

Competition-dependent incompatibility in *Phormium tenax*: does self-fertilisation provide reproductive assurance?

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Abstract New Zealand flax (*Phormium tenax*) has a reproductive system in which the early abscission of selfed flowers is determined by the degree of competition with crossed fruits on the same plant. It has been suggested that this functions as a form of reproductive assurance, allowing for high levels of outcrossing when pollinators are abundant, while ensuring seedset when pollinators are scarce. We performed experimental crosses and mating system estimation in natural populations to determine whether *P. tenax* can set seed in the absence of pollinators, and how pure and mixed pollen loads influence reproductive success and population-level selfing rates. *Phormium tenax* can set seed autonomously, although if other flowers on the plant are available for outcrossing, resources are preferentially allocated to maturing those fruits. Experimental crosses suggest that flowers pollinated with mixtures of selfed and outcrossed pollen can result in fruitset, although fruitset is reduced if flowers pollinated with mixed pollen loads compete with cross-pollinated flowers. Despite the potential for geitonogamy in *P. tenax*, outcrossing rates from five populations showed that these populations were universally outcrossing ($t = 0.83$ – 1.06). In *P. tenax*, self-compatibility in the absence of competition from

outcrossed pollen can provide some reproductive assurance, but in large populations, selfing and mixed mating rarely occur.

Keywords cryptic incompatibility; inbreeding depression; *Phormium tenax*; reproductive assurance; outcrossing rates

INTRODUCTION

Population-level estimates of outcrossing in natural plant populations have shown that more than half of animal-pollinated species produce a mixture of selfed and outcrossed progeny within a season (Barrett & Eckert 1990; Vogler & Kalisz 2001). In most cases mixed mating is likely a non-adaptive strategy that represents the inevitable consequences of large floral displays to attract pollinators or of population structure (Eckert 2000; Herlihy & Eckert 2004). For example, studies have suggested that pollination among flowers on an individual plant (geitonogamy) can be considerable, especially in clonal plants with large displays (Eckert 2000). In addition, mating between close relatives within populations (biparental inbreeding) will increase estimates of selfing (Griffin & Eckert 2003; Herlihy & Eckert 2004). However, adaptive strategies can result in mixed mating through the simultaneous selection of the two opposing targets of high seedset and frequent outcrossing. These “best of both worlds” strategies promote outcrossing when pollinators are abundant, but also are a form of reproductive assurance to provide some seedset when pollinators or mates are scarce (Darwin 1877; Müller 1883; Kalisz & Vogler 2003). Selection for these strategies will occur if the benefits of seedset, less the cost of inbreeding depression, are greater than the resources invested in mating (Lloyd 1980; Schoen & Brown 1991; Herlihy & Eckert 2002). Examples of strategies that can result in mixed mating include cryptic self-incompatibility (Bateman 1956; Weller & Ornduff 1977; Bertin et al. 1989; Becerra & Lloyd 1992), delayed selfing (Lloyd 1992; Kalisz et al. 1999),

and forms of herkogamy and dichogamy (Kalisz et al. 1999).

The deleterious effects of selfing or mixed mating may be mitigated to some degree by the preferential allocation of resources to higher quality offspring, especially in species that regularly produce more flowers than fruit (Charlesworth 1989). The selection of preferential offspring can occur post-zygotically through early-acting inbreeding depression in which selfed ovules are aborted (Charlesworth & Charlesworth 1990; Husband & Schemske 1996), or pre-zygotically through a genetically based recognition system either at the stigmatic surface, or in the style, or in the ovary (de Nettancourt 1977; Levin 1996). In many of these cases, the self-incompatibility is "cryptic" in that there is equal success of self- and cross-pollinations performed on different flowers, but self-pollen is less successful in competition with cross-pollen on the same stigma (Bateman 1956; Weller & Ornduff 1977; Bowman 1987). Cryptic self-incompatibility can occur though differential pollen tube germination, slower pollen tube growth rates of self-pollen and/or pollen tube attrition (Eckert & Allen 1997). In this way, more outcross pollen tubes are likely to fertilise the ovules in a flower, resulting in a higher proportion of outcrossed seeds.

