Rates and pattern of ovule abortion vis-à-vis *in situ* pollen germination in some populations of *Trifolium fragiferum* L.

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The present study is based on four populations of *Trifolium fragiferum* L. of the family Fabaceae growing at four different sites in Jammu region, India. The species, which grows as a common weed in the area of study, follows an annual life cycle of about 3½ month in the subtropical climates of Jammu region. While all of these populations were recorded in full bloom during February and March, they displayed a temporal scatter. Detailed studies revealed these population types to be morphologically similar but distinct in the many aspects studied. An interesting phenomenon noted for the plants of this species was *in situ* pollen germination, which was recorded in about 28.8% of the flowers studied. The species under investigation also showed an appreciable amount of ovule abortion. The ovule abortion in pistils was found to be non-random, with the peduncular ovule aborting at a higher rate than the stylar one. The rates and patterns of ovule abortion were studied vis-à-vis *in situ* pollen germination and were compared between different populations. Interesting results were obtained, indicative of the fact that precocious pollen germination does affect the ovule abortion in one way or other.

1. Introduction

In majority of the flowering plants, only a proportion of flowers and ovules develop fully to form fruits and seeds. A portion of them invariably aborts at various stages of development resulting in overall decrease in the fecundity. A number of theoretical implications have been extended to explain this bizzare behaviour (Lloyd 1980; Stephenson 1981; Bawa and Webb 1984; Lee 1988). Legumes have been a choice material for most of the studies on rates, patterns and causes of ovule abortion. This is because of several advantages associated with this group of plants (Ganeshaiah and Uma Shaanker 1988; Uma Shaanker and Ganeshaiah 1988; Rocha and Stephenson 1991a; Mohan Raju *et al.* 1996; Arathi *et al.* 1996; Rosallini *et al.* 2003). Most important among these being the linear arrangement of ovules and later seeds, which facilitates the study of ovule development in accordance with two distinct gradients within an ovary-maternal resource allocation from the peduncular towards the stylar end and pollen tube growth from the stylar end towards the peduncular end (Hossaert and Valero 1988; Rocha and Stephenson 1990, 1991a, b). Many members of the family Fabaceae have thus studied in relation to ovule abortion, Trifoliums being one of them.

Trifoliums are known to be prolific flower producers but poor seed setters, due to the wide occurrence of ovule abortion, irrespective of the range of breeding system operating in different species (Dhar *et al.* 2006). We tried to study the rates and patterns of ovule abortion in *Trifolium fragiferum* L.; a species forming natural populations in and around Jammu, India, and enjoying mixed mating system due to the prevalence of frequent insect visits and the incidence of *in situ* pollen germination in their anthers (Koul *et al.*, personal communication). Four different populations of the species were analysed and tagged. These bloomed at different times of the flowering season and varied in the frequency of *in situ* pollen germination in their flowers. During the present study, we tried to correlate the rates and pattern of ovule abortion vis-à-vis *in situ* pollen germination.

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2. Materials and methods

2.1 *Material*

Different populations of this species were tagged and scanned in and around Jammu University Campus, Jammu, and at some other sites in Jammu city during December to May (table 1). Plants in all these populations start blooming in February every year and flowering and fruiting continues till April. The temperature during these months fluctuates between 6.6°C and 41°C and RH is between 10% and 100%.

Four different populations were analysed in detail. Plants from each of these sites were transplanted in pots filled with a mixture of sand and earth and supplemented with natural fertilizer (cow dung). These pots were kept in University Botanical Garden and watered regularly.

2.2 *Anthesis and anther dehiscence*

Both anthesis and anther dehiscence were observed at regular intervals of time. The time taken by an individual inflorescence and the full plant to bloom was recorded. Flowers were collected at different times before and after anthesis to check for anther dehiscence. Also ,flowers showing some

peculiar mode of anther dehiscence were studied by staining essential whorls (stamens + pistil) in a drop of Lewis stain (Lewis 1979) (mixture consisting of 2 mL 1% aq. acid Fuchsin, 2 mL 1% aq. light green, 40 mL lactic acid and 46 mL distilled water).

2.3 *Stigma receptivity*

Stigma receptivity was checked from stigmas of different ages fixed in a mixture of 3 parts of absolute alcohol and 1 part of acetic acid (Carnoy's fixative) for 6–8 h. These stigmas were washed in distilled water, stained in Lewis stain (1979) and mounted in lactophenol (mixture consisting of lactic acid, distilled water, glycerine and phenol in the ratio 1:1:1:1). The stigmas with germinating pollen grains attached to their surface or showing copious exudations were considered receptive.

2.4 *Fruit- and seed-set*

Fruit- and seed-set were observed in different populations. Number of flowers/inflorescence, fruit-set/inflorescence and seed-set/inflorescence were counted. The fruit and seed set was calculated as follows:

$$\text{Percentage fruit} - \text{set/inflorescence}(\%) = \text{Average fruit count/inflorescence/Average flower count/inflorescence} \times 100$$

$$\text{Percentage seed} - \text{set/inflorescence}(\%) = \text{Total seed count/inflorescence/Total number of ovules of the inflorescence} \times 100$$

A few inflorescences were kept undisturbed and bagged for autoselfing. Fruit-set and seed-set on these bagged inflorescences has been treated as a measure of natural autogamy. The remaining inflorescences served as open-pollination control.

