Genetic Diversity in Narrow-Leafed Lupin Breeding After the Domestication Bottleneck

1

Wallace A. Cowling

Abstract

Narrow-leafed lupins (*Lupinus angustifolius* L.) were fully domesticated as a valuable grain legume crop in Australia during the mid-twentieth century. Pedigree records are available for 31 released varieties and 93 common ancestors from 1967 to 2016, which provides a rare opportunity to study genetic diversity and population inbreeding in a crop following a domestication bottleneck. From the 1930s–1960s, partially domesticated germplasm was exchanged among lupin breeders in eastern and western Europe, Australia, and USA. Mutants of two founder parents contributed to the first fully domesticated narrow-leafed lupin variety "Uniwhite" in 1967. Four Phases of breeding are proposed after domestication in the Australian lupin breeding program: Foundation (1967–1987), First Diversification (1987–1998), Exploitation (1998–2007), and Second Diversification (2007–2016) Phases. Foundation Phase varieties had only two or three founder parents following the domestication bottleneck and high average coefficient of coancestry ($f = 0.45$). The First Diversification Phase varieties were derived from crosses with wild lupin ecotypes, and varieties in this Phase had lower average coefficient of coancestry ($f = 0.27$). Population coancestry increased in varieties of the Exploitation Phase ($f = 0.39$). The rate of inbreeding (ΔF) between the First Diversification and Exploitation Phase (10 years) was 0.09 per cycle, which equates to 9% loss of alleles per cycle due to random drift and low-effective population size ($N_e = 5.4$), assuming two 5-year cycles. New genetic diversity was introduced in the Second Diversification Phase varieties ($f = 0.24$) following more crossing with wild lupins. Genetic progress in Australian lupin breeding so far has been substantial with improvements in grain yield and disease resistance, but narrow genetic diversity will limit future genetic progress. The pedigree of the latest varieties includes 39.1% from three founder varieties in the domestication bottleneck and 48.3% from 9 wild ecotypes that survived 50 years of selection. In terms of conservation genetics, the Australian lupin breeding program is a critically endangered population, and subject to excessive random drift. Migration of genetic diversity from wild lupins or exchange with international breeding programs will improve long-term genetic gain and effectiveness of genomic selection.

The original version of this chapter was revised: Figure 1.1 has been updated with part figure. The correction to this chapter is available at https://doi.org/10.1007/978-3-030-21270-4_13

W. A. Cowling (✉)
The UWA Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Perth, WA 6009, Australia
e-mail: wallace.cowling@uwa.edu.au

K. B. Singh et al. (eds.), *The Lupin Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-030-21270-4_1

1.1 Introduction

Narrow-leafed lupins (*Lupinus angustifolius* L.) provide a rare opportunity to study the impact of a recent domestication bottleneck on reducing genetic diversity and its subsequent recovery in a self-pollinating crop. Sweet narrow-leafed lupins were fully domesticated in the mid-twentieth century, following the discovery of domestication genes in different parts of the world and the exchange of germplasm among breeders in eastern and western Europe, USA, and Australia (Gladstones 1970). Pedigree records are available for 31 varieties released in Australia from 1967 to 2016 (Cowling 1999; IP Australia 2019), and 93 common ancestors. For more information on the history and attributes of narrow-leafed lupin breeding globally, readers are directed to several extensive reviews on the subject (Clements et al. 2005; Cowling and Gladstones 2000; Cowling et al. 1998b; Gladstones 1970, 1998; Święcicki et al. 2015).

Genome diversity is much lower in domesticated narrow-leafed lupins compared with their wild relatives, and wild and landrace *L. angustifolius* ecotypes provide a wealth of genetic and phenological diversity for potential use by lupin breeders (Berger et al. 2012; Mousavi Derazmahalleh et al. 2018; Cowling et al. 1998a). Wild narrow-leafed lupins have contributed to improved grain yield and disease resistance in sweet domesticated varieties (Cowling and Gladstones 2000; Stefanova and Buirchell 2010). The progeny of several wild × domesticated lupin crosses were fully fertile and released as improved varieties in the Australian lupin breeding program in the 1980s (Cowling 1999). The best lines from this round of crossing were recombined to produce high-performing varieties released in the 2000s (Stefanova and Buirchell 2010).

This chapter investigates genetic diversity and population inbreeding in the Australian lupin breeding program over 50 years from 1967 to 2016 based on pedigrees, and suggests methods for improving genetic diversity and the potential for long-term genetic gain, including the use of genomic and pedigree information and optimal contributions selection to achieve these goals.

1.2 Analysis of Genetic Diversity and Population Inbreeding

Pedigree records exist for 31 varieties released from 1967 to 2016 including 93 common ancestors in the pedigree (Cowling 1999; IP Australia 2019, Dr. Bevan Buirchell *pers. comm.*). This information was used to develop a pedigree including founder lines, varieties, and presumed or known common ancestors (Table 1.1). The number of generations of selfing in each line ("fgen") was used to calculate the level of inbreeding in each line. The value of fgen was assumed to be "0" for F_1 progeny, "5" for released varieties, and "10" for landraces or wild ecotypes (Table 1.1).

These records were used to construct a numerator relationship matrix (A-matrix) using ASREML software (VSN International, UK), and pedigrees were plotted in a pedigree chart (Fig. 1.1). The most significant feature of the lupin pedigree is the relatively small number of individuals which contribute to variety development over 50 years (total 124), compared with animal breeding where thousands of animals in the pedigree typically contribute to future performance (Goddard and Hayes 2009).

1.2.1 Coefficient of Coancestry and Inbreeding Coefficient

From pedigree records (Table 1.1), the coefficient of coancestry or kinship coefficient (*f*) between each pair of lines was calculated as ½ the numerator relationship value (*a*-value), which is the proportion of additive genetic variance that two individuals have in common. The coancestry of two individuals is "the probability that two gametes taken at random, one from each, carry alleles that are identical by descent" (Falconer and Mackay 1996), or put another way, the chance that a randomly chosen allele in two potential crossing parents is the same allele as in the common ancestor. In *L. angustifolius*, commercial varieties are homozygous at most loci, and therefore identity by descent represents the

Table 1.1 Pedigrees of Australian narrow-leafed lupin varieties during four Phases of variety release: Phase 1 (Foundation Phase, 1967–1987), Phase 2 (First Diversification Phase, 1987–1998), Phase 3 (Exploitation Phase, 1998–2007), and Phase 4 (Second Diversification Phase, 2007–2016). Key contributing ancestors are shown together with released varieties, indicated by date of release. Where parents are not known, the symbol "0" appears. "fgen" is the number of generations of selfing in the line or variety. "Var. no." is the number of the line or variety in temporal order of the pedigree. Wild ecotypes from the Australian Lupin Collection are indicated by the suffix "w", e.g. P22750w. Where numbers were not located in the records, these are replaced with "xx", e.g. 62Axx1 is a line derived from a cross made in 1962.

