

# The functional significance of synchronous protandry in *Alstroemeria aurea*

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## Summary

1. Synchronous dichogamy, the simultaneous change in sex phases of all flowers in an inflorescence or entire plant, has been proposed to reduce self-pollination between flowers within a plant (geitonogamy) and so should leave more pollen for export and/or reduce inbreeding. We tested these hypotheses with *Alstroemeria aurea* Graham, a self-compatible, clonal herb with synchronous protandrous inflorescences.

2. We manipulated *A. aurea* inflorescences to compare pollen export, pollen receipt and pollen-tube attrition in synchronous and asynchronous inflorescences. We constructed asynchronous inflorescences by tying together female- and male-phase ramets which were collected within 1 m of each other and so had a high probability of being genetically related.

3. Inflorescences with both male- and female-phase flowers received 27% more pollen than synchronous inflorescences, which was associated with higher pollen-tube attrition. The selfing suggested by these results would reduce genetic contributions to the next generation as a result of the strong inbreeding depression found in this species.

4. In contrast, we found no evidence that asynchronous inflorescences exported less pollen to companion female-phase inflorescences than did synchronous inflorescences.

5. Our results suggest that synchronous dichogamy evolves primarily as an anti-selfing mechanism, rather than to promote outcross siring success.

*Key-words:* pollen discounting, pollen receipt, pollen removal, pollen-tube attrition, self-pollination

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## Introduction

Hermaphroditic organisms have the opportunity to function simultaneously as female and male, but many function as only one sex at any particular time. Temporal separation of the sex roles played by an individual can occur between (Clay 1993; Buston 2003) or within breeding seasons (Cruden 1988). These patterns of separation of sex roles arise for different reasons. Between-season separation allows individuals to take advantage of changes in size to function most effectively as either females or males (Charnov 1982). In contrast, separation of sex roles within seasons has been proposed to reduce the incidence of self-mating and reduce gamete wastage, particularly through pollen discounting (Lloyd & Webb 1986).

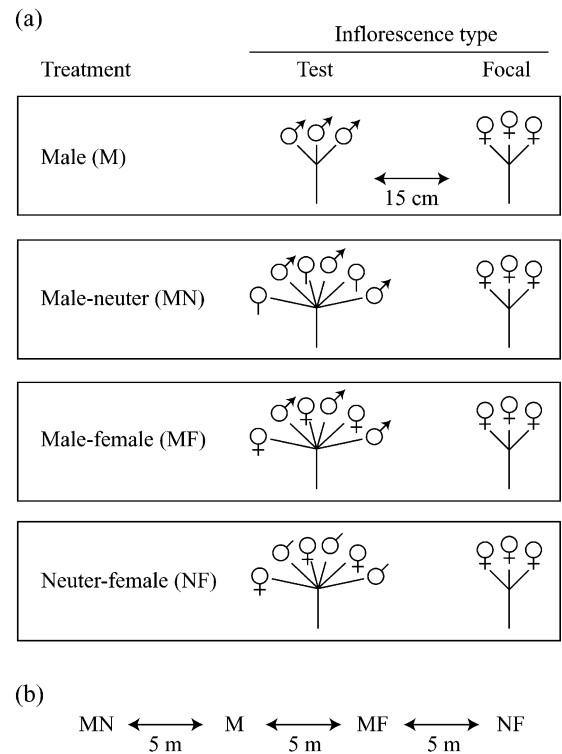
Separation of the sex roles within a reproductive season occurs widely among flowering plants, being found in species with perfect flowers in at least 17 angiosperm families (Cruden 1988). This synchronous dichogamy (Lloyd & Webb 1986) involves simultaneous

change in sexual phase among hermaphrodite flowers of the same inflorescence, ramet or plant. The most obvious consequence of synchronous dichogamy is the reduction of both within- and among-flower (geitonogamous) self-pollination (Cruden & Hermann-Parker 1977), which bears two potential advantages. Reduced self-pollination may leave more pollen to be exported to other plants (reduced pollen discounting; Harder & Barrett 1995), and reduced self-fertilization may diminish the proportion of offspring suffering from inbreeding depression (reviewed by Charlesworth & Charlesworth 1987). Despite recognition of these possible advantages of synchronous dichogamy, their occurrence and relative importance have not yet been quantified (Aizen & Basilio 1995).

