

## Reproductive biology of *Torilis arvensis* (Hudson) Link.

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**Abstract.** *Torilis arvensis* is an andromonoecious umbellifer which produces hermaphrodite and staminate flowers in the ratio of 1:0.36. This figure is recorded for the first time among andromonoecious umbellifers. The species practices geitonogamy on account of weak protandry and little visual impact of its umbels.

**Keywords.** *Torilis arvensis*; andromonoecy; protandry; geitonogamy.

### 1. Introduction

Reproduction in plants is accomplished through asexual or sexual processes. The former involves simple replication of the parental genotype and the latter brings forth new diversity in each generation. The main factors involved in producing diverse reproductive units are the breeding and the meiotic system of the species.

Umbellifers exhibit a variety of breeding systems *viz.*, andromonoecy, hermaphroditism, gynodioecy and dioecy. A good deal of literature has accumulated on breeding system of umbellifers (Braak and Kho 1958; Bell 1971; Lloyd 1973; Kumar 1977; Bell and Lindsey 1978; Webb 1979; Lovett Doust 1980; Koul *et al* 1984) but very little work has been published on the meiotic system. The present communication deals with the breeding and meiotic system of *Torilis arvensis*, an andromonoecious umbellifer inhabiting foothills of western Himalayas.

### 2. Material and methods

All studies were made using plant population growing at Bahu-Wali Rakh, Jammu (India) between January–April, 1984. Individual plants were tagged for studying flowering phenology and pollination mechanisms. The proportion of hermaphrodite and staminate flowers was worked out separately for different umbel orders as well as the whole plant. Observations on anthesis were made on different umbel orders of 5 individuals at different time intervals. Stigma receptivity was detected by calculating pollen load on stigmas of different developmental stages using a malachite green—acid fuchsin stain combination. Pollen counts were made by calculating the number of pollen produced per anther; aniline blue stained preparations were made for the purpose. The figures thus obtained were used for calculating pollen: ovule ratio per hermaphrodite flower, per umbel order and finally per individual (each hermaphrodite flower has 5 anthers and 2 ovules). Fruits set on umbels of different orders were counted, and then the percentage of fruit production was calculated.

For karyology seeds were germinated on moist filter paper at room temperature. Root tips, about 1.5 cm long, were pretreated with saturated aqueous solution of para-

dichlorobenzene at  $\pm 10^{\circ}\text{C}$  for 3 hr. The pretreated root tips were fixed in 1:3 acetic alcohol for 24 hr. For making chromosome preparations, root tips were hydrolysed at  $60^{\circ}\text{C}$ , in a mixture of 9 parts aceto-orcein and 1 part  $1\text{NHCl}$ , and squashed in 1% aceto-orcein.

For meiotic studies, young inflorescences were fixed in Carnoy's fluid (3 parts absolute alcohol: 1 part glacial acetic acid) for 24 hr and stored in 70% ethyl alcohol. Buds for appropriate stages were squashed in 1% aceto-orcein. All studies were made from temporary squashes.

### 3. Observations

Plants of *T. arvensis* are 20–60 cm tall and are annual. Leaves are 1–2 pinnate, leaf segments are lanceolate and coarsely toothed; the involucrel consists of linear bractlets and the flowers are white. Each population comprised 80–100 plants/m<sup>2</sup>.

#### 3.1 Flowering phenology

The main shoot develops as a leafy stem and terminates in the primary or first order umbel. Lateral shoots are formed within the leaf axils on the main shoot. These terminate in umbels of second order. Further umbels, upto the sixth order arise on lateral branches. The flowering shoot is composed of a sequentially arranged shoots, each terminating in an umbel (figure 1A). The primary umbel is invariably one, but the number of umbels in higher orders varies.

Umbels are concave and compound with 2–4 rays. Each ray terminates in an umbellet which consists of a mixture of hermaphrodite and staminate flowers (figure 1B). The number of umbellets produced by umbels of various orders is 2–3 ( $2.89 \pm 0.11$ ) in primary; 2–4 ( $2.70 \pm 0.61$ ) in secondary; 2–4 ( $2.87 \pm 0.05$ ) in tertiary; 2–4 ( $2.92 \pm 0.08$ ) in quarternary; 2–4 ( $2.57 \pm 0.09$ ) in fifth and 2–3 ( $2.30 \pm 0.13$ ) in sixth order umbels.

