

1 **In the right place at the right time: *Parnassia* resolves the herkogamy dilemma by**
2 **accurate repositioning of stamens and stigmas**

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16 Running head: Movement herkogamy versus pollination accuracy

17

1 **Abstract**

2 *Background and aims* Spatial (herkogamy) and temporal (dichogamy) separation of
3 pollen presentation and stigma receptivity have been interpreted as reducing
4 interference between male and female functions in hermaphroditic flowers. However,
5 spatial separation leads to a potential conflict: reduced pollination accuracy, where
6 pollen may be placed in a location on the pollinator different from the point of stigma
7 contact.

8 *Methods* To understand better how herkogamous flowers resolve this conflict, we
9 studied a subalpine herb, *Parnassia epunctulata*, the nectariferous flowers of which
10 exhibit sequential anther dehiscence (staggered pollen presentation) and stamen
11 movements; usually one newly-dehisced anther is positioned each day over the central
12 gynoecium, while the older stamens bend away from the central position.

13 *Results* The open flowers were visited by a variety of pollinators, most of which were
14 flies. Seed set was pollinator dependent (bagged flowers set almost no seeds) and pollen
15 limited (manual pollination increased seed set over open pollination).

16 Adaptive-accuracy analyses showed that coordinated stamen movements and style
17 elongation (movement herkogamy) dramatically increased pollination accuracy.

18 Specifically, dehiscing anthers and receptive stigmas were positioned accurately in the
19 vertical and horizontal planes in relation to the opposite sexual structure and pollinator
20 position. By contrast, the spatial correspondence between anthers and stigma was
21 dramatically lower before the anthers dehisced and after stamens bent outwards, as well
22 as before and after the period of stigmatic receptivity. *Conclusions* We show for the
23 first time that a combination of movement herkogamy and dichogamy can maintain
24 high pollination accuracy in flowers with generalized pollination. Staggered pollen and

1 stigma presentation with spatial correspondence can both reduce sexual interference
2 and improve pollination accuracy.

3

4 **Key words:** adaptive accuracy, Celastraceae, dichogamy, generalist pollination,
5 herkogamy, Parnassiaceae, staggered pollen presentation, sexual interference, stamen
6 movement

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1 INTRODUCTION

2 A key innovation in the evolution of seed plants was the origin of the hermaphroditic
3 flower, where both male and female sexual functions occur in the same complex
4 structure (the flower; Stebbins, 1974; Endress, 1996). However this innovation created a
5 significant problem: sexual conflict, where the function of one sex is compromised by
6 the proximity and function of the other (Lloyd and Webb, 1986; Barrett, 2002;
7 Armbruster *et al.*, 2009b). This led to a further fundamental challenge in the function of
8 animal-pollinated, hermaphroditic flowers: minimizing such sexual conflict while still
9 enabling the male and female fertile parts to contact pollinators in the same place. Two
10 solutions to the sexual conflict have been explored evolutionarily by plants: 1) spatial
11 separation of fertile parts (herkogamy), and 2) temporal separation of sexual functions
12 (dichogamy). The former may greatly increase the risk that the fertile parts no longer
13 contact pollinators in the same location, while the latter may preclude reproductive
14 assurance by self-pollination. Even in dichogamous flowers, the avoidance of sexual
15 interference often requires movement of fertile parts, such that the first functional
16 organs make way for the second.

17 Herkogamy and dichogamy are thus thought to function mainly to reduce sexual
18 interference between male and female function in hermaphroditic flowers (Lloyd and
19 Yates, 1982; Lloyd and Webb, 1986; Webb and Lloyd, 1986; Barrett, 2002). However,
20 successful pollen transfer depends on stigma contact at the site on a pollinator's body
21 where pollen grains are placed, which may be impossible when flowers are
22 herkogamous. Thus, selection for pollination accuracy may operate in the opposite
23 direction to selection against sexual interference. Other things being equal, we expect it
24 to reduce the spatial separation between anthers and stigmas in herkogamous flowers
25 (Armbruster *et al.*, 2009b). Although Lloyd and Webb (1986) recognized that avoidance

1 of sexual interference may involve a conflict with pollination accuracy, very few studies
2 have shown how herkogamy and dichogamy can interact in homostylous species to
3 reduce interference between pollen removal and pollen receipt and yet maintain a
4 degree of pollination accuracy (Armbruster *et al.*, 2009a; Armbruster *et al.*, 2009b).

