Flora morphology of the resurrection plant *Chamaegigas intrepidus* Dinter and some of its potential pollinators

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Dedicated to Prof. Dr. Stefan Vogel on the occasion of his 80th birthday

Abstract

The resurrection plant *Chamaegigas intrepidus* Dinter (Scrophulariaceae) is a rare endemic species growing in ephemeral rock pools on isolated granite outcrops in Central Namibia. Previous studies suggested a high degree of gene flow within individual pools. Therefore, floral morphology, pollination and potential pollinators of the plant species were studied while the plants were at full flower set.

The zygomorphous, intensively scenting flowers carry dense layers of trichomes (400–1600 mm⁻²) on the lower lip, similar to well-known oil-flowers. Four species of potential pollinators could be found. Two of them the Hymenoptera, *Apis mellifera* and *Liotrigona bottegoi*, were found to be rare, whereas beetles of the genus *Condylops* (e.g. *Condylops erongoensis* and a new species) showed up with numbers up to 50 individuals m⁻² in some pools, visiting the flowers most frequently. Individuals of *Liotrigona* and *Condylops* were proven to carry pollen of *Chamaegigas* after their flower visits. The results are discussed in relation to the genetic variability of the plant and the phenomenon of pollen limitation in rare plant species.

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Introduction

*Chamaegigas intrepidus* Dinter (formerly *Lindernia intrepidus* Obermeyer; Scrophulariaceae s.l., tribe Lindernieae) is a perennial hemi- or criptophyte that lives endemically in shallow ephemeral rock pools on isolated granite outcrops (Inselberge) of Central Namibia (Fischer, 1992). Only some 20 sites where the species occurs are known at present (Heilmeier et al., 2005). Its
poikilohydric nature, habitat conditions and adaptations to multiple environmental stresses (repeated desiccation and rewetting, high temperatures, exposure to a UV-rich radiation, large diurnal oscillations of the pH of the pool water, extreme nutrient deficiency) have been investigated within the last 15 years both at its natural site and under laboratory conditions (for references, see the review articles of Heilmeier and Hartung, 2001; Heilmeier et al., 2005).

The distribution range of *Chamaegigas intrepidus* lies within the semi-desert and savanna transition zone of Namibia (Giess, 1969, 1997). The shallow pools in which the species grows may either occur isolated on the slopes of the rock outcrops or clumped on flat tops of the outcrops. The plants either grow dispersed or in one to several dense patches (median size: 0.018 m²) per pool (Heilmeier et al., 2005). Thus distribution of *Chamaegigas intrepidus* is characterized by a highly hierarchical pattern of temporarily suitable patches within a rather hostile landscape matrix. The population densities at the growing sites are extremely variable, and the yearly flowering is highly uncertain and unreliable in space and time.

*Chamaegigas intrepidus* is a tiny aquatic plant with two types of leaves: 8–15 mm long lanceolate submerged basal leaves on a short main axis and two decussate pairs of sessile floating leaves on top of a thin, 1.5–10 cm long stem. Within the centre of the floating rosette, two flowers are produced (Heil, 1924). The flower corolla is white/pink (cf. Plate 3A). The bilabiate appearance is dominated by the larger (6–7 mm long and 5 mm wide) lower central lobe, whereas the lateral lobes in the upper lips spread outwards only 4 mm (length) by 3 mm (width). The flowers do not produce nectar, but are characterized by a distinct fragrance. Seeds are minute, about 1.5 mm long and very rugose (Smook, 1969).

Durka et al. (2004) have performed a pollination experiment with *Chamaegigas intrepidus* and investigated the genetic diversity of this species within pools, and within and among different outcrops (sites) using AFLP techniques. They found a high genetic variability within pools, but also among sites and concluded that gene flow must happen within pools and between sites. Such a gene flow may occur by seed dispersal and pollination. Besides a possible role of birds and mammals which might carry seeds to more distant sites, an important role was attributed to insects which could act as pollinators. In this paper, it is described that, besides a small stingless bee, small beetles of the genus *Condylops* could act as pollinators. In this context, a detailed study of the morphology of the flowers and the pollen of *Chamaegigas intrepidus* was performed to elucidate the degree of associations between floral attributes and possible insect pollinators.