Name of line or variety	Female parent	Male parent	fgen	Var. no	Phase of release
New Zealand Blue	0	0	10	V1	
Germany-iuc	0	0	10	V2	
Landrace-moll	0	0	10	V3	
Borre 1947	Germany-iuc	Landrace-moll	10	V4	
New Zealand Blue-le	New Zealand Blue	New Zealand Blue	5	V5	
New Zealand Blue-ta	New Zealand Blue	New Zealand Blue	5	V6	
New Zealand Blue-leuc	New Zealand Blue	New Zealand Blue	5	V7	
Borre-Ku	Borre 1947	Borre 1947	5	V8	
Borre-efl	Borre 1947	Borre 1947	5	V9	
62Axx1	New Zealand Blue-leuc	Borre 1947	2	V10	
62Axx2	New Zealand Blue-le	New Zealand Blue-ta	2	V11	
64Axx2	62Axx1	New Zealand Blue-ta	0	V12	
64Axx1	64Axx2	62Axx2	0	V13	
Rancher	0	0	5	V14	
66A001	64Axx1	Rancher	0	V15	
66Axx2	64Axx1	Borre-Ku	0	V16	
Uniwhite 1967	64Axx2	64Axx2	5	V17	Phase1
P20722w	0	0	10	V18	
P20723w	0	0	10	V19	
AB12	66Axx2	66Axx2	2	V20	
Borre-efl/Uw	Borre-efl	Uniwhite 1967	0	V21	
Borre-efl/Uh	Borre-efl	64Axx1	0	V22	
65G-251	0	0	5	V23	
70A61	P20722w	AB12	0	V24	
70A62	P20723w	AB12	0	V25	
71Axx1	Borre-efl/Uw	64Axx1	0	V26	
65G-251/Uh	65G-251	64Axx1	5	V27	
Pxxxx1w	0	0	10	V28	
P20639w	0	0	10	V29	
P22661w	0	0	10	V30	
72Axx1	65G-251/Uh	66A001	5	V31	
Uniharvest 1972	64Axx1	64Axx1	5	V32	Phase1
72A014	66A001	66Axx2	0	V33	
72A015	71Axx1	66A001	0	V34	
Unicrop 1973	66Axx2	66Axx2	5	V35	Phase1
Fest 1973	62Axx2	62Axx2	5	V36	Phase1

(continued)

Table 1.1 (continued)

Name of line or variety	Female parent	Male parent	fgen	Var. no	Phase of release
64A02	Uniwhite 1967	P20639w	5	V37	
73Axx1	Borre-efl/Uh	Uniharvest 1972	5	V38	
P22750w	0	0	10	V39	
P22872w	0	0	10	V40	
P22748w	0	0	10	V41	
P22721w	0	0	10	V42	
72A014-1	72A014	72A014	2	V43	
72A014-2	72A014	72A014	2	V44	
72A015-2	72A015	72A015	2	V45	
74Axx1	72Axx1	Unicrop 1973	0	V46	
74A003	74Axx1	Unicrop 1973	0	V47	
75A045	P22872w	72A014-1	0	V48	
75A054	P22721w	72A014-1	0	V49	
75A060	P22748w	72A014-1	0	V50	
75A061	P22750w	72A014-1	0	V51	
Unicrop-E	Unicrop 1973	Unicrop 1973	5	V52	
Marri 1976	66A001	66A001	5	V53	Phase1
CE2-1-1	Pxxxx1w	72A014-1	5	V54	
76A106-31	Unicrop 1973	P22661w	5	V55	
76A106-32	Unicrop 1973	P22661w	5	V56	
76A6-11-3-1-2	Marri 1976	Unicrop-E	5	V57	
79A078	70A62	70A61	0	V58	
Illyarrie 1979	72A014-1	72A014-1	3	V59	Phase1
Yandee 1980	72A014-2	72A014-2	3	V60	Phase1
Chittick 1982	72A015-2	72A015-2	3	V61	Phase1
75A061-3	75A061	75A061	2	V62	
75A054-5	75A054	75A054	2	V63	
79A078-14-10	79A078	79A078	5	V64	
84A086	75A061	CE2-1-1	0	V65	
84L528-18	CE2-1-1	76A106-31	2	V66	
84L551-13	76A106-32	76A6-11-3-1-2	2	V67	
75A054-5-8	75A054-5	75A054-5	2	V68	
75A061-3-1	75A061-3	75A061-3	2	V69	
84S019-96-2	79A078-14-10	84A086	4	V70	
P26672w	0	0	10	V71	
P22764w	0	0	10	V72	
84A086-12-17	84A086	84A086	4	V73	
84A086-73-10	84A086	84A086	4	V74	
Danja 1986	74A003	74A003	5	V75	Phase1
Wandoo 1986	73Axx1	64A02	5	V76	Phase1

(continued)

Table 1.1 (continued)

Name of line or variety	Female parent	Male parent	fgen	Var. no	Phase of release
83A025	75A061-3-1	75A054-5-8	0	V77	
Geebung 1987	73Axx1	64A02	5	V78	Phase1
Gungurru 1988	75A061	75A061	2	V79	Phase2
88L152-29	Gungurru 1988	P26672w	5	V80	
Yorrel 1989	75A045	75A045	5	V81	Phase2
Warrah 1989	75A060	75A060	5	V82	Phase2
75A045-10-8	Yorrel 1989	Yorrel 1989	1	V83	
Merrit 1991	Gungurru 1988	Gungurru 1988	2	V84	Phase2
84S019-96-2-11	84S019-96-2	84S019-96-2	1	V85	
84A086-73-10-37	84A086-73-10	84A086-73-10	0	V86	
84A041	Yorrel 1989	83A025	2	V87	
83A008-71-41(sel)	75A061-3-1	75A045-10-8	5	V88	
84S035-48-2	Yorrel 1989	84A086	3	V89	
84S035-48-4	Yorrel 1989	84A086	3	V90	
90A050	Merrit 1991	84S035-48-2	0	V91	
95L335-17-15	88L152-29	84S019-96-2-11	5	V92	
84S017	79A078-14-10	84A041	0	V93	
Myallie 1995	CE2-1-1	76A106-31	5	V94	Phase2
84S035-48-4-24	84S035-48-4	84S035-48-4	0	V95	
Wonga 1996	83A025	83A025	4	V96	Phase2
Kalya 1996	Warrah 1989	79A078-14-10	4	V97	Phase2
Tallerack 1997	84L528-18	84L551-13	5	V98	Phase2
84S017-50S-62	84S017	84S017	5	V99	
Tanjil 1998	Wonga 1996	Wonga 1996	2	V100	Phase2
Belara 1997	84S035-48-2	84S035-48-2	1	V101	Phase3
Moonah 1998	84S017	84S017	1	V102	Phase3
Quilinock 1999	84S019-96-2	84S019-96-2	1	V103	Phase3
90S085-107-33	Tanjil 1998	90A050	5	V104	
90S085-107-39	Tanjil 1998	90A050	5	V105	
91A047-58	Tanjil 1998	84A086-12-17	3	V106	
97L122-1	91A047-58	Kalya 1996	3	V107	
01LF1 bulk	90S085-107-39	0	0	V108	
01L576-108	P22764w	83A008-71-41(sel)	5	V109	
95L256-17	84A086-73-10-37	Quilinock 1999	0	V110	
97L182-5-7	84S017-50S-62	0	5	V111	
03A013R	95L335-17-15	0	0	V112	
04A010	97L182-5-7	03A013R	0	V113	
03LF1 bulk	0	95L335-17-15	0	V114	
Mandelup 2004	84A086-12-17	84S035-48-2	4	V115	Phase3

(continued)

Table 1.1 (continued)

Name of line or variety	Female parent	Male parent	fgen	Var. no	Phase of release
01A019R-23	Mandelup 2004	90S085-107-39	2	V116	
Coromup 2006	84S035-48-4-24	84A086-73-10	4	V117	Phase3
Jenabillup 2007	95L256-17	95L256-17	3	V118	Phase3
PBA Gunyidi 2012	90S085-107-39	01LF1 bulk	5	V119	Phase4
PBA Barlock 2013	97L122-1	90S085-107-33	5	V120	Phase4
06AF1 bulk	Jenabillup 2007	04A010	0	V121	
PBA Jurien 2015	03LF1 bulk	95L335-17-15	5	V122	Phase4
PBA Leeman 2016	01L576-108	Coromup 2006	5	V123	Phase4
PBA Bateman 2016	01A019R-23	06AF1 bulk	5	V124	Phase4

"end of the road" in terms of allelic variation at a locus. Progeny of a cross between two highly selfed parents that share an allele that is identical by descent will be fixed at that locus. The coefficient of coancestry between two parents is equal to the inbreeding coefficient (F) of their progeny.

