# Impact of introduced honey bees on native pollination interactions of the endemic Echium wildpretii (Boraginaceae) on Tenerife, Canary Islands

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Abstract

The aim of this study was to investigate effects of introduced honey bees (Apis mellifera) on native pollination interactions of Echium wildpretii ssp. wildpretii in the sub-alpine desert of Tenerife. We selected two study populations, one dominated by honey bees, while the other was visited by many native insects. During peak activity period of insects, nectar was nearly completely depleted in flowers of the first, but not the latter population. Thus, a high abundance of honey bees may have suppressed visitation by native animals due to exploitative competition. Honey bees stayed longer and visited more flowers on the same inflorescence than native bees, thus potentially promoting self-pollination of the plants. Level of seed set and viability was similar in the two study populations. However, we cannot rule out long-term changes in genetic population structure due to changes in gene-flow patterns caused by foraging behaviour of honey bees vs. native flower-visitors.

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Keywords: Disruption of native mutualisms; Interspecific competition; Apis mellifera; Conservation

## 1. Introduction

In recent years the impact of introduced honey bees (Apis mellifera L.) on native flora and fauna has been much debated. Results of some studies indicate that foraging patterns and abundance of native pollinators are altered in the presence of honey bees (Roubik, 1978; Schaffer et al., 1983; Sugden and Pyke, 1991; Paton, 1993; Wenner and Thorp, 1994; Vaughton, 1996; Gross and Mackay, 1998; Gross, 2001; Hansen et al., 2002). Although stressed as important by most researchers, it has been difficult to investigate potential detrimental effects of introduced honey bees on food storage or on reproduction of native bee species (Roubik, 1983; Sugden et al., 1996; Butz Huryn, 1997; Steffan-Dewenter and Tscharntke, 2000; Thorp et al., 2000). The impact of honey bees on the pollination of native flora includes effects on pollen dispersal and thus patterns of seed set and genetic structure of plant populations. Honey bees are often found to be less efficient pollinators compared to native flower-visiting animals (Schaffer et al., 1983; Taylor and Whelan, 1988; Westerkamp, 1991; Paton, 1993; Vaughton, 1996; Gross and Mackay, 1998; Hansen et al., 2002). However, other studies have found that A. mellifera does not adversely affect plant reproductive success, perhaps due to the numerical abundance of honey bees compared to native bees (Vaughton, 1992; Gross, 2001). Furthermore, effects of introduced honey bees on native flora or fauna are often difficult to assess due to a lack of suitable control sites (i.e., absence of A. mellifera). Lastly, patterns induced by honey bees may be swamped by demographic, stochastic, and environmental variation.

Two features of island pollination networks leave them susceptible to invasion by introduced generalist species, such as honey bees: Low species diversity (Kennedy et al., 2002) and the generalised nature of interactions (Olesen et al., 2002). Several studies of island ecosystems have reported a decline in both native bee and bird species visiting flowers in the presence of A. mellifera (Roubik, 1978; Kato, 1992; Wenner and

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Thorp, 1994; Kato et al., 1999; Hansen et al., 2002). Honey bees are widespread in the Canary Islands, and bee-keeping has been practiced for centuries (Méndez, 2000). The colonisation of the islands by A. mellifera is ancient, and honey bees are considered native based on mitochondrial DNA data (De la Rúa et al., 1998). However, A. mellifera is absent from the two eastern arid islands, Fuerteventura and Lanzarote, where the climate is dry and the flowering season too short to support perennial colonies of bees (De la Rúa et al., 2001). Similar climatic conditions prevail in the semi-arid, sub-alpine desert zone, found in the mountain regions above 2000 m a.s.l. on Tenerife. Combined with the geographical isolation provided by both crater rim and the surrounding pine forest, this suggests a natural absence of A. mellifera in this habitat. Few, but very distinct plant species inhabit these altitudes, exposed to high irradiation, drought, strong winds and extreme temperature ranges. Most of the native sub-alpine biota is confined within Teide National Park (18,900 ha), which is legally protected. However, apiculture is permitted. Every year bee-keepers bring thousands of beehives to the mountain areas of Tenerife during the short sub-alpine summer (Anon., 2000).

Observations in 2000 and 2001 showed a marked seasonal shift in the flower-visiting fauna of Echium wildpretii ssp. wildpretii Pearson ex Hook. f. (Boraginaceae) in Teide National Park. In early season, native passerine birds (Phylloscopus collybita (Vieillot) and Serinus canarius L.) and native insects visited the red, nectar-rich flowers. However, coinciding with a sudden increase in honey bee activity, the birds stopped foraging for nectar (Valido et al., 2002; unpubl. data from 2000). These observations indicate that the introduced honey bees may alter native pollination interactions. While the cessation of bird visits was easily observed, effects on native insects were less obvious. The main objective of this study is to investigate the relative importance of native insects versus introduced honey bees as flower-visitors and pollinators of E. wildpretii. National Park staff controls the placement and numbers of beehives, and we were thus able to obtain information about temporal and geographical distribution of hives throughout the flowering season.

The controlled framework of bee-keeping in Teide National Park provides a man-induced ecological experimental setup, allowing us to assess the impact of introduced honey bees on reproductive success, and hence long-term persistence of E. wildpretii. We chose two study populations, one population close to managed beehives within the caldera and an adjacent population on the outside of the crater rim. We hypothesise that honey bees, when present in large numbers, deplete flowers of nectar, thus leading to exploitative competition with native flower-visitors. Moreover, differences in foraging patterns of honey bees and natives could alter pollen flow, and thus affect seed set or seed viability of E. wildpretii. Therefore, we studied the following key aspects of the pollination interactions in the two study populations: (1) Diurnal and seasonal patterns of visitation by native and introduced flower-visitors. (2) Nectar secretion in animal-excluded flowers and flowers open to visitation. (3) Patterns of seed set.

## 2. Materials and methods

### 2.1. Study system

The study was carried out in Teide National Park, which is part of the region Las Cañadas, a volcanic alluvial plain delimited by a crater rim. The National Park (NP) covers an area of 18,900 ha and encompasses the high-altitude sub-alpine zone of Tenerife, Canary Islands. Vegetation is a low and sparse shrub dominated by a few species.

