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# Reproductive ecology of the Australian herb *Trachymene incisa* subsp. *incisa* (Apiaceae)

*Yvonne C. Davila*[A,B] *and Glenda M. Wardle*[A]

[A]Institute of Wildlife Research, School of Biological Sciences, University of Sydney, Sydney, NSW 2006, Australia.
[B]Corresponding author; email: ydavila@bio.usyd.edu.au

*Abstract.* Within the Apiaceae, subtle variation in reproductive characters such as dichogamy, pollinator specificity and umbel density may cause cryptic specialisation and be responsible for the diversity of life histories and gender expression in the family. To address the paucity of information for Australian species we investigated the reproductive ecology of the native perennial herb, *Trachymene incisa* Rudge subsp. *incisa*. *T. incisa* exhibits protandry within flowers and umbels; however, an overlap of 3 days in male and female phases among umbels of consecutive orders permits geitonogamous pollination. There are 72 ± 2.0 ($n$ = 74) white flowers per umbel and nectar is presented during the male and female phases. *Apis mellifera* appears to be the main diurnal pollinator. The pollen:ovule ratio is 1902:1, indicating that *T. incisa* is a facultatively xenogamous species. The long phase of pollen presentation and the low natural seed set of about 45% implies that many flowers are functioning as pollen donors only. Controlled pollination experiments showed that self-pollen led to lower seed set than cross, open and supplemental applications. Early and late-produced cohorts differed in days to emergence but not in seed mass or final percentage emergence.

## Introduction

The Apiaceae is a large cosmopolitan family with many well-known crop species, such as edible carrot *Daucus carota* L., celery *Apium graveolens* L. and parsnip *Pastinaca sativa* L. (Elliot 1990). Much of the work published on the Apiaceae is concerned with the effective pollination, seed set and quality of crop species, but there has also been some work on wild-growing populations of these species (e.g. Hendrix 1984; Hendrix and Sun 1989; Koul *et al.* 1989). However, numerous authors have commented on the absence of detailed observations and studies on native Apiaceae (Bell 1971; Keighery 1982; Lindsey 1982), especially in Australia.

The Apiaceae are characterised by a high degree of floral uniformity, with many small flowers grouped into umbels (Bell 1971). Most species are protandrous and andromonoecious, although some protogynous species have been reported (Bell 1971; Webb 1981). The plants have been termed 'promiscuous' because of their open floral systems that present no restrictions to nectar or pollen and the large diversity of visitors that apparently pollinate them indiscriminately (Bell 1971). While the Apiaceae appear to be unspecialised in terms of floral morphology (Bell 1971), subtle variation in characters exist, such as different degrees of dichogamy (Cruden and Hermann-Parker 1977), pollinator specificity and umbel density (Bell and Lindsey 1978), nectar constitution and nectar and pollen availability (Lindsey and Bell 1985). These subtle characters may give rise to different pollination patterns observed among genera, which Bell (1971) termed 'cryptic specialisation' and may be responsible for the large diversity of life histories and gender expression in the family.

Australia has 42 genera from the Apiaceae found in all states (Powell 1992). The only review of the reproductive strategies of Australian Apiaceae addressed 70 taxa from Western Australia (Keighery 1982). One of the genera studied in this review was *Trachymene* Rudge. There are about 55 species of *Trachymene*, with 37 of 38 Australian species also being endemic (J. M. Hart, unpubl. data). In Keighery's (1982) review, the *Trachymene* species studied (*T. caerulea* Benth., *T. anisocarpa* (Turcz.) B.L.Burtt and *T. croniniana* (F.Muell.) T.Durand & B.D.Jacks) were reported to be self-compatibile and autogamous, contain nectar, exhibit protandry and were visited by a number of insect orders, generalisations which are consistent with most Apiaceae.


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*Trachymene incisa* Rudge subspecies *incisa* is a native herb that grows in dry eucalypt woodland or scrub, on sandy soils that are very infertile (Benson and McDougall 1993). While *T. incisa* subsp. *incisa* is common and distributed throughout coastal New South Wales and Queensland, including western Sydney, other *Trachymene* species in the Sydney region have been described as rare (*T. anisocarpa*), very restricted [*T. scapigera* (Domin) B.L.Burtt] or possibly extinct [*T. procumbens* (F.Muell.) Benth.] (Benson and McDougall 1993). The information from reproductive studies on *T. incisa* subsp. *incisa*, while contributing to the small body of knowledge on Australian Apiaceae and native herbaceous dicotyledons in general (Trémont and McIntyre 1994), may be useful in elucidating factors contributing to its widespread distribution in contrast to other *Trachymene* species.