Table 1. Different populations and their site of collection

S. No.	Type of population	Collection site
1	Population-1	Lawns of University Botanical Garden
2	Population-2	University Campus
3	Population-3	Anand Nagar, Bohri (open fields)
4	Population-4	Beds in University Botanical Garden

2.5 *Ovule abortion*

Pistils were dissected out from the flowers and fixed in Carnoy's fixative for 6–8 h. The pistils were then washed in distilled water. These pistils were later stained with Lewis stain and mounted in a drop of lactophenol on a clean glass slide. Each prepared slide was observed for the position and number of ovules aborting within a carpel under a compound HB Olympus microscope. Ovules turned flaccid or shrunk and reduced in size were treated as aborted. Also, the percentage of pistils showing

ovule abortion (both peduncular and stylar) was calculated in different populations scanned as follows:

Total number of pistils with ovule abortion/Total number of pistils scanned $\times$ 100

2.6 *Photography*

Flowers and the details of reproductive apparatus were studied under stereomicroscope SM Z 800 (Nikon).

Prepared slides of ovule abortion, stigmatic germination and *in situ* pollen germination were photographed in the Central facility Lab of the Department of Botany with the help of a photomicrographic unit
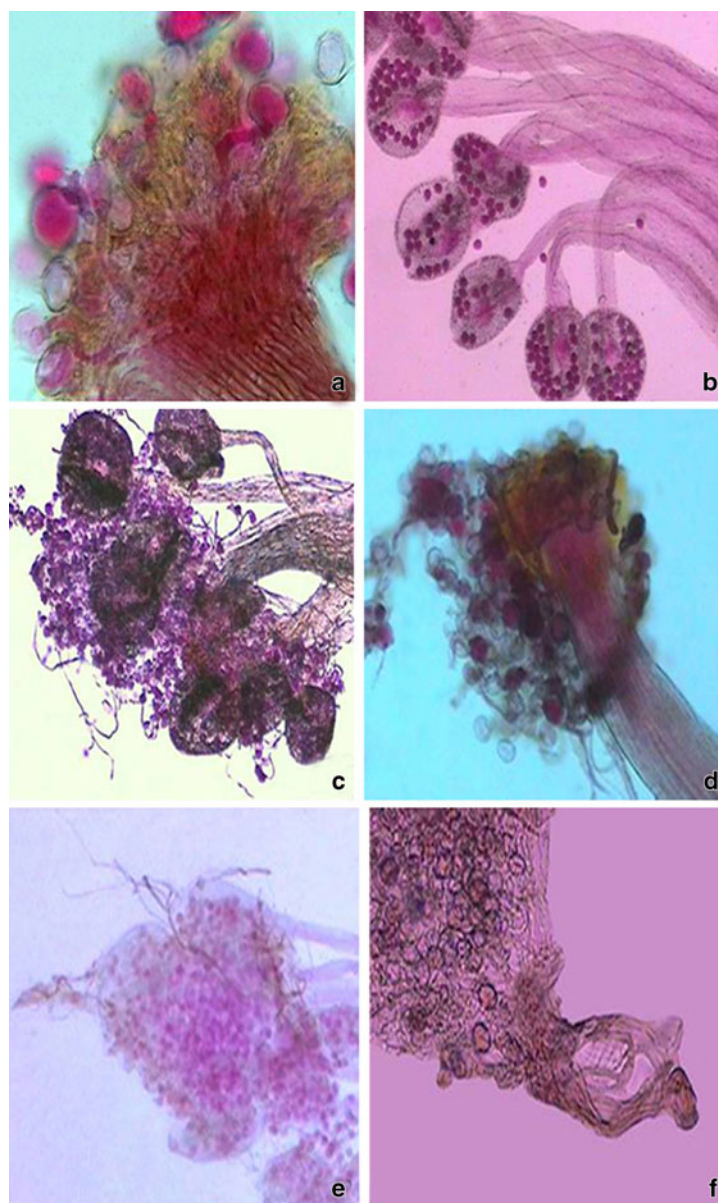

Figure 1. Receptive stigma of *Trifolium fragiferum* with normal stigmatic germination of pollen grains (X 370.4) (**a**), anthers showing pollen in suspended form (**b**), anthers showing the emergence of pollen grains germinated *in situ* (X 60.18) (**c**), stigma loaded with pollen with different sizes of pollen tubes formed *in situ* (**d**), and pollen tubes collectively emanating from anthers in the form of bundles (**e, f**).

