and temporal structure and differentiation of Pca populations that were tested further.

3.7 | Variability Within Populations

Based on *KW* dispersion estimates (Table 2), a very wide range of variability was seen within the six regional Pca populations during nine periods (α-variability). The least variable ($KW = 0.053$) were populations from V&T in 2017–2020 and WA in 2008–2010, whereas the most variable was the population from nNSW in 2021–2023 ($KW = 0.56$). Averaging across all nine periods, the most and least variable regional populations were those from nNSW and WA with $KW = 0.377$ and $KW = 0.137$, respectively (Table 2). Averaging across all six regions, the most and least variable populations were those in 2021–2023 and 1998–1999 with $KW = 0.396$ and $KW = 0.181$, respectively (Table 2). In general, all regional populations became more variable over time, and during all periods regional variability decreased along an east-to-west gradient.

The results obtained for dispersions within all 54 Pca populations (Table 2) as well as the UPGMA dendrogram for all populations (Figure 6) and for each separate region (data not shown) provided descriptive evidence of a notable transformation of the pathogen population at the beginning of the second decade of the 2000s. Analysing the pooled Australian Pca populations during the two periods 1998–2010 and 2011–2023, we established much larger variability within the populations of 2011–2023 in all regions as expressed by both the *KW* dispersion estimates and the normalised values of the effective number of different races *nENDR* (Table 3).

3.8 | Spatial (Regional) and Temporal Differentiation Among Populations

3.8.1 | Temporal Differentiation

Variability among Pca populations in the nine periods (temporal β-variability) was analysed for each separate region based on the *KB* distances between populations. The periodic effective

TABLE 2 | Variation in terms of *KW* dispersion within each of 54 *Puccinia coronata* f. sp. *avenae* populations in the six regions of Australia during the nine periods of survey from 1998 to 2023.

	Region						
Period	**Qld**	**nNSW**	**sNSW**	**V&T**	**SA**	**WA**	**Period average**
1998–1999	0.245	0.278	0.266	0.100	0.135	0.060	0.181
2000–2001	0.325	0.246	0.271	0.240	0.160	0.064	0.218
2002–2004	0.334	0.385	0.240	0.210	0.268	0.178	0.269
2005–2007	0.295	0.307	0.176	0.240	0.228	0.067	0.219
2008–2010	0.320	0.320	0.335	0.267	0.336	0.053	0.272
2011–2014	0.394	0.431	0.430	0.348	0.360	0.100	0.344
2015–2016	0.448	0.474	0.440	0.080	0.100	0.245	0.298
2017–2020	0.265	0.389	0.320	0.053	0.080	0.166	0.212
2021–2023	0.404	0.560	0.430	0.320	0.364	0.297	0.396
Region average	0.337	0.377	0.323	0.206	0.226	0.137	

Abbreviations: nNSW, northern New South Wales (NSW); Qld, Queensland; SA, South Australia; sNSW, southern NSW; V&T, Victoria and Tasmania; WA, Western Australia.

TABLE 3 | Variability within regional populations of *Puccinia coronata* f. sp. *avenae* in Australia during the two time periods 1998–2010 and 2011–2023.

		Period				
		1998–2010		**2011–2023**		
Region	***n*[a]**	***KW*[b]**	***nENDR*[c]**	***n***	***KW***	***nENDR***
Qld	129	0.321	0.298	101	0.419	0.382
nNSW	101	0.328	0.304	63	0.497	0.455
sNSW	110	0.301	0.281	54	0.433	0.383
V&T	40	0.222	0.196	42	0.326	0.285
SA	72	0.232	0.202	32	0.355	0.321
WA	27	0.107	0.080	78	0.219	0.184

Abbreviations: nNSW, northern New South Wales (NSW); Qld, Queensland; SA, South Australia; sNSW, southern NSW; V&T, Victoria and Tasmania; WA, Western Australia.
[a]*n*, number of races (as described in methods for clone-corrected data).
[b]*KW*, assignment-based dispersion.
[c]*nENDR*, normalised Effective Number of Different Races based on *KW* dispersion.

TABLE 4 | Variability among *Puccinia coronata* f. sp. *avenae* populations in the nine time periods between 1998 and 2023 within each of six regions of Australia.