In this study, the reproductive ecology of *Trachymene incisa* subsp. *incisa* was investigated in terms of floral phenology, floral architecture, diurnal insect visitors, breeding system and seed germination.

## Methods

### *Study species*

*Trachymene incisa* subsp. *incisa* (*T. incisa* hereafter) is an endemic erect perennial herb with a thick tuberous taproot (Watson 2000). It occurs in dry eucalypt woodland or scrub, sclerophyll forest and cleared areas along the coastal regions of New South Wales and Queensland (Powell 1992; Benson and McDougall 1993). It grows on sandy infertile soils and rock crevices, to a height of about 80 cm (Benson and McDougall 1993).

*Trachymene incisa* has bisexual flowers that are arranged in simple umbels. The umbels are arranged in a uniform branching pattern with the secondary umbels (second set to flower) branching off the flowering stalk of the terminal primary umbel (first to flower) and the tertiary umbels branching off the flowering stalk of the secondary umbels and so forth. Usually, there are up to four orders of umbels during a flowering season (Y. C. Davila, pers. obs.). In this study, the umbel was used as the unit for attraction and manipulation (i.e. for breeding system experiments).

### *Study site*

Field work was carried out from February to June 1999 and from January to April 2000, at Agnes Banks Woodland, in western Sydney, New South Wales, Australia (33°38′S, 150°41′E). Benson (1981) recognised five plant communities at Agnes Banks, including low woodlands on deep sand, with an understorey of heath and small areas of open scrub (Benson and Howell 1990; Benson *et al.* 1996). *T. incisa* is common on the nutrient-depleted sandy soils of Agnes Banks Nature Reserve (Benson and McDougall 1993). Plants used in this study were located near the entry to the nature reserve, bordered by the road and walking tracks. This side of the reserve was exposed to a fire in October 1998 (G. M. Wardle, pers. obs.), which scorched all understorey vegetation. *T. incisa* was among the first ground-cover species to re-sprout after the fire and was in high numbers and flowering by January 1999. In 2000, Agnes Banks Woodland (which covers approximately half of the nature reserve) was listed as an Endangered Ecological Community, with *T. incisa* as an identifying species (NSW NPWS 2000).

### *Floral, within-umbel and within-plant phenology and nectar availability*

Four pre-anthetic *T. incisa* plants and one with a single inflorescence were transplanted on 18 February 1999, from Agnes Banks Nature Reserve into pots containing soil from the site. Potted plants were placed in a laboratory with adequate sunlight and ambient temperatures and watered regularly.

Floral and within-umbel phenology was monitored in three umbels from the flowering transplant in 1999. Each umbel was divided into four circular sections (from outermost to innermost): outermost, intermediate 2, intermediate 1 and innermost. Three flowers in each section were marked with cotton string and monitored from anthesis. The onset and number of days the flowers in each section spent as male, quiescent and receptive female were recorded. The four non-flowering transplants from 1999 flowered during early 2000. The onset and number of days each umbel on each plant functioned as male, quiescent, receptive female and post-receptive female umbels were recorded.

Flowers and umbels with at least one dehiscent anther were designated as being male. The quiescent phase was characterised by the absence of stamens and the elongation of the style with a dry, non-bulbous tip. Stigma receptivity was judged as styles fully elongated with a fresh glistening appearance at the tip of the style and post-receptivity or post-pollination as a discoloration to the stylar tip (Cruden and Hermann-Parker 1977). Fruit set was determined by the loss of petals and swelling of the ovary and seed maturity as browning of the mericarps.

The onset and duration of the population flowering periods from 1998 to 1999 (flowering period following fire) and from 1999 to 2000 were casually observed.

The presence of nectar was observed on umbels from laboratory transplants and casually in the field. Presence of nectar was determined visually as a glistening at the base of the styles.

### *Floral architecture and reproductive effort*

Seventy-four umbels were collected throughout the 1999 flowering period and the number of flowers per umbel was counted. The number of umbels, floral stalks, leaves and proportion of umbels setting seeds were counted on 75 field plants chosen at random in 1999.

To assess potential differences in reproductive effort among umbel orders, 10 umbels from the primary, secondary and tertiary umbel positions were collected during the 1999 flowering period and the number of flowers per umbel counted.

### *Insect visitors*

Diurnal insect visitors to *T. incisa* umbels were collected opportunistically throughout the 1999 flowering season. Insects were caught in a sweep net and placed in an ethyl-acetate kill jar (Kearns and Inouye 1993). Insects were identified to family level or lower where possible by researchers at the School of Biological Sciences, University of Sydney and the Australian Museum.

