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The natural history of pollination and mating in bird-pollinated *Babiana* (Iridaceae)

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• *Background and Aims* Floral variation, pollination biology and mating patterns were investigated in sunbird-pollinated *Babiana* (Iridaceae) species endemic to the Western Cape of South Africa. The group includes several taxa with specialized bird perches and it has been proposed that these function to promote cross-pollination.

• *Methods* Pollinator observations were conducted in 12 populations of five taxa (*B. ringens* subspp. *ringens*, *australis*, *B. hirsuta*, *B. avicularis*, *B. carminea*) and geographic variation in morphological traits investigated in the widespread *B. ringens*. Experimental pollinations were used to determine the compatibility status, facility for autonomous self-pollination and intensity of pollen limitation in six populations of four taxa. Allozyme markers were employed to investigate mating patterns in four populations of three species.

• *Key Results* Sunbirds were the primary pollinators of the five *Babiana* taxa investigated. Correlated geographical variation in perch size, flower size and stigma–anther separation was evident among *B. ringens* populations. Experimental pollinations demonstrated that *B. ringens* and *B. avicularis* were self-compatible with variation in levels of autonomous self-pollination and weak or no pollen limitation of seed set. In contrast, *B. hirsuta* was self-incompatible and chronically pollen limited. Estimates of outcrossing rate indicated mixed mating with substantial self-fertilization in all species investigated.

• *Conclusions* Despite the possession of specialized bird perches in *B. ringens* and *B. avicularis*, these structures do not prevent considerable selfing from occurring, probably as a result of autonomous self-pollination. In eastern populations of *B. ringens*, smaller flowers and reduced herkogamy appear to be associated with a shift to predominant selfing. Relaxed selection on perch function due to increased selfing may explain the increased incidence of apical flowers in some populations.

Key words: *Babiana*, bird perch, sunbird pollination, floral variation, mating, pollen limitation, relaxed selection.

INTRODUCTION

Bird pollination (ornithophily) has evolved independently in many lineages of flowering plants. Approximately 65 families contain ornithophilous species with many independent origins of the pollination system, often from bee-pollinated ancestors (Stiles, 1981; Cronk and Ojeda, 2008; Wilson *et al.*, 2007; but see Martén-Rodríguez *et al.*, 2010). Three bird families (Trochilidae – hummingbirds, Nectariniidae – sunbirds, Meliphagidae – honey-eaters) are regarded as flower specialists (Proctor *et al.*, 1996), and different feeding strategies within these families have selected for contrasting suites of adaptive traits in ornithophilous plants (Westerkamp, 1990). Hummingbirds primarily hover during nectar feeding, whereas sunbirds and honeyeaters mainly perch on various vegetative or reproductive structures. A considerable literature documents the floral biology of hummingbird-pollinated species (reviewed in Grant and Grant, 1968; Thomson and Wilson, 2008), but less is known about the reproductive ecology and floral evolution of species pollinated by sunbirds and honeyeaters. Most sunbird-pollinated flowers are exploited by several species (Skead, 1967; Gill and Wolf, 1978),

although there is evidence for floral specialization to either long-billed malachite sunbirds or short-billed species among sunbird-pollinated plants in South Africa (Geerts and Pauw, 2009).

Adaptive radiation of pollination systems in African Iridaceae is closely associated with floral divergence and speciation (Goldblatt and Manning, 2006). *Babiana*, a genus of animal-pollinated perennial geophytes mainly from the winter rainfall zone of western South Africa, is one of the larger genera of Iridaceae consisting of approx. 92 species. Most species have showy flowers and are pollinated by insects of four different orders: Hymenoptera (mainly Apidae), Diptera (mainly Nemestrinidae), Coleoptera (mainly Scarabaeidae) and Lepidoptera (mainly Noctuidae). Goldblatt and Manning (2007*a*, *b*) proposed that there have been at least 14 shifts in pollination system in *Babiana*, including at least two transitions from insect to bird pollination. Five *Babiana* taxa endemic to southern Africa (*B. ringens* subsp. *ringens*, *B. ringens* subsp. *australis*, *B. hirsuta*, *B. avicularis* and *B. carminea*; Fig. 1) are pollinated by sunbirds, or have been inferred to be sunbird pollinated, based on floral morphology (Goldblatt and Manning, 2007*b*).
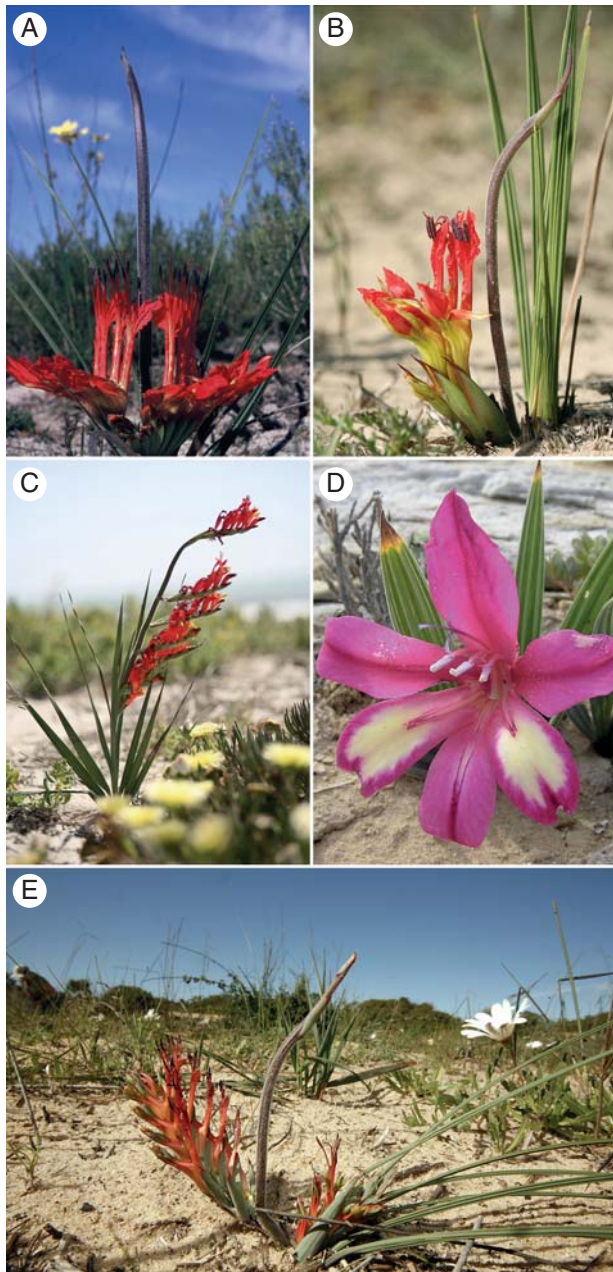
FIG. 1. Bird-pollinated species of *Babiana* (Iridaceae) from the Western Cape Province, South Africa: (A) *B. ringens* subsp. *ringens*; (B) *B. ringens* subsp. *australis*; (C) *B. hirsuta*; (D) *B. carminea*; (E) *B. avicularis*.

FIG. 2. Female malachite sunbird using the specialized bird perch of *Babiana ringens* subsp. *ringens* to feed from flowers.

A remarkable adaptation for bird pollination occurs in *B. ringens*. In this species the sterile inflorescence axis functions as a specialized bird perch for visiting malachite sunbirds (Fig. 2). Marloth (1898, 1915) first recorded sunbird visits to *B. ringens* flowers and suggested the function of the perch. However, until recently the function of the perch in *B. ringens* had not been investigated experimentally. Based on observations and manipulative studies of two populations of *B. ringens*, Anderson *et al.* (2005) proposed that the perch manipulates the position of sunbirds, during nectar feeding from the species' ground-level flowers, and that this facilitates contact with sexual organs promoting cross-pollination.

The recently described *B. avicularis* (Goldblatt and Manning, 2010) also possesses a naked inflorescence axis and ground-level flowers, raising the question of whether the inflorescence axis in this species has a similar function to the perch in *B. ringens*.

