Domestication of pasture and forage legumes for Australian Farming Systems; a personal perspective

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Abstract
Domesticated pasture legumes have played an important role in shaping broadacre farming systems in Australia. Past efforts have not only delivered novel plants species and cultivars but also substantial know-how about the processes of domestication that allows current efforts to be targeted and effective. This paper outlines these proven processes and provides a reminder of the range of resources and skills that are needed when embarking on such initiatives. The range and complexity of component activities involved in pasture legume domestication and the time scale over which these efforts occur distinguish this from most other agricultural research and development. This has important implications for the extent, resourcing and organisational structure that is required for future efforts.

Keywords
Howard medal, grazing, novel species, rhizobiology.

Introduction
The honour afforded by being awarded the AW Howard Medal allows me the opportunity to reflect on some career insights. I have chosen to focus on the broad issue of pasture legume domestication for Australia's mixed farming zone, this being the central theme of my much of my research efforts. This is a specialised aspect of plant breeding where Australian scientists have played a world leading role, guided by their motivation to deliver innovative farming system opportunities for Australian producers. While the recent outcomes from this work are transparent in the form of newly domesticated pasture legume genera, species and cultivars (Loi et al. 2005; Nichols et al. 2007; Nichols et al. 2012a), the detailed scientific processes that sit behind these outputs are less well understood and continue to evolve.

The lack of general appreciation of the complexity and time-scale of efforts involved in pasture legume domestication came home to me when I recently attended the formal release of Neptune, the first cultivar of the species *Melilotus siculus* (Turra) B.D. Jacks., which has the species common name messina. This is a plant with spectacular tolerance of waterlogging and salinity (Nichols et al. 2012b). During the release ceremony, much was made of the specific agronomic traits of the newly released cultivar, but next to nothing was explained about the decade or more of scientific effort undertaken by a multidisciplinary team to provide producers with a profitable tool to deal with salinity and waterlogging. As a practitioner in this area, I regarded this as a missed opportunity to celebrate very substantial scientific achievements and explain to a wider audience the complexity of the scientific processes involved.

This paper draws on the example of Neptune and other recent initiatives to highlight the scope and importance of pasture legume domestication. We now have an extensive body of knowledge and experience of effective pathways for legume domestication and these approaches were followed in the development of messina. The protocols used will be spelled out, including examples where I have personal insights. This information is relevant to the questions that currently hang over pasture legume innovation: whether to make further investment; what new targets are out there; how can this be funded and who might do the work?

Scientific and farming system context
The persistent use of leguminous pasture and forage legumes in rotations with crops has been a feature of southern Australian agricultural systems for many decades. This general land use strategy involving crops and pastures in rotation and its evolution over time has been widely documented (Puckridge and French 1983; Reeves and Ewing 1993; Ewing and Flugge 2004; Bell and Moore 2012). Changes in land use have occurred as part of a wider need to adapt farming systems to prevailing economic, technical and climatic circumstances. While under pressure from crop only systems, the 'mixed' or 'crop/livestock' farming system that integrates crop and livestock production within the boundaries of a farm has wide-scale application and continues to evolve. This system is the source of demand for new pasture legume domestication initiatives

and strong medium and long term drivers for such activities remain. The continuing decline in terms of trade in broad acre agriculture (Kingwell and Pannell 2005), changes in climate (Revell et al. 2013), development of new biotic stresses and soil sustainability threats such as acidification, salinity and soil nitrogen decline are some of the major drivers.

The pasture/forage legume component of these systems has been the result of the domestication of ‘wild’ legumes, primarily of Mediterranean origin. Initially, the systems were developed based on species that had become naturalised in Australia during the settlement phase. This activity matured over time to involve traditional breeding programs to improve the well-established species such as subterranean clover and annual medics. From the 1980s, active domestication of a much wider range of species (so called alternative species) has been a prominent area of research (Francis 1991) and has resulted in many new releases (Loi et al. 2005; Nichols et al 2007). The methodologies employed in these programs differ from the established pasture breeding programs (Ewing 1989). They have generally involved selection amongst accumulated germplasm or land races of promising species for a ‘cultivar’ with a set of characteristics delivering adaptation in Australian soils, climate and farming systems. Much of the accumulated germplasm has been the result of collections from the Mediterranean region with special emphasis on regions with closely similar climatic and soil conditions to the Australian context.