Table 2. Data on period of study of four populations vis-à-vis *in situ* pollen germination

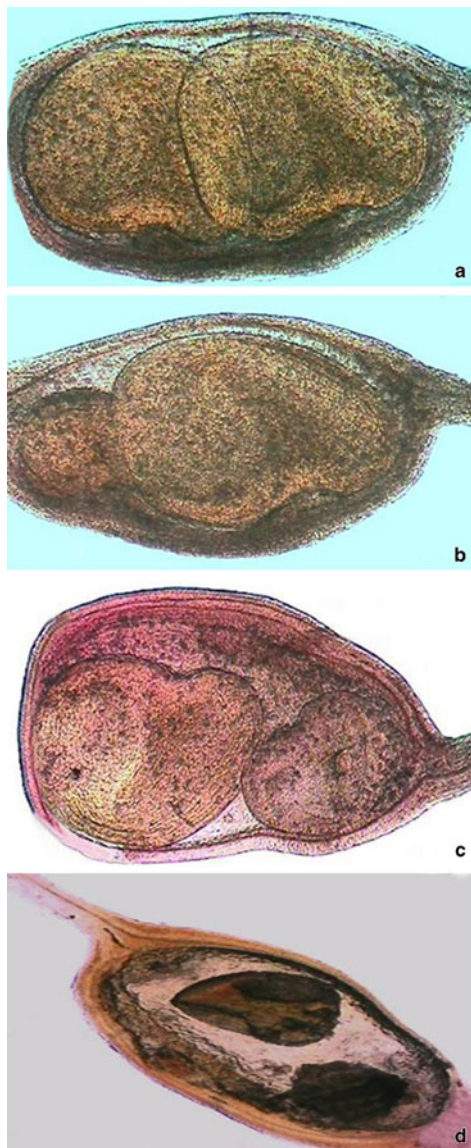| S.No.	Population type	Period of full bloom and study	Percentage *in situ* pollen germination	
			Open pollination	Unassisted selfing
1	Population-1	February	5	6.66
2	Population-2	mid-February to mid-March	11.66	15
3	Population-3	March	18.33	20
4	Population-4	March	31	40

Figure 2. A pistil with both developing ovules (stylar X 91.74×105.12; peduncular X 76.92×92.5) (**a**), a pistil showing the abortion of peduncular ovule (stylar X 106.42×100; peduncular X 42.12×60) (**b**), stylar ovule (stylar X 56.88×56.41; peduncular X 73.26×92.5) (**c**), and the abortion of both ovules (stylar X 60.55×35.89; peduncular X 45.78×32.5) (**d**).

(photographic camera attached to NIKON- Eclipse E 400 microscope).

2.7 Statistical analysis

Pearson's correlation: Data were analysed for the correlation (if any) between various parameters by using the system software – SPSS for Window 12.0.

3. Results

3.1 Floral biology and pollination

The flower of *Trifolium fragiferum* is typically papilionaceous. It is complete, zygomorphic, hypogynous and sessile. The corolla is irregular and 5 in number; the upstanding, the standard, the lateral two wings and the lower two united to form the keel. Stamens are diadelphous and held in a typical 9+1 arrangement and form a staminal tube around the pistil. Anthers are bithecous and dorsifixed. The pistil comprises of a single ovary with two campylotropous ovules varying slightly in their size, a long hollow style with a slight bent and capitate, papillate wet stigma covered with copious lipoidal exudations during the period of receptivity (figure 1a). Flowering in *T. fragiferum* commences from the first week of February and is at its peak during March. Plants show a marked decline in their blooming from April, and finally the highest incidence of fruit set is observed during May. The total flowering period of the species thus spans for a period of 2½ months.

Flowers are typically protogynous, so that the stigma turns receptive at least 1day before anther dehiscence. The stigma retains receptivity for 3–4 days after anthesis. Anthers dehisce either slightly before anthesis or at the time of anthesis. Usually during morning hours, anther dehiscence synchronizes with anthesis. In flowers that open during late hours, anther dehiscence precedes anthesis by 1–2 h.

Flowers in a population/inflorescence vary with respect to their mode of pollen dispersal following anther dehiscence. As the anthers mature, dehiscence in all occur by the formation of longitudinal slits, followed by pollen shedding or dispersal that occurs via two pathways. Out of a total number

of 824 flowers scanned at different times of blooming in four different populations of the species, 71.12% showed normal anther dehiscence and pollen shedding (figure 1b). In another group constituting 28.88% of the total flowers scanned in four populations, normal pollen dispersal did not occur. Instead, pollen grains germinated inside the pollen sac itself. In these flowers, anthers did form lateral slits and yet were not able to shed their pollen. Pollen continued to remain in the anther sac and thereafter germinated there (figure 1c). The way these germinating pollen grains come out through the dehiscence slit also varies among different flowers. Either the germinating pollen grains come out as such individually or the pollen tubes formed by the pollen grains *in situ* reach to the nearby stigma (figure 1d). These pollen tubes can travel as individual units or can also collectively join each other to form a bundle (figure 1e). In a bundle, these pollen tubes twist around one another (figure 1f) and the bundle of pollen tubes finally emerges out through the dehiscence slit, and finds its way to the nearby stigma.