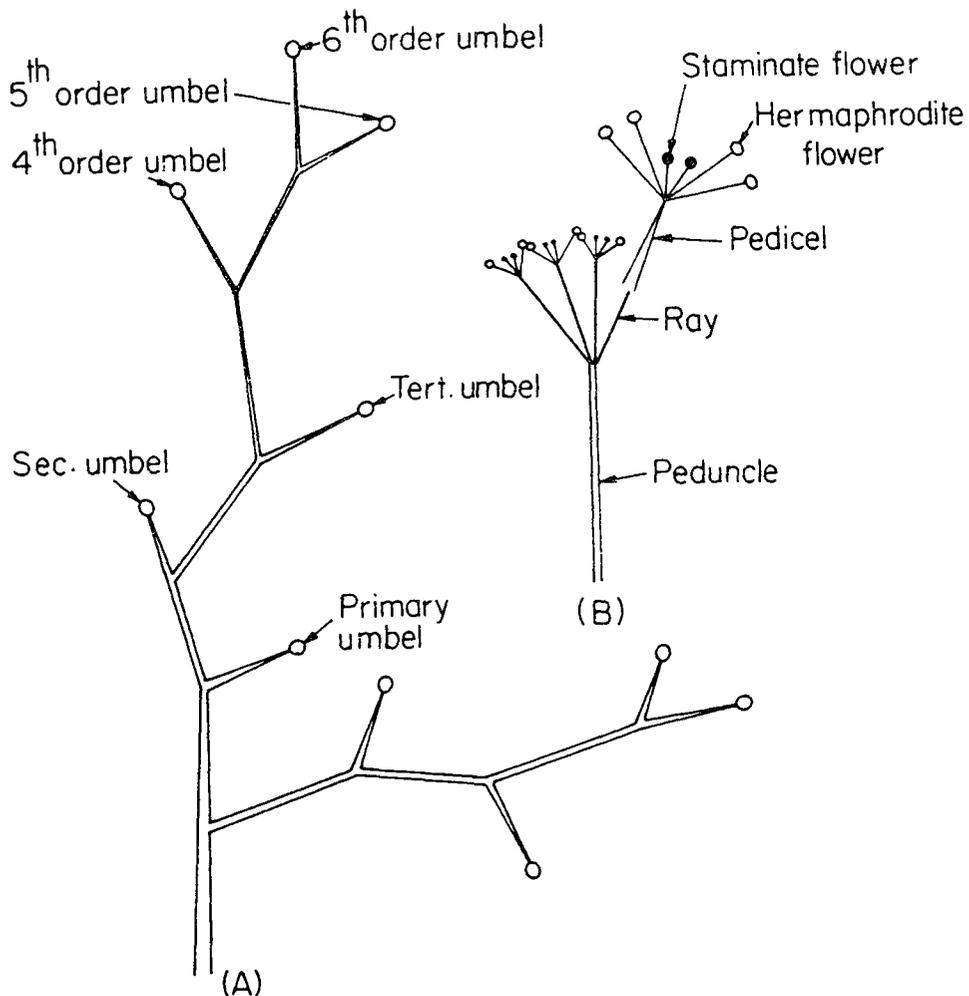
The outermost flowers of each umbellet are hermaphrodite and the inner are staminate. The former outnumber the latter in the mature plant (table 1); the ratio ( $\text{♂} : \text{♀}$ ) is 2.79. However, the actual ratios vary among umbels of different orders (table 2) on account of the gradual increase in the number of staminate flowers from primary to higher umbel orders.

#### 3.2 Flower morphology

The hermaphrodite flowers are larger than the staminate; they bear zygomorphic corolla, five stamens and two carpels (figures 2A,D). The anthers are white and basifixed. The ovary is inferior, bilocular, with a single pendulous ovule in each locule. A slightly swollen nectar secreting structure, the stylopodium, caps the ovary and the two styles emerge from within it.