### *Breeding system*

The breeding system was determined indirectly by pollen : ovule ratio and directly by experimental hand pollinations. In 1999, 20 non-dehisced anthers were collected from several male phase umbels and mounted in a drop of 30% glycerol and toluidine blue to stain viable pollen grains (Kearns and Inouye 1993). The number of pollen grains per anther was counted under ×100 magnification. The mean number of pollen grains per anther and pollen : ovule ratio were calculated and the breeding system estimated according to Cruden's classification (Cruden 1977).

A pilot study in early 1999 showed that mechanical autogamy was negligible; 52 umbels were covered with nylon mesh bags prior to anthesis to exclude natural pollinators and left until the post-female phase. Fifty umbels from the same plants were tagged as controls (left open to natural pollinators). Only three bagged umbels set any seed, with a mean percentage seed set of 2.9 ± 1.6 per umbel. Twenty control umbels set seed, with a mean percentage seed set of 34.6 ± 4.7 per umbel. The study also showed that covering umbels with a nylon mesh bag prior to flowering was efficient at excluding natural pollinators.

In 2000, 50 umbels, each from a different plant, were tagged and 10 umbels were randomly assigned to one of the following five treatments:

(1) hand self-pollination: umbels were bagged during the late male phase to exclude pollinators; when the umbel had entered the female phase, a male-phase umbel with pollen visible in anthers from the same plant was brushed 20 times over the stigmas;

(2) hand cross-pollination: umbels were bagged during the late male phase to exclude pollinators; when the umbel had entered the female phase a male-phase umbel, from a plant at least 5 m away, was brushed 20 times over the stigmas;

(3) mixed-load (self + cross) hand-pollination: umbels were bagged during the late male phase to exclude pollinators; when the umbel had entered the female phase, 10 brushes of pollen from a male-phase umbel from a plant at least 5 m away and 10 brushes of pollen from a male-phase umbel on the same plant were applied directly to the stigmas;

(4) open control: umbels were tagged and left open to natural pollinators; and

(5) supplementary pollination: umbels were tagged and left open to natural pollination; in addition, 20 brushes of pollen from an umbel located at least 5 m away were applied directly to receptive stigmas.

Selfed, crossed and mixed-load pollinated umbels were bagged immediately after hand-pollination and open treatments were bagged during seed ripening. Percentage seed set was determined once the seeds began to disperse in the bags and was calculated as

$$(\text{no. seeds} \times 100)/(\text{no. flowers} \times \text{no. ovules per flower}).$$

This experiment was executed twice during the peak flowering period of early 2000, using different plants.

*Germination of self-, cross- and open-pollinated seeds*

In 1999, 27 late male-phase umbels on different plants were bagged. Ten umbels were assigned to receive self-pollen and 17 umbels received cross-pollen, by brushing the male umbel onto the top of the female umbel. Following pollination, the umbels were bagged to exclude subsequent visitation and left until seed maturation. When experimental umbels were maturing fruit, several open-pollinated umbels at the same stage of maturity from the nearby plants were bagged. All umbels were collected at maturity, the seeds counted and weighed. For reliable germination percentages, only umbels with at least 10 healthy seeds were considered, which reduced the design to $n = 4$ umbels for self-pollination and $n = 6$ umbels for cross- and open-pollination treatments.

Between 10 and 33 healthy seeds per umbel were germinated on moistened filter paper in sealed Petri dishes. The dishes were divided into quarters and five seeds from four randomly chosen different umbels were placed in each quarter, so each dish had 20 seeds. When there were less than five seeds to be placed in a quarter, the density of 20 seeds per dish was maintained by placing excess seeds in that quarter. In each dish, all pollination treatments were represented and the identity and position of all seeds was recorded. This spreading of seeds over several dishes avoids confounding that would result if all seeds from the one umbel and/or treatment were to be germinated in the same dish. The dishes were placed randomly on a laboratory bench with natural light and ambient temperature and kept moist for the duration of the experiment. Germination was recorded as a visible shoot protruding from the top portion of the seed. Final percentage germination per umbel pooled over the dishes was determined after 7 weeks.