**Materials and methods**

Plant material of *Chamaegigas intrepidus* and insects were collected on an outcrop located on Otjua farm (Omaruru District, Central Namibia). The site is described in detail by Heilmeier et al. (2005), where a map of the site with the distribution and depth of the pools and cover by *Chamaegigas intrepidus* is given. Samples where collected on March 14, 2002, when plants were readily flowering, and stored in desiccated stage.

For scanning electron microscopy (SEM) flowers were fixed after rewetting in the laboratory in an ethanol/formalin/glacial acetic acid (90/5/5) mixture and the water was removed in an acetone series. After critical point drying samples were sputtered with platinum and investigated in a Zeiss DSM 962. Insects were sputtered without a dehydration pretreatment.

**Results**

**Flower morphology**

In Plate 1A a fully developed flower of *Chamaegigas intrepidus* is shown with its upper lip removed. The anthers with their pollen (cf. Plate 2A) are clearly visible. All the floral leaves carry large numbers of trichomes on their inner side. Most of these trichomes are found on the inner side of the lower lip along the veins with a density of up to 1600 mm⁻¹ (Plate 1B). Plate 1C shows a view into an intact flower with the stigma of the pistil (enlarged in Plate 1D). In Plate 1E and F a closed flower bud was opened before sputtering. Now the carpellate flower with pistil and stigma is clearly visible. The closed flower bud had already developed a high density of trichomes. The inner surfaces of the flower leaves resemble those of oil flowers with their oil producing elaiophores. Oil flowers have been described in the past in detail (Machado et al., 2002; Vogel, 1974, 1988; Vogel and Cocucci, 1995).

Flowers open typically during the night and are fully open at sunset. During this stage the anthers open. The stigma tends to open later, mostly until noon. Due to the slightly protandrous character of the flower, self-deposition of the granuliform, light-yellow pollen is possible. Flowering of each single flower lasts no longer than 1 day.

**Pollen morphology**

Plate 2A shows a pollen grain of an anther presented in Plate 1A. The spherical tricolpate pollen (~20 μm diameter) can also be detected between the hairs of the elytra of the *Condylops* beetles (Plate 2B–D) and on the
hindlegs of the bee Liotrigona bottegoi Magretti (Plate 2E and F), pollinators of Chamaegigas intrepidus described below. In the case of the latter pollen are glued to the legs with a cement-like material.

**Pollinators of Chamaegigas intrepidus**

During our investigations on the study site at Otjua farm rock pools with a dense cover of Chamaegigas have been observed for 10 days between 9 a.m. and 5 p.m. During this observation period four insect species could be observed, two bee species and two beetle species of the genus Condylops (Malachidae) which are commonly known as pollen eater and unspecialized pollinators (often abundantly appearing after rainfalls, Wesiak, pers. comm.).

A common honey bee (Apis mellifera) has been observed once. It is not clear whether this was a wild individual or a specimen of an apiary from the surrounding farm land. Apis mellifera does not seem to be a suitably adapted pollinator because its large size makes it very difficult to penetrate into a Chamaegigas flower. This insect could not be examined for Chamaegigas pollen; however, it cannot be excluded that *Apis mellifera* also carries some *Chamaegigas* pollen to other sites.

The small stingless bee Liotrigona bottegoi Magretti seems to be a more promising candidate for a pollinator. Two individuals have been observed a few times visiting several flowers per pool. Because of its small size the bee has no problems to completely invade the flowers. As shown in Plate 2E and F, it carries large numbers of pollen from *Chamaegigas intrepidus*. This bee species does not exhibit structures on its legs that are typical for oil collecting insects (Plates 2F and 3B).