1.2.2 Four Phases of Lupin Breeding Based on Coancestry

On the basis of average f-values, the 31 released varieties in the Australian lupin breeding program from 1967 to 2016 were allocated to four Phases of breeding: 11 varieties in the Foundation Phase (1967–1987), 9 varieties in the First Diversification Phase (1987–1998), 6 varieties in the Exploitation Phase (1998–2007), and 5 varieties in the Second Diversification Phase (2007–2016) (Table 1.1 and Figs. 1.1 and 1.2). The Foundation Phase is equivalent to breeding cycles one and two in Stefanova and Buirchell (2010), the First Diversification Phase to breeding cycles three and four, and the Exploitation Phase to breeding cycle five.

The average coefficient of coancestry in 11 varieties released during the Foundation Phase was high (average f = 0.45) (Fig. 1.2), as expected following a domestication bottleneck involving three main founders: "New Zealand Blue," "Borre," and "Rancher" (Fig. 1.1). "Rancher" from USA was incorporated into the breeding of several Foundation Phase varieties ("Marri," "Chittick," "Illyarrie," and "Yandee"). "65G-251" from USA forms a small proportion of the pedigree of "Danja" but did not contribute to pedigrees beyond this Phase. The high level of coancestry in the Foundation Phase varieties is almost equivalent to the mating of a noninbred individual with itself, that is, the first selfing of a cross progeny assuming no prior inbreeding (f = 0.5). On average, for two randomly mated foundation varieties in the Foundation Phase, there is a 45% chance that a randomly chosen allele is identical by descent, or put another way, the progeny of this mating will be fixed for ancestral alleles at 45% of loci by genetic drift alone. Such high levels of random drift are typical of "domestication bottlenecks" and dramatically reduce the potential for future genetic gain by selection (Falconer and Mackay 1996). The pedigrees of several varieties "Marri," "Chittick," "Wandoo," and "Danja" did not contribute beyond the Foundation Phase.

The average coefficient of coancestry decreased in the First Diversification Phase (f = 0.26) (Fig. 1.2), after intercrossing of 72A014 (the "Illyarrie" progenitor) with wild lupin ecotypes (Table 1.1 and Fig. 1.1). This level of coefficient of coancestry among varieties is equivalent to full-sib or parent-offspring mating, assuming unrelated parents.

Intercrossing of high-performing lines from the First Diversification Phase led to more population inbreeding in the Exploitation Phase, and

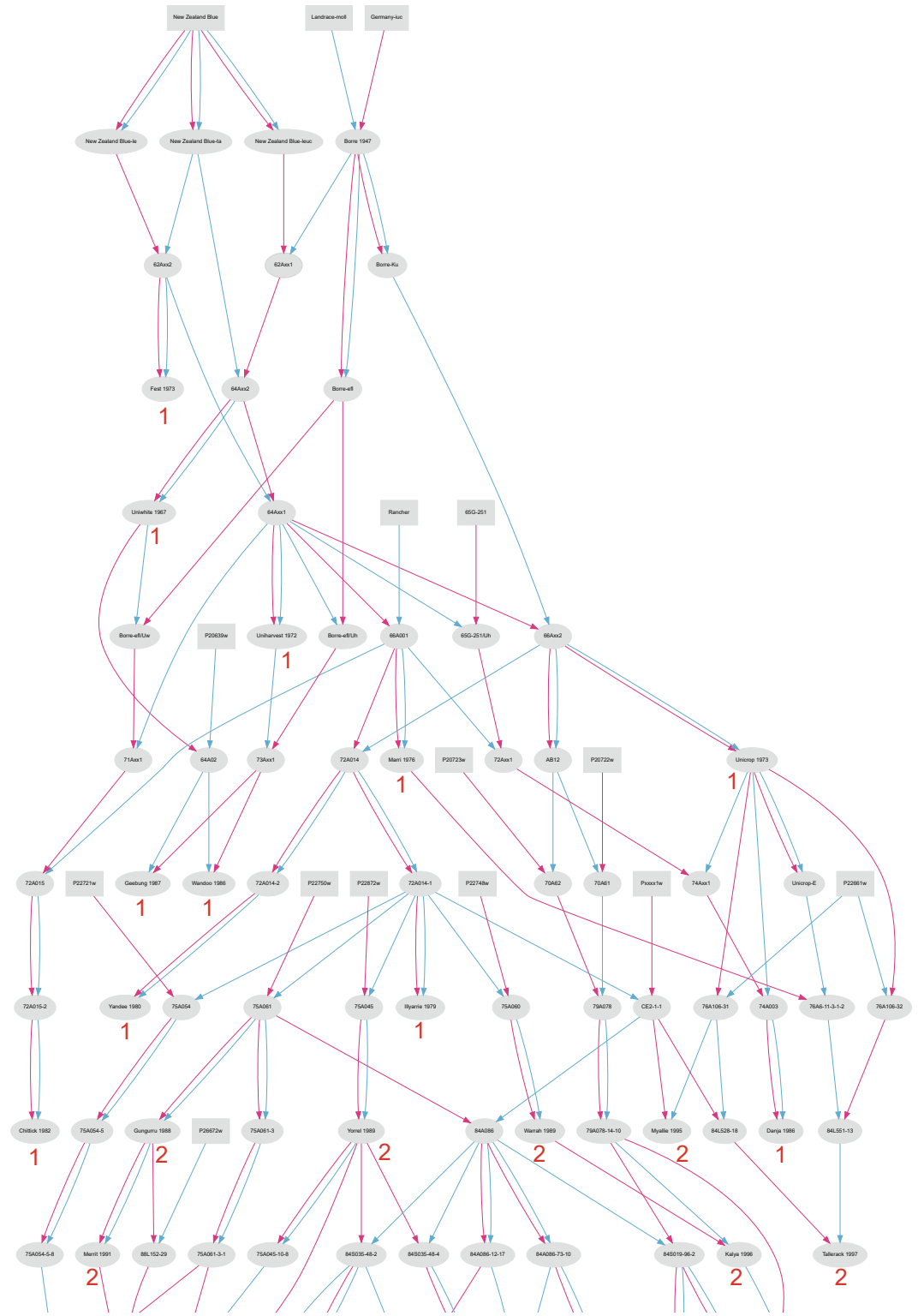

Fig. 1.1 Pedigree diagram of Australian narrow-leafed lupin varieties over four Phases of variety release, indicated by numerals 1, 2, 3, and 4 below the variety name. Female parents are indicated by red lines and male parents as blue lines. Selfing is indicated by both red and blue lines connecting from the parent