While cryptic self-incompatibility usually occurs within a flower, the preferential allocation of resources to outcrossed ovules can also occur between flowers on a plant. For example, Becerra & Lloyd (1992) demonstrated that while selfed and outcrossed flowers of *Phormium tenax* did not differ in seedset, the success of selfed fruits was lower if there was competition with crossed fruits already present on the plant ("competition-dependent abscission of selfed flowers"). Becerra & Lloyd (1992) suggested that closer proximity of flowers resulted in higher competition for resources. This competition-dependent abscission of the flower may represent a strategy that results in mixed mating, in that from year to year or plant to plant there will be variation in competition between selfed and outcrossed flowers. For whole-flower incompatibility to be a form of reproductive assurance, in years of poor pollinator visitation, seedset or fruitset must be increased by selfing. Furthermore, the costs of selfing through inbreeding depression, and ovules or pollen available for outcrossing, must not be pre-empted by selfing (see Herlihy & Eckert 2002).

While whole-flower incompatibility has been demonstrated to preferentially mature outcrossed fruits over selfed fruits, the effect of mixed pollinations is not known. Pollinator movements within

large floral displays often result in a mixture of both self and outcross pollen deposited onto a stigmatic surface (Waser & Price 1983; Thomson et al. 1986; Thomson & Thomson 1989). In *P. tenax*, pollinator behaviour may result in geitonogamous pollen deposition. Observations by Craig (1989a) showed that the vast majority of movements by pollinating birds (tui, *Prothemodera novaeseelandiae*) were between flowers on the same plant. After the first flower visited on a plant, pollen loads on subsequent flowers will inevitably be mixed, and so geitonogamous pollen deposition will result. This may have several possible outcomes: the amount of mixed mating in a population may increase, there may be a reduction in fruit or seed set in fruits that are fertilised by too many self-pollen tubes (e.g., through stigmatic clogging, and dilution of compatible pollen), or there may be preferential maturation of outcrossed seeds or fruits, either by differential rates of pollen tube growth or the preferential abortion of selfed ovules (for a review of the effects of geitonogamous pollination see Snow (1982)).

We were interested in examining whether competition-dependent flower abscission can act as a form of reproductive assurance in *P. tenax*, and how mixed pollen loads influence reproductive success and, subsequently, the mating system. We specifically addressed three questions: (1) can self-fertilisation occur in *P. tenax* when pollinators are absent? (2) do pollinations with mixes of self and outcrossed pollen within a flower result in flower abscission? (3) what are the levels of cross- versus self-fertilisation in natural populations of *P. tenax*?

MATERIALS AND METHODS

Non-pollinator mediated fruitset

We performed pollinator-exclusion experiments in a natural population of *P. tenax* at Taupo Swamp, north of Wellington, New Zealand (41°05'S, 174°52'E). *Phormium tenax* produces tall (up to 3 m high) paniculate inflorescences that consist of a main branch that supports 8–15 secondary peduncles (Becerra & Lloyd 1992). To test whether *P. tenax* is able to self autonomously, in December 2003 we bagged one secondary peduncle on each of 10 separate plants. Plants were selected haphazardly at the edge of the large population. Bags were held away from the flowers with either a small cone of plastic transparency film or wire to prevent bags rubbing the stigmas (see Craig & Stewart 1988). Fruits were

collected 4 weeks later, and we recorded whether a pod was formed (fruitset) and, if so, the number of seeds present in each pod (seedset). Pods include large viable and small inviable seeds. We tallied both seed types to estimate total ovule number. In addition, fruitset and seedset on an unbagged, randomly chosen secondary peduncle on each treatment plant were recorded to assess the effect of bagging, and fruitset on a similarly positioned secondary peduncle on each of 10 neighbouring plants was recorded to assess natural levels of fruitset.

Reproductive assurance predicts fruitset in times when outcrossed pollen is rare. To test for this, in December 2004 we selected 25 distinctly separate ramets (presumably entire genets) of *P. tenax* at Taupo Swamp and bagged one peduncle. For 20 of these plants, all other flowers and inflorescences on the ramet were removed to simulate no pollinator visitation. After 4 weeks we tallied fruitset. We compared this with fruitset in 20 randomly chosen open-pollinated inflorescences.

Pollen source and flower abscission

Between 10 December 2003 and 10 January 2004, we performed controlled pollinations in *P. tenax* at Taupo Swamp to determine the effect of competition between flowers for resources on fruit abscission and seedset. To assess whether competition-dependent flower abscission occurs in this population, one of three competition treatments was applied to all flowers on a secondary peduncle on each plant: (1) all flowers were self-pollinated (no competition), (2) all flowers were cross-pollinated (no competition), and (3) selfed and outcrossed pollinations were applied to separate flowers on the same peduncle (competition). Only one competition treatment was applied to a plant, and each treatment was performed on eight replicate plants (giving a total of 24 plants). Pollinations were performed on all receptive flowers available on a peduncle each day (generally 1–3), and on all flowers on each peduncle receptive over the experimental period (mean = 14 flowers per peduncle, range = 5–36, $N = 24$ peduncles).