Four groups of plants blooming at different times of the month were recognized and segregated during the present study. The populations studied reached the period of full bloom during different time periods in February and March (table 2). Population-1 was the first to bloom and was observed for *in situ* pollen germination in February. It showed *in situ* pollen germination in only 5% of the open pollinated flowers. Flowering in population-2 was initiated in February and plants were in full bloom in the middle of March. The percentage frequency of *in situ* was observed to be 11.66% on open pollination for this population. Populations-3 and 4 were in full bloom in March. An appreciable

percentage of *in situ* pollen germination was recorded in flowers of both these populations on open pollination. In population-3, 18.33% of the flowers showed *in situ* pollen germination. The highest incidence of *in situ* pollen germination was observed in population-4, where it averaged 31%. However, on unassisted selfing, the rate of *in situ* pollen germination increased in all the four populations studied (table 2).

3.2 *Ovule abortion*

As described earlier, pistil of *T. fragiferum* consists of two ovules per ovary. Within an ovary, both may develop (figure 2a), or one or both of these were seen to abort (figure 2b, c, d) in all populations of *T. fragiferum* scanned for the rates and patterns of ovule abortion. The same was detailed

1. On open pollination and on unassisted selfing in four populations scanned
2. According to the percentage of *in situ* pollen germination shown by each population

Plants as elaborated in observations on *in situ* pollen germination were categorized into four groups. All these four populations were also studied for the rate of ovule abortion in their pistils. The percentage of pistils showing ovule abortion in open pollinated flowers was 30% in population-4 and 5%, 13.3% and 25% for populations-1, 2 and 3 respectively (table 3a). However, on unassisted selfing, the rates soared and pistils with aborting ovules were

Table 3. Data on percentage ovule abortion*

(a) On open pollination

S. No.	Population type	Number of peduncular ovules aborted	Number of stylar ovules aborted	Total number of ovules aborted	Percentage of total ovule abortion
1	Population-1	3	0	3	5
2	Population-2	6	2	8	13.33
3	Population-3	11	4	15	25
4	Population-4	21	9	30	30

(b) On unassisted selfing

S. No.	Population type	Number of peduncular ovules aborted	Number of stylar ovules aborted	Total number of ovules aborted	Percentage of total ovule abortion
1	Population-1	7	2	9	15
2	Population-2	12	6	18	30
3	Population-3	14	9	23	38.33
4	Population-4	24	18	42	42

Sample size (N)= 60 (for populations-1, 2, 3)

Sample size (N)= 100 (for population-4)

*Period of study= February to March

Table 4. Data on percentage of fruits with variable seed number/inflorescence

S. No.	Character (type of fruit)	Average value (%)	Range
A	On open pollination		
1	Single-seeded	23.8±1.85*	11.11-43.47
2	Two-seeded	67.55±2.53	39.13-89.28
3	Empty	8.62±1.42	3.57-25.92
B	On unassisted selfing		
1	Single-seeded	41.98±4.35	8.33-93.75
2	Two-seeded	34.68±3.78	4.16-59.25
3	Empty	23.31±3.71	4.34-87.5

* Mean ± SE

Sample size (N)= 25 (for each character)

recorded as 42% for population-4 and 15%, 30% and 38.3% for populations-1, 2 and 3 respectively (table 3b). Ovule abortion is non-random in the plants of *T. fragiferum*, as the peduncular ovule gets aborted in much higher frequency in the pistils scanned than the stylar one both on open pollination as well as selfing (figure 3a and b; table 3). Many times, in some flowers both the ovules also get aborted, resulting in fruits that are empty (figure 3c and d). However, such empty fruits were found in quite low frequency in different populations scanned (table 4).

4. Discussion

4.1 *Mating system*

Plant mating systems show a complete range from outbreeding among unrelated individuals to inbreeding among relatives and self-fertilization (Shields 1993; Waser 1993). Although many of the species exhibit either of these two phenomena, a distinct minority exhibits a mixed-mating system in which both selfing and outcrossing co-occur and it is basically an evolutionarily stable strategy (ESS) (Maynard Smith 1982). The same enables a species to harness the benefits of immediate adaptability and assured seed set through selfing and to ensure evolutionary flexibility by occasional bouts of outcrossing. Holsinger (1992) opines that an ESS can be developed for mixed-mating as both selfing and outcrossing can confer fitness benefits to offspring. The most often cited hypothesis for these benefits is called as 'best of both worlds' (Cruden and Lyon 1989; Becerra and Lloyd 1992). This hypothesis enumerates that mixed mating evolved because it helps in promoting outcrossing and at the same time provides reproductive assurance in times when pollinators/mates are scarce, thus combining the advantages of both reproductive strategies

Figure 3. A pistil showing the abortion of peduncular ovule (**a**) and stylar ovule (**b**), healthy fruits of *Trifolium fragiferum* (X2) (**c**), and empty fruits with abortion in pods (X1.8) (**d**).