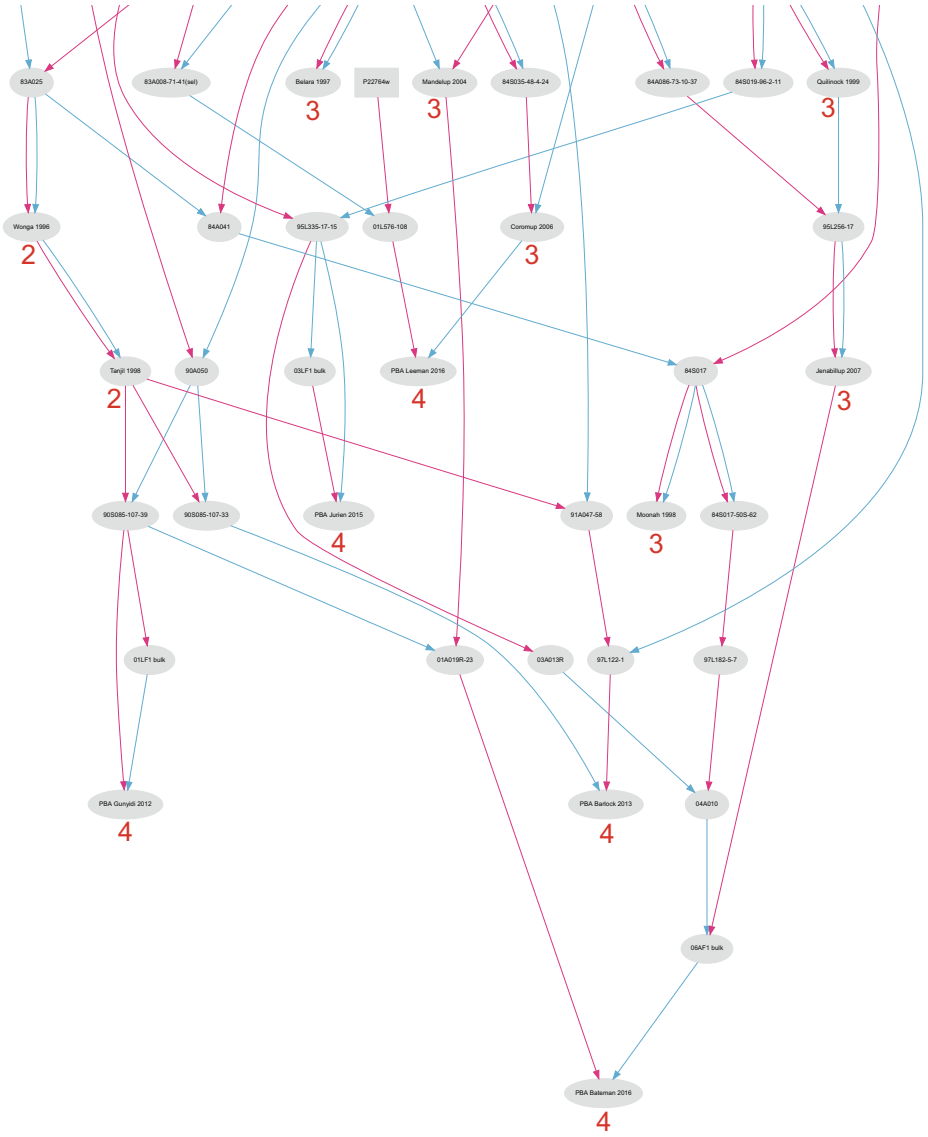

Fig. 1.1 (continued)

average coefficient of coancestry among the varieties in this Phase was again high ($f = 0.39$) (Fig. 1.2). On average, progeny of crosses in the Exploitation Phase were fixed for a common ancestor's allele at 39% of random loci. This level of kinship severely limits the potential for future genetic progress.

Another round of crossing with wild lupins helped to reduce population coancestry in varieties released during the Second Diversification Phase ($f = 0.24$). Second Diversification Phase varieties were based on three founder varieties "New Zealand Blue," "Borre," and "Rancher" and nine wild lupins (Fig. 1.2 and Table 1.2). The pedigree of varieties released in the Second Diversification Phase was dominated by three founder varieties "New Zealand Blue," "Borre," and "Rancher" and three wild lupin ecotypes (those used initially in formation of "Gungurru," "Yorrel," and "Myallie") (Fig. 1.2 and Table 1.2).

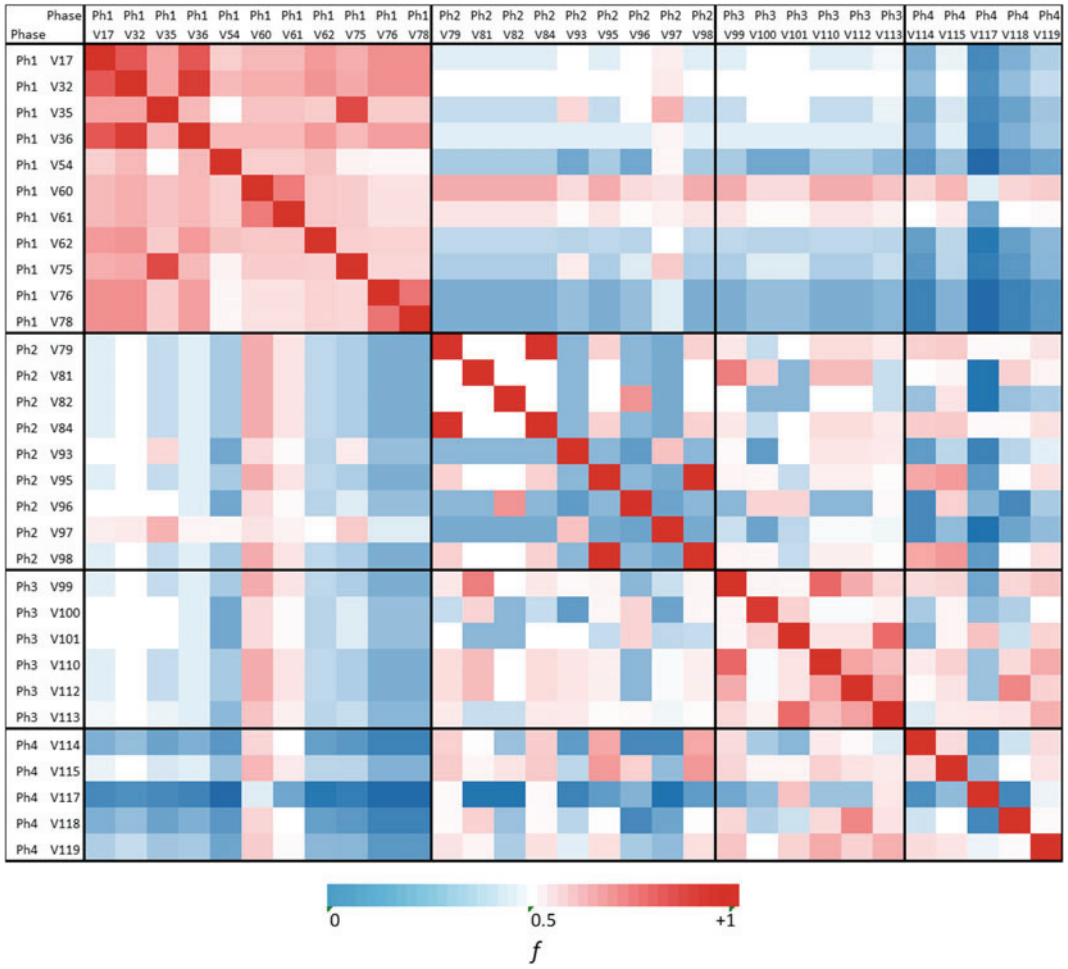

Fig. 1.2 Heat map of coefficients of coancestry (f) among 31 varieties released from the Australian lupin breeding program allocated during four Phases (Ph1 to Ph4) of variety release. Variety names associated with "V" numbers are listed in Table 1.1

1.2.3 Rate of Population Inbreeding

The rate of population inbreeding (ΔF) per cycle from the First Diversification Phase to the Exploitation Phase was calculated as follows (Falconer and Mackay 1996):

$$\Delta F = \frac{F_t - F_{t-1}}{1 - F_{t-1}}$$

From the data presented above for f (= F) in these two phases, and assuming two breeding cycles between these phases, then the average ΔF per cycle was 0.09, equivalent to 9% fixation of alleles per cycle due to random drift.

