Interspecific hybridization in perennial species of *Lathyrus* (fabaceae)

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Abstract – In an attempt to introduce new variation into *Lathyrus latifolius*, the perennial pea, a programme of interspecific hybridization with several perennial and one annual *Lathyrus* species was undertaken. New interspecific hybrid combinations were produced involving three of the perennial species (*L. latifolius*, *L. sylvestris*, *L. heterophyllus*). Crosses with the annual species, *L. gorgoni*, were unsuccessful. These new hybrids are important additions to the limited number of *Lathyrus* hybrids. Meiotic analysis of the new hybrids and in particular the analysis of their pachytene pairing from spread synaptonemal complexes show that the low fertility of these hybrids is not due to structural differences between the chromosomes of the parental species. Some of the new hybrids that were produced showed unusual karyotypes and variation in meiotic chromosome pairing. (© Inra/Elsevier, Paris.)

Lathyrus / chromosome analysis / interspecific hybridisation / synaptonemal complexes

Résumé – Hybridations interspécifiques entre espèces pérennes du genre *Lathyrus* (Fabaceae). Dans le but d'introduire une nouvelle variabilité chez *Lathyrus latifolius*, le pois pérenne, un programme d'hybridation interspécifique a été entrepris entre espèces de *Lathyrus*, plusieurs pérennes et une annuelle. De nouvelles combinaisons hybrides interspécifiques ont été obtenues, impliquant trois des espèces pérennes (*L. latifolius*, *L. sylvestris* et *L. heterophyllus*). Les croisements avec l'espèce annuelle, *L. gorgoni* furent infructueux. Ces nouveaux hybrides constituent un apport important au nombre limité d'hybrides de *Lathyrus* actuellement disponibles. L'analyse des méioses des nouveaux hybrides et en particulier l'analyse de l'appariement au pachytène à partir d'étalements de complexes synaptonémaux montre que la faible fertilité de ces hybrides n'est pas due à des différences structurales entre les chromosomes des espèces parentales. Quelques uns de ces nouveaux hybrides ont présenté des caryotypes atypiques et une variation dans l'appariement des chromosomes à la méiose. (© Inra/Elsevier, Paris.)

Lathyrus / analyse chromosomique / hybridisation interespèces / complexes synaptonémaux

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1. Introduction

The genus *Lathyrus* L. (Fabaceae) contains a number of important crop species such as *L. sativus* L., an important pulse and fodder crop and *L. odoratus* L., the sweet pea, a widely grown and valuable ornamental annual garden plant. Other species are economically less important but may have the potential to become significant new crops. One of these is *L. latifolius* L., the perennial pea, a perennial vine that is grown on a limited scale as an ornamental garden plant as well as occurring wild as a native of central and Mediterranean regions of Europe and as a garden escape in North America. Unlike the sweet pea, it lacks scent and a wide range of flower colours and forms but it is well adapted to hot, continental climatic regions, regions where sweet peas are not a viable proposition. If it were possible to introduce new characters such as variation in flower colour and scent into *L. latifolius*, it has the potential to become an important ornamental plant. It is, therefore, necessary to establish the limits of the *L. latifolius* gene pool by making interspecific hybrids with a range of other *Lathyrus* species. In *L. odoratus* the huge variation between cultivars has arisen by the accumulation and recombination of spontaneous mutations and hybridization within the species but more recently interspecific hybrids have also been used in this species in an attempt to introduce further new characteristics [14].

Although several cultivars of *L. latifolius* have been named that have pale pink or white rather than the characteristic deep pink flowers, the range of variation is limited. Interspecific hybridization could provide a more rapid means of introducing new characteristics into this species. Unlike many *Lathyrus* species, it has been reported to form natural hybrids with the related species *L. sylvestris* in northern France [2]. This is unusual as, despite the uniformity of chromosome number in the genus ($2n = 2x = 14$), interspecific hybrids are in general difficult to produce using conventional plant breeding methods [8, 21]. This can be seen from the fact that after decades of attempts at interspecific hybridization in a genus with over 150 species,

only 19 interspecific hybrids have been reported [6]. Also, there are several reports of unsuccessful interspecific hybridizations within this genus [8, 13, 21].