Region	**Dispersion[a]**	**Diversity[b]**	**Variability[c]**
Queensland	0.157	8.58	2.35
Northern NSW	0.149	8.73	2.30
Southern NSW	0.129	8.85	2.14
Victoria and Tasmania	0.129	8.73	2.13
South Australia	0.129	8.70	2.12
Western Australia	0.074	8.42	1.63

[a]Average difference within the set of the populations for nine time periods in each region based on the *KB* distances between the corresponding populations (ADW_{KB}).
[b]Effective number of equally distant populations in the set of populations for nine time periods in each region based on the *KB* distances between the corresponding populations (*ENEDP*[*KB*]; ranges between 1 and 9).
[c]Effective number of different populations in the set of populations for nine time periods in each region based on the corresponding ADW_{KB} dispersion and *ENEDP*(*KB*) diversity (*ENDP*[*KB*]; ranges between 1 and 9).

number of different populations (*ENDP*) was close to 2 (Table 4; *ENDP*(*KB*) may range between 1 and 9), with the largest values in the eastern regions (Qld and nNSW: 2.35 and 2.3, respectively) and the smallest value in the west (WA: 1.63). This indicates two significantly different Pca population periods across 1 April 1998–31 March 2023 in all regions.

The extent of temporal changes (measured by the *KB* distances) that occurred between Pca populations in the nine periods across two eastern regions (Qld and nNSW) showed strong similarity with a high Mantel correlation (coefficient 0.928; $p = 0.001$, Table 5). The changes in Qld and nNSW also correlated significantly with those in sNSW and WA, although weaker with the latter. Significant associations of changes were established between V&T and SA, and to a lesser degree between SA and WA (Table 5).

3.8.2 | Regional Differentiation

Variability among Pca populations in the six regions (spatial β-variability) was analysed for each separate survey period based

TABLE 5 | Mantel test of association between changes in populations of *Puccinia coronata* f. sp. *avenae* in six regions of Australia from 1998 to 2023.

	Region					
Region	**Qld**	**nNSW**	**sNSW**	**V&T**	**SA**	**WA**
Qld		0.928	0.712	0.217	0.291	0.509
nNSW	0.001		0.611	0.291	0.332	0.475
sNSW	0.005	0.01		0.097	0.252	0.513
V&T	0.1	0.07	0.3		0.742	0. 273
SA	0.08	0.055	0.07	0.002		0.457
WA	0.03	0.02	0.007	0.07	0.02	

Note: Mantel correlation coefficient between matrices of *KB* distances between the populations for nine time periods for each pair of the six regions is shown above the diagonal. Significance of correlation (*p*-value) is shown below the diagonal. Where $p \leq 0.05$, the corresponding correlation is considered statistically significant. Abbreviations: nNSW, northern New South Wales (NSW); Qld, Queensland; SA, South Australia; sNSW, southern NSW; V&T, Victoria and Tasmania; WA Western Australia.

TABLE 6 | Variability among *Puccinia coronata* f. sp. *avenae* populations in six regions of Australia within each of nine periods from 1998 to 2023.

Period	Dispersion[a]	Diversity[b]	Variability[c]
1998–1999	0.113	5.57	1.63
2000–2001	0.112	5.76	1.65
2002–2004	0.098	5.85	1.57
2005–2007	0.117	5.85	1.68
2008–2010	0.135	5.67	1.77
2011–2014	0.163	5.65	1.92
2015–2016	0.205	5.62	2.15
2017–2020	0.271	5.40	2.47
2021–2023	0.183	5.60	2.02

[a]Average difference within the set of six regional populations in each period based on the *KB* distances between the corresponding populations (ADW_{KB}).
[b]Effective number of equally distant populations in the set of six regional populations in each period based on the *KB* distances between the corresponding populations (*ENEDP*[*KB*]; ranges between 1 and 6).
[c]Effective number of different populations in the set of six regional populations in each period based on the corresponding ADW_{KB} dispersion and *ENEDP*(*KB*) diversity (*ENDP*[*KB*]; ranges between 1 and 6).

on the *KB* distances between populations. The regional *ENDP* generally increased over time and was in a relatively narrow range of 1.57–1.77 in the five periods from 1998 to 2010 (Table 6; *ENDP*(*KB*) may range between 1 and 6), whereas larger estimates of *ENDP* from 1.92 to 2.47 were obtained for the four periods in the second interval of the survey 2011–2023. This means that while the regional populations were more similar during the first period of the survey 1998–2010, they could be subdivided into two different groups in the second period of the survey 2011–2023.