Here, the natural history of pollination and mating in bird-pollinated *Babiana* species is investigated. The main focus is on *B. ringens* because this species has the widest distribution and displays considerable geographical variation in reproductive traits, particularly flower size (Goldblatt and Manning, 2010). The study had four main objectives. (1) To record flower visitors and their foraging behaviour in populations of each of the five *Babiana* taxa. No previous pollinator observations had been made for *B. avicularis* and *B. carminea*, so we wanted to confirm their putative status as ornithophilous species. (2) To investigate geographic variation in perch length, flower size, floral-tube length and stigma–anther separation in *B. ringens*. We wanted to determine whether variation in these traits was associated with differences among populations in sunbird visitation, both in terms of visitation rates and sunbird species. (3) To evaluate the compatibility status, facility for autonomous self-pollination and intensity of pollen limitation of populations. Pollinator specialization in flowering plants may come at a cost if visitation rates are unreliable, and we sought to determine the extent to which species were capable of maintaining fertility through self-compatibility and autonomous self-pollination. (4) To quantify mating patterns in populations using genetic markers to assess

TABLE 1. *Estimated population sizes and observations of sunbirds in 11 populations of five bird-pollinated* Babiana *taxa (*B. ringens *subsp.* ringens, B. ringens *subsp.* australis, B. hirsuta, B. avicularis, B. carminea) *during August–October 2009 in the Western Cape Province of South Africa*

| | | | | | | | No. of visits to single inflorescence | |
Taxon	Population	Estimated population size	No. of days observed	Total observation period (min)	Mean no. of inflorescences observed per day	Mean visitation rate (visits inflorescence^{-1} h^{-1}) (s.e.)	Malachite	Southern double-collared
B. ringens	MAM	150	3	330	33	0·25 (0·11)	41	0
subsp. *ringens*	ROND	40	2	240	11	0·20 (0·09)	9	0
	HOPE	75	3	360	9	0·15 (0·15)	7	0
B. ringens	SCAR	150	3	290	64	0·13 (0·03)	36	0
subsp.	DEK	50	2	105	15	0·00	0	0
australis	VIC1	500	3	540	30	0·004 (0·004)	0	1
	VIC2	300	1	180	15	0·00	0	0
B. hirsuta	VELD	>5000	3	460	44	0·65 (0·06)	6	220
	ELA	>1000	3	330	26	0·54 (0·14)	42	36
	LAM1	>1000	3	430	40	1·08 (0·9)	20	50
B. avicularis	DRAAI	300	3	360	24	0·07 (0·07)	0	14
B. carminea	VANR	90	2	300	18	0·08 (0·03)	8	0

whether specialized adaptations for bird pollination are associated with cross-pollination and high outcrossing rates.

MATERIALS AND METHODS

Study species and sites

Babiana ringens occurs on sandy flats and lower slopes in strandveld and fynbos vegetation. Populations are rarely large in size and are typically composed of scattered individuals that appear after fire (Table 1). Two subspecies have been described differing in geographical distribution and morphology (Goldblatt and Manning, 2010). *Babiana ringens* subsp. *ringens* (Fig. 1A) is patchily distributed along the West Coast region, and individuals have robust perches and large red flowers. In contrast, *B. ringens* subsp. *australis* (Fig. 1B) occurs on the southern Cape Peninsula and east to the region surrounding Albertinia. Individuals of this subspecies have smaller perches and flowers.

Babiana hirsuta (Fig. 1C) occurs on coastal sand flats and dunes along the west coast of South Africa (Goldblatt and Manning, 2007b). Although the species is distributed over approx. 500 km, it is rarely found >200 m from the shoreline. *Babiana hirsuta* is clonal with genets producing many showy inflorescences and hundreds of flowers and unlike the other species considered here population sizes can be very large.

The remaining two *Babiana* species are highly restricted in distribution and poorly known. Despite extensive fieldwork only a single population of each species was located. *Babiana avicularis* (Fig. 1E) is restricted to the west coast and near interior of the Western Cape and is reported from just four localities in strandveld (Goldblatt and Manning, 2010). Flowers of *B. avicularis* are orange to orange–green in colour and are produced on one to three horizontal basal branches. *Babiana carminea* (Fig. 1D) is limited to limestone outcrops in the Knersvlakte of southern Namaqualand where plants grow in crevices, and corms are tightly wedged in bedrock. The species is morphologically distinct from the preceding three species and possesses large carmine red flowers that arise from a tuft of leaves near ground level (Goldblatt and Manning, 2007b).

The locations of all populations investigated in the present study are mapped in Fig. 3 and details of localities are listed in Supplementary Data Table S1 (available online). With the exception of *B. hirsuta*, which was commonly encountered, all populations of the remaining taxa located were included in this study.

Pollinator observations

Pollinator observations were made during July–October 2009 at three populations of *Babiana ringens* subsp. *ringens* (HOPE, MAM and ROND), four populations of *B. ringens* subsp. *australis* (SCAR, DEK, VIC1 and VIC2), three populations of *B. hirsuta* (VELD, LAM1 and ELA), and one population each of *B. avicularis* (DRAAI) and *B. carminea* (VANR). Flower visitors and their behaviour were observed over a period of 1–3 d per site (mean = 2·6 d) for between 30 and 190 min each day (mean = 131·1 min d^{-1}) just after sunrise when sunbirds are most active. Observers took the same vantage point each day so as to view the maximum number of plants (mean 31·6 plants per population, range 8–80). The observers were far enough from plants so as not to deter birds. For each population the number and species of sunbirds visiting *Babiana* individuals were recorded. A visit was regarded as when a bird landed on a plant and probed flowers. Mean visitation rate per population was standardized as the number of visits per inflorescence observed per hour of observations over *n* number of observation days. Where possible it was also recorded whether sunbirds visited flowers legitimately, or whether they robbed nectar, and also if they were present in the surrounding plant community and not feeding on *Babiana*, either flying over the study population, or sitting and/or feeding on neighbouring vegetation. Finally, in all populations the number of open flowers per individual showing signs of nectar robbery, in the form of a narrow slit on the floral tube, was recorded.
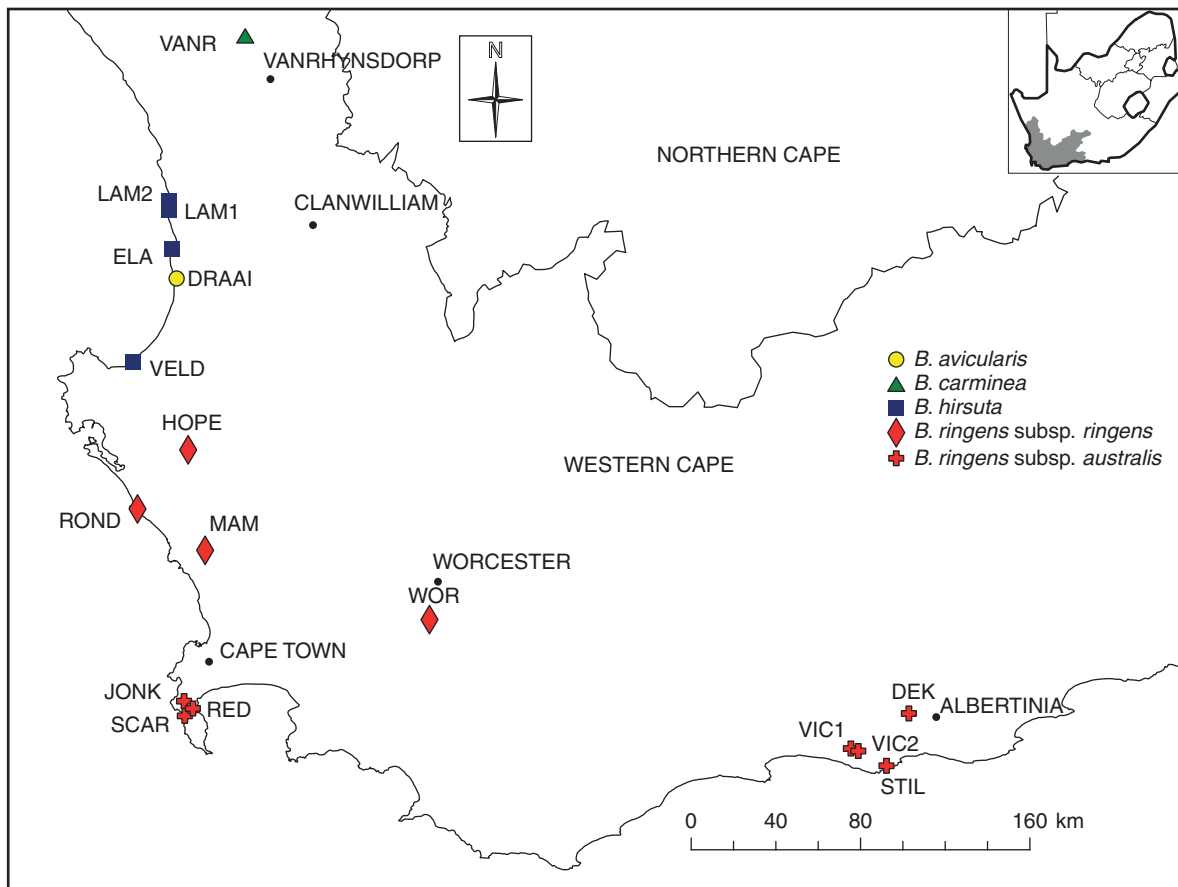
FIG. 3. Localities of bird-pollinated *Babiana* species from the Western Cape Province, South Africa that were investigated in this study.