*Germination of seeds pollinated at different times during the flowering period*

Mature seeds from three umbels were collected on each of 10 days between 18 February and 3 June 1999. The percentage seed set and total seed mass per umbel were calculated. While there were no significant differences among cohorts in terms of seed set and mean seed mass (Y. C. Davila and G. M. Wardle, unpubl. data), seed set tended to decrease throughout the flowering period and mean seed mass peaked in the middle of the flowering period. Seed set was negatively correlated with seed mass ($R = –0.619$, $n = 10$). Flowers that bloom at different times in the season are subject to different pollen and resource availability and weather regimes, and may differ in seed quality (Kearns and Inouye 1993; Nishikawa 1998). Intraspecific variation in seed mass, due to seed number, timing of umbel production and location of umbel, has been shown for several species of Apiaceae (Hendrix and Sun 1989). In addition, numerous studies show an advantage within species to seedlings from larger seeds in terms of emergence or early growth (see Haig and Westoby 1988). These patterns form the basis for testing for differences in emergence and other fitness traits among seeds pollinated and matured at different times during the flowering season.

Ten healthy seeds per umbel were sown separately into seedling-tray wells filled with sieved soil from Agnes Banks. Ten control wells, with sieved soil and no seed, were included. The trays were kept on a laboratory bench under natural light, ambient temperatures and were regularly watered. The seeds were censused every 2 or 3 days for 1 month, then every week for the following 6 weeks. The final percentage emergence (74 days after sowing) was determined per umbel family per cohort. First emergence date (from beneath the soil) and age in days of the seedling when the first leaf appeared were recorded and analysed for nine germinants per cohort (pooled across families).

*Statistical analyses*

All statistical tests were set with the probability of Type I error at $\alpha = 0.05$. Bonferroni tests (Data Desk 6.1) were used to determine differences post-hoc. Correlations were calculated by Pearson product–moment correlation. Means and standard errors are reported.

## Results

### *Floral, within-umbel and within-plant phenology and nectar availability*

Floral, within-umbel and within-plant phenology were similar for all monitored plants and results for one representative umbel and plant are presented. Flowers are protandrous with the male and female phases separated by a quiescent, non-sexual phase. During the male phase, anthers dehisce sequentially within the flower, while the styles remain curled and the stigmas non-receptive. Once the anthers are spent, the stamens fall off and the flower enters the quiescent phase, which varies in length depending on the position of the flower within the umbel (Figs 1, 2). The female phase begins once all flowers in the umbel have entered the quiescent phase and lasts about 4 days (Figs 1, 2). During this time, the styles are elongated and upright and the stigmas are bulbous and wet.

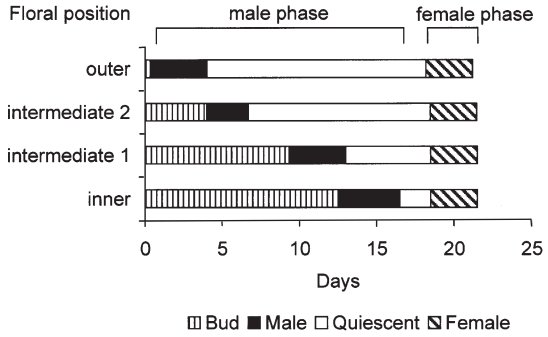


**Fig. 1.** Floral phenology within a representative tertiary umbel. Outer refers to outermost quarter ring of flowers within an umbel. Intermediate 2 refers to the next inner-quarter ring of flowers within the umbel and so on. Male and female phases refer to the functional phase of the umbel as a whole.

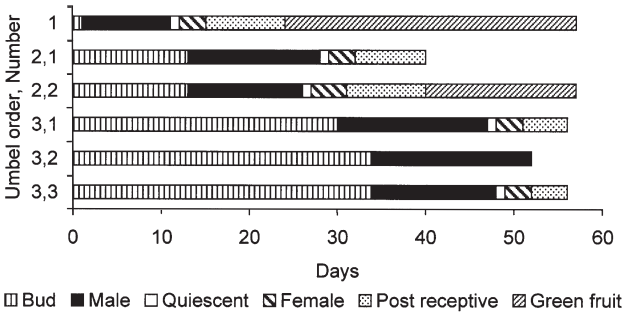


**Fig. 2.** Umbel phenology within one reproductive glasshouse plant during the 2000 flowering season. This plant had one inflorescence with six umbels (one primary, two secondary and three tertiary).

Within an umbel, flowers open centripetally causing pollen presentation in an umbel to be extended over approximately 16 days (Fig. 1). Male and female phases are also completely separated within an umbel. Male-phase umbels consist of pollen presenting flowers and, depending on how old the umbel is, buds and/or quiescent flowers (Fig. 3*a*). Female-phase umbels contain flowers with receptive stigmas only (Fig. 3*b*).