Table 5. Data on percentage ovule abortion vis-à-vis range of *in situ* pollen germination*

(a) On open pollination

S. No.	Population type	Range of *in situ* pollen germination (%)	Percentage of total ovule abortion	Percentage of peduncular ovule abortion	Percentage of stylar ovule abortion
1	Population-1	0–9	5	100	0
2	Population-2	10–15	13.33	75	25
3	Population-3	16–20	25	73.33	26.66
4	Population-4	30–40	30	70	30

(b) On unassisted selfing

S. No.	Population type	Range of *in situ* pollen germination (%)	Percentage of total ovule abortion	Percentage of peduncular ovule abortion	Percentage of stylar ovule abortion
1	Population-1	0–9	15	77.77	22.22
2	Population-2	10–15	30	66.66	33.33
3	Population-3	16 –20	38.33	60.86	39.13
4	Population-4	30–40	42	57.14	42.85

Sample size (N)= 60 (for populations-1, 2, 3)

Sample size (N)= 100 (for population-4)

*Period of study= February to March

(Goodwillie *et al.* 2005). The maintenance of these distinct alternative strategies within a population is tedious and may involve frequency-dependent selection or may show condition dependence. The tactics used by an individual is also likely to be greatly influenced by its physiological state or environmental circumstances (Maynard Smith 1982). This has led many authors to predict that the mixed-mating system that consists of both selfing and outcrossing should not be evolutionarily stable (Schoen 1984; Holsinger 1986, 1988; Charlesworth and Charlesworth 1987; Le Corff 1996; Culley 2000). However, if selfing and outcrossing lead to a progeny that differ in the pattern of seed dispersal or if the overall level of inbreeding depression is low enough to enable selfed progeny to survive, it may lead to a stable mixed-mating system (Kaul and Koul 2009).

Trifolium fragiferum L., a highly successful common lawn weed in Jammu, has beautifully evolved this stable mixed-mating system for its survival and propagation. The species is an annual weed with a brief life cycle of about 3½ months in the subtropical climates of Jammu. This legume is a profuse flower and fruit producer and is totally self-compatible. It sets seed by self-fertilization if undisturbed. Brisk insect visits during a part of flowering season, however, ensure some amount of outcrossing also (Koul 2010; personal observations communicated).

4.2 *In situ pollen germination*

An interesting phenomenon shown by a proportion of flowers (28% in a sample of 824 flowers) in the populations of this species explored in Jammu is *in situ* pollen germination.

This describes a condition wherein pollen grains germinate inside the anther and either the germinated pollen grains or the pollen tubes invade the stigma. This phenomenon, which results in obligate selfing, was first reported in cleistogamous flowers (Sablon 1900; Frisendahl 1927; Hanson 1943; Conners and Mathews 1977; Lee *et al.* 1979; Lord 1979; Anderson 1980; Mayers and Lord 1984; Philbrick 1984). Later on, several chasmogamous flowers were also found to show this phenomenon, *T. fragiferum* being a member of this group (Dhar 2004). The frequency of flowers showing this phenomenon, however, varies in different populations; blooming at different times.