In a separate experiment, we examined the effect of a mixture of self and crossed pollen applied to the same stigma (mixed pollination) on competition-dependent flower abscission. Two competition treatments were applied to flowers on a secondary peduncle: (1) self-pollination and mixed pollination, and (2) mixed pollination and cross-pollination. Twelve replicates of each treatment were used; each treatment consisted of pollination of all receptive flowers on one secondary peduncle on a plant (mean

= 15 flowers per peduncle, range = 8–59; $N = 22$ plants). Data from two plants were not collected due to weather damage.

For both experiments, before pollination, we bagged a secondary peduncle on each plant with fine curtain mesh to exclude pollinators and emasculated flowers prior to anther dehiscence. Bags were held away from the flowers using a small cone of plastic transparency film at the base of the peduncle. Cross-pollinations used 3–5 pollen donors for each flower that was pollinated. Anthers were collected from pollen donors at least 100 m from the experimental plants. For mixed pollinations, pollen was removed from three anthers collected from the sample plant and from one anther each from three plants at least 100 m away and mixed in an Eppendorf tube with a toothpick. The mixed pollen was then applied to the stigma with the toothpick until pollen could be seen with the naked eye. Stigmas were receptive 2–3 days after anther dehiscence (Craig & Stewart 1988). Levels in a treatment (selfed, mixed, or outcrossed pollen) were applied to a stigma at random as stigmas became receptive. We did not explicitly examine the effect of the two treatments on pairs of flowers within each peduncle (cf. Becerra & Lloyd 1992), as on any day between 1 and 5 flowers could become receptive and so occasionally only one treatment was applied on a day. Following pollination, flowers were rebagged for a 3–5 day period, although it was usually possible to assess fruitset 24–48 h after pollination. After 4 weeks, fruitset, seedset, and total ovule number was tallied.

We analysed data for fruitset using logistic general linear models (GLM) (Venables & Ripley 1994), coding successes as the number of fruit that set seed and failures as those that did not. Significance was tested against a chi-square distribution. For seedset we used general linear models with a Gaussian link function, tested against an F distribution. Data did not require transformation. All analyses were conducted using *R* (Ihaka & Gentleman 1996).

Inbreeding depression

We planted seeds from outcrossed, selfed, and open pollinated pods to determine the levels of inbreeding depression in *P. tenax*. Ten seeds from each of 10 plants of each treatment were sown in separate pots in propagating sand and kept in a greenhouse. Watering occurred for 10 min every 2 days so that the soil was generally moist. After 2 months, the number of seedlings that germinated was recorded and the length of each longest leaf was measured as a surrogate for plant height. Germination was

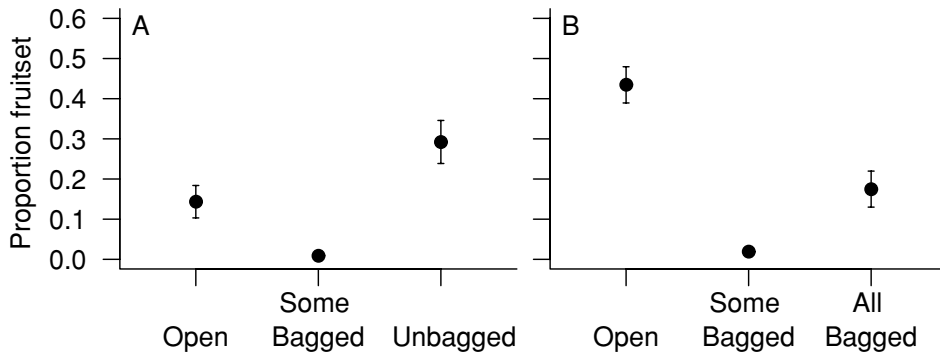


Fig. 1 The effect of experimental treatments on fruitset of other peduncles on the same plant. **A**, In 2004, fruitset from open-pollinated flowers (Open) was compared with fruitset from flowers where the peduncle was bagged to exclude pollinators (Some Bagged) and fruitset from flowers on another unbagged peduncle on the same inflorescence (Unbagged). **B**, In 2005, fruitset from open-pollinated plants (Open) was compared with fruitset from flowers where peduncles were bagged to exclude pollinators (Some Bagged) and fruitset from flowers in which the peduncle had been bagged and all other flowers on the ramet were removed (All Bagged). Values are mean \pm 1 SE.

analysed using logistic general linear models using the number of seeds germinated in each pot as successes and seeds not germinated as failures, while leaf length was analysed using one-way analysis of variance.

