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1 **SPECIAL ISSUE – INSECT-PLANT INTERACTIONS: HOST SELECTION,**  
2 **HERBIVORY, AND PLANT RESISTANCE**

3

4 **Style length variation in male and female figs: development, inheritance, and control of**  
5 **pollinator oviposition**

6

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19 **Running title:** *Style length variation in male and female figs*

20

21 **Key words:** dioecious, hybrid, *Ficus montana*, *Ficus asperifolia*, fig wasp, *Kradibia*  
22 *tentacularis*, *Kradibia hilli*, Moraceae, comparative ontogeny, Hymenoptera, Agaonidae

23

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25

**1 Abstract**

2 Fig trees (*Ficus* spp., Moraceae) depend on female fig wasps to transport their pollen and as a  
3 reward provide nutrition for pollinator fig wasp larvae. Most pollinators are host specific, but  
4 natural plant hybrids occur if atypical hosts are pollinated. Female fig wasps lay their eggs  
5 into ovaries of female flowers in receptive figs by inserting their ovipositors through the  
6 styles, and relative style and ovipositor lengths are believed to largely determine their ability  
7 to oviposit. In dioecious fig trees, flowers in receptive male figs have short styles and support  
8 larval development, whereas flowers in receptive female figs have long styles and only  
9 develop into seeds. Using the dioecious fig tree species *Ficus montana* Burm. f. and *Ficus*  
10 *asperifolia* Miq., we recorded the comparative ontogeny of style lengths to determine  
11 whether style growth in the two sexes is coordinated with the attraction of pollinators, and  
12 how maximum style lengths relate to the ovipositor lengths of their pollinators. F1 hybrids  
13 between these species produce viable seeds, but no pollinator offspring. We examined style  
14 lengths in F1s and backcrosses, relative to those of their parents, and whether coordination of  
15 style growth with pollinator attraction is disrupted. The ovipositor of *Kradibia tentacularis*  
16 (Grandi) (Hymenoptera: Agaonidae), the pollinator of *F. montana*, could reach some of the  
17 ovaries in female figs, but fails to lay eggs there. Styles in male F1 figs were intermediate in  
18 length between those of the two parents, but in female F1 figs styles grew longer than in  
19 either parent. Maximum style lengths in F1s were partially decoupled from receptivity,  
20 especially in female figs. Our results illustrate how timing of stigmal growth is coordinated  
21 with pollinator attraction, and that this coordination can be disrupted in hybrid individuals.  
22

## 1 **Introduction**

2 Mutualisms are interactions between species where the species involved receive mutual  
3 benefits, but in many mutualisms at any one time some individuals may be gaining from the  
4 interaction whereas others are gaining no benefits, or are even being harmed (conditionality  
5 sensu Cushman & Whitham, 1989; context dependency sensu Hoeksema & Bruna, 2015).  
6 Partners in a mutualism routinely have conflicts of interest, and if reproductive advantage can  
7 be achieved at the expense of other individuals, then selection will favour the evolution of  
8 ‘cheating’ (Bronstein, 1994; Herre et al., 1999; Sachs & Simms, 2006; Sachs, 2015). Some  
9 plant species achieve pollination by deception, without ever providing a reward, and in some  
10 dioecious species only one sex provides rewards to the pollinator (Willson & Agren,  
11 1989). The negative consequence of a failure by one sex to provide rewards to pollinators is  
12 particularly apparent for insects in nursery mutualisms. Here the expected reward is in the  
13 form of food for larval development (Dufay & Anstett, 2004) and in the extreme example  
14 provided by females of dioecious fig trees (*Ficus* spp., Moraceae), ‘cheating’ female  
15 inflorescences reduce pollinator lifetime reproductive success to zero.