The malachidae beetles Condylops erongoensis (Wittmer, 1985; Plates 2B, 3A and 3C) and Condylops nova spec. were most abundant on the rock pools. In pools with a cover of Chamaegigas of more than 70% some 50 individuals m⁻² were counted. They move across the rock pools on the floating leaves or around the pools along the pool margin. The beetles also visit the flowers of another resurrection plant, Craterostigma plantagineum, which grows around the margin of the granite outcrops. Thus, another source of pollen is available for them during periods when the pools are dry and *Chamaegigas* plants have withdrawn to the substrate.
While visiting the flowers, the two Condylops species fully penetrate the flower, similar to Liotrigona bottegoi (Plate 3A). This can happen from all sides of the flower. The beetles were never observed flying. However, they may be forced to do so when they have to find other flowers for harvesting pollen, as it can be the case with Craterostigma.

Ninety percent of the beetles investigated belong to the species Condylops erongoensis, the rest proved to be a new species of Condylops. This new species will be described in detail in a separate publication by Wesiak and Woitke (in preparation).

Discussion

Flowers of Chamaegigas intrepidus show a typical insect pollination syndrome: a zygomorphic flower shape, a distinct colouring and fragrance, and a rich floral reward (abundant pollen grains). A most striking feature is the huge number of trichomes. Thus, the morphology of the flowers of Chamaegigas intrepidus shows similarities with the elaiophore-bearing oil flowers, as described earlier by Vogel (1974, 1988). Oil flowers with their oil-collecting bees have also been observed in southern Africa, particularly within the Scrophulariaceae. Vogel and Cocucci (1995) give an overview of these Scrophulariaceae genera, known or suspected to have oil flowers with elaiophores that have been tested for oils. We do not know whether the trichomes of the strongly smelling Chamaegigas flowers produce oil that could be collected by bees. During the fixation and dehydration procedure for SEM any adhering oil would have been removed completely. However, Liotrigona bottegoi does not have any structures that are typical for oil-collecting bees (Plates 2B and 3B). It is therefore concluded that Chamaegigas does not live in an oil flower/oil-collecting bee symbiosis with Liotrigona bottegoi.

Other floral attributes like the zygomorphic flower shape indicate that bees might be pollinators of Chamaegigas intrepidus (Wyatt, 1983). Liotrigona bottegoi is commonly distributed in Africa. It lives in small colonies of 100–200 individuals (Brooks and Michener, 1988; Michener, 1990). However, only a small number of bees were found to visit Chamaegigas flowers in contrast to the two beetle species of the genus Condylops (Malachidae). The strong fragrance and pollen, but not nectar as a floral reward agrees with beetles as being the major pollinators. On the other hand, beetle-pollinated flowers are often actinomorphic and flat- to bowl-shaped (Wyatt, 1983). This inconsistency within the pollination syndrome suggests that, due to the harsh environment and the isolated distribution of suitable habitats within a hostile landscape matrix, plant–pollinator coevolution was less tight for Chamaegigas intrepidus than for other plant–insect systems under more favourable environmental conditions. Alternatively, beetles may have evolved as primary pollinators of Chamaegigas, since the bee fauna of southern Africa is relatively poor compared to the flora (Johnson, 2004).

Both bees and beetles may serve as effective pollinators of Chamaegigas as shown by the large number of pollen attached to their bodies’ surface. Although Condylops species are not specialized pollinators, rather the opposite, distinct pollen eaters, they may...
nevertheless pollinate exclusively *Chamaegigas intrepidus* during one foraging period, since most of the flowers in a pool and across an outcrop are opening nearly synchronously after the pools being flooded for several days (personal observations). Furthermore, apart from a few individuals of *Limosella grandiflora*, which grow mostly in deeper pools due to a lower desiccation tolerance (Heilmeier et al., 2005), *Chamaegigas intrepidus* is the only plant species flowering abundantly in the rock pools. The large number of beetles continuously visiting different flowers of *Chamaegigas intrepidus* could explain the high degree of genetic diversity within local *Chamaegigas* populations in single pools on an inselberg found by Durka et al. (2004).

