Ballistic seed dispersal and associated seed shadow in wild *Pisum* germplasm

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The genus *Pisum* belongs to a broad group of plant genera that have evolved ballistic mechanisms of seed dispersal. Central to the mechanism in *Pisum* is the dehiscent pod (single carpel fused along its edges) where the central pod suture undergoes an explosive rupturing along a line of weakness (dehiscence zone) beneath the dorsal and ventral sutures. The thickened cells of the exocarp and the sclerenchyma layers of the endocarp are elongated and arranged diagonally at perpendicular planes to each other (1). As the pod matures and the cells dry, the pod walls shrink in opposite directions generating stresses that are suddenly released when the sutures rupture. There is an orchestration of events during which the pod walls twist in opposite directions which projects the seeds on an upward trajectory. This is evidenced by a change in aspect of pods which are pendulous immediately prior to dehiscence and horizontal immediately after dehiscence. The effect of these events is to propel the seeds from the pod away from the plant. While the character has been observed by many, the authors could find no reference of recorded data as to the distances seeds were broadcast as a result of this process. This paper reports on an opportunity that arose to record such data for two forms of wild *Pisum* germplasm.

The presence of non-dehiscent pods is often cited as a primary discriminating factor between cultivated and wild forms (2) and possibly one of the most important traits in the domestication and widespread cultivation of pea as a crop (3). The genetics of the dehiscent pod trait was first reported by Marx (4) where he described pods as being tough and leathery and prone to dehiscence. The line he used which was subsequently adopted as the type line for the trait was L 1293=JI 64 *(Pisum sativum* ssp. *elatius).* He reported the trait as being semi-dominant and a simple monogenic trait *(Dpol* locus) which showed linkage to LG III. More recently, Weeden et al. (5), in investigations using recombinant inbred populations and a simplified correlation analysis reported an additional locus *(Dpo2),* mapping to LG V and a third locus that they postulated as being the *Gp* locus (yellow pod) that is described as being of restrictive width and prone to splitting along the suture (5, 6).

Materials and Methods

A test array of 56 *Pisum* accessions are grown out as part of a field demonstration each summer on the John Innes Centre experimental plots. This set of lines was originally developed to represent a broad range of the diversity of Pisum within a restricted set of lines. The inclusion of type lines from the literature to represent some of the taxa means that it is no coincidence that lines JI 64 (4) and JI 1794 (5) both feature as part of this restricted set. The lines have been the subject of a successive series of molecular marker studies to explore the underlying diversity within Pisum (7, 8). A phylogenetic tree of genetic distances based 101 AFLP and 276 SSAP markers (Fig. 1) was scaled up in a $10m^2$ grid and transcribed onto cultivated ground using colored twine. Due to the fact that the test array included both wild and cultivated forms, seeds of all lines were chipped and sown in small pots in the glasshouse to ensure even germination. On emergence the seedlings were transferred to cold frames to be hardened off before being planted out in the field. Six seedlings were transplanted in a circle with 10cm circumference and a 1m cane placed to mark the end of each line of the phylogenetic tree. The whole of the demonstration area is kept bare during the growing season by regular hoeing of emerging weeds (Fig. 1).

Figure 1. Phylogenetic tree of Pisum diversity planted out as a field demonstration.

The growing season in 2006 was hot and dry during June and July which lead to ideal conditions for pod dehiscence at maturity. Following a weekend of particularly hot dry weather with no wind, two lines (JI 1794 and JI 224) were observed to have broadcast their seeds which could be clearly observed lying on the bare soil surface (Fig. 2).

Figure 2. Plants of JI 1794 at time of recording showing (a) positions (dots) where seeds had fallen and (b) the aspect of the twisted pods after dehiscence.

The soil surface was dry and powdery to the depth of 2-3cm. The softness of the soil surface absorbed the impact of the seeds so there was no secondary travel due to bouncing, thus the distances recorded were true distances from the plants. JI 224 and JI 1794 matured before the remaining 54 lines and were of contrasting seed types and could, therefore, be readily distinguished from each other (Table 1).

Table 1. Seed characteristics of JI 224 and JI 1 794.

