

Pollinator networks, alien species and the conservation of rare plants: *Trinia glauca* as a case study

L. G. Carvalho¹, E. R. M. Barbosa¹ and J. Memmott^{1*}

¹School of Biological Sciences, Woodland Road Bristol, BS81UG, UK

Summary

1. Despite the essential role of pollination in the maintenance of many rare plant species, conservation management plans rarely consider the service of pollination.
2. This study identifies the main pollinators of a rare English plant species, *Trinia glauca* (Apiaceae), and provides recommendations for its conservation. A community-level approach is used, whereby a visitation network is constructed to identify the direct and indirect links between *T. glauca* and other members of the plant-visitor community.
3. A field experiment that excluded the main visitor species from female *T. glauca* showed that ants were the main pollinators of *T. glauca*. The network revealed that over the field season, 33% of the ants' visits to flowers were to alien plants, with *Cotoneaster horizontalis* making a particularly high contribution (58%) during the *T. glauca* flowering period.
4. The removal of alien plants is a part of the conservation management of *T. glauca*, and we simulated the likely consequences of this form of habitat management on *T. glauca* pollination, any effects being mediated by shared pollinators. Although positive or neutral effects are possible, a negative effect is also possible, whereby the removal of alien plants leads to a crash in ant populations, potentially reducing pollinator visits to *T. glauca* by up to 85.2%.
5. *Synthesis and applications.* Conserving the pollinators of rare plants is essential if their conservation is to be sustainable in the long term. Our data indicate that *T. glauca* is pollinated by ants and demonstrate that ants can also feed on alien plants, particularly during the flowering season of this rare plant. We suggest that management measures involving the removal of alien plants should consider the possible negative impacts on rare plants through changes in pollinator populations. In this case, a staged removal is likely to prove the best conservation approach, allowing the pollinators' response to be assessed before any serious negative effects occur.

Key-words: alien plants, conservation of rare plants, food web as a predictive tool, network, pollination by ants, rare plant, *Trinia glauca*

Introduction

Pollination plays a key role in the maintenance of genetic variability of plant species (e.g. Ohara *et al.* 1996; Paschke, Bernasconi & Schmid 2005), facilitating adaptation to environmental change (Lewontin 1974). Habitat fragmentation, one of the main threats to biodiversity worldwide, impacts strongly on pollination (Sih & Baltus 1987; Aizen & Feinsinger 1994; Valdivia, Simonetti & Henriquez 2006). However, some populations of rare plants maintain stable densities through long periods of time (Lawton 1995; Mace & Kershaw 1997). An understanding of the pollination system of these rare plant species, especially those with fragmented populations, is likely

to provide important pointers for the conservation of rare species in general.

Given that most plants use multiple pollinators and most pollinators visit multiple plants (e.g. Waser *et al.* 1996), pollination can be viewed at the level of an entire ecological community as a web, or network, of mutually beneficial (mutualistic) interactions between the species (nodes of a network) of two trophic levels. Therefore, an impact on a given species can propagate through the network affecting other species. Food web ecologists have developed methods to quantify the robustness of complex networks to the loss of species (Solé & Montoya 2001; Dunne *et al.* 2002; Memmott, Waser & Price 2004). This approach involves the simulation of primary extinction of species, by removing them from the network, and quantifying any ensuing secondary extinctions, for example, when primary plant extinctions leads to secondary

*Correspondence author. E-mail: jane.memmott@bristol.ac.uk

extinction of their specialist pollinators. The amount of extinction that can be tolerated before the network collapses (i.e. when all species are lost) can be remarkably small. For example, in a 154-species network, constructed by Memmott, Martinez & Cohen (2000), the simulation of a 10% species loss was sufficient to elicit a cascade of secondary extinctions which led to network collapse (Dunne, Williams & Martinez 2004). To date, one of the main limitations of these types of simulations is not considering the potential of adaptation, whereby consumers adjust their diet to the remaining available resources. Generalist species (i.e. species with large diet breaths) would be most likely to adapt, thereby reducing the impact of species loss (Dunne *et al.* 2002; Memmott *et al.* 2004) and increasing the stability of food webs (Kondoh 2003). Moreover, the possible existence of 'parametric thresholds' (Bascompte 2003) must be considered, whereby the impact of species loss is enhanced, due to extinction occurring before the total exhaustion of resources.

While alien species are usually undesirable in areas of high conservation status, they can be linked to many native species through shared pollinators (e.g. Olesen, Eskildsen & Venkatasamy 2002) and, therefore, there may be a negative effect on native species following their removal, at least in the short term. With two exceptions to date (Lopezaraiza-Mikel *et al.* 2007; Bartomeus, Vila & Santamarz ya 2008), ecologists have considered only the impact of removing native species from ecological networks. However, using network analysis to predict the impact of removing alien species at the community level could provide data to guide an eradication programme.

Our chosen rare plant, *Trinia glauca* (Apiaceae), commonly known as honewort, is common in several regions of Europe with its distribution limits in South England, Iberian Peninsula and South-west Asia (Constantinidis, Bareka & Kamari 2002; Nieto-Feliner, Jury & Herrero 2003). In England, this perennial monocarpic herb species is extremely rare, with a highly fragmented distribution, and is restricted to a few dry limestone sites (Lovatt 1982) in the Bristol/Somerset region (Preston *et al.* 2002). Being dioecious, it is an obligate out-breeder, it is also a poor disperser (Lovatt 1982), which combined with its rarity, puts the plant (theoretically at least) at a high threat of extinction in England. However, the population of this plant has remained relatively stable over many years, and in recent decades, there has even been a reported increase in abundance (Preston *et al.* 2003). Ants are believed to be important pollinators of *T. glauca* (Proctor & Yeo 1973; Lovatt 1982), based on observation of visitation (Lovatt 1982) rather than evidence of pollination. Its high dependency on cross-pollination (a result of its dioecy) makes pollinator conservation a key part of any management strategy.