#### 3.3 Anthesis and stigma receptivity

The umbels undergo anthesis in the same sequence in which they differentiate. Primary umbels bloom first followed by higher umbel orders. Each hermaphrodite flower takes 3–5 days and a staminate flower 2–4 days, to complete anthesis.



**Figure 1.** A. Diagrammatic representation of the ordering of umbels of *T. arvensis*. B. A peduncle showing the arrangement of umbellets in an umbel and the flowers within an umbellet.

The peripheral hermaphrodite flowers undergo anthesis prior to the staminate ones. In the hermaphrodite flowers, anthers dehisce before the stigma becomes receptive (figures 2B,C). At the level of the umbel, stigmas of peripheral flowers (which have already shed their pollen), become receptive first followed by those of the central ones. There is sufficient overlap between anthesis of central flowers and stigmatic receptiveness of the peripheral flowers. Similarly, the stigma receptivity and anthesis of other umbel orders overlap (figure 3).

### 3.4 Pollen: Ovule ratio

On an average, the total number of pollen grains produced per hermaphrodite and staminate flower is 1,633–2,001 ( $1,807.4 \pm 76.65$ ) and 1,194–1,398 ( $1,265.0 \pm 44.26$ )

**Table 1.** Mean values for staminate and hermaphrodite flowers and fruit set in a plant.

Character	Frequency Mean $\pm$ S D
Total number of flowers	183.78 $\pm$ 92.98 (56–417)
No. of hermaphrodite flowers	135.36 $\pm$ 71.45 (45–321)
Percentage of hermaphrodite flowers	73.65 $\pm$ 8.72
No. of staminate flowers	48.42 $\pm$ 28.46 (6–122)
Percentage of staminate flowers	26.35 $\pm$ 8.72
Ratio of ♀: ♂ flowers	2.79
Total no. of fruits formed	111.72 $\pm$ 53.71 (36–157)
Percentage fruit set of hermaphrodite flowers	83.43 $\pm$ 0.57

Mean  $\pm$  S D of 53 plants

Values in parentheses represent ranges.

respectively. The pollen:ovule ratio per hermaphrodite flower is  $903.5 \pm 29.15$  (797–1,047). Since the proportion of staminate and hermaphrodite flowers vary in umbels of different orders, the pollen-ovule ratios vary (table 3) accordingly. The pollen-ovule ratio for the individual averages 1,129.95.

### 3.5 Fruit production

Total fruit set of the plant is 83.43%. The fruit output increases from primary to fourth order umbels, and decreases thereafter (table 2).

### 3.6 Chromosome complement and meiosis

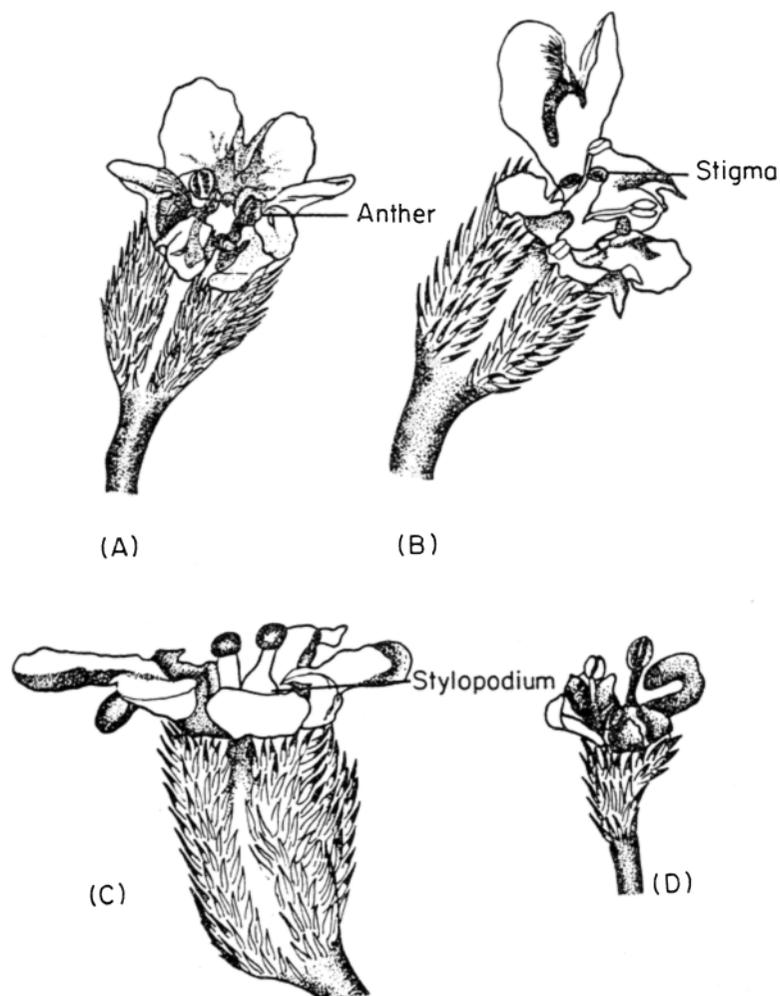
The somatic complement of the species comprises 12 chromosomes measuring 3.39–4.11  $\mu\text{m}$  (figure 4). On the basis of absolute length and arm ratio, these chromosomes form 6 pairs (figure 5); the first comprises the two longest chromosomes, both having sub-median primary constriction. The second consists of 2 sub-metacentric chromosomes, each having a small satellite at the distal end of the short arm. The third pair comprises 2 sub-metacentric chromosomes with an arm ratio of 2.33. Fourth pair consists of 2 metacentric chromosomes. The fifth consists of 2 chromosomes whose arm ratios approach 1.5. The two smallest chromosomes constituting the sixth pair have their centromere at subterminal position.