Variation in flower visitors were compared among populations using log-transformed visitation rate with an ANOVA model that included plant species and populations nested within plant species. Unless otherwise noted, this and all subsequent analyses were performed using JMP Version 8·02 (SAS, 2009).

Variation in morphological traits

To investigate intra-specific variation of perch length, tepal size, floral-tube length and stigma–anther separation in *B. ringens*, individuals were randomly sampled from a total of ten populations (four, *B. ringens* subsp. *ringens*; six, *B. ringens* subsp. *australis*) during peak flowering from August to October 2009. These traits were also measured in the single population of *B. avicularis*. In both species, the numbers of buds, flowers or fruits on the apical portion of perches were also recorded. For floral traits, one to three flowers per individual were sampled using calipers, and the mean used in subsequent analyses. Sample sizes for the four traits are given in Fig. 4. One-way ANOVAs were used to analyse morphological variation among populations and Tukey–Kramer tests to determine which populations differed significantly for a particular trait.

Factors influencing fertility

To investigate factors influencing fruit and seed set, controlled pollinations and caging treatments were performed in one

population of *B. ringens* subsp. *ringens* (MAM), two populations of *B. ringens* subsp. *australis* (SCAR and VIC1), two populations of *B. hirsuta* (LAM2 and VELD) and one population of *B. avicularis* (DRAAI) during July–September 2009. Not all types of pollination data were collected from each population because of sample size limitations, antelope grazing and vandalism. At best the following five treatments were conducted in each population: (1) hand self-pollination; (2) hand cross-pollination; (3) unmanipulated and caged; (4) removal of stamens; (5) open-pollination. When fruits were ripe, fruit and seed set per fruit were recorded in each treatment. Sample sizes are provided in Supplementary Data Table S2. Comparison of the fertility of (1) versus (2) provides evidence for self-compatibility versus self-incompatibility. Treatment (3) provides evidence for the occurrence of autonomous self-pollination. Treatment (4) indicates the amount of cross-pollination. Comparison of (2) versus (5) provides evidence for pollen limitation of seed set.

For cross-pollinations, all flowers on a plant were pollinated with a mixture of pollen from flowers of at least three pollen donors collected several metres away to reduce the incidence of inbreeding. In *B. hirsuta* populations, plants were covered with mesh wire cages following hand-pollination to prevent damage from antelope herbivory. To investigate whether plants were self-compatible, flowers were pollinated with self-pollen by rubbing dehisced anthers picked from either the same flower, or other flowers on the same plant. To investigate
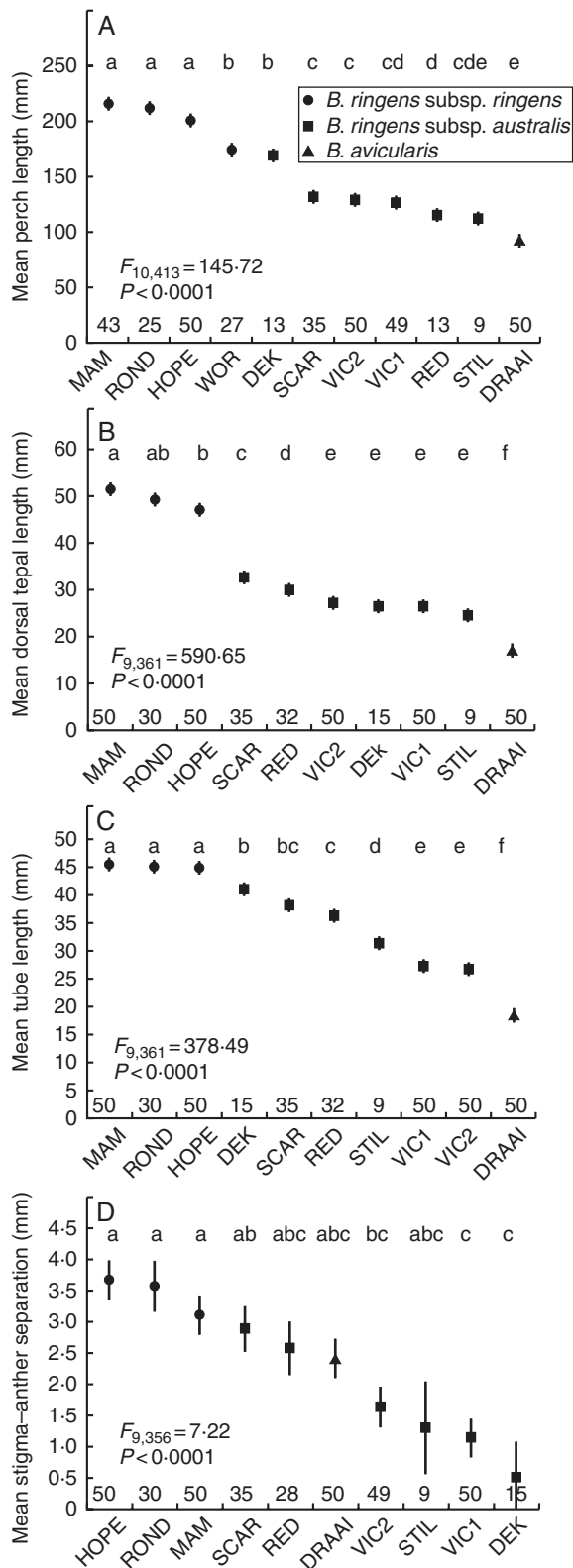
Fig. 4. Mean ($\pm$ s.e.) of (A) perch length, (B) dorsal tepal length, (C) tube length and (D) stigma–anther separation in populations of *Babiana ringens* subsp. *ringens* and *B. ringens* subsp. *australis*, and one *B. avicularis* population, as indicated. Means that share the same letter are not significantly different ($P < 0.05$). Sample sizes are provided for each trait.

the contribution of cross-pollen to fruit and seed set, self-pollination was prevented by removing undehisced anthers from mature buds with fine forceps the day before the bud was due to open, and this procedure was replicated in all buds on the inflorescence. It was only possible to emasculate flowers in two populations of *Babiana ringens* subsp. *australis*. To investigate the capacity for autonomous self-pollination, plants were covered with wire mesh cages before flowering to prevent sunbird visitation and the plants left undisturbed until fruits were mature. For populations of both *B. ringens* subspecies and *B. avicularis*, hand-pollinations were performed on three or four consecutive days, pollinating all newly opened flowers and any remaining small buds were removed after this period. In *B. hirsuta*, where floral displays were too large for supplemental pollinations of all flowers, a subset of flowers was pollinated and the base of the flowers marked with colour paint so that they could be identified at harvest.

The effects of the experimental treatments on proportion fruit set and seed set per fruit were analysed using ANOVA. Because not all experimental treatments were replicated in each population of *B. ringens*, variation among treatments was analysed separately in each population. The same experimental treatments were replicated in *B. hirsuta* populations and therefore population, treatment and their interaction were included in the ANOVA model. Tukey–Kramer HSD tests were used to examine whether treatment means differed significantly among *B. hirsuta* populations, but separately within each population of *B. ringens* and *B. avicularis*. To test for self-compatibility, autofertility and pollen limitation, proportion fruit and seed set per fruit were compared in specific contrasts of treatments, as outlined above using Tukey–Kramer tests.

Estimates of mating patterns

During July–October 2009, open-pollinated capsules were randomly sampled at maturity from plants in two populations of *Babiana ringens* subsp. *ringens* (HOPE, WOR), two populations of *B. ringens* subsp. *australis* (SCAR, VIC1), two populations of *B. hirsuta* (VELD, ELA) and one population each of *B. avicularis* (DRAAI) and *B. carminea* (VANR). Seeds were ground in a 0·02 M Na$_2$HPO$_4$ buffer (pH 7·4) with DL-dithiothreitol (1 mg mL^{-1}) and PVP-40. Extracts were adsorbed onto filter paper wicks and stored at -80 °C until electrophoresis was performed. Wicks were then inserted into 11–12 % starch gels. Enzymes were resolved on two buffer systems: (1) glutamate dehydrogenase, triose phosphate isomerase, alcohol dehydrogenase and phosphoglucoisomerase on lithium borate (pH 8·3); and (2) 6-phosphogluconate dehydrogenase, phosphoglucomutase, isocitrate dehydrogenase, malate dehydrogenase and acid phosphatase on histidine citrate (pH 6·5). Genotypes at a total of 15 allozyme loci were inferred based on segregation patterns characteristic of either dimeric or monomeric codominant enzymes; however, not all loci were polymorphic within populations and three loci (*PGD*-2, *ACP*-1 and *ACP*-3) were polymorphic but not consistently scoreable.