The aim of our work was to identify the pollinators of *T. glauca* in England in order to improve management recommendations. The objectives were threefold: (i) to use a visitation network to quantify and identify *T. glauca*'s flower visitors and to determine how these integrate into the local flower visitation network; (ii) to experimentally exclude putative key pollinators at two sites to determine their role in the pollination of *T. glauca*; (iii) to use a species-loss simulation

to predict the impact of removing alien plant species on flower visitation frequency of *T. glauca* and to use this information to make management recommendations.

Methods

FIELD SITES

Three of the seven populations of *T. glauca* recorded in England (Preston *et al.* 2002) were studied during this research. Visitation data were gathered in 2004 in the Avon Gorge (Grid Reference ST5674), an iconic field site well known for its rare plant populations (Marren 1999). Although 1 year of visitation data do not capture inter-annual variability, it does provide a detailed snapshot of how alien plants are integrated in the native flower visitation network. The field experiment was run in 2005 at two sites: Crook Peak (ST387558), an area of semi-natural grassland and scrubland, and Sand Point (ST315658), a small area of a south-facing headland with maritime grassland and scrub. Male plant abundance was higher than female plant abundance with the sex-ratio among adult plants being approximately 3:2 at both sites.

CONSTRUCTION OF THE VISITATION NETWORK

An area of 1480 m² was selected in the Avon Gorge covering a broad range of flowering plants and enclosing the site's entire population of *T. glauca*. A total of 11 survey visits were carried out from 10 May to 27 September 2004, this covering the main period of insect activity. Flower and insect surveys took place approximately every 14 days under dry conditions.

In each flower abundance survey, a stratified random design was used to select 1 m² quadrats in the study area. The area was divided into nine sub-areas based on habitat type and accessibility. Each sub-area was divided into 1 m² quadrats and 2.5% (37) of these were randomly selected per sampling occasion. In each quadrat, the number of floral units of each plant species was recorded, defined as the distance that a small bee (*c.* 1 cm length) would fly, rather than walk (Saville 1993). For example, in the Asteraceae, a flower unit is the entire inflorescence while in the Rosaceae, a flower unit is a single flower. Thus, the floral unit is defined from the bee's perspective rather than by flower anatomy. Rare flowers which were missed using this method were included in the food web data as rare species with an abundance of two flower units (which was the lowest number of units observed in the plot for any species).

In the insect surveys, an observation point was chosen for each flowering plant species by randomly selecting one of the quadrats where the species was present. All the flowering units that could be surveyed by a single observer (approximately a semi-circle with 1-m radius) were observed for 20 min. On consecutive sampling occasions, plant species were rotated through three time slots, the morning (09.00–12.00 h), early afternoon (12.00–15.00 h) and late afternoon (15.00–18.00 h), to allow each species to be observed equally over time. At least two floral units were observed per plant species per sample. All flower–visitor interactions were recorded, and all visitors observed were collected for identification. To estimate the overall abundance of each plant species, the average number of flower units per 1 m² quadrat was multiplied by the total area of the study site. To estimate the interaction frequency for each visitor–plant species pair, we divided the total number of visits recorded by the number of flower units observed (per 20 min) and then multiplied by the total number of floral units in the study plot. By collecting the insects, we did not

allow for repeated visits by the same individual; hence, some visitation frequencies may be underestimated. However, collecting specimens is essential for identification of most visitor species. Hymenoptera, Diptera, and Coleoptera were identified by taxonomists either to species or to morphospecies. Lepidoptera were identified to species by the authors and Heteroptera and parasitoids were morphotyped by the authors. To estimate the overall abundance of each insect species, we sum the overall number of visits per plant species. The data were used to draw a visitation web using software written in MATHEMATICA® (Wolfram Research, Inc.). Finally, given the importance of ants in *T. glauca* pollination (see Results), we calculated the contribution of alien plants to ants' floral diet in each survey visit.

THE FIELD EXPERIMENT

Pollinator exclusion

The visitation network (see Results) revealed that three ant species made up 85.2% of the insects visiting *T. glauca* in the Avon Gorge. This information, along with the fact that ants are reported to be pollinators of *T. glauca*, led us to exclude ants from *T. glauca* in the field experiment. Given that it was impossible to exclude each ant species separately, we excluded all ant species from the experimental plants. In each field site (Crook Peak and Sand Point), 70 female plants were selected: 35 plants at each site were allocated to an ant exclusion treatment and 35 were left as controls. To exclude ants from the experimental plants, a ring of fruit tree grease (approximately 15 mm wide) was painted 5–10 cm away from the base of the stem, prior to flowering. To maintain the effectiveness of the exclusion treatment, these plants were checked at least every 10 days, with grease reapplied where necessary. No crawling pollinators other than ants were observed at either of the two sites. The experiment encompassed the *T. glauca* flowering season (12 April to 24 May 2005). At the end of the experiment the number of seeds and the number of unfertilized ovules were counted for each *T. glauca* to estimate reproductive success and a two-way analysis of variance was applied to logit transformed data to test whether reproductive success varied among treatments and sites.

Insect visitation at Crook Point and Sand Point

Insect visitation to *T. glauca* was observed at both experimental sites to check whether ants were the main visitors here as in the Avon Gorge (see Results). Sites were visited once a week for 6 weeks. A maximum of 25 flowering *T. glauca* female plants were observed for 20 min per sampling occasion. To avoid interfering with visiting behaviour and to estimate visitation frequency, insects contacting the stigmas during the observation periods were only collected after leaving the observed plant and placed in tubes lined with paper to prevent transfer of pollen between insects. If a visitor could not be caught, the order of the species was recorded and they were classified as ants (distinguishing between *Lasius* sp. and *Myrmica* sp.) or flying visitors. For each flower visitor, the total number of flower units visited and the time spent on the observed plant were recorded. Visitation frequency was then calculated for each specimen by dividing the number of flower units visited by the time spent on the observed plant. Data on visitation frequency between ants and flying visitors were compared using Poisson regression models corrected for overdispersion, and *F* tests on changes in deviance (Sileshi 2006). To quantify the effectiveness of the exclusion treatment, the ant-excluded plants were also observed. All insects were identified

to species or morphotyped by taxonomists. All pollinator observations took place between 09.00 h and 19.00 h. A total of 107 observation periods were carried out on female plants.