16 Fig trees are characterised by having tiny flowers distributed inside the cavity of their  
17 inflorescences (figs, also known as syconia) (Anstett, 2001). The several hundred species of  
18 fig trees are distributed in warmer countries worldwide (Berg & Corner, 2005), and all are  
19 pollinated by tiny fig wasps belonging to the family Agaonidae (Hymenoptera). Each  
20 pollinator species is usually associated with a single species of fig tree, but pollination of  
21 atypical hosts can result in hybrid plants, and hybridisation may have been a significant force  
22 in the evolution of the genus (Kusumi et al., 2012). Fig trees and fig wasps are mutually  
23 dependent, with fig wasp females laying their eggs only in the female flowers of receptive  
24 figs and the figs only being pollinated by these fig wasps. Around half of all fig species are  
25 monoecious and the rest are dioecious. In monoecious species the relationship is consistently  
26 mutualistic with both seeds and fig wasp offspring developing within the same figs.  
27 However, in dioecious fig trees, some individuals have figs that produce pollen and support  
28 development of the fig wasps that will carry it (male trees) and others produce only seeds  
29 (female trees) and exploit pollinators without providing any reward.

30 Pollinators are attracted to their hosts by chemical signals released by receptive figs  
31 (Proffitt et al., 2009). After a female has landed on a receptive fig, she needs to squeeze  
32 through its narrow ostiole in order to reach the female flowers that line its inner surface. Once  
33 inside she attempts to insert her ovipositor down the full length of the styles, and if she  
34 reaches an ovary she can lay an egg (Weiblen, 2002). Larvae complete their development

1 inside the galled flowers. Males of the next generation of fig wasps emerge first, mate with  
2 the females that are still in their galls, then chew a hole through the fig wall to allow the  
3 females to depart in search of receptive figs, which are usually on other trees (Weiblen, 2002;  
4 Suleman et al., 2012). Before departing their natal figs the female fig wasps either become  
5 covered passively with pollen from dehiscent anthers, or actively collect pollen into thoracic  
6 pollen pockets (Kjellberg et al., 2001, 2014), which they later unload while ovipositing.

7 Many *Ficus* species are pollinated by a single fig wasp species (Ramirez, 1970;  
8 Bronstein, 1987), but increasing numbers of tree species are known to have two or more  
9 pollinators (Molbo et al., 2003; Compton et al., 2009; Lin et al., 2010). Host specificity is  
10 believed to be maintained by a combination of unique chemical cues (Grison-Pigé et al.,  
11 2001; Wang et al., 2013), the physical barrier generated by ostiole size and shape (van Noort  
12 & Compton, 1986), style lengths relative to the length of a pollinator's ovipositor (Nefdt &  
13 Compton, 1996; Liu et al., 2013), and the ability of the fig wasps to induce gall development  
14 (Ghana et al., 2015a). The relationship between style lengths in figs and the lengths of their  
15 pollinator's ovipositor are significant because pollinators insert their ovipositors down the  
16 length of the styles and they must have sufficient length to reach the ovules. The style lengths  
17 are typically distributed unimodally (Kathuria et al., 1995; Otero & Ackerman, 2002) and the  
18 ovipositors of a tree's pollinator are usually long enough to reach most or all of the ovaries in  
19 monoecious figs or male dioecious figs (Nefdt & Compton, 1996; Zhang et al., 2009).  
20 Variation in style lengths is linked to the fate of the ovules in monoecious figs, with  
21 longer-styled flowers tending to produce seeds, and flowers with shorter styles more likely to  
22 support fig wasp development (Compton et al., 1994; Kathuria et al., 1995; Nefdt &  
23 Compton, 1996; Jouselin et al., 2001; Yu et al., 2004; Dunn et al., 2008).

24 Dioecious fig trees have modified their relationship with pollinators by having seeds  
25 and pollinator offspring developing on different trees. In male figs the style lengths are short  
26 and ovipositors can easily access all the styles, whereas in female figs the styles of the  
27 flowers are typically longer than the ovipositor, so pollinators cannot reach the ovules to lay  
28 their eggs (Nefdt & Compton, 1996; Weiblen, 2004). The stigmas of female flowers in male  
29 and female figs also differ in shape, with the former facilitating oviposition and the latter  
30 adapted for pollen collection (Verkerke, 1987; Beck & Lord, 1988). Pollination of female  
31 dioecious figs is achieved by mutual mimicry between males and females, with receptive figs  
32 of the two sexes having a similar appearance and smell (Grafen & Godfray, 1991; Grison-  
33 Pigé et al., 2001). Pollinator females lose their wings when passing through the ostiole into a  
34 fig, so they have no chance of re-emerging to fly to other trees after they enter a female fig.