Mating system estimation

To determine multilocus outcrossing rates in *P. tenax*, we performed horizontal starch gel electrophoresis on open-pollinated seeds from the five populations across New Zealand. In February 2000, *Phormium* maternal seed families were collected from five populations throughout New Zealand, three in the South Island and two in the North Island. Lake Coleridge (43°20'S 171°42'E) is located in the mountains of the South Island (600–900 m above sea level), Rapahoe and Stillwater populations (39°25'S, 175°25'E, and 42°29'S 171°14'E, respectively) were located on coastal dunes in the South Island (12 and 52 m above sea level, respectively), while in the North Island, Taupo Swamp (42°29'S 171°22'E) was a coastal population (150 m above sea level) and Ohakune (41°05'S, 174°52'E) was alpine (300–600 m above sea level). One seed pod per plant was collected from between 9 and 24 parent plants in each population. Maternal families were haphazardly sampled within each population; however, to ensure as many genets as possible were sampled, pods were collected from obviously separate ramets.

We scored 9–24 families sampled from each population for allozyme variability. Allozyme activity could not be reliably resolved using seeds, so samples were taken from extracted leaf material.

Eighteen enzyme systems were originally screened for polymorphism following the methods of Glover & Barrett (1987). Two polymorphic enzyme systems (Pgi, Pgm) were reliably resolved on a histidine-citrate buffer system (pH = 6.2). Ritland's MLTR program (Ritland 1990) was used to estimate multilocus outcrossing rate (t_m) for each population. Standard errors were calculated as the standard deviation of 1000 bootstraps with the family as the unit of resampling. Differences in outcrossing rate between populations were assessed using pairwise comparisons of bootstrap estimates (see Eckert & Barrett 1994). Two populations were considered to differ significantly in outcrossing rate if 100(1- α PC/2) of the differences between randomly paired bootstrap estimates lay either all above or all below zero (where α PC is the Type-I error rate). The experimental error rate (α EW) was held at α EW = 0.05 using Sidak's correction for non-orthogonal contrasts.

RESULTS

Autonomous fruitset

Autonomous seedset in *P. tenax* was dependent on the levels of competition from outcrossed flowers on the plant. For treatments where a peduncle was bagged but all other flowers on the inflorescence were left unbagged, only 2 of the 146 flowers set fruit. In this situation, open-pollinated flowers on the same plants had significantly higher proportions of fruitset than open-pollinated flowers on plants not

Fig. 2 The effect of experimental pollinations and resource competition in *Phormium tenax*. **A**, Proportion fruitset of flowers on a peduncle where all flowers were either exclusively selfed or outcrossed (no competition). **B**, Proportion fruitset where selfed and outcrossed pollinations were applied to separate flowers on the same peduncle (competition). **C**, Mean seedset where all flowers on a peduncle were given the same pollination treatment (either exclusively selfed or exclusively outcrossed; no competition). **D**, Mean seedset where selfed and outcrossed treatments were applied to separate flowers on the same peduncle (competition). Values are mean \pm 1 SE.