Insect pollen loads

To count the number of pollen grains of *T. glauca* transported by each insect, up to 45 specimens per species per field site were collected on female flowers. Although some pollen may have been lost during flower visitation (i.e. pollen deposition had occurred), this will have affected all visitors equally. Each collected insect was systematically dabbed with a small cube (approximately $3 \times 3 \times 2$ mm) of glycerine jelly containing basic fuchsin stain (Kearns & Inouye 1993). After sampling the pollen, the jelly was placed on a microscope slide, melted and covered with a cover slip for analysis. Pollen grains were counted and identified by comparing them to a pollen reference collection made from flowers at the field sites. As a substantial proportion of the data on pollen grains count are zeros, the number of *T. glauca* pollen grains, and the number of other species' pollen grains, carried by each visitor species were compared using Poisson regression models corrected for overdispersion, and *F* tests on changes in deviance (Sileshi 2006).

THE SPECIES-LOSS SIMULATION

While the plant list from the Avon Gorge is predominantly native, there are a notable number of aliens (see Supplementary material Table S1). These species all grew in the study plot near to the *T. glauca* population. The removal of these species is currently one of the ongoing management techniques used in the conservation of the Avon Gorge (Higgins 2006). To predict the effect of this management on *T. glauca* pollination, the removal of alien plants was simulated *in silico*, using the subset of the network data that includes all the interactions of visitors to *T. glauca*.

The species-loss simulation quantifies how the removal of a given species will affect the other species in the network (Solé & Montoya 2001; Dunne *et al.* 2002). In our network, the removal of alien plants constituted the primary extinctions in this analysis, this being simulated by simply removing all the alien plants from the network data. Given that *T. glauca* visitors have generalized dietary requirements (see Results), it is likely that their visitor species will adjust their diet to fit the available resources following the removal of alien species. Therefore, we developed a mathematical expression that models how each flower visitor species adapts its diet to the remaining resources. Assuming that: (i) there is an equilibrium situation before alien removal, where ants foraging strategy is optimized according to resource conditions (e.g. availability of resource, energy supplied by resource); (ii) for the remaining resources, these conditions will not change; and (iii) ants' maximum foraging range was detected with the sampling method, the adaptation of ants to the removal of a resource will be mostly dependent on the saturation level of the remaining resources (i.e. whether a given plant species can or cannot support further insect visits). Therefore, the importance of a given remaining unsaturated resource to the consumer's diet, after the visitors' foraging strategy has adapted to the removal of alien plants, would be given by the original importance (proportion) of that resource to the consumers' diet plus a fraction of the importance of the lost (removed) alien resources:

$$\begin{cases} A_i = O_i + R \times \frac{O_i}{\sum_{j=1}^n (O_j)} & \text{if } i \text{ is unsaturated} \\ A_i = O_i & \text{if } i \text{ is saturated} \end{cases}, \quad \text{eqn 1}$$

where A_i is the proportion of a given remaining resource species i in the consumer's diet after the removal of alien plants, O_i is the original proportion of a given remaining resource species i in the consumer's diet, R is the proportion of the removed resource in the consumer's diet, before the removal of alien plants and n is the total number of unsaturated resources. For example, if a given visitor's diet consists of 40% of native plant species A (unsaturated resource), 10% of a native plant species B (unsaturated resource) and 50% by alien plant resources, after alien plants removal, plant A will make up 80% of the visitor's diet, while plant B will make up 20%. However, if native plant B is a saturated resource before alien plant removal, the final outcome will be plant A making up 90% of the visitor's diet, while plant B will remain at 10%. Although simplistic, this model can be used to simulate a range of scenarios after habitat management that leads to reduction of resource diversity (here removing four species of alien plants from the habitat). Moreover, if all remaining resources are saturated, adaptation will not be possible, and consequently, the final level of resources will be reduced. If these remaining resources remain below the minimum amount of available resource needed for the persistence of the populations of the species involved (the parametric threshold, see Bascompte 2003), local consumer populations may decline. Three scenarios were simulated, following the removal of alien plants:

Scenario 1 None of the native resources available in the foraging range is saturated; hence, ants forage on the remaining species in the same proportion as they did before, maintaining ant population abundance;

Scenario 2 *Trinia glauca* is saturated as a flower resource to ants but other native resources are not; hence, ants forage on *T. glauca* equally as before, but increase the visitation rates to unsaturated species (using those unsaturated resources in the same relative proportion as they used before alien removal), thereby maintaining ant abundance;

Scenario 3 All native resources available in the foraging range are saturated; thus, removal of alien resources leads to an overall reduction of the resources. If the remaining level of resource is lower

than the ants' parametric threshold, the ant population will decline, leading to local extinction.

While intermediate situations could be simulated depending on which resources are or are not saturated, these three scenarios reveal the range of possible effects on *T. glauca* that the removal of alien plants could elicit.

Results

THE VISITATION NETWORK

There is a complex network of interactions between plants and their flower visitors in the Avon Gorge that links together 260 species (81 plant species, 179 insect species and morphospecies). *T. glauca* is linked directly to six species of insects, which link it indirectly to many other species of plants and their insect visitors (Fig. 1). The species of insect that visited *T. glauca* were: the ants *Lasius alienus* ss Förster (59.3% of visitors), *Formica fusca* L. (18.5%) and *Temnothorax albipennis* Curtis (7.4%), the ant-mimicking heteropteran *Myrmecoris gracilis* R.F. Sahlberg (11.1%), the weevil *Phyllobius roboretanus* Gredler (3.7%) and Thysanoptera (< 0.1%). Both dominant ant species, *L. alienus* and *F. fusca*, were generalist visitors, visiting 10 and 15 other plant species present at the field site, respectively.