Four different populations of *T. fragiferum* were scanned in detail over a brief period of blooming commencing from February to March-end during the present work. These varied in their time of blooming as also in the frequency of flowers showing *in situ* pollen germination. Plants in population-1 were the first to flower with full bloom occurring in February. Only 5% of the flowers in this population showed *in situ* pollen germination. Plants in population-2 showed full bloom from mid-February to mid-March and had the prevalence of *in situ* pollen germination in 11.66% of their flowers. Plants in population-3 and 4 were the last to bloom in March. About 18.33% of the flowers in population-3 showed *in situ* germination of pollen, whereas population-4 showed the highest percentage of flowers (31%) showing *in situ* pollen germination. The same pattern was followed when a proportion of flowers in each group were bagged for seed-set on unassisted selfing. However, the frequency of flowers showing precocious pollen germination recorded a slight rise in the bagged inflorescences (table 2).
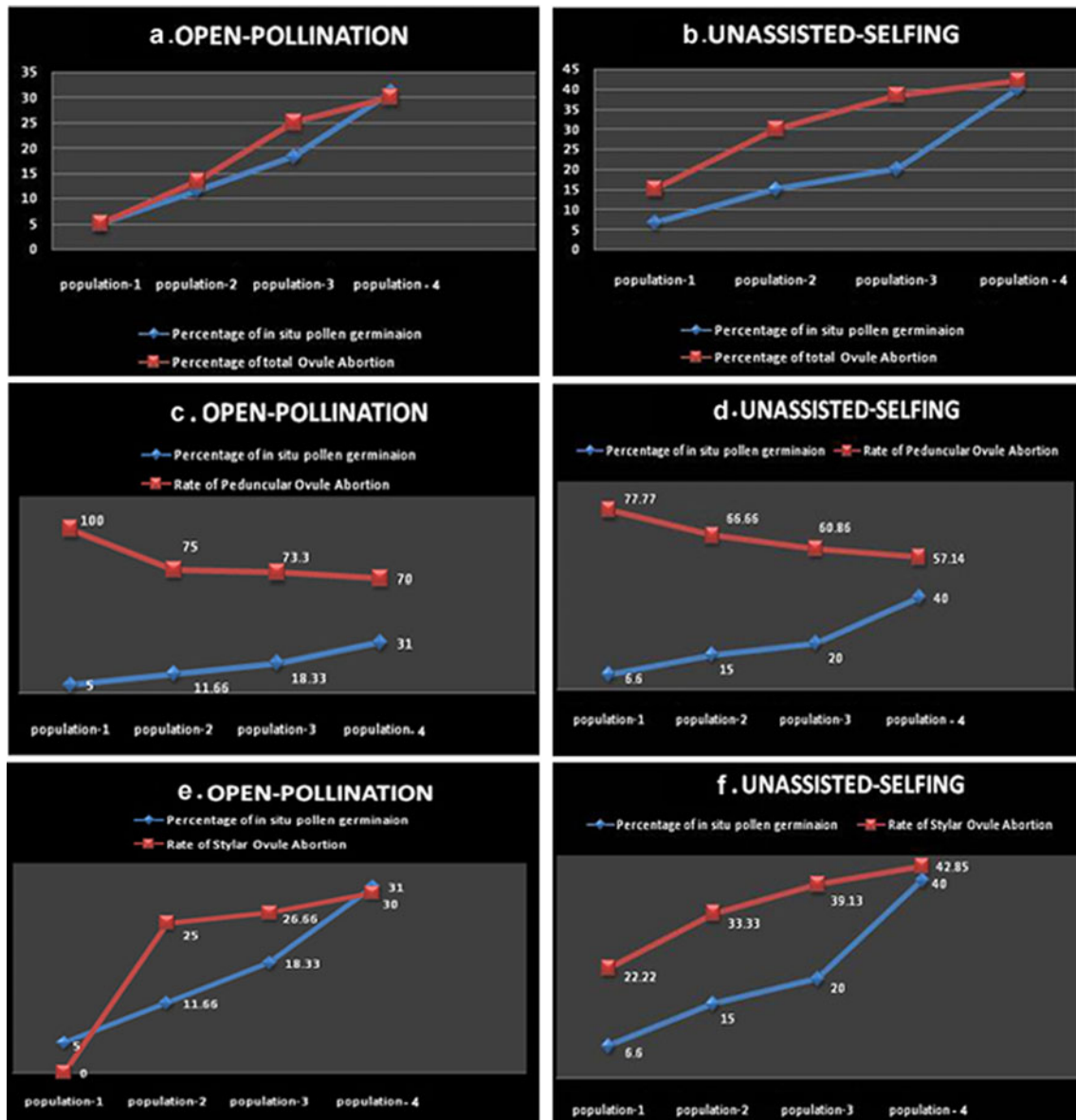
Figure 4. Line diagram showing a positive correlation between *in situ* pollen germination and ovule abortion both on open pollination (**a**) and on unassisted selfing (**b**); line diagram showing a negative correlation between *in situ* pollen germination and peduncular ovule abortion both on open pollination (**c**) and on unassisted selfing (**d**); line diagram showing a positive correlation between *in situ* pollen germination and stylar ovule abortion both on open pollination (**e**) and on unassisted selfing (**f**).

4.3 *Rates and pattern of ovule abortion*

All these populations were found to suffer from ovule abortion with an overall rate of nearly 30% pistils undergoing ovule abortion on open pollination and 42% on unassisted selfing in population-4. In populations-1, 2 and 3 the rates were recorded as 5%, 13.3% and 25% on open pollination and 15%, 30% and 38.33% on selfing respectively. The pattern of ovule abortion was found to be non random in the plants of *T. fragiferum*. Pistils scanned showed high

frequency of peduncular ovule abortion compared to the stylar one (table 5a and b).

4.4 *Correlation*

Detailed investigation on the ovule abortion in different populations as investigated on different times during a flowering period revealed an interesting pattern as the populations selected presently varied in the rate of *in situ* pollen germination and the rates of abortion of ovules. A series of correlations

were observed between the rates and pattern of ovule abortion in pistils and the type of pollination occurring in a flower. Some of these correlations are enumerated below.

4.4.1 An increase in percentage of *in situ* pollen germination was seen to be accompanied by an increase in the rate of ovule abortion in pistils, i.e. with a gradual increase in the range of *in situ* pollen germination from 0 to 40%, percentage ovule abortion increases from 5% to 30% on open pollination and 15% to 42% on unassisted selfing (table 5a and b; figure 4a and b). This clearly indicates that in this species enjoying mixed-mating system, ovule abortion results from inbreeding depression due to joining of genetically similar gametes after endogenous pollination, which causes the expression of deleterious recessive alleles described as mutational load (Wiens *et al.* 1989; Husband and Schemske 1996).

4.4.2 The pattern of ovule abortion also showed a distinct shift with rise in percentage of *in situ* pollen germination. As the range of *in situ* pollen germination increased, a decrease in percentage of pistils showing peduncular ovule abortion was recorded both on open pollination and unassisted selfing (figure 4c and d; table 5a and b). The percentage of pistils with stylar ovule abortion increased under the same set of range (figure 4e and f). These correlations are statistically significant as justified by Pearson's correlation. Both

positive and negative correlations were observed between different pairs of characters (X, Y). Pearson's coefficient of correlation (r) revealed different interpretations, exactly as observed in nature by us (figure 4; table 6a and b). On unassisted pollination (i.e. both on unassisted selfing as also during the time when pollinators were rare), the rate of stylar ovule abortion increases at much higher rates than on open pollination (table 5a and b).