The multilocus outcrossing rate (t) was estimated for each population using the computer program MLTR (version 3·4; Ritland, 1990). This program uses maximum-likelihood to infer the genotypes of maternal parents, allele frequencies in the pollen pool, and the proportion of progeny resulting from outcrossing. The standard deviation of 1000 bootstrap values was used to derive the standard errors of outcrossing rate estimates, and the seed family was used as the unit of resampling. Expectation-maximization iteration was used to estimate maximum-likelihood values of the outcrossing rate. Between two and six loci (mean = four) were used to estimate mean outcrossing rates in each population.

RESULTS

Sunbird visitation to flowers

During 65·4 h of field observations over 31 d in 12 populations of five *Babiana* taxa a total of 492 visits was recorded to individual inflorescences, of which 34·35 % were made by malachite sunbirds, and 65·24 % by southern double-collared sunbirds. Two visits by a cape sugarbird and a dusky sunbird were also recorded in population LAM1 of *B. hirsuta*. Small bees and flies were occasionally observed on flowers but they did not contact the reproductive organs and consequently they were not regarded as significant pollinators and are not considered further.

Sunbirds differed in their foraging behaviour depending on the species of *Babiana*. In the three taxa with specialized perches, sunbirds always alighted on the inflorescence axis, rotated so that their bodies were facing downwards, and then probed flowers (Fig. 2). In *B. hirsuta*, sunbirds commonly landed on the prominent bend in the inflorescence axis (see Fig. 1C) and then proceeded to probe flowers head down by moving along the inflorescence axis and by perching on the sturdy side branches. Owing to the diminutive stature of *B. carminea* and the absence of perching structures, visiting malachite sunbirds probed flowers from the ground with the top of their heads contacting sexual organs.

Visitation rates by sunbirds varied considerably from day to day and among *Babiana* species and populations. Species accounted for a significant fraction of the observed variation in visitation rate ($F_{4,19} = 3·55$, $P = 0·0251$). Two populations of *B. ringens* subsp. *australis* (VIC2 and DEK) received no visits and VIC1 received only a single visit by a southern double-collared sunbird (Table 1). All three *B. ringens* subsp. *ringens* populations (MAM, ROND, HOPE), *B. ringens* subsp. *australis* population SCAR, and the *B. carminea* population (VANR) were visited only by malachite sunbirds, whereas the *B. avicularis* population (DRAAI) was visited only by southern double-collared sunbirds. Both malachite and southern double-collared sunbirds were observed visiting flowers in all three *B. hirsuta* populations (VELD, ELA, LAM1).

Although 82 % of all visits to *B. hirsuta* flowers were made by southern double-collared sunbirds, most were not legitimate as these birds did not commonly make contact with the sexual organs of flowers. In contrast, malachite sunbirds probed flowers legitimately. Southern double-collared sunbirds positioned their heads to the side of the flower and gained access to nectar by making a distinctive slit at the bottom of the floral tube. The mean percentage of flowers ($\pm$ s.e.) per plant that showed signs of nectar robbery in the three populations of *B. hirsuta* were VELD 91·6 $\pm$ 5·0, $n = 11$; LAM1 44·5 $\pm$ 4·4, $n = 41$; ELA 60·3 $\pm$ 4·8, $n = 50$. There was also evidence of moderate levels of nectar robbery in *B. avicularis* (DRAAI 21·2 $\pm$ 4·5, $n = 49$) and limited amounts in populations of *B. ringens* subsp. *ringens* (HOPE 3·1 $\pm$ 1·71, $n = 50$) and *B. ringens* subsp. *australis* (DEK 0, $n = 14$; RED 0, $n = 4$; STIL 11·1 $\pm$ 11·1, $n = 9$; VIC1 1·9 $\pm$ 1·5, $n = 47$; VIC2 3·1 $\pm$ 1·7, $n = 50$).

Geographic variation in perch and flower traits

Measurements of perch length, flower size, floral-tube length and stigma–anther separation in 11 populations of *Babiana ringens* and *B. avicularis* revealed significant differences among populations. Three of the four *B. ringens* subsp. *ringens* populations (MAM, ROND and HOPE) had significantly larger perches than populations of *B. ringens* subsp. *australis* and *B. avicularis* (Fig. 4A). The fourth population of *B. ringens* subsp. *ringens* (WOR) was situated farther south-east (Fig. 3) and in this population perches were not significantly longer than in the *B. ringens* subsp. *australis* population DEK ($P = 0·99$). *Babiana avicularis* (DRAAI) had the smallest perches (mean = 92·1 mm, $n = 50$), less than half the size of those in *B. ringens* subsp. *ringens* populations, although not significantly smaller than perches in the STIL population of *B. ringens* subsp. *australis* ($P = 0·34$). Sunbird visitation rates were positively correlated with perch length in the seven populations of *B. ringens* from which data on these parameters ($R^2 = 0·62$, $F = 8·11$, $P = 0·046$) were collected.

Flower size also varied considerably over the geographical range of *B. ringens*. MAM, ROND and HOPE had significantly larger mean dorsal tepal lengths than all other *B. ringens* populations (Fig. 4B). *Babiana avicularis* (DRAAI) had significantly smaller flowers than all populations of *B. ringens*. Similar patterns were also evident for floral-tube length with the shortest floral tubes evident in eastern populations of *B. ringens* (Fig. 4C). Associated with this geographic variation in flower size of *B. ringens* was variation in the degree of herkogamy among populations. Populations of subsp. *ringens* on average exhibited significantly larger stigma–anther separations than populations of subsp. *australis* (Fig. 4D).

Apical flowers in Babiana ringens *and* B. avicularis *populations*

No plants were observed in the four *B. ringens* subsp. *ringens* populations (MAM, HOPE, ROND, WOR) with buds, flowers or fruits on the apical portions of perches. However, 6·63 % ($n = 392$) of the individuals of *B. ringens* subsp. *australis*, sampled in five of the six study populations (DEK, RED, STIL, VIC1, VIC2), produced either one or two functional flowers on the tips of perches (Fig. 5A, B). Although *B. avicularis* most commonly produced one to two side branches at ground level (mean = 1·67, $n = 138$ plants), plants were found in which the side branches were produced farther above ground level near the inflorescence terminus resulting in apical flowers, which set fruit as in *B. ringens* subsp. *australis* (Fig. 5C).
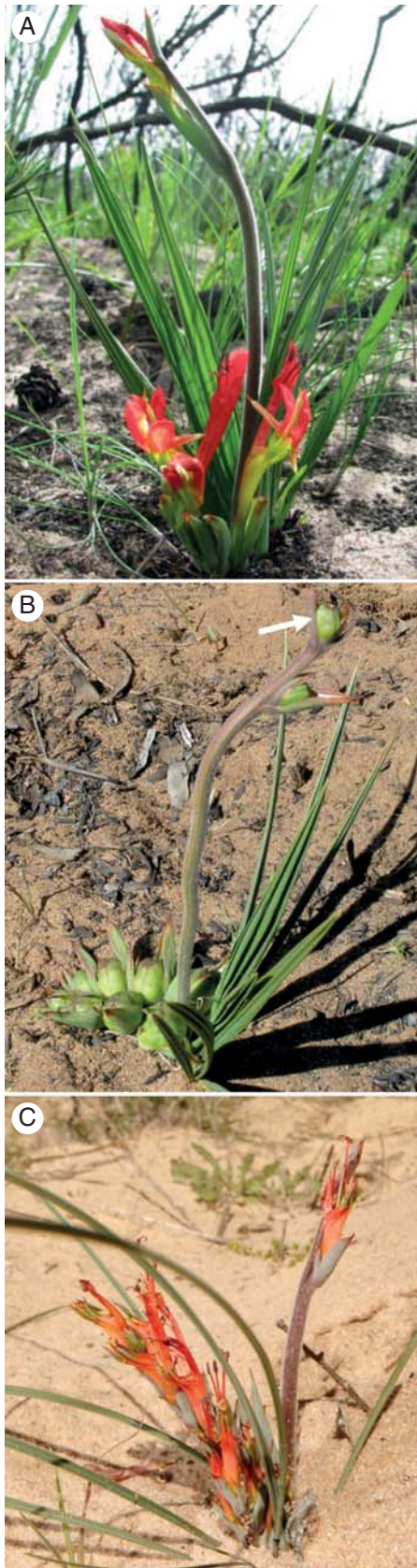
FIG. 5. Formation of apical flowers and fruits (A and B, respectively) in *Babiana ringens* subsp. *australis* and (C) *B. avicularis*.