In this paper we explore the consequences of synchronous dichogamy for self-pollination in *Alstroemeria aurea* Graham (Alstroemeriaceae). *Alstroemeria aurea* is a rhizomatous, herbaceous perennial of temperate forests in the south-central Andes of Argentina and Chile (Sanso 1996). Flowering *A. aurea* ramets produce a single, terminal, umbellate inflorescence, typically with two to eight large (>5 cm long), yellow-orange flowers. Inflorescences of most plants produce a single

whorl of flowers, which open synchronously and proceed through male (4–5 days), neuter ( $\approx 1$  day) and finally female phases (3–4 days) simultaneously (Aizen & Basilio 1995; Aizen 2001). Thus at any moment an entire inflorescence is either male, neuter or female, with no overlap between anther and stigma presentation. Some robust plants have more complicated, ‘multi-whorled’ inflorescences, in which the male phase of the second whorl can overlap briefly ( $\approx 1$  day) with the female phase of the first whorl. *Alstroemeria aurea* is pollinated by bumble bees (*Bombus*) and, to a lesser extent, other bees and nemestrinid flies (Aizen 2001; Aizen, Vázquez & Smith-Ramírez 2002). Bumble bees generally fly between nearest inflorescences (Souto 1999) and an inflorescence’s pollen receipt is influenced by the sex phase of its immediate neighbours (Aizen 1997). Bees deposit more pollen from a specific donor inflorescence on the first female flowers visited than on later flowers (Aizen 2001). *Alstroemeria aurea* is self-compatible; however, selfed plants set fewer fruits with smaller seeds as a result of early acting inbreeding depression (Aizen & Basilio 1995). Populations of *A. aurea* are genetically structured ( $F_{IS} \approx 0.5$ ) because of clonal growth and limited pollen and seed dispersal, so that ramets separated by 1 m are 10 times more likely to belong to the same genet than ramets separated by 100 m (Souto, Aizen & Premoli 2002). Consequently, crosses between neighbouring ramets result in higher pollen-tube attrition than crosses between distant plants (Souto *et al.* 2002).

Here we report the result of a field experiment that contrasts the pollination and post-pollination performance of typical synchronous *A. aurea* inflorescences and artificially constructed ‘asynchronous’ inflorescences. We also made supplementary observations of the behaviour of bumble bees visiting natural inflorescences. The experiment considered four treatments (Fig. 1), each of which involved a test inflorescence and a companion focal inflorescence, with three female-phase (hereafter ‘female’) flowers. The four test ‘inflorescences’ included either a single inflorescence with three male-phase (hereafter ‘male’) flowers (M), or two three-flowered inflorescences tied together to produce a six-flowered asynchronous inflorescence. These asynchronous inflorescences included: three male and three female flowers (MF); three male and three neuter-phase (hereafter ‘neuter’) flowers (MN); or three neuter and three female flowers (NF). The ramets used to produce asynchronous inflorescences were collected within 1 m of each other, and so had a high probability of being genetically related (Souto *et al.* 2002). Despite considerable genetic structure within populations, the sex phase of ramets is not spatially autocorrelated at any spatial scale (Aizen & Basilio 1995). Flowering probably becomes unsynchronized among ramets as death of the short-lived rhizomes fragments individual genets (M.A.A., unpublished data). In contrast to the inflorescences combined to form test inflorescences, test and focal inflorescences were collected from areas



**Fig. 1.** Layout of the experimental design showing (a) characteristics of paired inflorescences in M, MN, MF and NF treatments; (b) example of spatial arrangement of the four treatments in a plot. Symbols: ♀ female flowers, neutered flowers without +, ♂ male flowers, neutered flowers without arrow.

separated by 200 m, and so were unrelated. These conditions could result in three possible outcomes. First, geitonogamy should result in greater pollen receipt by female flowers in the test MF inflorescences than those in either the test NF or focal inflorescences. Second, self-pollination in MF inflorescences should reduce the pollen exported to their companion focal inflorescences (pollen discounting) compared to that seen in the M and MN treatments. Finally, because of higher attrition of self-pollen tubes (Souto *et al.* 2002), female flowers in MF inflorescences should have fewer pollen tubes at the bases of their styles than either their companion focal inflorescence, or NF inflorescences, after accounting for differences in pollen receipt.