During *T. glauca*'s flowering period, an alien, *Cotoneaster horizontalis* Decne (Rosaceae), made a particularly high contribution (57.7%) to the ant's floral diet of pollen and/or nectar (Table 1). Following the flowering period of *T. glauca*, the three dominant floral resources used by ants were a native plant, *Scabiosa columbaria* L. (Dipsacaceae), and two alien plant species, *Centranthus ruber* L. DC (Valerianaceae) and *Cotoneaster franchetti* Bois (Rosaceae).

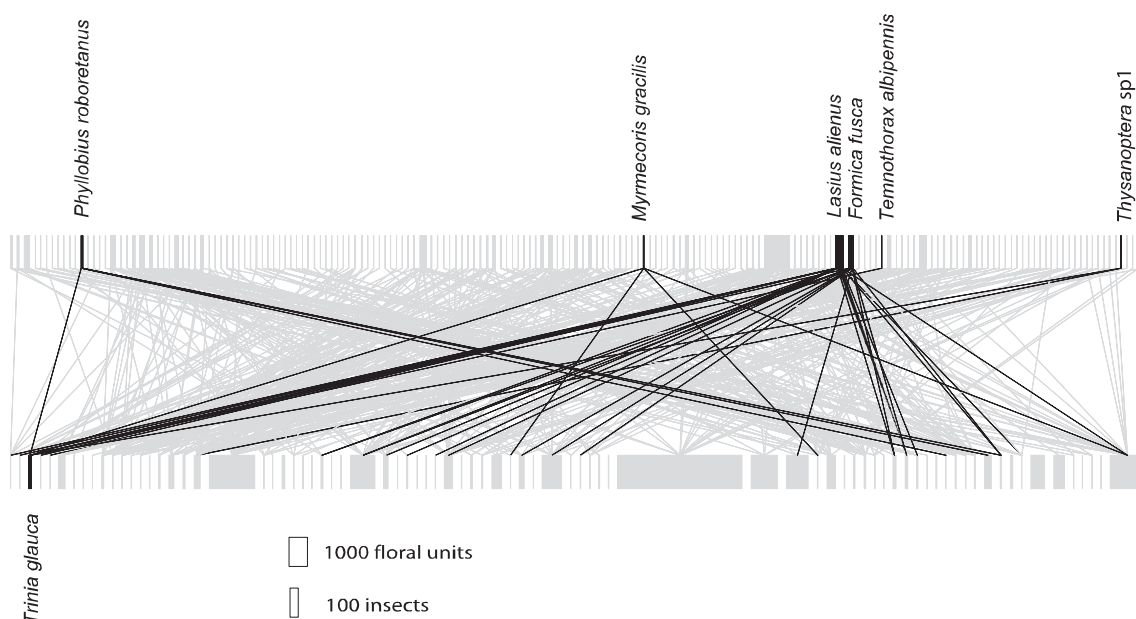


Fig. 1. Flower visitation web for plant community at Avon Gorge. Each species of plant and insect is represented by a rectangle. A list of plant and visitor species is provided in the Supplementary Material. The widths of the rectangles represent overall species abundance at the field site and the size of the lines connecting them represents the frequency of interaction in the study area. All interactions with *Trinia glauca* are shown in black. The scale bar represents the number of flower units present in the study area and the number of insects present in the study area.

Table 1. The flowering plant species used by the two main visitors of *Trinia glauca* in Avon Gorge (*Formica fusca* and *Lasius alienus*), through the main flowering season (May to September). The data in the table are the average number of ants 20 min⁻¹ visiting each flower species over the field season. The symbol '–' represents months when the plant species was not flowering

	Early May	Mid- May	Early June	Mid- June	Early July	Mid- July	Early August	Mid- August	Early September	Mid- September	End September	Total
Native resources												
<i>Trinia glauca</i> (L.) Dummort	35	51	–	–	–	–	–	–	–	–	–	86
Other native plant species	25	129	270	146	47	27	1	1	2	0	1	649
Alien resources												
<i>Centranthus ruber</i> (L.) DC	–	–	159	–	–	–	–	–	–	–	–	159
<i>Cotoneaster franchetti</i> Bois	–	–	10	8	69	31	–	–	–	–	–	118
<i>Cotoneaster horizontalis</i> Decne	81	–	–	–	–	–	–	–	–	–	–	81
<i>Smyrniolum olusatrum</i> L.	1	1	–	–	–	–	–	–	–	–	–	2
Total contribution of alien plants to ant's diet (%)	57.7	0.60	38.5	5.20	59.5	53.4	0	0	0	0	0	32.9

THE FIELD EXPERIMENT

Pollinator exclusion

The tree grease was 79% effective at excluding ants at Crook Peak and 66% effective at Sand Point (these figures being the percentage of ant-excluded plants on which ants were never seen). It was impossible to achieve 100% exclusion, as both sites are windy and bridges of vegetation were often blown across the grease barrier. At both field sites, 28 experimental and seven control plants were lost, chiefly by grazing sheep or rabbits. Fewer controls were lost because these were replaceable whereas the experimental plants could not be replaced (as the tree grease needed to be in position for the duration of the field season). Despite the reduced sample size there was a significant decrease in reproductive success between the control and ant-excluded plants (average reproductive success (mean \pm standard deviation): Control = 77.2% \pm 10.4, Experimental = 54.3% \pm 13.9; $F_{1,101} = 34.3$, $P < 0.0001$, Fig. 2). There was no effect of site on reproductive success ($F_{1,101} = 0.2$, $P = 0.6670$), and no significant interaction between treatment and site ($F_{1,101} = 1.9$, $P = 0.1680$).