1 Once inside, they continue to behave as if they were in a male fig, and pollination is achieved  
2 (Raja et al., 2008a).

3 *Ficus montana* Burm. f. is a small dioecious fig tree from Southeast Asia, where its  
4 pollinator is *Kradibia* (= *Liporrhopalum*) *tentacularis* (Grandi) (Hymenoptera: Agaonidae).  
5 *Ficus asperifolia* Miq. is a closely related species that in East Africa is pollinated by *Kradibia*  
6 *hilli* Wiebes. Hybrid seeds generated from crosses between the two *Ficus* species germinate  
7 readily, grow rapidly, and mature about as rapidly as their parents (Ghana, 2012). Together  
8 with their parent species they provide an opportunity to investigate patterns of inheritance  
9 and growth of style lengths in figs, whether style lengths are solely responsible for preventing  
10 the reproduction of fig wasps inside female figs and whether style lengths in F1s can reduce  
11 their reproductive success. Dissections of flowers in female figs of *F. montana* that had been  
12 entered by *K. tentacularis* females have shown that they cannot reproduce in female figs of  
13 their host because females of this wasp species do not lay any eggs there (Ghana et al.,  
14 2015b). In contrast, when wasps enter F1 male figs they do lay eggs, but larvae fail to  
15 complete development (Ghana et al., 2015a,b).

16 Here we address the following questions: (1) Does the maximum length of styles in  
17 male and female figs coincide with receptivity? (2) Are growth patterns similar in male and  
18 female figs and between hybrids and their parents? (3) What are the relationships between  
19 style lengths in male and female figs and the lengths of the ovipositors of the fig wasps that  
20 pollinate *F. montana* and *F. asperifolia*? (4) Do style lengths alone explain the lack of  
21 oviposition in female figs? And (5) do hybrids display style length characteristics  
22 intermediate between those of their parents?  
23

## 24 **Materials and methods**

### 25 **Study species**

26 This study was conducted in the glasshouses of the University of Leeds Experimental  
27 Gardens (Leeds, UK) using *F. montana*, *F. asperifolia*, their F1 hybrids, and backcrosses to  
28 *F. montana*. *F. montana* and its pollinator had been maintained at Leeds University since  
29 1995 (Suleman, 2007). The seeds of *F. montana* and mature figs containing *K. tentacularis*  
30 were obtained from the Centre of International Forestry Research (CIFOR) plantation near  
31 Bogor, Java and Rakata (Krakatau Islands) in Indonesia (Moore, 2001). Female *K.*  
32 *tentacularis* pollinate figs actively (Compton et al., 2010), can reemerge from the first figs  
33 they enter, and are capable of utilizing up to four additional figs nearby (Suleman et al.,

1 2013b), but after losing their wings on initial entry they cannot fly. Their offspring sex ratios  
2 are biased and they lay mostly male eggs when they first enter a fig (Moore et al., 2002; Raja  
3 et al., 2008b).

4 The *F. asperifolia* population at Leeds was established using seeds collected from  
5 Kibale Forest in Uganda in August 2004 (Ghana, 2012). Trees of this fig species produced  
6 few figs in winter. *Kradibia hilli* is the pollinator of *F. asperifolia* in East Africa, whereas in  
7 West Africa the plant is pollinated by another *Kradibia* species (Berg & Wiebes, 1992).  
8 There are large differences between the number of flowers in the figs from the two areas and  
9 they may eventually be recognised as distinct species (Verkerke, 1987; SG Compton,  
10 unpubl.).

11 Although they occur on different continents, *F. montana* and *F. asperifolia* are closely  
12 related within the *F. montana* species group in *Ficus* subgenus *Sycidium*, Section *Sycidium*  
13 (Berg & Corner, 2005). Hybrids were generated by crossing *F. montana* (paternal parent) and  
14 *F. asperifolia* (maternal parent) and backcrosses were between male *F. montana* and female  
15 F1s using *K. tentacularis* as the pollen vector. No live *K. hilli* were available to generate  
16 reciprocal crosses, and *K. tentacularis* cannot develop in F1 hybrid figs, which prevented  
17 breeding of F2s (Ghana et al., 2015b). Experimental crosses were achieved by placing *K.*  
18 *tentacularis* females from known male host plants at the ostiole of figs on female plants. The  
19 figs were bagged before and after wasp entry into individual figs to prevent entry by other fig  
20 wasps. F1s were generated in 2005 and backcrosses in 2006.