Flowering is nearly synchronous for umbels of the same order on a given flowering stalk. However, due to the sequential production of umbels on a floral stalk, male- and female-phase umbels from different umbel orders are often present on the same plant (Fig. 2).

In October 1998, part of Agnes Banks Nature Reserve was burnt by fire, delaying the onset of population flowering to about mid-December. The flowering season usually starts in spring (Benson and McDougall 1993). Peak flowering in that season was during March and flowering ceased at the beginning of June 1999. Population flowering in the following season returned to normal, with onset in about October 1999 and ceasing in June 2000. Peak flowering was during February.

Nectar secretion was observed during the male and female floral phases only, with no nectar observed during the quiescent phase or after female receptivity.

### *Floral architecture and reproductive effort*

There were 72.0 ± 2.0 ($n$ = 74) white flowers per umbel in 1999. There were 40.0 ± 3.8 umbels per plant, 4.0 ± 0.3 floral stalks per plant and 16.9 ± 1.3 leaves per plant ($n$ = 75) in 1999. In these plants, an average of 44.9 ± 2.3% of umbels set seeds. There were no differences among umbel orders in terms of number of flowers ($F_{2, 27}$ = 0.8468, $P$ = 0.4398), with primary, secondary and tertiary umbels bearing 70.9 ± 6.5, 81.3 ± 5.5 and 75.5 ± 4.8 flowers per umbel, respectively.

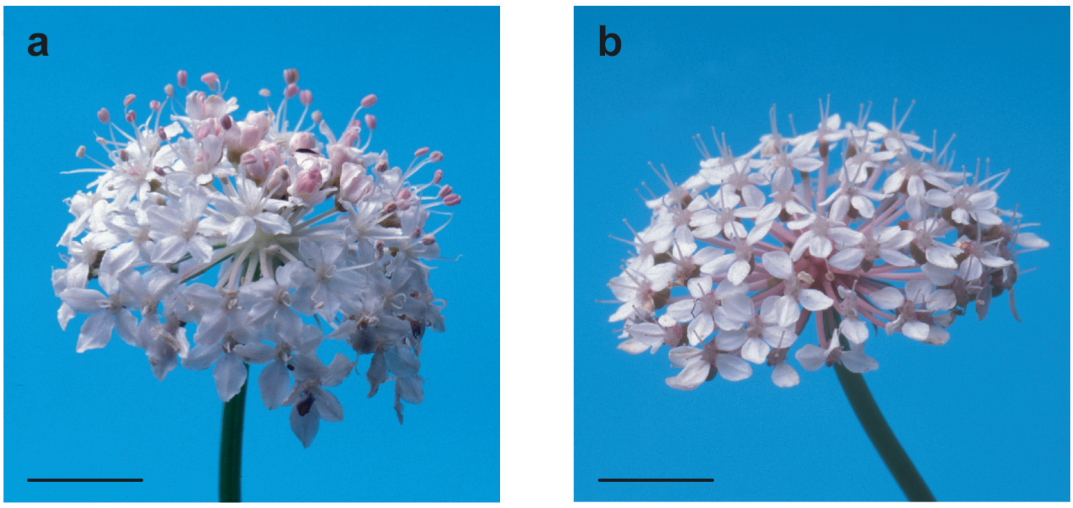


**Fig. 3.** *Trachymene incisa* subsp. *incisa* umbels from Agnes Banks Nature Reserve, flowering period summer 1999. (*a*) Male umbel with outermost flowers in the quiescent phase, intermediate flowers in the male phase and innermost flowers in bud phase; (*b*) female umbel with all flowers in female receptive phase. Scale bars = 5 mm.

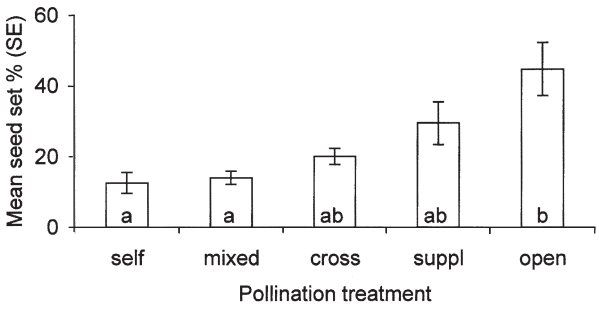


**Fig. 4.** The mean ± s.e. seed set per umbel for the following hand-pollination treatments: self-, cross-, mixed-load, supplementary (suppl) ($n$ = 8) and open pollination ($n$ = 6) of *Trachymene incisa* subsp. *incisa* umbels. Data are pooled across the two repetitions of the experiment conducted in January and February 2000. Different letters indicate significantly different values, determined by Bonferroni post-hoc tests.