Abortion of embryos that are farthest from the stigma, i.e. peduncular ovules, is the most common pattern found in legumes (Bawa and Buckley 1989). These ovules are generally the last to get fertilized just because of their position. Early fertilization gives a head start to the stigmatic ovules to draw resources towards themselves and this in turn may lead to the abortion of peduncular ovules, e.g. in *Lathyrus latifolius* and *L. sylvestris* (Hossaert and Valero 1988) and *Cassia fasciculata* (Lee and Bazzaz 1982a, b). This non-random pattern of ovule abortion can also be explained by the fact that basal positions are less likely to be reached by pollen tubes than stylar ones (Rocha and Stephenson 1990). In the flowers of *T. fragiferum* showing *in situ* pollen germination, when this barrier of differential pollen tube growth is removed, the peduncular ovules become less likely to face ovule abortion. Since pollen tubes have already gained a proximate length, these after passing through the style can easily fertilize both the ovules. This ultimately leads to a decrease in peduncular ovule abortion with an increase in *in situ* pollen germination.

Table 6. Data on correlations (r) between different pair of characters

(a) On open pollination			
S. No.	Characters	Pearson's Correlation (r)	Interpretation
	X — Y		
1	*In situ* — Total ovule abortion	+ 0.954*	Strong positive correlation
2	*In situ* — Peduncular ovule abortion	−0.792	Good negative correlation
3	*In situ* — Stylar ovule abortion	+0.792	Good positive correlation
4	Total ovule abortion — Peduncular ovule abortion	−0.863	Strong negative correlation
5	Total ovule abortion — Stylar ovule abortion	+0.863	Strong positive correlation
6	Peduncular ovule abortion — Stylar ovule abortion	−1.000**	Perfect negative correlation

(b) On unassisted selfing			
S. No.	Characters	Pearson's Correlation (r)	Interpretation
	X — Y		
1	*In situ* — Total ovule abortion	+0.859	Strong positive correlation
2	*In situ* — Peduncular ovule abortion	−0.880	Strong negative correlation
3	*In situ* — Stylar ovule abortion	+0.880	Strong positive correlation
4	Total ovule abortion — Peduncular ovule abortion	−0.999**	Strong negative correlation
5	Total ovule abortion — Stylar ovule abortion	+0.999	Strong positive correlation
6	Peduncular ovule abortion — Stylar ovule abortion	−1.000	Perfect negative correlation

*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).

References

Anderson WR 1980 Cryptic self fertilization in the Malphighiaceae. *Science* **207** 892–893

Arathi HS, Ganeshaiah KN, Uma Shaanker R and Hedge SG 1996 Factors affecting embryo abortion in *Syzygium cumini* (L.) skeels (Myrtaceae). *Int. J. Plant. Sci.* **157** 49–52

Bawa KS and Buckley DP 1989 Seed : ovule ratios, selective seed abortion and mating in Leguminosae; in *Advances in legume biology: Monograph of systematic botany 29* (eds) CH Stirton and JL Zarucchi (Missouri Botanical Gardens) pp 243–262

Bawa KS and Webb CJ 1984 Flower, fruit and seed abortion in tropical forest trees: implications for the evolution of paternal and maternal reproductive patterns. *Am. J. Bot.* **71** 736–751

Becerra JX and Lloyd DG 1992 Competition-dependent abscission of self-pollinated flowers of *Phormium tenax* (Agavaceae): a second action of self-incompatibility at the whole flower level. *Evolution* **46** 458–469

Charlesworth D and Charlesworth B 1987 The effect of investment in attractive structure on allocation to male and female functions in plants. *Evolution* **41** 948–968

Conners HE and Mathews BA 1977 Breeding system in New Zealand grasses, VII. *New Zeal. Bot.* **15** 531–534

Cruden RW and Lyon DL 1989 Facultative Xenogamy: examination of a mixed-mating system; in *The evolutionary ecology of plants* (ed) JH Bock and YB Linhart (Westview: Boulder, Co) pp 171–207

Culley TM 2000 Inbreeding depression and floral type differences in *Viola canadensis* (Violaceae), a species with chasmogamous and cleistogamous flowers. *Can. J. Bot.* **78** 1420–1429

Dhar R 2004 *Reproductive strategies of some clover species with emphasis on pattern and causes of ovule abortion*, PhD thesis, University of Jammu, Jammu

Dhar R, Sharma N and Sharma B 2006 Ovule abortion in relation to breeding system in four *Trifolium* species. *Curr. Sci.* **91** 4 482–485

Frisendahl A 1927 Birdie Entwicklung Chasmogamer and Kleistogamer Bl ten beider Gattung Elatine. *Acta Hort. Gothoburg* **3** 99–142

Ganeshaiah KN and Uma Shaanker R 1988 Seed abortion in wind-dispersal pods of *Dalbergia sissoo*, maternal regulation or sibling rivalry. *Oecologia* **77** 135–139

Goodwillie C, Kalisz S and Eckert CG 2005 The evolutionary enigma of mixed mating systems in plants: Occurrence, theoretical explanations, and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.* **36** 47–79

Hanson CH 1943 Cleistogamy and the development of embryo sac in *Lespedeza stipulata*. *J. Argic. Res.* **67** 265–272