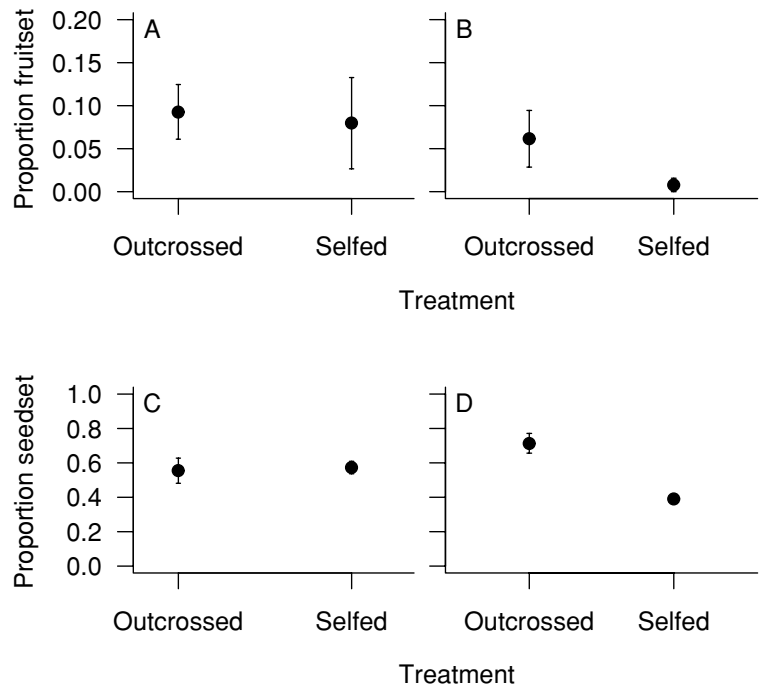


Table 1 Logistic general linear model of fruitset in *Phormium tenax*. A peduncle was either subjected to a no-competition treatment where all flowers received pollen from the same source (either entirely selfed or entirely outcrossed), or subjected to a competition treatment where some flowers on the peduncle were pollinated with self-pollen while other flowers were outcrossed. *P* (chi) is the probability tested against a chi-square distribution.

Source	d.f.	Deviance	<i>P</i> (chi)
Selfed versus outcrossed	1	1.56	0.21
Competition	1	2.40	0.12
Competition \times treatment	1	4.13	0.042
Residuals	21	40.82	

subjected to a bagging treatment (Fig. 1; Logistic GLM of fruitset: d.f. = 1, Deviance = 149.05, $P < 0.001$, $N = 19$). Removing all other flowers and inflorescences on a ramet increased fruitset in bagged flowers compared with plants where flowers were bagged but other open-pollinated flowers were left on the plant (Fig. 1). However, this fruitset was still significantly lower than open-pollinated fruitset (Logistic GLM of treatment on proportion fruitset: d.f. = 2, Deviance = 230.62, $P < 0.01$, $N = 20$). On average, proportional fruitset in bagged flowers with no competition from other flowers on the plant was 61% lower than open-pollinated plants, despite vast

differences in total flower number between the two treatments (unmanipulated flax plants produce an average of 209 flowers on an inflorescence, range = 132–507, $N = 10$ plants), whereas our treatments reduced total flower number to an average of 37 flowers (range = 16–60, $N = 20$ plants).

Compatibility relationships

Comparison of fruitset when all flowers on a peduncle were entirely selfed or outcrossed revealed no significant differences in fruitset or seedset (Fig. 2; Table 1). In contrast, when selfed and outcrossed pollinations were applied to separate flowers on

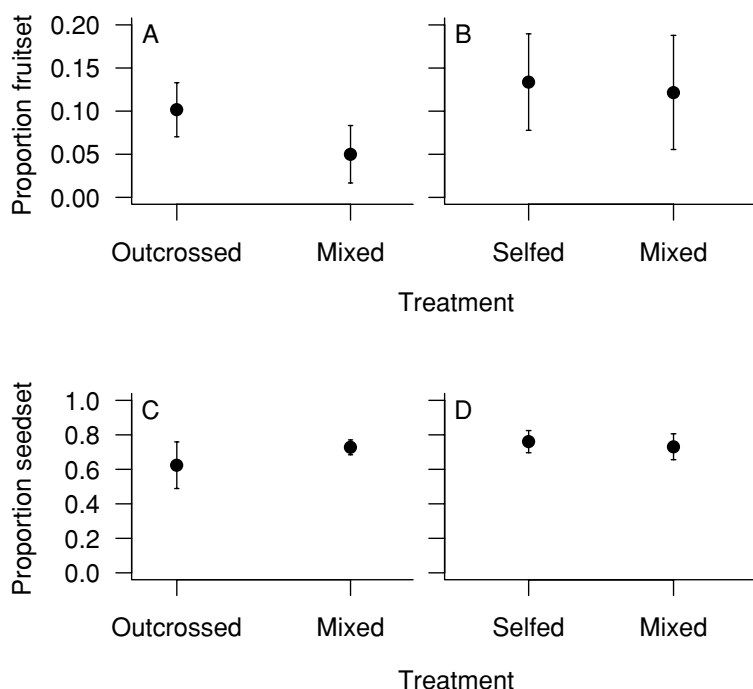


Fig. 3 The influence of pollen source on competition-dependent fruitset and seedset. **A**, Proportion fruitset on a peduncle where mixed pollinations and outcrossed pollinations were performed on separate flowers on the same peduncle. **B**, Proportion fruitset where mixed pollinations and selfed pollinations were performed on separate flowers on the same peduncle. **C**, Proportion seedset where mixed pollinations and outcrossed pollinations were performed on separate flowers located on the same peduncle. **D**, Proportion seedset where mixed pollinations and selfed pollinations were performed on separate flowers on the same peduncle. Values are mean \pm 1 SE.