The extent of regional changes (measured by the *KB* distances) that occurred between Pca populations in the six regions across the six periods during 1998–2014 was generally similar and statistically significant (in all but one case) with the Mantel correlation coefficients in a range of 0.475–0.929 and *p*-values less than 0.04 (Table 7). The changes across the three periods in 2015–2023 were also significantly correlated (correlation coefficients and *p*-values ranged in 0.732–0.938 and 0.001–0.01 intervals, respectively; Table 7). No or weak only association was generally established between the changes among the six regions in 1998–2014 versus 2015–2023.

3.8.3 | Two Distinct Periods Within the Course of the Survey

Comparison of the regional Pca populations resulted in a hypothesis of a major change in the population composition occurring in all regions between 1998 and 2010 compared to after that, with 2015 onward being significantly different. We tested this hypothesis by comparing populations of 1998–2010 versus 2011–2023 as well as populations of 1998–2014 versus 2015–2023 with differentiation statistics and the permutation test. The individual-based and population-based approaches (Table 8) together conclude that remarkable changes occurred in regional Pca populations in Qld, nNSW, sNSW and WA after 2011 or so, whereas populations of V&T and SA transformed later, around 2015.

4 | Discussion

The present study is only one of two to report and analyse long-term (over 25 years) pathogenic variability in Pca (see also Moreau et al. 2024), and the first to report long-term Australia-wide studies of pathogenic variability in Pca and the role of crown rust resistance in commercial oat cultivars in shaping Pca populations there. Our results revealed two significant changes in the composition of regional Australian populations of Pca between 1998 and 2023: first, rapid (average 3.4 years) adaptation to crown rust resistant cultivars that were released principally in Qld and nNSW; and secondly, large changes between regional populations in Qld, nNSW, sNSW and WA around 2011 and in V&T and SA around 2015 that were not related to resistance gene deployment.

TABLE 7 | Mantel test of association between differences in the regional populations of *Puccinia coronata* f. sp. *avenae* in Australia during nine periods from 1998 to 2023.

	Period								
Period	**1998–99**	**2000–01**	**2002–04**	**2005–07**	**2008–10**	**2011–14**	**2015–16**	**2017–20**	**2021–23**
1998–99		0.702	0.577	0.340	0.751	0.475	0.764	0.606	0.567
2000–01	0.03		0.771	0.602	0.761	0.656	0.463	0.368	0.407
2002–04	0.02	0.01		0.782	0.858	0.929	0.389	0.475	0.578
2005–07	0.10	0.04	0.01		0.702	0.846	0.399	0.589	0.683
2008–10	0.01	0.01	0.004	0.02		0.897	0.554	0.465	0.442
2011–14	0.04	0.02	0.002	0.008	0.004		0.337	0.408	0.476
2015–16	0.04	0.10	0.08	0.10	0.05	0.10		0.876	0.732
2017–20	0.04	0.12	0.04	0.04	0.07	0.07	0.001		0.938
2021–23	0.02	0.10	0.04	0.02	0.06	0.14	0.01	0.006	

Note: Mantel correlation coefficient between matrices of *KB* distances between the six regional populations for each pair of the nine periods is shown above the diagonal. Significance of correlation (*p*-value) is shown below the diagonal. Where $p \leq 0.05$, the corresponding correlation is considered statistically significant.

TABLE 8 | Differentiation among regional populations of *Puccinia coronata* f. sp. *avenae* in Australia across two time periods between 1998 and 2023.

Region	**1998–2010 versus 2011–2023**	**1998–2014 versus 2015–2023**
Individual-based[a]		
Queensland	0.001[b]	0.001
Northern NSW	0.001	0.001
Southern NSW	0.01	0.01
Victoria and Tasmania	ns	0.001
South Australia	ns	0.01
Western Australia	0.03	ns
Population-based[c]		
Queensland	0.001	0.03
Northern NSW	0.01	0.03
Southern NSW	0.03	0.03
Victoria and Tasmania	ns	0.02
South Australia	ns	0.05
Western Australia	0.03	0.001

[a]Significance (*p*-value) of differentiation was determined with the permutation tests (1000 random reshuffling of individuals) based on the differentiation statistics and the simple mismatch dissimilarity between pathotypes.
[b]*p*-value; ns, non-significant differentiation ($p > 0.05$).
[c]Significance (*p*-value) of differentiation was determined with the permutation test (126 and 84 random reshufflings of populations) based on the differentiation statistics and the *KB* distance between populations.