5 In their simple manifestations, dichogamy and herkogamy differ in their effects on
6 pollination accuracy in that dichogamy allows pollen and stigmas to be presented in
7 similar positions (at different times) within the flowers, whereas herkogamy often
8 results in separation of pollen and stigma positions on pollinators (inaccuracy), at least
9 when pollinators land directly on open flowers (vs. crawling down a tube, as in
10 approach herkogamy; Lloyd and Webb, 1986). However, even in open flowers,
11 accuracy may be increased by organ movement, as when “movement herkogamy”
12 (sensu Barrett, 2002--where some floral parts move; i.e. "temporal herkogamy" of
13 Willmer, 2011) is coupled with partial dichogamy: movement of stamens allows them at
14 the time of pollen release to occupy a position corresponding to the position of receptive
15 stigmas on older (or younger) flowers, and to be elsewhere during the female phase. In a
16 recent study, Ren and Tang (2012) observed that *Ruta graveolens* presented pollen to a
17 variety of pollinators by successively raising each of its eight stamens one by one to the
18 flower's centre, and then moving them back down later. Their results indicated that
19 pollen removal from the next stamen was significantly reduced when the previously
20 opening stamen was manipulated to remain at the flower centre, supporting
21 anther-anther interference as an adaptive explanation for the stamen movement, given
22 the temporal staggering of anther dehiscence (Ren and Tang, 2012). However, it
23 remains unclear how this behaviour affects female function and how accurately such
24 successive stamen movements position the anthers in the flower centre in relation to the
25 place on pollinators where pollen grains are likely to be picked up by stigmas.

1 An additional feature of sequential anther maturation and stamen movements is the
2 staggered presentation of pollen. Theory suggests that this can increase male fitness in
3 species in which seed set is pollen limited and pollinator visits are frequent, but
4 pollinators are inefficient in transporting pollen due to pollen feeding/grooming and/or
5 inconstancy (Harder and Thomson, 1989; Thomson and Thomson, 1992, Castellanos *et*
6 *al.*, 2006).

7 Sequential stamen movements have been observed in several families, including
8 Loasaceae, Rutaceae, Celastraceae/Parnassiaceae, and Tropaeolaceae (see Ren, 2010;
9 Henning and Weigend, 2012; Ren and Tang, 2012). In *Parnassia*, stamen movements
10 have been noted in *P. palustris* in Europe over two centuries, for example by Sprengel
11 (1793), Gris (1868), Bennett (1869), Arber (1913) and Martens (1936). These authors
12 observed that the five anthers presented pollen one by one at the flower centre on
13 different days and then bent away and down to overlie the petals before the stigma was
14 exposed. Recent pollination experiments on this protandrous herb showed that it was
15 self-compatible but fruit and seed production largely depended on pollination by a
16 variety of insects, especially flies (Martens, 1936; Sandvik and Totland, 2003).
17 However, the exact temporal and spatial pattern of pollen presentation and movement of
18 anthers has not been documented in any *Parnassia* species. Nor has the effect of such
19 complex movements on pollination accuracy been investigated previously.

20 Adaptive accuracy measures how close a population is to its adaptive optimum and/
21 or where it fits on the governing adaptive surface, while taking into account phenotypic
22 variation in the population (Armbruster *et al.*, 2004; Hansen *et al.*, 2006; Pelabon *et al.*,
23 2012). This concept is readily applied to floral morphology of animal pollinated plants
24 under the assumption that the optimum geometry of flowers is one that causes anthers
25 and stigmas to contact pollinators in the same location, promoting pollen transport to

1 stigmas and pollen receipt by stigmas, respectively (Armbruster *et al.*, 2004, 2009a).
2 Ignoring for the moment the potential negative effects of self-pollination and other
3 types of sexual interference, this assumption seems reasonable for most flowers,
4 including *Parnassia*. A significant advantage of using adaptive accuracy to analyse
5 phenotypes is that it allows us to compare the contributions of adaptive bias (departure
6 of a population mean from the optimum) versus imprecision (population variation) to
7 total adaptive inaccuracy. Here we use adaptive accuracy metrics to assess the adaptive
8 costs and benefits of movement herkogamy (*sensu* Barrett, 2002) and partial dichogamy
9 in *Parnassia* flowers (see Armbruster *et al.*, 2009b).

10 To evaluate the effect of partial dichogamy and movement herkogamy on
11 pollination accuracy in “generalist” flowers (flowers pollinated by a variety of animal
12 species), we investigated *Parnassia epunctulata* J.T.Pan, a plant with open, white
13 flowers, in a subalpine meadow in southwest China. The stamens of this species show a
14 remarkable pattern of repositioning, and dehisce one by one over several days before the
15 female phase. This feature permitted us to examine whether anthers and stigma are
16 positioned accurately, thus facilitating pollen removal and receipt. We describe the
17 progression of flowering and stamen and style movements in *P. epunctulata*, and we ask:
18 1) Does *P. epunctulata* meet the conditions in which gradual dosing through staggered
19 pollen presentation optimizes delivery (i.e., is seed set pollen limited and dependent on
20 pollinator visits, and do flowers receive frequent visits by wasteful pollinators;
21 Thomson and Thomson, 1992; Castellanos *et al.*, 2006)? 2) How accurate is the
22 positioning of stamens in relation to the position of the stigma and *vice versa*?

23

24 MATERIALS AND METHODS

1 *Parnassia* (Celastraceae/Parnassiaceae) comprises about 70 species of perennial herbs
2 occurring in arctic and temperate regions of the Northern Hemisphere, with nearly
3 three-quarters of the species being restricted to South and Southeast Asia (Wu *et al.*,
4 2009). We observed flowers of *Parnassia epunctulata* in July and August 2012 in the
5 Hengduan Mountains, Yunnan, China. Plants grew on well-drained areas in a marshy
6 subalpine meadow (altitude 3369 m a.s.l.) grazed by cattle, yaks and sheep located in
7 the Sicun Valley, Jiantong Town, in Shangri-La County, ca. 5 air miles east of
8 Shangri-La (previously named Zhongdian) (27°49.79' N, 99°45.69'E).