Table 2 General linear models of the effect of mixed pollinations on fruitset and seedset in *Phormium tenax*. Peduncle treatment is either outcrossed and mixed treatments or selfed and mixed treatments applied to a peduncle; flower treatment is selfed, mixed, or outcrossed pollen applied to a flower. The probability is tested against a chi-square distribution for fruitset and an *F* distribution for seedset.

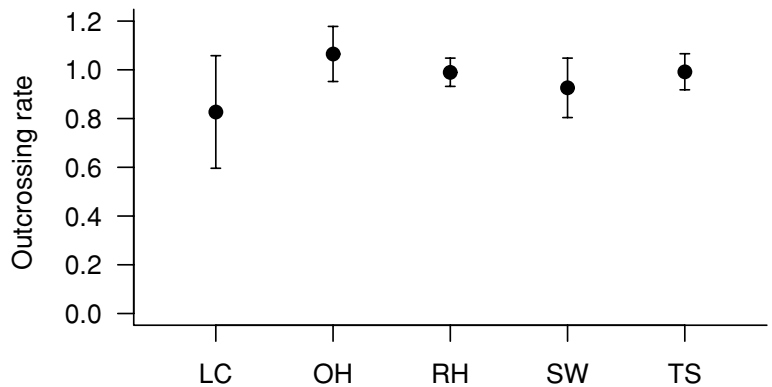
Source	d.f.	Deviance	<i>P</i> (chi or <i>F</i>)
Fruitset			
Peduncle treatment	1	1.187	0.27
Flower treatment nested within peduncle	5	20.83	0.001
Residuals	40	130.03	
Seedset			
Peduncle treatment	1	0.024	0.40
Flower treatment nested within peduncle	5	0.020	0.73
Residuals	11	0.3463	

the same peduncle, there was a reduction in fruitset in selfed pollinations (Fig. 2). This resulted in a significant competition \times treatment interaction (Table 1). As only one selfed fruit set seed, we did not statistically analyse the effect of competition on seedset; however, there was no difference in seedset between fruits from peduncles with entirely

outcrossed flowers and to those with entirely selfed flowers (General Linear Model: Treatment Deviance = 0.03, d.f. = 1, *P* = 0.23, *N* = 12).

Mixed pollinations exhibited moderate competition-dependent effects. Flowers pollinated with both self and outcross pollen set 50% fewer fruit when competing with neighbouring flowers pollinated

Fig. 4 Multilocus outcrossing rates (t_m) in five populations of *Phormium tenax*. LC, Lake Coleridge; OH, Ohakune; RH, Rapahoe; SW, Stillwater; TS, Taupo Swamp. Values are mean \pm 1 SE.



with only outcrossed pollen. Mixed-pollinated flowers did not significantly differ in fruitset from competing flowers that had been self-pollinated (Table 2; Fig. 3). There was no significant effect of treatment on seedset.

Inbreeding depression

Germination of selfed seeds was significantly less than germination of open-pollinated seeds and seeds from outcrossed experimental pollinations (mean % germination \pm 1 SE: selfed = 3.0 ± 1.5 ; outcrossed = 9.0 ± 4.8 ; open pollinated = 20.0 ± 7.3 ; logistic regression: $P = 0.0002$, d.f. = 2, $N = 30$). Germination of outcrossed seeds was not significantly different from open-pollinated seeds. There was also no significant difference between the heights of seedlings for any treatment (mean height (mm) \pm 1 SE: selfed = 49.6 ± 7.9 ; outcrossed = 29.8 ± 4.9 ; open pollinated = 44.5 ± 4.2 ; $F_{2,9} = 3.6$, $P = 0.08$).

Mating system estimation

The mating system of germinated seedlings of *P. tenax* is predominantly outcrossing. Multilocus outcrossing rates among the five populations ranged from $t_m = 1.065$ at Ohakune to $t_m = 0.827$ at Lake Coleridge (Fig. 4). There was no significant difference between estimates (e.g., proportion of pairwise differences between randomly paired bootstrap estimates lying above zero for $t_{\text{ohakune}} - t_{\text{lake coleridge}} = 0.081$).