About 35% of the 172 pathotypes identified were detected once only. Moreau et al. (2024) reported that about 88% of the pathotypes identified in the United States from 1993 to 2022 were isolated once only. Similarly, Chong et al. (2008) reported that 84.5% of the pathotypes of Pca identified in Canada between 2002 and 2006 were isolated once only, and Sowa and Paczos-Grzęda (2021) reported that about 83% of the pathotypes identified in Poland from 2017 to 2019 were isolated once only. The higher frequencies of pathotypes identified only once in North America and Poland compared to Australia probably reflect the occurrence of sexual recombination in Pca in those regions and its apparent absence in Australia. Year-round survival of Pca in the uredinial stage in oat crops and on wild oat or self-sown cultivated oat in Australia was also reflected in the persistence of pathotypes, with the two most common pathotypes (pts 7 and 61) being isolated regularly over the course of the study in most eastern Australian regions. There was also evidence of clonal lineage preservation, with Cluster 1 (Figure 1) apparently comprising a clonal lineage within which virulence complexity increased over the course of the study, paralleling resistance gene deployment in grazing oat in nNSW and Qld. Pathotype 162, first detected in WA in 2016, shared virulence/avirulence attributes of pathotypes in Clusters 2a and 2b. Genome sequencing provided strong evidence that it was the product of hybridisation between isolates from Cluster 2 (Lu 2024).

In Australia, oat is grown for grain or for forage/silage/hay. Forage oat has been grown traditionally in the subtropical region of eastern Australia, extending south from the coastal and inland areas from central Queensland to central NSW. Little is known of crown rust resistance in oat cultivars released in Australia prior to 1990. Cultivars Bond (*Pc3*+*Pc4*) and Victoria (*Pc2*, *Pc11*, *Pc12*) were the sole sources of resistance used in Australia before 1960 (Upadhyaya and Baker 1960). The dual purpose (mainly grain) cvs Cluan and Quamby (released in Tasmania in 1990) and the grazing oat cv. Amby II (Queensland in 1991) were the first Australian releases in a series of mainly grazing oat cultivars with crown rust resistance derived from *A. sterilis* or *A. magna* (*Pc36*,

Pc38, *Pc39/55/71*, *Pc50*, *Pc52*, *Pc56*, *Pc58/59*, *Pc61*, *Pc68* and *Pc91*). Virulence for *Pc36*, *Pc50*, *Pc56*, and *Pc68*, *Pc91* all increased rapidly following their deployment. The resistances *Pc38*, *Pc39/55/71*, *Pc52*, *Pc58/59* and *Pc61* were deployed before 1998, and virulence was common in Qld and nNSW in 1998. Several studies prior to 1998 showed that virulences for *Pc38*, *Pc39/55/71* and *Pc58/59* were low between 1985 and 1989, and began to increase from the early 1990s (Bonnett 1996; Oates 1985, 1989; Oates et al. 1996). Virulence for Cleanleaf (*Pc38*, *Pc39*, *Pc52*) was first detected in 1995 and increased rapidly from 1996 to 1998 (Park et al. 2000). Bonnett (1996) reported virulence for *Pc61* in samples collected from Qld (55%; 11 samples) and nNSW (20%; 5 samples) in 1993. The presence of virulence for *Pc61* in Qld, nNSW and sNSW from 1998 was most likely related to the release and cultivation of the grazing oat cultivars Amby II (1991; *Pc61*), Condamine (1994; *Pc61*) and Nobby (1994; *Pc58/59*, *Pc61*) in Qld and nNSW. Almost 75% of the isolates virulent for *Pc61* from those regions were pt. 61, which was one of the most commonly isolated pathotypes from Qld, nNSW and sNSW over the period 1998–2023. Pt. 61 was common in SA from 1998 to 2014, probably due to the release (1998) and cultivation of cv. Quoll, which was postulated to carry *Pc61*. The increase in frequency of virulence for *Pc61* in WA from 2016, and then in SA (2017–2020) and V&T (2021–2023) was due to the emergence of pathotypes 55, 56, 57, 58 and 59 in WA and the later detection of pathotypes 57 and 58 in SA and V&T. The increase in frequency of these pathotypes in these regions did not appear to be related to the resistance of cultivars grown there at the time.