Compatibility

Babiana ringens. Fruit and seed set did not differ significantly between hand cross- and self-pollination in VIC1, indicating strong self-compatibility [Tukey HSD; fruit set, $P = 0.35$ (Fig. 6A); seed set per fruit, $P = 0.72$ (Fig. 6B)]. Self-compatibility was also evident in MAM and SCAR because, in both populations, fruit and seed set was evident in caged flowers.

Babiana hirsuta. Hand self-pollination resulted in only 19 % and 23 % of the fruits and 57 % and 71 % fewer seeds per fruit compared with supplemental cross-pollination in VELD and LAM2, respectively [fruit set: VELD, $P < 0.0001$; LAM2, $P < 0.0001$ (Fig. 7A); seed set: VELD, $P = 0.023$; LAM2, $P < 0.0001$ (Fig. 7B)] indicating moderate self-incompatibility in both populations.

Babiana avicularis. Hand self- and cross-pollinations of plants at DRAAI produced abundant fruits and values were not significantly different ($P = 0.909$) indicating strong self-compatibility (Fig. 8).

Autonomous self-pollination

Babiana ringens. There was variation in the capacity for autonomous self-pollination among populations of *B. ringens*. Seed set was evident in caged flowers in populations where it was tested (MAM, SCAR and VIC1), although the amounts obtained were quite variable (Fig. 6B). The fruit set of caged flowers was significantly lower than open-pollinated flowers at MAM and SCAR (MAM, $P < 0.0001$; SCAR, $P = 0.0291$), and seed set per fruit was significantly lower in the autonomous selfing treatment compared with the open-pollinated treatment at MAM, but only marginally significant at SCAR (MAM, $P = 0.0068$; SCAR, $P = 0.0715$). However, at VIC1 there was no significant difference in fruit and seed set per fruit between hand self-pollinated and caged flowers (fruit set, $P = 0.19$; seed set, $P = 0.79$), indicating a well-developed capacity for autonomous self-pollination.

Babiana hirsuta. Caged flowers set only 3 % and 6 % of the fruits obtained by hand cross-pollination at VELD and LAM2, respectively. Compared with flowers that had been self-pollinated, fruit set of caged flowers in both populations was significantly lower, although in both cases only low levels of fruit set were evident (VELD, $P = 0.01$; LAM2, $P = 0.018$; Fig. 7A). Seed set per fruit did not differ significantly between hand self-pollinated and caged flowers in either population (VELD, $P = 0.525$; LAM2, $P = 0.140$; Fig. 7B).

Babiana avicularis. Caged flowers of *B. avicularis* set abundant fruit, demonstrating a high capacity for autonomous seed set at DRAAI. Caged flowers set 21 % less fruit and produced 38 % fewer seeds than was obtained from hand self-pollination [fruit set, $P = 0.01$ (Fig. 8A; seed set per fruit, $P < 0.0001$ (Fig. 8B)]. Fruit and seed set of caged flowers was 80 % and 84 %, respectively, of the fruit and seed set obtained by hand cross-pollination (fruit set, $P = 0.03$; seed set, $P < 0.0001$).
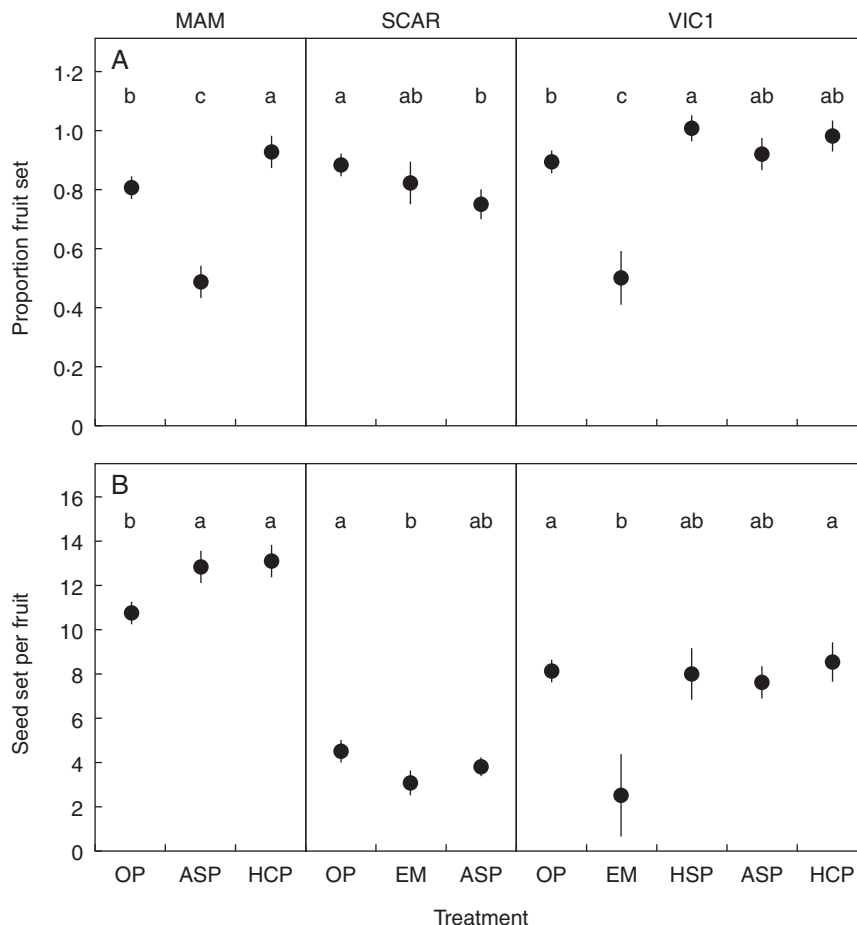
FIG. 6. Mean ($\pm$ s.e.) of (A) proportion fruit set and (B) seed set in populations of *Babiana ringens* subsp. *ringens* (MAM) and *B. ringens* subsp. *australis* (SCAR and VIC1) following experimental treatments: open-pollinated (OP), emasculation (EM), hand self-pollination (HSP), autonomous self-pollination (ASP) and supplementary cross-pollination (HCP). Means that share the same letter are not significantly different ($P < 0.05$) among treatments within each population.

Pollen limitation

Babiana ringens. The two populations of *B. ringens* investigated for pollen limitation of fruit and seed set differed in their response to the treatments (Fig. 6A, B). In MAM, fruit and seed set were 13 % and 18 % higher, respectively, in hand cross-pollinated flowers compared with open-pollinated flowers, demonstrating pollen limitation (fruit set, $P = 0.0341$; seed set per fruit, $P = 0.0023$). In contrast, at VIC1 the two treatments were not significantly different, indicating no pollen limitation of maternal fertility (fruit set, $P = 0.0887$; seed set per fruit, $P = 0.707$).

Babiana hirsuta. There was clear evidence of strong pollen limitation of fertility in both populations of *B. hirsuta*. Averaging across both populations, open-pollinated fruit set was only 8 % of that obtained from hand cross-pollination. At VELD, fruit set was significantly higher in the supplementary cross-pollination treatment than in the open-pollinated treatment, but there was no significant difference in seed set per fruit [fruit set, $P < 0.0001$ (Fig. 7A); seeds per fruit, $P = 0.341$ (Fig. 7B)]. At LAM2 the supplementary cross-pollination treatment resulted in significantly higher fruit and seed set (fruit set, $P < 0.0001$; seed set per fruit, $P < 0.0001$).

Babiana avicularis. There was no evidence of pollen limitation at DRAAI, as fruit and seed set per fruit did not differ significantly between the supplemental cross-pollinated and open-pollinated treatments [fruit set, $P = 0.50$ (Fig. 8A); seed set per fruit, $P = 0.762$ (Fig. 8B)].

Contribution of autogamy to self-fertilization

At the SCAR population of *B. ringens* ssp. *australis*, fruit set did not differ significantly between the emasculation and open-pollinated treatment, but seed set per fruit was 34 % lower in the emasculation treatment [fruit set, $P = 0.448$ (Fig. 6A); seed set per fruit, $P = 0.0055$ (Fig. 6B)]. At VIC1, both fruit set and seed set per fruit were significantly lower in the emasculation treatment compared with the open-pollinated treatment (fruit set, $P < 0.0001$; seed set, $P = 0.0029$).