Insect visitation at Crook Peak and Sand Point

At both field sites, the ant species *L. alienus* and *Myrmica sabuleti* (Meinert) were the most frequent visitors to female flowers of *T. glauca*. At Crook Peak, ants were responsible for 98% of visits, and at Sand Point, ants were responsible for 80.7% of visits. Seven other species were observed visiting *T. glauca* female flowers: two dipterans (*Delia pratensis* (Meigen) and another unidentified small Dipteran), three coleopterans (*Aphthona euphorbiae* (Schränk); *Cantharis rustica* Fallén; *Meligethes* sp.) and two hymenopterans (an Andrenidae sp. and a parasitoid wasp). However, with the exception of one of the dipterans, *D. pratensis*, all other species were recorded on just one or two occasions over the sampling season. There was no significant difference in average visitation frequency to *T. glauca* flowering female plants between ant and flying visitor specimens [$F_{1,151} = 0.0$, $P = 0.9475$, visitation frequency

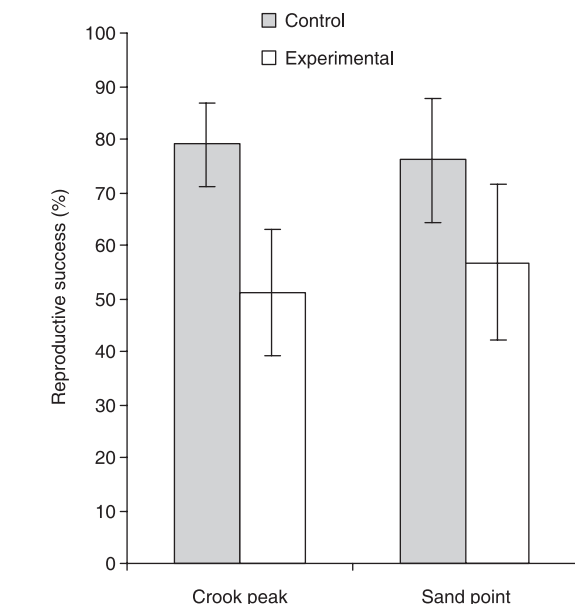


Fig. 2. Average reproductive success (% of developed seeds) in Control and Experimental (ant-excluded) female plants of *Trinia glauca* at Crook Peak and Sand Point. Error bars represent the standard deviation.

(ants) = 10.0 \pm 22.7, visitation frequency (other visitors) = 10.4 \pm 21.2]. Both species of ants as well as *D. pratensis* also visited male *T. glauca* plants.

Insect pollen loads

There was no significant difference in the number of *T. glauca* pollen grains carried by ants and other visitors [$F_{1,151} = 3.2$, $P = 0.0767$, average number of pollen grains (ants) = 45.9 \pm 153.9, average number of pollen grains (other visitors) = 121.8 \pm 303.6, n (ants) = 129, n (other visitors) = 25]. However, ants carried significantly less heterospecific pollen than flying visitors ($F_{1,151} = 37.3$, $P < 0.0001$, average number of pollen grains (ants) = 1.2 \pm 3.2, average number of pollen grains (other visitors) = 21.0 \pm 58.2).

THE SPECIES-LOSS SIMULATION

Alien plants were important components of the ants' floral diet in the Avon Gorge with 33% of ant visits being to alien plants. The alien species-loss simulation was run on the subsection of the network which describes the interactions between *T. glauca*, its visitors and its visitors' other host plants (see Fig. 3a). The outcomes were:

Scenario 1 Alien removal affects *T. glauca* positively if its floral resource is unsaturated with respect to pollinator visitors. If all other native resources are also unsaturated, ants may use those and *T. glauca* to compensate the loss of alien plants, increasing visitation frequency to all native plants pro rata (Fig. 3b). This leads to a 106.9% increase in visitation frequency to *T. glauca*. *Scenario 2* Alien removal has a neutral effect on *T. glauca* if its floral resource is saturated, and hence ants replace alien