The chromosomes pair into 6 bivalents during prophase of meiosis. Each bivalent has 1 or 2 randomly distributed chiasmata at diakinesis (figure 6). The average frequency of chiasmata at metaphase-I (figure 7) is 8.5 per cell and 1.41 per bivalent. Recombination index of the species averages 14.6. Segregation of chromosomes is regular, during anaphase-I as well as II (figure 8). Pollen viability exceeds 76.82%.

Table 2. Quantitative data (mean  $\pm$  S D) on staminate and hermaphrodite flowers and fruit set in umbels of different orders.

Umbel order Character	I	II	III	IV	V	VI
No. of umbels studied	53	98	84	42	22	15
Total number of flowers	20.15 $\pm$ 4.84 (9-25)	34.96 $\pm$ 20.15 (9-90)	37.80 $\pm$ 24.79 (7-89)	35.28 $\pm$ 19.10 (9-66)	28.42 $\pm$ 14.41 (8-53)	27.17 $\pm$ 11.24 (10-38)
Number of hermaphrodite flowers	17.66 $\pm$ 3.71 (8-23)	27.62 $\pm$ 14.38 (6-81)	28.20 $\pm$ 15.61 (4-69)	24.94 $\pm$ 11.43 (6-46)	19.02 $\pm$ 9.93 (5-36)	17.92 $\pm$ 8.05 (6-28)
Percentage of hermaphrodite flowers	87.64 $\pm$ 9.85	79.00 $\pm$ 9.74	74.60 $\pm$ 11.05	70.69 $\pm$ 8.21	66.92 $\pm$ 6.86	65.96 $\pm$ 6.47
Number of staminate flowers	2.49 $\pm$ 1.59 (0-6)	7.34 $\pm$ 4.29 (0-16)	9.60 $\pm$ 6.18 (0-24)	10.34 $\pm$ 4.98 (3-21)	9.40 $\pm$ 4.19 (3-17)	9.25 $\pm$ 3.59 (3-12)
Percentage of staminate flowers	12.36 $\pm$ 9.74	21.00 $\pm$ 9.74	25.40 $\pm$ 11.05	29.31 $\pm$ 8.21	33.08 $\pm$ 6.86	34.04 $\pm$ 6.47
Ratio of hermaphrodite: staminate flowers	1:0.14	1:0.27	1:0.34	1:0.41	1:0.49	1:0.51
Total no. of fruits formed	10.20 $\pm$ 5.48 (0-22)	20.08 $\pm$ 11.49 (5-54)	21.46 $\pm$ 15.11 (3-43)	18.08 $\pm$ 11.57 (2-40)	14.90 $\pm$ 7.17 (4-23)	11.00 $\pm$ 4.24 (3-16)
Percentage fruit set	70.62 $\pm$ 30.77	80.80 $\pm$ 15.60*	86.13 $\pm$ 7.24*	89.30 $\pm$ 10.04*	86.12 $\pm$ 8.29*	83.69 $\pm$ 12.58*

Ranges in parentheses. \*Significant at 0.001 probability against first order umbel.