As a research participant in this area from the early 1980s onwards and subsequently as a leader and manager of pasture legume ‘breeding’ initiatives, there has been an on-going challenge to design and undertake research that was both relevant and potentially adoptable by producers. Importantly, such initiatives must use the limited research resources available to deliver good returns on that investment. This implies an in-depth understanding of the key factors driving the costs and benefits of new research initiatives including the likely scale of future adoption of the plant, the profit advantage likely to be generated and the time delay before new technologies becomes available. The relevant profit advantage for pasture legumes relates to their farm level impact through the length of an entire rotation rather than profit delivered in a pasture year or even during a complete pasture phase. Given the historical evidence that a decade is a minimum time scale for product delivery, it becomes clear that in this dynamic farming system context to which we are exposed, any benefit/cost evaluation needs to take account of likely parallel innovations in competing land uses, mainly crops. This applies to likely advances with legume crops and oil seeds as well as cereals. Acquisition of the information needed to evaluate alternative pasture legume investment has been a major driver for the development of farming systems analysis including tools such as the MIDAS model (Morrison et al. 1986) where these complex bio-economic issues can be tackled.

Once commercially available, producers have been active and innovative in trialling and adapting new pasture technologies to make them useful as part of their individualised systems. New pasture and forage legumes, mainly the result of domestication of novel species have been an important part of evolving patterns of land use. However, despite these innovations, pastures and pasture legumes have generally diminished in importance as part of trends that have seen greater emphasis placed on crop production. While these trends are real, they are slow and circumstances may arise that reverse the trends towards further cropping intensification (Bell and Moore 2012). There are currently very few resources allocated to pasture legume development initiatives. Lack of investment in pasture innovation may limit our ability to drive the changes to the mixed farming systems that are needed for future prosperity.

Pasture legume domestication processes

Experience has demonstrated that there is a set of generalised steps followed in most pasture legume domestication initiatives and these are spelled out in Table 1. The intensity of research effort involved in each stage varies depending on circumstances. Although there is a natural progression between the stages outlined, in practice stages can overlap.

Plant breeding following the path of domestication differs from more traditional crop and pasture breeding in two main ways. The first is the extent of potential system disruption. Traditional breeding generally targets incremental change while new species domestication aims to delivers tools to facilitate major system adjustments.

The second main difference is in the generation and manipulation of genetic diversity. Traditional breeders are attempting to change a crop by increasing yield, overcoming a constraint or increasing the range of adaptation of the crop. They do this by crossing elite parents and assessing the outcome of the gene rearrangements. The results tend to be incremental changes that do not strongly perturb the farming system within which these crops are grown. For instance, changing the wheat variety from Westonia to Mace has been very important for the profitability of some Australian grain producers but this has had little or no impact on the farming system or general production strategy.

With pasture legume domestication, the primary objective is typically to identify plants that can perform a farming systems role not currently filled by an existing plant. When contemplating a new initiative, the implied question is: can a change to the current farming system involving the inclusion of a new pasture legume make the system more profitable; and, what key characteristics would the plant need to succeed in that role? At its core, the plant breeding question becomes: has a plant genus/species/accession evolved in the wild with a genetic makeup that closely enough matches the identified list of traits needed to fill the farm system niche identified and can we access it?

Table 1. Sequence of processes required to domesticate a novel pasture legume (Adapted from Dear and Ewing 2008).