## Materials and methods

### STUDY AREA

We studied *Alstroemeria aurea* in a mature *Nothofagus pumilio* forest in the upper Challhuaco Valley, Parque Nacional Nahuel Huapi, Argentina (41°8' S, 71°19' W). At this site intermixed clones of *A. aurea* form extensive, dense stands ( $>10$  flowering ramets  $m^{-2}$ ). During our study in 2002 the introduced bumble bee, *Bombus ruderatus*, visited *A. aurea* most abundantly, although the native *Bombus dahlbomii* also commonly visited.

## POLLINATOR BEHAVIOUR

The pollination effects of the experimental manipulations described below depend on the behaviour of pollinators while they visit *A. aurea* inflorescences. To quantify relevant components of this behaviour, we observed 49 bumble bees feeding on unmanipulated plants for a total of 152 min. We observed each bee feeding on 1–51 inflorescences for a total of 434 inflorescences. For each inflorescence we counted the number of open flowers of each sex phase, and for each sex phase we recorded the number of flowers visited, number of flowers revisited, and number of flowers that a bee bypassed while moving around the inflorescence.

## EXPERIMENTAL TREATMENTS

We assessed the consequences of synchronous protandry for pollination of *A. aurea* with four contrasting treatments: M, MN, MF and NF (Fig. 1a). Each treatment involved a pair of cut inflorescences: a three-flowered, focal female inflorescence (F) and a test inflorescence. On asynchronous test inflorescences, for which we tied together a three-flowered male and a three-flowered female inflorescence (MN, MF and NF), we intermixed the two flower types (Fig. 1a). To create neuter flowers on asynchronous inflorescences we removed either the anthers from male flowers (NF inflorescences) or the stigmas from female flowers (MN inflorescences). All inflorescences used in this experiment were collected either before anther dehiscence (male and neutered male flowers) or during the neuter phase (female and neutered female flowers). As a result, flowers with unaltered stigmas or anthers became functionally female or male, respectively, shortly after an experimental trial began and remained in that sex phase during the remaining 4 days of the trial. Inflorescences were placed in water-filled test tubes and exposed to pollination for 4 days. *Alstroemeria aurea* inflorescences remain fresh for many days after cutting, with their flowers proceeding normally through their phenology (Aizen 1997). *Alstroemeria* varieties are favoured by florists because of their resilience after cutting (Healy & Wilkins 1986).

The four different treatment pairs were arranged randomly along a transect with 15 cm between the focal and test inflorescences and 5 m between adjacent pairs (Fig. 1). We distributed 20 such transects within the forest so that transects were separated from each other and from natural stands of *A. aurea* by at least 10 m. These transects were located at least 100 m from the areas at which we collected ramets for both test and focal inflorescences. This arrangement was repeated twice (23–27 January, 28 January–1 February 2002) using the same 20 transects, resulting in 40 replicates of each treatment pair. Bumble bees and other insects were observed to visit our artificial arrangements and move frequently between test and focal companion inflorescences.

## POLLINATION SUCCESS

After an experimental trial, we collected styles and anthers from the experimental inflorescences to quantify pollination success. The entire styles from the three female flowers on each test (MF and NF) and all focal inflorescences were placed in individual microcentrifuge tubes and preserved in 70% ethanol until pollen receipt and the number of pollen tubes at the base of the style could be quantified in the laboratory. We similarly preserved all dehiscent anthers from the three male flowers on each M, MF and MN test inflorescence in individual microcentrifuge tubes for later counting of the number of remaining pollen grains. Finally, we collected one undehiscent anther from each male flower on each M, MF and MN test inflorescence and placed them in an empty microcentrifuge tube. After these anthers dehiscent in the open tube, we preserved them in 70% ethanol until the pollen could be counted as a measure of pollen production.

We assessed both pollen production by undehiscent anthers and the pollen remaining in anthers exposed to pollinators with a Micromeritics Elzone 5380 electronic particle analyser. Before analysis the microcentrifuge tubes with preserved pollen were sonicated in an ultrasonic water bath for 10 min to dislodge the pollen from the anthers. The sample in a tube was then washed into a counting vessel with 0.63% NaCl solution and the anthers were removed and counted. Enough NaCl solution was then added to the sample to increase the total volume to 25 ml. Three subsamples were drawn from each mixed sample, and the included pollen was counted with the particle analyser as described by Harder (1990). To estimate pollen production by the anthers that opened during the experiment, we multiplied the pollen count for the undehiscent anthers by  $A_R/A_P$ , where  $A_P$  and  $A_R$  are the numbers of undehiscent anthers and anthers exposed to pollinators that we collected from an inflorescence.