**Figures 2.** A-B. Hermaphrodite flowers at various stages of anthesis ( $\times 28$ ). C. A hermaphrodite flower showing receptive stigma ( $\times 28$ ). D. A staminate flower at anthesis ( $\times 30$ ).

**Table 3.** Pollen: ovule ratios in different umbel orders.

Umbel order	Pollen grains produced by hermaphrodite flowers	Pollen produced by staminate flowers	Total pollen grain production	Ovules produced	Pollen: ovule ratio
Primary	31,918.68	3,149.85	35,068.53	35.52	987.28
Secondary	49,920.39	9,285.00	59,205.39	55.24	1,071.78
Tertiary	50,968.68	12,144.00	63,112.68	56.40	1,119.02
Quarternary	45,076.56	13,080.10	58,156.66	49.88	1,165.93
Fifth	34,376.75	11,891.00	46,267.75	38.04	1,216.29
Sixth	32,388.61	11,701.25	44,089.86	35.84	1,230.19
Plant	2,44,649.66	61,251.30	3,05,900.96	270.72	1,129.95

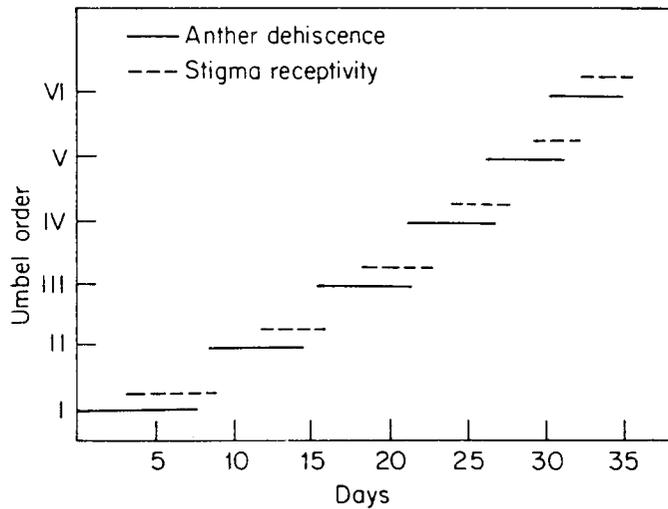


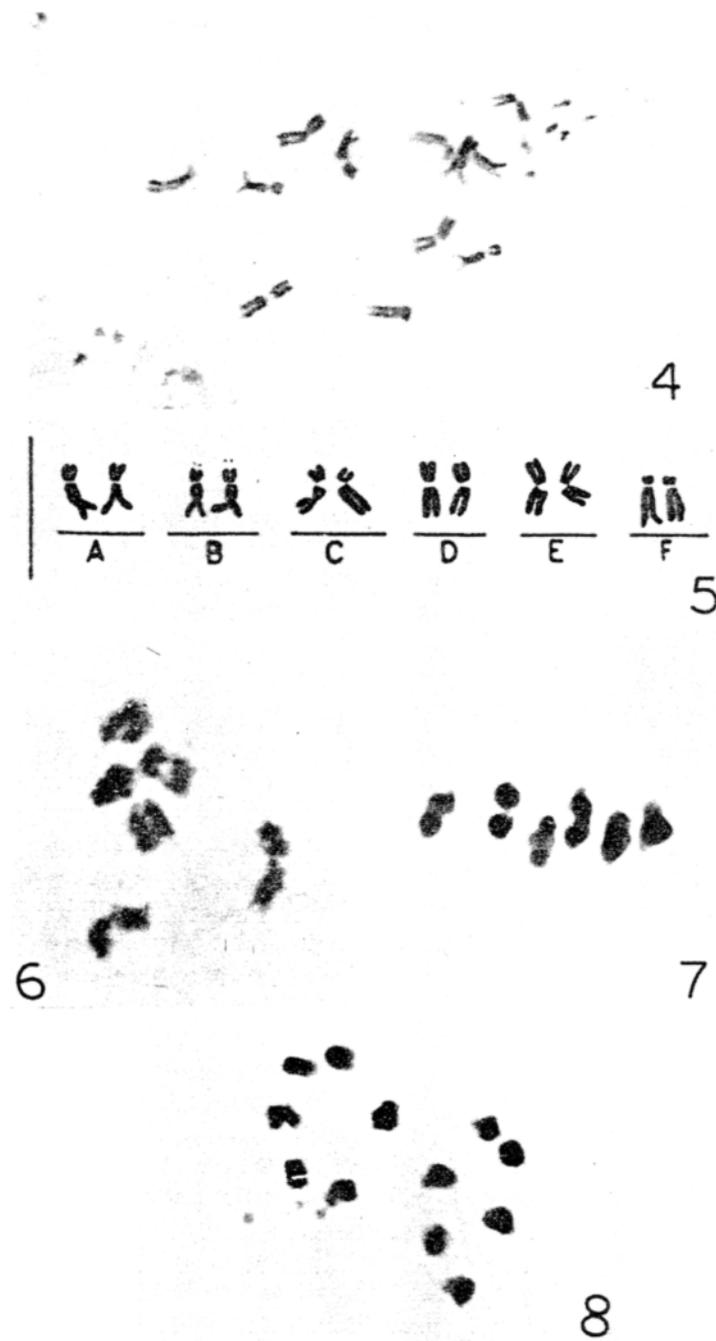
Figure 3. Graphic representation of anthesis and stigma receptivity in various umbel orders.

#### 4. Discussion

Umbellifers exhibit different types of sex expressions, of which andromonoecism is very common. The andromonoecious species vary in the proportion of staminate and hermaphrodite flowers in umbels of different orders. In some taxa (*Daucus carota*, *Anthriscus sylvestris*, *Pastinaca sativa*, *Torilis leptophylla*) there is gradual decrease in the frequency of hermaphrodite flowers from the primary to higher umbel orders; in others (*Thaspium barbinode*, *Zizia* sp.), the primary umbel bears the maximum number of staminate flowers. Although the frequency of staminate flowers increases gradually from primary to higher umbel orders in *Torilis arvensis* as well as *T. leptophylla*, hermaphrodite flowers outnumber the staminate flowers in the former (table 4).