Stage	Plant breeding activity	Comment on activities and skill sets required
1	Farming system and agro-ecological analysis to identify gaps in existing farming systems and quantify their potential scale	Preliminary benefit cost analysis can be made at this point to evaluate the likely impact of the investment *Key skills: farming systems analysis, economic analysis*
2	Identify necessary traits for the target opportunity and conduct scoping reviews to identify suitable species	Some traits such as ease of establishment and seed harvest are generic while others such as soil stress tolerance will be case specific. *Key skills: Pasture agronomy and ecology*
3	Germplasm acquisition – assemble appropriate genetic diversity	Key sources are likely to be Genetic Resources Centres, international collaborators or targeted collection – includes rhizobial diversity linked to the host plant species. *Key skills: Genebank curation, plant and rhizobial ecology and collection*
4	Conduct desktop weed risk assessment	This process may identify key information gaps requiring further data collection *Key skills: pasture and weed ecology*
5	Seed multiplication	Seed quantities available from genebanks are typically small and multiplication is required. *Key skills: Pasture agronomy*
6	Small plot scale evaluation of representative accession of highest priority species	This step is a low-cost filter to reject poorly adapted material and allow later stages of evaluation to be undertaken with smaller numbers. Matched rhizobia are needed *Key skills: Pasture agronomy and breeding*
7	Evaluation of cohorts of elite species in the target environment using anticipated management system.	This is typically proceeded by a further seed multiplication step. Involves phenotyping the material for key production and persistence traits with management including realistic defoliation *Key skills: Pasture agronomy and pasture breeding*
8	Livestock performance evaluation	Involves quantification of the chemical feed value, grazing performance and anti-nutritional compound status. Where grazing systems evaluation is required, additional seed increase may be necessary *Key skills: Plant chemistry, animal production and pasture agronomy*
9	Seed increase of elite material targeted for commercialisation	Seed crops require high input management *Key skills: Pasture agronomy*
10	Development of appropriate management packages	Often run in parallel with seed increase and needs to cover agronomic and grazing issues. A special issue is herbicide tolerance and this information is helpful for managing seed production. *Key skills: Pasture agronomy, animal production and weed science*

Farming systems context and analysis

Given this preamble, it becomes clear that a prerequisite to effective targeting of research resources in pasture legume domestication is a sophisticated understanding of existing farming systems with a special emphasis on identification of unprofitable existing elements, constraints or unsustainable practices. From such an understanding comes the opportunity to spell out the potential for a hypothetical new plant to be used to overcome or by-pass these recognised constraints.

Farming system analysis is also central to predicting the likely spatial extent of use of a potentially successfully innovation, at both farm level adoption as well as the projected uptake regionally or nationally. This information is a key driver of the benefit/cost analysis that should precede any substantial physical investment. These are the key activities outlined as Stage 1 in the development of a novel plant (Table 1).

The importance of farming system understanding and analysis as a backdrop to pasture legume domestication was a primary motivation in the development of the bio-economic farming system analytical tool MIDAS (Morrison et. al. 1986; Pannell 1995; Bathgate et al. 2009). This represents a major step forward in allowing the direct and indirect impacts of legumes on farm profitability to be quantified and valued. It has also allowed an analysis of the economic impact of hypothetical new plant options to be explored in the absence of full experimental data sets. Analytical processes use sensitivity analysis where a range of possible experimental outcomes are tested for economic impact. These techniques can also be used to evaluate the implications of changes in key underlying but dynamic drivers of system profit such as product prices or technical developments in competing technologies. These are important considerations given the potential for system evolution in the decade or more that it takes to develop a new farming system. The commitment of resources to sound pre-experimental contextual analysis has emerged as a key success factor with pasture legume domestication initiatives. Useful examples of a comprehensive approach to these matters include the two most recent initiatives to identify novel pasture legumes, these being:

- saline and waterlogging environments (*M. siculus,* common name messina cv. Neptune)
- drought tolerant perennial legumes (*Bituminaria bituminosa* C.H.Stirt var. *albomarginata*, common name tedera, a yet to be named cultivar).

In both case, substantial reviews and scoping studies underpinned the selection of the targeted areas for investment (Rogers et al. 2003; Ewing and Dolling 2003) and detailed *ex ante* economic analysis highlighted the potential for farming system change and profit advantages that could follow from successful plant development initiatives (O'Connell and Young 2002; Finlayson et al. 2012). Careful and patient targeting analysis needs to be undertaken before embarking on the time-consuming efforts associated with identifying the necessary traits and the plants needed for successful adaptation (Stages 2 and 3, Table 1).