**Table 1. Diversity of diurnal visitors to *Trachymene incisa* between February and May 1999**

Identification is to family level, unless a lower taxonomic level was known

| Order | Family | No. of morpho-species |
|---|---|---|
| Hymenoptera | Apidae | 1 (*Apis mellifera* L.) |
| | Colletidae | 1 |
| | Formicidae | 2 |
| | Halictidae | 1 |
| | Sphecidae | 4 |
| | Vespidae | 1 |
| Lepidoptera | Arctidae | 1 |
| | Hesperiidae | 2 |
| | Lycaenidae | 2 |
| | Oecophoridae | 1 |
| Diptera | Chloropidae | 1 |
| | Tiphiidae | 2 |
| | Sarcophagidae | 1 |
| | Asilidae | 1 |
| | Tephritidae | 1 |
| | Tipulidae | 2 |
| | Syrphidae | 1 |
| | Muscidae | 3 |
| | Therevidae | 1 |
| | Tabanidae | 1 |
| Neuroptera | Chrysopidae | 1 |
| Hemiptera | Aphididae | 1 |
| Thysanoptera | Phlaeothripidae | 1 |
| Araneae | Thomisidae | 1 |

*Insect visitors*

Invertebrates from seven orders and 24 families were observed visiting flowering umbels (Table 1). The most abundant and frequent visitor was *Apis mellifera* L., the European honeybee. This introduced honeybee was observed to collect *T. incisa* pollen, as were the less-common native bees Halictidae. Honeybees foraged on several umbels for a short period of time (about 2 s per umbel) in a foraging flight, often visiting male and female umbels from the same plant. On the basis of casual observation, it appears that *A. mellifera* is the main diurnal pollinator of *T. incisa* in this population at present. Although several invertebrates visit flowering umbels, the effectiveness of these visitors as pollinators was not assessed and the native pollinator and most effective pollinators remain unknown.

*Breeding system*

There were 760 ± 26.5 ($n$ = 20) pollen grains per anther, which converts to a pollen:ovule ratio of 1902:1. This corresponds to the facultative xenogamous breeding system in Cruden's classification (Cruden 1977).

Some umbels in the controlled pollination experiment were not pollinated properly (stigmas not receptive at time) or were damaged after pollination, reducing the design to $n$ = 4 for all treatments except open pollination, for which $n$ = 3. Variances were homogeneous at $P < 0.05$, determined by Bartlett's test for unbalanced data. There was a significant difference in percentage seed set among treatments ($F_{4, 4}$ = 11.565, $P$ = 0.0180), with no difference in time the experiment was performed ($F_{1, 28}$ = 0.103, $P$ = 0.750) and no interaction ($F_{4, 28}$ = 0.672, $P$ = 0.617). Bonferroni post-hoc tests revealed that the selfed and mixed-load treatments differed significantly from the open-pollination treatment ($P < 0.05$) (Fig. 4). Supplementary pollination did not increase seed set.

*Germination of self-, cross- and open-pollinated seeds*

There were four umbels in the self-pollinated treatment and six umbels in the cross- and open-pollinated treatments. There were no differences among treatments in terms of percentage germination ($F_{2, 13}$ = 0.234, $P$ = 0.795). The mean values (and range) of percentage germination for self-, cross- and open-pollinated umbels were 52.4 ± 14.1% (14.3–76.9%), 62.9 ± 10.1% (30.4–92.3%) and 59.2 ± 8.2% (29.4–86.7%), respectively. There were no differences in mean seed mass among the treatments ($F_{2, 13}$ = 1.408, $P$ = 0.280). The mean seed masses for self-, cross- and open-pollinated umbels were 1.23 ± 0.09 mg, 1.29 ± 0.12 mg and 1.05 ± 0.11 mg, respectively.

*Germination of seeds pollinated at different times during the flowering period*

There were no significant differences among cohorts of seeds collected throughout the dispersal period, in terms of final percentage emergence and the age of germinant at first leaf (Table 2). The percentage emergence across all 30 umbels ranged from 0 to 100%, with a mean of 63.7 ± 5.2%. The percentage emergence across the 10 cohorts ranged from 46.7 to 90%, with a mean of 63.7 ± 5.0%. The mean age of germinants at first leaf was 38.0 ± 0.7 days and ranged from 34.0 to 44.6 days across cohorts.