Despite the difficulty of making interspecific hybrids in *Lathyrus*, our group has been successful in producing a number of new combinations [6, 13, 15]. In general these new hybrid combinations have been more readily achieved between species that are taxonomically closely related and have similar karyotypes [5, 6, 13, 21]. However, it is interesting that many of these hybrids have very low fertility despite the similarity in parental karyotype [6]. In some cases, sterility can be correlated with irregularities of chromosome pairing and segregation at meiosis [6] but in others, chromosome pairing is regular yet the plants are virtually sterile [6]. Thus, it would be useful to know whether the cause of this reduced fertility is genetic or due to cryptic chromosome differences [20]. One way to determine this is to make detailed studies of chromosome pairing at pachytene in suitable hybrids using synaptonemal complex spreading techniques [1, 12].

Preliminary work on karyotypes of the species of section *Lathyrus* has shown that the karyotypes of *L. gorgoni* Parl., *L. sylvestris* L., *L. cirrhosus* Ser. and *L. heterophyllus* L. are similar to that of *L. latifolius* and therefore we chose those species as candidates for our hybridization programme. We were particularly interested in using *L. gorgoni*, since it has yellow–orange flowers rather than pink ones that are found in the other species of this group. This paper reports the results of this hybridization programme and on the chromosome behaviour of some of the hybrids that we have produced.

2. Materials and methods

The plant material used in this study was as follows: one accession of *L. cirrhosus* (University of Southampton no. 830156), one of *L. heterophyllus* (Chiltern Seeds, Ulverston, Cumbria, UK), eight of *L. latifolius* (four wild collections from the USA – Lompoc, California; Flathead, Montana; Florence,

Oregon; Budd Inlet, Washington; three from Europe – Lessines, Belgium; D'arc la Fontaine (DFL), France; Avion, France and the cultivar 'White Pearl (WP)' (Thompson and Morgan, Ipswich, UK), two of *L. sylvestris* – Royal Horticultural Society, Wisley, UK; Urdos, Belgium and four of *L. gorgoni*, all wild collections from Syria – ICARDA 580, Homs, ICARDA 596 Tartous, ICARDA 606 Tartous, ICARDA 646 Lattakia.

All perennial species were grown outside either in pots or in the field. *L. gorgoni*, which is an annual, was grown in a glasshouse. Buds were emasculated 2–3 d before pollination for perennials and 1 d before pollination for *L. gorgoni*. All European accessions of *L. latifolius* and *L. sylvestris* were bagged after emasculation and were kept bagged for 2–3 days after pollination as initial crosses showed that unbagged flowers were cross pollinated by bumble bees. The methods used for interspecific hybridization, analysis of pollen tube growth and preparation and staining of mitotic and meiotic chromosomes were those outlined in Nandini [16], Murray and Hammett [13] and Murray et al. [15]. Pollen viability was assessed using the method of Heslop-Harrison and Heslop-Harrison [9]. Synaptonemal complexes were prepared using the technique outlined in Loidl [12] with a few minor modifications in the timing of the various stages. Ovules were cleared using the technique described in Herr [7].

3. Results

3.1. Karyotype analysis

The karyotypes of the five species show a superficial similarity when stained with orcein. They all have $2n = 14$ with one large and one smaller meta-centric pair plus five pairs of acrocentrics, one of which has a secondary constriction in the short arm (figure 1). However, when differential staining with Giemsa, DAPI or silver nitrate is applied clear differences between the species become apparent. As an example, following silver nitrate staining, *L. latifolius*, *L. heterophyllus* and *L. sylvestris* (Wisley) all had a single band at the secondary constriction, whereas *L. cirrhosus* and *L. sylvestris* (Urdos) had this band at the secondary constriction and additional ones at all centromeres (figure 2). *L. gorgoni* had the interstitial band, bands at four centromeres in the acrocentric chromosomes plus telomeric bands on the short arms of four pairs and

Figure 1. Karyotypes of (a) *L. latifolius*, (b) *L. sylvestris*, (c) *L. heterophyllus*, (d) *L. cirrhosus* and (e) *L. gorgoni*. Scale = 10 μ m.