The effective population size (N_e) in the Exploitation Phase was estimated in an idealized population as follows (Falconer and Mackay 1996):

$$\Delta F = \frac{1}{2N_e}$$

With average ΔF per cycle equal to 0.09, N_e in the Exploitation Phase was estimated to be 5.4.

Table 1.2 The average proportion of ancestral founder varieties and wild types (WT) in the pedigree of varieties released in each Phase of narrow-leafed lupin breeding in Australia is based on average coefficient of coancestry from 4 founders and 11 wild types. The total proportion of founder varieties and WT in the pedigree is close to unity in Phases 1, 2, and 3, indicating that most of the ancestral parents are accounted for, but 0.12 of the pedigree remains unknown in Phase 4

Phase	No. varieties	Variety name:	New Zealand Blue	Borre	Rancher	65G-251							Proportion founders	Number founders
		Variety number	VI	V4	V14	V23								
		Variety status	Founder	Founder	Founder	Founder								
1	11		0.579	0.250	0.117	0.006							0.952	4
2	9		0.225	0.181	0.106	0.000							0.512	3
3	6		0.219	0.182	0.097	0.000							0.498	3
4	5		0.171	0.133	0.086	0.000							0.391	3

Phase	No. varieties	Variety name	P20722w	P20723w	Pxxxx1w	P20639w	P22661w	P22750w	P22872w	P22748w	P22721w	P26672w	P22764w	Proportion WT	Number WT
		Variety number	V18	V19	V28	V29	V30	V39	V40	V41	V42	V71	V72		
		Variety status	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT		
1	11		0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	1
2	9		0.014	0.014	0.042	0.000	0.056	0.167	0.056	0.083	0.056	0.000	0.000	0.486	8
3	6		0.052	0.052	0.135	0.000	0.000	0.146	0.104	0.000	0.010	0.000	0.000	0.500	6
4	5		0.021	0.021	0.063	0.000	0.000	0.178	0.052	0.012	0.045	0.041	0.050	0.483	9

The published values of N_e in Thoroughbred horses was close to 100 (Corbin et al. 2010) and 95 in the Meatlinc sheep breeding program in the UK (Avendaño et al. 2003). The median effective population size in zoo animals was 22.6, which was considered low (Boakes et al. 2007). The effective population size in Australian narrow-leafed lupin breeding seems very low in comparison to animal breeding programs. In genetic conservation of endangered species, an effective population size of less than 50 is considered detrimental to long-term persistence; such populations are in danger of extinction (Rutledge et al. 2017). While self-pollinating crops are not in danger of extinction (inbreeding depression is not an issue), the rate of loss in genetic diversity of narrow-leafed lupin breeding is very high and detrimental to long-term genetic gain. The efficiency of molecular genetic techniques such as marker-assisted selection, gene mapping, genome-wide diversity analysis and genomic selection will decay rapidly as allelic diversity is removed from the population by random drift.

1.2.4 Founders and Migrants Contributing to the Pedigree

Another approach to estimating effective population size is to consider the number of founders or migrants in the pedigree of crop varieties. In varieties released during the Second Diversification Phase of Australian narrow-leafed lupin breeding (2007–2016), 68% of the pedigree was based on three founders and three migrant wild ecotypes (Table 1.2). This is similar to the situation reported in soybean in the USA, where five introductions accounted for 55% of the pedigree of public cultivars in the 1990s (Gizlice et al. 1994), and canola breeding in Australia, where four founder parents in 1970 made up more than 50% of the pedigrees of varieties released in 2000, and 11 founders made up 98.7% of the pedigree (Cowling 2007). The effective population size in these self-pollinating crops is much smaller than in animal breeding examples cited above.

1.3 Characteristics of Varieties Released in the Australian Narrow-Leafed Lupin Breeding Program 1967–2016

Narrow-leafed lupins are unusual because they were domesticated in the mid-twentieth century, and because pedigrees are mostly in the public record for 31 varieties from the first domesticated variety "Uniwhite" in 1967 to "PBA Bateman" in 2016 (Table 1.1 and Fig. 1.1). The history of breeding in the Australian narrow-leafed lupin breeding program is well documented (Gladstones 1970, 1975; Cowling 1999).

1.3.1 Domestication (Pre-1967)

International exchange of lupin germplasm in the 1950s and 1960s was very important for the domestication of *L. angustifolius* and its successful development as a competitive grain legume crop (Gladstones 1970). Scientists in eastern and western Europe, USA, and Australia exchanged valuable germplasm and research results, including domestication and disease resistance genes, despite major geographical and political barriers. Such international germplasm exchange remains vitally important for increasing the genetic diversity and future breeding prospects of international lupin breeding programs.

German researchers discovered a low-alkaloid natural mutant *iuc* in the 1930s. This gene was combined with a permeable seed natural mutant *moll* in the Swedish sweet forage variety "Borre" (1947) (Gladstones 1975). *Ku*, an early flowering natural mutant discovered in Australia in "Borre", was important for adaptation of narrow-leafed lupins to the climate of southern Australia (Gladstones 1970). A similar early-flowering gene *Julius* (*Jul*) was identified by Polish breeders in the Russian variety Krasnolistny (Mikołajczyk 1966), and this permitted spring sowing of narrow-leafed lupins in northern Europe. Recently, *Jul* and *Ku* were shown to have unique deletions at the same *LanFTc1* locus (Taylor et al. 2019). Other interesting deletion

alleles were also discovered recently at this locus in wild ecotypes of narrow-leafed lupin (Taylor et al. 2019).

Gladstones (1970) also discovered natural mutants for non-shattering pods (*le*, *ta*) and white flowers and seeds (*leuc*) in the bitter fodder variety "New Zealand Blue". In effect, two foundation varieties "New Zealand Blue" and "Borre" contributed all the genes in the first fully domesticated sweet narrow-leafed lupin varieties "Uniwhite" (1967) (*iuc*, *mollis*, *ta*, *leuc*), "Uniharvest" (1971) (*iuc*, *mollis*, *ta*, *le*, *leuc*), and Unicrop (1973) (*iuc*, *mollis*, *ta*, *le*, *leuc*, *Ku*) (Table 1.1 and Fig. 1.1). Later varieties in the Foundation Phase incorporated disease resistance from USA variety "Rancher" (Gladstones 1975).

Global collaboration in lupin breeding allowed the domestication of sweet narrow-leafed lupins in Australia and in several other countries in the 1960s and 1970s. This set the stage for the Foundation Phase of narrow-leafed lupin breeding in Australia.

1.3.2 Phase 1: Foundation (1967–1987)

The Foundation Phase includes varieties released from 1967 to 1987. This Phase includes the first fully domesticated varieties "Uniwhite" (1967), "Uniharvest" (1971), and "Unicrop" (1973) (Fig. 1.1). Improved varieties "Marri" (1976), "Illyarrie" (1979), "Yandee" (1980), and "Danja" (1986) included contributions of disease resistance and yield from "Rancher" and frost tolerant germplasm "65G-251" from the USA. "Rancher" has survived in the pedigree of recent varieties (Figs. 1.1 and 1.2). A chemical mutant for mid-season flowering time (*efl*) was selected from "Borre" and used to breed mid-season flowering "Chittick" (1982) and "Wandoo" (1986) (Cowling 1999); the latter variety was withdrawn immediately due to susceptibility to cucumber mosaic virus (Cowling 1999). Finally, late-flowering "Geebung" (1987) was released in eastern Australia to replace "Uniharvest". Several successful varieties from the Foundation Phase, such as "Marri," "Chittick," and "Danja" did not contribute as parents to the First Diversification Phase (Fig. 1.1).