We quantified pollen receipt and the number of pollen tubes reaching the base of the style. Styles were cleared overnight in 10 mol l<sup>-1</sup> NaOH and stained with 0.1% aniline blue in 0.1 mol l<sup>-1</sup> K<sub>3</sub>PO<sub>4</sub>, squashed, and examined with an epifluorescence microscope (Martin 1959). We counted both the number of pollen grains still attached to the stigma, and the number of pollen tubes reaching the base of the style.

## STATISTICAL ANALYSES

We analysed the proportion of flowers that bees visited on individual inflorescences by maximum-likelihood analysis of a generalized linear model (McCullagh & Nelder 1989; Genmod procedure of SAS version 8.2, SAS 2001). This proportion is a binomial variable, so we applied the logit transformation before analysis. The linear model considered bee species (*B. dahlbomi* or *B. ruderatus*) and inflorescence type (female, male or neuter) as categorical independent variables, and the

number of open flowers per inflorescence (ln-transformed) as a covariate. Initially the analysis also considered all interactions between the categorical factors and the covariate, but they were omitted from the analysis described here because they did not significantly affect the proportion of flowers visited ( $\alpha = 0.05$ ). Significance testing involved likelihood-ratio ( $G$ ) tests.

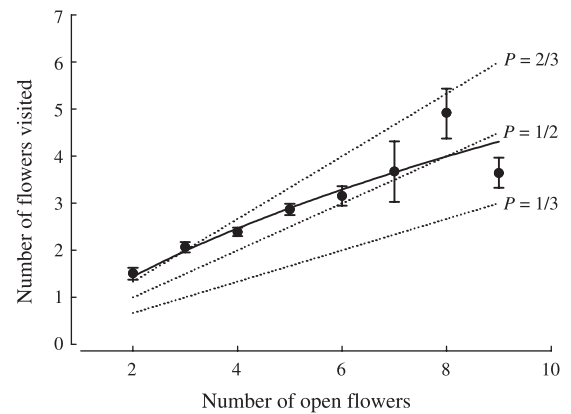
Our analyses of pollen availability, pollen removal, pollen receipt and pollen-tube growth involved mixed-effects, general linear models (Neter *et al.* 1996: Mixed procedure of SAS version 8.2, SAS 2001). All analyses considered site as a random factor and used restricted maximum likelihood to estimate the associated variance component. Denominator degrees of freedom for  $F$ -tests of fixed effects were calculated by Kenward & Roger's (1997) approximation, which frequently resulted in fractional degrees of freedom. Because of non-normality and heterogeneous variances, all analyses considered ln-transformed data. To assess whether pollen discounting reduced pollen export from MF test inflorescences, we contrasted pollen receipt by focal inflorescences in the MF treatment with the average receipt by focal inflorescences in the M and MN treatments. *A posteriori* contrasts that considered all pairwise comparisons employed Tukey's procedure for controlling the experiment-wise type I error rate to  $\alpha = 0.05$ , whereas contrasts involving other families of comparisons used the Dunn–Šidák procedure (Kirk 1995).

Although all analyses considered transformed data, we present the results for back-transformed means and standard errors. Back transformation results in asymmetric standard errors.

## Results

### POLLINATOR BEHAVIOUR

Bumble bees visited several flowers per inflorescence on average (Fig. 2). The number of flowers visited increased with the number available; however the proportion visited declined with increasing display size ( $G_1 = 7.36$ ,  $P < 0.01$ ). In particular, bees visited an average of two flowers on three-flowered inflorescences and three flowers on six-flowered inflorescences (Fig. 2). The number of flowers visited did not vary significantly with either inflorescence type (female, male or neuter;



**Fig. 2.** Relation of least-squares mean ( $\pm$  SE) number of flowers visited per *Alstroemeria aurea* inflorescence by bumble bees to number of open flowers. Solid line, regression relation; dashed lines, fixed proportions of visited flowers.