9 The late floral development and organ movements were monitored for 70 tagged
10 flowers in various stages of development; we recorded the position of each anther and
11 the stigma daily at about the same time for five successive days. After deleting records
12 for flowers that were lost, damaged or diseased, we were left with records for 59
13 flowers spanning the whole 5-day period. On a further set of 25 flowers, we measured
14 the lengths of one anther filament in each of three positions (when present): (1)
15 immature stamens below the level of the staminode tips, (2) stamens that had emerged
16 to overlie the stigma, and (3) stamens that had bent outwards, with anthers clear of the
17 stigma. We also measured in a series of flowers (at one time) the heights and positions
18 of dehisced and undehisced anthers and receptive and unreceptive stigmas in order to
19 calculate the accuracy of pollen and stigma positioning in various phases of late floral
20 development.

21 The excess temperature in sunlit flowers was measured with a digital
22 thermometer (Digitron Instrumentation Ltd) and a fine thermocouple. For
23 measurements in the flower the thermocouple was positioned about 2 mm above the
24 centre of the flower, and for corresponding ambient temperature measurements, it was
25 about 5 cm away from the flower at flower height. Each datum was the mean of a set of

1 ten measurements taken in rapid succession. Each set of ten measurements taken in the
2 flower alternated with a set of ten measurements of ambient temperature outside the
3 flower.

4 Insect observations were made by four observers in 50 10-minute watches, each
5 covering 9-13 flowers, between 1025 and 1445 h on 4, 5 and 6 August 2012. Individual
6 flowers were first mapped, and classed as pistillate (with the stigma exceeding the
7 staminodes and no anthers in the central position) or staminate (with at least one anther
8 over the unreceptive stigma). Insects were categorised as small (up to 3 mm long),
9 medium (up to 6 mm) or large (over 6 mm) flies, or bees (mostly small halictids), wasps
10 (mostly parasitic hymenopterans), ants, butterflies or moths. During 29 of the 10-minute
11 sessions, we recorded whether each visitor was seen to touch the anthers or stigma.

12 To test for autogamy whole plants in three sites not more than 200 m apart were
13 bagged on 5 August with fine-mesh polyester bags when the flowers were in bud, to
14 exclude insects, and another flower within 2 m of each bag, in a corresponding stage,
15 was labelled as a control. To test for pollen limitation, on 13 August one pistillate
16 flower (with the last anther having moved outwards and the stigma having emerged
17 above the level of the staminode tips) near each control was hand pollinated with mixed
18 pollen from multiple donors. To maintain phenological synchrony, this was done eight
19 days after bagging, when many of the control flowers had reached a similar stage.
20 Fifteen days later, on 28 August, the fruits were harvested and the seeds and
21 undeveloped ovules were counted. Reallocation of resources from other flowers on the
22 same plant was not likely to have caused a problem in this experiment because most
23 experimental plants bore only one flower in the year of the study. In the three
24 experimental sites 91%, 96% and 84% of plants bore a single stem with one flower; the
25 remaining plants bore two, three or four flowering stems.

1 Adaptive inaccuracies were calculated for all three floral-organ stages
2 (pre-receptive, receptive, and post-receptive) in order to gain insights into the extent that
3 floral movements improve pollination accuracy, if at all. Inaccuracies were calculated
4 by assuming that the optimal position of a fertile structure (anther releasing pollen or
5 receptive stigma) is the position of the opposite fertile structure (receptive stigma or
6 anther releasing pollen, respectively). The adaptive *inaccuracy* was calculated as:

$$7 \quad \text{Inaccuracy} = (\text{Adaptive Bias})^2 + \text{Imprecision}$$

8 where the adaptive bias is the difference between the population mean and the optimum
9 and the imprecision is the population variance in the structure's position. In order to
10 be able to compare male and female inaccuracies, the adaptive target variances were not
11 included in our calculation (cf. Armbruster *et al.*, 2009a). For comparisons between
12 structures of different sizes, inaccuracies were scaled to the square of the population
13 mean (see Armbruster *et al.*, 2004, 2009a; Hansen *et al.*, 2006; and Pelabon *et al.*, 2012
14 for further details).

15 Statistical analyses were performed in R (R Core Team, 2012) and with IBM
16 SPSS Statistics 20 (IBM-SPSS 2013).

17

18 **RESULTS**

19 Flower morphology and organ movements

20 Flowers of *Parnassia epunctulata* (Fig. 1) have five sepals and five white, clawed
21 petals, weakly fimbriate towards the base, forming a horizontal disc. The tripartite
22 (rarely quadripartite) stigma at the flower centre is surrounded by the insertion of five
23 stamens, alternating with five flattened staminodes, each with three blunt lobes. The
24 staminodes together form a tight cup-like ring, enclosing the immature anthers and the
25 stigma. We detected no measurable nectar, but it was presumably present. Visiting

1 insects probed with their proboscides between the staminodes and the gynoecium; their
2 behaviour indicated that, as in *P. palustris* (Sprengel, 1793; Sandvik and Totland,
3 2003), the staminodes bear nectar near the base of the inner surface (Fig. 2).