1.3.3 Phase 2: First Diversification (1987–1998)

The varieties of the First Diversification Phase were mostly the progeny of 1975 crosses of foundation variety 72A14-10 (the "Illyarrie" progenitor) with wild lupin ecotypes (Table 1.1). Domestication genes were reselected in the selfed progeny of each cross, which required substantial commitment inside the breeding program to select for soft seeds, low alkaloids, white flowers and seeds, and non-shattering pods. The first variety to be released was "Gungurru" in 1988 followed by "Yorrel" (1989), "Warrah" (1988), and "Merrit" (1991) (Cowling and Gladstones 2000). At least two ecotypes contributed to "Wonga" (1996), "Tanjil" (1998), "Myallie" (1995), "Kalya" (1996), and "Tallerack" (1997). A late flowering variety "Jindalee" was also registered for release in this Phase, but has an unknown pedigree (IP Australia 2019). It was not used in further cross-breeding and is not included in this analysis.

Selection for disease resistance was a feature of the First Diversification Phase. "Gungurru" (tested as breeding code 75A61-3) was moderately resistant to Phomopsis (Cowling and Wood 1989), and stubble derived from "Gungurru" displayed reduced lupinosis toxicity to sheep (Cowling et al. 1988). Phomopsis resistance removed a major impediment to the adoption of lupins by farmers—prior to "Gungurru," lupinosis was a serious mortality risk to sheep grazing lupin stubbles (Cowling and Gladstones 2000).

"Yorrel" and "Warrah" were progeny of 72A14-10 crossed to a different wild ecotype; each showed unique attributes derived from the wild parent, and each had improved resistance to Phomopsis. "Merrit" (1991) was a reselection from "Gungurru".

Strong selection occurred in the First Diversification Phase for anthracnose resistance, "Wonga" and its single plant selection "Tanjil" were resistant, "Kalya" was moderately resistant,

"Gungurru" and "Merrit" were moderately susceptible, and "Myallie" was susceptible (Garlinge 2005).

Resistance to brown spot and Pleiochaeta root rot were also selected during this Phase (Cowling et al. 1997), and moderate resistance was found in "Myallie," "Kalya," and "Tallerack" (Garlinge 2005).

Moderate resistance to seed transmission of cucumber mosaic virus was found in "Danja," whereas "Yorrel" and "Gungurru" were moderately susceptible and "Wandoo" was susceptible (Jones and Cowling 1995).

Aphid susceptibility was found in some varieties, and "Tallerack" was not promoted due to its susceptibility.

Disease resistance and higher yield contributed to the expansion of narrow-leafed lupin production in Australia in the 1990s (Cowling 1999). In 2004, approximately one million tonnes of lupins were produced in Western Australia, and 97% of the lupin area was sown to First Diversification Phase varieties (Garlinge 2005).

1.3.4 Phase 3: Exploitation (1998–2007)

Varieties released in the Exploitation Phase such as "Belara" (1997), "Moonah" (1998), "Quilinock" (1999), "Mandelup" (2004), "Coromup" (2006), and "Jenabillup" (2007) were derived from intermatings of high-yielding and disease-resistant parents from the First Diversification Phase. Every variety in the Exploitation Phase included ancestry of at least two wild lupin ecotypes. However, several successful varieties in the First Diversification Phase did not contribute as parents to the Exploitation Phase (Stefanova and Buirchell 2010) (Fig. 1.1).

Varieties released in this Phase showed substantial genetic improvements in grain yield, resistance to anthracnose (Stefanova and Buirchell 2010), and moderate tolerance of the herbicide metribuzin was selected following mutation breeding (Si et al. 2011). The exception was "Quilinock" which was very susceptible to anthracnose (Garlinge 2005).

1.3.5 Phase 4: Second Diversification (2007–2016)

Varieties released after 2007 such as "PBA Gunyidi" (2012), "PBA Barlock" (2013), "PBA Jurien" (2015), "PBA Leeman" (2016), and "PBA Bateman" (2016) include new wild ecotypes in the pedigree and complex pedigrees. Some of these varieties were selected for specific traits such as moderate metribuzin herbicide tolerance and disease resistance. There is some missing pedigree information due to complex pedigrees (Table 1.1 and Fig. 1.2). The missing information is due to complex crossing within the current pedigree, so the level of inbreeding is most likely underestimated in this part of the analysis.

1.3.6 Move to the Private Sector

Recently, the Australian narrow-leafed lupin breeding program was transferred from the public sector to the private sector, and no information exists on the genetic diversity retained for crossing in the private breeding program. This follows a trend in Europe of privatization of lupin breeding into companies in Germany and Poland. Genomic analysis could be used in future to estimate coancestry and effective population size, if relevant germplasm is made available to researchers.

1.4 Genetic Progress in Lupin Breeding

1.4.1 Australia

Substantial genetic progress has been achieved for yield and disease resistance in narrow-leafed lupin breeding in Australia during the 50 years of this study. Improvements are evident in grain yield, and resistance to Phomopsis, brown spot, CMV, and anthracnose (Cowling and Gladstones 2000). There was an 81% increase in grain yield in 34 years from "Unicrop" (1973) to "Mandelup" (2004) in trials of historical lupin varieties

(Stefanova and Buirchell 2010), although the yield improvement of new cultivars was only evident at high seeding rates (Cowling et al. 1998b). Tolerance to the herbicide metribuzin was included in the Second Diversification Phase (Si et al. 2011; Stefanova and Buirchell 2010). It appears that small-effective population size has not limited genetic progress in lupin breeding. This begs the question, then, why should we be concerned about low-effective population size in lupin breeding?

The number of breeding cycles during 50 years of this study was between 8 and 10 (range 4–8 years per cycle). There was a high rate of population inbreeding between Phases 2 and 3 (2 cycles). The rapid rate of population inbreeding ($\Delta F = 0.09$) in this period suggests an effective population size of approximately five, and a rapid approach to a genetic improvement plateau. It is also noted that a total of 15 "founder" or "migrant" genotypes contributed to the pedigree of released varieties after 50 years of lupin breeding, and 7 of these genotypes contributed to 73% of the pedigree in Phase 4 (Table 1.2). Compared with typical animal breeding programs (Goddard and Hayes 2009), very few individuals contribute to the lupin pedigree.

The proportion of founder varieties in the pedigree decreases over time as the proportion of wild types increases. However, even in Phase 4, the average coefficient of coancestry with founder varieties is 0.391, or 39.1% of the alleles are identical by descent to founder alleles (Table 1.2).

The major reason for concern about the small-effective population size in lupin breeding is the limit this imposes on long-term genetic progress. In a stochastic model of pea breeding for several traits in an economic index (Cowling et al. 2017), the model with high selection pressure and truncation selection appeared to have equal genetic gain as the model with optimal contributions selection for the first 10 cycles. After 10 cycles, equivalent to 40 or 50 years of lupin breeding, the high selection pressure scenario based on truncation selection reached a premature plateau in genetic improvement. In the pea model with high selection pressure there were 40 founder parents (Cowling et al. 2017), but only 4 founder parents and 11 wild types contributed to released varieties in the Australian lupin breeding program during 50 years (Table 1.2). Lupin breeding is therefore facing the risk of a premature plateau in genetic improvement, and new genetic diversity should be incorporated into the program as soon as possible.