Figure 2. Mitotic metaphase chromosomes of (a) *L. latifolius* and (b) *L. sylvestris* stained with 50 % silver nitrate. Scale = 10 μ m.

on both arms of two pairs of chromosomes. Giemsa and DAPI staining also revealed different banding patterns and when taken together the different sorts of bands obtained showed that each of the species had a unique karyotype [16].

3.2. Interspecific hybridization

3.2.1. Interspecific crosses involving *L. gorgoni*

One-hundred and sixty-nine reciprocal crosses between four accessions of *L. gorgoni* and six accessions of *L. latifolius* failed to produce any seed. Pod formation was seen in approximately 12 % of the crosses, but none contained seed. None of the ovules from the crosses that formed pods contained embryos except for four crosses between *L. gorgoni* (596) and *L. latifolius* 'White Pearl'. These showed normal, well-developed embryos comparable to the embryos from selfs and turned out to be the result of self-fertilization. A smaller number (14) of crosses were made reciprocally between *L. gorgoni* and *L. sylvestris* and between *L. gorgoni* and *L. cirrhosus* (25 crosses) but none of these set any seeds. No crosses were made with *L. heterophyllus*.

3.2.2. Pollen tube growth in crosses involving *L. gorgoni*

To investigate possible causes of seed failure, cross pollinations (94 in total) of the four accessions of *L. gorgoni* were made with *L. cirrhosus*, *L. heterophyllus* and four accessions of *L. latifolius* and analysed for pollen germination and tube growth in the stylar tissue. Three features were common to all the observations made. First, pollen germination was observed in the majority of the cross-pollinated stigmas (88.3 %), but only 43.6 % of the styles contained pollen tubes. Second, twisting and coiling of pollen tubes was observed in all the pistils examined. Finally, none of the gynoecia observed showed any pollen tubes at the base of the style or entering the micropyle of any ovule. In the nine crosses involving *L. gorgoni* (646) as a female parent and *L. latifolius* (Lompoc) as the pollen donor, the pollen tubes exhibited abnormal branching and swelling at the tips (figure 3). In addition, only six of these nine crosses showed any pollen tube growth in the style and the pollen tubes grew no more than a third of the length of the style.

3.2.3. Crosses between the other species

Seven out of 21 (33.3 %) crosses between *L. latifolius* and *L. heterophyllus*, resulted in both pod

Figure 3. Pollen tube growth in the cross between *L. gorgoni* (646) and *L. latifolius* (Lompoc) showing swelling (A) and branching (B) of pollen tubes.

and seed formation. No significant reciprocal difference in the success of these crosses was seen. However, differences appear to be present in the crossability of specific *L. latifolius* accessions with *L. heterophyllus*. One third of the reciprocal crosses with 'White Pearl' were successful, whereas only 13.3 % of the reciprocal crosses with Lompoc and none of the crosses with DLF were successful.

Ninety-two reciprocal crosses were made between various accessions of *L. latifolius* and *L. sylvestris*. Of the 28 crosses involving *L. latifolius* as the female parent, only seven (25 %) resulted in pod and seed set and from these several hybrid plants were raised, three of which were studied in detail. Of the 64 crosses where *L. sylvestris* was the female parent, 22 set pods containing seeds, the majority of which were hybrid. During the course of these hybridizations, it became clear that some of the unbagged crosses were setting self seed. It is significant that this only occurred when the European accessions of the two species were used as the female parent in the cross. Fifteen out of 27 unbagged crosses involving *L. sylvestris* (Urdos) as the female parent resulted in pod and seed formation, but only two out of the 20 plants raised from these seeds were hybrid. Of the 17 bagged crosses, two of the 11 between *L. sylvestris* (Urdos) and 'White Pearl' resulted in

seed and pod formation. All plants raised from these seeds appear to be hybrids.