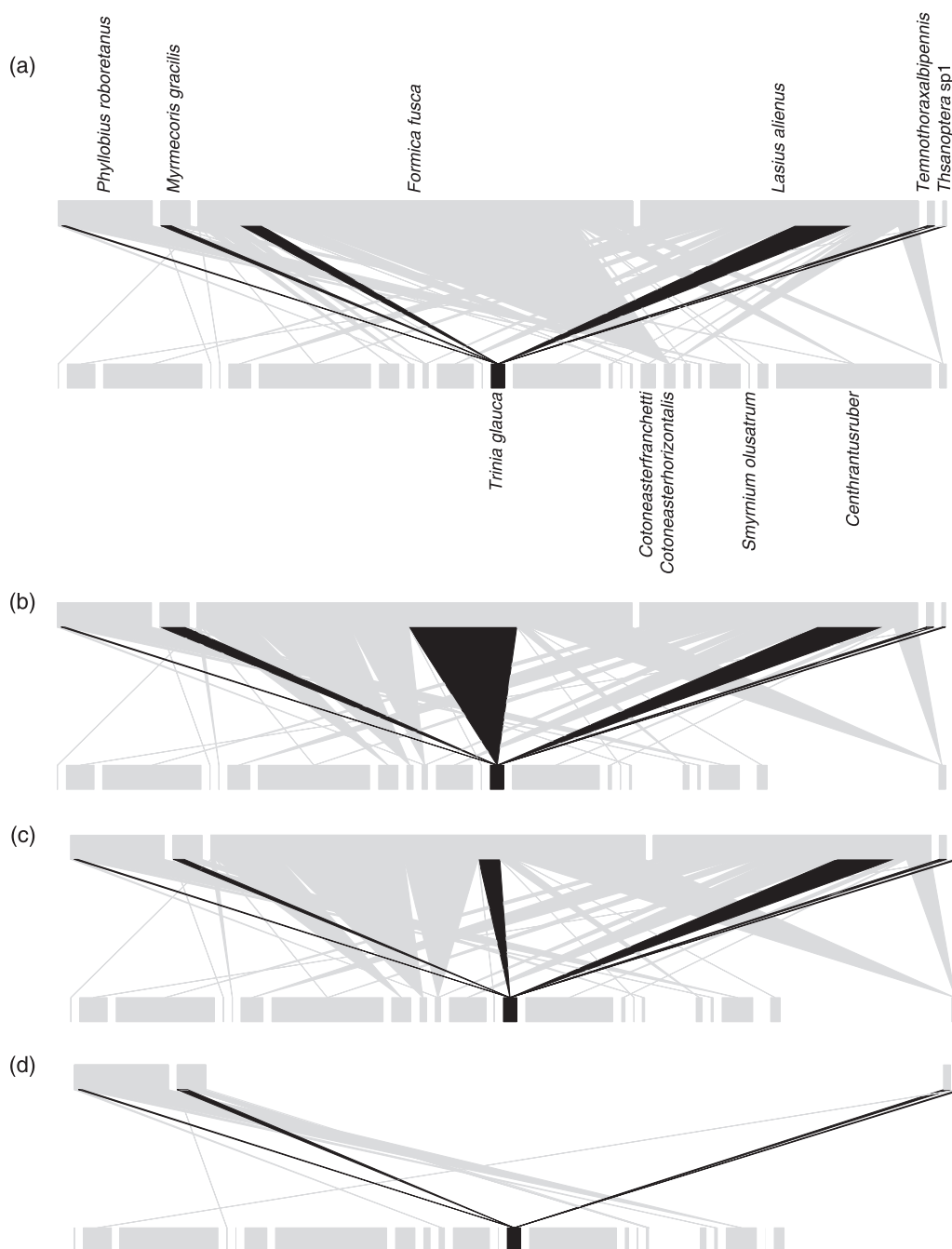


Fig. 3. Simulation of the consequences of the removal of alien plants in a subset of the Avon Gorge flower visitation web with all plant species that share flower visitors with *Trinia glauca*: (a) Actual flower visitation data in 2004, showing *T. glauca* and the alien plant species; (b) scenario 1: positive effect with an increase of visitation by ants to native plants, (c) scenario 2: neutral effect due to saturation of *T. glauca* as food source and (d) scenario 3: negative effect due to decline of the ant population. Each species of plant and insect is represented by a rectangle, with the aliens removed from Fig. 3b–d. All interactions with *T. glauca* are shown in black. For explanation of the figure see Fig. 1.

species by native unsaturated resources other than *T. glauca* (Fig. 3c). In this case, visitation frequency to *T. glauca* would remain unchanged.

Scenario 3 Alien removal affects *T. glauca* negatively as ant populations crash due to saturation of the remaining resources. Ants cannot compensate the lost resources and this leads to an 85.2% reduction in the number of visitors to *T. glauca* (Fig. 3d).

Discussion

Trinia glauca, in common with many plants, is embedded in a complex network of plant–flower visitor interactions in the Avon Gorge. Our food web approach identified ants as the likely pollinators of this rare plant, and this was verified by the field experiment. A species-loss simulation approach was used to predict the likely effects of removing alien species (a key management strategy in many nature reserves worldwide) on the rare plant's reproductive success and showed that one potential outcome is an 85.2% reduction in visitation to *T. glauca*, which according to the results of the pollinator exclusion experiment may lead to a significant reduction in seed set.

THE VISITATION NETWORK

Our visitation network adds to a growing collection of plant–pollinator networks, joining the much smaller subset of data in which species abundance along with the frequency of interactions between the species are quantified. The network shows that six species of insects visited *T. glauca*, with ants making up the majority of these visits. The ant species feed on a broad range of flowering plants and adapted their diet according to availability of the flower resource (Table 1). Alien plants comprised a substantial part of the ants' diet, especially during *T. glauca* flowering season. Since these alien species are very nectar-rich and given that nectar is one of the most important components of a number of ant species' diet (Ricogray 1993; Koptur & Truong 1998), it is possible that the removal of aliens will have at least a short-term impact on ant populations.

THE FIELD EXPERIMENT

Although ants are often considered ineffective pollinators (e.g. Beattie *et al.* 1985; Peakall & Beattie 1991), the reduction in *T. glauca* reproductive success clearly shows that they are important pollinators of this plant. There are other examples of pollination by ants especially in plant species with a low, dense flowering stature (Gomez & Zamora 1992; Gomez *et al.* 1996). However, due to the relatively small radius of movement around their nests, pollen transfer by ants can lead to a restricted gene flow (Peterson, Bartish & Peterson 2002). Consequently, sporadic visits by flying visitors, such as bees and flies, may be very important for the maintenance of genetic diversity within a plant population. *Delia platura* (Diptera: Anthomyiidae) was the only flying visitor species

whose frequency of visitation was sufficiently high at the field sites for it to be considered a potential pollinator. Nevertheless, it was still a rare visitor, being recorded in only one of the sites (Sand Point) and with all its visits recorded in one single day. In contrast, ants were reliable visitors, being present at all three field sites and visiting *T. glauca* throughout its flowering season. Furthermore, flying visitors' pollen loads contained significantly more heterospecific pollen grains in comparison to ants and the deposition of foreign pollen on stigmas by flying visitors could negatively affect pollination (e.g. Brown & Mitchell 2001).

Even allowing for the fact that the ant-exclusion treatment was not 100%, the reproductive success obtained when ants were excluded was surprisingly high (51.2% at Crook Peak and 56.8% at Sand Point), particularly when considering that the plant is dioecious. Therefore, some of the seeds must be pollinated by other means. The very low number of flying visitors makes it unlikely that these are responsible for the remainder of the pollination. Given that members of the plant family Apiaceae do not possess the characteristics of Lepidoptera-pollinated flowers (Proctor, Yeo & Lack 1996), nocturnal visitors such as moths are unlikely to be making up the difference. The two *T. glauca* populations at the experimental sites were located in steep and windy areas and were at a high population density, with male and female plants adjacent, and it is possible that wind or the physical brushing together of flowers under windy conditions leads to pollination at the two experimental sites. However, the *T. glauca* population in Avon Gorge is located in a sheltered gully and plants are sparsely distributed; therefore, it is likely to be much more dependent on its pollinators for reproduction, and hence would suffer higher losses in seed set in the absence of ant pollination. Another possible explanation for the unexpectedly high reproductive success is the occurrence of agamospermy (the asexual formation of embryos and seeds without the occurrence of fertilization), which is a common occurrence in many angiosperm taxa, including Apiaceae (Plitmann 2002), albeit unrecorded for *T. glauca*. Interestingly, although the control plants had higher reproductive success, it never reached 100%, which may be due to pollen limitation or other ecological factors, such as limited nutrients.