4 When the bud first opened the anthers were immature, with short filaments (1.12
5 ± 0.086 mm, mean \pm s.e.m., $n = 15$), and located within the ring of staminodes and
6 below the staminode tips. Each day the filament of usually one stamen extended and
7 curved inward, bringing the anther into a position above the pistil (filament length 4.26
8 ± 0.089 mm, $n = 18$) (Table 1, Fig. 1). In the field, fresh pollen was available only on
9 this centrally-placed anther, those elsewhere still being closed (younger) or depleted
10 (older). Usually the next day the stamen bent outwards clear of the stigma, often losing
11 its shrivelled anther, overlying the petals or descending between them into the hollow of
12 a sepal, and its filament extended further (to 4.68 ± 0.088 mm, $n = 25$). After the last
13 stamen had bent out of the way, the style elongated and elevated the newly receptive
14 stigma (now with divergent lobes) to a location close to that previously occupied by the
15 pollen-bearing anthers (see accuracy section below). The flowers were long-lived; some
16 flowers remained apparently receptive and retained petals for 11 days or more after the
17 beginning of the pistillate phase (and for some time after the ovary had begun to swell),
18 giving an estimated flower lifetime of at least 16 days [longer than most (10/11) species
19 studied by Ashman and Schoen (1994)].

20 In two flowers that opened on plants in a small patch of turf kept indoors, the
21 stamens behaved differently. In the absence of insect visitors they retained apparently
22 fresh pollen until day 5, and they often remained in position above the stigma for more
23 than one day (so that there were two or three anthers above the stigma on 4 out of 6
24 flower-days). After straightening to lift the anther away from the stigma the filaments

1 sometimes remained vertical for one or two days before bending outwards horizontally,
2 a situation that was rare in the field.

3

4 Insect visits

5 Most floral visitors probed the base of the staminodes, presumably for nectar, thrusting
6 the proboscis or the head down between the inner surface of the staminodes and the
7 ovary, and often touching the stigma or the central anther in doing so (Fig. 2). Some
8 visitors collected pollen. One tenthredinid sawfly visited flowers to capture other floral
9 visitors; it caught a pollinating fly and consumed it. Some insects rested in the flowers,
10 perhaps because these were warmer than the ambient air. In ten sequences of ten
11 measurements inside and outside a sunlit flower, the mean air temperature in the centre
12 of the flower was always higher (by up to 2.95°C) than the ambient temperature.

13 Of a total of 561 insect visits to *Parnassia* flowers in 50 10-minute sessions,
14 most (84%) were made by Diptera, most of which were more than 3mm long (Table 2).
15 Small bees made a further 12% of the total, and the remaining 20 visits were made by
16 wasps (most of them parasitic hymenopterans, but including one aculeate wasp), ants
17 and lepidopterans. In 29 of the 10-minute sessions we recorded whether or not the insect
18 touched the anthers or stigmas. Flies more than 3 mm long were seen to touch the anther
19 or stigma on over 75% of their visits, bees on 60%, and wasps more than 3 mm long on
20 83% of their (few) visits. Small flies and wasps, less than 3 mm long, were seen to
21 touch less often. Pistillate flowers and staminate flowers received a mean of 7.2 ± 1.00
22 and 5.9 ± 0.65 visits/flower/hour respectively (mean \pm s.e.m., $n = 39$ sessions).

23 Insect visits were thus numerous; flies were the most frequent visitors, mostly
24 obtaining nectar, and many of the larger visitors touched the anthers or stigma and were
25 therefore potentially capable of transferring pollen from one flower to another. Thrips

1 (Thysanoptera; including *Thrips* sp.) were present in many of the flowers. They may be
2 capable of transferring pollen, as Martens (1936) suggested in *P. palustris*, but they are
3 unlikely to be important pollinators here in the presence of such frequent visits from
4 larger insects.

5

6 Accuracy and precision

7 Receptive stigmas were positioned accurately in the vertical plane in relation to stamen
8 position; inaccuracy was calculated as only 1.59%, of which 18.9% was due to bias and
9 81.1% due to imprecision. In comparison, inaccuracy would have been dramatically
10 higher were the stigmas receptive in the pre-receptive phase (26.72%; 93.6% due to bias
11 and 6.4% due to imprecision) and modestly higher in the post-receptive phase (4.83%;
12 36.9% due to bias and 63.1% due to imprecision; Fig.3).

13 Dehiscing anthers were positioned very accurately in the vertical plane in relation
14 to stigma position; inaccuracy was calculated as only 1.41%, of which 23.9% was due
15 to bias and 76.1% due to imprecision. In comparison, the spatial correspondence
16 between anthers and style was dramatically lower before anther dehiscence (inaccuracy
17 in pre-dehiscence phase: 361.2%; 98.1% due to bias and 1.9% due to imprecision), and
18 after stamens bent outwards (inaccuracy in post-dehiscence phase: 200.9%; 99.5% due
19 to bias and 0.5% due to imprecision; Fig. 3).