Observations of pollen tube growth in crosses involving the perennial species were more difficult to make than those with *L. gorgoni*. The gynoecia of the perennials are very hairy and despite trying a number of modifications to the fluorescence technique for observing pollen tube growth, such as varying the times in aniline blue and sodium hydroxide, weak fluorescence made it difficult to observe pollen tubes in the style or ovary. Pollen germination was observed in 83 % of the crosses. Cleared ovules 6 days after pollination were studied to check for embryo formation in a small number of crosses involving *L. latifolius* as the female parent, since pollen tube growth could not be observed. Although the embryo sac could be seen, no obvious post-fertilization changes in the form of embryo or endosperm development were observed in any of the ovules.

3.3. Analysis of hybrids

3.3.1. Karyotypes

Most of the hybrids studied showed karyotypes that clearly combined the haploid chromosome sets of their parents. However, karyotype repatterning was observed in some of the hybrids that were produced. In *L. latifolius* × *L. heterophyllus* (WP × het3), two new chromosome types, not present in either parent, were observed. One of these was an acrocentric and the other an extra metacentric (figure 4a). Another hybrid between these two species showed a high percentage of polyploid cells (70 %). In one hybrid between *L. latifolius* and *L. sylvestris* (4) there were several interesting features. First, only one NOR was expressed in approximately 25 % of the cells studied, with only one silver-stained band in metaphase cells. Second, the chromosomes appeared fuzzy and third, one of the largest metacentric chromosomes had a constriction close to the telomere (figure 4b).

3.3.2. Meiotic analysis

Detailed observations were made at pachytene in two of the hybrid combinations, *L. sylvestris*

Figure 4. Mitotic metaphase chromosome of (a) *L. latifolius* × *L. heterophyllus* (WP × het3), note the odd acrocentric (arrowed) and metacentric chromosomes; (b) *L. sylvestris* × *L. latifolius* (Urdo × WP 4) showing the additional secondary constriction on a metacentric chromosome (arrowed). Scale = 10 µm.

(Urdo) × *L. latifolius* 'White Pearl' (five cells) and *L. heterophyllus* × *L. latifolius* 'White Pearl' (two cells). In both combinations SC formation appeared normal and there was complete pairing

with no evidence of unpaired regions, loops or multivalents (figure 5).

The meiotic metaphase (MI) behaviour of the parental species was regular, meiosis was highly synchronized between pollen mother cells (pmcs) of an anther, no univalents were observed and they had a high chiasma frequency, between 16 and 19.7 per cell (table I). Meiosis in the hybrids was more variable with low frequencies of univalents in some plants and lower chiasma frequencies than the parental species. There was also variation between different hybrid plants derived from different combinations of the same species pairs. Some had poorly synchronized meiosis with a wide variety of stages in an anther loculus and the chromosomes were sometimes very fuzzy in appearance, despite using a variety of fixatives. This latter feature made the accurate determination of chiasma frequency very difficult and in these plants it was only possible to identify rod and ring bivalents. On the basis of their MI pairing, the hybrids appear to fall into groups. *L. latifolius* (Lompoc) $\times$ *L. sylvestris* (Urdos) showed regular bivalent formation in all the cells examined but the 'sticky' nature of the chromosomes did not allow the accurate determination of chiasma frequency. Three *L. latifolius* 'White Pearl' $\times$ *L. sylvestris* (Wisley) hybrids were analysed and the mean number of univalents (0–2.9), chiasma frequency (7.9–17.41) and percentage pollen fertility (20–95) differed between plants. For the five *L. sylvestris* (Urdos) $\times$ *L. latifolius* 'White Pearl' hybrids analysed, the frequency of univalents, chiasma frequency and pollen fertility were also variable. In both these combinations there was no clear relationship between chiasma and univalent frequency and pollen fertility. The two hybrids between *L. latifolius* 'White Pearl' and *L. heterophyllus* had a low frequency of univalents and very similar pollen fertilities.

Anaphase I bridges without fragments were seen in all of the hybrids. For every 50 cells at anaphase I examined the number of cells with bridges and no fragments ranged from 1 to 3 (2–6 %). Pentads, hexads and heptads were also seen occasionally. In *L. sylvestris* (Urdos) $\times$ *L. latifolius* 'White Pearl'

Figure 5. Electron micrograph of a surface spread synaptonemal complex of the hybrid *L. latifolius* $\times$ *L. heterophyllus* (Urdos $\times$ WP). Scale = 5 μ m.

and *L. latifolius* 'White Pearl' $\times$ *L. heterophyllus* one in every ten tetrads was abnormal.