DISCUSSION

Forms of cryptic self-incompatibility have been considered to be a “best of both worlds” strategy where variation in the expression of self-incompatibility may function as a mechanism that optimises

offspring number and quality in uncertain pollination environments. While selfing can occur from both within-flower and between-flower pollinations, only autonomous pollinations will result in reproductive assurance in the absence of pollinators. In this study, we investigated the potential for autonomous selfing and mixed pollinations (as can occur in geitonogamous transfer) to result in fruitset in *P. tenax*, and relate this to the mating system of germinated seedlings.

Can self-fertilisation occur in *Phormium tenax* when pollinators are absent?

Tests of the reproductive assurance hypothesis require a demonstration that selfing increases fruitset or seedset when pollinators or mates are scarce, and that the costs of selfing (e.g., inbreeding depression and pollen and seed discounting) are not greater than the fitness benefits of selfing in occasional years of low availability of outcross pollen. Bagging flowers and removing all other flowers on a ramet of *P. tenax* resulted in fruitset, suggesting that *P. tenax* can set seed in the absence of pollinators. However, plants with bagged secondary peduncles increased fruitset in peduncles elsewhere on the inflorescence. This provides evidence that resources can be reallocated between peduncles and, perhaps, inflorescences to provision better quality offspring.

Our experimental studies revealed significantly less germination of selfed seeds than open-pollinated seeds. Craig (1989b) showed that selfed seeds of *P. tenax* were smaller, with less endosperm than those resulting from open pollinations, which may be one explanation for less germination. Reproductive assurance can still select for autonomous selfing even if inbreeding depression is strong, provided that selfing increases seed production when the opportunity

for outcrossing is limited, and that inferior selfed ovules do not pre-empt ovules that would otherwise have been outcrossed (seed discounting) (Schoen & Brown 1991; Herlihy & Eckert 2002; Kalisz et al. 2004). Herlihy & Eckert (2002) found in *Aquilegia canadensis* that the advantages to selfing were outweighed by the loss of high quality outcross seed. This discounting of outcrossed seed is not likely to occur in *P. tenax* as the preferential retention of outcrossed fruits over mixed or selfed pollinations would reduce the prevalence of ovules fertilised by selfed pollen grains. Further, we found that open-pollinated peduncles set significantly more fruit if another peduncle on the same inflorescence was bagged, suggesting that resources for ovule maturation are not being pre-empted by selfed ovules, but are reallocated within an inflorescence towards higher quality offspring.

Do pollinations with mixes of self and outcrossed pollen within a flower result in flower abscission?

If variation in the expression of whole-flower incompatibility acts as a mechanism for reproductive assurance, then there may be selection against fruits resulting from geitonogamous pollinations as some of the ovules fertilised will be inferior, yet there is no benefit in years of low pollinator service (Lloyd 1992; Lloyd & Schoen 1992). This might predict that mixed pollen loads on a flower should result in whole-flower abscission if there is competition from outcrossed fruits. Our study demonstrated a moderate effect of mixed pollination on fruitset but not seedset. Experimental pollinations resulted in reduced fruitset when flowers pollinated with mixed pollen competed with outcross flowers, but there was no difference in fruitset when selfed flowers competed with mixed-pollinated flowers.

The discrimination between outcrossed and mixed pollen sources, but not between selfed and mixed sources, and the resulting abscission within 24–48 h of pollination, indicates that the early detection of self pollen grains or pollen tubes may play a role in the abscission of the flower. Preliminary observations of cleared stigmas of *P. tenax* pollinated with self pollen revealed that self pollen tube germination occurs readily (L.K. Jesson and R.H. Brookes unpubl. data), yet the rates of growth and fertilisation success of selfed and outcrossed pollen tubes are unknown. Experiments examining pollen-tube growth in flowers with and without competition for resources are required to investigate this further.