The Teide bugloss, Echium wildpretii ssp. wildpretii (Boraginaceae) (E. wildpretii hereafter) was the focal species of our study. This endemic plant is almost exclusively confined to the sub-alpine mountain zone of Tenerife (Bramwell and Bramwell, 1990). Although this subspecies is categorised as rare (Gómez Campo, 1996), it is locally abundant and the large red-flowered inflorescences are conspicuous in the landscape during the flowering season. The plant is monocarpic and grows as a rosette for 5–10 years before producing a single flowering shoot. The columnar inflorescence is ca. 0.5–2.5 m in height, a basal diameter of 10–70 cm, tapering towards the top. Flowers are borne in cymes, which are arranged spirally on the inflorescence. Cymes have a total of four (in apical cymes) to 30 flowers (in basal cymes) with 1–3 open flowers at a time. The flower is protandrous and is open for 2.5–3 days (Olesen, 1988). Each flower contains four ovules, developing into a maximum of four nutlets. Individual plants flower for 3–5 weeks, but since the time of flowering varies among individuals, the total flowering period of a population is ca. 1–1.5 months. In addition to the passerine birds and honey bees, 16 native insect species and juveniles of the endemic lacertid lizard Gallotia galloti Duméril & Bibron have been observed visiting the flowers (Valido et al., 2002).

### 2.2. Experimental design

Beehives are brought to the park during the flowering season of the nectar-rich plant species, mainly Descurainia bourgeauana Webb ex O.E. Schulz (Brassicaceae), Spartocytisus supranubius (L.) Webb & Berth (Fabaceae) and E. wildpretii. The timing of flowering varies between years and is tracked by the bee-keepers. Generally, a maximum of 2500 hives at 25 sites are allowed in the NP

each year. Each hive houses 10,000–70,000 bees. However, due to drought and hence scarcity of floral resources, the actual number of beehives placed in the NP was lower than the allowed maximum in 2001, the year of our experiment. A total of 1393 beehives were placed at 17 sites in Teide NP from April 27 to June 10 2001. Beehives were removed again from May 25 and onwards. By July 12th 2001 all hives had been removed (Fig. 1). Information about numbers of beehives placed and removed at each site throughout the flowering season was obtained from Teide NP office. Due to the limited area of the national park, all populations of E. wildpretii inside the caldera are located few kilometres from beehives, and thus visited heavily by honey bees. Only E. wildpretii populations isolated from bee hives by a geographical barrier were visited by fewer honey bees. For this reason, we selected two study populations ca. 2 km apart, separated by the crater rim surrounding Las Cañadas.

Population 1 (hereafter Pop1) was located at 'Cementerio de los Tajinastes' (28°13′N, 16°38′W, 2050 m a.s.l.), on the inner slope of the crater rim. Within 5 km of Pop1, there were a total of 356 beehives in five locations. Pop1 was expected to be heavily exploited by honey bees, as 90% of foraging trips by honey bees are within a 5-km radius of the colony (Visscher and Seeley, 1982). Vegetation at the study site was dominated by S. supranubius. Other abundant species were Scrophularia glabrata Aiton (Scrophulariaceae), Pimpinella cumbrae Buch ex DC (Apiaceae), Erysimum scoparium (Brouss.) Wettst. (Brassicaceae), Tolpis webbii Sch. Bip. (Asteraceae), and E. wildpretii. Approximately 120 flowering individuals of E. wildpretii were found at this site. Population 2 (hereafter Pop2) was located at 'Valle de los Tajinastes' (28°12′N; 16°37′W, 2150 m a.s.l.), less than 2 km from Pop1, but on the outer side of the crater rim. This study site was isolated from nearby beehives in the NP by a 400-m high mountain ridge. Moreover, Pop2 is isolated from beehives at lower altitudes by pine forest, a uniform vegetation type consisting of few species. We have no information about bee-keeping activity outside the NP in the nearby pine forest. However, feral colonies are unlikely to persist in this zone due to the arid conditions. Thus, Pop2 was predicted to have a low visitation by A. mellifera. Vegetation was dominated by S. supranubius and Carlina xeranthemoides L. fil (Asteraceae). Due to severe drought only very few plant individuals set flowers in 2001, and most species were only observed in vegetative condition. E. wildpretii, represented by 60–70 flowering individuals, was the most conspicuously flowering species and the only species offering large amounts of floral resources to animals.

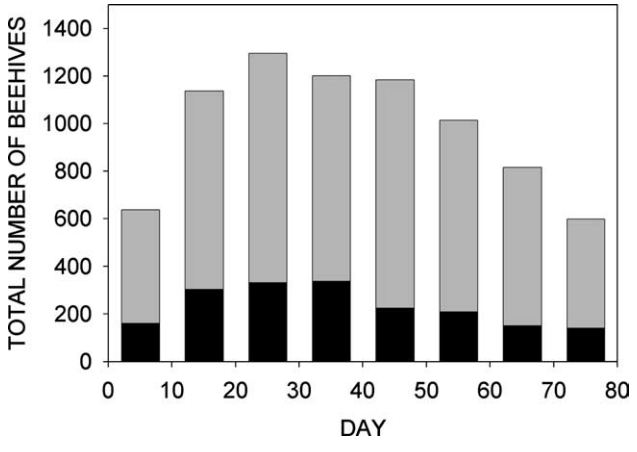


Fig. 1. Columns indicate total number of beehives within the area of Teide National Park during 10-day periods (days 1–10, 11–20, etc.). Number of hives within 5 km of Pop1 is shown in black. Day 1 corresponds to April 27, 2001. The last beehives were removed on day 77 – July 12, 2001.

To compare plant characteristics of Pop1 and Pop2, we measured height of flowering plants, size of inflorescences (length, basal diameter and surface approximated to a cone), flower density, number of flowers per cyme and total number of flowers per inflorescence. Since number of flowers per cyme varied along the length of the inflorescence, 20 cymes from each of the lower, mid and upper part of an inflorescence were used to calculate the average number of flowers per cyme. Furthermore, we measured the distance to the three nearest neighbouring conspecific flowering plants and the size of these. Data from the two study populations were compared by t-tests when assumptions of parametric tests were met. Otherwise the non-parametric Mann–Whitney U-test was used.