20

21 Pollination experiments

22 A two-way ANOVA showed a significant effect of treatment on seed set ($p < 0.0001$),
23 but no significant effect of site ($p = 0.14$), or interaction between site and treatment ($p =$
24 0.49). Seed set was very low in flowers kept bagged from the bud stage to exclude
25 insects (mean seeds per ovule 0.010 ± 0.0008 , mean \pm s.e.m.; $n = 15$). No ovules

1 developed at all in 13 of the 15 bagged flowers. Seed set was significantly higher in the
2 open-pollinated controls (mean seeds per ovule 0.279 ± 0.055 , $n = 24$, $p < 0.0001$,
3 Welch two-sample t test). We conclude that bagging to exclude insects reduced seed set.
4 Seed set was significantly higher in hand-pollinated flowers (mean seeds per ovule
5 0.531 ± 0.034 , $n = 22$) than open-pollinated flowers ($p < 0.001$, Welch two-sample t
6 test), indicating that seed set was limited by the availability of pollinator-transported
7 pollen (“pollen limitation”).

8 **DISCUSSION**

9 Our observations indicate that *Parnassia epunctulata* is protandrous and shows
10 elaborate stamen movements comparable with those of *P. palustris* (Sprengel, 1793;
11 Martens, 1936) and *Ruta graveolens* (Ren and Tang, 2012), with gradual presentation of
12 pollen, generally by one fresh anther each day over a period of about five days. We
13 found that, as in *P. palustris* (Sandvik and Totland, 2003), seed set requires pollinator
14 visits and is pollen limited. Insect visitors were numerous and diverse, were often
15 generalist feeders, had low constancy (WSA, unpubl. observations), and were
16 presumably inefficient. In this respect, we can expect staggered pollen presentation to
17 be selected for (Harder and Thomson, 1989; Thomson and Thomson, 1992; Castellanos
18 *et al.* 2006; Ren and Tang, 2012).

19 It would be interesting to explore the unconfirmed suggestion, arising from our
20 indoor observations, that this may be a “dynamic dispensing schedule” (Harder and
21 Wilson, 1994), responsive to local conditions, as seen in other species. In *P. palustris*,
22 stamen movement may be delayed if pollen is not removed (Martens, 1936) so that the
23 anthers remain in the optimal position for pollen dispersal, and so that, in the absence of
24 insect visitors, anthers with fresh pollen may remain near the stigma, perhaps providing

1 reproductive assurance through autogamy. A comparable dynamic schedule has been
2 described in *Ruta* (Ren and Tang, 2012).

3 The movement of the anthers into the “correct” position for dehiscence, by
4 elongation of the filament, dramatically increases the male adaptive accuracy (Fig. 3).
5 In contrast, if the anthers still contained pollen, post-dehiscence movement would
6 dramatically decrease adaptive accuracy (Fig. 3). This would be another factor
7 favouring condition-dependent organ movement describe above. Elongation of the style
8 just before apparent stigma receptivity also markedly increases female adaptive
9 accuracy compared to an un-elongated style. The inaccuracy measure then again
10 increases moderately in the post receptive period with further elongation of the pistil
11 (Fig. 3), although , because the fruit is already developing, this “inaccuracy” no longer
12 has an adaptive cost.

13 One characteristic that distinguishes *P. epunctulata* from many other *Parnassia*
14 species is that the staminodes are tightly integrated into a cuplike ring rather than being
15 showy and spreading (as in, for example, *P. palustris* and *P. aff. simaoensis/wightiana*
16 of north western Yunnan (Baishui Terraces); Fig. 4). Because the nectar is apparently
17 secreted at the bottom of this cupule, the staminodes control, to some extent, which
18 insects can gain access to it, and what orientation they must assume, forcing them up
19 against the pollen-bearing anthers or receptive stigmas as they probe for nectar (Fig. 2).
20 In this way the integration of the staminodes into a ring further increases the accuracy of
21 pollination by forcing nectar feeders of the “right” size to behave appropriately for
22 consistent pollen placement and pick-up. Thus *P. epunctulata* appears to have achieved
23 higher pollination accuracy than its relatives with divergent staminodes, if perhaps at
24 the cost of being less visually attractive to flies (see Faegri and van der Pijl, 1979). In a
25 species from south western Sichuan, *P. aff. leptophylla* Handel-Mazzetti, the petals,

1 rather than the staminodes, form the walls of the cup-like chamber (at least distally) into
2 which pollinators must insert their proboscides, heads or bodies to obtain nectar. The
3 gap between the chamber wall and the single centrally located anther or stigma
4 influences which insects can reach nectar and/or contact the fertile structures (WS
5 Armbruster unpublished observation; for an analogous situation in *Linum*, see
6 Armbruster *et al.* 2006, 2009a). This represents an example of transfer of function
7 between floral structures as Stebbins (1974) described for dispersal mechanisms.