$G_2 = 0.64$ ,  $P > 0.7$ ), or between bee species ( $G_1 = 0.30$ ,  $P > 0.5$ ).

Bumble bees generally foraged very consistently while visiting *A. aurea* inflorescences. Individual bees revisited at least one flower during only 6.4% of visits to the 404 inflorescences that we observed. In addition, bees moved exclusively between adjacent flowers on 89.4% of these inflorescences.

### POLLINATION SUCCESS

Neither pollen production nor pollen removal differed significantly among different types of test inflorescences with male flowers (M, MF and MN; Table 1). On average, plants produced more pollen during the second trial (mean = 175 448, lower SE = 6716, upper SE = 6983 grains, based on ln-transformed data) than during the first trial (mean = 156 906, LSE = 6053, USE = 6296 grains). At the end of the experiment an average of 20 908 pollen grains (9% of production) remained in flowers (LSE = 1486, USE = 1600 grains). The amount of pollen remaining in anthers varied positively with a plant's original production (partial regression coefficient  $\pm$  SE =  $0.835 \pm 0.262$ ; Table 1), but did not additionally differ among treatments or between trials (Table 1).

**Table 1.** Results of ANOVAS comparing pollen production, pollen removal, pollen receipt and number of pollen tubes between experimental treatments and trials

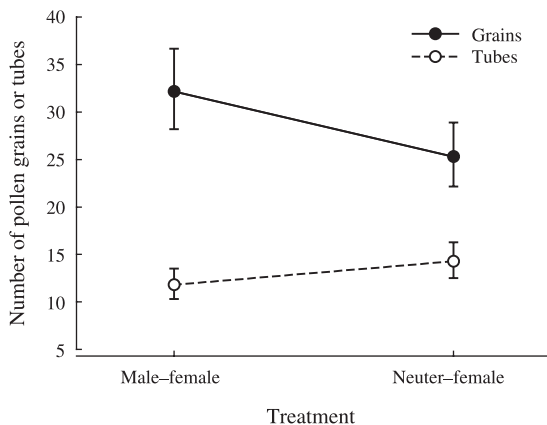
	Pollen production	Pollen remaining	Pollen on stigmas		Pollen tubes	
			Focal inflorescences	Test inflorescences	Focal inflorescences	Test inflorescences
Treatment	$F_{2,105} = 1.86$	$F_{2,104} = 0.04$	$F_{3,150} = 0.52$	$F_{1,76} = 1.65$	$F_{3,150} = 0.52$	$F_{1,76} = 1.02$
Trial	$F_{1,105} = 4.06^*$	$F_{1,105} = 0.88$	$F_{1,150} = 9.31^{**}$	$F_{1,76} = 2.26$	$F_{1,150} = 9.69^{**}$	$F_{1,76} = 2.38$
Treatment $\times$ trial	$F_{2,105} = 0.61$	$F_{2,104} = 2.08$	$F_{3,150} = 0.38$	$F_{1,76} = 0.52$	$F_{3,150} = 0.08$	$F_{1,76} = 0.59$
Pollen production		$F_{1,78.6} = 10.14^{**}$				

\* $P < 0.05$ ; \*\* $P < 0.01$ .

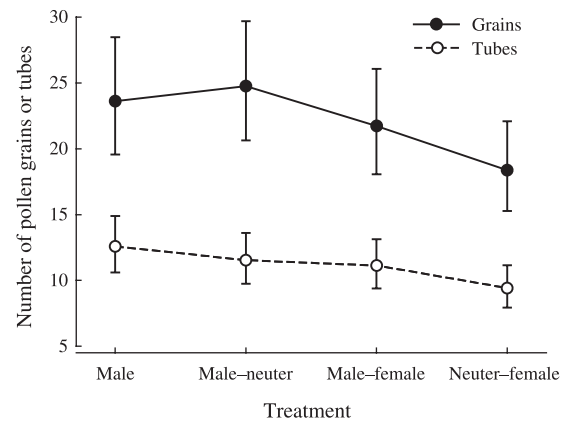
Overall, stigmas of experimental inflorescences received an average of 24 pollen grains (LSE = 1.62, USE = 1.73 grains). In general plants received more pollen during the first trial than during the second, although this difference was statistically significant only for focal inflorescences (Table 1). The difference in pollen receipt between the two trials occurred consistently for all treatments (no significant treatment  $\times$  trial interaction; Table 1).