Cruden (1976) reported a uniform ratio of 1 hermaphrodite: 4 staminate flowers at the level of whole plant in 4 umbellifers growing in open habitat. In plants of *Heracleum lanatum* inhabiting a forest site, the production of staminate flowers was considerably higher. This skewness towards the production of more staminate flowers by this species was attributed to its location in the forest where the foraging activity of bees is high which calls for increased pollen production. In *Scandix pecten-veneris* the ratio is 1 hermaphrodite:1.07–1.20 staminate flowers (P Koul, A K Koul and I A Hamal, unpublished results) *T. leptophylla* bearing medium sized umbels falls in between, with the ratio of 1 hermaphrodite:2.73 staminate flowers (Koul *et al* 1984). In an allied species, *T. arvensis*, the staminate flowers are still fewer; the actual ratio between hermaphrodite and staminate flowers being 1:0.36.

Bell and Lindsey (1978) have correlated differences in the ratio of hermaphrodite to staminate flowers with the differences in the degree of dichogamy. The strongly protandrous umbellifers produce more hermaphrodite flowers in the first formed umbels, but in the strongly protogynous taxa the primary umbel has mostly the staminate flowers. In *T. arvensis* there is no difference between the first and the later formed umbels, the hermaphrodite flowers predominate in all. Similar situation



**Figures 4–8.** 4. A root tip cell showing 12 chromosomes. 5. Karyoidiogram of the cell in figure 4. 6 and 7. Pollen mother cells at diakinesis and metaphase-I. 8. A pollen mother cell at anaphase-I with 6 chromosomes at each pole. (Bar = 10  $\mu$ m).