### 2.3. Flower visitation

To investigate diurnal and seasonal patterns of visitation, we measured visitation rate of native insects and honey bees from sunrise (ca. 8:00 h) to sunset (ca. 21:00 h) from early to late in the flowering season. We observed bird visits with a binocular from a hideout >10 m away, this is treated in further detail in another paper, Valido et al. (2002). In Pop1 insect visitation rates were recorded on nine observation days (1–27 May) and in Pop2 on five observation days (15–30 May) interspersed regularly through the flowering season. To capture variation due to differences in floral display, spatial location and stage of flowering between individual plants, visitation rate was recorded alternately in 20 study plants in Pop1 and in six plants in Pop2. Each plant was observed for 10-min periods consisting of five 2-min intervals. Depending on the level of visitation, one side of the whole inflorescence, a proportion (1/2, 1/3 or 1/4) or a square of 10 × 10 cm was observed. At the beginning of each 2-min census period, all insect individuals present on the observed part of the inflorescence were

counted, and during the 2 min all newcomers were added to this number. One visit was defined as an individual landing on the inflorescence foraging for nectar and/or pollen until its departure from the inflorescence. Since insect individuals were not marked, repeated visits by the same individual were counted as new visits. Thus, this census method could lead to an overestimate of visitation rate especially in the 10 × 10 cm squares, since an insect individual may arrive and depart from the square several times during a single visit to the whole inflorescence. For this reason we made simultaneous measurements by two observers, recording visitation rate in a whole (or half) inflorescence and in 10 × 10 cm squares in 32 2-min periods. Based on these data we constructed regression equations, which were subsequently used to convert visitation rate measured in 10 × 10 squares to visitation rate of the whole inflorescence. Visitation rates recorded in 1/2, 1/3 or 1/4 of an inflorescence were multiplied by 2, 3 and 4, respectively, to estimate visitation rate per plant. When insects were counted only in a proportion of the inflorescence, we alternately observed lower, mid and upper parts of the inflorescences. Spatial differences in visitation rate were investigated using seven different plants in Pop2, for which visits to the upper and lower halves of the inflorescence were recorded. During all visitation observation periods, we monitored air temperature at the surface of the inflorescence at mid-inflorescence height.

To investigate differences in foraging behaviour between honey bees and native insects, we observed the behaviour of individual honey bees, Anthophora alluaudi Perez and Eucera gracilipes Perez (Apidae). These two endemic anthophorid type bees were the most abundant native visitors. For each bee individual we recorded the time spent and the number of flowers probed per visit to an inflorescence.

### 2.4. Nectar

To investigate diurnal patterns of nectar secretion and the influence of flower-visitors on nectar availability in E. wildpretii, we measured nectar volumes using micro-capillary tubes and sugar concentration using a hand-held Bellingham–Stanley refractometer. Sampling was done during peak flowering season, when the activity of flower-visiting animals was high (May 13–14 in Pop1, 15 and 17 May in Pop2). Two adjacent plants of approximately equal size and stage of flowering were selected in each study population. The inflorescence of one plant was covered with fine nylon mesh prior to the activity period of flower-visitors to exclude animals (''excluded''). Flowers of the other plant were left non-manipulated to be visited by flower-visiting animals (''open''). Nectar volume per flower and sugar concentration were measured from sunrise (8:00 h) to sunset (21:00 h). Sugar weight in sucrose-equivalents was calculated as volume × concentration. In each sampling period, nectar characteristics were measured in 10 flowers of each treatment, five in male and five in female phase. The light regime (sun or shade) of the sampled flowers was also recorded. When possible, equal numbers of flowers in sun and shade were sampled in each period. Flowers were removed from the inflorescence after sampling to avoid repeated sampling. An estimate of the total lifetime nectar amount offered by an E. wildpretii individual was calculated as total nectar production per plant, based on number of flowers per plant (using plants of average size and maximum size, respectively), average nectar volume per flower per day and an average nectar production period of 2 days per flower.

### 2.5. Seed set and viability

After flowering had ended and fruit development started, the level of seed set was recorded in 22 plants in Pop1 and 12 plants in Pop2. In each plant, the infructescence was divided into three parts (lower, mid and upper). In each part, seed set per flower was counted in flowers of 20 randomly selected cymes. Seed set was calculated as a percentage of maximum seed set, which is four seeds per flower. To assess the importance of animal pollinators for seed set, we excluded animal visitors by bagging whole inflorescences of six plants in Pop1 and four in Pop2 before flowering. Using metal wire and fine nylon mesh, we constructed cages around each inflorescence, to avoid the mesh from touching (and thus pollinating) the flowers. At the end of the flowering season, cages and bags were removed and seed set recorded in 60 randomly selected cymes per plant.

Mature seeds from 19 plants of Pop1 and 14 plants of Pop2 were collected and tested for viability using a 2,3,5 triphenyl tetrazolium chloride (TTC) enzyme activity test (Heydecker, 1965, 1968). For each plant, 30 seeds were collected from the lower, mid and upper parts of the infructescence. Seeds were cut into halves, placed in petri dishes with the 2,3,5 TTC solution and left in darkness for 24 h. Seeds were considered viable if the embryo turned red, a reaction indicating enzyme activity.

### 2.6. Data analysis

Average visitation rate per 2-min period was calculated for each 10-min census period for both honey bees and native insects. Diurnal patterns were analysed by dividing visitation rate observations into 2-h periods from 7:00 to 21:00 h, pooling data from all observation dates.

To investigate diurnal changes in nectar availability of open and excluded flowers, the mean volume and concentration in ten flowers sampled at each time were used to represent nectar characteristics (volume,

concentration and sugar weight) at a given time of the day. All analyses were run separately for Pop1 and Pop2.

To analyse patterns of variation in final seed set (log transformed) at different levels, we carried out an ANOVA (GLM procedure) with the levels: populations, plants within populations, infructescence parts (lower, mid and upper) within plants and cymes within parts. All these variables were included in the model as random effects and the Type III Sum of Squares were calculated (Shaw and Mitchell-Olds, 1993).