The pollen viability of all the hybrids was lower than that of the parents and the hybrid plants appear to fall into three groups with 0–25 %, 26–70 % and 71–95 % fertile pollen. In addition, the viable pollen in the *L. sylvestris* (Urdos) $\times$

Table I. Chromosome configurations at meiotic metaphase I (I = univalents, cII = chain bivalents, oII(2), oII(3), oII(4) = ring bivalents with 2, 3 or 4 chiasmata, respectively), chiasma frequency and pollen viability in parental species and hybrids of *Lathyrus*.

Species	Code	Mean meiotic configurations per cell frequency with range in brackets					Mean chiasma viability per cell	% Pollen	
		I	cII	oII(2)	oII(3)	oII(4)			
<i>L. latifolius</i>	WP	0 (0)	1.12 (0-2)	2.25 (1-4)	2.13 (1-3)	1.50 (0-2)	18.0	97	
	Montana (0)	0 (1-3)	1.47 (1-4)	2.65 (1-4)	2.12 (0-2)	0.76	16.0	96	
<i>L. heterophyllus</i>		0 (0)	0.29 (0-1)	2.43 (1-3)	2.57 (2-4)	1.71 (0-3)	19.7	75	
<i>L. sylvestris</i>	Urdos	0 (0)	1.44 (1-3)	1.67 (1-3)	1.78 (1-3)	2.11 (1-3)	18.6	96	
Hybrid									
<i>L. latifolius</i> × <i>L. sylvestris</i>	WP × Wisley	1	2.9 (0-14)	0.33 (0-2)	3.77 (0-7)	0 (0)	0 (0)	7.9	20
		2	0.13 (0-2)	0.31 (0-1)	6.50 (6-7)	0 (0)	0 (0)	!	75
		3	0 (0)	0.75 (0-2)	2.75 (1-4)	2.83 (2-4)	0.67 (0-2)	17.4	95
<i>L. sylvestris</i> × <i>L. latifolius</i>	Urdos × WP	1	0.21 (0-2)	1.14 (0-2)	2.71 (2-3)	2.57 (2-3)	0.35 (0-2)	15.4	5-25
		2	0.12 (0-2)	0.14 (0-2)	6.70 (5-7)	0 (0)	0 (0)	!	92
		3	0.29 (0-2)	0.38 (0-2)	6.30 (5-7)	0 (0)	0 (0)	!	78
		4	0.40 (0-4)	2.10 (1-3)	2.90 (2-4)	1.50 (1-5)	0 (0)	12.4	84
		5	0 (0)	0.13 (0-2)	6.87 (5-7)	0 (0)	0 (0)	!	88
		Lompoc × Urdos 1		0 (0)	0 (0)		7* (7)		!
<i>L. latifolius</i> × <i>L. heterophyllus</i>	WP × het	1	0.50 (0-4)	2.00 (1-2)	3.28 (3-4)	1.21 (0-2)	0 (0)	12.2	67
<i>L. heterophyllus</i> × <i>L. latifolius</i>	het × WP	1	0.20 (0-2)	0.37 (0-3)		6.43* (7)		!	65

! Chiasmata frequency could not be determined; * number of chiasmata in bivalents could not be determined.

L. latifolius 'White Pearl' hybrids showed a marked variation in size. Different hybrid plants showed from 2 to 18 % of grains that were smaller in size, yet still showed normal fluorescence, compared to the rest.

The morphology of the hybrids that we have produced was unexceptionable. In general the plants were very vigorous and were intermediate between the parental species. No new flower colours were produced.

4. Discussion

The present study shows that attempts to produce hybrids between these *Lathyrus* species have had mixed results. All the crosses with *L. gorgoni* failed and observations on pollen tube growth indicated that it is strongly isolated by pre-fertilization hybridization barriers from the perennial species. Hybrids were produced between two different pairs of perennials and this is the first report of artificially produced hybrids involving *L. heterophyllus*. Hybridization between *L. latifolius* and *L. sylvestris* has been reported previously [2, 3]. It is interesting that the hybrids described by Chaib et al. [2] were stunted, chlorotic and lethal at a very early stage in their development; this is in stark contrast to those produced in this study which were very vigorous. This difference in hybrid viability together with the differential success of different genotypes in hybridization programmes in *Lathyrus*, are both consistent with the idea that the success of hybridization in *Lathyrus* may be influenced by the genotypes of the parental species.