Identifying target germplasm and assembling genetic diversity

Knowledge of Mediterranean ecosystems, their flora and its distribution in relation to climate, soils and land uses is central to the task of identifying target germplasm for evaluation (Stage 3, Table 1). This specialised knowledge has typically been acquired by those involved directly in plant collection or in evaluating and preserving the collections of others as part of a systematic genetic resource preservation process. Australian pasture scientists have been extensively involved in plant collections. Some of these ventures have been comprehensive in terms of the species targeted and others have been narrow focussing on a single genus or a combination of climatic and soil conditions at the sites of collection. Scientists engaged in this work have typically collected sufficient details of each collection site and plant population to allow subsequent cross referencing to sites in Australia with similar environmental conditions. Importantly, those involved have often interpreted and published their observations of individual collection missions (Francis and Gillespie 1981; Ewing and Fortune 1990) or drawn on their composite of experience to provide general guidance on likely promising opportunities (Francis 1999). A thorough approach to the job of identifying useful genetic diversity typically involves;

- Literature reviews
- Interrogation of Genetic Resource Centre (GRC) data bases (local and international centres)
- Consultation with experts, especially curators of GRCs (local and international centres) and plant collectors
- *In-situ* studies of plant distribution in environments analogous to those being targeted.

Establishing and maintaining networks with genebanks and scientists working in other parts of the world with similar climates has been an important success factor in identifying and subsequently accessing useful genetic diversity.

The size and generic diversity of the list in Table 2 highlights the challenges associated with the next stage, assembling representative examples of potentially useful diversity (Stage 3, Table 1). Table 2 is an example of the list of prospective species that was prepared in advance of efforts to identify legumes with tolerance of salinity and waterlogging stresses (Rogers et al. 2003) .The scale of this assembly task is substantially increased for legumes because of the need to identify suitable strains of root nodule bacteria for each of the species. The selected organisms must exhibit high nitrogen fixation ability and possess adaptation to the soil environment for which the legume is targeted (Howieson 1999).

Table 2. List of legumes with known salinity and/or waterlogging tolerance used as the basis for assembling genetic diversity for testing for field adaptation to theses stresses (Rogers et al. 2003).

Genus	Species
Astragalus	*A. adsurdens*
Ceratoides	*C. lateens*
Glycyrrhiza	*G. acanthocarpa, G. glabra*
Hedysarum	*H. carnosum, H. coronarium*
Lotus	*L. angustissimus, L. corniculatus, L. creticus, L. maroccanus, L. subbiflorus, L. tenuis*
Medicago	*M. cancellata, M. carstiensis, M. ciliaris, M. hispida, M. littoralis, M. marina, M. polymorpha, M. scutellata, M. tornata*
Melilotus	*M. albus, M. indicus, M. italica, M. siculus, M. officinalis, M. segetalis. M. sulcatus*
Swainsona	*S. lessertifolia, S. procumbrans, S. purpurea, S. swainsonioides*
Trifolium	*T. alexandrinum, T. angulatum, T. blancheanum, T. clusii, T. fragiferum, T. isthmocarpum. T. ligusticum, T. michelianum, T. ornithopoides, T. palaestinum, T. philistaeum, T. resupinatum, T. squamosum, T. stipulaceum, T. striatum, T. tomentosum, T. variegatum, T. wormskiddii*
Trigonella	*T. balansae*, *T. suavissima*
Viminaria	*V. juncea*

Typically, acquisition of genetic material for a domestication initiative has two phases. Initially, representative accessions of species thought likely to deliver appropriate traits are assembled. These are then evaluated in small plot nurseries, preferably in the target environment. The aim of early evaluation is to identify a much smaller sub-set of genera/species with all or most of the key required traits. Subsequently, additional efforts are required to acquire a more diverse collection of the elite performing species, undertake seed multiplication, and then expose the plants to a second rigorous evaluation in the likely target environment (soil and climate). It is important to subject them to the management regime likely to be applied when used as part of future systems. In the case of the messina project, a targeted plant collection mission to south-west Spain (with financial support from the AW Howard Memorial Trust) was undertaken in 2009 to collect addition diversity of plant and rhizobial diversity. Messina had emerged from the first stage of testing as clearly the most promising species. Importantly, collections of root nodule bacteria were also made to address a problem with soil adaptation of existing commercial rhizobial strains selected because of their effectiveness on closely related species. These strains proved to be inadequate because although they provided effective symbiosis in the year of introduction they failed to persist in the saline soil environment and to nodulate regenerating seedlings (Bonython et al. 2011). Tedera followed a similar pattern (Real et al. 2008) with targeted collection being undertaken in the Canary Islands, centre of origin of the plant, in collaboration with Spanish scientists once it had been identified as the most promising drought tolerant perennial pasture legume.