21

## 22 **Style length in figs of different diameters**

23 Fig development is generally characterised using the stages described by Galil & Eisikowitch  
24 (1968) and Valdeyron & Lloyd (1979). Phase B figs are receptive and attract adult female  
25 wasps (loaded with pollen) to enter them. Phase C female figs contain developing seeds,  
26 whereas phase C male figs contain wasp larvae developing in galled ovules. Male figs at D  
27 phase release the next generation of fig wasps, with female pollinators then flying away in  
28 search of receptive figs to enter. The subsequent E phase male figs wither and eventually  
29 abort. Female figs have an extended C phase, which ends when the figs become attractive to  
30 seed dispersers (E phase).

31 *Ficus montana*, *F. asperifolia*, F1s, and backcrosses were selected haphazardly from  
32 the plants available in the general glasshouse population, with six plants from each group and  
33 three plants from each sex. Figs from each plant were collected at a range of diameters from  
34 2.5 to 7.5 mm (one fig representing each 0.5 mm increment, if available, from each plant).

1 Pollination of the figs once they were receptive was allowed to occur naturally. The figs were  
2 examined under a binocular microscope (after being split longitudinally) and the style lengths  
3 of all the female flowers from a quarter of each fig were measured under a compound  
4 microscope (40× magnification) using an ocular scale. The measurements followed Nefdt &  
5 Compton (1996), where style length was taken as the distance between the top of the stigma  
6 and the end of the style, where it is connected to the ovule.

7

### 8 **Style lengths in receptive phase figs**

9 Thirty-one pre-receptive (A phase) figs from three male plants and 33 figs from three female  
10 plants of *F. montana* were chosen haphazardly. The figs were bagged until they became  
11 potentially receptive and were then tested for receptivity by introducing wasps to the ostiole  
12 area. Any attempt by the wasps to enter the figs was considered to indicate that the figs were  
13 receptive. These figs were collected and fig diameter was measured to record the range of  
14 sizes of figs at receptivity and then dissected under a binocular microscope. All the flowers in  
15 the figs were removed and positioned horizontally on slides to measure the styles, as  
16 described above.

17 With F1s, backcrosses, and *F. asperifolia*, three plants from each sex were chosen  
18 haphazardly and five figs of varying sizes from each plant were scored for receptivity. Due to  
19 the absence of its own pollinator, potentially 'receptive' figs of *F. asperifolia* were tested by  
20 first rubbing the ostiole of receptive *F. montana* figs onto the ostiole of *F. asperifolia* figs  
21 before introducing *K. tentacularis* females to the area of the ostiole. Successful entry of the  
22 pollinators was used as an indicator of receptivity. The figs were then divided longitudinally  
23 into four pieces, with the flowers counted and styles measured as indicated above.

24 Ovipositors of *K. tentacularis* were measured by collecting females as they emerged  
25 from 25 mature (D phase) *F. montana* figs obtained from five trees (five figs from each  
26 plant). Up to 10 wasps from each fig were collected, slide mounted, and ovipositors were  
27 measured under a compound microscope using an ocular scale after displacement of the  
28 sheaths (Nefdt & Compton, 1996). In addition, the ovipositors of *K. hilli*, the pollinator of *F.*  
29 *asperifolia*, were also measured. These were reared from their host figs by SG Compton & C  
30 Nuttman in Kibale Forest, Uganda.