8 It is thus clear that the coordinated movement of pre- and post-dehiscent anthers
9 into and out of the central position in the ring of nectariferous staminodes not only
10 reduces stamen-stamen interference (see Ren and Tang, 2012) and interference with
11 pollen arrival to receptive stigmas, but also leads to maximal positional accuracy for
12 placing pollen in the “right” place on the “right” species of pollinators. Similarly,
13 elongation of the style only just before stigma receptivity not only keeps the stigma out
14 of the way of the pollen-bearing anthers, reducing or precluding self-pollination and
15 pollen discounting, but also puts the stigma into the right place at the right time for
16 receiving cross-pollen, thus raising the adaptive accuracy of pollination.

17 Successive stamen movements have been reported previously in the Loasaceae,
18 Parnassiaceae, Rutaceae and Tropaeolaceae (Ren, 2010; Henning and Weigend, 2012;
19 Ren and Tang, 2012). The phenomenon of staggered pollen presentation through
20 sequential movement of anthers into a similar position probably occurs in additional
21 species with open-shaped flowers. At least one other family, Saxifragaceae, can be
22 added into the list of those with successive stamen movements. We observed the ten
23 stamens of *Saxifraga* sp. in Shangri-La Alpine Botanical Garden, SW China presenting
24 pollen sequentially 1-2 at a time in a position subsequently occupied by the stigmas,
25 behaviour very like that seen in *Parnassia*. Indeed, an early note described sequential

1 stamen movements in *Saxifraga sarmentosa* (Todd, 1880) but the adaptive advantages
2 have not been explored experimentally. Other examples may include Ranunculaceae;
3 Weber (1993) described sequential movements of the 50-70 stamens in *Nigella arvensis*,
4 and corresponding subsequent positioning of the styles.

5 Although several previous studies have suggested that movement herkogamy is
6 the result of selection to avoid sexual interference within hermaphrodite flowers (Lloyd
7 and Yates, 1982; Webb and Lloyd, 1986; Ren and Tang, 2012), none has previously
8 considered the role of the opposing selection for pollination accuracy. Our
9 measurements of adaptive accuracy in *P. epunctulata* show how movement herkogamy
10 maintains high accuracy while reducing sexual interference, and, more generally, they
11 shed light on the functioning of flowers that are hermaphrodite, which is the dominant
12 condition in angiosperms.

13

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7

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1 **Figure legends**

2 **Fig. 1.** A. mean numbers of anthers dehiscid (mature or shrivelled) on successive
3 days, in flowers with 1 (closed circles), 2 (open circles), 3 (closed squares) or 4
4 (open squares) matured anthers on day 1 (n = 10, 7, 3 and 8 respectively). B. A
5 flower of *Parnassia epunctulata* after removal of one petal, two staminodes and two
6 sepals, to show the tripartite staminodes and four of the five stamens, of which two
7 are immature, one (with a dehiscid anther and fresh pollen) is positioned over the
8 pistil and one (with a shrivelled anther) has bent down between two petals to lie
9 against a sepal. Black: anthers and cut surfaces; stipple: calyx and gynoecium.

10 **Fig. 2.** Diverse insects, mostly generalist pollinators, visiting male-phase (A, C, D)
11 and female-phase (B, E, F) flowers of *Parnassia epunctulata*, showing sequential
12 movement of the five stamens with one dehiscing anther at the centre of the flower.
13 (A) A syrphid fly obtaining nectar from an early male-phase flower; the second
14 anther to dehisce is at the flower's centre. Note yellow pollen grains on the fly's
15 back; (B) a tephritid fly obtaining nectar from a female-phase flower; (C) a halictid
16 bee collecting both nectar and pollen from a male-phase flower, surrounded by a
17 whorl of greenish-yellow staminodes; (D) a butterfly (*Melitaea jezabel*) obtaining
18 nectar from a male-phase flower; (E) a small fly obtaining nectar from a
19 female-phase flower; the five dehiscid stamens have moved down between the
20 petals; (F) a halictid bee obtaining nectar from a female-phase flower, where it is
21 likely to deposit pollen on the stigma. Dehiscid and spent stamens are marked with
22 arrows.

23 **Fig. 3.** Adaptive inaccuracies (scaled to square of trait means) for stigma position
24 (blue) and anther position (red) of *Parnassia epunctulata* flowers at various stages
25 of development. Flowers are protandrous; pre-receptive stigmas occur during the

1 male phase of the flowers and post-receptive stigmas after ovaries begin to swell.
2 Pre-receptive anthers are those not yet dehisced, receptive anthers are those on the
3 first or second day after dehiscence, and post-receptive anthers are on older stamens
4 that have bent away from the centre of the flower (see Fig. 1). The inaccuracy of
5 this last category reflects deviation from the optimum position in two dimensions
6 (vertical plus horizontal planes) and was calculated using the Euclidean distances.