Mating patterns

Of the eight populations of bird-pollinated *Babiana* species that were screened for allozyme polymorphisms, only four populations of three species provided sufficient variation to
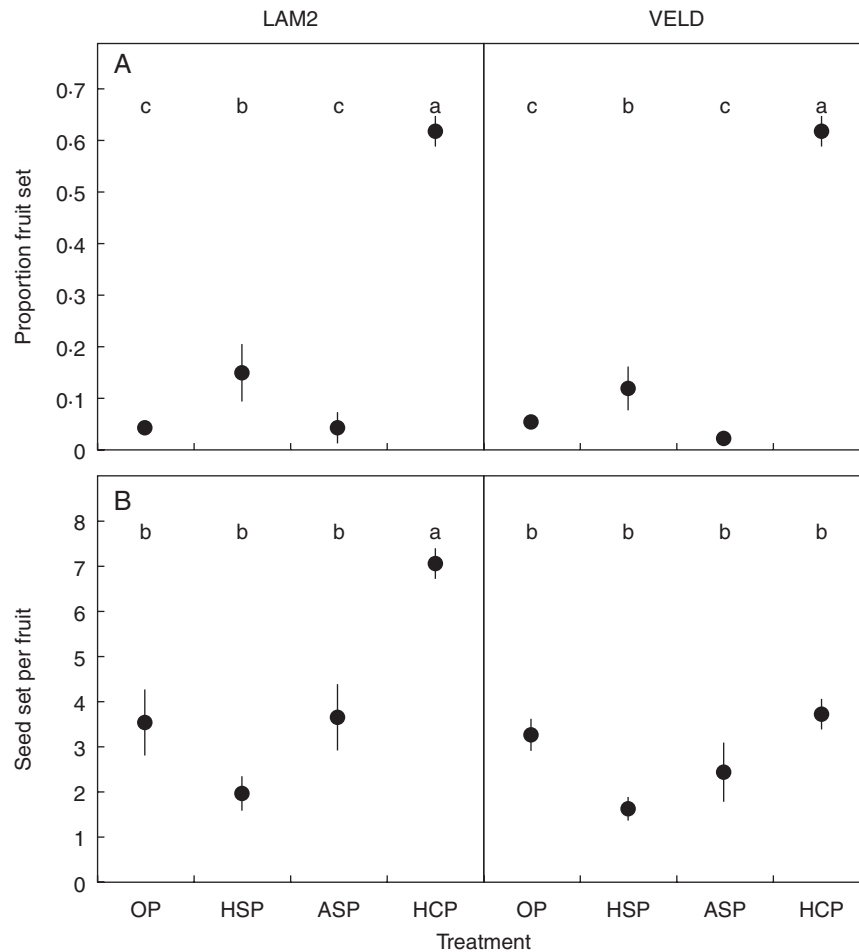
Fɪɢ. 7. Mean ($\pm$ s.e.) of (A) proportion fruit set and (B) seed set in populations of *Babiana hirsuta* (LAM2 and VELD) following experimental pollination treatments: open-pollinated (OP), hand self-pollination (HSP), autonomous self-pollination (ASP) and hand cross-pollination (HCP). Means that share the same letter are not significantly different ($P < 0.05$) among treatments across populations.

estimate mating patterns. All populations displayed mixed mating with low to moderate frequencies of outcrossing: *B. ringens* subsp. *ringens* HOPE ($t_m = 0.32 \pm 0.10$; $n = 14$ families/11·4 seeds per family; $n = 5$ loci); *B. hirsuta* VELD ($t_m = 0.44 \pm 0.19$; $n = 25$ families/11·4 seeds per family; $n = 2$ loci) and ELA ($t_m = 0.25 \pm 0.05$; $n = 20$ families/9·4 seeds per family; $n = 3$ loci), and *B. carminea* VANR ($t_m = 0.49 \pm 0.12$; $n = 23$ families/9·6 seeds per family; $n = 6$ loci). The remaining populations were monomorphic at most loci, or if they possessed polymorphic loci they exhibited strongly unbalanced allele frequencies making estimates of mating parameters unreliable.

DISCUSSION

This study provides novel information on pollination and mating in four *Babiana* species endemic to the Cape region of South Africa. The pollinators of this group appear to be exclusively sunbirds that forage in distinct ways, depending on the plant species they encounter. Three taxa possess specialized bird perches that facilitate nectar feeding from

flowers. Correlated geographic variation was found in perch size and floral traits among *B. ringens* populations, with smaller-flowered populations characterized by very low sunbird visitation. The experimental pollinations demonstrated that *B. ringens* and *B. avicularis* were self-compatible with either weak or no pollen limitation of seed set. In contrast, *B. hirsuta* was moderately self-incompatible, with the fertility of populations strongly pollen limited. The marker-based estimates indicated mixed mating, including substantial self-fertilization in all populations that were investigated. Below, the ecological and evolutionary significance of these findings is discussed and explanations provided for several seemingly unexpected results obtained that have general implications for mating system inference.

Bird pollination in Babiana

The observations of *B. ringens* subsp. *ringens* confirm previous reports (Goldblatt *et al.*, 1999; Anderson *et al.*, 2005) that this taxon is pollinated by malachite sunbirds (Table 1). In contrast, of the four *B. ringens* subsp. *australis* populations
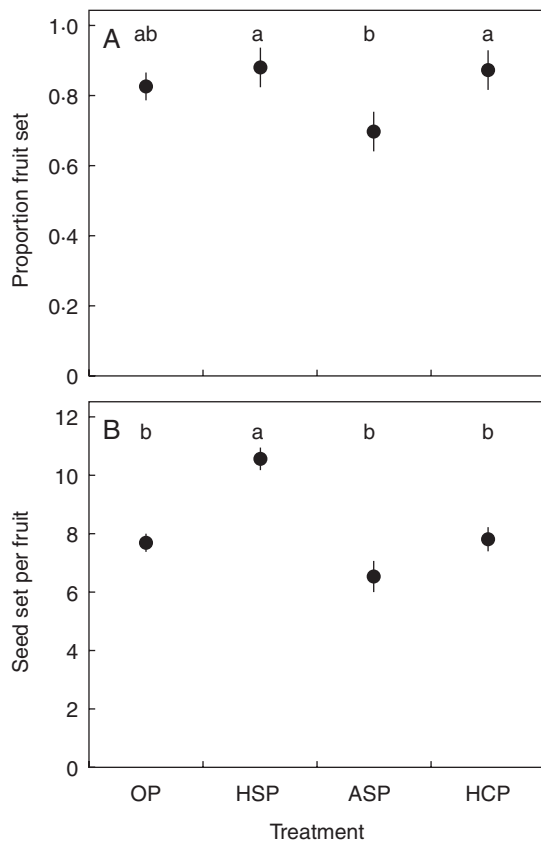
FIG. 8. Mean ($\pm$ s.e.) of (A) proportion fruit set and (B) seed set in a population of *Babiana avicularis* (DRAAI) following four experimental treatments: open-pollinated (OP), hand self-pollination (HSP), autonomous self-pollination (ASP) and supplementary cross-pollination (HCP). Means that share the same letter are not significantly different ($P < 0.05$) among treatments.

double-collared sunbird. The larger malachite sunbird was not observed visiting *B. avicularis*, although it was present in the habitat, raising the possibility of pollinator-driven differentiation in flower and perch size and the partitioning of pollination resources among bird-pollinated *Babiana* species.

The present study also provides the first direct evidence that sunbirds pollinate the large-flowered *B. carminea* (Table 1), confirming the predictions of Goldblatt and Manning (2007*b*). The morphology and presentation of *B. carminea* flowers differed markedly from the other bird-pollinated *Babiana* species. Pale markings on the tepals of *B. carminea* flowers reminiscent of nectar guides may reflect a recent shift from long-tongued fly to bird pollination (Goldblatt and Manning, 2007*b*). A shift in the opposite direction seems unlikely, as *B. carminea* is the only bird-pollinated species nested within a clade of insect-pollinated flowers (Schnitzler *et al.*, 2011). It is possible that an ecological shift by the ancestor of *B. carminea* to the highly specialized habitats now occupied by this species may have triggered a transition in pollination system. Because *B. carminea* individuals do not have perches, visiting malachite sunbirds probe flowers while on the ground, or when perching on surrounding rocks, a relatively unusual feeding position for sunbirds, although one that is occasionally observed in *Babiana* (Goldblatt and Manning, 2007*a*; see fig. 1C in Anderson *et al.*, 2005) and other bird-pollinated plants (e.g. *Calceolaria uniflora*; Sérsic and Cocucci, 1996). An elongated bird perch may have been difficult to evolve in *B. carminea* if ancestral populations resembled most long-tongued fly and bee-pollinated *Babiana* species, which do not possess flowers supported by a sturdy inflorescence.