## 3. Results

### 3.1. Plant characteristics

Although plants of Pop1 and Pop2 did not differ in total height, inflorescences of plants in Pop2 were significantly shorter, and thus had a smaller surface area. Moreover, flower density was lower in Pop2, and plants of this population generally had fewer flowers per inflorescence per day compared to Pop1, although the difference was not significant. Furthermore, number of flowers per cyme was significantly lower in Pop2 (Table 1). Hence, in conclusion, plants in Pop2 had a lower number of flowers, both on a per day and on a seasonal basis. Spatial patterns of plants (nearest neighbours) did not differ between Pop1 and Pop2, although neighbouring plants in Pop2 were smaller, reflecting the general difference in plant size between populations (Table 1).

### 3.2. Flower visitation

A total of 16 native insect species and the introduced A. mellifera were observed visiting E. wildpretii (see Table 1 in Valido et al., 2002). Of these, the most dominant species were A. mellifera, A. alluaudi and E. gracilipes (Apidae). Diurnal visitation patterns of both honey bees and native insects were strongly influenced by temperature in both study populations: Visitation started at sunrise (when temperatures were 10–11 °C) and increased steadily until ca. 11:00 h, when the air temperature levelled off at ca. 23–24 °C (Figs. 2 and 3). Insect activity decreased again around 18:00 h, when the temperature decreased. A marked difference in composition of the flower-visitor pool was found between Pop1 and Pop2. In Pop1, no significant difference was found in levels of visitation by honey bees and native insects in the early season (before the 8th of May). However, in mid and late season (after the 8th of May), visitation by honey bees increased suddenly, significantly exceeding visitation by native insects (Fig. 4). This increase was coincident with an increase in numbers of beehives placed within the National Park, including the sites close to Pop1 (Fig. 1). In contrast, in Pop2, level of visitation by native insects was higher than that of honey bees both diurnally (Fig. 3) and throughout the season (although only significantly so in mid season) (Fig. 4).

Honey bees, A. alluaudi and E. gracilipes, differed in foraging patterns, both in time spent per inflorescence (ANOVA: $N = 95$, $F = 31{:}31$; $P < 0{:}0001$, $R^2 = 0{:}40$) and number of flowers probed per visit (ANOVA: $N = 95$, $F = 21{:}59$, $P < 0{:}0001$, $R^2 = 0{:}32$) (Table 2).

Table 1
Plant characteristics for E. wildpretii at the two study sites

| Characteristic | Pop1 | Pop2 | Statistics[c] |
|---|---|---|---|
| Total plant height (cm) | 188.5 ± 34.9 (55) | 182.5 ± 34.6 (22) | $t = 0{:}61^{n.s.}$ |
| Height of inflorescence (cm) | 139.6 ± 33.8 (55) | 111.4 ± 26.0 (25) | $t = 3{:}84^{+++}$ |
| Basal diameter of inflorescence (cm) | 28.8 ± 11.3 (55) | 27.6 ± 6.7 (25) | $t = 1{:}10^{n.s.}$ |
| Surface of inflorescence ($cm^2$) | 6795.3 ± 4236.6 (55) | 5087.9 ± 2424.9 (25) | $t = 3{:}05^{++}$ |
| Density of flowers (no. of flowers/$cm^2$) | 0.40 ± 0.09 (54) | 0.32 ± 0.08 (10) | $t = 2{:}89^{++}$ |
| Open flowers per plant per day | 3301 ± 2241 (20) | 1836 ± 762 (10) | $t = 1{:}97^{n.s.}$ |
| Total number of flowers per cyme[a] | 17.3 ± 4.7 (1320) | 15.5 ± 5.5 (660) | $U = 7{:}21^{+++}$ |
| Open flowers per cyme per day | – | 1.76 ± 0.52 (250) | – |
| Distance to nearest neighbour (m) | 6.0 ± 8.1 (22) | 13.8 ± 13.6 (12) | $U = 1{:}27^{n.s.}$ |
| Mean distance to three nearest neighbours (m) | 9.1 ± 7.6 (22) | 16.5 ± 13.9 (12) | $U = 1{:}32^{n.s.}$ |
| Surface of nearest neighbour ($cm^2$) | 4750.9 ± 3877.9 (22) | 5942.6 ± 2370.1 (12) | $t = 2{:}43+$ |
| Nectar volume per flower per day (**µl**)[b] | 9.79 ± 4.61 (111) | 4.99 ± 2.13 (80) | $U = 7{:}95^{+++}$ |
| Male phase flowers | 8.73 ± 3.38 (55) | 4.88 ± 1.40 (40) | $t = 7{:}35^{+++}$ |
| Female phase flowers | 10.82 ± 4.77 (56) | 5.09 ± 2.69 (40) | $U = 5{:}91^{+++}$ |
| Nectar sugar concentration (%) | 15.5 ± 4.1 (111) | 15.3 ± 3.3 (76) | $t = 0{:}09^{n.s.}$ |
| Male phase flowers | 13.4 ± 1.9 (55) | 14.0 ± 3.2 (36) | $t = 0{:}89^{n.s.}$ |
| Female phase flowers | 16.7 ± 4.6 (56) | 16.6 ± 2.9 (40) | $U = 1{:}22^{n.s.}$ |

All values are given as means ± SD (N).

[a] Mean of 20 cymes from each of the upper, middle and lower parts of the inflorescence.

[b] Excluded flowers, based on data from May 13 to 14 (Pop1) and May 15 and 17 (Pop2).

[c] Pop1 and Pop2 were compared using t-tests when data conformed to assumptions of parametric tests, and the non-parametric Mann–Whitney U-test otherwise. Significance level: $^{++}$, $P < 0{:}005$, $^{+++}$, $P < 0{:}0005$, n.s., $P > 0{:}05$.