1.4.2 Global Genetic Advances

A few narrow-leafed lupin breeding programs exist globally, normally alongside other lupin or pulse breeding programs.

There is renewed interest in lupin production and breeding in Europe due to the need for locally-grown plant protein and sustainable cropping (Lucas et al. 2015). The focus of breeding in northern Europe has been on early maturing *L. angustifolius* (Murphy-Bokern et al. 2017). Active breeding and release of varieties occurs in Germany at Saatzucht Steinach, where the most recent variety is "Mirabor" (www.saatzucht.de/english/grossleguminosen/leguminosengross.html). In France, the company Jouffray-Drillaud (https://www.jouffray-drillaud.com/accueil-en.html) lists two white lupin (*L. albus*) varieties. An EU research project "LIBBIO" is focused on selection in Andean lupin, *L. mutabilis* (https://www.libbio.net/).

In Poland, Poznan Plant Breeders (https://phr.pl/en/) and HR Smolice (https://www.hrsmolice.pl/pl/) have several varieties of narrow-leafed and yellow lupin in their portfolios. Out of the total grain legume acreage in Poland of 200,000 ha, yellow lupin occupies 15% and narrow-leafed lupin 30% (Prof Wojciech Swiecicki, *pers. comm.*).

In Russia, narrow-leafed lupin breeding is carried out mainly by State institutions. The State Register 2018 lists 25 varieties of *L. angustifolius*, 10 varieties of *L. albus*, and 10 varieties of *L. luteus* (Dr. Galina Gataulina, *pers. comm.*). Breeding institutes include the Former

All-Russian Research Institute of Lupin (Briansk region), The Russian State Agrarian University—Moscow Timiryazev Academy, All-Russia Research Institute of Grain Legumes and Groat crops, and Leningrad Research Institute of Agriculture. The sown area of lupin in the Russian Federation in 2017 amounted to 120,000 hectares, 5 times more than in 2012 (Dr. Galina Gataulina, *pers. comm.*).

In Chile, three breeding programs exist at Campex Baer (*L. angustifolius*, *L. albus,* and *L. mutabilis*), INIA Carillanca (*L. albus*), and Centro de Genómica Nutricional Agroacuícola (CGNA, *L. luteus*). Minor breeding programs on *L. mutabilis* occur in Peru, Ecuador, and Bolivia. The salmon industry generates a strong demand for lupin in Chile (Dr. Erik von Baer, *pers. comm.*).

1.5 Technologies to Improve Long-Term Genetic Gain

Four factors are important for long-term genetic gain for several commercially-important traits—genetic diversity, accurate prediction of breeding values, moderate selection pressure based on an economic index, and optimal mating designs (Cowling et al. 2017). Accuracy of prediction of breeding values has increased with the use of best linear unbiased prediction (BLUP) and GBLUP (genomic BLUP). However, while BLUP selection accelerated genetic gain compared with pre-BLUP breeding, it also increased the rate of population inbreeding (Avendaño et al. 2003). Independent culling on phenotype, the most common method of selection in crop breeding, is not conducive to long-term genetic gain (Cowling and Li 2018). The best outcome in the long run will be achieved with some form of optimal contributions selection for an economic index composed of weighted BLUP values for all traits (Cowling et al. 2017).

The first challenge in lupin breeding is to increase genetic diversity without reducing performance in the elite breeding program. Non-adapted germplasm may contain potentially valuable alleles, but how can these alleles be accessed without reducing commercial value in the breeding program through linkage drag? The answer is to migrate a small portion at a time of the genome of non-adapted lines into the elite breeding program. A BC_2-based migration scheme was proposed for introducing small portions of wild lupin genome into elite germplasm (Cowling et al. 2009). This also permitted selection of the key domestication traits during the BC_2-procedure. The scheme was introduced into the Australian lupin breeding program where it helped to increase protein content in near-elite BC_2 lines from crosses with high protein wild lupins (Buirchell 2008; Berger et al. 2013).

A major recommendation of this Chapter is that small isolated breeding programs, such as the Australian narrow-leafed lupin breeding program, while achieving good genetic progress after the domestication bottleneck, should increase their effective population size through migration from the large and diverse wild and landrace germplasm pool (Berger et al. 2012) or from contemporary breeding programs in other countries. Without much larger allelic diversity in the program, new technologies such as genomic selection will be economically inefficient. New genetic diversity should be incorporated into the elite program in small proportions (Cowling et al. 2009) to avoid reducing genetic gain in the breeding program. This process will be greatly assisted by optimal contributions selection to optimize mating designs for long-term genetic gain (Cowling et al. 2017; Cowling and Li 2018).

1.6 Conclusions and Recommendations

This Chapter describes population coancestry during 50 years of breeding in the Australian narrow-leafed lupin breeding program after the domestication bottleneck in the mid-twentieth century. In the Foundation Phase, immediately after the domestication bottleneck (1967–1987), the effective population size was very low (2 or 3 founder varieties in the pedigree). Several wild ecotypes were crossed with Foundation Phase

varieties, and this reduced population coancestry in the First Diversification Phase (1987–1998). Population coancestry increased in the Exploitation Phase (1998–2007), and more wild lupins were introduced in the Second Diversification Phase (2007–2016). The genetic diversity available in the Australian narrow-leafed lupin breeding program in 2016 is relatively low compared with animal breeding programs, but similar to other crop breeding programs such as canola breeding in Australia. This raises a major challenge for lupin breeders—how to increase genetic diversity and improve long-term genetic gain without reducing the performance of the elite breeding program? The most promising approach is to use low rates of migration based on BC_2-derived progeny of crosses with wild lupins or with breeding lines exchanged between international breeding programs, and optimized mating designs based on optimal contributions selection.

Collaboration and germplasm exchange among lupin breeders and geneticists in the mid-twentieth century promoted the domestication of narrow-leafed lupins. The three founder varieties of Australian narrow-leafed lupin breeding were from Europe and USA. Since the domestication bottleneck, there is no evidence of migration of germplasm from international breeding programs. However, active narrow-leafed lupin breeding continues in Germany, Poland, Russia, and South America. It is highly likely that all international lupin breeding programs have low-effective population size, but most have unique genetics—hence, it would be mutually beneficial for all programs to undertake regular germplasm exchange. Even low levels of international germplasm exchange would improve long-term prospects for lupin genetic improvement in the twenty-first century. International exchange helped narrow-leafed lupins through the domestication bottleneck, and international exchange is the key to the recovery of effective population size after the domestication bottleneck.

Acknowledgements I received substantial help from Australian and international colleagues who provided material for this chapter. Dr. Bevan Buirchell kindly filled in some gaps in the pedigree record. Mr Paul McGowan, Senior Technical Officer (Bioinformatics) Agri-Science Queensland, kindly developed the pedigree diagram shown in Fig. 1.1. I thank international colleagues who sent substantial amounts of information, including Dr. Fred Stoddard (University of Helsinki, Finland), Dr. Galina Gataulina (The Russian State Agrarian University—Moscow Timiryazev Academy), Dr. Erik von Baer (Semillas Baer, Chile), and Prof. dr. hab. Wojciech Święcicki (Institute of Plant Genetics, Polish Academy of Sciences).