Comparison of the MF and NF treatments provides equivocal evidence for self-pollination when female and male flowers are open simultaneously. Test inflorescences (MF and NF combined) received an average of 28.5 pollen grains (LSE = 2.57, USE = 2.81 grains), which significantly exceeded the mean of 20.0 grains (LSE = 2.30, USE = 2.56 grains) received by their corresponding focal inflorescences ( $F_{1,76} = 4.12$ ,  $P < 0.05$ ). Much of this greater receipt by test inflorescences resulted because those in the MF treatment received 27% more pollen than test inflorescences in the NF treatment, on average (Fig. 3, solid symbols), although pollen receipt by test inflorescences did not differ significantly between treatments (Table 1). 'Self-pollination' in MF test inflorescences is also suggested by a tendency for pollen receipt to vary positively with pollen removal for these inflorescences ( $r^2 = 0.10$ ,  $P = 0.07$ ), but not for the corresponding focal inflorescences ( $r^2 = 0.01$ ,  $P > 0.6$ ).

Pollen receipt by focal inflorescences provides little evidence for pollen discounting. Stigmas of focal inflorescences received an average of 21.9 pollen grains (LSE = 1.96, USE = 2.14 grains) during the experiment. However, pollen receipt by focal inflorescences did not differ among treatments (Table 1; Fig. 4, solid symbols). Specifically, we found no evidence that average pollen receipt by focal inflorescences of the MF treatment differed from that of focal inflorescences in the M and MN treatments ( $t_{150} = 0.50$ ,  $P > 0.5$ ), as would be expected if pollen discounting significantly reduced pollen export.



**Fig. 3.** Least-squares mean ( $\pm$  SE) number of pollen grains on stigmas and number of pollen tubes in styles of male-female and neuter-female test inflorescences.

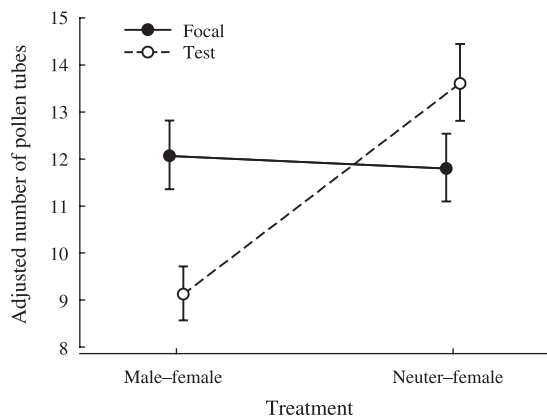


**Fig. 4.** Least-squares mean ( $\pm$  SE) number of pollen grains on stigmas and number of pollen tubes in styles of focal inflorescences in the four inflorescence treatments.

#### POST-POLLINATION SUCCESS

The number of pollen tubes at the bases of styles varied little among treatments and inflorescence types when we did not account for variation in pollen receipt (Table 1; Figs 3 and 4, open symbols). On average flowers in focal inflorescences had 11.1 pollen tubes (LSE = 0.907, USE = 0.981 tubes) at the bases of their styles. As with pollen receipt, we found 69% more pollen tubes in flowers of focal inflorescences during the first trial than during the second (Table 1). Flowers of test inflorescences had an average of 13.0 pollen tubes (LSE = 1.176, USE = 1.284 tubes) at the bases of their styles. *Alstroemeria aurea* flowers produce 17–26 ovules (Aizen & Basilio 1995), so the pollination realized during our experiment would not have resulted in complete fertilization.

Our results support negative effects of inbreeding in inflorescences with asynchronous dichogamy, despite little variation in the absolute number of pollen tubes. An analysis comparing the numbers of male gametophytes at two locations in the styles of test MF and NF inflorescences (pollen grains on stigmas *vs* pollen tubes at the bases of the styles), detected a strong interaction between treatment and location ( $F_{1,76} = 21.02$ ,  $P < 0.001$ ). This interaction resulted because of greater attrition of pollen tubes in MF test inflorescences than in NF test inflorescences (Fig. 3). Furthermore, an analysis of covariance that accounted for variation in pollen receipt found a significant interaction between inflorescence treatment (MF *vs* NF) and inflorescence type (focal *vs* test:  $F_{1,74.6} = 10.14$ ,  $P < 0.005$ ) in the number of pollen tubes reaching the base of the style. As expected with inbreeding effects, test inflorescences in the MF treatment had fewer pollen tubes than either their adjacent focal inflorescence or both types of inflorescences in the NF treatment (Fig. 5;  $P < 0.05$  in all cases). Stigmas on test and focal inflorescences in the NF treatment had equivalent numbers of pollen tubes ( $P > 0.5$ ).