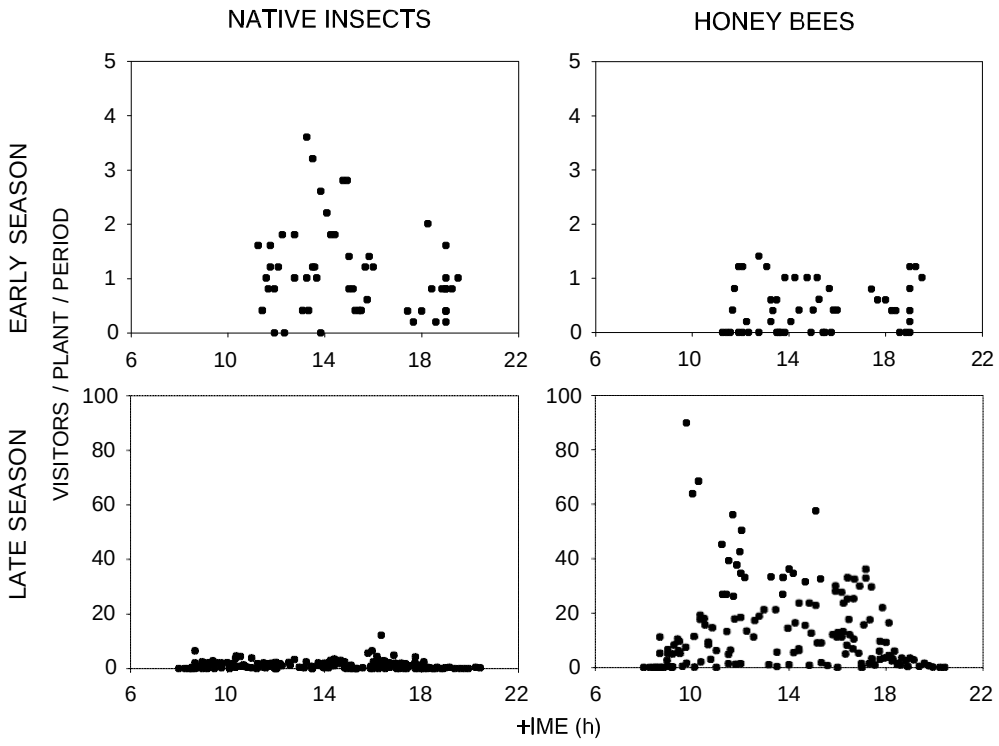


Fig. 2. Diurnal variation in visitation rates (individuals/2 min/inflorescence) of native insects and honey bees in Pop1 in early (1–8 May) and late season (8–27 May). Notice differences in levels of visitation between early and late season (different scales of the Y-axis).

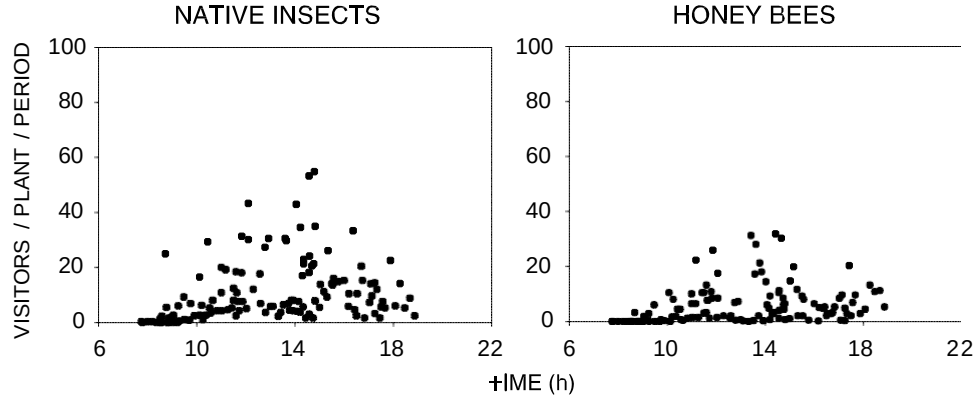


Fig. 3. Diurnal variation in visitation rates (individuals/2 min/inflorescence) of native insects and honey bees in Pop2.

Pair-wise comparisons revealed that honey bees spent significantly more time and visited significantly more flowers per inflorescence than the native bees. However, A. alluaudi and E. gracilipes did not differ in foraging pattern (Tukey–Kramer test). Furthermore, native insects visited the upper part of the inflorescence much more frequently than the lower part (Wilcoxon signed ranks test, visitation rate of upper versus lower: U ¼ 141:5; P < 0:001, N ¼ 28). In contrast, visitation rate of honey bees was higher in the lower part than in the upper part of an inflorescence (t-test: t ¼ —2:31, P ¼ 0:029, N ¼ 28).

Two native species of passerine birds, the Common chiff-chaff (P. collybita) and the Canary (S. canarius), were commonly observed visiting the flowers for nectar in early season in both populations. On one occasion we observed a pair of another passerine, the Blue tit (Parus caerulius), visiting six inflorescences for nectar in Pop2. Bird visits continued occasionally in Pop2 throughout the flowering season. However, after May 8 no bird visitors were observed in Pop1. The disappearance of birds was coincident with the increase in honey bee activity (Fig. 4).

### 3.3. Nectar

In Pop1, nectar volume of excluded flowers remained at a constant level throughout the day (regression:

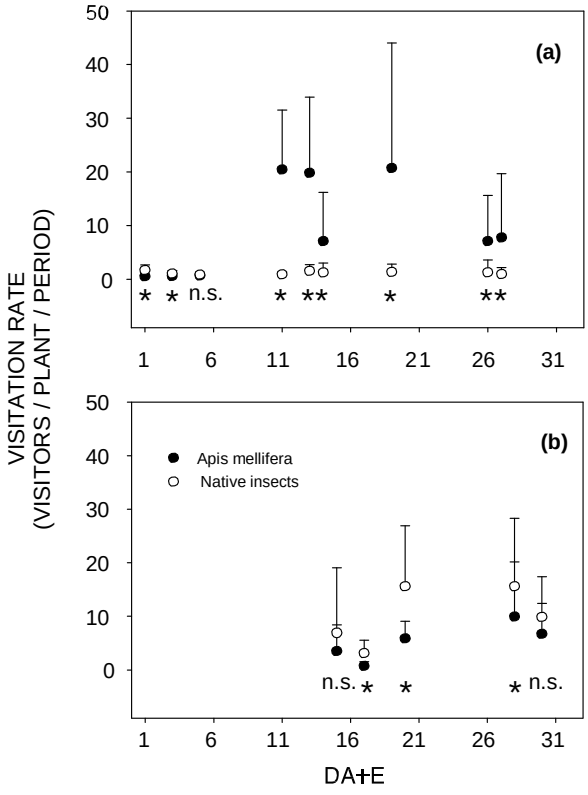


Fig. 4. Seasonal visitation pattern. Daily averages + SD of visitation rates of honey bees and native insects in (a) Pop1 and (b) Pop2. Wilcoxon signed ranks tests were used for comparisons. Significant differences are indicated (* for $P < 0:05$, n.s. for non-significance). Numbers on the X-axis indicate dates in May 2001.