Oates et al. (1983) found that pathotypic diversity and virulence complexity (number of differentials for which an isolate was virulent) were higher in the north than in the south of NSW from 1975 to 1979. A later study from 1977 to 1980 found that pathotypic diversity and virulence complexity were higher in Qld and nNSW than in sNSW, Victoria, SA and WA (Brouwer and Oates 1986). This difference was attributed at least in part to the cultivation of oats with resistance to Pca in Qld and nNSW and of oats lacking effective resistance to Pca in SA and WA (Brouwer and Oates 1986). Limited virulence surveys in 1993 (Bonnett 1996) and 2022 (Henningsen et al. 2024) similarly found that pathotypes of Pca from eastern Australia were virulent on more differential lines than those from WA. By studying pathotypic diversity and virulence complexity over a period of 26 years and understanding the resistance of oat cultivars grown over that time, we were able to examine long-term relationships among different regions of Australia in the context of resistance gene deployment. From 1998 to 2001, SA and WA were distinct from all other regions in terms of prevailing pathotypes, and from 2002 to 2014, WA was distinct from all other regions (Figure 6). Starting from 2011, populations in WA began to change significantly with the emergence and increase of a group of pathotypes typified by virulence on *Pc64*. The effectiveness of *Pc64* in Australia was first assessed by Bonnett (1996), who found virulence in crown rust samples collected in 1993 from nNSW and Victoria. Virulence for *Pc64* was again detected in 1995 in all regions except Victoria at low levels (Oates et al. 1996). In the present study, low levels of virulence for *Pc64* were detected in 1998 (sNSW and SA) and in 1999 (Victoria), but from 2000 to 2010, it was not detected at all in any region until 2011, when two pathotypes were detected in WA, one that appeared to be the same as that detected in 1998 and 1999 (pt. 20), and one that differed in being avirulent on Swan (pt. 6). Of the 16 *Pc64*-virulent pathotypes detected between 1998 and 2023, 12 were first detected in WA, and of these, three were subsequently detected in eastern Australia. The spread of these pathotypes to eastern Australia resulted in populations in sNSW, V&T and SA becoming more similar to those in WA and more distinct from those in Qld and nNSW. Given that gene *Pc64* has not been deployed in Australia and that the 16 *Pc64*-virulent pathotypes detected do not appear to carry virulence or virulence combinations not already present in other pathotypes, the increase in frequency of this group of pathotypes suggests that they are more aggressive and could be in the process of displacing older pathotypes. Such selective continental sweeps have been documented in Australia in the wheat stem rust pathogen Pgt (Zwer et al. 1992), the wheat leaf rust pathogen *P. triticina* (Park et al. 1995) and the wheat stripe rust pathogen Pst (Ding et al. 2021). The apparent absence of the *Pc64*-virulent pathotypes in Qld and nNSW most likely relates to their inability to overcome resistance genes in the grazing oat cultivars grown there (e.g., *Pc50*, *Pc68*, *Pc91*).

Studies of pathogenic variability in all three wheat rust pathogens (Park 2008; Wellings 2007) and the barley leaf rust pathogen *P. hordei* (Park 2003) in Australia have clearly established limited gene flow between the eastern and western cereal-growing regions, primarily in a west-to-east direction on prevailing winds. The initial detections of pt. 162 and three distinct pathotypes of Pca characterised by virulence on *Pc64* in WA (pts 6, 57, 58) and their subsequent detection in eastern Australia provide four further examples of west-to-east migration of a cereal rust pathogen. The detection of pt. 61 in WA in 2021 and 2022, previously detected only in eastern Australia, was unusual given that the frequency of east-to-west migration of cereal rusts is much lower (Watson and Cass Smith 1962).