Holsinger KE 1986 Dispersal and plant mating systems: the evolution of self fertilization in subdivided populations. *Evolution* **40** 405–413

Holsinger KE 1988 Inbreeding depression doesn't matter: the genetic basis of mating system evolution. *Evolution* **42** 1235–1244

Holsinger KE 1992 Ecological models of plant mating systems and the evolutionary stability of mixed mating systems; in *Ecology and evolution of plant reproduction: new approaches* (ed) R Wyatt (New York: Chapman and Hall) pp 169–191

Hossaert M and Valero M 1988 Effect of ovule position in the pod on patterns of seed formation in two species of *Lathyrus* (Leguminosae : Papilionoideae). *Am. J. Bot.* **75** 1714–1731

Husband BC and Schemske DW 1996 Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50** 54–70

Kaul V and Koul AK 2009 Sex expression and breeding strategy in *Commelina benghalensis* L. *J. Biosci.* **34** 6 977–990

Koul M 2010 *Studies on the incidence and effects of in situ pollen germination in some populations of Trifolium fragiferum L.*, M Phil dissertation, University of Jammu, Jammu

Le Corff J 1996 Establishment of chasmogamous and cleistogamous seedlings of an ant-dispersed understory herb, *Calathea micans* (Marantaceae). *Am. J. Bot.* **83** 155–161

Lee TD 1988 Patterns of fruit and seed production; in *Plant reproductive biology: Patterns and strategy* (eds) JL Doust and LL Doust (New York: Oxford University Press) pp 179–202

Lee TD and Bazzaz FA 1982 a Regulation of fruit and seed production in an annual legume *Cassia fasciculata*. *Ecology* **63** 1363–1373

Lee TD and Bazzaz FA 1982 b Regulation of fruit maturation pattern in an annual legume *Cassia fasciculata*. *Ecology* **63** 1374–1388

Lee CW, Erickson HT and Janik J 1979 Cleistogamy in *Salpiglossis sinuata*. *Amer. J. Bot.* **66** 626–632

Lewis D 1979 *Sexual incompatibility in plants* (London: Edward Arnold)

Lloyd DG 1980 Sexual strategies in plants I. A hypothesis of serial adjustment of maternal investment during one reproductive session. *New Phytol.* **86** 69–79

Lord EM 1979 The development of cleistogamous and chasmogamous flowers in *Lamium amplexicaule* (Labiatae): an example of hetroblastic inflorescence development. *Bot. Gaz.* **140** 544–577

Mayers AM and Lord EM 1984 Comparative flower development in the cleistogamous species

Maynard Smith J 1982 *Evolution and the theory of games* (Cambridge: Cambridge University)

Mohan Raju B, Uma Shaanker R and Ganeshaiah KN 1996 Intrafruit seed abortion in a wind dispersed tree *Dalbergia sissoo* Roxb.: proximate mechanisms. *Sex. Plant Reprod.* **9** 273–278

Philbrick CT 1984 Pollen tube growth within tissue of *Callitriche* (Callitrichaceae). *Am. J. Bot.* **71** 882–886

Rocha OJ and Stephenson AG 1990 Effect of Ovule position on seed production, seed weight, and progeny performation in *Phaseolus coccineus* L. (Leguminosae). *Am. J. Bot.* **77** 1320–1329

Rocha OJ and Stephenson AG 1991a Effects of non-random seed abortion on progeny performance in *Phaseolus coccineus* L. *Evolution* **45** 1198–1208

Rocha OJ and Stephenson AG 1991b Order of fertilization within the ovary of *Phaseolus coccineus* L. (Leguminosae). *Sex. Plant Reprod.* **4** 126–131

Rosallini D, Ferranti F and Veronesil F 2003 Ovule sterility and seed set in alfa alfa (*www.naaic.org/TAG*)

Sablon LD 1900 Recherches sur les fleus cleistogamous. *Rev. Gen. Bot.* **12** 305–318

Schoen DJ 1984 Cleistogamy in *Microlaena polynoda* (Gramineae): an examination of some model predictions. *Am. J. Bot.* **71** 711–719

Shields WM 1993 The natural and unnatural history of inbreeding and outbreeding; in *The natural history of inbreeding and outbreeding* (ed) NM Thornhill (Chicago: University of Chicago Press)

Stephenson AG 1981 Flower and fruit abortion: proximate causes and ultimate functions. *Annu. Rev. Ecol. Syst.* **12** 253–279

Uma Shaanker R and Ganeshaiah KN 1988 Bimodal distribution of seeds per pod in *Caesalpinia pulcherrima* Parent-offspring conflict. *Evol. Tr. Pl.* **2** 91–98

Waser NM 1993 Sex, mating systems, inbreeding and outbreeding; in *The natural history of inbreeding and outbreeding* (ed) NM Thornhill (Chicago: University of Chicago Press)

Wiens D, Nickrent DL, Daven CI, Calvin CL and Viverette NJ 1989 Development failure and loss of reproductive capacity in the rare palaeoendemic shrub *Dedeckera eurekensis*. *Nature* **338** 65–67