Many studies have demonstrated that mixed or prior self pollinations result in a reduction in seed or fruitset, suggesting that male and female functions of a flower can interfere with each other (e.g., Bertin & Sullivan 1988; Waser & Price 1991; Broyles & Wyatt 1993). This may be due to “stigmatic clogging” where selfed pollen physically prevents outcross pollen tubes from entering the style, or to biochemical interactions involved in pollen tube growth or in the incompatibility process (Snow et al. 1996; Barrett 2002). That mixed pollinations reduce fruitset in *P. tenax* suggests that this is a form of sexual interference between male and female functions of a flower (Lloyd & Webb 1986; Webb & Lloyd 1986; Barrett 2002). Self-pollen deposition within a flower will be reduced to some degree through anti-interference mechanisms including herkogamy and dichogamy (Lloyd & Webb 1986; Webb & Lloyd 1986) but these mechanisms are unlikely to reduce self-fertilisation between flowers on the same plant to the same extent (and see Craig 1989a). Stigmatic clogging can also occur from self-pollen sourced from other flowers on the same plant; thus, geitonogamous pollination can be considered a form of sexual interference (Barrett 2002). Moreover, geitonogamous pollination not only wastes ovules, it also has potential to waste male gametes that could be exported to other plants (Harder & Barrett 1996; Harder et al. 2000). In *P. tenax*, geitonogamous pollinations are likely to be a strong source of sexual interference, as whole-flower incompatibility means geitonogamous pollen deposition can result in the wastage of all ovules in a flower.

What are the levels of cross- versus self-fertilisation in natural populations of *Phormium tenax*?

Our results showed that seedlings of *P. tenax* were almost exclusively outcrossed. This may be due either to strong competition from outcrossed flowers or to the marked inbreeding depression found at the stage of seedling germination. Most inbreeding depression is evident in the first stages of selection in outcrossing species, whereas selfers frequently exhibit severe inbreeding depression at later life stages (Husband & Schemske 1996), providing further evidence that *P. tenax* is a highly outcrossed species. In the five populations used for mating system estimation, seed germination ranged from 35 to 75% (data not shown). While it is not known how much of this difference in germination was due to inbreeding depression or to environmental factors, if levels of inbreeding depression are similar for the other

populations as for the Taupo Swamp population, then the lower germination of selfed seeds would have upwardly biased the estimation of outcrossing rates in *Phormium*.

Many aspects of the life history of *P. tenax* are likely to have important influences on its mating system. *Phormium tenax* is a masting, clonal plant with water-dispersed seeds. In many clonal species, geitonogamous pollinations can result in very high levels of selfing. Eckert (2000) found in *Decodon verticillatus* that more than 80% of all selfed seed is a result of geitonogamous pollinations, yet strong inbreeding depression prevents selfed offspring from reaching reproductive maturity (Eckert & Barrett 1994). Geitonogamy is also significant in large clones of water-pollinated *Zostera marina* (Zosteraceae), likely as a result of restricted pollen dispersal (Reusch 2000). In *P. tenax*, despite considerable between-flower visitation (L.K. Jesson pers. obs.; Craig 1989a), most seedlings were highly outcrossed, suggesting that any increases in geitonogamous pollen transfer is not reflected in the seedling stage. It is possible that year-to-year variation in mast flowering influenced levels of geitonogamous pollen transfer. For example, Craig (1989a) found that pollinating birds visiting *P. tenax* in heavy flowering years visited fewer flowers on the same inflorescence than in lower flowering years. The 1999/2000 flowering year (after which seeds were collected for isozyme analysis) was very poor across the country (R. Brookie unpubl. data), which likely increased the potential for geitonogamous pollinations or intrafloral selfing, yet any selfing was not reflected at the seedling stage.

Does self-fertilisation provide reproductive assurance in *Phormium tenax*?

Phormium tenax can self autonomously when no flowers on the inflorescence are outcrossed. Despite this, the populations we studied were highly outcrossing. Thus, it seems that when in competition, partially and fully selfed fruits are selected against, and selfing rarely occurs. In these situations self-fertilisation does not assure reproduction. However, lack of evidence for reproductive assurance is not evidence that reproductive assurance does not occur at all. In small populations or habitually colonising species, outcrossed pollinations can be limited either through reduced attractiveness to pollinators or through lack of access to unrelated mates (Baker 1955; Jain 1976; Lloyd 1980). Theoretical work by Pannell & Barrett (1998) and Morgan et al. (2005) also suggests that the advantages to reproductive

assurance for a selfer are greatest when a species is uncommon across the landscape. Tisch (1996) simulated high and low mast-flowering years in *P. tenax* by comparing bird visitation between populations of differing densities and isolated plants. While isolated plants of *P. tenax* received 80% fewer visits than control plots, fruitset was only 33% less, suggesting that seedset might be increased in these isolated plants by autogamous selfing. It may be in *P. tenax* that selection for competition-dependent incompatibility occurs in small populations or in isolated individuals. Comparisons of pollinator visitation, outcrossing rates, and the effects of emasculation on fruit and seed set across populations of different sizes would further elucidate factors selecting for whole-flower incompatibility in *P. tenax*.

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