THE SPECIES-LOSS SIMULATION

The species-loss simulation revealed how *T. glauca* could be affected by the removal of alien plants from Avon Gorge, this impact being mediated indirectly by shared pollinators. Further field experiments are needed to determine which of the three scenarios actually occurs in the field. This could be done within the programme of alien-plant removal currently running in the Avon Gorge. Ideally, such experiments should include measuring the impact of alien plant removal on *T. glauca* seed production and recruitment in the short term (immediately after alien removal) and the long term (after several insect generations).

IMPLICATIONS FOR THE MANAGEMENT OF *TRINIA GLAUCA*

Given that *T. glauca* is a rare plant species with a highly fragmented distribution, it could be at high risk of extinction (Lawton 1995; Aguilar *et al.* 2006; Pocock *et al.* 2006). The maintenance of its pollinators is essential if its conservation is to be sustainable in the long term. As alien plants represent an important component of the ants' floral diet, they may be important for *T. glauca* populations in the short term. While we are not suggesting that removal of alien plants is an inappropriate management tool, we suggest that their impact on pollinators is at least considered: without the pollinators, the conservation of the rare plants in the Avon Gorge cannot be considered a sustainable conservation programme. Management plans that involve removal of alien plants need to consider the unintended, indirect, short-term negative impact, as well as the intended long-term positive gains. In the case of *T. glauca*, a cautious approach would be recommended whereby part of the alien plant population is removed and ant behaviour and abundance are closely monitored. This approach would enable 'beneficial' insects, such as the ants, that currently rely on the alien plants to adapt to the change of resources.

Conclusion

Pollinators are undergoing a widespread decline in Europe which is believed to be leading to declines in insect-pollinated plants (Biesmeijer *et al.* 2006). Consideration of the importance of pollinators in conservation management plans is a laudable aim, but one that is rarely implemented in practice (Memmott *et al.* 2007). Here we have shown that ants are effective and important pollinators in the reproduction of a rare plant. A network approach is a novel tool in conservation research and management practices, but one that could prove very useful as it reveals how rare species are linked to abundant and invasive species. By identifying likely pollinators and making predictions concerning the consequences of management practices at the site, our work provides an example of how network data can inform conservation management decisions. Testing the predictions provided by a network approach using rigorous experiments is possible within many conservation programmes involving removing alien species and is likely to provide new perspectives on the management of both natural and managed ecosystems.

Acknowledgements

We thank Libby Houston for help with field site selection; Bristol City Council for permission to work on Avon Gorge; Ashley Robertson for *Sorbus* identification; Ruth Boada, Kate Henson, Audrey Collings, Nick Boase and Della Craven for help in data collection; Glenda Orledge and Cedric Collingwood for identification of ant species, using the separating characters described by Czechowski, Radchenko & Czechowska (2002) and by Alexander & Orledge (2006); John Deeming, Brian Levey and Mark Pavott for insect identification; Phil Quinn for help with plant identification; Michael Pocock for help on statistics; Mariano Devoto and four anonymous referees for comments on the manuscript; Fundação para a Ciência e Tecnologia (Portugal) for funding (L.G.C.).

References

- Aguilar, R. *et al.* (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters*, **9**, 968–980.
- Aizen, M.A. & Feinsinger, P. (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology*, **75**, 330–351.
- Alexander, K.N.A. & Orledge, G.M. (2006) *Lasius platythorax* Seifert (Hymenoptera: Formicidae) in Ireland, with notes on the distinctions between *Lasius platythorax* and *Lasius niger* (L.). *Irish Naturalists' Journal*, **28**, 249–252.
- Bartomeus, I., Vila, M. & Santamarz a, L. (2008) Contrasting effects of invasive plants in plant-pollinator networks. *Oecologia*, **155**, 761–770.
- Bascompte, J. (2003) Extinction thresholds: insights from simple models. *Annales Zoologici Fennici*, **40**, 99–114.
- Beattie, A.J. *et al.* (1985) The vulnerability of pollen and fungal spores to ant secretions – evidence and some evolutionary implications. *American Journal of Botany*, **72**, 606–614.
- Biesmeijer, J.C. *et al.* (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, **313**, 351–354.
- Brown, B.J. & Mitchell, R.J. (2001) Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia*, **129**, 43–49.
- Constantinidis T., Bareka, E.P. & Kamari, G. (2002) Karyotaxonomy of Greek serpentine angiosperms. *Botanical Journal of the Linnean Society* **139**, 109–124.
- Czechowski, W., Radchenko, A. & Czechowska, W. (2002) *The Ants (Hymenoptera: Formicidae) of Poland*. Museum and Institute of Zoology PAS, Warsaw, Poland.
- Dunne, J.A. *et al.* (2002) Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters*, **5**, 558–567.
- Dunne, J.A., Williams, R.J. & Martinez, N.D. (2004) Network structure and robustness of marine food webs. *Marine Ecology Progress Series*, **273**, 291–302.
- Gomez, J.M. & Zamora, R. (1992) Pollination by ants: consequences of the quantitative effects on a mutualistic system. *Oecologia*, **91**, 410–418.
- Gomez, J.M. *et al.* (1996) Experimental study of pollination by ants in Mediterranean high mountain and arid habitats. *Oecologia*, **105**, 236–242.
- Higgins, R. (2006) *Avon Gorge and Leigh Woods Management Plan, Bristol Side Management Proposals*, Wessex Ecological Consultancy, Bristol, UK.
- Kearns, C.A. & Inouye, D.W. (1993) *Techniques for Pollination Biologists*. University Press of Colorado, Niwot, Colorado, USA.
- Kondoh, M. (2003) Foraging adaptation and the relationship between food-web complexity and stability. *Science*, **299**, 1388–1391.
- Koptur, S. & Truong, N. (1998) Facultative ant-plant interactions: nectar sugar preferences of introduced pest ant species in south Florida. *Biotropica*, **30**, 179–189.
- Lawton, J.H. (1995) Population dynamics principles. *Extinction Rates* (eds J.H. Lawton & R.M. May), pp. 147–163. Oxford University Press, Oxford, UK.
- Lewontin, R.C. (1974) *The Genetic Basis for Evolutionary Change*. Columbia University Press, New York.
- Lopezaraiza-Mikel, M.E. *et al.* (2007) The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters*, **10**, 539–550.
- Lovatt, C.M. (1982) *The history, ecology and status of the rare plants and the vegetation of the Avon Gorge, Bristol*. PhD Thesis. University of Bristol, Bristol, UK.
- Mace, G.M. & Kershaw, M. (1997) Extinction and rarity on an ecological timescale. *The Biology of Rarity: Causes and Consequences of Rare-Common Differences* (eds W.E. Kunin & K.J. Gaston), pp. 130–149. Chapman & Hall, London.
- Marren, P. (1999) *Britain's Rare Flowers*. Poyser, London.
- Memmott, J., Gibson R.H., Carvalho, L.G., Heleno, R., Henson, K.S.E., Lopezaraiza, M.E. & Pearce, S. (2007) The conservation of ecological interactions. *Insect Conservation Biology* (eds A.J.A. Stewart, O.T. Lewis & T.R. New), pp. 226–244. CABI Publishing, Wallingford, UK.
- Memmott, J., Martinez, N.D. & Cohen, J.E. (2000) Predators, parasitoids and pathogens: species richness, trophic generality and body sizes in trophic food webs. *Journal of Animal Ecology*, **69**, 1–15.
- Memmott, J., Waser, N.M. & Price, M.V. (2004) Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society, Series B*, **271**, 2605–2611.
- Nieto-Feliner, G., Jury, S.L. & Herrero, A. (2003) *Flora Ib rica, Plantas vasculares de la Pen nsula Ib rica, e Islas Baleares, Vol. X. Araliaceae-umbelliferae*. Real Jard n Bot nico, C.S.I.C. Madrid, Spain.
- Ohara, M. *et al.* (1996) Variations in the breeding system and the population genetic structure of *Trillium kamschatcicum* (Liliaceae). *Heredity*, **76**, 476–484.