This high genetic variability can only be explained by an outbreeding behaviour. On the other hand, the pollen/ovule ratio of 99 (Durka et al., 2004) is characteristic of a predominantly selfing species (Cruzen, 1977). Increased self-compatibility may be a strategy of rare plants to reduce reliance on pollinator visitation (Brigham, 2003). Pollination experiments with *Chamaegigas intrepidus*, however, yielded a reduced fruit set after artificial selfing compared to outcrossing, suggesting inbreeding depression or a weak incompatibility reaction (Durka et al., 2004). Consequently, *Chamaegigas intrepidus* is considered to be a predominantly outcrossing species with bees and beetles favouring high genetic diversity within a population on a single outcrop.

On the other hand, Durka et al. (2004) found a high genetic differentiation between individual outcrops, and genetic differentiation was not related to geographic distance. This indicates that gene flow between isolated sites within an inhabitable matrix is too low to counteract differentiation. In view of the distance between individual outcrops (minimum ca. 10 km, average ca. 25 km), Heilmeier et al. (2005) concluded that pollinators will hardly move between single inselbergs.

Small, isolated populations may not be attractive resources for pollinators, causing low visitation rates (Oostermeyer, 2003). Pollinator limitation may result in higher levels of selfing (e.g. Cruzan, 2001). In a number of cases, seed production may be reduced, which can lead to demographic consequences (Ward and Johnson, 2005). On the other hand, historically rare species may have developed adaptations for maintaining pollinator service while at low density or in small population size (Brigham, 2003). Thus, for the rare granite endemic shrub *Verticordia staminosa* rates of pollination and the subsequent proportion of flowers producing viable seeds were independent of subpopulation size (Yates and Ladd, 2004). Species that are frequently at low abundance may also use generalist pollinators who will respond to the density of flowering neighbours (Brigham, 2003). We assume that this is an important mechanism of *Chamaegigas intrepidus* which relies to a large extent on the rather unspecialized pollinators from the genus *Condylops* having been observed to visit flowers of *Craterostigma* as well. Alternatively, some rare species may reduce their reliance on pollinator visitation by increasing self-compatibility. Thus, endemic species may be preferentially cross-pollinated under ideal conditions of pollinator visitation, but can revert to self-pollination in the event of outcross failure (e.g. *Brachycome muelleri*, Jusaitis et al., 2003). For *Agalinis neoscutica* (Scrophulariaceae) precocious self-pollination in buds was found, assuring reproduction in the absence of pollinators (Stewart et al., 1996). The pollen–ovule ratio of *Agalinis neoscutica* was rather similar to the one found for *Chamaegigas intrepidus* in the present study.

The aquatic resurrection plant *Chamaegigas intrepidus*, growing in ephemeral water-filled rock pools on isolated granite outcrops within a rather unfavourable landscape matrix, represents a naturally fragmented (meta-) population. Fragmentation, being naturally or anthropogenically induced, results in low population sizes and restricted flow of pollen or seeds (Kwak et al., 1998; Sork et al., 1999). For the naturally fragmented clonal plant *Geum reptans* Pluets and Stöcklin (2004) emphasized the importance of gene flow among different populations for avoiding genetic depletion. In contrast, *Chamaegigas intrepidus*, growing in a rather irregular spatial arrangement of suitable patches within a landscape matrix with low connectivity, avoids pollen limitation and maintains a high genetic diversity within a population, apart from genetic drift and possibly high mutation rates due to excessive UV radiation (Heilmeier et al., 2005), by living in a habitat that is also attractive for an abundance of potential pollinators.

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**References**