Southern double-collared sunbirds were observed robbing nectar from flowers of *B. hirsuta* by making conspicuous slits at the bottom of the floral tube. Nectar robbing is commonplace in flowering plants, although it is more commonly reported for bee-pollinated species and may have diverse ecological and evolutionary consequences (reviewed in Irwin *et al.*, 2010). In contrast to southern double-collared sunbirds, malachite sunbirds visiting *B. hirsuta* probed for nectar in a legitimate manner. This contrast in feeding behaviour most likely occurs because of differences between the two species in bill and tongue length (Rebelo, 1987), with the shorter-billed southern double-collared sunbird unable to access nectar by probing from the mouth of the flower. On average 34 % of the flowers in *B. hirsuta* populations showed signs of nectar robbing. These results corroborate a recent study of *B. hirsuta* by Geerts and Pauw (2009) reporting that all visits by southern double-collared sunbirds were illegitimate, whereas those by malachite sunbirds during the same observation period were legitimate. Nectar robbing may alter the behaviour of malachite sunbirds, as birds commonly avoid nectar-robbed flowers in other systems (Irwin *et al.*, 2010). For a self-incompatible species such as *B. hirsuta* this could have important reproductive consequences, and nectar robbing may have contributed to the very low female fertility observed (Fig. 7).

Geographic patterns of trait variation in Babiana ringens

The patterns of geographic variation in morphological traits of *B. ringens* that were detected largely reflect the taxonomic differentiation between subsp. *ringens* in the western portion

included in the present study, only SCAR was visited by malachite sunbirds. In the remaining three eastern populations only a single southern double-collared sunbird was observed visiting flowers in a single population. The lack of visitation in these populations was not due to an absence of sunbirds in the habitats they occupied, as malachite, southern double-collared and orange-breasted sunbirds were observed at the sites. At VIC1 and VIC2 large patches of flowering proteaceous shrubs, most notably *Leucospermum praecox*, occurred adjacent to *Babiana* populations and were commonly visited by sunbirds. Thus, competition for pollinators may explain the absence of visitation to *B. ringens* in these populations. The diminutive stature, relatively low rewards and sparse density of individuals of this taxon may make populations especially vulnerable to competition from larger and more abundant Proteaceae with abundant nectar rewards.

This is the first study to report sunbird pollination in *B. avicularis*, making it one of the smallest flowered bird-pollinated species in the Iridaceae (Goldblatt and Manning, 2010). Southern double-collared sunbirds used the perches of this species when foraging for nectar in a similar way to malachite sunbirds visiting *B. ringens*. It is plausible that the smaller flowers of *B. avicularis* (and perhaps also *B. ringens* subsp. *australis*) have resulted, in part, from selection imposed by smaller sunbird species, particularly the southern

of the range and subsp. *australis* in the east (Goldblatt and Manning, 2010). In general, plants became more diminutive in stature moving eastwards and this was associated with correlated changes in perch length, flower size, floral-tube length and stigma–anther separation. It seems likely that this variation has a selective basis, but the mechanisms responsible are not known. It will be particularly important to determine the relative importance of abiotic versus biotic factors governing the origin and maintenance of this variation. Less reliable rainfall (Cowling and Lombard, 2002), regional variation in soil fertility, and differences in the intensity of competition for sunbird pollination resulting from co-occurring flowering plant species could all play a role. Interestingly, disjunct populations of subsp. *australis* on the Cape Peninsula (SCAR, RED) had somewhat larger flowers than were typical in eastern populations of this subspecies (Fig. 4B); but whether this pattern results from greater rainfall or more reliable pollination by sunbirds, including the larger malachite sunbird, or both, is unclear. Common garden and reciprocal transplant studies would be valuable to investigate these problems further.

Several lines of evidence suggest that populations of *B. ringens* subsp. *australis* in which sunbird visitation was low or absent experience higher rates of selfing compared with the larger-flowered *B. ringens* subsp. *ringens*. First, stigma–anther separation in subsp. *australis* is significantly less than in subsp. *ringens* (Fig. 4D), facilitating autonomous selfing in the absence of sunbird pollination. Reduced herkogamy is commonly associated with increased selfing rates in flowering plants (Barrett and Shore, 1987; Takebayashi *et al.*, 2006). Secondly, observed average heterozygosity in subsp. *australis* was substantially lower (0·004 ± 0·001, $n = 7$ populations) than in subsp. *ringens* (0·027 ± 0·008, $n = 4$ populations), based on a survey of 13 allozyme loci (C. de Waal, B. Anderson and S. C. H. Barrett, unpubl. res.). Finally, the failure to suppress apical branch development in perches of *B. ringens* subsp. *australis* (Fig. 5) suggests that natural selection maintaining the integrity of perches may be relaxed (Lahti *et al.*, 2008), as one might expect in populations that have shifted to high rates of selfing. If this is the case, we may be observing the evolutionary dissolution of the perch as a result of the failure of *B. ringens* subsp. *australis* to compete for sunbird pollination.

Factors influencing female fertility

The results of experimental pollinations confirm a previous report of self-compatibility in *B. ringens* subsp. *ringens* (Anderson *et al.*, 2005). The present results extend this finding by demonstrating that *B. ringens* subsp. *australis* (Fig. 6) and *B. avicularis* (Fig. 8) are also highly self-compatible and populations have the ability to autonomously self-pollinate to different degrees. Through comparisons of supplemental cross- and open-pollinations it was also demonstrated that populations of *B. ringens* exhibited either weak or no pollen limitation of seed set. Maternal fertility in *B. avicularis* also showed no evidence of pollen limitation. In these taxa it seems likely that the capacity for autonomous self-pollination may function as a mechanism of reproductive assurance when sunbird visitation is unreliable.

Controlled pollinations of *B. hirsuta* revealed moderately strong self-incompatibility in both populations, although the extent to which early-acting inbreeding depression might also contribute to low seed set following self-pollination is unknown (see Husband and Schemske, 1996). The two populations of *B. hirsuta* investigated were chronically pollen limited. Populations can suffer from pollen limitation when floral densities are far in excess of the number of effective pollinators (e.g. Larson and Barrett, 1999). Given the large floral displays in *B. hirsuta* populations it seems likely that this is the main reason for the very low fruit set recorded, as visitation by malachite sunbirds was substantially lower than by the primarily nectar-robbing southern double-collared sunbirds at the two sites studied for pollen limitation (Table 1). In addition, nectar robbing may also potentially damage the styles of *B. hirsuta* flowers and negatively affect female function. However, the incidence of pollen limitation can vary significantly in both space and time (e.g. Baker *et al.*, 2000) and future population studies over several years are desirable before firm conclusions can be reached. Obtaining this information is of particular importance because of earlier claims of pervasive pollen limitation of fruit set in the Cape Floristic Region (Johnson and Bond, 1997).

Self-incompatibility is commonly associated with clonality in plants (reviewed in Vallejo-Marín *et al.*, 2010). Clonal species often exhibit large floral displays resulting in considerable geitonogamous self-pollination (e.g. Eckert, 2000). It seems likely that self-incompatibility in *B. hirsuta* is maintained in populations to limit fitness costs (e.g. inbreeding depression and pollen discounting) associated with geitonogamy. Clones of *B. hirsuta* often consist of many flowering ramets and daily floral displays are orders of magnitude larger than in the other bird-pollinated *Babiana* species. Consequently, the extent of geitonogamous self-pollination seems likely to be much higher in *B. hirsuta* and the authors' own observations indicated that sunbirds frequently probed many flowers on clones before departing. Variation in the pollination environment will, however, have less influence on lifetime fitness of *B. hirsuta* clones compared with the non-clonal *Babiana* species because their greater longevity should buffer them against annual variation in outcross pollen receipt.