7 **Fig. 4.** Showy, spreading staminodes (arrows) of a Chinese species of *Parnassia*, *P.*
8 *aff. simaoensis* YY Qian (= *P. wightiana* Wallich ex Wight and Arnott s.l.
9 complex), which do not restrict pollinator access to nectar or position them relative
10 to the pollen-bearing anthers and receptive stigmas. Note one pollen-bearing
11 anther at the centre of the flower, one spent anther at circumference and three
12 undehisced anthers appressed to the ovary. This species appears to follow the same
13 basic pattern of stamen movement as observed in *P. epunctulata*.

Table 1. Number (and %) of flower-days on which given numbers of stamens made the transition (a) from the immature position (below the level of the staminode tips) to the position over the style, and (b) from there outwards to a peripheral position away from the flower centre. The usual condition is shown in bold.

| | (a) Immature to over style | (b) Over style to peripheral |
|--------------------------|----------------------------|------------------------------|
| 0 stamens | 8 (11%) | 16 (16%) |
| 1 stamen | 45 (64%) | 57 (57%) |
| 2 stamens | 16 (23%) | 22 (22%) |
| 3 stamens | 1 (%) | 4 (4%) |
| Number of flower-days | 70 | 99 |

Table 2. Insect visits to *Parnassia epunctulata* flowers. Visits were recorded in 50 10-min sessions, and in 29 of those sessions (367 visits) the recorder also noted whether or not the visitor touched the stigma or anthers.

| | Number (%) of visits | Number (%) of visits that touch |
|-----------------|----------------------|---------------------------------|
| Diptera > 6 mm | 137 (24) | 76 (78) |
| Diptera 3-6 mm | 211 (38) | 119 (76) |
| Diptera < 3 mm | 126 (22) | 18 (28) |
| Apoidea | 67 (12) | 21 (60) |
| Other Apocrita: | | |
| Wasps 3-6 mm | 9 (2) | 5 (100) |
| Wasps < 3 mm | 5 (1) | 0 (0) |
| Others | 6 (1) | 3 (75) |
| Total | 561 visits | 242 touching visits |

Figures

Fig. 1.

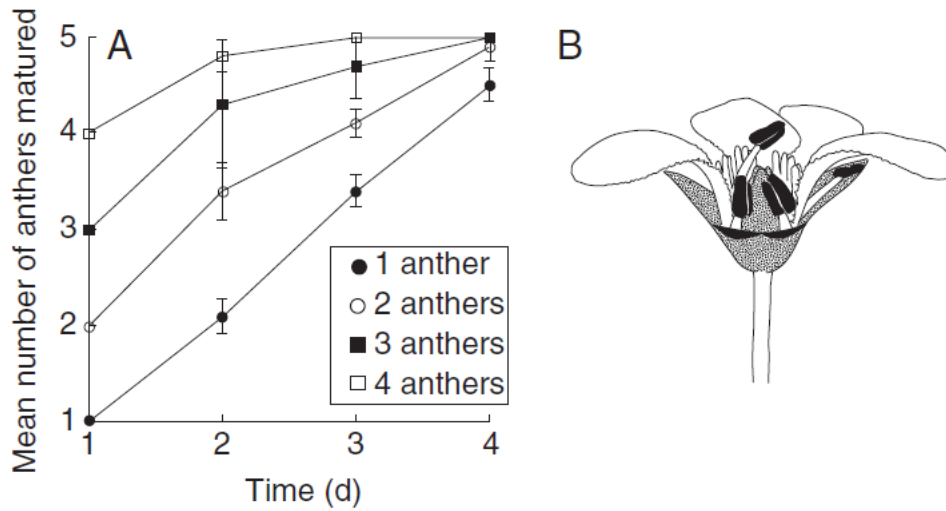


FIG. 1. (A) Mean numbers of anthers dehiscent (mature or shrivelled) on successive days in flowers with one, two, three or four matured anthers on day 1, as indicated in the key ($n = 10, 7, 3$ and 8 respectively). (B) A flower of *Parnassia epunctulata* after removal of one petal, two staminodes and two sepals, to show the tripartite staminodes and four of the five stamens, of which two are immature, one (with a dehiscent anther and fresh pollen) is positioned over the pistil and one (with a shrivelled anther) has bent down between two petals to lie against a sepal.

Black, anthers and cut surfaces; stipple, calyx and gynoecium.

Fig. 2.