31

### 32 **Data analysis**

33 All statistical analyses were performed in R (v.2.12.2). We used Wilcoxon's rank sum tests to  
34 compare ovipositor lengths of the pollinators of *F. montana* and *F. asperifolia* (*K.*

1 *tentacularis* and *K. hilli*, respectively). Nested ANOVA (figs within plants) was used to  
2 compare differences in style lengths of female flowers at receptivity, with trees nested within  
3 groups (parental species and offspring generations). F tests (R procedure ‘variance’) were  
4 used to compare the equality of variances in style lengths between sexes.  
5

## 6 **Results**

### 7 **Style lengths during fig development**

8 In figs of both sexes, and throughout their development, variance in the lengths of the styles  
9 was low (Figure 1). The mean style lengths of female flowers in female figs of *F. montana*  
10 were higher than in male figs at all stages of fig growth from early phase A to phase C, when  
11 seeds and the next generation of fig wasps are developing (Figure 1A). In male figs the  
12 maximum mean style length was 0.38 mm, at a time when fig diameter was 5.0 mm, towards  
13 the end of the receptive phase (when diameters were 4.0–5.5 mm). In female figs the mean  
14 maximum style length was 1.15 mm, recorded at a fig diameter of 6.5 mm, which was just  
15 after the end of the receptive phase (diameters 4.0–6.0 mm). In general, the styles in male and  
16 female figs of *F. montana* started to shrink when receptivity ended, at the time when the fig  
17 wasp offspring and seeds would have started developing.

18 *Ficus asperifolia* figs were available only from early phase A to late phase B, because  
19 these figs were rarely entered by *K. tentacularis* females. Style lengths in female figs of *F.*  
20 *asperifolia* were again longer than in male figs throughout (Figure 1B). The maximum mean  
21 style length in male figs was 0.43 mm, reached at a fig diameter of 7.5 mm, which coincided  
22 with the end of the putative receptive phase (the range at which pollinators would attempt  
23 entry was 5.5–7.5 mm). In female figs the maximum mean style length was 1.05 mm, at a fig  
24 diameter of 6.5 mm, which again coincided with the putative receptive phase (range 6.0–7.5  
25 mm).

26 In very young figs on F1 plants, style lengths in female figs were marginally shorter  
27 than in male figs of the same diameter, but they had become much longer by the receptive  
28 phase, as in both parents (Figure 1C). The maximum mean style length in male figs was 0.39  
29 mm, at a diameter of 6.5 mm, which was after the receptive phase (diameters 4.0–5.5 mm). In  
30 female F1 figs the maximum mean style length was 1.18 mm, at a diameter of 7.0 mm, which  
31 again was well after the end of the receptive phase (range 4.0–5.5 mm).

32 On backcross plants, the mean style lengths in female figs were longer throughout  
33 (Figure 1D). In male figs the maximum mean style length was 0.5 mm at a diameter of 6.0

1 mm, during the receptive phase (4.5–6.5 mm). Maximum mean style length in female figs  
 2 reached 1.0 mm at a fig diameter of 6.5 mm, towards the end of the receptive phase (5.0–6.5  
 3 mm).

4 In summary, fig diameters during the receptive periods of male and female *F.*  
 5 *montana*, *F. asperifolia*, their F1s, and backcrosses were generally the same, or at least  
 6 largely overlapped. The styles in female figs were generally slightly longer even in very  
 7 young figs, and then elongated much more quickly. Style lengths in both sexes were  
 8 generally at their longest during the receptive period, before starting to shrink after  
 9 receptivity, but there was an apparent disconnect in development in F1s, where in both sexes  
 10 styles continued to elongate after the receptive period.

11

### 12 **Style lengths of receptive figs in relation to ovipositor lengths**

13 There was a consistent unimodal distribution in style lengths in both male and female figs  
 14 and the ovipositor lengths of the pollinators. On average, styles in receptive-phase male figs  
 15 were consistently much shorter than in female figs. The ovipositors of *K. tentacularis* females  
 16 ranged from 0.64 to 0.92 mm, and they were much longer than the styles of any flowers in  
 17 male figs of *F. montana* (range 0.25–0.46 mm; mean  $\pm$  SE = 0.33  $\pm$  0.0007 mm, n = 2 306;  
 18 Figure 2A). The styles in female figs of *F. montana* were much longer than in male figs  
 19 (range 0.61–1.41 mm; mean  $\pm$  SE = 0.98  $\pm$  0.002 mm, n = 3 342; Figure 2A) and the variance  
 20 in style lengths of male and female figs was also significantly different ( $F_{2305,3341} = [MP1]0.06$ ,  
 21  $P < 0.001$ ), with more variation amongst styles in female figs[sc2]. Although styles in female  
 22 figs were mostly longer than the *K. tentacularis* ovipositors, up to 33% of the female flowers  
 23 in these figs were potentially reached by ovipositors of *F. montana* females (based on the  
 24 longest recorded ovipositor) and 8.9% of the styles were shorter than the mean length of the  
 25 fig wasps' ovipositors.