	JI 224	JI 1794
	Pisum fulvum	*Pisum humile*
Seed wt (mg ± S.D.)	**61.5 ± 10.9**	**129 ± 25.2**
Hilum size	**Small**	**Small**
Testa background colour	**Pale brown**	**Green**
Testa marbling *(M)*	**Absent**	**Present**
Anthocyanin spotting (Fs)	**Absent**	**Present**
Gritty Testa *(Gty)*	**Present**	**Present**

The direction of travel was recorded for a limited number of seeds and did not appear other than random. Limited time precluded continuing with angle measurements. A linear measure of the ground distance from the outer edge of the circle of plants of each particular line to where the seed lay on the soil surface was recorded. The continuing warm weather enabled all 56 accessions to be scored for pod dehiscence.

Results

Pod dehiscence was recorded in the four *P. fulvum* accessions and all but two accessions of *P. elatius.* All accession of *P. sativum ssp. sativum* and the four accession of *P. abyssinicum* failed to dehisce (Fig. 3).

The range of seed distances was recorded as 260cm for JI 224 and 356cm for JI 1794 (Table 2a). The minimum distance for JI 224 was 14cm and 1cm for JI 1794 and the maximum distances were 274cm for JI 224 and 357cm for JI 1794. Both distributions were positively skewed and significantly non-normal. While the mean distance for the two lines differed (71cm for JI 224 and 86cm for JI 1794), the medians were very close (54cm for JI 224 and 53cm for JI 1794). Two subsets of seeds of JI 1794 were found in straight lines, strongly suggestive of the seeds having originated from the same pod. The distances for these two sets were 29, 31, 35, 36, and 38cm for subset 1; and 178, 183 and 217cm for subset 2.

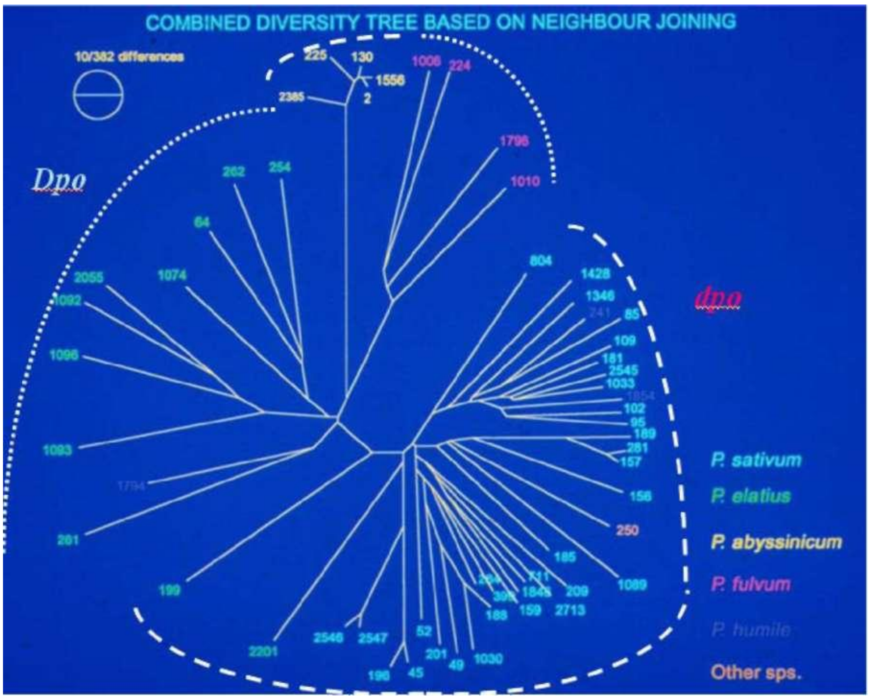


Figure 3. Phylogenetic tree based on 382 marker band differences in 56 accession of Pisum showing accessions which exhibited pod dehiscence (Dpo) and accessions that failed to dehisce (dpo).