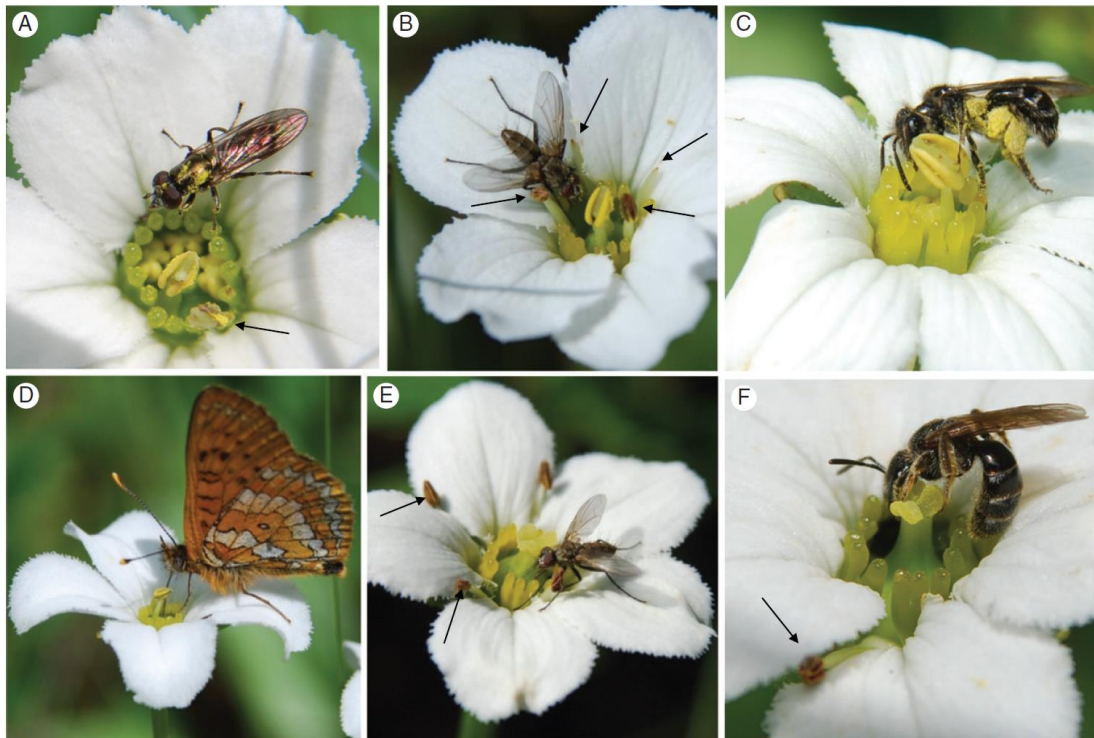


FIG. 2. Diverse insects, mostly generalist pollinators, visiting male-phase (A, C, D) and female-phase (B, E, F) flowers of *Parnassia epunctulata*, showing sequential movement of the five stamens with one dehiscent anther at the centre of the flower. (A) A syrphid fly obtaining nectar from an early male-phase flower; the second anther to dehisce is at the flower's centre. Note yellow pollen grains on the fly's back. (B) A tephritid fly obtaining nectar from a female-phase flower. (C) A halictid bee collecting both nectar and pollen from a male-phase flower, surrounded by a whorl of greenish-yellow staminodes. (D) A butterfly (*Melitaea jezabel*) obtaining nectar from a male-phase flower. (E) A small fly obtaining nectar from a female-phase flower; the five dehiscent stamens have moved down between the petals. (F) A halictid bee obtaining nectar from a female-phase flower, where it is likely to deposit pollen on the stigma. Dehiscent and spent stamens are marked with arrows.

Fig. 3.

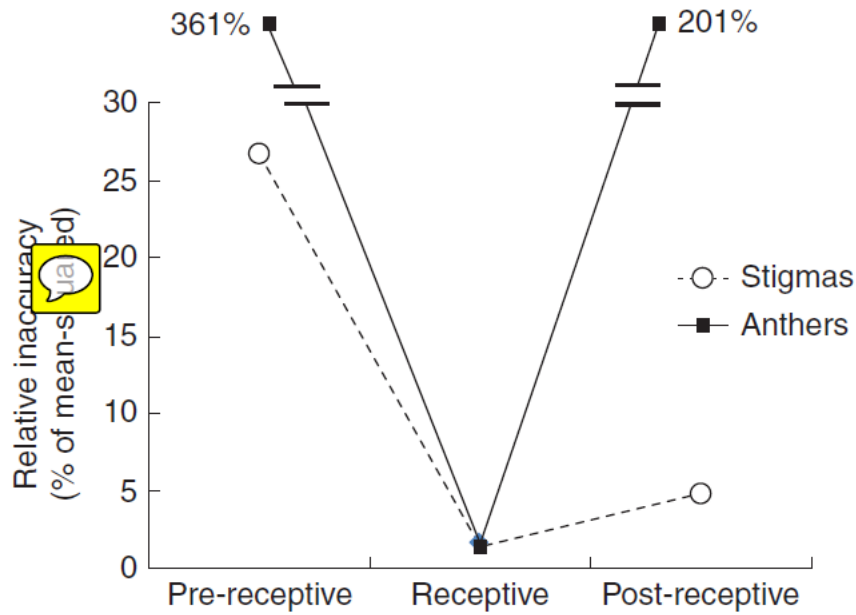


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Fig. 4.



FIG. 4. Showy, spreading staminodes (arrows) of a Chinese species of *Parnassia*, *P. aff. simaoensis* YY Qian (= *P. wightiana* Wallich ex Wight and Arnott *s.l.* complex), which do not restrict pollinator access to nectar or position them relative to the pollen-bearing anthers and receptive stigmas. Note one pollen-bearing anther at the centre of the flower, one spent anther at the circumference and three undehisced anthers appressed to the ovary. This species appears to follow the same basic pattern of stamen movement as observed in *P. epunctulata*.