A key constraint in these processes is the resources required to recover existing collections from gene banks and multiply the seed to produce quantities sufficient large for use in nurseries (Stage 3, Table 1) and plots (Stage 7, Table 1) where they can be effectively phenotyped. For legume pastures, a mini sward that is of sufficient size to allow defoliation is typically required.

In most pasture legume domestication programs, a livestock performance evaluation will be necessary and logistics determine that this can only realistically occur when the numbers of accessions to be tested has been reduced to a very small number. Realistic grazing treatments (Stage 8, Table 1) require plot sizes that allow extended periods of grazing and the main experimental constraint is typically a shortage of seed to establish suitably sized areas.

Duty of care
In domesticating a novel pasture legume, it is important that steps are taken to minimise the risk of adverse outcomes emerging from its use. The concept of 'duty of care', now widely embedded in the culture and systems of plant domestication, embodies the need to take reasonable steps to ensure this does not occur. Institutions involved in plant development activities are increasingly risk averse when confronting their role in approving the public release of a new cultivar (Revell and Revell 2007). An explicit and well documented strategy needs to be in place to investigate known risks and to provide details of fault detection and risk mitigation.

The risk that an introduced plant might become a weed of native ecosystems is a primary concern. The Australian quarantine system has been strengthened in recent times to prevent the introduction of high risk species, and plants cannot now be introduced without a comprehensive weed risk evaluation. In the case of tedera, access from international sources was delayed for a year while a weed risk assessment was undertaken. Many institutions now have adopted protocols that require internal weed risk assessments before preliminary evaluation (Stage 4, Table 1) and again prior to commercial release.

Knowledge of and capacity to monitor the chemical constituents of plants are important aspects of duty of care. Many wild species contain chemicals with known or suspected negative impacts on grazing animals. Identification of known chemical risk factors is an important aspect of pre-evaluation literature reviews. Some plant material may be rejected prior to testing because of its high-risk status. Sampling and targeted chemical analysis is typically undertaken with material included in advanced evaluation. For example, some *Melilotus* spp. are known to contain coumarins. These compounds are associated with tainting the flavour of milk and meat and under some circumstances lead to adverse animal health outcomes. Comprehensive testing of messina has established that coumarins are only present in negligible concentrations. Subsequent grazing trials were conducted with Neptune and no adverse effects on livestock were detected (Babiszewski and Edwards, unpublished data). The genus *Bituminaria bituminosa* is known to contain furanocoumarins some of which are linked in other species to photosensitisation in animals. As a result, comprehensive chemical testing and grazing evaluations have been undertaken as part of the evaluation of tedera to demonstrate that the material under test does not contain threatening concentrations. Animal performance when grazing tedera has been in line with expectations based on plant quality measurements and no adverse animal health symptoms have been detected (Oldham et al. 2013).

The importance of root nodule bacteria in legume pasture domestication has been highlighted in the context of the need to identify strains with the capacity for high nitrogen fixation and adaptation to soil conditions in the target environment. However, duty of care obligations dictate additional layers of investigation. Active research steps also need to be taken to ensure that there are no negative impacts of newly introduced strains on non-target host legumes. It is also important to establish that any strain likely to progress to commercial application is stable in soil, in culture and in storage. Further, if a strain is to be used beyond experimental scale, it must demonstrate a capacity to grow and survive in the inoculant carrier (Howieson 1999).

Structural issues influencing pasture legume domestication efforts
Resourcing
Current low levels of investment in new pasture legumes highlight some of the issues involved with the funding of pasture research in general and legume breeding and domestication efforts. Pastures, unlike crops, have no endpoint royalty mechanism for commercial capture of investments in breeding. Long term revenue flows from seed sales are typically compromised by rights of producers to grow their own seed, based on the 'farmers' rights' provisions of Plant Breeders Rights rules. This state of affairs severely limits the commercial incentive of private breeders to participate in innovative, long term and resource-intensive activities such as new pasture legume domestication. This reflects Australian experience to date and, without structural changes, this is unlikely to change (Revell et al. 2013).