**Fig. 5.** Least-squares mean ( $\pm$  SE) number of pollen tubes in styles of male-female and neuter-female test and focal inflorescences after adjustment for variation in number of pollen grains on stigma.

## Discussion

### SYNCHRONOUS DICHOGAMY IN *ALSTROEMERIA AUREA*

Simultaneous presentation of female and male flowers within *A. aurea* inflorescences would significantly reduce reproductive performance compared to the synchronous protandry exhibited by this species. We observed fewer pollen tubes at the bases of styles in MF test inflorescences than in those of inflorescences that lacked anthers, after accounting for pollen receipt, indicating that a smaller proportion of pollen tubes successfully entered the ovary. Stigmas of MF test inflorescences received almost 30% more pollen than similar test inflorescences with neuter, rather than male flowers (NF). Although this difference was not statistically significant, the combination of this result with the strong difference in post-pollination performance indicates that asynchronous protandry would result in self-fertilization. Such selfing would reduce a plant's genetic contributions to the next generation, due to the strong inbreeding depression found in this species (Aizen & Basilio 1995). On the other hand, our experiment provided little evidence that synchronous protandry enhances paternal outcrossing in *A. aurea* by reducing pollen discounting.

Synchronous dichogamy increases the number of flowers open simultaneously compared to plants with asynchronous dichogamy that produce the same number of flowers. Such large displays could enhance pollination by increasing the attractiveness of plants to pollinators (Ohashi & Yahara 2001); however we found no evidence for such a benefit. Three-flowered M test inflorescences and six-flowered MN test inflorescences experienced equivalent pollen removal. Similarly, three-flowered focal inflorescences (female flowers only) and six-flowered NF test inflorescences received similar stigmatic pollen loads (Figs 3 and 4). Therefore increased display size was probably a minor influence

on the evolution of synchronous dichogamy in *A. aurea*.

Selection of synchronous dichogamy seems more likely to result from self-pollination and related consequences. The strongest result of our experiment involved the relatively high attrition of pollen tubes in styles on MF test inflorescences (Fig. 5), which we interpret as the result of deposition of 'self-pollen' and poor post-pollination performance of inbred pollen. The female and male inflorescences used to create MF test inflorescences were collected within 1 m of each other and so had a high probability of being genetically related (Souto *et al.* 2002). Hand-pollination between ramets within this distance results in half as many pollen tubes reaching the ovary as pollinations between plants separated by 100 m (Souto *et al.* 2002). Therefore the high attrition of pollen tubes can be attributed to geitonogamous pollination and the genetic similarity of flowers in MF test inflorescences, compared with all other inflorescences with female flowers in our experiment, which could receive pollen only from more distant sources. Pollen-tube attrition would probably have been more severe than we observed if we had been able to construct all MF test inflorescences with female and male ramets with the same genotype (Souto 1999). Therefore synchronous dichogamy protects *A. aurea* from self-pollination within ramets and from the consequences of self-fertilization. In particular, in *A. aurea* inbreeding depression is expressed soon after zygote formation and reduces fruit production and seed weight (Aizen & Basilio 1995). Such early acting inbreeding depression is characteristic of long-lived, clonal species which accumulate large mutation loads as they age (Klekowski 1988). Because of the life-history consequences of reduced early performance, such species should experience strong selection for mating systems that enforce outcrossing (Husband & Schemske 1996).