There was a difference among cohorts in terms of days to emergence, with a late-season cohort (27 May) taking

**Table 2. Summary table of single-factor ANOVA's on differences among 10 cohorts in terms of final percentage emergence, days taken to emerge and the age at first leaf**

Pearson product–moment correlation coefficients ($R$) for final percentage emergence, days taken to emerge and age at first leaf, correlated with mean seed mass

| Source | ANOVA | | | Correlation with |
|---|---|---|---|---|
| | d.f. | $F$-value | $P$-value | seed mass $R$ |
| Final emergence (%) | 9, 20 | 0.8735 | 0.5633 | 0.519 |
| Days to emergence | 9, 80 | 4.4115 | 0.0001 | 0.726 |
| Age at first leaf | 9, 80 | 1.7201 | 0.0979 | –0.839 |

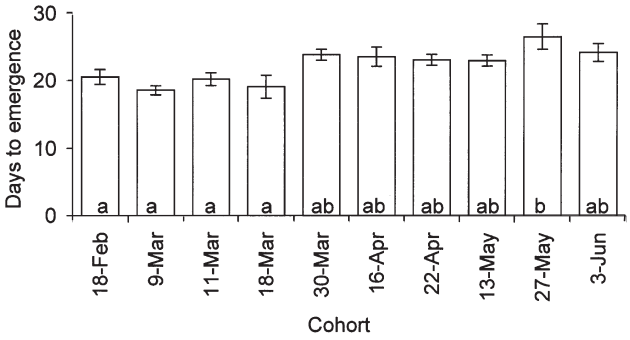


**Fig. 5.** The mean ± s.e. days taken to emerge for 10 cohorts (nine germinants per cohort) of seeds matured and collected during 1999. Different letters indicate significantly different values, determined by Bonferroni post-hoc tests.

significantly longer to emerge (6–8 days longer) than early season cohorts (Table 2, Fig. 5). Final percentage emergence and days to emergence were positively correlated with mean seed mass and the age at first leaf was negatively correlated with seed mass (Table 2).

## Discussion

The separation of male and female phases, through protandry, is complete both within flowers and within umbels, preventing mechanical self-pollination. However, despite the sequential expression of gender on a single floral stalk there is substantial overlap of at least 3 days in male and female phases among umbels of consecutive orders. Plants average four floral stalks over the 6-month flowering period, some of which flower at the same time but are often not synchronised across the stalks in gender expression. This unavoidable overlap of gender within a plant means that geitonogamous pollination is possible. For *T. incisa*, it is the architecture of the flowering stalks combined with the phenology of gender expression that influences the potential for geitonogamy, rather than the absolute size of the floral display as noted in other studies (de Jong *et al.* 1993; Harder and Barrett 1996; Snow *et al.* 1996). Therefore, quantifying the level of geitonogamous pollination in natural populations would be worthwhile, particularly because the most abundant diurnal visitor, *Apis mellifera*, was observed to visit male and female umbels on the same plant in the same foraging bout.

Protandry may also serve to prolong pollen presentation, avoid pollen–stigma interaction and optimally position pollen for dispatch and reception (Charlesworth and Charlesworth 1987; Imbert and Richards 1993; Snow and Grove 1995). Our laboratory results show that there is a long phase of pollen presentation within an umbel (16 days) compared with the average of 4 days of female receptivity. This is consistent with field observations of umbels that took 20 days from bud to the female receptive and/or post-receptive phase (Y. C. Davila, pers. obs.). This pattern is also observed in several North American Apiaceae; for example, in *Pastinaca sativa* the primary umbels are male for 6 days then female for about 2 days and the secondary umbels are male for 7 days then female for 2 days (Cruden and Hermann-Parker 1977). This extended male phase may be related to pollinator reward (Webb 1981; Koul *et al.* 1993), because at least two insect visitors to *T. incisa* were pollen-collecting bees. That umbels are not pollen limited, coupled with the low percentage of umbels setting seeds per plant and of ovules developing into seeds, consequently means that many flowers are functioning as pollen donors only.

*Trachymene incisa* plants have the potential to produce large numbers of small seeds. There are on average 40 umbels per plant, of which 44.9% set seed; there are 72 flowers per umbel and 2 ovules per flower, with mean natural seed set per umbel of 44.6% (Fig. 4). This equals a total seed production of approximately 1153 seeds per plant. The high degree of floral uniformity among umbels in *T. incisa* is characteristic of the Apiaceae (Webb 1981). It should be noted that these results are from a flowering season directly following fire, where there were few other flowering species and the relative density of plants and the size of plants were greater than prior to fire and in areas not burnt (G. M. Wardle, pers. obs.).