Investment has historically been dominated by public funding from state government agencies and federally from CSIRO and through mechanisms such as Cooperative Research Centres. Public funding has been complemented by industry investments by the Rural Development Trust Funds. Given that the benefits from pasture legume innovation flows to the crop, livestock (wool and meat), fodder and seeds industries, collaboration in funding the associated research by Australian Wool Innovation (AWI), Meat and Livestock Australia (MLA), Grains Research and Development Corporation (GRDC) and Rural Industries Research and Development Corporation (RIRDC) would seem desirable. In fact, although some collaboration has taken place in past programs such as the National Annual Pasture Legume Improvement Program (NAPLIP), current co-ordination and collaboration operates at low and informal levels. The commitment of resources to agricultural research from public sources is in decline and there is unlikely to be a major reversal. However, the influence of industry co-investment in maintaining public investment should not be overlooked. This is particularly important for maintaining capacity in key scientific infrastructure such as gene banks for pasture plants and their associated root nodule bacteria.

Continued innovation in pasture legume research and plant domestication will rely heavily on the investment strategies of industry funders, and the importance of inter-industry co-investment mechanisms deserves more attention. Better coordination depends on being realistic about maintaining co-investment arrangements in place for the gestation period of novel plant development, typically greater than a decade. Despite these real dilemmas, it is possible to break the domestication process up into a series of linked projects. Stages 1-6 (Table 1) fit naturally as a project to deliver a narrow target for subsequent detailed evaluation, and that can be further divided into pre-experimental and experimental sub-projects. Stages 7 and 8 cover the focussed activities required to deliver an elite cultivar or cultivars. Stages 9 and 10 in Table 1 are essential to an effective commercial outcome. This is a natural point for commercial engagement with special value coming from commercial expertise in seed production systems. Conceiving the pasture legume domestication process as a long-term investment in several linked phases also allows for points of review to be introduced. Progress towards the desired commercial outcome can be assessed and projects terminated if progress is inadequate. Not all identified gaps are sufficiently important or demonstrate enough technical feasibility to allow progress past the initial review stage. In other circumstances, nothing genetically useful might emerge from the pre-screening phase. These negative outcomes should not be regarded as research failures but as sensible management of the risks associated with highly innovative research.

Research and Development team and research capacity
Table 1 includes details of the diverse array of skills and capacities that are drawn on through the course of a pasture legume domestication process. In the past, research capacity to fill the full array of required skills was present in multiple research organisations, mainly state agencies. This is no longer the case, so current and future initiatives need to involve high levels of institutional collaboration, and relationships will need to be maintained over extended periods. While the identification and maintenance of relevant skills sets is vital to success, so is the sponsoring and resourcing of team leadership and coordination. Individuals who take on leadership roles are most effective when they have a comprehensive understanding of all aspects of the domestication process. Minimum required skills include farming system analysis, economic analysis, plant ecology, plant breeding, rhizobiology, plant chemistry, animal science and agronomy. To this list, we should also add the role of salesman given the importance of accessing required resources and funder relations. Active strategies need to be put in place to ensure on-going access to all the required skills and to identify and support potential program leaders.

Conclusions
Australia has a proud history of pasture legume domestication. A high level of subsequent cultivar adoption has occurred, and typically success has been linked to a large target market, low seed cost, a high relative advantage compared to existing technologies and a low complexity of agronomic and grazing management.

Pasture legume domestication is special variant of plant breeding that requires the integrated application of a much wider set of skills into operating teams than traditional crop breeding initiatives. Substantial know-how and human capacity has been established in operational processes. Application of the established know-how enhances research efficiency, reduces research risks and increases the likelihood that outcomes from research will benefit producers.

Australia has invested effectively in the past to establish useful repositories of plant and rhizobial genetic diversity, GRCs. Institutions and individuals have also established collaborative international networks that have facilitated access to necessary genetic diversity and information from overseas. This past investment will only be useful if it is maintained and drawn into active project initiatives. International collaboration requires a two-way flow of benefits and Australia's interests require that active research projects allocate resources to ensure relevant linkages are maintained.

Despite a reduction in emphasis on pasture legumes in mixed farming, evolution of systems is ongoing. In the process, opportunities are being created for new technological components. Systematic evaluation of the potential offered by novel pasture legumes should encourage funders to commit the long-term investments needed to create novel opportunity for producers where there is evidence that they can be profitable. Communication between research funders and providers is essential to ensure that the resources, infrastructure and human capacity is available to support these long-term investments, often requiring teams that cross regional, discipline and institutional boundaries.

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