Based on marker screens and rust phenotyping of three accessions of the grazing oat cv. Volta, Nguyen et al. (2024) concluded that one carried *Pc91*, a second possibly *Pc50* and *Pc91*, and a third accession obtained from a breeder's source *Pc50* only. This raises the question as to what resistance gene(s) are present in the selection of Volta that was released in 2003. Multipathotype tests conducted in 2004 using seed of both parents of Volta and two pure seed sources of Volta provided by the breeder showed the presence of *Pc91* in a parental source, and of *Pc50* in the second pure seed stock (Table S2d). Excluding Volta, the first deployment of *Pc50* in Australia was in cv. Qantom, and of *Pc91* in cv. Drover, both released in Qld in 2006. Monitoring of virulence on *Pc50* began in 1977, and of virulence on *Pc91* in 1993. Between 1977 and 2007, virulence for *Pc50* was detected only once in 1976 (Victoria) and twice in Qld in 1999 (Bonnett 1996; Brouwer 1983; Table S4a–f). It was then detected in Qld in 2008 in pt. 100, a presumed single-step derivative of pt. 101 (Table S4a), after which it increased in frequency there and became common in nNSW (Table S4b; Figure S2e). Virulence for *Pc91* was first detected in 2012, in a single pathotype in Qld (pt. 130, Table S4a), after which it also increased in frequency and was common in nNSW (Table S4b; Figure S2a). The earlier detection and build-up of virulence for *Pc50* (2008) than for *Pc91* (2012) is consistent with the presence of *Pc50* in the genotype of Volta that was grown in Qld and nNSW following its release in 2003. Volta was included in the differential set from 2008 using the seed source that was postulated to carry *Pc50*. It displayed exactly the same response

as the Pendek/Pc50 isoline differential to all 1203 isolates examined from 2008 to 2023 (Figure S2e,f), providing strong evidence that it lacks other crown rust resistance genes.

Virulence for *Pc36* (syn. *Pc47*, Simons et al. 1978) was common in the United States over the period 1993–2022 and showed a steady increase from 2013 to frequencies of 100% by 2021 (Moreau et al. 2024). Leonard et al. (2004) were unable to account for the common occurrence of virulence on *Pc36* in the United States from 1991 to 1996. The Australian oat cv. Gwydir was selected from the cross Panfive/Q18994 and released in Qld in 1999. The Panfive parent was developed at the University of Minnesota in 1964 from the cross Lodi/PI 267989, the latter being an accession of *A. sterilis* that carries *Pc36* (Irwin 1991). Our multipathotype testing provided strong evidence for the presence of *Pc36* in Gwydir (Table S2c). Monitoring of virulence for *Pc36* in Australia began in 1993 when it was detected in Qld, NSW and Victoria (Bonnett 1996), and Gwydir was included in the differential set from 1999. Virulence on Gwydir was first detected in 2001 in Qld and nNSW. All Gwydir-virulent pathotypes detected were also virulent on *Pc36*, and the increase in frequency of virulence for *Pc36* and on Gwydir showed a similar pattern (Figure S2c,d). However, several *Pc36*-virulent pathotypes were avirulent on Gwydir, indicating that it must carry one or more resistance genes in addition to *Pc36*.

Virulence for the PC36 differential was strongly correlated with that of the differential X716 (Table S5a; Figure S2c,j). The latter is a backcross line produced by Browning and colleagues at the Iowa Agriculture and Home Economics Station and USDA-ARS of an unknown recurrent parent (C649) and an unknown *A. sterilis* crown rust resistance donor (Leonard et al. 2004). Bonnett (1996) found that X716 was resistant to an array of historical Australian isolates of Pca but did detect virulence among samples collected in 1993 from Qld, NSW and Victoria. Of the 172 pathotypes we identified, 166 were either avirulent or virulent on both PC36 and X716, and six were virulent on PC36 but avirulent on X716. The low infection type resulting from incompatibility on X716 varied from to 12CN, the latter being typical of the low infection type observed with avirulence on PC36. These results, combined with those from multipathotype testing under standardised conditions (Table S2b,c), suggest the presence of *Pc36* in X716 along with one or more unknown resistance gene(s) conferring a low infection type of.

The successive failure of ASR genes for crown rust resistance documented in the present study has also been documented in the United States (Moreau et al. 2024) and in Canada (McCallum et al. 2007). Our study established that Pca spreads rapidly throughout Australia, even between the eastern and western cereal belts, and that it moves freely between wild oat communities and grazing, hay, and milling oat crops. Future efforts to achieve sustained crown rust control should therefore focus on the use of durable resistance in oats of all end-uses.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.