Although the high pollen:ovule ratio indicates a facultatively outcrossing breeding system and the high visitation rates by *Apis mellifera* and the non-significant difference in seed set between open-pollinated and supplemented umbels indicate that pollen is not limited in this population, the potential for geitonogamous pollination means that it is worth considering the relative success of self- *v.* cross-pollen in terms of seed set, percentage germination and mean seed mass. Self-pollination resulted in viable seed set, which is consistent with previous reports of self-compatibility in the Apiaceae (Bell 1971; Keighery 1982; Lindsey 1982). However, our hand-pollination experiment showed that self-pollen led to lower seed set than cross-, open- and supplemental-pollen applications. This implies that self-pollen is less effective at fertilising ovules, perhaps through late-acting self-incompatibility, or that the plant is selectively aborting selfed seeds because they are unfavourable (Lee 1988; Mahy and Jacquemart 1999). To

determine which mechanism is operating requires observations of pollen-tube growth, ovule fertilisation and seed development (Mahy and Jacquemart 1999). Also, umbels pollinated with a mixed load had intermediate percentage seed set, between self- and cross-pollen applications. This is further indication of less-effective fertilisation and seed production by self-pollen.

Self-pollinated seeds may be smaller (e.g. Mandujano *et al.* 1996) or have lower percentage germination (e.g. Mandujano *et al.* 1996; Ramsey and Vaughton 1996; Donohoe 1998) than outcrossed seeds, due to inbreeding depression (Charlesworth and Charlesworth 1987; Willis 1993). There were, however, no differences in mean seed mass (*c.* 1.0–1.3 mg) or percentage germination (*c.* 50–60%) between self- and cross-pollinated seeds. There was a trend for lower percentage germination for self- compared with outcrossed seeds, but this was not statistically significant. Estimates of selfing rates from a range of population sizes and over multiple years would be useful for determining the relative importance of selfing in this species.

*Trachymene incisa* has a lengthy flowering period, which means that seeds are being matured on plants from the end of summer into early winter. There is, therefore, the potential for the proximate environmental conditions during seed maturation to affect seed mass, seed provisioning or to alter germination cues (Kearns and Inouye 1993; Nishikawa 1998). We detected no significant differences among seeds from different flowering cohorts in mean seed mass or in final percentage emergence. However, there were differences between early maturing cohorts and a later-maturing cohort in days to emergence. These timing differences can lead to seedlings experiencing vastly different selective environments (Kalisz 1986) and to selection on time of emergence (Kelly 1992; Stratton 1992; Wardle 1995). Experiments using early and late-germinating cohorts of *Lesquerella fendleri* (A.Gray) S.Wats, a short-lived perennial desert mustard, have demonstrated that timing of germination and germination environment can also affect the genetic structure of emerging plant populations (Cabin *et al.* 1997). Cohorts of *T. incisa* with larger seeds tended to have higher final percentage emergence and to develop their first leaf earlier. However, they were slightly delayed in their time to emergence compared with smaller seeds. Selection for increased seed size was strong in most populations of *Prunella vulgaris* L. (Winn 1988); however, a review of studies that have investigated the effect of seed size on germination revealed that larger seeds may have higher percentage germination (27 species), lower percentage germination (11 species), or germination may be independent of seed size (7 species) (Baskin and Baskin 1998). In a study of parsnip, *Pastinaca sativa*, the mean emergence time, spread of emergence and the variability of seedling weight were more closely related to embryo length than to seed mass. The differences were attributed to differences in maturity of later-produced seeds on the higher umbel orders (Gray and Steckel 1985). Field studies on the effects of seed size and emergence times on fitness, and variation in the selective environment, would complement studies to determine the significance of a mixed mating system in *T. incisa*.

This study has provided some much-needed information on the reproductive ecology of *T. incisa*. It has proven an interesting breeding system, with the interaction among floral phenology, floral architecture and pollinator behaviour allowing geitonogamy, despite strong protandry within umbels. The plant is self-compatible but exhibited reduced seed set in self-pollinated umbels, suggesting that progeny should be screened at later life stages for any fitness differences resulting from inbreeding depression. The identity of pollinators among the native visitors remains unclear and the only previous record is of *Exoneura*, a species of social bee, on an unidentified *Trachymene* species in Lane Cove, Sydney (probably *T. incisa*) (Rayment 1951). While *T. incisa* is visited by a number of insect species, it has been shown in other species of Apiaceae that not all visitors are equally effective as pollinators (e.g. Lindsey 1984). It would be interesting to see how changes in pollinator guild, such as the introduction of the European honeybee, have altered pollination, seed set and gene flow in different populations of *T. incisa*.

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