Our previous studies in the genus (summarized in Murray and Hammett [14]) have shown that hybrids are most easily made between species that are taxonomically close, based on the treatment of Kupicha [10], and that have similar karyotypes. The results of the present study also support this conclusion. *L. gorgoni* is an annual that is thought to be taxonomically distant from the perennials used here [10] and although it and the perennial species all had a superficially similar karyotype, there were significant differences in chromosome banding patterns between them. Genome size in *L. gorgoni* is also significantly smaller (25–30 %) compared to the perennial species [17]. Amongst the perennials, *L. latifolius* crossed relatively freely with *L. heterophyllus* and *L. sylvestris* suggesting a close genetic relationship. The karyotypes and genome sizes of these three species were more similar to each other than between *L. gorgoni* and the perennials, and the relative ease of hybridization and significant level of fertility of the hybrids also suggests that these species are closely related. Kupicha [10] also places them next to each other in her classification.

Despite the relatively normal degree of chromosome pairing at meiosis, the fertility of some of the

hybrids that we have produced is relatively low. It is not unusual for hybrids to show high pairing at meiosis but still have reduced fertility or even sterility [20] and this is seen in a number of other *Lathyrus* hybrids [5, 6]. Stebbins [20] suggested that cryptic structural hybridity could explain these sorts of results, and this has been used by several authors [6, 18, 19] to explain the low fertility of a variety of high pairing hybrids. However, with the present observation of complete pachytene pairing in the two hybrid combinations of *Lathyrus* reported here, it would appear that the cause of the reduced fertility is not cryptic structural hybridity but probably is due to the segregation of genetically unbalanced combinations in the hybrids [20].

Karyotype studies of the hybrids between the perennial species have also demonstrated the presence of chromosome instability, both structural and numerical, and differential amphiplasty. In *L. latifolius* × *L. heterophyllus* (WP × het3) there has been a repatterning of the karyotype with new metacentric and acrocentric chromosomes. At this stage it has not been possible to elucidate the origin of these new chromosomes as this hybrid has failed to flower, even after several years of cultivation, and consequently no meiotic analysis could be made. The presence of a high frequency of polyploid cells in another *L. latifolius* × *L. heterophyllus* hybrid (WP × het2) is a reflection of numerical chromosome instability which has been reported for several other *Lathyrus* species and hybrids [4, 6, 11]. The suppression of NORs observed in the hybrid between *L. sylvestris* (Urdo) and *L. latifolius* (WP) has also been reported earlier in other hybrids of *Lathyrus* [6]. The sporadic occurrence of these phenomena suggests that different genotypes of the parental species interact in different ways depending on their combinations. The fuzzy appearance of the chromosomes at meiosis in some of the hybrids is possibly another reflection of genic disharmony between parental combinations.

In all the anaphase I cells where bridges were observed, no acentric fragments were seen suggesting that either chromosome stickiness, failure of chiasmata to terminalize or delayed separation of chromosomes rather than paracentric inversion may be the cause of the phenomenon.

The difference in the size of the viable pollen grains of the *L. sylvestris* (Urdo) × *L. latifolius* (WP) hybrids was unusual. There is a difference in genome size between the two parental species, although this is small (23.28 pg in *L. sylvestris* (Urdo) and 21.87 pg in *L. latifolius* (WP) [17]. It is possible, though perhaps not very likely, that this variation in pollen size comes about through the segregation of chromosomes of different sizes. However, we have no other explanation for the phenomenon at present.

All the hybrids produced in this study had pink flowers of varying shades and consequently we have not been able to extend the range of colours in *L. latifolius*. It now seems likely that sexual hybridization is unlikely to bridge the gap between *L. latifolius* and its relatives and that somatic hybridization may be the most appropriate future avenue to pursue.

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