In contrast to the post-pollination consequences of self-pollination, we found no evidence that synchronous dichogamy in *A. aurea* improves outcross siring ability. Our experiment provided conditions that should have resulted in pollen discounting for MF test inflorescences, as self-pollination could occur only geitonogamously. Lloyd (1988, 1992) proposed that geitonogamy should always reduce outcross siring, because it involves the same pollinator-mediated processes as pollen export. This proposal has been confirmed empirically (Harder & Barrett 1995), and dichogamy has been demonstrated to reduce the incidence of pollen discounting (Harder, Barrett & Cole 2000). In contrast, we did not find that focal inflorescences in the MF treatment received less pollen than those in the M and MN treatments. The apparent absence of pollen discounting could have resulted for at least two reasons. The most likely explanation is that total pollen receipt by a single focal inflorescence provides an imprecise measure of pollen export from test inflorescences, given the stochasticity of pollen dispersal and the tendency of pollinators to disperse pollen from a single

plant to many recipients (reviewed by Harder & Barrett 1996). Partial discrimination by bees against MF test inflorescences, resulting in lower visitation than for other experimental inflorescences, could also obscure pollen discounting (Harder *et al.* 2000), although we have no evidence for such bee behaviour. Whatever the cause for the lack of evidence for pollen discounting, our results suggest that synchronous dichogamy serves *A. aurea* more by eliminating the consequences of selfing than by promoting pollen export.

#### GENERAL IMPLICATIONS FOR THE EVOLUTION OF SYNCHRONOUS DICHOGAMY

The conclusion that synchronous dichogamy acts primarily as an anti-selfing mechanism may apply more generally. After reviewing all forms of dichogamy in angiosperms, Bertin (1993) reported that dichogamy occurs with equal frequency among self-compatible and self-incompatible species, leading to the conclusion that dichogamy does not generally evolve as a means of selfing avoidance. For example, asynchronous dichogamy in bee-pollinated species with vertical inflorescences serves as an inflorescence-level mechanism that reduces pollen discounting (Harder *et al.* 2000; Routley & Husband 2003). In contrast to Bertin's result, Cruden (1988) found that synchronous dichogamy occurred significantly more often in self-compatible species. This contrast suggests that the conditions that promote synchronization of sex phases among flowers differ from those that typically lead to temporal separation of sex function within flowers.

The common occurrence of synchronous dichogamy in families with umbellate inflorescences – e.g. Apiaceae (Molano-Flores 2001); Araliaceae (Thomson & Barrett 1981); Alstroemeriaceae (Snow & Grove 1995); Butomaceae (Bhardwaj & Eckert 2001) – suggests circumstances that may foster the evolution of synchronous dichogamy. Umbellate inflorescences lack much structure in the three-dimensional arrangement of flowers, so that inflorescence architecture can have little influence on the sequences of pollinator movements among flowers within an inflorescence. For example, bumble bees visiting *A. aurea* inflorescences moved between adjacent flowers around the ringed inflorescence, presumably moving clockwise and anticlockwise with equal frequency. As a result, no specific arrangement of female- and male-phase flowers would limit the incidence of geitonogamy within inflorescences if species with umbellate inflorescences had asynchronous dichogamy. The resulting selection for synchronous dichogamy in umbellate species is probably facilitated by the relatively equitable distribution of resources among flowers, which enables synchronization for floral development (e.g. Aizen & Basilio 1998).

Synchronous dichogamy could also evolve in species with non-umbellate inflorescences in association with a shift in pollinators from species that forage stereotypically within inflorescences to those that forage

unpredictably. For example, *Delphinium cardinale* differs from other *Delphinium* species in having synchronous dichogamy (Grant & Grant 1965). Most *Delphinium* species are pollinated by bees, which typically move upward on the racemose inflorescences, visiting lower female-phase flowers before upper male-phase flowers (Pyke 1978). In contrast, *D. cardinale* is visited by hummingbirds (Grant & Grant 1965) which move equally upward and downward on vertical inflorescences (Gross 2003). For this species the pattern of asynchronous protandry seen in bee-pollinated delphiniums would not limit geitonogamy, so that the transition from bee- to hummingbird-pollination probably imposed selection for synchronous floral development within inflorescences.

Complete protection against self-mating can result from sexual systems that preclude self-pollination (synchronous dichogamy and dioecy) or from physiological incompatibility systems that prevent self-fertilization. This paper provides the first experimental evidence of the advantages of synchronous dichogamy in selfing avoidance in a self-compatible species. Such an advantage may significantly influence the evolution of synchronous dichogamy in species with 'unstructured' inflorescences or pollinators that move unpredictably, so that inflorescence architecture cannot control the sequence of visits to female and then male flowers.

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