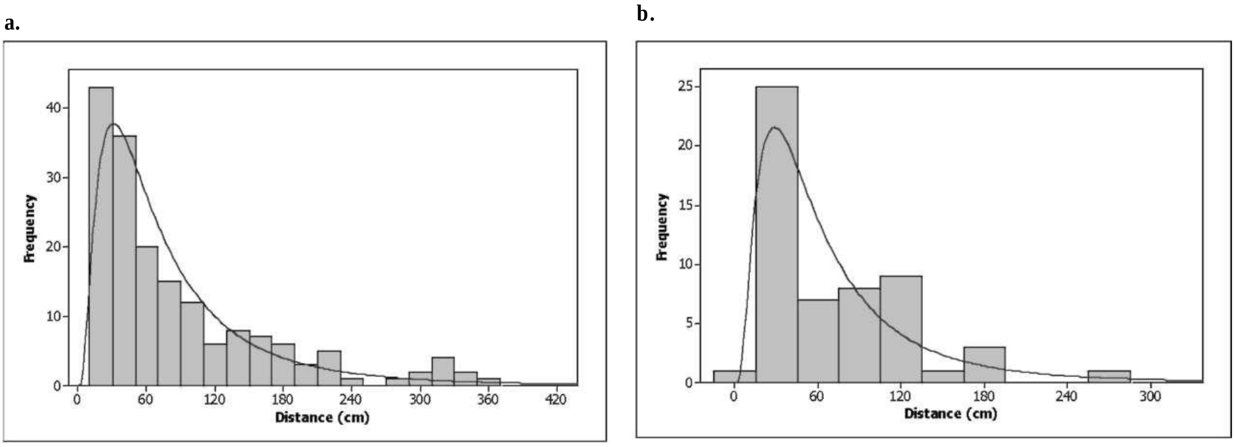
A number of transformations were investigated to try to identify the underlying nature of the distributions. Of these log and 2 parameter exponential models came the closest to normalising the distribution with the lognormal model having a marginally better fit. Descriptive statistics for the $\log_{10}$ transformation are presented in Table 2. Fitted curves and parameter estimates for lognormal

Table 2. Summary statistics for seed distance from plants of JI 1794 (a) and JI 224 (b).

a. JI 1794 (Plant height: 85cm)			b. JI 224 (Plant height: 32cm)		
	cm	Log_{10}		cm	Log_{10}
Mean	85.8	(1.75)	Mean	71.0	(1.72)
Median	52.5	(1.72)	Median	54.0	(1.73)
Minimum	1.0	(0.00)	Minimum	14.0	(1.15)
Maximum	357.0	(2.55)	Maximum	274.0	(2.44)
Range	356.0	(2.55)	Range	260.0	(1.29)
Lower quartile	29.0	(1.46)	Lower quartile	25.0	(1.40)
Upper quartile	114.0	(2.06)	Upper quartile	103.8	(2.02)
Standard deviation	81.6	(0.43)	Standard deviation	55.9	(0.34)
Variance	6657.5	(0.19)	Variance	3120.2	(0.12)
Number of observations	172		Number of observations	55	
Coefficient of variation	95		Coefficient of variation	78.7	
Skewness	2	(-0.73)	Skewness	1.3	(0.091)
Kurtosis	2	(2.24)	Kurtosis	1.8	(-1.158)

distributions are presented in Fig. 4. Due to the limited size of the two datasets attempts to fit more complicated solutions such as polymodal models was not pursued.

Figure 4. Lognormal distribution curves and parameter estimates for seed distances in JI 1794 (a) and JI 224 (b).



Parameter estimates for Lognormal distribution
Location 4.121 ± 0.0635 (s.e.)
Scale 0.8348 ± 0.0449 (s.e.)
Anderson- Darling test 1.953**

Parameter estimates for Lognormal distribution
Location 3.966 ± 0.1054 (s.e.)
Scale 0.7890 ± 0.0746 (s.e.)
Anderson Darling test 0.856*

Discussion

The performance of the diverse set of *Pisum* germplasm confirmed the findings of previous authors who reported pod dehiscence confined to wild germplasm of *P. fulvum* and *P. elatius* germplasm. Working with wild material over many years, the expression of *Dpo* has been frequently observed; nevertheless, it came as some surprise that the recorded dispersal range of the two accessions were 356 and 260cm given the relatively large heavy seeds of the lines, 62 and 129mg, respectively. These values are considerably heavier than those of other legume species exhibiting ballistic dispersal that has previously been reported, eg. *Lathyrus sativa* and *Cytisus scoparius.*

A lognormal probability density function (PDF) provided adequate fits to both datasets. Lognormal and exponential PDFs have been reported for other legume species (9). Exploration of the data and the incomplete fit of the lognormal PDF to the datasets gave the suggestion that the data might be composed of more than one population but the datasets were not large enough to explore this possibility further. Studies of seed dispersal curves have increasingly focused on the tails of the distributions, which are considered at least as important as the modal portion of the curve (10). The tails for both *Pisum* accessions in this study were well estimated by the lognormal models.