lnðvolÞ ¼ 2:00–0.25 h, F ¼ 0:03, P ¼ 0:87, $R^2$ ¼ 0:002). In contrast, nectar volume of open flowers decreased significantly during the day (regression: lnðvolÞ ¼ 3:99–0.26 h, F ¼ 7:67, P ¼ 0:01, $R^2$ ¼ 0:34). Rapid decrease

Table 2
Foraging patterns of two native bee species and the introduced honey bee visiting Echium wildpretii

| Species | N | Seconds/visit | Flowers/visit |
|---|---|---|---|
| Eucera gracilipes | 62 | 16.5 T 23.8 | 6.7 T 8.2 |
| Anthophora alluaudi | 13 | 18.7 T 10.8 | 9.9 T 9.5 |
| Apis mellifera | 20 | 137.1 T 131.7 | 34.5 T 29.6 |

Values are given as means T SD.

to near-zero level occurred from sunrise to ca. 12:00 h, after which nectar volume remained at a constant low level until ca. 20:00 h, where a slight increase was observed (Fig. 5). Hence, sugar weight of open flowers was significantly lower than the level found in excluded flowers from 09:50 h onwards (Mann–Whitney U-tests, all $P < 0:05$). In Pop2, nectar volume of excluded flowers tended to increase slightly during the day (regression: vol ¼ )0.06 + 0.46 h, F ¼ 5:61, P ¼ 0:06, $R^2$ ¼ 0:48), albeit only to about half the level of that found in Pop1 (Table 1). In open flowers, nectar volume was constant throughout the day (regression: vol ¼ 3.65 – 0.07 h, F ¼ 0:15, P ¼ 0:71, $R^2$ ¼ 0:024) (Fig. 5). Overall, average values in the four groups (excluded and open in Pop1 and Pop2, respectively) illustrate the effects of drought and nectar exploitation by visitors: Differences in nectar level between excluded flowers of Pop1 and Pop2 can be explained by extreme drought in Pop2, while differences between open flowers reflect exploitation by a flower-visitor fauna dominated by honey bees (Pop1) versus native insects (Pop2) (Fig. 5, dashed lines).

Nectar secretion in excluded flowers in Pop1 was significantly influenced by their sexual phase: nectar of flowers in female phase had a higher sugar concentra-

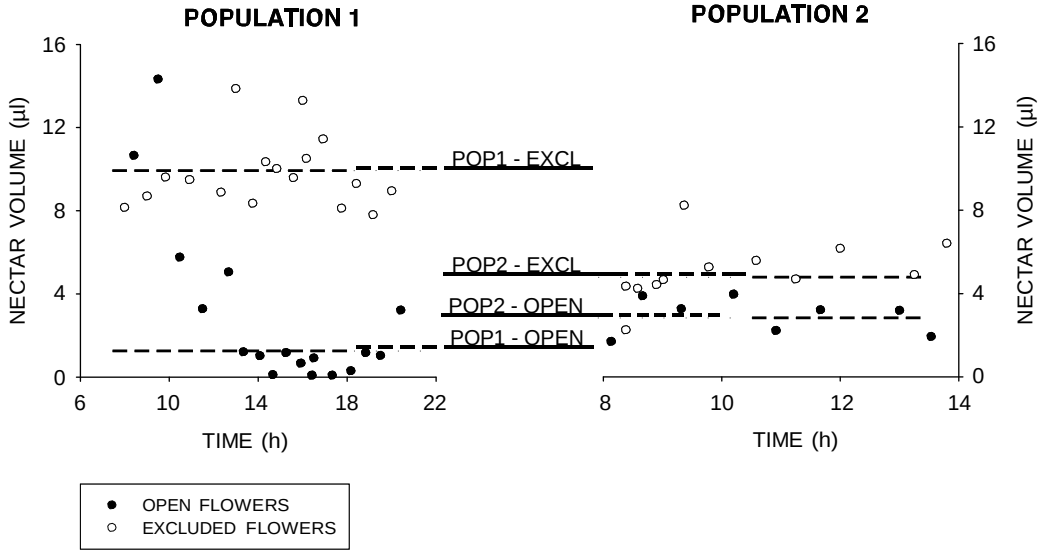


Fig. 5. Diurnal variation in nectar volume of animal-excluded and open (non-excluded) flowers of E. wildpretii. Each point represents the mean of 10 flowers. Dashed lines indicate the mean daily levels of nectar in the four groups, plants with excluded and open flowers in Pop1 and Pop2, respectively. Mean of open flowers in Pop1 was calculated excluding data before 10:00 h, when visitation was low.