- Olesen, J.M., Eskildsen, L.I. & Venkatasamy, S. (2002) Invasion of pollination networks on oceanic islands: importance of invader complexes and endemic super generalists. *Diversity and Distributions*, **8**, 181–192.
- Paschke, M., Bernasconi, G. & Schmid, B. (2005) Effects of inbreeding and pollen donor provenance and diversity on offspring performance under environmental stress in the rare plant *Cochlearia bavarica*. *Basic and Applied Ecology*, **6**, 325–338.
- Peakall, R. & Beattie, A.J. (1991) The genetic consequences of worker ant pollination in a self-compatible, clonal orchid. *Evolution*, **45**, 1837–1848.
- Peterson, A., Bartish, I.V. & Peterson, J. (2002) Genetic structure detected in a small population of the endangered plant *Anthericum liliago* (Anthericaceae) by RAPD analysis. *Ecography*, **25**, 677–684.
- Plitmann, U. (2002) Agamospermy is much more common than conceived: a hypothesis. *Israel Journal of Plant Sciences*, **50**, S111–S117.
- Pocock, M.J.O. *et al.* (2006) Ecological correlates of range structure in rare and scarce British plants. *Journal of Ecology*, **94**, 581–596.
- Preston, C.D. *et al.* (2003) *The Changing Distribution of the Flora of the United Kingdom: Technical Report*. Center for Ecology and Hydrology–Natural Environment Research Council, Huntingdon, Cambridgeshire, UK.
- Preston, C.D., Pearman, D.A. & Dines, T.D. (2002) *New Atlas of the British and Irish Flora*. Oxford University Press, Oxford, UK.
- Proctor, M. & Yeo, P. (1973) *The Pollination of Flowers*. Collins, Glasgow, Scotland.
- Proctor, M., Yeo, P. & Lack, A. (1996) *The Natural History of Pollination*. Timber Press, Portland, OR.
- Ricogray, V. (1993) Use of plant-derived food resources by ants in the dry tropical lowlands of coastal Veracruz, Mexico. *Biotropica*, **25**, 301–315.
- Saville, N.M. (1993) *Bumblebee ecology in woodlands and arable farmland*. PhD Thesis, University of Cambridge, Cambridge, UK.
- Sih, A. & Baltus, M.S. (1987) Patch size, pollinator behavior, and pollinator limitation in catnip. *Ecology*, **68**, 1679–1690.
- Sileshi, G. (2006) Selecting the right statistical model for analysis of insect count data by using information theoretic measures. *Bulletin of Entomological Research*, **96**, 479–488.
- Solé, R.V. & Montoya, J.M. (2001) Complexity and fragility in ecological networks. *Proceedings of the Royal Society, Series B*, **268**, 2039–2045.
- Valdivia, C.E., Simonetti, J.A. & Henríquez, C.A. (2006) Depressed pollination of *Lapageria rosea* Ruiz et pav. (Philesiaceae) in the fragmented temperate rainforest of southern South America. *Biodiversity and Conservation*, **15**, 1845–1856.
- Waser, N.M. *et al.* (1996) Generalization in pollination systems, and why it matters. *Ecology*, **77**, 1043–1060.

Received 30 November 2007; accepted 22 May 2008

Handling Editor: Jan Leps

Supplementary material

The following supplementary material is available for this article:

Table S1. Plant species in the food web

Table S2. Visitor species in the food web

This material is available as part of the online article from:
<http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2664.2008.01518.x>
 (This link will take you to the article abstract).

Please note: Blackwell Publishing is not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.