References

Avendaño S, Villanueva B, Woolliams JA (2003) Expected increases in genetic merit from using optimized contributions in two livestock populations of beef cattle and sheep. J Anim Sci 81:2964–2975

Berger JD, Buirchell BJ, Luckett DJ, Nelson MN (2012) Domestication bottlenecks limit genetic diversity and constrain adaptation in narrow-leafed lupin (*Lupinus angustifolius* L.). Theor Appl Genet 124:637–652

Berger JD, Clements JC, Nelson MN, Kamphuis LG, Singh KB, Buirchell BJ (2013) The essential role of genetic resources in narrow-leafed lupin improvement. Crop Pasture Sci 64:361–373

Boakes EH, Wang J, Amos W (2007) An investigation of inbreeding depression and purging in captive pedigreed populations. Heredity 98:172–182

Buirchell BJ (2008) Narrow-leafed lupin breeding in Australia -where to from here? In: Palta JA, Berger JB (eds) Lupins for health and wealth. Proceedings of the 12th international lupin conference, Fremantle, Western Australia, 14–18 Sept 2008, pp 226–230. https://www.cabdirect.org/cabdirect/abstract/20103116770. Accessed 6 May 2019

Clements JC, Buirchell BJ, Yang H, Smith PMC, Sweetingham MW, Smith CG (2005) Lupin, Chapter 9. In: Singh RJ, Jauhar PP (eds) Genetic resources, chromosome engineering, and crop improvement: grain legumes, vol I. CRC Press, pp 291–396

Corbin LJ, Blott SC, Swinburne JE, Vaudin M, Bishop SC, Woolliams JA (2010) Linkage disequilibrium and historical effective population size in the Thoroughbred horse. Animal Genetics 41:8–15

Cowling WA (1999) Pedigrees and characteristics of narrow-leafed lupin cultivars released in Australia from 1967 to 1998. Bulletin 4365, Agriculture Western Australia

Cowling WA (2007) Genetic diversity in Australian canola and implications for crop breeding for changing future environments. Field Crops Res 104:103–111

Cowling WA, Allen JG, Wood PMcR (1988) Resistance to Phomopsis stem blight reduces the lupinosis toxicity of narrow-leafed lupin stems. Aust J Exp Agric 28:195–202

Cowling WA, Buirchell BJ, Falk DE (2009) A model for incorporating novel alleles from the primary gene pool

into elite crop breeding programs while reselecting major genes for domestication or adaptation. Crop Pasture Sci 60:1009–1015

Cowling WA, Buirchell BJ, Tapia ME (1998a) Lupin. Lupinus spp. Promoting the conservation and use of underutilized and neglected crops. 23. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy, 105 pp

Cowling WA, Huyghe C, Swiecicki W (1998b) Lupin breeding, Chapter 4. In: Gladstones JS, Atkins CA, Hamblin J (eds) Lupins as crop plants—biology, production and utilization. CAB International UK, pp 93–120

Cowling WA, Gladstones JS (2000) Lupin breeding in Australia. In: Knight R (ed) Linking research and marketing opportunities for pulses in the 21st century. Kluwer Academic Publishers, Netherlands, pp 541–547

Cowling WA, Li L (2018) Turning the heat up on independent culling in crop breeding. In: Hermesch S, Dominik S (eds) Breeding focus 2018—reducing heat stress. Animal Genetics and Breeding Unit, University of New England, Armidale, Australia, pp 119–134

Cowling WA, Li L, Siddique KHM, Henryon M, Berg P, Banks RG, Kinghorn BP (2017) Evolving gene banks: improving diverse populations of crop and exotic germplasm with optimal contribution selection. J Exp Bot 68:1927–1939

Cowling WA, Sweetingham MW, Diepeveen D, Cullis BR (1997) Heritability of resistance to brown spot and root rot of narrow-leafed lupins caused by *Pleiochaeta setosa* (Kirchn.) Hughes in field experiments. Plant Breed 116:341–345

Cowling WA, Wood PMcR (1989) Resistance to Phomopsis stem and pod blight of narrow-leafed lupin in a range of environments and its association with reduced Phomopsis seed infection. Aust J Exp Agric 29:43–50

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Harlow, UK

Garlinge J (2005) 2005 crop variety sowing guide for Western Australia. Department of Agriculture and Food, Western Australia, Perth. Bulletin 4655. https://researchlibrary.agric.wa.gov.au/cgi/viewcontent.cgi?referer=&httpsredir=1&article=1156&context=bulletins. Accessed 6 May 2019

Gizlice Z, Carter TE Jr, Burton JW (1994) Genetic base for North American public soybean cultivars released between 1947 and 1988. Crop Sci 34:1143–1151

Gladstones JS (1970) Lupins as crop plants. Field Crop Abstr 23:123–148

Gladstones JS (1975) Lupin breeding in Western Australia: the narrow-leaf lupin (*Lupinus angustifolius*). J Dep Agric West Aust, Series 4, 16:44–49. https://researchlibrary.agric.wa.gov.au/journal_agriculture4/vol16/iss2/4. Accessed 6 May 2019

Gladstones JS (1998) Distribution, origin, taxonomy, history and importance, Chapter 1. In: Gladstones JS, Atkins CA, Hamblin J (eds) Lupins as crop plants—biology, production and utilization. CAB International UK, pp 1–39

Goddard ME, Hayes BJ (2009) Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nat Rev Genet 10:381–391

IP Australia (2019) Plant breeders rights—search plant breeders rights. https://www.ipaustralia.gov.au/plant-breeders-rights. Accessed 6 May 2019

Jones RAC, Cowling WA (1995) Resistance to seed transmission of cucumber mosaic virus in narrow-leafed lupins (*Lupinus angustifolius*). Aust J Agric Res 46:1339–1352

Mikołajczyk J (1966) Genetic studies in *Lupinus angustifolius*. Part III. Inheritance of the alkaloid content, seed hardness and length of the growing season in blue lupin. Genet Pol 7:181–196

Mousavi-Derazmahalleh M, Bayer PE, Nevado B, Hurgobin B, Filatov D, Kilian A, Kamphuis LG, Singh KB, Berger JD, Hane JK, Edwards D, Erskine W, Nelson MN (2018) Exploring the genetic and adaptive diversity of a pan-Mediterranean crop wild relative: narrow-leafed lupin. Theor Appl Genet 131:887–901

Murphy-Bokern D, Stoddard FL, Watson CL (2017) Legumes in cropping systems. CAB International UK

Rutledge LY, Desy G, Fryxell JM, Middel K, White BN, Patterson BR (2017) Patchy distribution and low effective population size raise concern for an at-risk top predator. Divers Distrib 23:79–89

Si P, Pan G, Sweetingham M (2011) Semi-dominant genes confer additive tolerance to metribuzin in narrow-leafed lupin (*Lupinus angustifolius* L.) mutants. Euphytica 177:411–418

Stefanova KT, Buirchell B (2010) Multiplicative mixed models for genetic gain assessment in lupin breeding. Crop Sci 50:880–891

Święcicki W, Kroc M, Kamel KA (2015) Lupins, Chapter 6. In: de Ron AM (ed) 'Grain Legumes', Handbook of Plant Breeding, vol 10. Springer NY, pp 179–218

Taylor CM, Kamphuis LG, Zhang W, Garg G, Berger JD, Mousavi-Derazmahalleh M, Bayer PE, Edwards D, Singh KB, Cowling WA, Nelson MN (2019) INDEL variation in the regulatory region of the major flowering time gene *LanFTc1* is associated with vernalisation response and flowering time in narrow-leafed lupin (*Lupinus angustifolius* L.). Plant Cell Environ 42:174–187