26 Styles of female flowers in receptive male figs of *F. asperifolia* (not a natural host for  
 27 *K. tentacularis*) were again all shorter than the fig wasp's ovipositor (style lengths 0.25–0.62  
 28 mm; mean  $\pm$  SE = 0.47  $\pm$  0.001 mm, n = 914) and much shorter than the styles in female figs  
 29 (0.74–1.36 mm; mean  $\pm$  SE = 1.03  $\pm$  0.002 mm, n = 1 157; Figure 2B). As in figs of *F.*  
 30 *montana*, the variance in style lengths was also greater in female figs ( $F_{913,1155} = 4.76$ ,  
 31  $P < 0.001$ ). Up to 13.7% of the ovules of female flowers could be reached by the ovipositor of  
 32 *K. tentacularis* (based on the longest recorded ovipositor), but only 0.4% were accessible for  
 33 a female with an ovipositor of average length.

34 *Kradibia hilli* is the routine pollinator of *F. asperifolia* in East Africa. Its ovipositor

1 (mean  $\pm$  SE = 0.80  $\pm$  0.005 mm; n = 44) is significantly longer than that of *K. tentacularis*  
 2 (mean  $\pm$  SE = 0.76  $\pm$  0.003 mm; n = 214; Wilcoxon test: W = 1 838, P<0.001) and potentially  
 3 reaches all the ovules in male figs of *F. asperifolia* (Figure 2C). It could also reach 1.5% of  
 4 the ovules in female figs (based on its mean ovipositor length).

5 All ovules in male F1 hybrids were accessible to *K. tentacularis* (style length range  
 6 0.32–0.57 mm; mean  $\pm$  SE = 0.45  $\pm$  0.001 mm, n = 862; Figure 3A). Style lengths of flowers  
 7 in female figs were again much longer (range 0.87–1.36 mm; mean  $\pm$  SE = 1.11  $\pm$  0.003 mm,  
 8 n = 795; Figure 3A) and the variance between style lengths of female flowers in male figs  
 9 and female figs was again significantly different ( $F_{794,861} = 3.87$ , P<0.001). Around 1.9% of  
 10 female flowers in female figs of F1 plants could be reached with the longest recorded  
 11 ovipositor of *K. tentacularis*, but all the female flowers in female figs were longer than the  
 12 mean ovipositor length.

13 Styles of female flowers in male figs of backcrosses were again all shorter than the  
 14 ovipositor of *K. tentacularis* (range 0.3–0.47 mm; mean  $\pm$  SE = 0.38  $\pm$  0.001 mm, n = 651;  
 15 Figure 3B). The much longer styles in female figs (0.67–1.31 mm; mean  $\pm$  SE = 0.99  $\pm$  0.006  
 16 mm, n = 558; Figure 3B) again displayed a larger variance in length than in male figs ( $F_{650,557}$   
 17 = 25.76, P<0.001). Around 36% of the ovaries of female flowers in female figs could be  
 18 reached with the longest recorded ovipositor of *K. tentacularis* and 8.2% of the female  
 19 flowers were shorter than its mean ovipositor length.

20 Styles of female flowers in male figs of *F. montana*, *F. asperifolia*, and its hybrids  
 21 were generally at least twice as long as in the corresponding male figs (Figure 4). In male figs  
 22 of F1s, style lengths were intermediate between those of the parents and so were longer than  
 23 those of *F. montana*, but shorter than those of *F. asperifolia* (the maternal parent). Styles in  
 24 the backcrosses were again longer than those of *F. montana* but were shorter than those of the  
 25 F1s (the maternal parent). There was a significant overall difference in style lengths among  
 26 male figs of the parental and other generations (nested ANOVA:  $F_{3,4728} = 457.86$ , P<0.001).  
 27 Using pairwise t tests for differences between groups, significant differences were found  
 28 among all four groups (all P<0.001).