Observations of pod angles after dehiscence showed them to be consistently horizontal (90° from vertical), whereas prior to dehiscence there was a range of angles from 20° to 90°. Based on the physics of the motion of projectiles it is possible to pose the basic question how does range vary with angle of projection by plotting the distribution of distances for a range of pod angles. The upward velocity is *v* sin6, where *6* is the angle above the horizontal. The height above the point of projection at time *t* is:

$$h = v(\sin\theta)t - gt^2/2$$

The time taken for the seed to reach the starting is *T* where:

$$T = \frac{2v\sin\theta}{g}$$

The horizontal distance (*D*) travelled in this time is:

$$D = vT\cos\theta$$
$$D = 2v^2\sin\theta\cos\theta/g$$
$$D = 2v^2\sin 2\theta$$

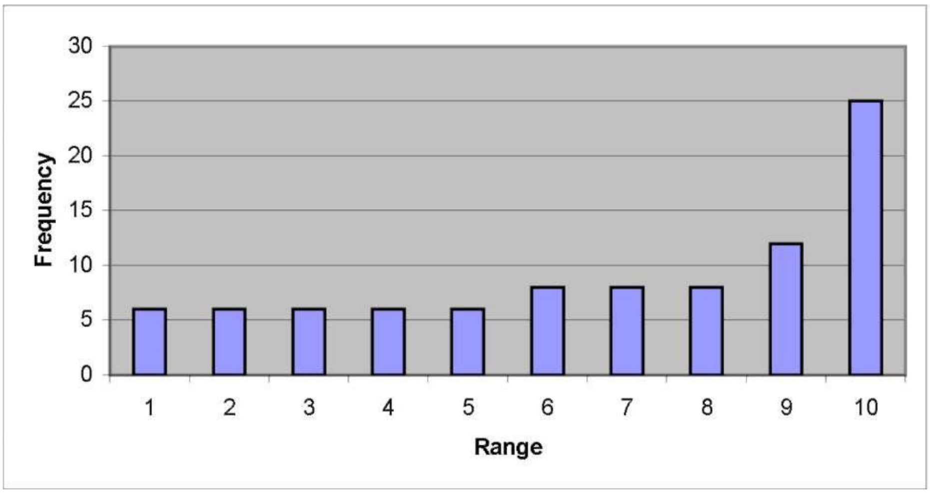


Figure 5. Calculated distribution of dispersal range (arbitrary units) for a uniform distribution of pod angles.

if *v* is constant, then *D* is proportional to sin2*6*. For a range of uniformly distributed pod angles from 20° to 90° a distribution of ranges results, with the maximum distance for an angle of 45°. The frequency distribution is negatively skewed with a higher frequency of longer distances (Fig. 5). This is the opposite of the actual distribution of seed distances observed suggesting that there is likely to be a relatively tight distribution of pod angles at dehiscence and suggesting this would be an interesting parameter to measure in different taxa of *Pisum.* Garrison et al. (11) who measured fruit angle orientation in the field for *Vicia sativa* have indeed shown a narrow range (30-45°)

which varied from *Corton capitatus* which also exhibited a narrow but different range of 80-90°. Such a convergence of fruit orientation angles they suggest is a specific adaptation for seed projection distance.

This was an opportunistic data collection exercise and not a planned experiment to study seed dispersal in *Pisum.* The dispersal distances are clearly an integration of many variables including the unequal distribution of pods up the plant and the angle of pod orientation which have been found to be correlated by others (11). Further variables that may play a role in dispersal distance in *Pisum* include, variation in thickness of the sclerenchyma layer in the pod walls, distribution of pod angle prior to dehiscence, pod length, initial speed of transit through the air, and the possible role of the rough seed coat or gritty testa *(Gty)* which might play in lowering the resistance during transit.

Further observations are planned to capture data on more variables in coming seasons. Despite the limitations, it is hoped that the detailed descriptive statistics in this report will provide a useful data set for others in further exploring the mechanisms and evolution of ballistic dispersal in legumes.

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