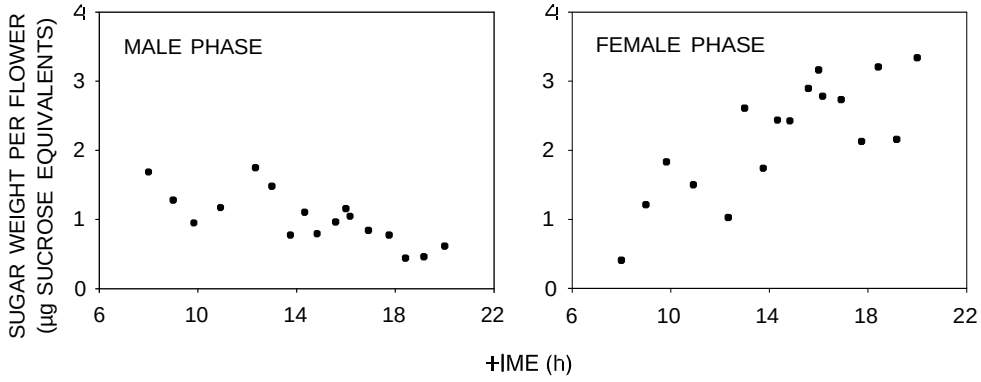


Fig. 6. Diurnal variation in sugar weight of nectar of excluded flowers in male and female phase. Each point represents the mean of five flowers. Data were only obtained for Pop1.

tion, larger volume, and hence higher sugar weight than flowers in male phase (Mann–Whitney U-tests, all $P < 0:0001$) (Table 1). Sugar weight decreased during the course of the day in male phase flowers and increased steadily in female phase flowers (Fig. 6). In Pop2, female phase flowers also had a larger sugar concentration ($U = 3:55$, $P < 0:001$, $N = 76$) and sugar weight ($U = —1:99$, $P < 0:05$, $N = 80$) than male phase flowers. However, differences in nectar volumes were not significant ($U = —1:12$, $P = 0:26$, $N = 80$). Light regime (sun or shade) did not affect any nectar characteristics of flowers in Pop1 or Pop2 (Mann–Whitney U-tests, all $P > 0:05$).

Total lifetime nectar production differed greatly between populations and among plants within each population. In Pop1, an average-sized plant produced an estimated amount of 0.54 L during its lifetime, while in Pop2 this amount was 0.16 L. However, inter-plant variation was considerable due to variation in size. The largest plant in Pop1 was estimated to produce 1.75 L, and in Pop2 the largest lifetime production of a plant was 0.43 L.

### 3.4. Seed set and viability

Seed set per flower per plant was slightly higher in Pop2 than Pop1 (Table 3), although the difference was not significant (Table 4). Considerable variation in seed set was found between plant individuals within the populations, and seed set was positively correlated with plant height (Pearson $r = 0:42$, $P = 0:01$, $N = 34$). However, seed set did not differ between populations when using plant height as a covariate ($F = 3:12$, $P = 0:11$). Furthermore, all the interactions terms calculated in the GLM analysis were highly significant, i.e., seed set varied among plants within populations, among parts of the infructescence within single plants and among cymes within parts of an infructescence (Table 4). Seed set of infructescences, which had been excluded from flower-visiting animals, revealed that plants set some seeds even in the absence of animal pollen vectors (Table 3). However, seed set was much lower than in the

Table 3
Seed set and viability in open pollinated flowers, and flowers excluded from flower-visitors

| Parameter | Pop1 | Pop2 |
|---|---|---|
| Seed set[b], open | 48.0 ± 27.1 (22,732) | 54.2 ± 23.7 (10,955) |
| Seed set[b], excluded | 23.3 ± 27.2 (6020) | 29.6 ± 30.7 (3744) |
| Seed viability[c], open | 84.4 ± 9.1 (57) | 83.9 ± 10.2 (48) |

Means ± SD (N)[a].

[a] Seed viability N, number of viability tests. Seed set N, number of flowers.

[b] Percentage of maximum seed set (four seeds per flower).

[c] Percentage viable seeds in viability tests of 30 seeds.

Table 4
Results of the ANOVA analysis (GLM procedure using Type III Sum of Squares) of seed set (log transformed) at different levels (population, plant individual, part of the inflorescence and cyme)

| Source of variation | SS | df | MS | F | P |
|---|---|---|---|---|---|
| Population | 0.49 | 1 | 0.49 | 0.15 | 0.709 |
| Plant | 93.57 | 20 | 4.68 | 1.47 | 0.263 |
| Part | 1.4 | 2 | 0.70 | 6.94 | 0.002 |
| Cyme | 0.72 | 19 | 0.04 | 0.96 | 0.527 |
| Population × Plant | 34.14 | 10 | 3.41 | 129.81 | <0.001 |
| Plant × Part | 4.21 | 40 | 0.10 | 4.00 | <0.001 |
| Part × Cyme | 1.53 | 38 | 0.04 | 1.53 | 0.020 |
| Error | 882.47 | 33,556 | 0.03 | | |

All variables were treated as random effects. The obtained model was statistically significant ($F = 2912:8$; $P = 0:008$).

controls for both Pop1 (U ¼—58:71, P < 0:00005, N ¼ 28; 752) and Pop2 (U ¼—44:26, P < 0:00005, N ¼ 14; 695).

There were no significant differences in seed viability between populations (Table 3). Viability differed significantly among plants in Pop2 (ANOVA: df ¼ 15, F ¼ 3:84, P ¼ 0:0007, $R^2$ ¼ 0:64), but not in Pop1 (df ¼ 18, F ¼ 1:68, P ¼ 0:09, $R^2$ ¼ 0:44).

## 4. Discussion

### 4.1. Impact of honey bees on native insects and birds

The potential impact of introduced A. mellifera in native systems is a major concern, but few studies provide quantitative data and clear-cut evidence of the effects of honey bees (Sugden et al., 1996; Butz Huryn, 1997; Kearns et al., 1998). Results of this study show that the site dominated by honey bees is characterised by low visitation rate by native insects throughout the season. In contrast, a high level of visitation by native insects is maintained during the flowering season in Pop2, which had a lower level of honey bee visitation. The high abundance of honey bees may have resulted in exploitative competition between honey bees and native insects, as nectar standing crops were reduced to near-zero levels in Pop1, but not in Pop2, despite the more arid conditions prevailing there (Fig. 5). On the other hand, the level of visitation by native insects remained constant throughout the flowering season in Pop1, and thus appeared to be unaffected by the emergence of honey bees (Fig. 4). Two alternative scenarios may explain this pattern: (1) abundance of native insects was limited by low temperatures in early season, and later suppressed by honey bee dominance or (2) because extensive honey bee keeping has been practiced for centuries in Las Cañadas (Méndez, 2000), we may be observing the 'ghost of competition past', i.e., numbers of native flower-visitors may already have been reduced by honey bee dominance in Pop1. It would be interesting to address this question in a future study.