29 In female figs, the styles were longer than in both parents (Figure 4), whereas  
 30 backcrosses were intermediate between *F. montana* (paternal parents) and the F1s (maternal  
 31 parents). Differences between groups in style lengths in female figs were again highly  
 32 significant (nested ANOVA:  $F_{3,5846} = 218.73$ , P<0.001). Pairwise t tests detected significant  
 33 differences among all four groups (all P<0.002).

34 Styles in female figs were consistently more variable in length than in male figs

1 (Figure 3), but coefficients of variation were almost similar between the sexes (Table 1). This  
2 suggests that the higher variance recorded in female figs can largely be attributed to their  
3 longer length.

## 4 **Discussion**

5 The styles in male and female figs of *F. asperifolia* and *F. montana* reached a maximum  
6 length at about the time that the figs were receptive, and then began to shrink. Growth of the  
7 styles before receptivity was much more rapid in female figs, resulting in them having much  
8 longer maximum lengths, but the ovipositors of *K. tentacularis* females were sufficiently long  
9 to reach some of their ovules in female figs of both the normal and atypical host figs. In  
10 contrast to the parental species, styles in F1 hybrids continued to grow after the receptive  
11 period, especially in female figs. Mean style lengths during receptivity were intermediate  
12 between the parents in male F1s, but longer than either parent in female F1s. Backcrosses to  
13 *F. montana* were consistently intermediate between F1s and *F. montana*.

14  
15 Female figs of *F. montana* and *F. asperifolia* are receptive to pollinators at the same  
16 diameter as male figs, as is to be expected given their need for mutual mimicry (Grafen &  
17 Godfray, 1991; Moore et al., 2003). Pollinators that enter female figs will always fail to  
18 produce any offspring and this should generate strong selection to avoid these figs. Although  
19 the emission of similar (though not always identical) volatile blends from receptive figs is a  
20 key element of this deception (Grisson-Pigé et al., 2001), pollinators searching for oviposition  
21 sites could also respond to visual cues if receptive figs were of different sizes.

22 Styles at receptivity in male figs were shorter than in female figs, as has been  
23 described for all other dioecious fig tree species (Weiblen, 2002; Shi et al., 2006; Ma et al.,  
24 2009). At the time of receptivity, styles in female figs are also much more variable in length  
25 than those in male figs. This is likely to be advantageous in terms of pollen capture, whereas  
26 the less variable and shorter styles in male figs facilitate oviposition by the pollinators.  
27 Development of the longer styles in female figs starts when they are very small, and  
28 accelerates up until receptivity, by which point the styles stop growing and then start to  
29 shrink. In *F. montana*, *F. asperifolia*, and backcrosses to *F. montana* the period of receptivity  
30 corresponds with the cessation of growth by the styles in both male and female figs. This was  
31 not the case with F1 figs, where receptivity ended at a time when the styles were still  
32 continuing to grow. *Krabidia tentacularis* cannot reproduce in male figs of *F. asperifolia*,  
33 F1s, and most backcrosses, a failure that seems linked to an inability to induce gall  
34 development, because eggs are laid in all three groups of male figs (Ghana et al., 2015a). The

1 slight disconnect between style growth and receptivity in male F1s could possibly be linked  
2 to a failure to respond to gall induction, if responses to galling can only be initiated in flowers  
3 where the styles have reached their maximum length, although at present there is no evidence  
4 to support this claim. Female F1 figs (and backcrosses) produce viable seed without any  
5 apparent costs resulting from the lack of synchrony between maximum style length and  
6 receptivity (Ghana, 2012).

7         Styles in male figs of all groups were much shorter than the ovipositor of *K.*  
8 *tentacularis*, and an ability to reach the ovules has been confirmed by the presence of eggs  
9 laid in *F. asperifolia*, F1, and backcross figs (Ghana et al., 2012). The longer ovipositor of *F.*  
10 *asperifolia*'s pollinator *K. hilli*, compared with *K. tentacularis*, is likely to reflect the slightly  
11 longer mean style length in its male figs (Nefdt & Compton, 1996). *Kradibia hilli* potentially  
12 reach all the flowers in male figs of *F. montana* and would also be able to reach more ovules  
13 inside female figs of *F. montana* than *K. tentacularis*, if the opportunity ever arose.