**Table 4.** Percentage of hermaphrodite flowers in various umbel orders of *T. arvensis* and *T. leptophylla*.

Name of species	Umbel orders					
	I	II	III	IV	V	VI
<i>T. arvensis</i>	87.64	79.00	74.60	70.69	66.92	65.96
<i>T. leptophylla</i> <sup>a</sup>	43.27	28.51	22.17	26.16	–	–

<sup>a</sup>Figures from Koul *et al* (1984).

prevails in the weakly dichogamous taxon *Osmorhiza* (C R Bell, personal communication).

In strongly dichogamous umbellifers the staminate phase in all flowers of an umbel is completed before the onset of the pistillate phase (Cruden and Hermann-Parker 1977). Although the flowers in *T. arvensis* are protandrous, there is considerable overlap between the female phase of the peripheral and male phase of the central flowers of the umbel. Obviously, autogamy does not occur on account of dichogamy, conditions for geitonogamy prevail within individual umbels. Production of many hermaphrodite flowers, coupled with very weak dichogamy, increase the chance of geitonogamy in *T. arvensis*.

Although structural features of the flower, existence of unisexual flowers besides the hermaphrodite type, and dichogamy are contrivances for outbreeding plants of *T. arvensis* do not offer much visual attraction to the pollinators on account of small umbel size. Barring some unspecialized ants, which crawl over the umbels, no other visitors were observed on the umbels during the present investigation. Wind also plays a negligible role in the pollination of umbellifers (Proctor and Yeo 1973). Allogamy is therefore, ruled out. Weak dichogamy leading to sufficient overlap between the male and female phases of the flowers of an umbel as well as among the umbels of different orders indicates the operation of geitonogamy. Low pollen: ovule ratio (1,129:95) of the plant is yet another indicator of the geitonogamous nature of the species.

Except for the variability released through the meiotic system, the species does not generate much variability. Low chromosome number and their small size leads to low recombination index. Chiasmata are randomly formed; average chiasmata frequency per cell and per bivalent at metaphase I is 8.5 and 1.41 respectively. Therefore, the individual populations of the species display phenotypic uniformity. Since *T. arvensis* is an annual species, low variability ensures greater stability to its populations.

### Acknowledgement

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### References

- Bell C R 1971 Breeding systems and floral biology of the Umbelliferae; In *The Biology and Chemistry of Umbelliferae* (ed.) V H Heywood (London: Academic Press) pp 93–108  
 Bell C R and Lindsey A H 1978 The umbel as a reproductive unit in the Apiaceae; in *Actes du 2<sup>e</sup>eme*

- symposium international sur les ombellifères - Contribution pluridisciplinaires à la systématique*, Perpignan, France, pp 739-747
- Braak J P and Kho Y O 1958 Some observations on the floral biology of the carrot (*Daucus carota* L.); *Euphytica* **7** 131-139
- Cruden R W 1976 Intraspecific variation in pollen-ovule ratios and nectar secretion—preliminary evidence of ecotypic adaptation; *Annals of Missouri Botanical Garden* **63** 277-289
- Cruden R W 1977 Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants; *Evolution* **31** 32-46
- Cruden R W and Hermann-Parker S M 1977 Temporal dioecism: an alternative to dioecism?; *Evolution* **31** 863-866
- Koul P, Koul A K and Hamal I A 1984 Floral biology of *Torilis leptophylla* (L.) Reichenb. f; *Proc. Indian Acad. Sci. (Plant Sci.)* **93** 449-454
- Kumar C R 1977 Floral biology and breeding system of Bulgarian Coriander (*Coriandrum sativum* L.); *New Botanist* **1-4** 131-135
- Lloyd D G 1973 Sex-ratios in sexually dimorphic umbelliferae; *Heredity* **31** 239-249
- Lovett Doust J 1980 Floral sex-ratios in andromonoecious umbelliferae; *New Phytol.* **85** 265-273
- Proctor M and Yeo P 1973 *The pollination of flowers* (London: Collins and Sons)
- Webb C J 1979 Breeding systems and the evolution of dioecy in New Zealand apoid Umbelliferae; *Evolution* **33** 662-672