Exploitative competition may also be acting between honey bees and passerine birds. In two consecutive years we observed that nectar-feeding birds ceased flower-visitation in Pop1 when honey bees became abundant. At this stage it is not possible to determine if this pattern is connected to depletion of nectar or to a seasonal change in the diet of birds related to, e.g., availability of insects or breeding activity. E. wildpretii is one of the largest nectar resources in the otherwise very dry and sparsely vegetated environment of Las Cañadas. Thus, overexploitation of E. wildpretii could force native flower-visitors to switch to other, less profitable floral resources. Furthermore, preliminary observations of flower-visiting insects in a population of E. wildpretii ssp. trichosiphon on La Palma Island revealed a lower abundance of A. mellifera, higher levels of standing nectar crop and a higher diversity of native insects compared to the honey bee dominated population on Tenerife (unpubl. data).

As mentioned in Section 1, other studies have shown that introduced honey bees negatively affect visitation rate and species diversity of native flower-visitors (Kato, 1992; Wenner, 1993; Kato et al., 1999; Wenner et al., 2000; Gross, 2001; Hansen et al., 2002). An interesting question is whether this pattern translates into reduced reproductive output of native insects, and hence threatens their long-term persistence in Teide National Park. In a long-term study, Roubik and Wolda (2001) found no decreases in population size of native insects in the presence of africanised honey bees in a Central American rain forest. On the other hand, an experimental study in an Australian tree-grass plain showed population declines of the native bee Exoneura asimillima in the presence of managed bee hives, possibly due to resource competition with honey bees (Sugden and Pyke, 1991). Resource level has also been shown to affect reproduction in the leaf-cutter bee Megachile apicalis (Kim, 1999). Obviously, the long-term impact of A. mellifera on a native pollination system varies between regions and habitat types, and thus extrapolation of above results to desert areas like Las Cañadas should be made with extreme caution (Paton, 1993). More long-term studies are clearly necessary to assess the impact of A. mellifera at the population level of native flower-visitors.

### 4.2. Impact of honey bees on E. wildpretii

Introduced honey bees are known to reduce fitness of some native plant species (Gross and Mackay, 1998). However, in other cases, seed set has been shown to be unaffected (Vaughton, 1992) or pollination even augmented by the presence of honey bees (Paton, 1993; Gross, 2001). In our study, seed set was not significantly different in the study population dominated by honey bees and the population visited predominantly by native insects, the level of seed set being only slightly lower in the former. Hence, the effect of A. mellifera on seed production is minor, if any. Some studies have shown that honey bees are poorer pollinators than native species (Westerkamp, 1991; Freitas and Paxton, 1998; Gross and Mackay, 1998; Hansen et al., 2002), but a high abundance may compensate for lowered pollination efficiency (Butz Huryn, 1995; Kraemer and Schmitt, 1997; England et al., 2001). Moreover, breeding system of the plant influences level of seed set. E. wildpretii was capable of producing a considerable number of seed without being visited by animals, and thus may to some extent be pollinated by wind or even set seeds by autogamy. On the other hand, open pollination increases

seed set, and most aspects of anthesis seem to be related to animal pollination: Most flowers open in the morning, live for 2.5 days, with approximately 1 day in male phase and 1 day in female phase (Olesen, 1988). Our study showed that flowers had two peaks in sugar weight of nectar, one in male phase and one in female phase, separated by a minimum during the sexual transition phase (Fig. 6). Thus, flowers are most attractive to flower-visitors during peak pollen presentation and again during stigma receptivity.

It is difficult to assess the influence of animal visitation for variation in seed set, superimposed upon the background reproductive output by spontaneous autogamy and wind pollination. Our analyses indicate a positive correlation between plant size and seed set, which is concordant with a preference of animals to visit larger plants (Valido et al., 2002). However, the same pattern could be explained by resource allocation in semelparous organisms, larger plants having accumulated more resources before reproduction.

### 4.3. Future perspectives

Although level of seed set can be slightly affected by foraging patterns of the visitors, the primary impact of introduced honey bees may be changes in pollen flow, and thus genetic structure of the plant population. In contrast to native insects and birds, honey bees visited many flowers on each inflorescence and rarely moved between plants, which is likely to promote geitonogamy and hence inbreeding. However, how and if this affects long-term persistence of the plant population is unknown. We found no differences in seed viability between plants pollinated mainly by honey bees or native insects. One explanation could be that E. wildpretii is relatively unaffected by inbreeding, since it is capable of producing a large number of seeds by selfing alone (up to 50% of the seed set level of open pollinated plants). Plants in Las Cañadas have been pollinated by honey bees since these were introduced in the 16th century (Méndez, 2000). Thus, honey bees have influenced the pattern of pollen transfer in 80–100 plant generations, which may have contributed to purging of deleterious alleles expressed through inbreeding. On the other hand, inbreeding effects in life stages other than seed viability cannot be ruled out. In a close relative, Echium vulgare L., no effect of selfing was found at the stage of seed production (Mensler et al., 1997). Yet, late-acting inbreeding depression in male and female function of offspring derived from selfing has been reported (Mensler et al., 1999). Future studies should address patterns of genetic variation in populations of E. wildpretii pollinated predominantly by introduced honey bees versus those pollinated by native animals. For instance, in Grevillea macleayana (Proteaceae), which is visited mainly by native birds and introduced honey bees, outcrossing rate was reduced significantly when birds were excluded from the inflorescences (England et al., 2001). Furthermore, an interesting question is the role of birds as pollinators and potential long-distance pollen vectors of E. wildpretii in early flowering season, before the onset of bee-keeping activities.

Many studies call for further investigation of the effects of honey bees on the reproductive output of plants and long-term persistence of native flower-visiting animal populations. However, impact of honey bees are difficult to disentangle from confounding biotic and abiotic factors. Las Cañadas offers a unique and simple study system. The total flowering season of the sub-alpine desert system is short (ca. 2 months) and the plant–flower-visitor network is simple, consisting of a few species isolated by the crater rim and surrounding pine forest (Dupont et al., 2003). Furthermore, placement of beehives can be controlled both spatially (as in Paton, 1993) and temporally, creating gradients of honey bee visitation pressure over the season and between plant populations.

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