14         The female flowers in male figs of F1s had styles that were intermediate in length  
15 between those of its parents, but closer to those of *F. asperifolia*. Whether this was linked to  
16 *F. asperifolia* being the maternal parent is unclear, but the overall volatile profile of F1s  
17 during receptivity is also more like that of *F. asperifolia* than that of *F. montana* (C Soler,  
18 pers. comm.). Phenotypic characters in hybrids are not always intermediate between those of  
19 their parents (Arnold & Hodges, 1995; Rosas et al., 2010) and styles in female F1 figs are an  
20 example of this. They attained longer mean lengths than those seen in either parent,  
21 apparently as a result of style lengths continuing to grow post-receptivity. Style lengths in  
22 both sexes of backcrosses were intermediate between their parents (*F. montana* and F1s).

23         The ovipositors of the largest *K. tentacularis* individuals appear capable of reaching  
24 up to one third of the ovaries in female figs of *F. montana* and backcrosses to *F. montana*,  
25 and fewer ovaries in female figs of *F. asperifolia* and F1s. Despite this, no eggs were  
26 detected in female figs of *F. montana* (Ghana et al., 2012). Success in laying eggs in female  
27 figs may be influenced by stigma shape and the lack of a clearly defined synstigma (the  
28 platform formed from the tops of the stigmas, on which pollinators stand during oviposition),  
29 which are likely to make it harder for the fig wasps to insert the full length of their  
30 ovipositors to reach the ovules (Galil, 1973; Verkerke, 1986; Jousset & Kjellberg, 2001).  
31 These factors, in combination with style length per se, appear to prevent oviposition by the  
32 pollinators when they enter female figs. The shape and presentation of the stigmas of fig  
33 flowers varies considerably between taxonomic groups (and sexes) within *Ficus* (Verkerke  
34 1989; Berg & Corner, 2005). The significance of this variability may have been

1 underestimated.

2

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8

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13  
 14

#### 15 **Figure captions**

16 **Figure 1** Changes in the style lengths of female flowers in male (diamond-shaped symbols)  
 17 and female (squares) figs of (A) *Ficus montana*, (B) *F. asperifolia*, (C) F1s, and (D)  
 18 backcrosses to *F. montana*. The horizontal lines indicate the receptive phase when *Kradibia*  
 19 *tentacularis* females attempted entry into the figs. Standard errors were too small to be visible  
 20 in the figure.

21

22 **Figure 2** Distribution of ovipositor lengths (hatched bars) of (A, B) *Ficus montana*'s  
 23 pollinator (*Kradibia tentacularis*) and style lengths (at receptive phase) in male (white bars)  
 24 and female (black bars) figs of (A) *F. montana* and (B) *F. asperifolia*, and of (C) *F.*  
 25 *asperifolia*'s pollinator (*K. hilli*) and style lengths in male and female figs of *F. asperifolia*.

26

27 **Figure 3** Distribution of ovipositor lengths (hatched bars) of *Ficus montana*'s pollinator  
 28 (*Kradibia tentacularis*) and style lengths (at receptive phase) in male (white bars) and female  
 29 (black bars) (A) F1 hybrid figs and (B) backcrosses to *F. montana*.

30

31 **Figure 4** Mean ( $\pm$  SE) style lengths in receptive male (white bars) and female (grey bars) figs  
 32 of *Ficus montana*, *F. asperifolia*, F1 hybrids, and backcrosses to *F. montana*.

33

1 **Table 1** Style length (mm) in receptive male and female figs of *Ficus montana*, *F.*  
 2 *asperifolia*, F1 hybrids, and backcrosses to *F. montana*

Ficus	Sex	No. figs	Style length (mm)		
			Mean	Variance	CV
<i>F. montana</i>	Female	33	0.977	0.020	0.146
	Male	31	0.334	0.001	0.104
<i>F. asperifolia</i>	Female	15	1.030	0.009	0.090
	Male	15	0.469	0.002	0.090
F1	Female	15	1.109	0.008	0.079
	Male	15	0.455	0.002	0.097
Backcross	Female	15	0.994	0.022	0.148
	Male	15	0.376	0.001	0.077

3 CV = Coefficient of variation.

4