Cautionary lessons on mating system inferences

It is often assumed that specialized pollination systems function, in part, to promote high outcrossing rates and improve progeny quality. However, it has recently been recognized that the flowers of many specialized pollination systems often have the ability to self-pollinate autonomously, presumably because of the risks of specialization in an uncertain pollination environment (Zhang *et al.*, 2005; Fenster and Martén-Rodríguez, 2007). The present results on mating patterns in *B. ringens* suggest that this species may represent such a case. The large showy red flowers and specialized perches of *B. ringens* subsp. *ringens* are clearly adaptations for promoting cross-pollination by sunbirds. This initially led us to the naïve expectation of high outcrossing rates in populations. Yet, estimates from three populations of this species indicate relatively high levels of self-fertilization in each

(mean $s = 0.63$; Anderson *et al.*, 2005; this study). Similarly, half of the seeds produced by *B. carminea* resulted from self-fertilization despite its large showy bird-pollinated flowers. It seems probable that mating patterns in these species are highly sensitive to rates of sunbird visitation. In years with reliable visitation, outcrossing may be higher than reported here but, when visitation is low, autonomous self-pollination may provide reproductive assurance. However, the role that autonomous self-pollination plays in providing reproductive assurance will depend on the magnitude of inbreeding depression. Unfortunately, as is the case in most plant species with mixed mating, nothing is known in *Babiana* about the relative fitness of selfed and outcrossed offspring produced by open-pollinated plants.

Populations with chronic pollen limitation pose similar challenges for mating system inference. Controlled self- and cross-pollinations of *B. hirsuta* demonstrated that this species has a moderately strong self-incompatibility system also leading to an expectation of high outcrossing rates. However, the present estimates of mating patterns indicated significant amounts of selfing in both populations (VELD, $s = 0.56$; ELA, $s = 0.75$). This apparent contradiction can be reconciled by considering that the open-pollinated fruit set used for mating-system analysis represented only 5 % and 4 % of the potential fruit set for VELD and LAM2, respectively, because of substantial pollen limitation in both populations. Self-incompatibility in *B. hirsuta*, as in many species, is not absolute allowing some seed to be produced from self-pollination (Fig. 7). As a result, most of the open-pollinated fruits used for mating-system estimates probably originated from flowers with pollen loads composed of significant amounts of self-pollen due to the scarcity of cross-pollen deposition. Despite the recent burgeoning literature on mating systems (reviewed in Goodwillie *et al.*, 2005) and pollen limitation (reviewed in Harder and Aizen, 2010) in plant populations, most studies on these topics have been conducted in isolation from one another. Future empirical work on the quantitative relationships between the intensity of pollen limitation and variation in mating patterns are long overdue.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxford-journals.org and consist of the following. Table S1: specific locations with GPS coordinates of all *Babiana* populations investigated in this study. Table S2: samples sizes for the experimental pollination treatments.

ACKNOWLEDGEMENTS

LITERATURE CITED

Anderson B, Cole WW, Barrett SCH. 2005. Specialized bird perch aids cross-pollination. *Nature* **435**: 41–42.
Baker AM, Barrett SCH, Thompson JD. 2000. Variation of pollen limitation in the early flowering Mediterranean geophyte *Narcissus assoanus* (Amaryllidaceae). *Oecologia* **124**: 529–535.
Barrett SCH, Shore JS. 1987. Variation and evolution of breeding systems in the *Turnera ulmifolia* complex (Turneraceae). *Evolution* **41**: 340–354.
Cowling RM, Lombard AT. 2002. Heterogeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. *Diversity and Distributions* **8**: 163–179.
Cronk Q, Ojeda I. 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* **59**: 715–727.
Eckert CG. 2000. Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. *Ecology* **81**: 532–542.
Fenster CB, Martén-Rodríguez S. 2007. Reproductive assurance and the evolution of pollination specialization. *International Journal of Plant Sciences* **168**: 215–228.
Geerts S, Pauw A. 2009. Hyper-specialization for long-billed bird pollination in a guild of South African plants: the malachite sunbird pollination syndrome. *South African Journal of Botany* **75**: 699–706.
Gill FB, Wolf LL. 1978. Comparative foraging efficiencies of some montane sunbirds in Kenya. *Condor* **80**: 391–400.
Goldblatt P, Manning JC. 2006. Radiation of pollination systems in the Iridaceae of sub-Saharan Africa. *Annals of Botany* **97**: 317–344.
Goldblatt P, Manning JC. 2007a. Floral biology of *Babiana* (Iridaceae: Crocoideae): adaptive floral radiation and pollination. *Annals of the Missouri Botanical Garden* **94**: 709–733.
Goldblatt P, Manning JC. 2007b. A revision of the southern African genus *Babiana*, Iridaceae: Crocoideae. Section 2·2 Antholyzoides. *Strelitzia* **18**. South African National Biodiversity Institute, Pretoria and Missouri Botanical Garden, Missouri.
Goldblatt P, Manning JC. 2010. New taxa of *Babiana* (Iridaceae: Crocoideae) from coastal Western Cape, South Africa. *Bothalia* **40**: 47–53.
Goldblatt P, Bernhardt P, Manning JC. 1999. Evidence of bird pollination in the Iridaceae of southern Africa. *Adansonia* **3**: 25–40.
Goodwillie C, Kalisz S, Eckert CG. 2005. The evolutionary enigma of mixed mating in plants: occurrence, theoretical explanations, and empirical evidence. *Annual Review of Ecology, Evolution and Systematics* **36**: 47–79.
Grant V, Grant KA. 1968. *Hummingbirds and their flowers*. New York, NY: Columbia University Press.
Harder LD, Aizen MA. 2010. Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society of London Series B* **365**: 529–543.
Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**: 54–70.
Irwin RE, Bronstein JL, Manson JS, Richardson L. 2010. Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution and Systematics* **41**: 271–292.
Johnson SD, Bond WJ. 1997. Evidence for widespread pollen limitation of fruiting success in Cape wildflowers. *Oecologia* **109**: 530–534.
Lahti DC, Johnson NA, Ajie BC et al. 2008. Relaxed selection in the wild. *Trends in Ecology & Evolution* **24**: 487–496.
Larson BMH, Barrett SCH. 1999. The ecology of pollen limitation in buzz-pollinated *Rhexia virginica* (Melastomataceae). *Journal of Ecology* **87**: 371–381.
Marloth R. 1898. Die Ornithophilie in der Flora Südafrikas. *Berichte der Deutschen Botanischen Gesellschaft* **19**: 176–179.
Marloth R. 1915. *Flora of South Africa*. Cape Town: Darter Brothers.
Martén-Rodríguez S, Fenster CB, Agnarsson I, Skog LE, Zimmer EA. 2010. Evolutionary breakdown of pollination specialization in a Caribbean plant radiation. *New Phytologist* **188**: 403–417.
Proctor M, Yeo P, Lack A. 1996. *The natural history of pollination*. Portland, OR: Timber Press.
Rebelo AG. 1987. Bird pollination in the Cape Flora. In: Rebelo AG. ed. *A preliminary synthesis of pollination biology in the Cape Flora*. Pretoria: CSIR, 83–108.

Ritland K. 1990. A series of FORTRAN computer programs for estimating plant mating systems. *Journal of Heredity* **81**: 235–237.

SAS. 2009. JMP, Version 8·02. 1989–2009. SAS Institute Inc, Cary, NC.

Schnitzler J, Barraclough TG, Boatwright JS *et al.* **2011.** Causes of plant diversification in the Cape biodiversity hotspot of South Africa. *Systematic Biology* **60**: 343–357.

Sérsic AN, Cocucci AA. 1996. A remarkable case of ornithophily in C*alceolaria*: food bodies as reward for a non-nectarivorous bird. *Botanica Acta* **109**: 172–176.

Stiles FG. 1981. Geographical aspects of bird–flower coevolution, with particular reference to Central America. *Annals of the Missouri Botanical Garden* **68**: 323–351.

Skead CJ. 1967. *The sunbirds of Southern Africa*. Cape Town, South Africa: Cape Transvaal Printers.

Takebayashi N, Wolf DE, Delph LF. 2006. Effect of variation in herkogamy on outcrossing within a population of *Gilia achilleifolia*. *Heredity* **96**: 159–165.

Thomson JD, Wilson P. 2008. Explaining evolutionary shifts between bee and hummingbird pollination: convergence, divergence, and directionality. *International Journal of Plant Sciences* **169**: 23–28.

Vallejo-Marín M, Dorken ME, Barrett SCH. 2010. The ecological and evolutionary consequences of clonality for plant mating. *Annual Review of Ecology, Evolution and Systematics* **41**: 193–213.

Westerkamp C. 1990. Bird-flowers: hovering versus perching exploitation. *Botanica Acta* **103**: 366–371.

Wilson P, Wolfe AD, Armbruster WS, Thomson JD. 2007. Constrained lability in floral evolution: counting convergent origins of hummingbird pollination in *Penstemon* and *Keckiella*. *New Phytologist* **176**: 883–890.

Zhang L, Barrett SCH, Gao JY *et al.* **2005.** Predicting mating patterns from pollination syndromes: the case of "sapromyiophily" in *Tacca chantrieri* (Taccaceae). *American Journal of Botany* **92**: 517–524.